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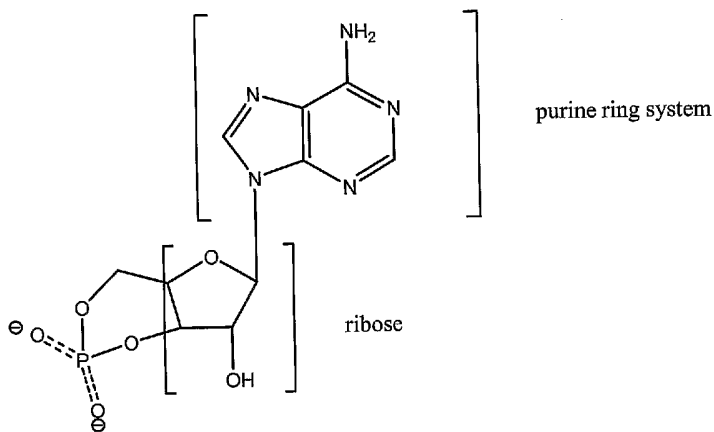
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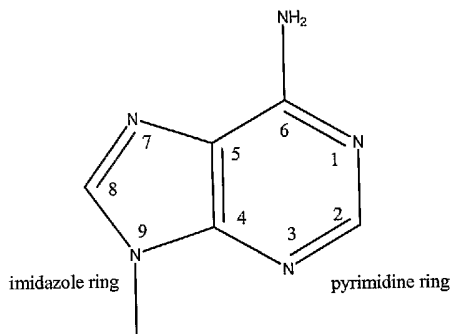
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(54) Title: COMPOSITIONS AND METHODS FOR EX VIVO PRESERVATIONS OF ORGANS



cAMP



(57) Abstract: The present invention relates to methods for the activation of cAMP-dependent protein kinase (PKA) activity and/or cGMP-dependent protein kinase (PKG) activity in organs and portions thereof suitable for transplantation as a means for preservation/maintenance of the organs and portions thereof prior to transplant into the recipient. The present invention also relates to compositions comprising an activator of PKA and/or PKG kinase activity for use in the methods of the invention.



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COMPOSITIONS AND METHODS FOR *EX VIVO* PRESERVATION OF ORGANS

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All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described herein.

1. FIELD OF THE INVENTION

20

The present invention provides methods for the activation of cAMP-dependent protein kinase activity and/or cGMP-dependent protein kinase activity in organs and functional portions thereof suitable for transplantation as a means for the preservation/maintenance of the organs and portions thereof prior to transplant into the recipient. The present invention also provides compositions comprising an activator of

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cAMP-dependent protein kinase activity and/or cGMP-dependent protein kinase activity for use in *ex vivo* preservation/maintenance of organs and functional portions thereof suitable for transplantation.

2. BACKGROUND OF THE INVENTION**2.1 Organ Preservation Solutions**

30

Adequate preservation of organs intended for transplantation is critical to the proper functioning of the organ following implantation. Long preservation times are desired to enable cross-matching of donor and recipient to improve subsequent survival, as well as to allow for coast to coast and international transportation of organs to expand the donor and

recipient pools. Organ preservation requires preservation of both the structure and function of the organ, including the specialized cells of the organ as well as the blood vessels and other cells found in the organ that together are responsible for its function.

Many different organ preservation solutions have been designed, as investigators
5 have sought to lengthen the time that an organ may remain extra-corporeally, as well as to maximize function of the organ following implantation. Several of the key solutions that have been used over the years include: 1) the Stanford University solution [see, *e.g.*, Swanson *et al.*, 1988, *Journal of Heart Transplantation*, 7(6):456-467]; 2) a modified Collins solution [see, *e.g.*, Maurer *et al.*, 1990, *Transplantation Proceedings*, 22(2):548-550;
10 Swanson *et al.*, *supra*]; and 3) the University of Wisconsin solution (U.S. Patent No. 4,798,824, issued to Belzer *et al.*). Further, U.S. Patent Nos. 5,552,267 and 5,370,989 to Stern *et al.* and a publication by Kayano *et al.*, 1999, *J. Thoracic Cardiovascular Surg.* 118:135-144 describe an organ preservation solution known as the Columbia University solution.

15 In addition to the composition of the organ preservation or maintenance solution, the method of organ preservation also affects the success of preservation. Several methods of cardiac preservation have been studied in numerous publications: 1) warm arrest/cold ischemia; 2) cold arrest/macrop perfusion; 3) cold arrest/microp erfusion; and 4) cold arrest/cold ischemia. The first method involves arresting the heart with a warm cardioplegic
20 solution prior to explantation and cold preservation, but this method fails because of the rapid depletion of myocardial energy store during the warm period. The second method, which involves arresting the heart with a cold preservation solution, is better; but continuous perfusion of the heart with preservation solution during the storage period fails because of the generation of toxic oxygen radicals. In addition, the procedure of the second
25 method is cumbersome and does not lend itself to easy clinical use. The third method, called "trickle perfusion", is better but also cumbersome. The fourth method of preservation is that of a cold cardioplegic arrest followed by a period of cold immersion of the heart. The fourth method is currently the standard method of cardiac preservation. This fourth method reliably preserves hearts for periods of up to six hours, but less than four hours is
30 considered ideal for this method.

2.2 Coronary Artery Bypass Graft (CABG)

Coronary artery disease is a major medical problem in throughout the world. Coronary arteries, as well as other blood vessels, frequently become clogged with plaque,

impairing the efficiency of the heart's pumping action, and inhibiting blood flow which can lead to heart attack and death. In certain instances, these arteries can be unblocked through relatively noninvasive techniques such as balloon angioplasty. In other cases, a bypass of the blocked vessel is necessary.

5 A coronary artery bypass graft ("CABG") involves performing an anastomosis on a diseased coronary artery to reestablish blood flow to an ischemic portion of the heart muscle. Improved long-term survival has been demonstrated bypassing the left anterior descending artery with a left internal mammary artery and this encouraged surgeons to extend revascularization with arterial grafts to all coronary arteries.

10 Since the internal mammary artery can only be used for two CABG procedures (using right and left internal mammary arteries, respectively), where multiple-vessels need to be bypassed, other arteries or veins have to be used. Such other arteries or veins that have been used include the right gastroepiploic artery, the inferior epigastric artery, the internal mammary artery (also known as the internal thoracic artery), the radial artery, and
15 the saphenous vein. The internal mammary artery is the most common arterial conduit used for CABG; yet, despite its widespread use and superior patency when compared to the saphenous vein (Grondin *et al.*, 1984, *Circulation*, 70 (suppl I): I-208-212; Camereon *et al.*, 1996, *N Engl J Med*, 334: 216-219), the saphenous vein continues to be one of the most popular conduits for CABG (Roubos *et al.*, 1995, *Circulation*, 92 (9 Suppl) II31-6).

20 During a typical coronary artery bypass graft procedure using the saphenous vein, a section of the saphenous vein is surgically removed from the leg and the graft is retained *ex vivo* (out of the body) for a length of time prior to attachment to another blood vessel within the body (Angelini and Jeremy, 2002, *Biorheology*, 39 (3-4): 491-499). In a bypass operation involving such a venous graft, the graft is harvested by a surgically invasive
25 procedure from the leg of the patient and then stored for up to about four hours *ex vivo* as the heart surgery is conducted. Although there are variations in methodology in surgical preparation of the heart, the first part of the procedure typically requires an incision through the patient's sternum (sternotomy), and in one technique, the patient is then placed on a bypass pump so that the heart can be operated on while not beating. In alternative
30 techniques, the heart is not stopped during the procedure. Having harvested and stored the saphenous vein or arterial blood vessel conduit and upon completion of the surgery to prepare the heart for grafting, the bypass procedure is performed. A precise surgical procedure is required to attach the bypass graft to the coronary artery (anastomosis), with the graft being inserted between the aorta and the coronary artery. The inserted

venous/arterial segments/transplants act as a bypass of the blocked portion of the coronary artery and thus provide for a free or unobstructed flow of blood to the heart. More than 500,000 bypass procedures are performed in the United States every year.

5 The overall short and long term success of the CABG procedure is dependent on several factors including the condition of the graft that is to be inserted which itself depends on any form of damage during the removal of the graft from the body or deterioration or damage of the graft due to storage conditions. In such circumstances, the short term detrimental effect can be potentially lethal thrombotic disease as a result of contact of flowing blood with a changed phenotype of the graft due to its deterioration or damage
10 during the removal or storage stage. Possible long term detrimental effects include the vein graft itself becoming diseased, stenosed, or occluded, similar to the original bypassed vessel. In this case, the diseased or occluded saphenous vein grafts are associated with acute ischemic syndromes necessitating some form of intervention. It is, therefore, of critical importance not only that care be taken in the surgical procedure to remove the blood
15 vessel to be used as the graft in surgical bypass procedures including CABG, but, also that no deterioration or damage occurs in the storage period of the graft prior to attachment to another blood vessel and the resumption of blood flow in that vessel.

2.3 Cyclic cAMP and cGMP-dependent Protein Kinases

20 The cAMP dependent protein kinase is, as its name infers, a kinase, which by definition transfers a phosphate moiety to various substrates. It is a key effector limb of the cAMP signaling cascade, in that when cAMP binds to it, its kinase activity is activated. Agents which stimulate the cAMP pathway can do so at any of a number of levels in the pathway. For instance, at the cell surface, the pathway is potently stimulated by beta
25 adrenergic receptor agonists, such as norepinephrine, dobutamine, or any of a host of other endogenous or synthetic compounds. The pathway can also be stimulated by inhibiting inhibitory G-proteins within the cell membrane (with an agent such as pertussis toxin), or by directly stimulating the catalytic moiety which generates cAMP (adenylate cyclase), with an agent such as forskolin. The pathway then converges inside the cell with the generation of
30 cAMP, which activates the cAMP-dependent protein kinase (also termed protein kinase A, or PKA), which then exerts a myriad of effects. The cAMP-dependent protein kinase has several isozymes (for instance, PKA I and PKA II). These isozymes are regulated by the alpha-subforms (RI alpha and RII alpha) of the regulatory subunits of PKA I and PKA II, and for the alpha- and beta-subforms (C alpha and C beta) of the catalytic subunits of PKA.

The cGMP cascade is stimulated by different initial mediators, for instance, nitric oxide or carbon monoxide stimulate an enzyme called guanylate cyclase, which can be either soluble or particulate, which mediates formation of cGMP. cGMP and cAMP share many similar features, sometimes activating similar or even the same kinases. The cyclic
5 GMP-dependent protein kinase, PKG, can be stimulated with a variety of analogs, as can the cAMP dependent protein kinase, and it is subjected to similar regulation. Therefore, by analogy with cAMP, its activity can be stimulated or suppressed by agents which activate (or inhibit) the catalytic or regulatory (stimulatory or inhibitory) subunits of the enzyme.

PKG exhibits two primary intrasubunit cGMP binding sites. Both of these binding
10 sites show high specificity for the cyclic part of the molecule containing the ribose sugar and attached phosphate. There is much less binding exhibited for the guanine moiety of cGMP. Modifying the pyrimidine part of guanine, especially at C-1, increases selectivity for the rapidly exchanging site whereas modifications to guanine at C-7 and C-8 more effectively increases selectivity for the slowly exchanging site. The potency of cGMP are
15 most potent (in terms of kinase activity) when they bind both binding sites than either site alone.

In order to decrease the likeliness of short and long term detrimental consequences of grafting organs and blood vessels in surgical procedures, including coronary arterial bypass grafting (CABG), and consequently to improve the overall outcome of patients
20 undergoing such procedures, there is a need for improved storing conditions for such organs and vascular grafts during the time period from harvesting of the organ or graft to implanting the organ or attaching the graft to another blood vessel in the patient.

Citation or identification of any reference in Section 2 or in any other section of this application shall not be construed as an admission that such reference is available as prior
25 art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is directed to a solution comprising an activator of adenosine 3',5'-cyclic monophosphate (cAMP) dependent protein kinase (PKA) activity, which activator:

30 (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of adenosine 3',5'-cyclic monophosphate (cAMP), Sp-adenosine 3',5'-cyclic monophosphate (Sp-cAMPS) (the S isomer of cAMP), dibutyryl adenosine 3',5'-cyclic

monophosphate (db cAMP) and 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP);

(b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP;

5 and/or

(c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively,

10 for preserving an organ, or a portion thereof, removed from an individual.

In a specific embodiment, the foregoing activator of PKA kinase activity also (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (8-CPT cAMP) and N⁶-Benzoyladenosine 3',5'-cyclic monophosphate (6-NHC(O)C₆H₅-cAMP or 6-Bnz cAMP);

15 (b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of 8-CPT cAMP and 6-Bnz cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively.

20 The present invention is also directed to a solution comprising an activator of guanosine 3',5'-cyclic monophosphate (cGMP) dependent protein kinase (PKG) activity, which activator:

(a) activates PKG, and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP;

25 (b) activates PKG, and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or

(c) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP,

30 respectively,

for preserving an organ, or a portion thereof, removed from an individual.

In a particular embodiment, the present invention is directed to a solution comprising an activator of PKA and an activator of PKG. The organ or portion thereof can be used as a transplant in the same or different individual from which the organ was

harvested. In certain embodiments of the present invention, the organ is selected from the group consisting of heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessel. In a particular embodiment, where the organ is a blood vessel or a functional portion thereof, the blood vessel is one or a combination of a mammary artery, a radial artery, an internal mammary artery (an internal thoracic artery), right gastroepiploic artery, inferior epigastric artery, or a saphenous vein.

Not to be limited to a particular mechanism of action, it is believed that the use of an activator of PKA or PKG enhances the viability of the organs and will result in less damage or phenotypic change of the organ as a result of storage conditions. Thus, it is believed that organs so treated will improve short and long term outcomes of transplant procedures, including heart transplants and vascular bypass procedures involving blood vessel grafts, including, but not limited to, coronary artery bypass, abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, or popliteal aneurysm repair. In one embodiment of the present invention, the preservation solution does not contain adenosine 3',5'-cyclic monophosphate (cAMP), Sp-adenosine 3',5'-cyclic monophosphate (Sp-cAMPS) (the S isomer of cAMP), dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP), 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP), guanosine 3',5'-cyclic monophosphate (cGMP), 8-bromo-guanosine 3',5'-cyclic monophosphate (8-bromo cGMP) and/or 8-(4-chlorophenylthio)-guanosine 3',5'-cyclic monophosphate (8-CPT cAMP). In yet another embodiment of the present invention, the preservation solution also does not contain 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (8-CPT cAMP) and/or N⁶-Benzoyladenosine 3',5'-cyclic monophosphate (6-NHC(O)C₆H₅-cAMP or 6-Bnz cAMP).

Accordingly, the present invention provides a solution comprising an activator of cAMP-dependent protein kinase (PKA) and/or cGMP-dependent protein kinase (PKG).

The activator of PKA (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of adenosine 3',5'-cyclic monophosphate (cAMP), Sp-adenosine 3',5'-cyclic monophosphate (Sp-cAMPS) (the S isomer of cAMP), dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP) and 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP); (b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively. In a specific embodiment, the foregoing activator of PKA

kinase activity also (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of 8-CPT cAMP and 6-Bnz cAMP; (b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of 8-CPT cAMP and 6-Bnz cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an
5 at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively. In a preferred embodiment, the activator of PKA is an analogue of cAMP.

The activator of PKG (a) activates PKG, and is at least 25% more cell membrane
10 permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (b) activates PKG, and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (c) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase
15 activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively. Alternatively, the present invention provides a solution consisting of an activator of cAMP-dependent protein kinase (PKA) and/or cGMP-dependent protein kinase (PKG). In a preferred embodiment, the activator of PKG is an analogue of cGMP.

In an alternative embodiment, the solutions can further comprise or consist of
20 heparinized blood. The present invention also provides a method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG. In another embodiment, the solution employed in the method further comprises heparinized blood. The present invention also provides a method of preserving an organ comprising contacting an isolated organ or
25 portion thereof *ex vivo* with a solution consisting of an activator of PKA and/or PKG. Optionally, the solution consists of the activator and heparinized blood.

An activator of PKA for use in the present invention (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of adenosine 3',5'-cyclic monophosphate (cAMP), Sp-adenosine 3',5'-cyclic monophosphate (Sp-cAMPS) (the S
30 isomer of cAMP), dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP) and 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP); (b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase

activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively. In a preferred embodiment, the activator binds with at least 25% greater affinity to the regulatory subunit of PKA and results in an at least 25% greater level of kinase activity as compared to each of cAMP, Sp-cAMPS, db cAMP, and 8-bromo cAMP. In a specific embodiment, the foregoing activator of PKA kinase activity also (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of 8-CPT cAMP and 6-Bnz cAMP; (b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of 8-CPT cAMP and 6-Bnz cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively. In another preferred embodiment, the activator of PKA is an analogue of cAMP.

In specific embodiments, the increased cell membrane permeability is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the cell membrane permeability of each of cAMP, db cAMP and 8-bromo cAMP. In other specific embodiments, the increased binding affinity to the regulatory subunit is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the binding affinity of each of cAMP, db cAMP and 8-bromo cAMP. In yet other specific embodiments, the increased kinase activity is at least at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the level of kinase activity resulting from exposure of PKA to the same amount of each of cAMP, db cAMP and 8-bromo cAMP, respectively.

In other specific embodiments, the increased cell membrane permeability also is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the cell membrane permeability of each of 8-CPT cAMP and 6-Bnz cAMP. In other specific embodiments, the increased binding affinity to the regulatory subunit also is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the binding affinity of each of 8-CPT cAMP and 6-Bnz cAMP. In yet other specific embodiments, the increased kinase activity also is at least at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the

level of kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively.

An activator of PKG for use in the present invention (a) activates PKG, and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (b) activates PKG, and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (c) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively. In a preferred embodiment, the activator binds with at least 25% greater affinity to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP. In another embodiment, the activator of PKG is an analogue of cGMP.

In specific embodiments, the increased cell membrane permeability is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the cell membrane permeability of each of cGMP, 8-bromo cGMP and 8-CPT cGMP. In other specific embodiments, the increased binding affinity to the regulatory subunit is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the binding affinity of each of cGMP, 8-bromo cGMP and 8-CPT cGMP. In yet other specific embodiments, the increased kinase activity is at least at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the level of kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

In other embodiments of the present invention, an isolated *ex vivo* organ or portion thereof is provided, which organ or portion thereof is in contact with a solution comprising an activator of PKA and/or PKG. The present invention also provides an isolated *ex vivo* isolated organ or portion thereof in contact with a solution comprising heparinized blood and an activator of PKA and/or PKG. In certain embodiments of the present invention, the organ is selected from the group consisting of heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessel. In a particular embodiment, the organ is a blood vessel or a functional portion thereof is one or a combination of an internal mammary artery (internal thoracic artery), a radial artery, right gastroepiploic artery, inferior epigastric artery, or a saphenous vein. As used herein, a functional portion of an organ is a portion of

sufficient size for transplantation. As used herein, a functional portion of a blood vessel is a portion of a blood vessel of sufficient size as to be able to act as a vascular graft.

The present invention also provides a container containing an organ or portion thereof in contact with a solution comprising an activator of PKA and/or PKG.

5 Alternatively, the container contains the blood vessel or portion thereof in contact with a solution comprising heparinized blood and an activator of PKA and/or PKG.

Further, the present invention provides a method of using an organ in a transplantation procedure comprising contacting an isolated organ or functional portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG; and
10 transplanting the organ into a patient. The present invention also provides a method for performing an organ transplantation in a patient comprising removing from contact with an organ or functional portion thereof a solution comprising a an activator of PKA and/or PKG; and transplanting the organ or functional portion thereof into the patient.

Alternatively, the present invention provides a method of using a blood vessel as a
15 vascular graft comprising contacting an isolated blood vessel or functional portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG; and inserting the blood vessel into a patient so as to form a vascular graft in the patient. The present invention also provides a method for performing a coronary artery bypass graft in a patient comprising removing from contact with a blood vessel or functional portion thereof a solution
20 comprising a an activator of PKA and/or PKG; and grafting the blood vessel or functional portion thereof into the patient so as to serve as a coronary bypass graft.

4. BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1B. Figure 1A shows the chemical structure of adenosine 3',5'-cyclic monophosphate (cAMP) and specifically points out the purine ring system and the ribose
25 moiety of the molecule. Figure 1B shows the chemical structure of the purine ring system of cAMP and the imidazole and pyrimidine ring sub-parts of the purine ring system.

5. DETAILED DESCRIPTION OF THE INVENTION

It is believed that the use of an activator of PKA or PKG of the invention enhances the viability of the organs and will result in less damage or phenotypic change of the organ
30 as a result of storage conditions. Thus, it is believed that organs so treated will improve short and long term outcomes of transplant procedures, including heart transplants and vascular bypass procedures involving blood vessel grafts, including, but not limited to,

coronary artery bypass, abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, or popliteal aneurysm repair. Exemplary organs that can be so isolated include heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessels. In particular embodiments where the organ is a blood vessel, such blood vessels that can be isolated include, but are not limited to, a saphenous vein, a mammary artery, a renal artery, and a radial artery.

5.1 Preservation Solutions

The present invention is directed to use of a solution comprising an activator of cAMP-dependent protein kinase (PKA) activity. The present invention is also directed to a solution comprising an activator of cGMP-dependent protein kinase (PKG) activity. In specific embodiments, the solution can contain more than one activator of PKA and/or PKG. The solution can be an aqueous solution or a semi-solid gel. In a preferred embodiment, the solution is an aqueous solution. Any physiologic solution to which the activators are added can be used in the present invention so long as the solution does not damage the organ tissue that is placed in it. For example, the solution comprising the activator can be saline, buffered saline, phosphate buffered saline. The solution can also be Hank's Balanced Salt solution (HBSS), which typically comprises 1.26 mM CaCl_2 , 5.36 mM KCl, 0.44 mM KH_2PO_4 , 0.81 mM MgSO_4 , 136 mM NaCl, 0.42 mM Na_2HPO_4 , 6.1 mM glucose, 20 mM HEPES-NaOH, at pH 7.4 (Herreros *et al.*, 2000, *J. Neurochem* 74(5):1941-1950) or modified HBSS, which typically comprises 143 mM NaCl, 5.6 mM KCl, 2mM MgCl_2 , 10 mM HEPES, 10 mM glucose, 0.2 mM CaCl_2 , and 0.4% BSA, at pH 7.2 (Bridson *et al.*, 1999, *Blood* 93:3847-3855). The solution can also be Ringer's Lactate, which typically comprises 155 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 2 mM NaH_2PO_4 , 10 mM HEPES, and 10 mM glucose, at pH 7.2 (Sturgill-Koszycki and Swanson, 2000, *J Exp. Med.* 192:1261-1272). The solution can also be Tyrodes buffer, which typically comprises 137 mM NaCl, 12 mM NaHCO_3 , 26 mM KCl, 5.5 mM glucose, 0.1% BSA, and 5.0 mM Hepes at pH 7.35 (Kasirer-Friede *et al.*, 2002, *J. Biol. Chem.*, 277:11949-11956). The solution can also be Krebs's buffer, which typically comprises 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.17 MgSO_4 , 25 mM NaHCO_3 , 1.18 mM KH_2PO_4 , 0.026 mM EDTA, and 5.5 mM glucose (Knock *et al.*, 2002, *J. Physiology* 538:879-890).

The solutions of the present invention can also contain a variety of additional additives, such as vasodilators, as detailed *infra*. In a preferred embodiment, the solution comprises heparinized blood and the activator of PKA and/or PKG kinase activity.

Alternatively, the solution consists of an activator of PKA and/or PKG in heparinized blood. In a preferred embodiment, the solution is sterilized.

In an embodiment of the present invention, the preservation solution further comprises a vasodilator in an amount sufficient to maintain vascular homeostasis.

5 Preferably, the vasodilator is cell membrane permeable. The vasodilator can be selected from the group including, but not limited to, adenosine 3',5'-cyclic monophosphate (cyclic adenosine monophosphate, cyclic-AMP, or cAMP), guanosine 3',5'-cyclic monophosphate (cyclic guanosine monophosphate, cyclic-GMP, or cGMP), additional analogues of cyclic nucleotides (additional to those analogues of the present invention described herein),
10 nitroglycerin, adenosine, and pertussis toxin. Suitable combinations of the vasodilators may be used. The additional analogues of cAMP can be selected from the group including, but not limited to, dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP), and 8-bromo-adenosine 3',5'-cyclic monophosphate. Other suitable additional analogues of cAMP may also be used. Exemplary analogues of cAMP are listed in the catalog at the website of
15 BIOLOG Life Science Institute, Bremen, Germany, the address of which is BIOLOG.de. Preferably, the additional analogue is cell membrane-permeable. The optimal concentration of db cAMP is believed to be about 2 mM, though in specific embodiments, db cAMP concentrations of about 1 mM, or of about 2 to 4 mM can be used. It is known that db cAMP concentrations higher than about 4 mM become toxic to endothelial cells. Hence, 2
20 mM is considered to be the optimal concentration of db cAMP. In a preferred embodiment, the concentration of db cAMP ranges from about 1 mM to about 4 mM. The term "about" as used herein is intended to cover the range of experimental variation.

An exemplary solution of the invention further comprises db cAMP, alone or in combination with nitroglycerin and adenosine. In another embodiment, a solution of the
25 invention further comprises 8-bromo cAMP, alone or in combination with nitroglycerin and adenosine. In a specific embodiment, the concentration of nitroglycerin ranges from about 0.05 g/l to about 0.2 g/l. In another specific embodiment, the concentration of adenosine ranges from about 3 mM to about 20 mM.

In certain embodiments, the solutions of the present invention also comprise a sugar,
30 for example, D-glucose, *e.g.*, in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics. In a preferred embodiment, the concentration of the sugar ranges from about 50 mM to about 80 mM. The solutions can also comprise magnesium ions, *e.g.*, in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics. In a preferred embodiment, the concentration of

magnesium ions ranges from about 2 mM to about 10 mM. In particular, the magnesium ions can be derived from magnesium sulfate, magnesium gluconate, or magnesium phosphate, or suitable combinations thereof. The magnesium ions can also be derived from some other suitable magnesium containing compound. D-Glucose, adenosine, and
5 magnesium ions are substrates for adenosine triphosphate (ATP) synthesis. Metabolic substrates such as D-glucose and perhaps adenosine for ATP formation are probably important for maintaining the small degree of anaerobic metabolism that occurs during *ex vivo* preservation of blood vessels. Basal energy metabolism (even during hypothermia) can be supported by the anaerobic metabolism of D-glucose. The presence of magnesium ion
10 allows for the proper functioning of the enzymes needed for adenosine triphosphate (ATP) synthesis. In general, substrates for ATP synthesis are helpful to allow intracellular function and maintenance of cellular bioenergetics.

In other embodiments, the preservation solution also comprises a macromolecule of molecular weight greater than 20,000 daltons, *e.g.*, in an amount sufficient to maintain
15 endothelial integrity and cellular viability. In a preferred embodiment, the macromolecule of molecular weight greater than 20,000 daltons is a macromolecule having a molecular weight greater than about 100,000 daltons, a polysaccharide, or a polyethylene glycol. Other suitable macromolecules can be used. The macromolecule of molecular weight greater than 20,000 daltons can be a colloid. In a preferred embodiment, the polysaccharide
20 is a dextran. Furthermore, in a preferred combination, the dextran is a dextran molecule having a molecular weight of 308,000 daltons. Macromolecules of molecular weight greater than 20,000 daltons are believed to be helpful in reducing trans-endothelial leakage and subsequent intracellular and interstitial edema in the reperfusion period, by serving to plug small endothelial leaks which may occur. Macromolecules may thus also prevent the
25 extravasation of intravascular contents into the pericellular space, thus helping to prevent cellular swelling and rupture during the preservation and recovery periods.

The osmolarity of the preservation solution of the invention is also a factor in helping to prevent cellular swelling and rupture. The osmolarity of the solution should be greater than the cellular osmolarity. Cellular osmolarity is about 290 mOSm/l. In a
30 preferred embodiment, the osmolarity ranges from about 315 mOSm/l to about 340 mOSm/l.

The preservation solution also optionally comprises potassium ions, preferably in a concentration greater than about 110 mM. The potassium ions can be derived from potassium sulfate, potassium gluconate, monopotassium phosphate (KH_2PO_4), or suitable

combinations thereof. The potassium ions can also be derived from some other suitable potassium containing compound. In a preferred embodiment, the concentration of potassium ions ranges from about 110 mM to about 140 mM.

In other embodiments, the preservation solution also comprises a buffer in an amount sufficient to maintain the average pH of the solution during the period of blood vessel preservation at about the physiologic pH value. In a preferred embodiment, the buffer is monopotassium phosphate (KH_2PO_4). However, other suitable buffers may be used. The buffering capacity should be adequate to buffer the organic acids that accrue during ischemia. Because basal metabolism results in the generation of acid, preferably a buffering system is used. The pH of the solution can decline during prolonged storage times that can be employed with this solution. In a preferred embodiment, the initial pH of the preservation solution is adjusted to the alkaline side of normal physiologic pH because then the average pH during storage of the blood vessel in the preservation solution remains physiologic. Normal physiologic pH is about 7.4. A preferred embodiment of the preservation solution has a pH range of about 7.3 to about 7.6. The pH may be adjusted to the desired value with the addition of a suitable base, such as potassium hydroxide (KOH). Hence, during the period of preservation, the pH of the preservation solution starts on the alkaline side of physiologic pH, and may drift slowly down to the acidic side of physiologic pH. But the average pH of the preservation solution during the period of preservation is preferably about the physiologic value.

In other embodiments, the preservation solution may further comprise impermeant anions, *e.g.*, in an amount sufficient to help maintain endothelial integrity and cellular viability. The impermeant anion can be the gluconate anion or the lactobionate anion. Other suitable impermeant anions can be used. In a preferred embodiment, the concentration of the gluconate anion ranges from about 85 mM to about 105 mM. The gluconate anion can be derived from potassium gluconate or magnesium gluconate. The gluconate anion can also be derived from some other suitable gluconate containing compound. Impermeant anions are large anions that cannot cross cell membranes, so that sodium is at least in part prevented from diffusing down its concentration gradient into the cell during the preservation period. Impermeant anions thus help to prevent cellular edema.

The preservation solution may further comprise an anticoagulant, *e.g.*, in an amount sufficient to help prevent clotting of blood within the capillary bed of the blood vessel. The anticoagulant can be heparin or hirudin. Other suitable anticoagulants may be used. In a preferred embodiment, the concentration of heparin ranges from about 1000 units/l to about

100,000 units/l. Anticoagulants are believed to help in preventing clotting of blood within the capillary bed of the preserved blood vessel. Specifically, anticoagulants are believed to help prevent a total no-reflow phenomenon at the level of the microcirculation, which would be undesirable following re-implantation and could result in graft failure.

- 5 Anticoagulants are believed to be helpful in ensuring that thrombosis does not occur during or after preservation, so that nutrient delivery and toxin removal can proceed.

In yet another embodiment, the preservation solution may further comprise an antioxidant, *e.g.*, in an amount sufficient to help decrease reperfusion injury secondary to oxygen free radicals. The antioxidant can be butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Vitamin C, Vitamin E, or suitable combinations thereof. Other
10 suitable antioxidants can be used. In a preferred embodiment, the antioxidant is butylated hydroxyanisole (BHA) at a concentration range from about 25 μ M to about 100 μ M, alone or in combination with butylated hydroxytoluene (BHT) at a concentration range from about 25 μ M to about 100 μ M. The preservation solution can further comprise a reducing
15 agent, *e.g.*, in an amount sufficient to help decrease reperfusion injury secondary to oxygen free radicals. Any suitable reducing agent can be used.

Optionally, the preservation solution may further comprise N-acetylcysteine in an amount sufficient to help cells produce glutathione. In a preferred embodiment, the concentration of N-acetylcysteine ranges from about 0.1 mM to about 5 mM. N-
20 acetylcysteine is an agent which can enter cells and is believed to play a role in helping cells to produce glutathione, which is a reducing agent. During blood vessel preservation, glutathione is lost. Simply adding glutathione to the preservation solution, however, would likely be of little to no help, because it is now known that glutathione in solution does not enter easily into the cell.

25 In another optional embodiment, the preservation solution may further comprise an agent that helps prevent calcium entry into cells in an amount sufficient to help prevent calcium entry into cells. Agents that help prevent calcium entry into cells include so-called calcium channel blockers, as well as other agents that serve the described function. An agent that helps prevent calcium entry into cells that can be used is verapamil. Other
30 suitable agents that help prevent calcium entry into cells may be used. In a preferred embodiment, the concentration of verapamil ranges from about 2 μ M to about 25 μ M. Agents that help prevent calcium entry into cells are believed to play a role in preventing calcium overload.

Optionally, the solution does not contain sodium. The absence of sodium in the solution is preferred, since any sodium which may enter the cells during the period of preservation (when energy currency is low and the normal trans-cellular gradient may not be well maintained) may 1) lead to cellular swelling, 2) cause calcium entry by facilitated diffusion (following re-implantation), and 3) sodium load the cell, such that a high amount of energy is required following reestablishment of blood flow before a normal membrane potential can be re-established.

In other embodiments, the preservation solution can further comprise a bacteriostat, in an amount sufficient to help inhibit the growth of, or destroy, bacteria. The bacteriostat can be cefazolin or penicillin. Other suitable bacteriostats or antibiotics can be used. In a specific embodiment, the concentration of cefazolin ranges from about 0.25 g/l to about 1 g/l. The addition of an antibiotic to the solution is a surgical consideration, due in one embodiment to the practical inability of sterilizing the solution completely, as the high molecular weight solutes would not pass through a 0.2 micron membrane filter which may be used in the preparation of the preservation solution. It is believed that gamma irradiation may be used to better sterilize the solution.

In other embodiments, the solution can be any organ preservation solution known in the art in combination with the activator(s) of PKA and/or PKG kinase activity. Illustrative examples of organ preservation solutions include, but are not limited to, the Euro-Collins solution, the University of Wisconsin solution, the low-potassium dextran glucose solution (Perfadex™), the Celsior™ solution, and the Columbia University solution. In general, these solutions contain electrolytes and, optionally, sugars. One illustrative solution comprises a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics, *e.g.*, glucose, D-glucose; magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics; potassium ions; and a buffer, *e.g.*, a monopotassium phosphate or bicarbonate buffer, in an amount sufficient to maintain the average pH of the solution at about the physiologic pH value, *i.e.*, about pH 7.3 to about pH 7.6. The specific composition of certain known solutions is listed below.

The Euro-Collins solution is described in Maurer *et al.*, 1990, *Transplantation Proceedings* 22:548-550 and in Swanson *et al.*, 1988, *J. Heart Transplantation* 7(6):456-467, and typically comprises 115 mM potassium, 10 mM sodium, 8 mM magnesium, 10 mM bicarbonate, 100 mM phosphate, and 120 mM glucose, at a pH of about 7.4 and with an osmolality of about 452 mOsm/L.

The University of Wisconsin solution is described in U.S. Patent No. 4,798,824 to Belzer *et al.*, and typically comprises 125 mM potassium, 30 mM sodium, 5 mM magnesium, 25 mM phosphate, 5 mM sulfate, 100 mM lactobionate, 50 mM hydroxyethyl starch, 5 mM adenosine and 1 mM allopurinol, at a pH of about 7.4 and with an osmolality of about 327 mOsm/L.

The low-potassium dextran glucose solution (Perfadex™, commercially available from VitroLife, Gothenberg, Sweden), and typically comprises 4 mM potassium, 165 mM sodium, 2 mM magnesium, 101 mM chloride, 34 mM phosphate, 2 mM sulfate, 20 mM Dextran-40, and 56 mM glucose, at a pH of about 7.4 and with an osmolality of about 335 mOsm/L.

The Celsior™ solution is commercially available from Sangstat Medical Corporation, Freemont, CA, and typically comprises 60 mM mannitol, 80 mM lactobionic acid, 20 mM glutamic acid, 30 mM histidine, 0.25 mM calcium, 15 mM potassium, 13 mM magnesium, 100 mM sodium hydroxide, and 3 mM reduced glutathione, at a pH of about 7.3 and with an osmolality of about 320-360 mOsm/L.

The Columbia University solution is described in U.S. Patent Nos. 5,370,989 and 5,552,267 to Stern *et al.*, and typically comprises 120 mM potassium, 5 mM magnesium, 25 mM phosphate, 5 mM sulfate, 95 mM gluconate, 50 mM Dextran 50, 67 mM glucose, 5 mM adenosine, 2 mM dibutyladenosine 3',5'-cyclic monophosphate (db cAMP), 0.1 mg/ml nitroglycerin, 50 µM butylated hydroxyanisole, 50 µM butylated hydroxytoluene, 0.5 mM N-acetylcysteine, 10 U/ml heparin, and 10 µM verapamil, at a pH of about 7.6 and with an osmolality of about 325 mOsm/L.

In a specific embodiment, a preservation solution of the present invention comprises, or alternatively consists of, an activator of PKA and/or PKG kinase activity; a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics; magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics; a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability; potassium ions in a concentration greater than about 110 mM; and a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting step at about the physiologic pH value. Optionally, the preservation solution further comprises a vasodilator in an amount sufficient to maintain vascular homeostasis, wherein the vasodilator is cAMP, db cAMP, 8-bromo cAMP, cGMP, 8-bromo cGMP, 8-CPT cGMP, nitroglycerin or pertussis toxin.

In another specific embodiment, a preservation solution of the present invention comprises, or alternatively consists of, an activator of PKA and/or PKG kinase activity; 67.4 mM D-glucose; 5 mM magnesium sulfate (MgSO_4); 25 mM monopotassium phosphate (KH_2PO_4); 50 g/l dextran (molecular weight 308,000 daltons); 95 mM potassium gluconate (K-gluconate); 50 μM butylated hydroxyanisole (BHA); 50 μM butylated hydroxytoluene (BHT); 0.5 mM N-acetylcysteine; 5 mM adenosine; 0.1 g/l nitroglycerin; 10 μM verapamil; 2 mM dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP); 10,000 units heparin; and 0.5 g/l cefazolin. The pH is adjusted to 7.6 with potassium hydroxide.

The amount of the preservation solution required in a surgical procedure, such as organ transplantation or a cardiac arterial bypass graft (CABG), would be clear to one who is skilled in such surgical procedures, and depends, *inter alia*, upon the size of the organ and the particular container used to hold the organ and solution.

The preservation solution is suitable for use at the low temperatures that may be required or desired during vascular bypass, *e.g.*, CABG, or other surgical procedure. For instance, temperatures of about 0.5 to about 10 degrees Centigrade, preferably 4°C, may be used during CABG or other surgical procedure.

The present invention is also directed to a container containing a preservation solution of the present invention. In a preferred embodiment, the container is one of certain dimensions useful in preserving an organ such as a liver or heart and lungs. In a more preferred embodiment, the container is one of certain dimensions useful in preserving a blood vessel. In another embodiment, the container also contains the organ or a portion thereof, in contact with the solution. In yet another embodiment, the container also contains the blood vessel or functional portion thereof, intended for use as a vascular graft, in contact with the solution.

5.2 Cyclic cAMP- and cGMP-dependent Protein Kinase Activators

The preservation solutions of the present invention comprise an activator of cAMP-dependent protein kinase (PKA) activity and/or an activator of cGMP-dependent protein kinase (PKG) activity.

An activator of PKA for use in the present invention (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of adenosine 3',5'-cyclic monophosphate (cAMP), Sp-adenosine 3',5'-cyclic monophosphate (Sp-cAMPS) (the S isomer of cAMP), dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP) and 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP); (b) activates PKA, and

binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively. In a preferred embodiment, the activator binds with at least 25% greater affinity to the regulatory subunit of PKA and results in an at least 25% greater level of kinase activity as compared to each of cAMP, Sp-cAMPS, db cAMP, and 8-bromo cAMP.

In a specific embodiment, the foregoing activator of PKA kinase activity also (a) activates PKA, and is at least 25% more cell membrane permeable as compared to each of 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (8-CPT cAMP) and N⁶-Benzoyladenosine 3',5'-cyclic monophosphate (6-NHC(O)C₆H₅-cAMP or 6-Bnz cAMP); (b) activates PKA, and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of 8-CPT cAMP and 6-Bnz cAMP; and/or (c) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively. In another specific embodiment, the activator of PKA kinase activity is an analogue of cAMP. In another embodiment, the activator of PKA kinase activity is an analogue of cAMP, with the proviso that the analogue is not Sp-cAMPS, db cAMP or 8-bromo cAMP. In yet another embodiment, the activator of PKA kinase activity is an analogue of cAMP, with the proviso that the analogue is not Sp-cAMPS, db cAMP, 8-bromo cAMP, 8-CPT cAMP or 6-Bnz cAMP.

In specific embodiments, the increased cell membrane permeability is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the cell membrane permeability of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP. In other specific embodiments, the increased binding affinity to the regulatory subunit is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the binding affinity of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP. In yet other specific embodiments, the increased kinase activity is at least at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the level of kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

In other specific embodiments, the increased cell membrane permeability also is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the cell membrane permeability of each of 8-CPT cAMP and 6-Bnz cAMP. In other specific embodiments, the increased binding affinity to the regulatory subunit also is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the binding affinity of each of 8-CPT cAMP and 6-Bnz cAMP. In yet other specific embodiments, the increased kinase activity also is at least at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the level of kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively.

An activator of PKG for use in the present invention (a) activates PKG, and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (b) activates PKG, and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (c) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively. In a preferred embodiment, the activator binds with at least 25% greater affinity to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP. In another embodiment, the activator of PKG is an analogue of cGMP.

In specific embodiments, the increased cell membrane permeability is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the cell membrane permeability of each of cGMP, 8-bromo cGMP and 8-CPT cGMP. In other specific embodiments, the increased binding affinity to the regulatory subunit is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the binding affinity of each of cGMP, 8-bromo cGMP and 8-CPT cGMP. In yet other specific embodiments, the increased kinase activity is at least at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% greater or more as compared to the level of kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

Further, as used herein, kinase activity of PKA or PKG refers to the enzymatic ability of PKA or PKG, respectively, to phosphorylate a substrate, *i.e.*, another molecule or itself (auto-phosphorylation). Any method known in the art can be used to measure the kinase activity of PKA or PKG. Illustrative methods that can be used for measuring PKA or PKG kinase activity resulting from exposure to an activator are described in Øgreid *et al.*, 1983, J. Biol. Chem. 258:1041-1049; De Gunzburg *et al.*, 1984, Biochemistry 23:3805-3812; Øgreid *et al.*, 1985, Eur. J. Biochem. 150:219-227; and Vaandrager *et al.*, 1997, J. Biol. Chem. 272:11816-11823. For example, a substrate, *e.g.*, a protein or a synthetic peptide, is contacted with PKA or PKG in the presence of a putative PKA or PKG activator and γ -[³²P] ATP under conditions appropriate for kinase activity of PKA or PKG. The substrate is then separated from the reaction mixture and the amount of radioactivity in the substrate is determined, *e.g.*, with a scintillation counter. The amount of ³²P transferred to the substrate is directly proportional to the kinase activity of PKA or PKG. Data are compared to the results of the same assay performed with a known, control activator, *e.g.* cAMP, db cAMP, 8-bromo cAMP, cGMP, 8-bromo cGMP or 8-CPT cGMP, to establish the relative ability of the putative activator to activate PKA or PKG kinase activity.

As used herein, increased binding affinity to the regulatory subunit of the kinase (PKA or PKG) includes increased binding to the subunit A binding site and/or increased binding to the subunit B binding site. Binding affinity to the regulatory subunit A or B site can be measured by any method known in the art. For example, the affinity of a putative activator of PKA or PKG kinase activity for either site can be deduced by the ability of the putative activator to compete with labeled cAMP or cGMP, *e.g.*, [³H]cAMP or [³H]cGMP, respectively, for binding. For example, PKA or PKG or the respective regulatory subunit is mixed with the putative activator to be tested and [³H]cAMP, [³H]db cAMP, [³H]8-bromo cAMP, [³H]cGMP [³H]8-bromo cGMP or [³H]8-CPT cGMP, as required, in the appropriate buffer for a time sufficient to reach equilibrium. The amount of radioactivity that separates with the protein fraction of the mixture is then inversely proportional to the affinity of the putative activator. Data are compared to the results of the same assay performed with an activator of known affinity for the subunit, *e.g.* cAMP, db cAMP, 8-bromo cAMP, cGMP, 8-bromo cGMP or 8-CPT cGMP, to establish the relative affinity of the tested putative activator. Illustrative methods that can be used for measuring such binding affinity are described in Øgreid *et al.*, 1983, J. Biol. Chem. 258:1041-1049; Døskeland *et al.*, 1983, Biochemistry 22:1094-1101; Øgreid *et al.*, 1985, Eur. J. Biochem. 150:219-227; and Dostmann *et al.*, 1990, J. Biol. Chem. 265:10484-10491.

Additionally, any method known in the art for measuring cell membrane permeability can be employed in identifying activators of the present invention. The ability of a compound to pass through the cell membrane is a function of its lipophilicity, or the ability to dissolve in a lipid phase when an aqueous phase is also present. Traditionally this has been measured by determining the compound's partition coefficient, *i.e.*, the relative concentration of the compound at equilibrium in the two distinct phases of an octanol/water system. While such methods may be used, such methods are, however, both cumbersome and difficult. The more modern approach uses reversed-phase high-performance liquid chromatographic (RP-HPLC) methods to assess the ability of a compound to be eluted from water by a water-miscible organic solvent, *e.g.*, methanol. Illustrative methods for measuring such ability are described in Braumann *et al.*, 1985, J. Chromatogr. 350:105-118 and Krass *et al.*, 1997, Anal. Chem. 69:2575-2581.

In a specific embodiment, the activator is present in the solution in a concentration range of about 0.05 μM to about 250 μM , preferably in a range of about 0.1 μM to about 100 μM . In an embodiment, the concentration range refers to the concentration of a single activator in the solution. For example, in one embodiment wherein the solution comprises an activator of PKA kinase activity and an activator of PKG kinase activity, the concentration of the PKA activator and the PKG activator is each about 250 μM in the solution. The optimal concentration of the activator in the solution to achieve an increase in kinase activity can be determined by standard techniques.

In a specific embodiment, the activator of PKA kinase activity is an analogue of cAMP. In another embodiment, the activator of PKA kinase activity is an analogue of cAMP, with the proviso that the analogue is not Sp-cAMPS, db cAMP or 8-bromo cAMP. In yet another embodiment, the activator of PKA kinase activity is an analogue of cAMP, with the proviso that the analogue is not Sp-cAMPS, db cAMP, 8-bromo cAMP, 8-CPT cAMP or 6-Bnz cAMP. In yet another embodiment, the activator of PKG kinase activity is an analogue of cGMP. In yet another embodiment, the activator of PKG kinase activity is an analogue of cGMP, with the proviso that the analogue is not 8-bromo cGMP or 8-CPT cGMP. Analogues of cAMP/cGMP that can be used according to the invention are those that have modifications to the purine ring system, to the ribose, or to the phosphate group. See Figures 1A-1B showing the chemical structure of cAMP and the subparts of the molecule, *i.e.*, phosphate group, purine ring, imidazole ring, pyrimidine ring. The purine ring system is the most commonly studied site for modification as it is essential for cyclic nucleotide recognition by its dependent kinase. Øgreid *et al.*, 1985, Eur. J. Biochem.

150:219-227; Corbin *et al.*, 1986, J. Biol. Chem. 261:1208-1214; Øgreid *et al.*, 1989, Eur. J. Biochem. 181:19-31. Modifications to the purine ring system can be made in either the pyrimidine portion or the imidazole portion. For example, modifications to the pyrimidine portion of the ring system (positions 1, 2 or 6) alter binding affinity in direct correlation to the changes in tertiary structure or hydrophilic interactions; in contrast, modifications to the imidazole portion of the system (position 8) seem to regulate binding through a combination of electronic, steric and hydrophobic forces. Corbin *et al.*, 1986, J. Biol. Chem. 261:1208-1214. Although most substituents at position 8 reduce the affinity of the analog for its respective kinase, a few, notably 8-Br cAMP and 8-Br cGMP, have the opposite effect. Øgreid *et al.*, 1989, Eur. J. Biochem. 181:19-31. This is thought due either to electronic effects in the case of electron withdrawing groups or the direct interaction of the substituent with the binding site. Corbin *et al.*, 1986, J. Biol. Chem. 261:1208-1214.

Analogues of cAMP or cGMP also comprise simultaneous modifications to the purine ring system, the ribose or to the phosphate group. For example, modifications to the either the purine ring system or the ribose are often combined with a substitution of one of the exocyclic oxygens of the phosphate group by sulfur. Sulfur replacement at either the equatorial or axial position (Sp or Rp isomer, respectively) increases not only the lipophilicity of the compound but also induces its resistance to hydrolysis by phosphodiesterase. Braumann *et al.*, 1985, J. Chromatogr. 350: 105-108; Eckstein, 1985, Annu. Rev. Biochem. 54:367-402; Schaap *et al.*, 1993, J. Biol. Chem. 268:6323-6331. This modification also allows limited influence over the activity of the target kinase; when compared to the level of activation by the parent cyclic nucleotide, Sp isomers tend to be weakly agonistic while Rp isomers are antagonistic. O'Brian *et al.*, 1982, Biochemistry 21:4371-4376.

Specific activators of PKA kinase activity that activate PKA and are at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP are known in the art, *see, e.g.*, Braumann *et al.*, 1985, J. Chromatogr. 350:105-118. Illustrative examples of such activators include, but are not limited to N⁶-Mono-tert-butylcarbamoyladenosine-3',5'-cyclic monophosphate (6-MBC cAMP); 8-(4-Chlorophenylthio)adenosine-3',5'-cyclic monophosphate (8-CPT cAMP); 5, 6- Dichloro-1-β-D-ribofuranosylbenzimidazole-3',5'-cyclic monophosphorothioate, Sp-isomer (Sp-5,6-DCl₂-cBIMPS); or a mixture of any two or more of the foregoing.

Specific activators of PKA kinase activity that activate PKA and bind with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-

cAMPS, db cAMP and 8-bromo cAMP are known in the art, *see, e.g.,* Øgreid *et al.*, 1989, Eur. J. Biochem. 181:19-31. Illustrative examples of such activators include, but are not

limited to N⁶-Phenyladenosine-3',5'-cyclic monophosphate (6-NHC₆H₅-cAMP or 6-Phe cAMP); 8-Methylaminoadenosine-3',5'-cyclic monophosphate (8-MA cAMP); 8-NH₂

cAMP (8-amino cAMP); 2-Cl,8-NH₃-cAMP; 8-n-Hexylaminoadenosine-3',5'-monophosphate (8-HA cAMP); 8-(6-Aminohexyl) aminoadenosine-3',5'-cyclic

monophosphate (8-AHA cAMP); 8-(4-Chlorophenylthio) adenosine-3',5'-cyclic monophosphate (8-CPT cAMP); N⁶-Benzoyladenosine-3',5'-cyclic monophosphate (6-

NHC(O)C₆H₅-cAMP or 6-Bnz cAMP); 6-NHC(O)NHC(CH₃)₂ cAMP; 6-

NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉-8-

bromo cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-

NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*i*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-

SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*l**p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP (8-azido cAMP); 2-Cl,8-NHCH₃ cAMP; or a mixture of any two or more of the foregoing.

Specific activators of PKA kinase activity that bind to the regulatory subunit of the kinase and result in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively, are known in the art, *see, e.g.,* Øgreid *et al.*, 1985, Eur. J. Biochem. 150:219-227. Illustrative examples of such activators include, but are not limited to, 2-chloroadenosine-3',5'-cyclic monophosphate (2-Cl cAMP); 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-Methylaminoadenosine-3',5'-cyclic monophosphate (8-MA cAMP); 8-NH₂ cAMP (8-amino cAMP); N⁶-Phenyladenosine-3',5'-cyclic monophosphate (6-Phe cAMP); 6-NHnC₈H₁₇ cAMP; 6-NHnC₄H₉-8-Benzylthioadenosine-3',5'-cyclic monophosphate (8-BT cAMP); 6-NHCH₂CH=C(Cl)CH₃ cAMP; N⁶-Diethyladenosine-3',5'-cyclic monophosphate (6-(C₂H₅)₂ cAMP); and a mixture of any two or more of the foregoing.

Specific activators of PKG kinase activity that activate PKG and are at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP are known in the art, *see, e.g.,* Vaadrager *et al.*, 1997, J. Biol. Chem. 272:11816-11823; Genieser, 1995, *Proceedings of the 9th International Conference on Second Messengers & Phosphoproteins*, p.104. Illustrative examples of such activators include, but

are not limited to 8-(4-Chlorophenylthio)guanosine-3',5'-cyclic monophosphate, Sp-isomer, (Sp-8-pCPT cGMPS); β -Phenyl-1, N^2 -etheno-8-bromoguanosine-3',5'-cyclic monophosphate (8-Br-PET cGMP); 8-Bromoguanosine-3',5'-cyclic monophosphorothioate, Sp-isomer (Sp-8-Br cGMPS); or a mixture of any two or more of the foregoing.

5 Specific activators of PKG kinase activity that activate PKG and bind with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP can be determined by methods known in the art, *see, e.g.*, Corbin *et al.*, J. Biol. Chem. 261:1208-1214.

10 Specific activators of PKG kinase activity that bind to the regulatory subunit of PKG and result in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively, are known in the art, *see, e.g.*, Corbin *et al.*, J. Biol. Chem. 261:1208-1214; Sekhar *et al.*, Mol. Pharmacol. 42:103-108. Illustrative examples of such activators include, but are not limited to, 8-(2,4-Di-OH-phenylthio)
 15 guanosine-3',5'-cyclic monophosphorothioate (8-(2,4-Di-OH-PT)-cGMP); 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1, N^2 -(4-CH₃O-PET)-cGMP; 8-Br-1, N^2 -PET-cGMP; 1, N^2 - $\square$ NET-cGMP; 8-Br-1, N^2 -PMET-cGMP; 8-(4-OH-PT)-1, N^2 -PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

20 5.3 Methods of Preservation and Use of Preserved Organs

 The invention also provides a method of preserving or maintaining an organ comprising contacting the organ with a solution of the present invention comprising an activator of cyclic cAMP-dependent protein kinase (PKA) and/or cyclic cGMP-dependent protein kinase (PKG). The contacting comprises immersing, infusing, flushing, or
 25 perfusing. Other suitable procedures of contacting can be used. The method can be used wherein the organ is a blood vessel that is intended for transplantation for a vascular bypass procedure, *e.g.*, abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, popliteal aneurysm repair, or for a coronary arterial bypass (CABG). Hence, the preservation solution may be used to preserve a blood vessel or functional portion thereof
 30 prior to use in such vascular transplantation procedures.

 Any known organ or portion thereof can be preserved *ex vivo* in a solution of the present invention. The organ can be heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, or a blood vessel. In preferred embodiments, any known blood vessel or a functional portion thereof can be preserved *ex vivo* in solution of the invention, preferably

prior to use as a vascular graft. The blood vessel can be an artery or a vein. Exemplary blood vessels include, but are not limited to, the internal mammary artery (also known as the internal thoracic artery), the renal artery, the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein, or a functional portion thereof.

5 Preferably, the blood vessel is a saphenous vein or functional portion thereof. For example, where the blood vessel graft is for a coronary arterial bypass, the blood vessel can be the internal mammary artery (also known as the internal thoracic artery), the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein, or a functional portion of the artery or vein. Preferably, the blood vessel for use as a graft is the
10 saphenous vein or a functional portion thereof. In another example, where the graft is for abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, or popliteal aneurysm repair, the blood vessel is the renal artery or functional portion thereof, or the saphenous vein or a functional portion thereof. Preferably, the graft is isolated from the saphenous vein or a functional portion thereof.

15 As used herein, a "functional" portion of an organ refers to a portion that is able to act as a graft. The organ can be isolated from and used in transplantation procedures in, *e.g.*, any mammal including primates, pigs, dogs, cats. Preferably, the organ is isolated from a human, *e.g.*, human child (less than 18 years old), or human adult (18 years or older).

As used herein, a "functional" portion of a blood vessel refers to a portion that is
20 able to act as a vascular graft. The blood vessel can be isolated from and used as a vascular graft in, *e.g.*, any mammal including primates, pigs, dogs, cats. Preferably, the blood vessel is isolated from a human, *e.g.*, human child (less than 18 years old), or human adult (18 years or older). Preferably, the blood vessel is isolated from the patient in which it is subsequently used as a vascular graft.

25 One embodiment of the invention is directed to a method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG kinase activity in a solution comprising heparinized blood. Another embodiment is directed to a method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution consisting
30 of an activator of PKA and/or PKG in heparinized blood. In a preferred embodiment, the method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG, wherein the solution is at a temperature in the range of about 0.5 to about 10°C, preferably at 4°C, during the

contacting step. In another preferred embodiment, the contacting is for a time period of not longer than 4 hours, preferably not longer than 2 or 3 hours.

In another embodiment, the invention is directed to a method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG kinase activity in a solution comprising heparinized blood. Another embodiment is directed to a method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution consisting of an activator of PKA and/or PKG in heparinized blood. In a preferred embodiment, the method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG, wherein the solution is at a temperature in the range of about 0.5 to about 10°C, preferably at 4°C, during the contacting step. In another preferred embodiment, the contacting is for a time period of not longer than 4 hours, preferably not longer than 2 or 3 hours.

Optionally, prior to use of the organ or blood vessel or functional portion thereof, the method further comprises a step of removing the solution from contact with the organ or blood vessel or portion thereof. Preferably, the removing of the solution comprises flushing, immersing, infusing, or perfusing the blood vessel or portion thereof with a second solution that lacks the activator. In a preferred embodiment, the second solution is appropriate for maintaining cardiovascular homeostasis *in vivo*, e.g., the solution lacks potassium. An exemplary solution is saline or Ringer's Lactate.

In another embodiment, the present invention is directed to an isolated *ex vivo* organ or functional portion thereof in contact with a solution comprising an activator of PKA and/or PKG, at a temperature in the range of about 0.5 to about 10°C, preferably 4°C. The present invention is also directed to an isolated *ex vivo* isolated organ or functional portion thereof in contact with a solution comprising, or alternatively consisting of, (a) heparinized blood and (b) an activator of PKA and/or PKG. Preferably, the contacting is at a temperature in the range of about 0.5 to about 10°C, and more preferably at 4°C. Any known organ or a functional portion thereof can be preserved *ex vivo* in solution of the invention. Exemplary organs include, but are not limited to, heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessel. Exemplary blood vessels include, but are not limited to, the internal mammary artery (also known as the internal thoracic artery), the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein. As used herein, a "functional" portion of a blood vessel refers to a portion

that is able to act as a vascular graft. The organ, including a blood vessel, can be isolated from, *e.g.*, any mammal including primates, pigs, dogs, cats. Preferably, the organ is isolated from a human, *e.g.*, human child (less than 18 years old), or human adult (18 years or older).

5 In another embodiment, the invention is directed to a container containing a solution of the invention comprising the activator of PKA and/or PKG and the organ, blood vessel or functional portion thereof. Preferably, the organ or blood vessel or portion thereof is a human organ, blood vessel or portion thereof.

10 In yet another embodiment, the present invention is directed to a method of using an organ in a transplant operation comprising contacting an isolated organ or functional portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG, and; inserting the organ or portion thereof into a patient. In one embodiment, the solution further comprises heparinized blood. Preferably, the temperature of the solution ranges from about 0.5°C to about 10°C, and more preferably is about 4°C. Preferably, the contacting is for a
15 time period not longer than four hours. The method can further comprise a step before inserting the organ or portion thereof of removing the solution from contact with the organ or portion thereof, wherein the removing step comprises flushing, immersing, infusing, or perfusing the organ or portion thereof with a second solution that lacks the activator of the invention.

20 In yet another embodiment, the present invention is directed to a method of using a blood vessel as a vascular graft comprising contacting an isolated blood vessel or functional portion thereof *ex vivo* with a solution comprising an activator of PKA and/or PKG, and; inserting the blood vessel into a patient so as to form a vascular graft in the patient. In one embodiment, the solution further comprises heparinized blood. Preferably, the temperature
25 of the solution ranges from about 0.5°C to about 10°C, and more preferably is about 4°C. Preferably, the contacting is for a time period not longer than four hours. The method can further comprise a step before inserting the vessel of removing the solution from contact with the blood vessel or portion thereof, wherein the removing step comprises flushing, immersing, infusing, or perfusing the blood vessel or portion thereof with a second solution
30 that lacks the activator of the invention. Preferably, the second solution is appropriate for maintaining cardiovascular homeostasis *in vivo*, *e.g.*, the solution lacks potassium. An exemplary solution is saline or Ringer's Lactate.

Any known blood vessel or a functional portion thereof can be preserved *ex vivo* in a solution of the invention, preferably prior to use as a vascular graft. Exemplary blood

vessels include, but are not limited to, the internal mammary artery (also known as the internal thoracic artery), the renal artery, the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein. Preferably, the blood vessel or functional portion thereof is a saphenous vein or functional portion thereof. As used herein, a

“functional” portion of a blood vessel refers to a portion that is able to act as a vascular graft. The blood vessel can be isolated from, *e.g.*, any mammal including primates, pigs, dogs, cats. Preferably, the blood vessel is isolated from a human, *e.g.*, human child (less than 18 years old), or human adult (18 years or older). Further, the blood vessel or portion thereof is isolated from the same patient receiving the graft, *i.e.*, the graft is autologous.

In specific embodiments, the solution is the Columbia University solution further comprising said activator, or the Euro-Collins solution further comprising said activator, or the University of Wisconsin solution further comprising said activator, or the low-potassium dextran glucose solution further comprising said activator, or the Celsior™ solution further comprising said activator. Optionally, the solution further comprises a vasodilator.

Exemplary vasodilators include, but are not limited to an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate, such as dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP) or 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP). Preferably, the analog is cell membrane-permeable. Other vasodilators include, but are not limited to nitroglycerin, adenosine, pertussis toxin.

In yet another embodiment, the present invention is directed to a method for performing a coronary artery bypass graft in a patient comprising, removing from contact with a blood vessel or functional portion thereof a solution comprising an activator of cAMP-dependent protein kinase (PKA) activity and/or cGMP-dependent protein kinase (PKG) activity; and grafting the blood vessel or functional portion thereof into the patient so as to serve as a coronary bypass graft. Preferably, the patient is a human patient and the blood vessel or portion thereof was isolated from the same patient. Alternatively, the blood vessel is isolated from a non-human animal.

Another embodiment of the invention is directed to a pharmaceutical pack or kit comprising one or more containers filled with a solution of the invention comprising an activator of PKA and/or PKG kinase activity. For example, the kit can comprise a container containing the low-potassium dextran glucose solution (Perfadex™) further comprising an activator of PKA kinase activity. Optionally associated with such container(s) can be instructions for use of the kit and/or a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products,

which notice reflects approval by the agency of manufacture, use or sale for human administration.

The following series of examples are presented by way of illustration and not by way of limitation on the scope of the present invention.

6. EXAMPLES

6.1 In carrying out a coronary bypass using a saphenous vein as the vascular graft, the patient is first anesthetized and a portion of the saphena is excised from either leg. The excised saphenous vein is placed in contact with a preservation solution comprising An activator of PKA and/or PKG kinase activity of the present invention in a kidney dish such that the solution is both inside and outside the vein and the dish is placed on ice. The excision in the leg is closed, and, concurrently, the chest is opened to allow access to the heart. The patient is placed on life support with a cardiac bypass machine and the heart is stopped. The saphenous vein is removed from the solution and is rinsed (flushed) with buffered saline lacking the activator and potassium ions. The saphenous vein is cut to size for the bypass area and is grafted onto the cardiac tissue. The inserted venous segment acts as a bypass of the blocked portion of the coronary artery, and, thus, provides for a free or unobstructed flow of blood to the heart. The patient's heart is restarted and the chest is closed.

6.2 The following experimental vein graft procedure is used to assess the efficacy of a composition of the invention in *ex vivo* preservation of a blood vessel to be used as a vascular graft.

Vein graft procedure - intrapositional saphenous vein anastamosis

Female sheep, dogs or pigs are purchased from Charles River (Charles River, MA). The investigation will conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The surgical procedures used are standard; animals are anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). An incision is made on the left and right legs to expose the saphenous vein and femoral artery. A segment of the saphenous vein (19 cm long) is transected after ligation at both ends with 8-0 sutures. This segment is washed with saline solution containing 100 U/ml of heparin, and stored at 25°C for up to 2 hours in heparinized saline or in an experimental solution of heparinized

saline containing a cAMP or cGMP analogue of the invention, such as 6-Phe cAMP or Sp-8-pCPT cGMPS.

A portion of the graft before these incubation is cut (0.5cm) and placed in formaldehyde fixative (10%); after incubation for up to 2 hours, 0.5 cm sections are also cut and placed in formaldehyde fixative (10%), both for later immunohistochemical analysis as described, *infra*. After incubation in control or experimental solutions as described, a segment of the femoral artery is temporally occluded at two places with a microvascular clamp (Roboz Surgical Instrument Co., Gaithersburg, MD), and a circular incision (of about the same size as the vein in diameter) is made. The anastomosis in loop is repaired by suturing the prepared vein into the clamped femoral artery with an 11-0 continuous suture around vein graft artery anastomosis. Contact between the instruments and the vein graft endothelium is avoided as much as possible throughout the procedure. After the vascular clamp is removed, the vein is inspected for adequacy of repair. Surgery is considered successful if strong pulsation is confirmed in both the graft and native artery without significant bleeding. If there is no pulsation or pulsations are diminished within a few minutes of restoration of blood flow, the procedure is considered a surgical failure. Cefazolin (50 mg/kg,) is administered and the skin incision is closed with a 6-0 nylon suture. Buprenorphine (2.5 mg/kg) is given subcutaneously for postoperative analgesia. The duration of the entire procedure is approximately 30 minutes. One leg in each animal is for the experimental solutions; the contralateral leg is always used for the control solution. In order to verify intimal hyperplasia, both phorbol myristate acetate (PMA, Sigma, St. Louis, MO) and lipopolysaccharide (LPS, Sigma, St. Louis, MO) at 1.0 uM is used to incubate saphenous vein segments for up to 2 hours as positive controls.

Morphology

Animals are sacrificed at various time points after surgery and perfusion-fixed using 10% formaldehyde at physiological pressure. The grafts, together with a short segment of the native femoral artery, are harvested and cut at the center. The specimens are embedded in medium (OCT compound), and frozen at -80°C. The section (5 µm) at the mid portion of each composite graft is stained with hematoxylin and eosin (H&E) or Van Gieson's elastic stain (Sigma, St. Louis, MO), and the degree of neointimal expansion is analyzed quantitatively using a Zeiss microscope and image analysis system (Media Cybernetics, Silver Spring, MD). The consistency of neointimal formation in the central portion of the graft is histologically confirmed by analyzing serial sections from the center to the proximal and distal ends of the graft. The neointima of the vein graft is defined as the region between

the lumen and the adventitia. Neointimal cell number is calculated by counting the number of nuclei visible in sections stained with H&E. The percentage of neointimal expansion is calculated as $100 \times (\text{neointimal area} / \text{neointimal area} + \text{luminal area})$. These quantifications are performed by an observer blinded to the experimental circumstances.

5 Masson Trichrome stain is performed according to the manufacture's instructions (Sigma, St. Louis, MO).

En face immunofluorescence

The procedure used in this study is similar to that reported by Zou *et al.*, 2000, *Circ Res* 86:434-440 and Dietrich *et al.*, 2000, *Arterioscler Thromb Vasc Biol* 20:343-352. The
10 vein patch is retrieved 24 hours after surgery and mounted onto a glass slide with endothelium side up, and air-dried for 1 hour at room temperature. The segments are fixed in cold acetone (-20°C) for 10 minutes and rinsed in PBS. The segments are then incubated with rat monoclonal antibody to MAC-1 (1:25, Pharmingen, San Diego, CA) for 30 minutes and visualized with FITC-labeled rabbit anti-rat IgG (1:25, Sigma, St. Louis, MO). MAC-1
15 positive sells are blindly counted at x 400 magnification in 10 fields of each segment.

Immunohistochemistry

Representative sections (5 mm) are immunostained with rat anti PECAM-1 (CD31) antibody (1:100, Pharmingen, San Diego, CA), rat anti-MAC-1 antibody (1:50, Pharmingen, San Diego, CA), and hamster anti-ICAM-1 antibody (1:100, Pharmingen, San
20 Diego, CA). Sections are blocked with hydrogen peroxide (0.3%) in methanol for 10 min. Blocking is performed with goat serum (4%) and bovine serum albumin (1%) in PBS. Primary antibodies are added to slides, and incubated overnight at 4°C. Secondary antibodies (1:100; anti- hamster or rat IgG, Phamingen, San Diego, CA) are added for 30 min at room temperature. Sections are reacted with horseradish peroxidase conjugated
25 streptavidin (1:100, Sigma, St. Louis, MO) for 30 min at room temperature and developed with 3,3'-diaminobenzidine (DAB substrate kit, Vector, Burlingame, CA).

Statistical analysis

All data are expressed as mean +/- SEM. Student's unpaired t test for a comparison between two groups, or ANOVA with post hoc analysis using the Bonferroni/Dunn test for
30 a comparison among more than two groups are used to determine significant difference. P values of less than 0.05 are considered statistically significant. All analyses are performed using the Statview statistical package, version J5.0 (Abacus Concepts Inc., Berkeley, CA).

7. REFERENCES CITED

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. Such modifications are intended to fall within the scope of the appended claims.

All references, patent and non-patent, cited herein are incorporated herein by reference in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

WHAT IS CLAIMED IS:

1. A method of using an organ in a transplantation procedure comprising:
 - (a) contacting an organ or portion thereof *ex vivo* with a solution comprising an activator of cAMP-dependent protein kinase activity, which activator:
 - (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP;
 - (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or
 - (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively, and;
 - (b) transplanting the organ or portion thereof into a patient.
2. The method of claim 1, wherein the activator is an analogue of cAMP.
3. The method of claim 1, wherein the activator activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP.
4. The method of claim 3, wherein said activator is selected from the group consisting of 6-MBC cAMP; 8-CPT cAMP; Sp-5,6-DCI₂-cBIMPS; and a mixture of any two or more of the foregoing.
5. The method of claim 3, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.
6. The method of claim 1, wherein the activator activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP.
7. The method of claim 6, wherein said activator is selected from the group consisting of 6-Phe cAMP; 8-MA cAMP; 8-amino cAMP; 2-Cl,8-NH₃-cAMP; 8-HA

cAMP; 8-AHA cAMP; 8-CPT cAMP; 6-Bnz cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; and a mixture of any two or more of the foregoing.

8. The method of claim 6, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

9. The method of claim 1, wherein the activator binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

10. The method of claim 9, wherein said activator is selected from the group consisting of 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

11. The method of claim 9, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

12. A method of using an organ in a transplantation procedure comprising:
- (a) contacting an organ or portion thereof *ex vivo* with a solution comprising an activator of cGMP-dependent protein kinase activity, which activator:
 - (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP;
 - (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or

- (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively, and;
- (b) transplanting the organ or portion thereof into a patient.
13. The method of claim 12, wherein the activator is an analogue of cGMP.
14. The method of claim 12, wherein the activator activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP.
15. The method of claim 14, wherein said activator is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; and a mixture of any two or more of the foregoing.
16. The method of claim 14, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.
17. The method of claim 12, wherein the activator binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.
18. The method of claim 17 wherein said activator is selected from the group consisting of 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.
19. The method of claim 17 wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.
20. The method of claim 1 or 12, wherein the organ is selected from the group consisting of heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessel.

21. The method of claim 20, wherein the blood vessel is one or a combination of: an internal mammary artery, a radial artery, right gastroepiploic artery, inferior epigastric artery, or a saphenous vein.

22. The method of claim 21, wherein the blood vessel is a saphenous vein.

23. The method of claim 1 or 12, wherein the patient is a human.

24. The method of claim 23, wherein the organ or portion thereof is from the patient.

25. The method of claim 1 or 12, wherein the temperature of the solution ranges from about 0.5 to about 10° C.

26. The method of claim 1 or 12, wherein said contacting step is for a time period not longer than four hours.

27. The method of claim 1 or 12, which further comprises before step (b) a step of removing the solution from contact with the organ or portion thereof.

28. The method of claim 27, wherein said removing step comprises flushing the organ or portion thereof with a second solution lacking said activator.

29. The method of claim 28, wherein said second solution is buffered saline or Ringer's Lactate.

30. The method of claim 1 or 12, wherein said solution further comprises heparinized blood.

31. The method of claim 1 or 12, wherein said solution further comprises buffered saline.

32. The method of claim 1 or 12, wherein said solution is the Columbia University solution further comprising said activator.

33. The method of claim 1 or 12, wherein said solution is the Euro-Collins solution further comprising said activator.
34. The method of claim 1 or 12, wherein said solution is the University of Wisconsin solution further comprising said activator.
35. The method of claim 1 or 12, wherein said solution is the low-potassium dextran glucose solution further comprising said activator.
36. The method of claim 1 or 12, where said solution is the Celsior™ solution further comprising said activator.
37. The method of claim 1 or 12, wherein said solution further comprises nitroglycerin.
38. The method of claim 1 or 12, wherein said solution further comprises Phosphate Buffered Saline (PBS), Hanks' Balanced Salt Solution (HBSS), HBSS (Modified), Ringer's Lactate, Tyrodes buffer, Krebs buffer, Euro-Collins solution, University of Wisconsin solution, low-potassium dextran glucose solution, Celsior™ solution, or Columbia University solution.
39. The method of claim 1 or 12 wherein said solution further comprises:
- (a) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
 - (b) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
 - (c) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;
 - (d) potassium ions in a concentration greater than about 110 mM; and
 - (e) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting step at about the physiologic pH value.
40. A method of using a blood vessel as a vascular graft comprising:

(a) contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising an activator of cAMP-dependent protein kinase activity, which activator:

- (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP;
 - (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or
 - (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively, and;
- (b) transplanting the blood vessel or portion thereof into a patient so as to form a vascular graft.

41. The method of claim 40, wherein the activator is an analogue of cAMP.

42. The method of claim 40, wherein the activator activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP.

43. The method of claim 42, wherein said activator is selected from the group consisting of 6-MBC cAMP; 8-CPT cAMP; Sp-5,6-DCI₂-cBIMPS; and a mixture of any two or more of the foregoing.

44. The method of claim 42, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

45. The method of claim 40, wherein the activator activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP.

46. The method of claim 45, wherein said activator is selected from the group consisting of 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 8-CPT cAMP; 6-Bnz cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-

NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*i*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*l*pCl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP (8-azido cAMP); 2-Cl,8-NHCH₃ cAMP; and a mixture of any two or more of the foregoing.

47. The method of claim 45, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

48. The method of claim 40, wherein the activator binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

49. The method of claim 48, wherein said activator is selected from the group consisting of 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

50. The method of claim 48, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

51. A method of using a blood vessel as a vascular graft comprising:

(a) contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising an activator of cGMP-dependent protein kinase activity, which activator:

(i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP;

(ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or

- (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively, and;
- (b) transplanting the blood vessel or portion thereof into a patient so as to form a vascular graft.

52. The method of claim 51, wherein the activator is an analogue of cGMP.

53. The method of claim 51, wherein the activator activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP.

54. The method of claim 53, wherein said activator is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; and a mixture of any two or more of the foregoing.

55. The method of claim 53, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

56. The method of claim 51, wherein the activator binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

57. The method of claim 56, wherein said activator is selected from the group consisting of 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

58. The method of claim 56, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μ M to about 100 μ M.

59. The method of claim 40 or 51, wherein the temperature of the solution ranges from about 0.5 to about 10° C.

60. The method of claim 40 or 51, wherein said contacting step is for a time period not longer than four hours.
61. The method of claim 40 or 51, which further comprises before step (b) a step of removing the solution from contact with the blood vessel or portion thereof.
62. The method of claim 61, wherein said removing step comprises flushing the blood vessel or portion thereof with a second solution lacking said specific inhibitor.
63. The method of claim 62, wherein said second solution is buffered saline or Ringer's Lactate.
64. The method of claim 40 or 51, wherein the blood vessel is a saphenous vein, a mammary artery, or a radial artery; and the vascular graft is a coronary artery bypass graft.
65. The method of claim 64, wherein the blood vessel is a saphenous vein.
66. The method of claim 40 or 51, wherein said solution further comprises heparinized blood.
67. The method of claim 40 or 51, wherein said solution further comprises buffered saline.
68. The method of claim 40 or 51, wherein said solution is the Columbia University solution further comprising said activator.
69. The method of claim 40 or 51, wherein said solution is the Euro-Collins solution further comprising said activator.
70. The method of claim 40 or 51, wherein said solution is the University of Wisconsin solution further comprising said activator.
71. The method of claim 40 or 51, wherein said solution is the low-potassium dextran glucose solution further comprising said activator.

72. The method of claim 40 or 51, where said solution is the Celsior™ solution further comprising said activator.

73. The method of claim 40 or 51, wherein said solution further comprises nitroglycerin.

74. The method of claim 40 or 51, wherein said solution further comprises Phosphate Buffered Saline (PBS), Hanks' Balanced Salt Solution (HBSS), HBSS (Modified), Ringer's Lactate, Tyrodes buffer, Krebs buffer, Euro-Collins solution, University of Wisconsin solution, low-potassium dextran glucose solution, Celsior™ solution, or Columbia University solution.

75. The method of claim 40 or 51 wherein said solution further comprises:

- (a) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
- (b) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
- (c) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;
- (d) potassium ions in a concentration greater than about 110 mM; and
- (e) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting step at about the physiologic pH value.

76. The method of claim 40 or 51, wherein the patient is a human.

77. The method of claim 76, wherein the blood vessel or portion thereof is from the patient.

78. A solution comprising (a) an activator of cAMP-dependent protein kinase activity, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same

amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively; and (b) heparinized blood.

79. The method of claim 78, wherein the activator is an analogue of cAMP.

80. The solution of claim 78, wherein the activator activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP.

81. The solution of claim 80, wherein said activator is selected from the group consisting of 6-MBC cAMP; 8-CPT cAMP; Sp-5,6-DCI₂-cBIMPS; and a mixture of any two or more of the foregoing.

82. The solution of claim 80, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

83. The solution of claim 78, wherein the activator activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP.

84. The solution of claim 83, wherein said activator is selected from the group consisting of 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 8-CPT cAMP; 6-Bnz cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*i*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; and a mixture of any two or more of the foregoing.

85. The solution of claim 83, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μ M to about 100 μ M.

86. The solution of claim 78, wherein the activator binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

87. The solution of claim 86, wherein said activator is selected from the group consisting of 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄pNO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

88. The solution of claim 86, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

89. A solution comprising (a) an activator of cGMP-dependent protein kinase activity, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively; and (b) heparinized blood.

90. The method of claim 89, wherein the activator is an analogue of cGMP.

91. The solution of claim 89, wherein the activator activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP.

92. The solution of claim 91, wherein said activator is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; and a mixture of any two or more of the foregoing.

93. The solution of claim 91, wherein the concentration of the activator ranges from about 0.1 μM to about 100 μM.

94. The solution of claim 89, wherein the activator binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

95. The solution of claim 94, wherein said activator is selected from the group consisting of 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

96. The solution of claim 94, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

97. A solution consisting of (a) an activator of cAMP-dependent protein kinase activity, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively; and (b) heparinized blood.

98. The method of claim 97, wherein the activator is an analogue of cAMP.

99. The solution of claim 97, wherein the activator activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP.

100. The solution of claim 99, wherein said activator is selected from the group consisting of 6-MBC cAMP; 8-CPT cAMP; Sp-5,6-DCl₂-cBIMPS; and a mixture of any two or more of the foregoing.

101. The solution of claim 99, wherein the concentration of the activator ranges from about 0.1 μM to about 100 μM.

102. The solution of claim 97, wherein the activator activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP.

103. The solution of claim 102, wherein said activator is selected from the group consisting of 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 8-CPT cAMP; 6-Bnz cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*l**p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP (8-azido cAMP); 2-Cl,8-NHCH₃ cAMP; and a mixture of any two or more of the foregoing.

104. The solution of claim 102, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

105. The solution of claim 97, wherein the activator binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

106. The solution of claim 105, wherein said activator is selected from the group consisting of 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NH*n*C₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

107. The solution of claim 105, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

108. A solution consisting of (a) an activator of cGMP-dependent protein kinase activity, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as

compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively; and (b) heparinized blood.

109. The method of claim 108, wherein the activator is an analogue of cGMP.

110. The solution of claim 108, wherein the activator activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP.

111. The solution of claim 110, wherein said activator is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; and a mixture of any two or more of the foregoing.

112. The solution of claim 110, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

113. The solution of claim 108, wherein the activator binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

114. The solution of claim 113, wherein said activator is selected from the group consisting of 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²- $\square$ NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

115. The solution of claim 113, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μ M to about 100 μ M.

116. An isolated *ex vivo* organ or portion thereof in contact with a solution comprising an activator of cAMP-dependent protein kinase (PKA) activity, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each

of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

117. An isolated *ex vivo* organ or portion thereof in contact with a solution comprising an activator of cGMP-dependent protein kinase (PKG) activity, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

118. The organ or portion thereof of claim 116 or 117, wherein the organ is selected from the group consisting of heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessel.

119. The organ or portion thereof of claim 118, wherein the blood vessel is one or more of: an internal mammary artery, a radial artery, right gastroepiploic artery, inferior epigastric artery, or a saphenous vein.

120. The organ or portion thereof of claim 118, wherein the blood vessel is a saphenous vein.

121. The organ or portion thereof of claim 116 or 117, which is a human organ or portion thereof.

122. The method of claim 116, wherein the activator is an analogue of cAMP.

123. The organ or portion thereof of claim 116, wherein the activator activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP.

124. The organ or portion thereof of claim 123, wherein said activator is selected from the group consisting of 6-MBC cAMP; 8-CPT cAMP; Sp-5,6-DC1₂-cBIMPS; and a mixture of any two or more of the foregoing.

125. The organ or portion thereof of claim 123, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

126. The organ or portion thereof of claim 116, wherein the activator activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP.

127. The organ or portion thereof of claim 126, wherein said activator is selected from the group consisting of 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 8-CPT cAMP; 6-Bnz cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄OCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*i*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP (8-azido cAMP); 2-Cl,8-NHCH₃ cAMP; and a mixture of any two or more of the foregoing.

128. The organ or portion thereof of claim 126, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μ M to about 100 μ M.

129. The organ or portion thereof of claim 116, wherein the activator binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

130. The organ or portion thereof of claim 129, wherein said activator is selected from the group consisting of 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

131. The organ or portion thereof of claim 129, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μ M to about 100 μ M.

132. The method of claim 117, wherein the activator is an analogue of cGMP.

133. The organ or portion thereof of claim 117, wherein the activator activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP.

134. The organ or portion thereof of claim 133, wherein said activator is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; and a mixture of any two or more of the foregoing.

135. The organ or portion thereof of claim 133, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

136. The organ or portion thereof of claim 117, wherein the activator binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

137. The organ or portion thereof of claim 126, wherein said activator is selected from the group consisting of 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²- $\square$ NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

138. The organ or portion thereof of claim 136, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μ M to about 100 μ M.

139. The organ or portion thereof of claim 116 or 117, wherein said solution comprises heparinized blood.

140. The organ or portion thereof of claim 116 or 117, wherein said solution is buffered saline further comprising said activator.

141. The organ or portion thereof of claim 116 or 117, wherein said solution is the Columbia University solution further comprising said activator.

142. The organ or portion thereof of claim 116 or 117, wherein said solution is the Euro-Collins solution further comprising said activator.

143. The organ or portion thereof of claim 116 or 117, wherein said solution is the University of Wisconsin solution further comprising said activator.

144. The organ or portion thereof of claim 116 or 117, wherein said solution is the low-potassium dextran glucose solution further comprising said activator.

145. The organ or portion thereof of claim 116 or 117, wherein said solution is the Celsior™ solution further comprising said activator.

146. The organ or portion thereof of claim 116 or 117, wherein said solution further comprises:

- (a) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
- (b) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
- (c) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;
- (d) potassium ions in a concentration greater than about 110 mM; and
- (e) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof at about the physiologic pH value.

147. An isolated *ex vivo* organ or portion thereof in contact with a solution consisting essentially of (a) a solvent; and (b) an activator of cAMP-dependent protein kinase (PKA) activity, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from

exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

148. An isolated *ex vivo* organ or portion thereof in contact with a solution consisting essentially of (a) a solvent; and (b) an activator of cGMP-dependent protein kinase (PKG) activity, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

149. A container containing the organ or portion thereof of claim 116 or 117.

150. A container containing the organ or portion thereof of claim 147 or 148.

151. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising an activator of cAMP-dependent protein kinase A (PKA) activity and heparinized blood, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

152. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising an activator of cGMP-dependent protein kinase (PKG) activity and heparinized blood, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from

exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

153. The method of claim 151 or 152, wherein said contacting is for a time period not longer than four hours.

154. The method of claim 151 or 152, wherein the organ is selected from the group consisting of heart, kidney, liver, lung, skin, pancreas, intestine, stomach, muscle, and blood vessel.

155. The method of claim 154, wherein the blood vessel is one or a combination of: an internal mammary artery, a radial artery, right gastroepiploic artery, inferior epigastric artery, or a saphenous vein.

156. The method of claim 154, wherein the blood vessel is a saphenous vein.

157. The method of claim 151 or 152, wherein the organ or portion thereof is a human organ or portion thereof.

158. The method of claim 151, wherein the activator is an analogue of cAMP.

159. The method of claim 151, wherein the activator activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP.

160. The method of claim 159, wherein said activator is selected from the group consisting of 6-MBC cAMP; 8-CPT cAMP; Sp-5,6-DCI₂-cBIMPS; and a mixture of any two or more of the foregoing.

161. The method of claim 159, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

162. The method of claim 151, wherein the activator activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP.

163. The method of claim 162, wherein said activator is selected from the group consisting of 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 8-CPT cAMP; 6-Bnz cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄*p*Cl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*l**p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; and a mixture of any two or more of the foregoing.

164. The method of claim 162, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

165. The method of claim 151, wherein the activator binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

166. The method of claim 165, wherein said activator is selected from the group consisting of 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

167. The method of claim 165, wherein the concentration of the activator that binds the regulatory subunit ranges from about 0.1 μM to about 100 μM.

168. The method of claim 152, wherein the activator is an analogue of cGMP.

169. The method of claim 152, wherein the activator activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP.

170. The method of claim 169, wherein said activator is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; and a mixture of any two or more of the foregoing.

171. The method of claim 169, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

172. The method of claim 152, wherein the activator binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

173. The method of claim 172, wherein said activator is selected from the group consisting of 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²- $\square$ NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

174. The method of claim 172, wherein the concentration of the activator ranges from about 0.1 μ M to about 100 μ M.

175. The method of claim 151 or 152, wherein the temperature of the solution ranges from about 0.5 to about 10° C.

176. The method of claim 151 or 152, which further comprises a step of removing the solution from contact with the organ or portion thereof.

177. The method of claim 176, wherein said removing step comprises flushing the organ or portion thereof with a second solution lacking said activator.

178. The method of claim 151 or 152, wherein said solution further comprises buffered saline.

179. The method of claim 151 or 152, wherein said solution is the Columbia University solution further comprising said activator.

180. The method of claim 151 or 152, wherein said solution is the Euro-Collins solution further comprising said activator.

181. The method of claim 151 or 152, wherein said solution is the University of Wisconsin solution further comprising said activator.

182. The method of claim 151 or 152, wherein said solution is the low-potassium dextran glucose solution further comprising said activator.

183. The method of claim 151 or 152, wherein said solution is the Celsior™ solution further comprising said activator.

184. The method of claim 151 or 152, wherein said solution further comprises:

- (a) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
- (b) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
- (c) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;
- (d) potassium ions in a concentration greater than about 110 mM; and
- (e) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting at about the physiologic pH value.

185. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution consisting of an activator of cAMP-dependent protein kinase (PKA) activity and heparinized blood, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively.

186. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution consisting of an activator of cGMP-dependent protein kinase (PKG) activity and heparinized blood, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

187. The method of claim 1 or 40, wherein the solution further comprises an activator of cGMP-dependent protein kinase (PKG) activity, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively.

188. The method of claim 1, 12, 40, 51, 151, 152, 185 or 186, wherein the contacting comprises immersing, infusing, flushing, or perfusing.

189. A method for performing an organ transplantation in a patient comprising,
(1) removing from contact with an organ or portion thereof a solution comprising:

(A) an activator of cAMP-dependent protein kinase A (PKA) activity and heparinized blood, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from

exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively; and/or

(B) an activator of cGMP-dependent protein kinase (PKG) activity and heparinized blood, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively; and

(2) transplanting the organ or portion thereof into the patient.

190. The method of claim 189, wherein the patient is a human patient.

191. A method for performing a coronary artery bypass graft in a patient comprising,

(1) removing from contact with a blood vessel or portion thereof a solution comprising:

(A) an activator of cAMP-dependent protein kinase (PKA) activity and heparinized blood, which activator (i) activates PKA and is at least 25% more cell membrane permeable as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo-cAMP; (ii) activates PKA and binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in an at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of cAMP, Sp-cAMPS, db cAMP and 8-bromo cAMP, respectively; and/or

(B) an activator of cGMP-dependent protein kinase (PKG) activity and heparinized blood, which activator (i) activates PKG and is at least 25% more cell membrane permeable as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; (ii) activates PKG and binds with at least 25% greater affinity to the regulatory subunit of PKG as compared to each of cGMP, 8-bromo cGMP and 8-CPT cGMP; and/or (iii) binds to the regulatory subunit of PKG and results in an at least 25% greater level of PKG kinase activity as compared to the PKG kinase activity resulting from exposure of PKG to the same amount of each of cGMP, 8-bromo cGMP and 8-CPT cGMP, respectively; and

(2) grafting the blood vessel or portion thereof into the patient so as to serve as a coronary bypass graft.

192. The method of claim 191, wherein the patient is a human patient.

193. The method of claim 189 or 191, wherein said removing step comprises flushing the organ or portion thereof with a second solution lacking said activator.

194. A method of using an organ in a transplantation procedure comprising:

(a) contacting an organ or portion thereof *ex vivo* with a solution comprising a compound selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCI₂-cBIMPS; 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄OC₁ cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*l**p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHInC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing; and

(b) transplanting the organ or portion thereof into a patient.

195. A method of using an organ in a transplantation procedure comprising:

(a) contacting an organ or portion thereof *ex vivo* with a solution comprising a compound selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,*N*²-(4-CH₃O-PET)-cGMP; 8-Br-1,*N*²-PET-cGMP; 1,*N*²-□-NET-cGMP; 8-Br-1,*N*²-PMET-cGMP; 8-(4-OH-PT)-1,*N*²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing; and

(b) transplanting the organ or portion thereof into a patient.

196. A method of using a blood vessel as a vascular graft comprising:

(a) contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising a compound selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCI₂-cBIMPS; 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*i*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*l*pCl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing; and

(b) transplanting the blood vessel or portion thereof into a patient so as to form a vascular graft.

197. A method of using a blood vessel as a vascular graft comprising:

(a) contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising a compound selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,*N*²-(4-CH₃O-PET)-cGMP; 8-Br-1,*N*²-PET-cGMP; 1,*N*²-□NET-cGMP; 8-Br-1,*N*²-PMET-cGMP; 8-(4-OH-PT)-1,*N*²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing; and

(b) transplanting the blood vessel or portion thereof into a patient so as to form a vascular graft.

198. A solution comprising (a) a compound; and (b) heparinized blood, which compound is selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCI₂-cBIMPS; 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-

NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

199. A solution comprising (a) a compound; and (b) heparinized blood, which compound is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,*N*²-(4-CH₃O-PET)-cGMP; 8-Br-1,*N*²-PET-cGMP; 1,*N*²-□NET-cGMP; 8-Br-1,*N*²-PMET-cGMP; 8-(4-OH-PT)-1,*N*²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

200. An isolated *ex vivo* organ or portion thereof in contact with a solution comprising a compound selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCl₂-cBIMPS; 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

201. An isolated *ex vivo* organ or portion thereof in contact with a solution comprising a compound selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-

PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□-NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

202. A container containing the organ or portion thereof of claim 188 or 189.

203. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising a compound and heparinized blood, which compound is selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCI₂-cBIMPS; 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄OCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*Ip*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(Cl)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing.

204. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising a compound and heparinized blood, which compound is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□-NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing.

205. A method for performing an organ transplantation in a patient comprising,
(1) removing from contact with an organ or portion thereof a solution comprising:

(A) a compound and heparinized blood, which compound is selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCI₂-cBIMPS; 6-Phe cAMP; 8-MA

cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*Ip*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing; and/or

(B) a second compound and heparinized blood, which second compound is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,*N*²-(4-CH₃O-PET)-cGMP; 8-Br-1,*N*²-PET-cGMP; 1,*N*²-□NET-cGMP; 8-Br-1,*N*²-PMET-cGMP; 8-(4-OH-PT)-1,*N*²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing; and

(2) transplanting the organ or portion thereof into the patient.

206. A method for performing a coronary artery bypass graft in a patient comprising,

(1) removing from contact with a blood vessel or portion thereof a solution comprising:

(A) a compound and heparinized blood, which compound is selected from the group consisting of 6-MBC cAMP; Sp-5,6-DCI₂-cBIMPS; 6-Phe cAMP; 8-MA cAMP; 8-NH₂ cAMP; 2-Cl,8-NH₃-cAMP; 8-HA cAMP; 8-AHA cAMP; 6-NHC(O)NHC(CH₃)₂ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHCH₂C₆H₅ cAMP; 8-SeC₂H₅ cAMP; 2-nC₄H₉,8-Br-cAMP; 6-NHC(O)NHC(CH₃)₃ cAMP; 6-NH(*i*C₃H₇)₂ cAMP; 6-NH(CH₂)₂C₆H₃-3',4'-(OCH₃)₂ cAMP; 6-NHC(O)NH(CH₂)₂CH(CH₃)₂ cAMP; 6-NHC(O)NHC₆H₅ cAMP; 6-NHCH(CH₃)(CH₂)₂C₆H₅ cAMP; 6-NHC(O)NHC₆H₄oCl cAMP; 6-NH-(1-adamantyl) cAMP; 6-NH*t*C₄H₉ cAMP; 6-NHC(O)NHCH(CH₃)₂ cAMP; 6-piperidino cAMP; 8-SCH₂CH₃ cAMP; 8-SC₆H₄*p*Cl cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 2-CF₃ cAMP; 1-deaza cAMP; 8-SC₆H₄*Ip*Cl cAMP; 8-SCH₃ cAMP; 8-N₃ cAMP; 2-Cl,8-NHCH₃ cAMP; 2-Cl cAMP; 8-SiC₃H₇ cAMP; 8-SCH₂C₆H₄*p*NO₂ cAMP; 8-MA cAMP; 8-

NH₂ cAMP; 6-Phe cAMP; 6-NHnC₈H₁₇ cAMP; 8-BT cAMP; 6-NHCH₂CH=C(CL)CH₃ cAMP; 6-(C₂H₅)₂ cAMP; and a mixture of any two or more of the foregoing; and/or

(B) a second compound and heparinized blood, which second compound is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing; and

(2) grafting the blood vessel or portion thereof into the patient so as to serve as a coronary bypass graft.

207. A method of using an organ in a transplantation procedure comprising:

- (a) contacting an organ or portion thereof *ex vivo* with a solution comprising a compound selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing; and
- (b) transplanting the organ or portion thereof into a patient.

208. A method of using a blood vessel as a vascular graft comprising:

- (a) contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising a compound selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing; and
- (b) transplanting the blood vessel or portion thereof into a patient so as to form a vascular graft.

209. A solution comprising (a) a compound; and (b) heparinized blood, which compound is selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing.

210. An isolated *ex vivo* organ or portion thereof in contact with a solution comprising a compound selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing.

211. A container containing the organ or portion thereof of claim 210.

212. A method of preserving an organ comprising contacting an isolated organ or portion thereof *ex vivo* with a solution comprising a compound and heparinized blood,

which compound is selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing.

213. A method for performing an organ transplantation in a patient comprising,
(1) removing from contact with an organ or portion thereof a solution comprising:

(A) a compound and heparinized blood, which compound is selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing; and/or

(B) a second compound and heparinized blood, which second compound is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing; and

(2) transplanting the organ or portion thereof into the patient.

214. A method for performing a coronary artery bypass graft in a patient comprising,

(1) removing from contact with a blood vessel or portion thereof a solution comprising:

(A) a compound and heparinized blood, which compound is selected from the group consisting of 8-CPT cAMP; 6-Bnz cAMP; and a mixture of the foregoing; and/or

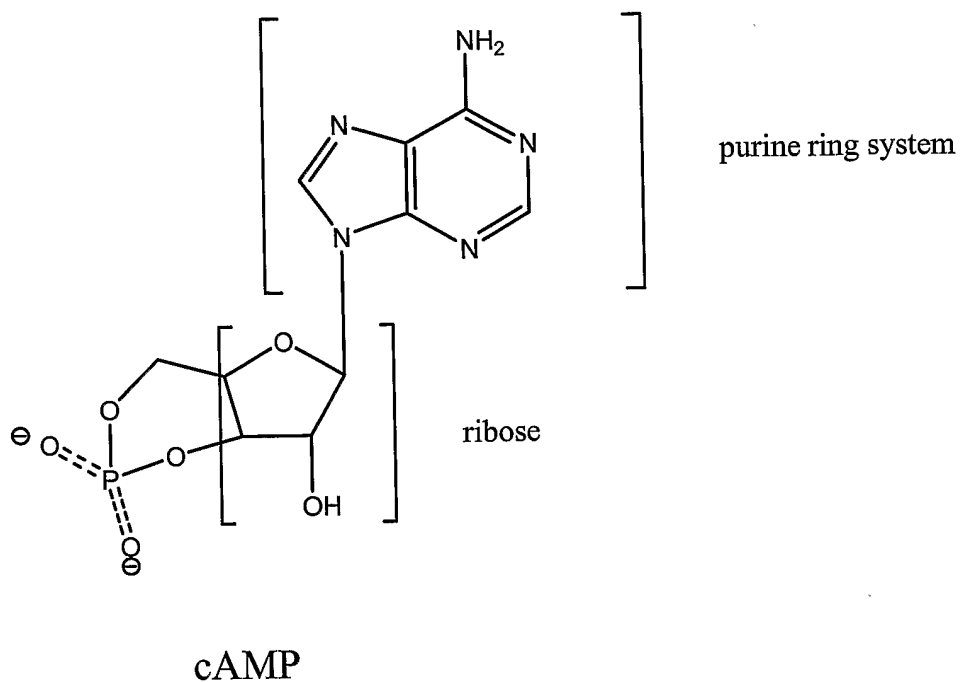
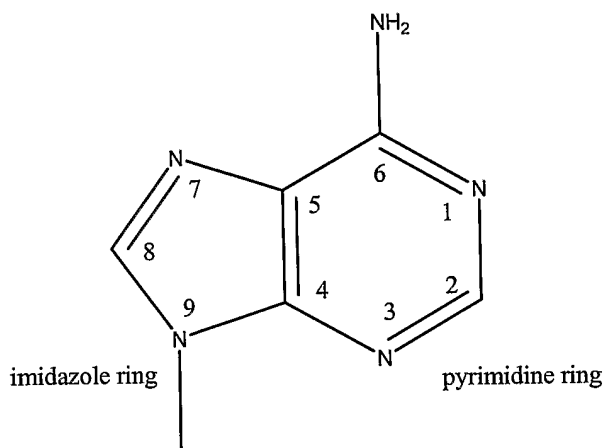
(B) a second compound and heparinized blood, which second compound is selected from the group consisting of Sp-8-pCPT cGMPS; 8-Br-PET cGMP; Sp-8-Br cGMPS; 8-(2,4-Di-OH-PT)-cGMP; 8-(2-NH₂-PT)-cGMP; 8-(4-OH-PT)-cGMP; 8-(4-NH₂-PT)-cGMP; 1,N²-(4-CH₃O-PET)-cGMP; 8-Br-1,N²-PET-cGMP; 1,N²-□NET-cGMP; 8-Br-1,N²-PMET-cGMP; 8-(4-OH-PT)-1,N²-PET-cGMP; 8-I-cGMP; and a mixture of any two or more of the foregoing; and

(2) grafting the blood vessel or portion thereof into the patient so as to serve as a coronary bypass graft.

215. The method of claim 1, 40, 151, 185, 189, 191, wherein the activator also (i) is at least 25% more cell membrane permeable as compared to each of 8-CPT cAMP and 6-

Bnz cAMP; (ii) binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of 8-CPT cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively.

216. The solution of claim 78, 97, wherein the activator also (i) is at least 25% more cell membrane permeable as compared to each of 8-CPT cAMP and 6-Bnz cAMP; (ii) binds with at least 25% greater affinity to the regulatory subunit of PKA as compared to each of 8-CPT cAMP; and/or (iii) binds to the regulatory subunit of PKA and results in at least 25% greater level of PKA kinase activity as compared to the PKA kinase activity resulting from exposure of PKA to the same amount of each of 8-CPT cAMP and 6-Bnz cAMP, respectively.

A**B****FIGURES 1A-1B**