AUSTRALIA
PATENTS ACT 1990
NOTICE OF ENTITLEMENT

We, **Atsuo F. Fukunaga** AND **Alex S. Fukunaga**
of **5411 Littlebow Road, Rancho Palos Verdes, CA, 90274, USA,**
the applicants/Nominated Persons in respect of Application No. 12920/95, state the following:-

The Nominated Persons are entitled to the grant of the patent because the Nominated Persons are the actual inventors.

The Nominated Persons are entitled to claim priority from the basic applications listed on the Patent Request form because the Nominated Persons are the applicants of the applications listed on the Patent Request form. The applications listed in the declaration under Article 8 of the PCT are the first applications made in a Convention country in respect of the invention.

DATED this TWENTY-NINTH day of NOVEMBER 1996

.....

a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the
applicant(s)

(DCC ref: 1815074)

AU9512920

(12) PATENT ABRIDGMENT (11) Document No. AU-B-12920/95
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 688835

(54) Title
PURINE COMPOSITIONS AND METHODS FOR ADMINISTRATION

International Patent Classification(s)
(51)[6] A61K 031/70 A61K 031/55 A61K 031/135

(21) Application No. : 12920/95 (22) Application Date : 21.11.94

(87) PCT Publication Number : WO95/14478

(30) Priority Data

(31) Number	(32) Date	(33) Country
158012	24.11.93	US UNITED STATES OF AMERICA
341668	17.11.94	US UNITED STATES OF AMERICA

(43) Publication Date : 13.06.95

(44) Publication Date of Accepted Application : 19.03.98

(71) Applicant(s)
ATSUO F. FUKUNAGA; ALEX S. FUKUNAGA

(72) Inventor(s)
ATSUO F. FUKUNAGA; ALEX S. FUKUNAGA

(74) Attorney or Agent
DAVIES COLLISON CAVE , 1 Little Collins Street, MELBOURNE VIC 3000

(56) Prior Art Documents
US 5104859

(57) Claim

1. A method for administering a purine compound to a mammal, comprising the steps of:

combining a purine compound with a counteractive agent, wherein said purine compound induces a desired effect and an undesired effect when administered in an effective amount to a mammal without administering said counteractive agent, and said counteractive agent counteracts at least a portion of said undesired effect upon administration of the combination to a mammal; and

administering said combination to a mammal in an amount effective to induce said desired effect in said mammal without inducing said undesired effect to the same extent said undesired effect would occur in the absence of said counteractive agent, wherein said counteractive agent is a catecholamine compound.

8. A method for administering a catecholamine to a mammal, comprising the steps of:

combining a catecholamine with a counteractive agent, wherein said catecholamine induces a desired effect and an

.../2

undesired effect when administered in an effective amount to a mammal without administering said counteractive agent, and said counteractive agent reduces said undesired effect upon administration of the combination to a mammal; and

administering said combination to a mammal in an amount effective to induce said desired effect in said mammal while reducing said undesired effect.

13. A pharmaceutical composition, comprising:

a purine compound, and a catecholamine compound, said compounds being in a pharmaceutically acceptable carrier, wherein the ratio of said purine compound to said catecholamine compound permits said purine compound to induce a desired effect when administered in an effective amount to a mammal without inducing an undesired effect to the same degree said undesired effect would have been induced by said purine compound had said catecholamine compound not been present in said composition.

OPI DATE 13/06/95 APPLN. ID 12920/95
AUJP DATE 03/08/95 PCT NUMBER PCT/US94/13412

AU9512920

(51) International Patent Classification 6 :
A61K 31/70, 31/55, 31/135

A1

(11) International Publication Number: WO 95/14478

(43) International Publication Date: 1 June 1995 (01.06.95)

(21) International Application Number: PCT/US94/13412

(22) International Filing Date: 21 November 1994 (21.11.94)

(30) Priority Data:
08/158,012 24 November 1993 (24.11.93) US
08/341,668 17 November 1994 (17.11.94) US

(71)(72) Applicants and Inventors: FUKUNAGA, Atsuo, F. [JP/US]; 5411 Littlebow Road, Rancho Palos Verdes, CA 90274 (US). FUKUNAGA, Alex, S. [US/US]; 5411 Littlebow Road, Rancho Palos Verdes, CA 90274 (US).

(74) Agents: PARKHURST, David, G. et al.; Fulwider Patton Lee & Utecht, 10th floor, 10877 Wilshire Boulevard, Los Angeles, CA 90024 (US).

(81) Designated States: AU, BG, BR, CA, CN, FI, HU, JP, KR, NO, NZ, PL, RU, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

688835

(54) Title: PURINE COMPOSITIONS AND METHODS FOR ADMINISTRATION

(57) Abstract

A purine compound, which has a desired and an undesired effect when a dosage sufficient to induce the desired effect is administered to a mammal, is combined with a counteracting agent, wherein the counteracting agent can reduce the undesired effect when the combination containing an effective amount of the purine compound is administered to a mammal. In a preferred embodiment, an adenosine compound is combined in vitro with a catecholamine in a predetermined ratio to form an adenosine composition. Very high dosages of a purine compound, such as adenosine, ATP or their analogs can be administered to a mammal via administration of compositions containing the purine compound and a counteracting agent, while reducing the dangerous, undesired effects associated with administering the same dosage of purine compound without first combining it with the counteracting agent. New catecholamine compositions are also taught. The purine and catecholamine compositions of the present invention pioneer new therapies which take advantage of the diversity of physiological effects of purine and catecholamine compounds.

PURINE COMPOSITIONS AND METHODS FOR ADMINISTRATION

RELATED APPLICATIONS

This is a continuation-in-part of Serial No. 08/158,012 filed November 24, 1993; and is related to Serial No. 08/083,214, which is incorporated by reference herein. Serial No. 08/083,214 is a continuation of Serial No. 07/756,480, which is a continuation-in-part of Serial No. 07/521,529.

FIELD OF THE INVENTION

The present invention is directed to medicinal compositions and methods for their administration, and more particularly is directed to purine containing compositions, methods for producing purine containing compositions, and methods for administration of same. In another aspect, the present invention is directed to catecholamine containing compositions, methods for producing catecholamine containing compositions, and methods for administration of same.

BACKGROUND OF THE INVENTION

Purine compounds are found in mammalian organisms both intracellularly and extracellularly, and play vital roles in metabolic processes. A nonlimiting example of the ubiquitous nature of purine compounds in mammalian systems is the purine containing nucleoside adenosine, which was reported over 60 years ago to relax coronary vascular smooth muscle and to impair atrioventricular conduction; adenosine has also been found to have antinociceptive properties and has recently been proven to be useful as an anesthetic. The widespread actions of adenosine include effects on the cardiovascular, nervous,

respiratory, gastrointestinal, renal and reproductive systems, as well as on blood cells, adipocytes, and immune systems. Very small doses of adenosine (0.01-0.25 mg/kg), provided as a single bolus injection, have been suggested for the treatment of supraventricular tachycardia. A continuous intravenous infusion of up to 0.2 mg/kg/min adenosine for a duration of about 6 minutes has been also suggested for use in diagnostic myocardial imaging. Likewise, the phosphorylated adenosine nucleoside, or adenosine nucleotide, has also been found useful in inducing an anesthetic effect (a phosphorylated nucleoside is a nucleotide). Use of adenosine compounds in anesthesia is discussed in more detail in co-pending U.S. patent application 08/083,214, entitled THERAPEUTIC USE OF ADENOSINE COMPOUNDS. The method described in application serial no. 08/083,214 involves a great improvement in anesthesia by administering up to 5 mg/kg/min adenosine or ATP to a mammal via a continuous infusion; the dosage is adjusted in response to cardiovascular changes which are due to surgical stimulation. At the dosages used, the anesthetic effect is slowly induced, and the patient must be carefully monitored. It is believed that the slow induction of an anesthetic effect is due to the low dosage of adenosine provided, but it was not believed safe to increase the dosages to more quickly achieve an anesthetic effect.

It is believed that the activity of purine compounds is mediated by cell surface receptors specific for a particular purine compound. Depending on a compound and its receptor, binding of the compound to the receptor can be reversible, and have a variety of effects. Further, it is believed that certain compounds can bind to more than one receptor in a competitive fashion with other compounds. In a process, sometimes referred to as biofeedback, the binding of a first compound to a particular receptor or the presence of a first compound

may induce the body to produce another agent which counteracts one or more of the effects of the first compound. For example, endogenous substances known as catecholamines, such as those produced by the nerve endings and the adrenal glands, may be released in response to a stressful situation (e.g., norepinephrine, epinephrine, and dopamine). For example, the endogenous production/release of tiny amounts of catecholamines causes increased heart rate and vasoconstriction, which the body responds to by the production of tiny amounts of adenosine and ATP which are believed to counteract certain of the effects of the increased endogenous catecholamines by different receptor mechanisms.

Considerable research has been directed to purine compounds since Drury and Szent-Gyorgyi reported in 1929 on the physiological actions of adenosine on cardiovascular function. Several classes of purine receptors have been identified, and adenosine and adenosine triphosphate, ATP, have been demonstrated as endogenous protective substances. Although certain purine compounds have significant beneficial physiological capabilities, the aforementioned ubiquitous nature and effects of purine compounds also tends to make it difficult to use them therapeutically. In other words, administration of purine compounds to a mammal will have both desired and undesired effects depending on patient physiology and the dosages provided.

Furthermore, because these purine compounds such as adenosine are considered toxic at concentrations that have to be administered to a patient to maintain efficacious extracellular therapeutic level, the administration of adenosine alone has been considered of no use or limited therapeutic use. Therefore, pharmacologists have directed their efforts to achieving high local extracellular level of adenosine by a) inhibiting the uptake of adenosine with reagents that

specifically block adenosine transport; b) prevention of the metabolic degradation of adenosine; c) the use of adenosine analogs which will bind to specific adenosine receptors; and recently d) the use of adenosine via its precursor, AICA riboside, which has been the subject of a number of publications and patents (U.S. Patent 5,082,829; 5,132,291; 5,187,162; 5,200,525; 5,236,908). However, the above approaches still have major disadvantages associated with their use. The metabolic and uptake blocker strategy is very much restricted in character due to the limited ability of tissue to generate purine compounds, and the adenosine agonist approach has the substantial peripheral side effects associated with these agents, such as hypotension, bradycardia, etc. Thus, despite all the intense efforts in basic sciences and pharmaceutical research, to this date, there has been little success in developing agents that can be used as therapeutic drugs to fully activate purine receptors without side effects. Therefore, until now, there has been no successful medical treatment for prevention or posttreatment of ischemic damage.

For more information on purine compounds and purine receptor agonists, see Ely et al., "Protective Effects of Adenosine in Myocardial Ischemia", Circulation, 85: 893-904 (1992); Miller et al., "Therapeutic Potential for Adenosine Receptor Activation in Ischemic Brain Injury," J. Neurotrauma, 9: 563-77 (1992); Williams, "Adenosine Receptors as Drug Targets: Fulfilling the Promise?," in Jacobson et al., Ed., Purine in Cellular Signaling: Targets for New Drugs, New York, Springer-Verlag (1990)(See particularly page 175); Lawson et al., "Preconditioning: State of the Art Myocardial Protection," Cardiovascular Research, 27: 542-50 (1993); Rudolphi, "Manipulation of Purinergic Tone as Mechanism for Controlling Ischemic Brain Damage," in Phillis, J. W., Ed., Adenosine and Adenine Nucleotides as Regulators

of Cellular Function, Boca Raton, CRC Press (1991); Berne, R., "Adenosine - a Cardioprotective and Therapeutic Agent," Cardiovascular Research, 27:2 (1993); Phillis et al., "Roles of Adenosine and Adenine Nucleotides in the Central Nervous System," in Daly et al., Eds., Physiology and Pharmacology of Adenosine Derivatives, Raven Press, New York (1983); Galinanes, "Should Adenosine Continue To Be Ignored As A Cardioprotective Agent In Cardiac Operations?," Journal of Thoracic and Cardiovascular Surgery, 105: 180-183 (1993); Jacobson et al., "Novel Therapeutics Acting Via Purine Receptors," Biochemical Pharmacology 41: 1399-1410 (1991); U.K. Patent 797,237; U.S. Patent 4,514,405; U.S. Patent 4,590,180; U.S. Patent 4,605,644; U.S. Patent 4,673,563; U.S. Patent 4,880,783; U.S. Patent 4,880,918; U.S. Patent 5,049,372; U.S. Patent 5,070,877; U.S. Patent 5,104,859; Daval et al., "Physiological and Pharmacological Properties of Adenosine Therapeutic Implications," Life Sciences 49: 1435-1453 (1991); Dubyak et al., Eds., Biological Actions of Extracellular ATP, Annals of the New York Academy of Sciences v. 603, New York, N.Y. Academy of Sciences (1990); Imai et al., Eds., Role of Adenosine and Adenine Nucleotides in the Biological System. Metabolism, Release, Transport. Receptors, Transduction Mechanisms and Biological Actions, Amsterdam, Elsevier (1991); Ribeiro, Ed., Adenosine Receptors in the Nervous System, London, Taylor & Francis (1989); Williams, Ed., Adenosine and Adenosine Receptors, Clifton, N.J. The Humana Press (1990); Tsuchida et al., "Pretreatment with the adenosine A_1 selective agonist, 2-chloro-N6-cyclopentyladenosine (CCPA), causes a sustained limitation of infarct size in rabbits," Cardiovascular Research, 27:652-66 (1993); Fukunaga et al., "Hypotensive effects of adenosine and adenosine triphosphate compared with sodium nitroprusside," Anesthesia and Analgesia 61:273-278

(1982); Fukunaga et al., "Effects of intravenously administered adenosine and ATP on halothane MAC and its reversal by aminophylline in rabbits," Anesthesiology, 71:A260 (1989); Drury et al. "The physiological activity of adenine compounds with special reference to their action upon the mammalian heart", Journal of Physiology (London) 68:213-37 (1929); Olsson et al. "Cardiovascular purinoceptors," Physiological Reviews, 70:761-809 (1990); Downey et al., Ed. "Spotlight on the cardioprotective properties of adenosine", Cardiovascular Research, v. 27, No. 1 whole issue (1993); Rudolphi et al., Neuroprotective role of adenosine in cerebral ischemia," Trends in Pharmacological Sciences, 13:439-45 (1992); and Williams, "Purinergic pharmaceuticals for the 1990s," Nucleosides & Nucleotides, 10:1087-99 (1991); U.S. Patent 5,082,829; 5,132,291; 5,187,162; 5,200,525; 5,236,908; Homeister et al., "Combined adenosine and lidocaine administration limits myocardial reperfusion injury," Circulation 82:595-608 (1990); Mullane K, "Acadesine: the prototype adenosine regulating agent for reducing myocardial ischaemic injury," Cardiovascular research 27:43-7 (1993); Van Belle H, "nucleoside transport inhibition: a therapeutic approach to cardioprotection via adenosine?," Cardiovascular Research 27:68-76 (1993); all of which are incorporated by reference. Perhaps the greatest problem with attempts to utilize purine compounds as therapeutic agents is due to the undesired and often fatal side effects associated with providing sufficient amounts of a purine compound to a patient to induce a desired effect. For example, it has been well documented that adenosine plays a key role in the endogenous defenses of the brain against the damaging effects of ischemia. Moreover, adenosine has been reported to protect the heart when given both prior to ischemia and at reperfusion; however, intravenous administration of adenosine or even an A_1 selective

agonist has been shown to cause profound hypotension (A_1 represents one of the purported adenosine receptors in mammalian systems). In another example, anesthesia is induced in a mammal by administering large amounts of adenosine or ATP; however, anesthetically effective dosages can be fatal to the recipient if extreme care is not followed in administering same (e.g., titration of adenosine in response to accurate monitoring of patient vital signs) or if a counteracting agent is not provided promptly in response to dangerous patient vital function levels. Even with prior or subsequent provision of agents to counteract certain undesired effects of administering the large dosages of adenosine, or an adenosine analog, sufficient to induce anesthesia, dangerous variations in vital functions can result. This "pendulum effect" on patient physiological processes, which is reflected in large changes in patient vital signs, such as but not limited to blood pressure, heart rate, and respiration, discourages the therapeutic use of purine compounds.

Therefore, there is a need for purine compositions comprising a purine compound which can be more easily and safely administered in a sufficient amount to induce a desired effect without inducing an undesired effect which is usually associated with administering the same amount of purine compound alone. Most prior attempts to counteract the undesired effects of administering a purine compound have involved the use of receptor specific antagonists. Furthermore, it was believed that, due to the dissimilar structure and function of the purine compounds with respect to the agents which counteract certain undesired effects of administering the purine compounds, that the purine compounds and counteracting agents could not be simultaneously used or mixed together in-vitro and still be safely administered for therapeutic purposes.

SUMMARY OF THE INVENTION

Briefly, and in general terms, the present invention provides for purine compositions and methods of administering the purine compositions, in which a synergistic and unexpected beneficial result is obtained by combining a purine compound with a counteractive agent, wherein the pendulum effect or radical variation in certain patient vital functions have been greatly reduced, and high dosages of a purine compound, previously believed to cause a dangerous or fatal undesired effect, can be safely administered to induce a desired effect while reducing an undesired effect.

Thus, the present invention is directed to purine compositions, and methods of administering the purine compositions. The purine composition preferably comprise a purine compound and a counteractive agent, in which the purine compound induces a desired effect and an undesired effect when administered in an effective amount to a mammal without administering the counteractive agent, and the counteractive agent, when combined with the purine compound prior to administration of the effective amount of the purine compound, reduces an undesired effect of the effective amount of the purine compound upon administration of the combination to a mammal.

In a preferred embodiment, purine compounds capable of inducing a desired effect, such as but not limited to central nervous system inhibition, neuroprotection, autonomic nervous system modulation or inhibition, cardiac protection, and respiratory protection, analgesia/anesthesia but which also induce an undesired effect, such as but not limited to severe hypotension and cardiodepression, are combined in vitro with a counteractive agent which reduces an undesired effect while permitting the purine compound to induce a desired effect when the mixture is administered. Compositions in accordance with the present invention can be formulated batchwise long periods of time in advance of

administration, or, for example, can be mixed in a suitable fitting attached to an IV set just prior to passage into a patient, or the components of the composition can be simultaneously administered. In a preferred embodiment, the purine compound is selected from the group consisting of adenosine, adenosine analogs, phosphorylated adenosine, and phosphorylated adenosine analogs, and is combined with a counteractive agent. In a preferred embodiment, the counteractive agent is a catecholamine, such as but not limited to epinephrine, norepinephrine, dopamine, dobutamine, and phenylephrine.

In another aspect, a purine composition is formed by combining a purine compound, a counteractive agent, and a purine compound potentiator and/or a CNS depressant. The potentiator may be a compound which inhibits uptake of the purine compound, or a compound which interferes with the ability of endogenous enzymes to metabolize or otherwise degrade the purine compound, or a compound which enhances adenosine release or a combination of any of them. The potentiator may be compounds such as but not limited to an adenosine uptake (transport) inhibitor (e.g., dipyridamole); an adenosine deaminase inhibitor (e.g., deoxycoformycin, and erythro-9-(2-hydroxy-3-nonyl)adenine; a precursor (e.g. AICA riboside); a CNS depressant (e.g., a benzodiazepine, such as diazepam, midazolam, and flumazenil, an opioid, such as morphine, fentanyl, and sufentanil, or a barbiturate, such as thiopental, and methohexital, etomidate, propofol); an adrenergic α_2- agonist (e.g., clonidine, and dexmedetomidine); or a non-steroidal anti-inflammatory drug (e.g. aspirin, ibuprofen, ketorolac).

In another aspect, the present invention has led to the discovery that the multiple effects of the present composition can be used in concert with other drugs producing synergistic effects of their combined activity.

AUSTRALIAN PATENT OFFICE

The present composition can be used as a carrier of other drugs such as antibiotics, antipyretics, anti-viral, anti-cancer, anti-toxin, chemotherapeutic agents, potassium channel openers, and the like. The effects of the present composition as a blood flow regulator/modulator will selectively target pathological tissues/organs and will enhance the desirable effects of other drugs as well. For example, the affirmative and desirable effects of opioids, benzodiazepines and the like, can be enhanced while the side effects and/or undesirable effects of such drugs can be counteracted. Thus the combined use of various drugs as in the present composition can act as catalysts. For example, by the methods and compositions according to the invention, the respiratory depression effects caused by the opioids and the benzodiazepines can be counteracted, while the salutary effects such as the analgesic and sedative effects can be potentiated.

In another aspect, the present invention has also led to the discovery that surprisingly large dosages of a catecholamine compound, previously thought sufficient to induce dangerous or fatal side effects, can be administered by combining the catecholamine compound with a counteractive agent prior to administration, wherein the catecholamine compound can induce a desired effect upon administration in an effective amount, while the counteractive agent reduces an undesired effect or effects upon administration of the combination to a mammal. In a preferred embodiment, the catecholamine is combined with a purine compound to form a catecholamine composition capable of inducing a desired catecholamine effect while reducing one or more undesired effects which would result if the catecholamine had been administered without first being combined with the purine compound.

In another aspect, it has been surprisingly discovered that high dosages of a purine compound or a

AUSTRALIAN PATENT OFFICE

AMENDED SHEET

catecholamine compound can now be safely administered to a mammal by mixing the appropriate ratio of purine compound or catecholamine compound with a counteracting agent. For example, higher dosages of a purine compound and a catecholamine compound can be safely administered to a mammal than previously thought possible by combining the purine compound and the catecholamine compound in predetermined ratios prior to administration. Appropriate ratios of purine compound or catecholamine compound to counteracting agent in pharmaceutical compositions according to the present invention can be readily determined by one of ordinary skill in the art by performing a few routine tests, which involve the monitoring of vital signs of interest (e.g., blood pressure, heart rate, respiration) while administering varying ratios of purine compound or catecholamine compound to counteracting agent, and at varying compound dosages.

By way of a nonlimiting example, initially administering low dosages (e.g., dosages known to be safe) of a purine compound combined with a counteracting agent in varying ratios will enable determination of the appropriate ratio of purine compound to counteractive agent which begin to induce a desired effect while reducing an undesired effect; dosages can then be increased and the ratio of purine compound to counteracting agent adjusted to optimize the desired effect achieved while minimizing an undesired effect. In a preferred embodiment, a purine composition, comprising between about 1 part by weight norepinephrine combined with about 25 to 2,000 parts by weight adenosine can be administered to a mammal to induce a desired effect while reducing an undesired effect, wherein the undesired effect would occur to a much greater degree were it not for the presence of the norepinephrine in the composition. Other nonlimiting examples of preferred

inventive compositions include compositions comprising 1 part epinephrine combined with between about 50 and about 4,000 parts by weight adenosine, compositions comprising one part by weight phenylephrine combined with about 10 to about 200 parts by weight adenosine, and compositions comprising one part by weight dopamine combined with about two to about five parts by weight adenosine. The preceding compositions can have varying dosages of adenosine.

Catecholamine compositions in accordance with the present invention can be formed by combining a catecholamine compound with a counteracting agent and determining the appropriate dosages and ratios for obtaining the desired effect while minimizing undesired effects.

The same principle can be applied for the use of adenosine analogs that have much longer effects. For this, longer acting catecholamines can be combined in the composition, or a separate, infusion of counteractive catecholamines can be administered in one or more stages, preferably in a continuous infusion commencing at some time following infusion of an initial mixture of an adenosine analog and catecholamine. In addition, the composition can be formulated with an additive that can make it absorbable from gastrointestinal tracts/organs, and be easily ingested (taken orally, enterally).

One of ordinary skill in the art will recognize that the ratios of catecholamine compound or purine compound to counteracting agent can be adjusted depending on patient physiology, vital signs, and the therapeutic purpose (e.g., a hypotensive and/or bradycardic patient will require less adenosine to induce normotension, while a hypertensive and/or tachycardiac patient will require more adenosine to induce the same effect). Likewise, dosage will depend on the desired effect and patient physiopathology. The compositions of the present

invention may be formed in combination with pharmaceutically acceptable carriers, and stored in accordance with standard procedures and precautions for medicinal compositions. The present invention pioneers the use of purine compounds to efficaciously activate purine receptors for therapeutic purposes.

The present invention pioneers the therapeutic use of high dosages of purine compounds and catecholamine compounds for a wide variety of uses, as well as pioneers the use of new and useful purine and catecholamine compositions formed by combining a purine compound or a catecholamine compound with a counteracting agent. For example, purine compositions prepared in accordance with the present invention can be administered in an amount effective to induce anesthesia and analgesia faster and/or more safely than present anesthetic methods. Further, administration of purine compounds according to the present invention has demonstrated CNS inhibitory effects, and the effects of modulation of the autonomic nervous system and modulation of circulation, respiration as well as homeostatic metabolism. It may be used in cardioprotection, neuroprotection, pulmonary protection, metabolic homeostasis preservation, sedation, anethesia, antipyretic, antihypertensive treatment, and prevention and/or treatment of ischemia/hypoxia.

The invention is further described and illustrated by the following detailed description and nonlimiting examples.

DESCRIPTION OF THE DRAWINGS

Figures 1(a)-(d) are blood pressure (in mmHg) recordings over time following administration of bolus injections of a catecholamine alone, adenosine alone, or varying combinations of adenosine with a catecholamine;

Figs. 2(a)-(b) are blood pressure (mmHg) tracings from a rabbit administered bolus injections of adenosine alone, norepinephrine alone, or a combination of adenosine and norepinephrine;

Fig. 3 illustrates sedative and antinociceptive thresholds in response to electrical tail stimulation, ETS, before and after administration of a mixture of adenosine, catecholamine, and benzodiazepine;

Fig. 4(a) is a blood pressure tracing over time which illustrates the effects of administering bolus injections of ATP and norepinephrine;

Fig. 4(b) is a blood pressure tracing over time which illustrates the reduction in the blood pressure pendulum effect when high dosages of ATP combined with norepinephrine are administered from a pre-mixed solution;

Fig. 5 illustrates the duration of the sedative and analgesic effects after administration of diazepam (2 mg/kg) and AC (ATP: 200 mg/kg combined with NE:0.8 mg/kg); Figs. 6(a)-(j) illustrate the cardio-respiratory and metabolic assessment following high dose-fentanyl administration in two groups pretreated with: a) saline, or b) AC (ATP:100 mg/kg and NE:0.2 mg/kg);

Figs. 7(a)-(d) illustrate the rates of mortality and pulmonary edema over time due to infusion of norepinephrine as a cardiotoxic stimulant with and without adenosine/catecholamine compositions of the invention, showing the cardiopulmonary protective effects of adenosine/catecholamine compositions of the invention;

Figs. 8(a)-(b) illustrate the rates of mortality and pulmonary edema over time due to infusion of epinephrine as a cardiotoxic stimulant with and without adenosine/catecholamine or ATP/catecholamine compositions of the invention, showing the cardiopulmonary protective effects of adenosine/catecholamine compositions of the invention;

Fig. 9 is a chart illustrating effects on metabolic acidosis due to stress and the effects on maintaining metabolic homeostasis and protection from ischemia by administration of adenosine/catecholamine compositions of the invention;

Fig. 10 is a blood pressure recording over time following an initial administration of a longer lasting adenosine analog, R-PIA, along with norepinephrine, and subsequent administration of norepinephrine, illustrating a method of administering longer lasting adenosine analogs with catecholamine according to the invention;

Fig. 11 is a blood pressure recording over time following an initial administration of a longer lasting adenosine analog, R-PIA, along with norepinephrine, and subsequent administration of norepinephrine, illustrating another method of administering longer lasting adenosine analogs with catecholamine according to the invention; and

Fig. 12 is a blood pressure recording over time following an initial administration of a longer lasting adenosine analog, NECA, along with norepinephrine, and subsequent administration of norepinephrine, illustrating another method of administering longer lasting adenosine analogs with catecholamine according to the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It has been surprisingly discovered that, despite the dissimilar structure and function of purine compounds and their counteracting agents, that they have sufficiently similar pharmacokinetics to be used simultaneously or to be combined together in vitro and administered, so that certain of the undesired effects of administering purine compounds alone can be offset by the coadministered counteracting agents. Further, it has been surprisingly

discovered that the in-vitro combination of the purine compounds with counteracting agents does not result in an adverse reaction in-vitro, or in-vivo following administration, and that surprisingly improved results can be achieved by administering a mixture of a purine compound with a counteractive agent. In fact, it is amazing that such unforseen synergistic effects of two potent and antagonistic substances when used simultaneously or combined together in vitro could have such enhanced and significant biological effects. For the purposes of this disclosure, a purine compound is defined as a compound including the purine functionality (by way of nonlimiting example, adenosine), a purine analog, or a purine receptor agonist, which has at least one desired effect and at least one undesired effect upon administration to a mammal in an amount sufficient to induce a desired effect ("purine effect"). A catecholamine compound is defined herein as a catecholamine (by way of nonlimiting example, norepinephrine), a catecholamine analog, or a catecholamine receptor agonist having at least one desired effect ("catecholamine effect") and at least one undesired effect upon administration to a mammal of an amount sufficient to induce a desired effect. A counteracting agent is defined as an agent which is capable of reducing an undesired effect caused by administration to a mammal of an effective amount of a purine compound or a catecholamine compound. As used hereinafter, the term "AC" refers to a combination or simultaneous administration of adenosine, adenosine analogs, phosphorylated adenosine, or phosphorylated adenosine analogs, and catecholamine, and "ACB" is the combinational use of AC and benzodiazepine.

As shown in the figures for purposes of illustration and as discussed above, the present invention has tremendous benefits to medicine, and pioneers new

purinergic therapies and adrenergic therapies. This is partly because larger doses of purine compounds and catecholamine compounds than were previously thought possible can now be safely administered to a mammal to induce a desired effect while reducing at least one undesired effect previously associated with administering such a dosage. As is illustrated by the blood pressure, BP, tracings in Figure 1(a), provision of a purine compound, such as adenosine, or a catecholamine compound, such as norepinephrine, alone, induces severe alterations in patient vital functions. It is noted that, although blood pressure is primarily used in this disclosure to demonstrate this phenomenon, other patient vital functions can be monitored as well to illustrate the beneficial effects of the present invention. For example, in addition to blood pressure, other patient vital functions which can be monitored include but are not limited to electrocardiogram EKG, respiratory rate, RR (breaths per minute), heart rate, HR (beats per minute, BPM), body temperature, and blood gas data: $PaCO_2$ and PaO_2 for respiratory parameters, pH, and base excess (BE) for metabolic parameters.

The present invention enables the therapeutic use of purine and catecholamine compounds by reducing the severe side effects associated with administering a sufficient dosage of a purine compound or a catecholamine compound to induce a desired effect. The attenuation or dampening of undesired radical alterations in certain patient vital functions by administration of compositions prepared in accordance with the present invention is made clear by the following nonlimiting examples.

As a nonlimiting example of how one of ordinary skill in the art would determine the appropriate ratio of a purine compound combined with a counteracting agent in a composition formed in accordance with the present invention, the following steps can be followed: A desired

effect of administering a purine compound can be achieved by administering a sufficient amount of the purine compound to a mammal to induce the desired effect. For example, adenosine can be administered to a patient to induce an analgesic/anesthetic effect provided a sufficient amount of adenosine is administered to the patient. However, administering a dosage of adenosine to a mammal sufficient to induce analgesia/anesthesia will also induce severe hypotension and cardio-depression, which can be monitored by blood pressure recording devices and heart rate (EKG) monitors. The degree of hypotension and cardio-depression can be sufficient to cause irreversible damage to patient vital organs, or may even induce death. Therefore, it is necessary to first determine the appropriate ratio of adenosine to counteracting agent in the purine compound composition to be administered to the patient. In order to do this, the patient vital functions of interest, for example, the heart rate and blood pressure, can be monitored prior to and during administration of compositions containing varying ratios of the purine compound to the counteracting agent (e.g., adenosine to catecholamine ratio).

Initially, only small dosages of the purine compound which are known not to cause dangerous side effects should be administered in combination with a counteracting agent which is also provided at a dosage sufficiently small that it is known to cause no adverse side effects. The ratios of the purine compound to the counteracting agent can then be titrated to attenuate radical fluctuations in the vital function of interest. Thereafter, the combined dosages of purine compound and counteracting agent can be gradually increased, with the ratio of the purine compound combined with counteracting agent adjusted to optimize patient vital functions of interest.

Because of the similarity of physiological response to purine compounds and catecholamine compounds in humans and in rabbits, rabbits provide an ideal source of information on the appropriate ratios of purine compound or catecholamine compound to counteracting agent in compositions to be administered to a human. Those of ordinary skill in the art will immediately recognize that dosages and ratios may vary from patient to patient depending upon the type of therapy desired and on the particular patient. As with the administration of any drug, those of ordinary skill in the art should follow normal procedures for minimizing the risk of adverse reactions when supplying compositions in accordance with the present invention to a patient receiving other drugs or therapies. See Gilman et al., Eds., Goodman and Gilman's. The Pharmacological Basis of Therapeutics, 9th ed., New York, Pergamon Press (1990); and Katzung, Ed., Basic and Clinical Pharmacology, 5th Ed., Norwalk, Appleton & Lange (1992).

Compositions of the present invention may be administered with pharmaceutically acceptable carriers, and may be combined with a potentiator, which may increase or prolong the beneficial effects of the purine or catecholamine compound. In the event of contraindications, dosages may be adjusted by a physician administering compositions of the present invention, or additional amounts of a purine compound, a catecholamine compound, and/or a counteracting agent may be administered.

Some patients have reported discomfort, such as headaches, flushing, and angina-like chest pain following administration of purine compounds, such as adenosine. In order to minimize this discomfort, a central nervous system, CNS, depressant may be administered to the patient first, or may be combined with a purine composition or a catecholamine composition prepared in

accordance with the present invention. Suitable CNS depressants include but are not limited to benzodiazepines, opioids, barbiturates, and propofol.

In a preferred embodiment, adenosine compounds can be combined with an adenosine potentiator, such as but not limited to an adenosine uptake inhibitor (e.g., dipyridamole, dilazep, benzodiazepine), and/or an adenosine deaminase inhibitor (e.g., 2'deoxycoformycin, and erythro-9-(2-hydroxy-3-nonyl)adenine. Thus, in an alternative embodiment, a purine compound or catecholamine compound combined with a counteracting agent is also combined with a purine compound or catecholamine compound potentiator. In yet another embodiment, a purine compound or a catecholamine compound combined with a counteracting agent are also combined with a CNS depressant. In another embodiment, a purine compound is combined with a counteracting agent, a CNS depressant, and a purine compound potentiator. In yet another embodiment, a catecholamine compound is combined with a counteracting agent, a CNS depressant, and a catecholamine compound potentiator.

The protective effects of administering a purine compound, such as adenosine, combined with a counteracting agent, such as a catecholamine, have also been clearly demonstrated by administration of a purine composition prepared in accordance with the present invention to a mammal suffering from severe respiratory depression and seizure activity caused by high dose opioids like fentanyl. Administration of a purine composition in accordance with the present invention also protects mammals from noxious stimulation by inducing both sedative and potent analgesic effects, while protecting the cardiovascular and metabolic functions which are usually affected by stressful conditions, such as excessively high plasma catecholamine levels and pain.

AUSTRALIAN PATENT OFFICE

With reference to Figs. 10-12, the method of the invention for administering a purine compound in combination with a catecholamine counteractive agent can also further comprise the administration of one or more separate infusions of additional catecholamine counteractive agent following the initial infusion of purine compound and catecholamine counteractive agent. Adenosine analogs that can be used in the compositions and method of the invention include, but are not limited to, 5'-N-ethylcarboxamidoadenosine (NECA), R(-)N^6-(2-phenylisopropyl) adenosine (R-PIA), 2-chloroadenoine (2-CADO), N^6-cyclopentyladenosine (CPA), and N^6-cyclohexyladenosine (CHA), for example. Such adenosine analogs can have longer lasting effects in the body than adenosine, and in particular can have longer lasting effects in the body than catecholamines typically co-administered with adenosine, such as norepinephrine, for example, so that the co-administration of catecholamine with an adenosine analog can further include judicious administration of at least one separate infusion of a selected catecholamine as the effects of a selected adenosine analog continue even beyond administration is stopped. The additional infusion of the selected catecholamine counteractive agent following the initial infusion of the mixture is preferably administered by a separate, continuous infusion of the catecholamine, and in one preferred embodiment, the additional infusion of catecholamine is administered in stages of progressively reduced dosages over time, as the selected adenosine analog is gradually metabolized.

The beneficial effects of administering the purine compositions and catecholamine compositions of the present invention to mammals are further illustrated by the following nonlimiting examples.

PCT/US 94/13412
IPEA/US 08 JAN 1996

EXAMPLE 1

Hemodynamic Effects of Intravenous Administration of a Combination of Adenosine-Catecholamine (AC)

Materials and Methods:

Drugs: Adenosine and ATP (adenosine 5'-triphosphate, disodium salt) were obtained from Kyowa Hakko Kogyo Co., Tokyo, Japan, and dissolved in standard saline solution. Norepinephrine bitartrate injection (LEVOPHED) was obtained from Winthrop Pharmaceuticals, and Midazolam hydrochloride was obtained from Roche Laboratories.

Unmedicated, healthy New Zealand white rabbits (male and female), weighing 2.5-2.7 Kg were studied. Rabbits were chosen because they are an excellent indicator of how these drugs and methods will work in humans. Anesthesia was initially induced with halothane 3-4 % in oxygen using a face mask, and the animals were allowed to breath spontaneously. A tracheostomy was performed on each rabbit, and a 3.5 F (French size) cuffed pediatric endotracheal tube was inserted into the trachea. The inhaled concentration of halothane was then lowered, and maintained with 1.5-2 % halothane in 100% oxygen during the preparation. Local infiltration with lidocaine (1 % solution) was done when a tracheostomy and femoral cut down were performed. An ear marginal vein and a central artery were cannulated with 22 and 24 gauge plastic catheters for drug and fluids administration and for blood sampling. After intravenous access was established, lactated Ringer's solution was started at 5 ml/kg/hr for fluid maintenance. The femoral artery was cannulated with a polyethylene catheter (PE 120) which was placed with its tip in the mid-thoracic aorta to measure central arterial blood pressure. The catheter was well secured and the skin was closed. The heart rate was continuously monitored via percutaneous leads (II)

AUSTRALIAN PATENT OFFICE

electrocardiograph (EKG), connected to a Hewlett Packard 78304A polygraph and recorded on a Hewlett Packard 78172A recorder. Body temperature was continuously monitored by means of a rectal probe, and maintained between 38.5-39.5°C with the aid of a heating lamp.

After completion of the experimental preparation, halothane was discontinued and the rabbits were placed in a sling in a natural, physiological posture which allowed the animal's head and legs freedom to move. After complete recovery from the halothane anesthesia, the following control measurements were taken from the unanesthetized animals: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), Respiratory Rate (RR), Heart Rate (HR), ECG, Body Temperature (BT), arterial blood gases: $PaCO_2$, PaO_2, pH, and base excess (BE). Blood gases were measured with a Radiometer ABL 30 blood gas analyzer.

Results and Conclusions:

In order to determine the optimal concentration ratio of the components of the adenosine-catecholamine, AC, composition, various concentrations of an adenosine compound (A) and a catecholamine (C) alone were separately injected, and then mixtures of adenosine with catecholamine having varying ratios of adenosine to catecholamine were tested in the in vivo experimental animal model. The results of these experiments are shown in Figures 1 and 2 which illustrate the effects on blood pressure (mmHg) over time from administering a purine compound or catecholamine alone, or from administering varying compositions of a purine compound combined with a catecholamine.

The above process was repeated several times for each animal, and with various combinations of adenosine and a catecholamine respectively, as illustrated in Figs

1(a)-(d). Fig. 1(a) has six separate tracings which result from administration of purine and catecholamine compounds as follows: Fig. 1(a), tracing (a)1: 20 μg norepinephrine; (a)2: 5 mg adenosine; (a)3: 10 mg adenosine; (a)4: 20 mg adenosine; (a)5: 40 mg adenosine; and (a)6: 20 mg adenosine. Figure 1(b) illustrates four tracings which result from administration of the following compositions formed from 20 mg adenosine combined with varying amounts of a catecholamine: (b)1: 1 part norepinephrine mixed with 250 parts adenosine (i.e., 20 mg adenosine combined with 0.08 mg norepinephrine); (b)2: 1 part norepinephrine mixed with 500 parts adenosine; (b)3: 1 part norepinephrine mixed with 1,000 parts adenosine; (b)4: 1 part norepinephrine mixed with 2,000 parts adenosine. Figure 1(c) illustrates four tracings which result from administration of the following compositions formed from 20 mg adenosine combined with varying amounts of a catecholamine: (c)1: 1 part epinephrine mixed with 500 parts adenosine; (c)2: 1 part epinephrine mixed with 1,000 parts adenosine; (c)3: 1 part epinephrine mixed with 2,000 parts adenosine; (c)4: 1 part epinephrine mixed with 4,000 parts adenosine. Figure 1(d) illustrates four tracings which result from administration of the following compositions formed from 20 mg adenosine combined with varying amounts of a catecholamine: (d)1: 1 part phenylephrine mixed with 25 parts adenosine; (d)2: 1 part phenylephrine mixed with 50 parts adenosine; (d)3: 1 part phenylephrine mixed with 100 parts adenosine; (d)4: 1 part phenylephrine mixed with 200 parts adenosine. Parts are given as parts by weight.

The tracings in Figure 2(a) illustrate the effects on blood pressure from administration of the following: (a)1: 10 mg/kg adenosine; (a)2: a mixture of 10 mg/kg adenosine with 0.01 mg/kg norepinephrine (ratio of 1000/1 adenosine to norepinephrine); (a)3: 0.01 mg/kg

norepinephrine. Figure 2(b) is a blood pressure tracing which results from administration of a mixture of 100 mg/kg adenosine with 0.1 mg/kg norepinephrine (ratio of 1000/1 adenosine to norepinephrine).

Once the adequate concentration ratio was determined for each animal, a large dose of the AC mixture solution was injected to test the blood pressure responses during the administration (Fig. 2(b)). A series of nine experiments were carried out in the rabbit model to estimate and determine the AC concentration ratio that showed minimal blood pressure changes, and to determine the effectiveness of the AC compositions having varying dosages and ratios of adenosine compounds to catecholamines. The cardio- respiratory vital signs of the rabbits were continuously monitored during and after administration of the AC mixture. Table 1 summarizes the effective concentration ratio of adenosine and 4 different catecholamines in mixtures administered to 9 animals which demonstrated minimum variations in blood pressure.

Table 1

Determination of Concentration Ratios of Adenosine (A) and Catecholamines (C) which demonstrated minimum blood pressure fluctuations. (Parts by weight Adenosine to 1 part by weight of designated catecholamine.)

	Catecholamine/Adenosine			
Rabbit #	**Norepinephrine**	**Epinephrine**	**Dopamine**	**Phenylephrine**
1	1/1000	1/2000	1/5.0	1/200
2	2000	4000	5.0	200
3	1000	2000	5.0	100
4	500	1000	2.5	50
5	1000	2000	5.0	100
6	500	1000	2.5	50
7	500	1000	2.5	50
8	1000	2000	5.0	100
9	1000	2000	5.0	100
Mean ± SD	944±464	1889±928	4.2±1.3	106±59
C/A Ratio	1/944	1/1889	1/4.2	1/106

Through these in vivo tests, the concentration ratios which cause minimum fluctuations in blood pressure can be determined for each combination of purine compound and counteracting agent. Fig. 2(a) shows the blood pressure changes when adenosine (ADO), norepinephrine (NE) and their combination (AC) is injected. The recordings demonstrate that administration of adenosine only (10 mg/kg, Tracing 1) causes profound hypotension. Likewise norepinephrine only (0.01 mg/kg, Tracing 3) causes excessive hypertension. However, the fluctuations (up and down) in the blood pressure are minimal after injection of the same dosages of adenosine (10 mg/kg) and norepinephrine (0.01 mg/kg) combined in-vitro prior to administration (ratio of 1000/1 adenosine to norepinephrine). The blood pressure recording in Fig. 2b illustrates the stability of a mammal's blood pressure during administration of a large dose of AC (ADO: 100 mg/kg, and NE: 0.1 mg/kg, ratio of 1000/1 adenosine to norepinephrine) manually administered over a duration of about 10 minutes.

Table 2 summarizes the hemodynamic, respiratory and metabolic data obtained before and after intravenous injection of an ACB (Adenosine-Catecholamine-Benzodiazepine) combination in spontaneously breathing rabbits (the benzodiazepine added in this example is midazolam, which acts as a CNS depressant as well as an adenosine uptake inhibitor).

Table 2

Cardiovascular, Respiratory and Metabolic Data Before and After Intravenous Injection of Large Doses of ACB in Spontaneously Breathing Rabbits

	Pre-injection (5 min before)	Post-injection (5 min after)	Δ Change	P Value
Blood Pressure (mmHg)				
Systolic (SBP)	116±7	113±7	-3	NS
Diastolic (DBP)	85±6	76±11	-9	NS
Mean (MAP)	95±6	90±9	-5	NS
Heart Rate (HR)				
(beats/min)	240±31	239±37	-1	NS
Arterial Blood Gases				
pH	7.40±0.09	7.33±0.08	-0.07	0.015
PCO_2 (mmHg)	28±4	32±10	+4	NS
PO_2 (mmHg)	583±21	574±19	-9	NS
BE (mEQ/L)	-5.4±5.1	-8.0±4.2	-2.6	0.005
Respiratory Rate (RR)				
(breath/min)	71±22	71±30	0	NS
Body Temperature (BT)				
°C	38.5±0.05	38.2±0.6	-0.3	NS

(ACB (Adenosine/Catecholamine/Benzodiazepine) in 0.9% saline); Adenosine (117±41 mg/kg); Norepinephrine (0.106±0.05 mg/kg); Midazolam (1.05±0.43 mg/kg); N=9; Mean±SD.

As can be appreciated from the data in Table 2, the administration of huge doses of adenosine: 117±41 mg/kg, norepinephrine: 0.106±0.051 mg/kg, and midazolam: 1.05±0.43 mg/kg caused minimal changes in all of the hemodynamic, respiratory and metabolic parameters of the subjects. These data and the recordings in Fig. 2(a) and 2(b) clearly demonstrate that large doses of adenosine and norepinephrine, combined in vitro in accordance with the invention, can be safely administered in order to induce a desired effect (e.g., analgesia, sedation, etc...) without causing deleterious cardiovascular, respiratory, or metabolic conditions.

The model system represented in the above example is designed to test the BP responses of the healthy, normotensive animal. However, the present method or principle is expected to be applicable to humans as well, particularly in view of the known effects of administering dosages of purine compounds and catecholamine compounds to humans.

In addition, administration of large dosages of adenosine or ATP combined with the appropriate ratio of a catecholamine can be injected to more quickly induce a desired effect, e.g., anesthesia, than by slowly infusing low dosages of adenosine or ATP alone. It has also been surprisingly discovered that, while the vasodilating effects of anesthetically effective amounts of purine compounds, such as adenosine or ATP, last about as long as the purine compounds remain at the effective concentrations in the blood plasma, certain effects, such as analgesia, last for much longer time periods. Thus, a patient administered a purine composition in accordance with the present invention to induce anesthesia may not require any, or as much, pain reducing drugs following surgery. Further, administration of purine compositions formed in accordance with the present invention reduces the release of endogenous catecholamines in response to

trauma (such as that induced in surgery). Thus, administration of a purine composition of the present invention is believed to reduce the need for an anesthesiologist to administer drugs to counteract endogenous catecholamine induced effects during surgery.

EXAMPLE 2

Central Nervous System (CNS) Inhibition by Administration of ACB (Adenosine-Catecholamine-Benzodiazepine)

The broad depressant effects on the CNS of exogenously administered adenosine, adenosine analogs and adenine nucleotides are well documented; those related to antinociception, reduction in sensing pain, have been reviewed extensively. It is believed that a major problem of the hypotensive effects of adenosine may complicate the systemic routes of administration to a point where therapeutic considerations are limited. Therefore, the present study was undertaken to find out whether intravenous administration of ACB could attain CNS inhibitory actions, such as sedative and analgesic effects, without causing severe hypotension.

Materials and Methods:

Unmedicated, healthy New Zealand white rabbits were studied. The animals were prepared as in Example 1. The sedative and antinociceptive effects were tested following the methodology described in Example 1 of copending U.S. patent application Serial No 08/083,214 which is useful for testing and screening the analgesic and anesthetic effects of adenosine compounds.

A pair of stimulating needle electrodes were placed at the base of the shaved tail of each rabbit. After the

AUSTRALIAN PATENT OFFICE

from anesthesia, electrical current (noxious stimuli) was delivered through a nerve stimulator (Grass S48 Stimulator); in addition, conventional tail clamping (a standard test for anesthetic effects) was done. The control values were measured and recorded. No other drug was used, and the animals were allowed to breath 100% O_2 spontaneously without mechanical ventilatory assistance. Blood pressure changes were continuously monitored and recorded. Neurobehavioral responses, including degree of sedation, arousal responses (eye opening and head lifting), and antinociceptive responses (purposeful escape movement) were carefully observed and recorded throughout the experiment.

A large dose of ACB (Adenosine: 100 mg/kg, Norepinephrine: 0.1 mg/kg, Midazolam: 1 mg/kg) was slowly injected into a peripheral ear vein over a duration of about 10 minutes. After 20 minutes, three types of electrical stimulation, 2 Hz, 5 Hz, and 50 Hz, were delivered to the rabbits. By changing the voltage intensity, two behavioral responses were recorded for each test: a) head lift (HL), an arousal response shown by opening the eyes and lifting the head (hypnotic/sedative index); and b) purposeful escape movement, as in trying to run, or escape movement (EM) away from the noxious stimulus (analgesic index). Noxious stimuli were delivered every 30 minutes, and the sedative and nociceptive thresholds were recorded. Also, the blood pressure (BP), the heart rate (HR), EKG, respiratory rate, blood gases ($PaCO_2$ and PaO_2), and blood pH and base excess (BE) were recorded.

Results and Conclusions.

The administration of ACB caused minimal blood pressure changes similar to the BP changes illustrated in Fig. 2(b) where the same dosage of 100 mg/kg adenosine and

0.1 mg/kg norepinephrine was administered. In addition, the animals were all well sedated, which is supported by the elevation of the sedative (HL) responses to electrical stimulation. The antinociceptive (EM) as well as the sedative (HL) thresholds were consistently elevated in all three types of electrical stimulation after administration of ACB. The animals also did not respond to tail clamping, indicating a potent CNS mediated depressant effect. Furthermore, such sedative and analgesic activity was sustained for at least three hours after administration, as is illustrated in Fig. 3. At all three ETS levels (2 Hz, 5 Hz & 50 Hz), the thresholds for both escape movement and head lift were consistently elevated following administration of the ACB composition.

Fig. 4(a) is a blood pressure tracing (mm Hg) over time, that shows, moving left to right, the effects of administering bolus injections of 0.1 mg/kg adenosine triphosphate (ATP), 1.0 mg/kg ATP, 10 mg/kg ATP, and 10 μg/kg norepinephrine, (NE). Fig. 4(b) shows the blood pressure, BP, recording obtained during continuous infusion of a very large dose of AC (ATP:200 mg/kg, and Norepinephrine:0.67 mg/kg) which was initiated 10 minutes after administration of 2 mg/kg diazepam (a sedative). The mixture of ATP and catecholamine is also referred to as AC. A total dosage of 200 mg/kg ATP and 0.67 mg/kg norepinephrine is supplied via a continuous infusion of AC (ratio of ATP to NE of 300/1). The continuous infusion of AC was initiated at ATP 100 μg/kg/min, then increased to 3200 μg/kg/min where it was maintained for about 30 minutes, and thereafter the dosage was decreased gradually toward the end of the infusion. This further shows that large doses of ATP and norepinephrine combined in vitro in accordance with the invention can be administered while maintaining stable blood pressure at variable rates of infusion for a long time.

The above BP recording illustrated in Fig. 4(a) demonstrates the BP swings during administration of ATP and Norepinephrine alone. Notice that 0.1, 1.0, and 10 mg/kg of ATP caused hypotensive effects in a dose dependent manner. Likewise, a small dose of Norepinephrine (0.01 mg/kg) caused excessive elevation of the BP. However, Fig. 4 (b) illustrates the reduction in the blood pressure pendulum effect which would otherwise occur from administration of ATP or NE separately, and the BP changes are minimal when the AC combination is administered, despite the huge dosage of ATP and Norepinephrine.

Fig. 5 illustrates that following the administration of diazepam (2mg/kg) and AC (ATP: 200 mg/kg combined with NE: 0.67 mg/kg), analgesic effects can be sustained for at least 5 hours. In Fig. 5, the vertical axis represents sedative and analgesic thresholds in response to electrical tail stimulation (ETS) in voltage (V). The horizontal axis represents time in minutes and the time at which drugs were administered. AC was administered as a continuous infusion over 60 minutes. Figure 5 illustrates that sedative and analgesic effects are sustained for over five hours after administration of AC, despite administration of flumazenil, a diazepam antagonist. Aminophylline did not completely antagonize the escape movement (EM) antinociceptive response but lowered the head lift (HL) arousal response. The cardiovascular, respiratory and metabolic changes are shown in Table 3.

Table 3

Cardiovascular, Respiratory and Metabolic Data Before, During and After AC Administration

	Control	Diazepam 10 min. After	During AC Infusion	Post AC Administration				
				30 min	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
BP (mmHg)	89	92	77	106	106	105	102	99	107
HR (beats/min)	214	236	226	198	211	208	200	206	234
$PaCO_2$ (mmHg)	34.6	31.0	32.8	21.2	29.4	31.2	32.1	34.7	32.4
PaO_2 (mmHg)	548	490	551	517	502	549	508	498	435
BE	-6.7	-6.4	-1.1	-5.8	-1.0	-0.2	0.4	1.2	2.0
RR (breath/min)	40	50	180	140	160	130	90	90	100
BT (°C)37.8	36.9	36.9	36.6	36.4	37.4	37.8	37.8	37.7	38.0

AC (ATP:200 mg/kg, Norepinephrine: 0.67 mg/kg); Diazepam: 2mg/kg; BP: Blood Pressure; HR Heart Rate; RR: Respiratory Rate; BT: Body Temperature.

SUBSTITUTE SHEET (RULE 26)

The above studies demonstrate that AC, or AC combined with a sedative, can be effectively administered without the side effects of physical discomfort and/or hypotension while achieving CNS inhibitory effects of sedation and analgesia, and without respiratory depression or metabolic deterioration.

EXAMPLE 3

Protection from High-Dose Opioid-Induced Cardio-Respiratory and Metabolic Disturbances with Pre-treatment by Administration of AC to Spontaneously Breathing Rabbits.

Administration of high doses of opioids has been the most frequently used anesthetic technique for open-heart surgery. However, the use of high doses of synthetic opioids (e.g., fentanyl, sufentanil, alfentanil) has been reported to cause central seizure activity, and to increase both sympathetic and parasympathetic (vagal) activities with excessively elevated plasma catecholamine levels. In addition, opioids may cause profound respiratory depression and serious signs of cardiopulmonary dysfunctions, including ischemic EKG abnormalities, hemorrhagic pulmonary congestion and left ventricular failure of the heart. In contrast, exogenously administered adenosine has been reported to stimulate ventilation and has been found to have potent analgesic effects. We hypothesized that, if sufficiently large dosages of AC (e.g., adenosine-norepinephrine) could be administered safely, this could protect mammals from the above mentioned deleterious effects of high opioid dosages. Therefore, the present study investigated whether intravenous administration of AC could protect against the cardio-pulmonary and metabolic disturbances caused by the administration of high doses of fentanyl.

Materials and Methods:

The experimental preparation was done as in Example 1. Tracheotomized and cannulated rabbits were each placed in a suspended sling. The animals were divided into 2 groups. Each group consisted of 4 animals: The rabbits were pretreated with (a) saline or (b) AC (combination of ATP:100 mg/kg and NE: 0.2mg/kg). A high dose of fentanyl: 100 μg/kg (Janssen Pharmaceutica, N.J.) was administered twice. The first injection was 10 minutes after the pretreatment with saline or AC, and the second dose was administered after 40 minutes. Cardiovascular and blood gas data were recorded right after the pretreatment drug was administered, and then at 5, 10, 20 and 30 minutes following administration of fentanyl. Also, the neuro-behavioral and the nociceptive thresholds were assessed as in Example 2 by tail clamping and electrical tail stimulation.

Results and Conclusion:

Data are summarized in Figures 6 (a)-(h). Fent (fentanyl: 100 μg/kg) was first injected 10 minutes after pretreatment with either pretreatment a or b, and a second injection of Fent was given at 40 minutes. Cont1 and Cont2 represent control data before injection of drugs. Fig. 6(a) provides MAP: Mean Arterial Pressure; 6(b) provides HR: Heart Rate; 6(c) provides $PaCO_2$: arterial carbon dioxide tension; 6(d) provides PaO_2: arterial oxygen tension. Notice that administration of fentanyl produced severe respiratory depression which resulted in a progressive increase in $PaCO_2$ and a decrease in PaO_2, as can be seen in 6(c) and (d). When hypercapnia became severe after the second administration of fentanyl, the AC pretreated group suddenly increased respiratory rate as seen in 6(e) resulting in a decrease of $PaCO_2$ as seen in

6(c) and an increase in PaO_2 as seen in 6(d), while the metabolic parameters of pH as seen in 6(g) and BE: Base Excess as seen in 6(h) were ameliorated.

As can be appreciated from Figs. 6 (a)-(b), the fluctuations in the blood pressure and heart rate due to the fentanyl injections are attenuated in the animals administered the AC composition compared to those administered saline. The incidence of seizure activity and the degree of skeletal muscle rigidity were less in the AC group than in the saline group (see Fig. 6(i)-(j)). The threshold responses to noxious stimulation in the form of
electrical stimulation and tail clamping were also higher in the AC group. These results demonstrate the beneficial effects rendered by the pretreatment with the AC composition by protecting the patients from the cardio-respiratory and metabolic disturbances caused by administering a high dosage of an opioid (e.g., fentanyl). In addition, AC rendered neuroprotection from seizure activities.

EXAMPLE 4

Cardiopulmonary protective effects of the AC (Adenosine/Catecholamine) composition

The protective and homeostatic actions of adenosine are well accepted. Extensive studies on the involvement of endogenous and exogenous adenosine in myocardial ischemia protection have been reported. The protective effects mediated by activation of adenosine receptors can be rendered by administration of adenosine. However, administering efficacious amounts of adenosine to attain the beneficial effects has been hampered by the insurmountable obstacle of the side effects of adenosine and has hindered a practical therapy based on the

administration of adenosine. We hypothesized that administering the invented AC composition would allow administration of effective dosages of adenosine to attain the desirable beneficial effects in the heart and the lungs while attenuating the undesirable side effects of administering adenosine or catecholamine alone.

The pathogenesis of the catecholamine-induced myocardial necrosis has been the subject of many research papers. The cardiotoxicity effects of excessive catecholamines are well known. Sustained infusions or excessive doses of catecholamines administered to experimental animals produce myocardial dysfunction, ischemic lesions, necrosis and in addition, hemorrhagic pulmonary congestion, edema and ultimately death. This experimental model has had wide acceptance to prove the protective effects of various drugs, and has clinical relevance in syndromes including myocardial ischemia, infarction and pulmonary edema. Opioids are believed to attenuate the cardiovascular responses to surgical stress. It is thought that the cardio-pulmonary detrimental effects of catecholamines can be suppressed by opioids. Thus, the use of high doses or opioids including sufentanil is a common practice in cardiopulmonary bypass surgery. The study was undertaken to determine whether the present ACB composition could prevent cardiac damage, pulmonary edema and death induced by challenging rabbits with high doses of catecholamines. The effects were compared to those of sufentanil.

Materials and Methods:

Drugs:

For Protocol A: ACB (Adenosine-Catecholamine-Benzodiazepine) Composition:

A=Adenosine: 100 mg/kg, C=Norepinephrine: 0.1 mg/kg, B=Midazolam: 1 mg/kg. Sufentanil: 15 μg/kg (Janssen Pharmaceutica, Titusville, N.J).

For Protocol B: Epinephrine ratio to Adenosine or ATP was 1/160, 8-PT (8-Phenyltheophylline) 25 mg/kg from Research Biochemicals International, Natick, MA.

Unmedicated, healthy adult New Zealand white rabbits of either sex, weighing 2.5-2.7 kg were studied and the preparation was done as in Example 1. After the prepared and tracheotomized rabbits were placed in the sling with all the monitoring in place, the hemodynamic blood gas and metabolic parameters were measured for the control values. There were two experimental protocols. The protective effects of the present composition, AC, were compared with those of Sufentanil and Saline (control) in Protocol A, and with those of Saline (control) and 8-PT in Protocol B. The adverse functional effects of catecholamines, norepinephrine (NE) and epinephrine (Epi) were studied in the in vivo animal experimental model. This was done by a continuous infusion into the marginal ear vein of high doses of the above catecholamines which provided cardiotoxic stimulation. The infusion of the drugs was slowly done in spontaneously breathing rabbits except for the sufentanil group in which ventilation was mechanically controlled. Using a Travenol Flo-Gard 8000 volumetric infusion pump, the catecholamines, NE or Epi were continuously infused into the marginal ear vein for two and three hours respectively.

Protocol A:

The study was divided in two groups, both groups received high doses of two hours continuous infusion of norepinephrine (NE) as a cardiotoxic stimulant. In Group I, the rabbits were subjected to 20 μg/kg/min (NE), and in Group II, the rabbits were subjected to 40 μg/kg/min (NE).

In each group, the animals were randomly assigned to a subgroup (n=6 for each subgroup): a) Saline, b) Sufentanil, c) ACB composition. All of the studied drugs (Saline, Sufentanil, ACB composition) were given as a pre-treatment drug prior to starting the NE infusion. Midazolam was used to sedate the animals in order to avoid excitation due to discomfort during administration of the drugs.

Protocol B:

In this study, the animals were subjected to 10 μg/kg/min of Epi as a cardiotoxic stimulant. In the AC and ATPC groups, epinephrine and adenosine or ATP were mixed at a ratio of 1/160. The mixed solutions were continuously infused for 3 hours. At the beginning of the infusion, the doses were titrated to the cardiovascular responses, and gradually increased. The animals were randomly assigned in subgroups of a) Saline (n=6), b) 8-PT (n=6), AC (Adenosine/Epinephrine, n=8), ATPC (ATP/epinephrine: n=2). In order to antagonize the endogenous adenosine, 8-PT (8-Phenyltheophylline), an adenosine receptor antagonist was used.

Results and Conclusion:

The results for Protocol A are summarized in Fig. 7 (a) - (d). Figs 7(a) and 7(b) illustrate the mortality rate, and Figs. 7(c) and 7(d) illustrate the rate that developed pulmonary edema (PE) when rabbits in Group I, Figs. 7(a) and 7(c), were subjected to 20μg/kg/min NE, and rabbits in Group II, Figs 7(b) and 7(d), were subjected to 40μg/kg/min NE. The figures show that in Group II, Fig 7 (b) and (d), where higher doses of NE (40 μg/kg/min) were infused, 6/6, 100% of the animals died in the Sufentanil group, 5/6 or 83% in the Saline, and 2/7 or 28% in the ACB group died within 3 hours. In the Group I, Fig. 7 (a)

where the animals were challenged by 20 μg/kg/min of NE, all the ACB pretreated animals survived (100%) for two hours, and one died after 150 minutes. Compared to the Saline group where the mortality rate was 3/6 (50%) or the Sufentanil group where the mortality rate was 4/6 (67%) within 180 minutes. The number of animals that developed pulmonary congestion and edema are also much higher in the Saline or Sufentanil groups than in the group administered with the ACB composition (Fig. 7 (c)-(d)). The blood gas data was progressively deteriorated in both the Saline and Sufentanil groups. However, no significant blood gas and metabolic changes were shown in the survived animals of the ACB groups.

The results are summarized in Fig. 8 (a)-(b) for Protocol B in which animals were challenged by high doses of Epi. Fig 8 (a) shows the mortality rate, and Fig 8(b) shows the rate of rabbits that developed pulmonary edema (PE). As can be seen from Figs. 8 (a) and (b), almost 70% of the animals developed pulmonary edema and died within 30-60 minutes in the 8-PT and Saline groups. In contrast, administration of the present composition, AC or ATPC could effectively prevent the development of pulmonary edema and death in most of the studied animals. The myocardial and pulmonary ischemic insult appeared to be involved in the catecholamine-induced damage and death which was apparent in the ECG and blood gas changes. The above results clearly demonstrate that administration of the present composition, AC, ACB or ATPC resulted in a significant reduction of pulmonary congestion and edema as well as cardiovascular damage and death in both protocols. The present composition effectively protected the heart and the lungs during acutely induced stressful and cardiotoxic stimulation challenged by large doses of catecholamine infusions.

EXAMPLE 5

Metabolic homeostasis maintaining and protection of ischemia by administration of the AC composition

Homeostasis, the biologic responses necessary to maintain a steady state in the internal environment, is necessary for survival. Maintenance of the body's internal milieu is the major function of buffering systems, while oxygen transport and the successful preservation of aerobic metabolism are key components in maintaining cellular integrity. As normal aerobic metabolism is compromised or as the ratio of buffering elements is altered, disturbances in acid-base homeostasis occur. Lactate accumulation in extracellular fluid is due to an imbalance between oxygen supply and metabolic demand. Lactic acidosis is associated with tissue hypoxia and impaired oxidative metabolism. Tissues that normally can use oxygen to produce ATP from glucose will resort to the less energy-efficient glycolytic pathway if oxygen is unavailable. Under anaerobic conditions, lactate production will therefore increase, and since lactate is readily diffusible across cell membranes, the concentration of lactate in the blood will increase. This is the basis of blood lactate as a marker of tissue ischemia/hypoxia. A practical indicator is the hydrogen ion level as expressed in base excess (BE) determined by the arterial blood sample.

Lactic acidosis is a metabolic derangement associated with a variety of pathological states including excessive levels of stress caused by intense stimulation like major body injury, surgical or accidental. The degree of increase in the lactate level seems to correlate directly with the severity of levels of stress. Moreover, increases in lactate may reflect increased activity of the sympathetic nervous system and increase catecholamine

release due to stress. Thus, responses to excess lactate are believed directly related to activation of the sympathetic nervous system after a variety of stresses, including anxiety, hypotension and major injuries. The degree of sympathetic nervous system activity and consequent release of endogenous catecholamines can directly influence the responses observed, since both epinephrine and norepinephrine result in increased blood levels of lactate and increased rates of anaerobic glycolysis in many tissues/organs. These considerations are particularly relevant in considering the responses to ischemia, hypoxia, anesthesia, surgery, hemorrhage, trauma and shock which are so dependent upon sympathetic nervous system activation and release of catecholamines.

Severe stress caused by stimuli such as surgical intervention induces acute disorders in endocrine, hormonal and cardiovascular systems. For example, traction and manipulation of the viscera during abdominal surgery in addition to the general biologic response to stress are known to be associated with marked increase in circulating catecholamines, mesenteric vasoconstriction and a decrease in gastrointestinal blood flow which may cause ischemia-reperfusion injury in various splanchnic organ systems, resulting in compromised organ function and increase in lactate levels (lactic acidosis). We designed an experimental model that can mimic the above conditions of intense sympathetic activation, release of catecholamines, severe vasoconstriction that may be transient but likely to cause ischemia-reperfusion injury in the splanchnic organ/tissues. This could be induced by delivering stressful intra-abdominal electrical stimulation. The stress response to noxious stimulation further yields a subsequent increase in oxygen demand which would worsen the imbalance of oxygen supply/demand. The measurement of blood gas/acid-base metabolic status as a useful tool in the assessment of critically ill patients

and patients undergoing severe stress like trauma or surgery is well recognized. For example, elevation of blood lactate level often alerts the clinician for the need to rapidly institute appropriate monitoring and potentially lifesaving therapy.

It is thought that adenosine acting via adenosine receptor activation can play a homeostatic role, that adenosine functions as a retaliatory metabolite in response to tissue trauma, hypoxia and ischemia. Under such conditions, tissue levels of adenosine are markedly increased because of ATP breakdown. It is also believed that the anti-adrenergic effects of adenosine can be beneficial to inhibit detrimental sympathetic activation and that administration of adenosine could be beneficial. Therefore, we sought to determine whether the invented AC (adenosine/norepinephrine) composition could attenuate or prevent the metabolic disturbances caused by stressful noxious stimuli in the intestine, and inhibit the sympathetic responses which may lead to mesenteric vasoconstriction with subsequent ischemia-reperfusion injury.

Methods and Materials

Drugs: AC (Adenosine/Norepinephrine ratio: 800/1 dissolved in saline) AC infusion, adenosine: 400 μg/kg/min; 8-Phenyltheophylline (8-PT): 25 mg/kg; Glibenclamide: 15 mg/kg.

The preparation of the animals was done as in Example 1. The prepared and tracheotomized rabbits were placed in a sling which allowed easy observation of the behavioral responses without restraining the animals. The cardiovascular, respiratory and metabolic monitoring was instituted. The baseline control values were then taken. EKG and hemodynamic changes were continuously monitored throughout the experiments. In addition, intermittent

blood gases and the metabolic changes were measured before and after noxious stimulations. The electrical stimulation was applied at 20 minutes intervals (3 series). As is illustrated in Fig. 9, three groups of rabbits were studied: a) AC group (n=5), b) Saline group (n=7); c) 8-PT+Glibenclamide group (n=5). In group (c), 8-PT and Glibenclamide were used in order to block endogenous release of adenosine and its effects on ATP sensitive K ion channels. 8-PT is an adenosine receptor antagonist, and Glibenclamide is an ATP-dependent K+ channel blocker. In group (c) 8-PT was administered first, and after 15 minutes, Glibenclamide was administered. In all the 3 groups (a,b,c), anesthesia was maintained with 1.4% isoflurane throughout all the experiments. In groups (a) and (b), noxious electrical visceral stimulation (EVS) was applied after 1 hour continuous infusion of AC, (adenosine, 400 μg/kg/min) or saline. The AC infusion was continued throughout the tested stimulation (EVS #1 - #3). Electrical current was delivered through a nerve stimulator via electrodes that were introduced into the rectum about 10-13 cm. Electric current at predetermined intensities of 50 Hz, 80 volts were applied for 40 seconds. Behavioral responses such as bodily movement and the hemodynamic responses were carefully monitored and recorded. The blood gas variables were measured right after stimulation and every 5 minutes afterwards.

Results and Conclusion:

Despite the fact that the animals were anesthetized with 1.4% isoflurane, when high intensity electric current was delivered, there was marked increase in blood pressure, heart rate, and the animals hyperventilated. The animals also moved violently, particularly in the saline and 8-PT+Glibenclamide groups. In contrast, these behavioral and the hemodynamic responses were quite

inhibited in the AC group. As Fig. 9 shows, the metabolic acidosis (decrease in BE) was progressively deteriorated and exaggerated particularly in the 8-PT+Glibenclamide group (c), where the endogenous adenosine release and the K+ ATP channel activities were blocked. The metabolic conditions of these animals progressively worsened as time passed, after each stimulation (See Fig. 9), and ultimately all the animals died in group (c). In comparison, the metabolic disturbances (decrease in BE) in the AC group were minimal, and did not show any pathological condition. Twenty minutes after the last stimulation (EVS #3), the AC group animals completely recovered to normal ranges.

The results indicate that intravenous administration of the present AC composition effectively inhibited excessive sympathetic activities and mesenteric vasoconstriction caused by intense noxious stimulation, and could greatly attenuate metabolic derangements in the animals exposed to severe stressful conditions. Therefore, it can be concluded that protection against trauma and subsequent ischemia-reperfusion injury in the splanchnic organs/tissues occurred.

Although the above example was indirectly assessing splanchnic organs/tissues ischemia-reperfusion injury possibly caused by the traumatic stimulation and excessive visceral vasoconstriction, the present method is expected to be applicable to any tissue/organ that has suffered from ischemic/hypoxic damage. In addition, the AC composition is expected to be useful in critically ill patients where lactic acidosis is common and is usually caused by inadequate tissue perfusion that does not meet metabolic demand. The present composition may be beneficial to aid metabolic adjustments following accidental trauma. The present method may also be beneficial to accelerate general recovery in patients in the ICU (Intensive Care Unit). The present method is also

expected to be applicable to other situations which include but are not limited to: stroke, in vivo organ preservation and transplant, trauma and shock which results from poor circulatory conditions, and a variety of pathological states resulting from metabolic disturbances.

EXAMPLE 6

Intravenous Administration of a Combination of Adenosine Analog (R-PIA)-Catecholamine

Unmedicated, healthy New Zealand white rabbits were prepared as in Example 1. Anesthesia was maintained with isoflurane 1.4% throughout all the experiments. A mixture of 5.0 mg. of an adenosine analog compound, R(-)N^6-(2-phenylisopropyl) adenosine (R-PIA) and 0.1 mg of norepinephrine (NE) was administered by intravenous infusion over a period of about 10 minutes. The initial blood pressure of about 100 mmHg gradually dropped during the infusion. When the initial infusion was stopped, blood pressure began to fall, as the norepinephrine was metabolized more quickly than the R-PIA, and resulting in increased hypotension. To counter this hypotensive condition, a separate continuous infusion of 4 μg/kg/min of norepinephrine (NE) was administered for a period of about 5 minutes to support blood pressure at about 75-80 mmHg, followed by administration of a continuous infusion of 2 μg/kg/min of norepinephrine to maintain and stabilize blood pressure near normotensive levels. The results of the initial infusion of the mixture of R-PIA and norepinephrine, followed by a staged, separate continuous infusion of norepinephrine are shown in the blood pressure tracing of Fig. 10.

EXAMPLE 7

Intravenous Administration of a Combination of Adenosine Analog (R-PIA)-Catecholamine

Unmedicated, healthy New Zealand white rabbits were prepared as in Example 1. Anesthesia was maintained with isoflurane 1.4% throughout all the experiments. A mixture of 5.0 mg. of an adenosine analog compound, R(-)N^6-(2-phenylisopropyl)adenosine (R-PIA) and 0.1 mg of norepinephrine was initially administered by intravenous infusion over a period of about 13 minutes, as in Example 6. However, administration of a separate continuous infusion of 4 μg/kg/min of norepinephrine (NE) was begun about halfway through the initial infusion of the mixture, and was continued for a period of about 10 minutes to stabilize blood pressure. This was followed by reduction of the continuous infusion of norepinephrine to 2 μg/kg/min to maintain and stabilize blood pressure near normotensive levels. The results of the initial infusion of the mixture of R-PIA and norepinephrine, accompanied by a staged, separate continuous infusion of norepinephrine, are shown in the blood pressure tracing of Fig. 11.

EXAMPLE 8

Intravenous Administration of a Combination of Adenosine Analog (NECA)-Catecholamine

Unmedicated, healthy New Zealand white rabbits were prepared as in Example 1. Anesthesia was maintained with isoflurane 1.4% throughout all the experiments. A mixture of 1.25 mg. of an adenosine analog compound, 5'-N-ethylcarboxamidoadenosine (NECA) and 0.2 mg of norepinephrine (NE) was initially administered by

intravenous infusion over a period of about 13 minutes. Administration of a separate continuous infusion of 60 μg/kg/min of norepinephrine (NE) was begun about 1-2 minutes following the commencement of infusion of the mixture, and was continued to maintain and stabilize blood pressure at normotensive levels. The results of the initial infusion of the mixture of NECA and norepinephrine, accompanied by a separate continuous infusion of norepinephrine, are shown in the blood pressure tracing of Fig. 12.

While the principles and exemplary embodiments of the present invention have been discussed herein, many variations and modifications can be made to the invention as disclosed without departing from the spirit and scope of the invention.

Docket No. 36154

CLAIMS

WE CLAIM:

1. A method for administering a purine compound to a mammal, comprising the steps of:

combining a purine compound with a counteractive agent, wherein said purine compound induces a desired effect and an undesired effect when administered in an effective amount to a mammal without administering said counteractive agent, and said counteractive agent counteracts at least a portion of said undesired effect upon administration of the combination to a mammal; and

administering said combination to a mammal in an amount effective to induce said desired effect in said mammal without inducing said undesired effect to the same extent said undesired effect would occur in the absence of said counteractive agent, wherein said counteractive agent is a catecholamine compound.

2. The method of claim 1, wherein:

said desired effect is selected from the group consisting of analgesia, anesthesia, metabolic disturbance protection, ischemia protection, antipyretic action, hypertension prevention or protection, central nervous system inhibition or protection, autonomic nervous system modulation, cardiac protection, respiratory protection, stress reduction, muscle relaxation and seizure activity reduction.

3. The method of claim 1, wherein said purine compound is selected from the group consisting of adenosine, adenosine analogs, 5'-N-ethylcarboxamidoadenosine, R(-)N^6-(2-phenylisopropyl) adenosine, 2-chloroadenosine, N^6-cyclopentyladenosine, N^6-cyclohexyladenosine phosphorylated adenosine, and phosphorylated adenosine analogs.

AMENDED SHEET

AUSTRALIAN PATENT OFFICE

4. The method of claim 1, wherein said catecholamine compound is selected from the group consisting of epinephrine, norepinephrine, dopamine, dobutamine, and phenylephrine.

5. The method of claim 1, further comprising combining a purine compound potentiator with said purine compound and said counteractive agent prior to administration to a mammal, said purine compound potentiator being selected from the group consisting of benzodiazepines, dipyridamole, deoxycoformycin, erythro-9-(2-hydroxy-3-nonyl)adenine, AICA riboside, opioids, a potassium channel opener, etomidate, propofol, adrenergic α_2-agonists, barbiturates, and non-steroidal anti-flammatory drugs.

6. The method of claim 1, further comprising, in addition to administering said combination, the step of administering to said mammal an infusion of said counteractive agent subsequent to said step of administering said combination.

7. The method of claim 6, wherein said additional step of administering said infusion of said counteractive agent comprises altering the dosage of said counteractive agent to control said undesired effect.

8. A method for administering a catecholamine to a mammal, comprising the steps of:

combining a catecholamine with a counteractive agent, wherein said catecholamine induces a desired effect and an undesired effect when administered in an effective amount to a mammal without administering said counteractive agent, and said counteractive agent reduces said undesired effect upon administration of the combination to a mammal; and

AUSTRALIAN PATENT OFFICE

administering said combination to a mammal in an amount effective to induce said desired effect in said mammal while reducing said undesired effect.

9. The method of claim 8, wherein said catecholamine is selected from the group consisting of epinephrine, norepinephrine, dopamine, dobutamine, and phenylephrine.

10. The method of claim 8, wherein said counteractive agent is a purine compound selected from the group consisting of adenosine, adenosine analogs, 5'-N-ethylcarboxamidoadenosine, R(-)N^6-(2-phenylisopropyl) adenosine, 2-chloroadenosine, N^6-cyclopencyladenosine, N^6-cyclohexyladenosine phosphorylated adenosine, and phosphorylated adenosine analogs

11. The method of claim 8, further comprising, in addition to administering said combination, the step of administering to said mammal an infusion of said counteractive agent subsequent to said step of administering said combination.

12. The method of claim 8, wherein said additional step of administering said infusion of said counteractive agent comprises administering said counteractive agent in a plurality of stages of continuous infusion of progressively decreasing dosages of said counteractive agent.

13. A pharmaceutical composition, comprising:

a purine compound, and a catecholamine compound, said compounds being in a pharmaceutically acceptable carrier, wherein the ratio of said purine compound to said catecholamine compound permits said purine compound to induce a desired effect when administered in an effective amount to a mammal without inducing an undesired effect to the same degree said undesired effect would have been

AUSTRALIAN PATENT OFFICE

induced by said purine compound had said catecholamine compound not been present in said composition.

14. The composition of claim 13 wherein the desired effect is selected from the group consisting of analgesia, anesthesia, metabolic disturbance protection, ischemia protection, antipyretic action, hypertension prevention, central nervous system inhibition or protection, autonomic nervous system modulation, cardiac protection, respiratory protection, stress reduction, muscle relaxation and seizure activity reduction.

15. The composition of claim 13, wherein:

said purine compound is selected from the group consisting of adenosine, adenosine analogs, 5'-N-ethylcarboxamidoadenosine, R(-)N^6-(2-phenylisopropyl) adenosine, 2-chloroadenosine, N^6-cyclopentyladenosine, N^6-cyclohexyladenosine phosphorylated adenosine, and phosphorylated adenosine analogs.

16. The composition of claim 13, wherein said catecholamine compound is selected from the group consisting of epinephrine, norepinephrine, dopamine, dobutamine and phenylephrine.

17. The composition of claim 13, wherein said purine compound is adenosine, and:

when said catecholamine compound is norepinephrine, said composition comprises about one part by weight norepinephrine to about 25 to about 2000 parts by weight adenosine;

when said catecholamine compound is epinephrine, said composition comprises about one part by weight epinephrine to about 50 to about 4000 parts by weight adenosine;

when said catecholamine compound is phenylephrine, said composition comprises about one part by weight

AUSTRALIAN PATENT OFFICE

phenylephrine to about 10 to about 200 parts by weight adenosine; and

when said catecholamine compound is dopamine, said composition comprises about one part by weight dopamine to about 2 to about 50 parts by weight adenosine.

18. A pharmaceutical composition, comprising:

a purine compound and a catecholamine compound in a pharmaceutically acceptable carrier.

19. The composition of claim 18, wherein the ratio of said purine compound to said catecholamine compound permits said composition to induce a desired effect when said composition is administered in an effective amount to a mammal without inducing an undesired effect to the same degree said undesired effect would have been produced if said purine compound and said catecholamine compound were not administered together in said composition.

20. An *in vitro* pharmaceutical composition capable of inducing or maintaining one or more desired effects selected from the group consisting of anesthesia, analgesia, and ischemia protection, said composition comprising a purine compound and a catecholamine compound in a pharmaceutically acceptable carrier, wherein said purine compound is selected from the group consisting of adenosine, phosphorylated adenosine, 5'-N-ethylcarboxamidoadenosine, R(-) N^6-(2-phenylisopropyl) adenosine, 2-chloroadenosine, N^6-cyclopentyladenosine and N^6-cyclohexyladenosine; and said catecholamine compound is selected from the group consisting of epinephrine, norepinephrine, dopamine, dobutamine, ephedrine, methoxamine, and phenylephrine;

wherein administration of said *in vitro* composition to a mammal in an amount sufficient to induce or maintain one

AUSTRALIAN PATENT OFFICE

or more of said desired effects will not reduce blood pressure to the same degree blood pressure would be reduced in the absence of said catecholamine; wherein:

when said purine compound is adenosine, and when said catecholamine compound is norepinephrine, said combination comprises about one part by weight norepinephrine to about 25 to about 2000 parts by weight purine compound;

when said catecholamine compound is epinephrine, said combination comprises about one part by weight epinephrine to about 50 to about 4000 parts by weight purine compound;

when said catecholamine compound is phenylephrine, said combination comprises about one part by weight phenylephrine to about 10 to about 200 parts by weight purine compound; and

when said catecholamine compound is dopamine said combination comprises about one part by weight dopamine to about 2 to about 5 parts by weight purine compound, wherein an amount of said composition sufficient to induce one of said desired effects would cause hypotension in the absence of said catecholamine.

DATED this THIRTIETH day of DECEMBER 1997

Atsuo F. Fukunaga AND Alex S. Fukunaga

By DAVIES COLLISON CAVE

Patent Attorneys for the applicant

AUSTRALIAN PATENT OFFICE

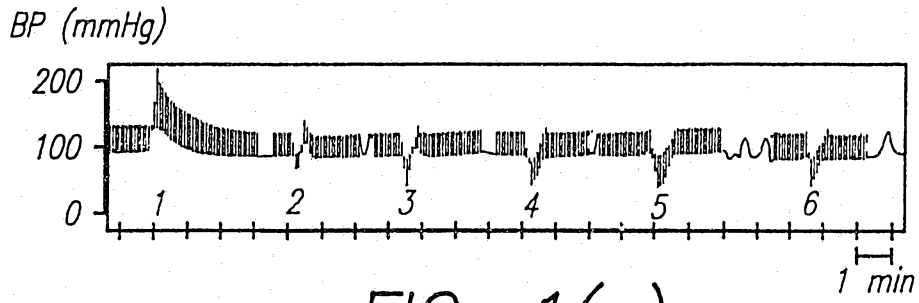


FIG. 1(a)

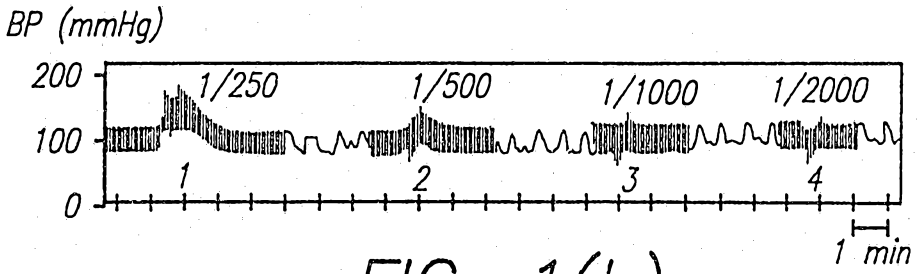


FIG. 1(b)

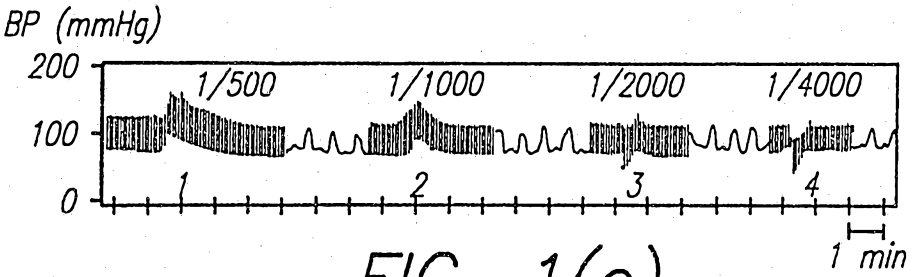


FIG. 1(c)

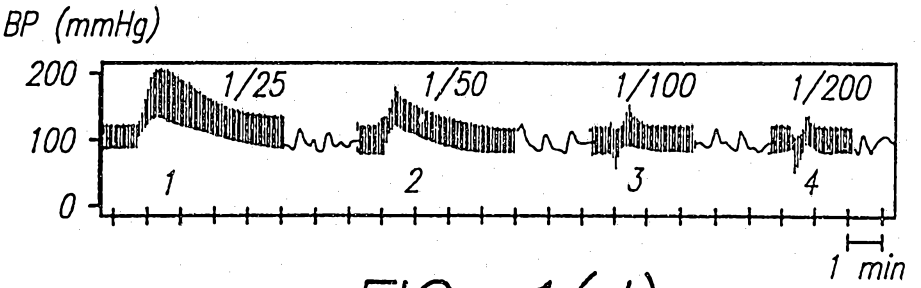


FIG. 1(d)

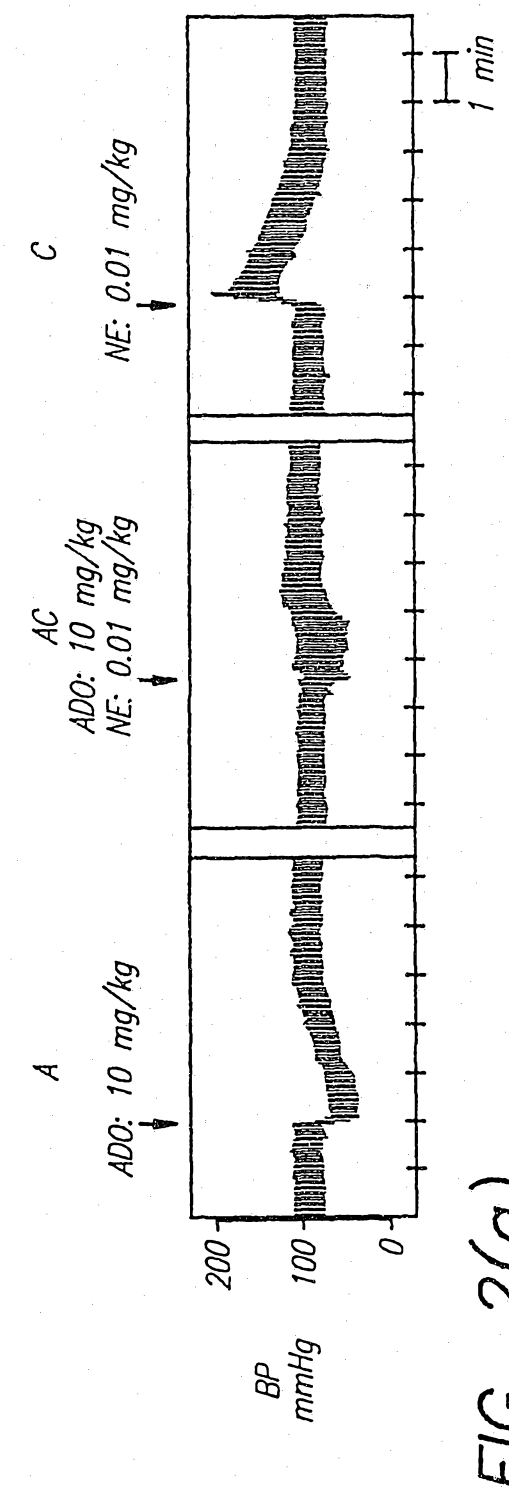

A
ADO: 10 mg/kg
AC
ADO: 10 mg/kg
NE: 0.01 mg/kg
C
NE: 0.01 mg/kg
1 min
200
100
0
BP
mmHg

FIG. 2(a)

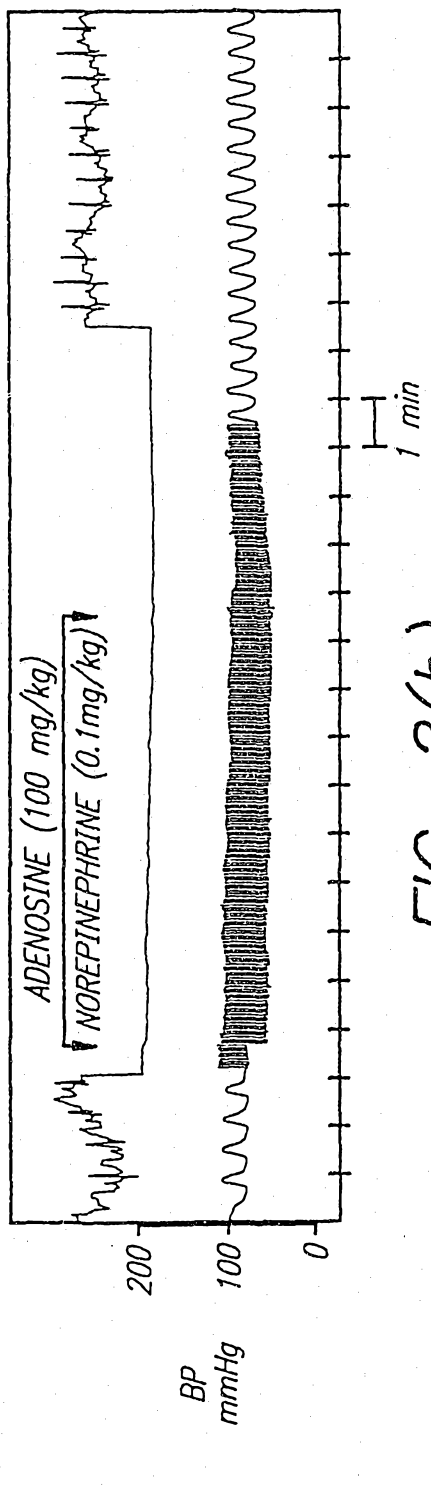

ADENOSINE (100 mg/kg)
NOREPINEPHRINE (0.1mg/kg)
1 min
200
100
0
BP
mmHg

FIG. 2(b)

FIG. 3

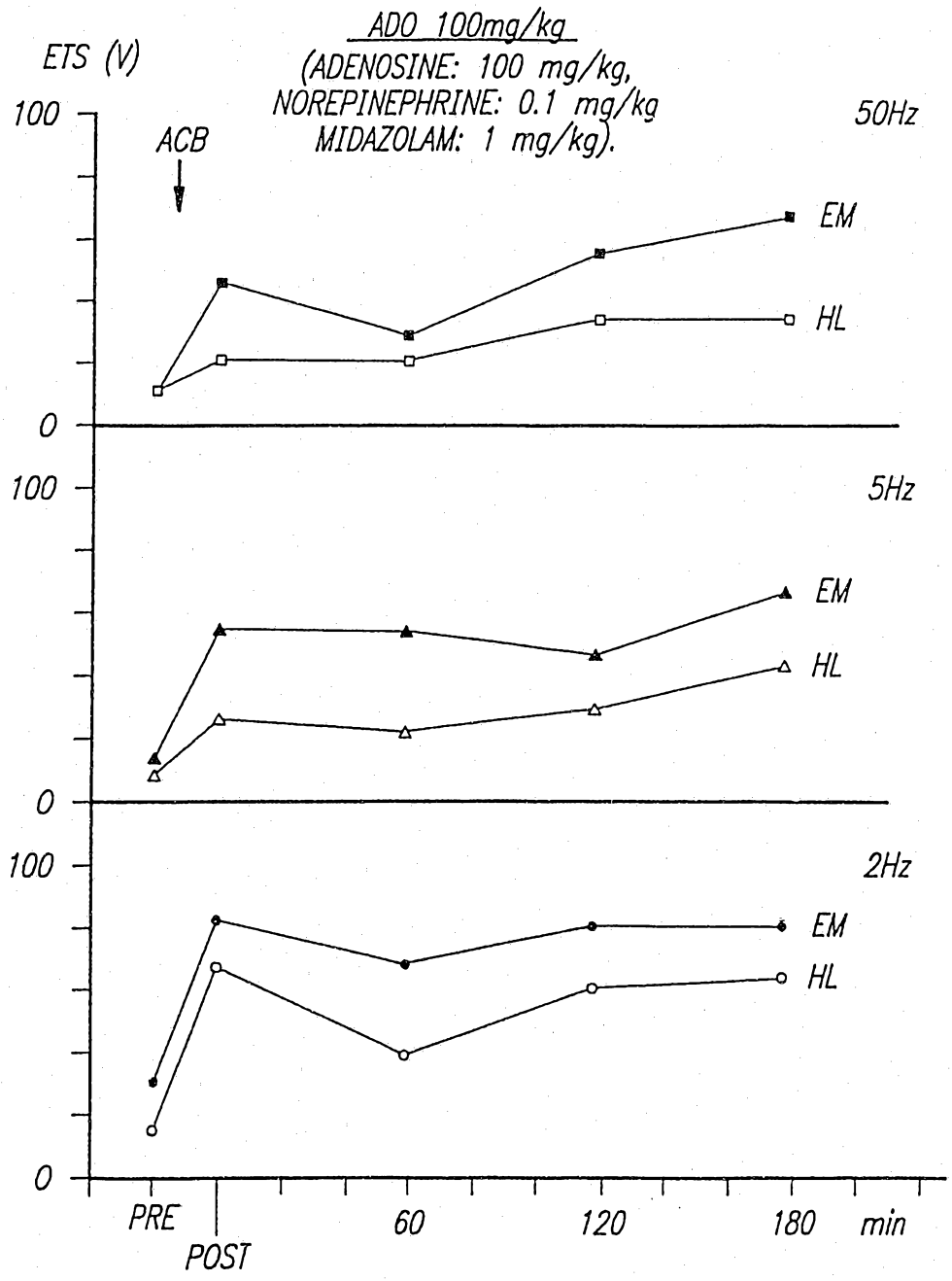

ADO 100mg/kg
(ADENOSINE: 100 mg/kg,
NOREPINEPHRINE: 0.1 mg/kg
MIDAZOLAM: 1 mg/kg).
ETS (V)
100
ACB
50Hz
EM
HL
0
100
5Hz
EM
HL
0
100
2Hz
EM
HL
0
PRE
POST
60
120
180
min

FIG. 4(a)

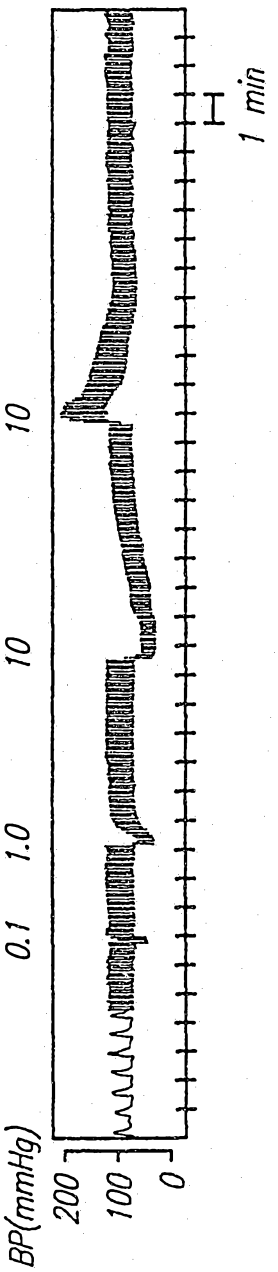

BP(mmHg)
200
100
0
ATP (mg/kg)
0.1
1.0
10
NE (μg/kg)
10
1 min

FIG. 4(b)

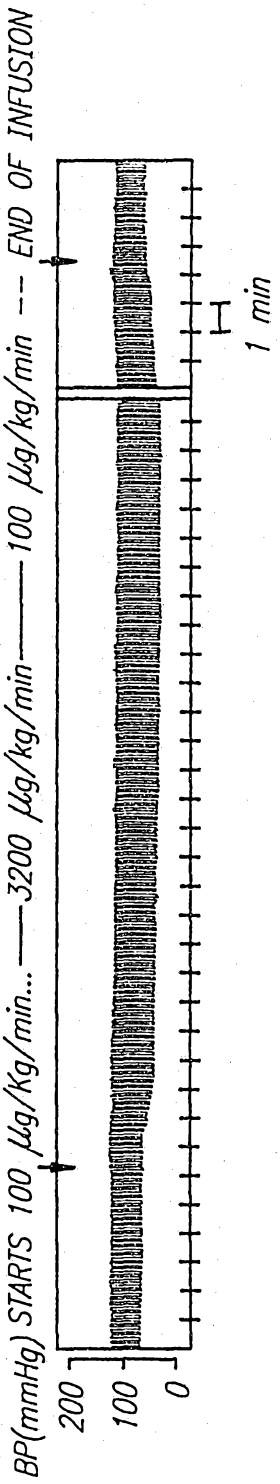

GRADUALLY INCREASING
CONSTANT INFUSION
GRADUALLY DECREASING
BP(mmHg)
STARTS 100 μg/kg/min...
3200 μg/kg/min
100 μg/kg/min -- END OF INFUSION
200
100
0
1 min

FIG. 5

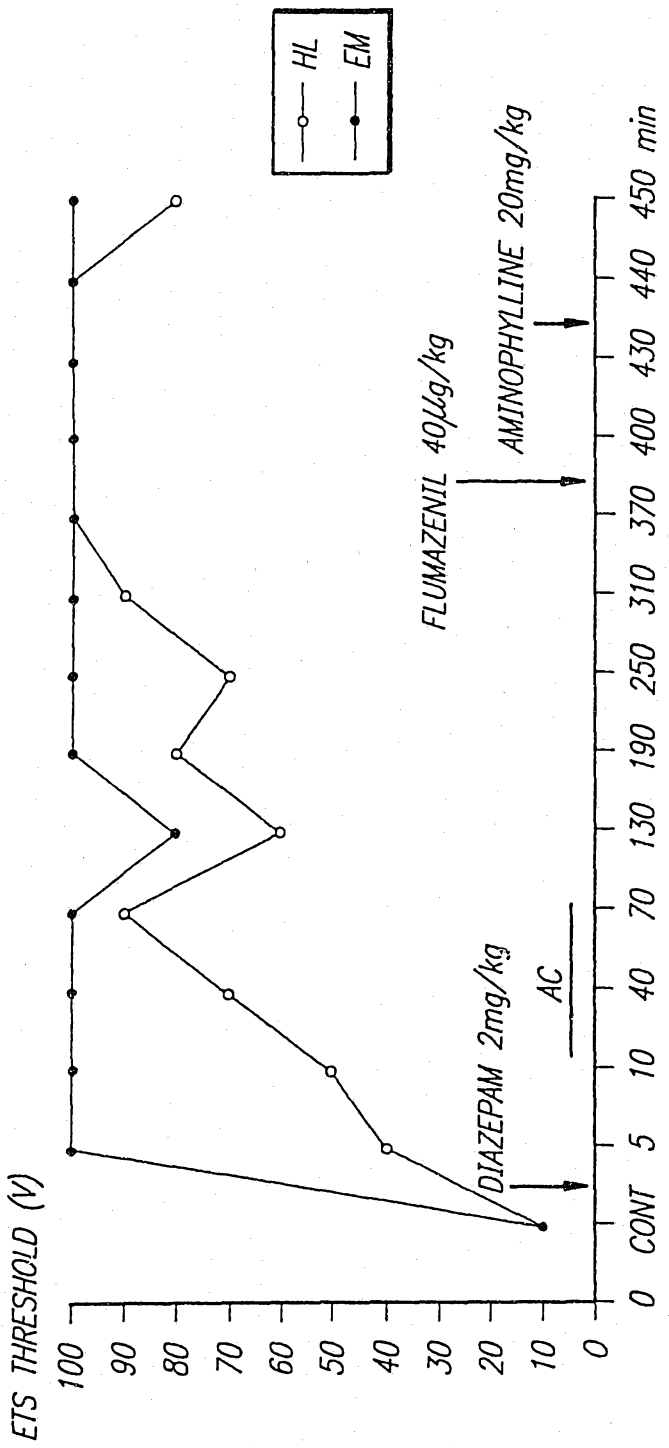

HL
EM
ETS THRESHOLD (V)
100
90
80
70
60
50
40
30
20
10
0
0 CONT 5 10 40 70 130 190 250 310 370 400 430 440 450 min
DIAZEPAM 2mg/kg
AC
FLUMAZENIL 40μg/kg
AMINOPHYLLINE 20mg/kg

FIG. 6(a)

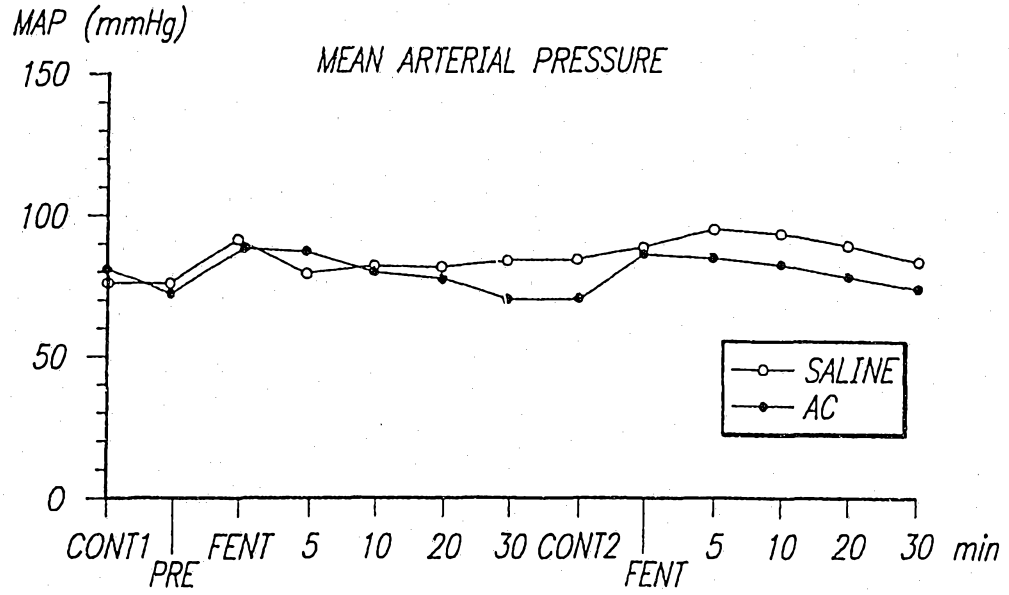

MAP (mmHg)
MEAN ARTERIAL PRESSURE
150
100
50
0
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(b)

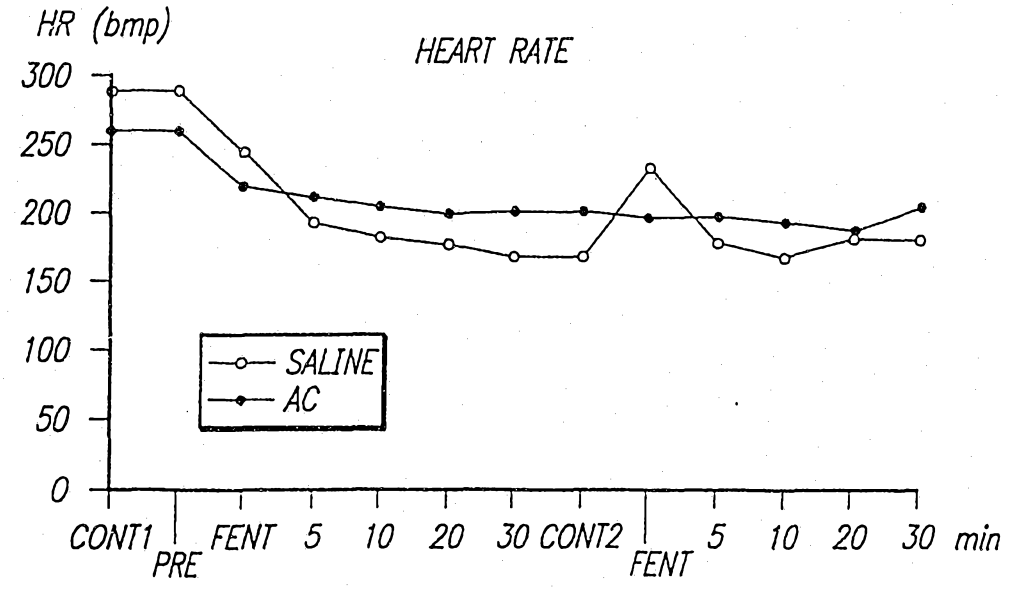

HR (bmp)
HEART RATE
300
250
200
150
100
50
0
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(c)

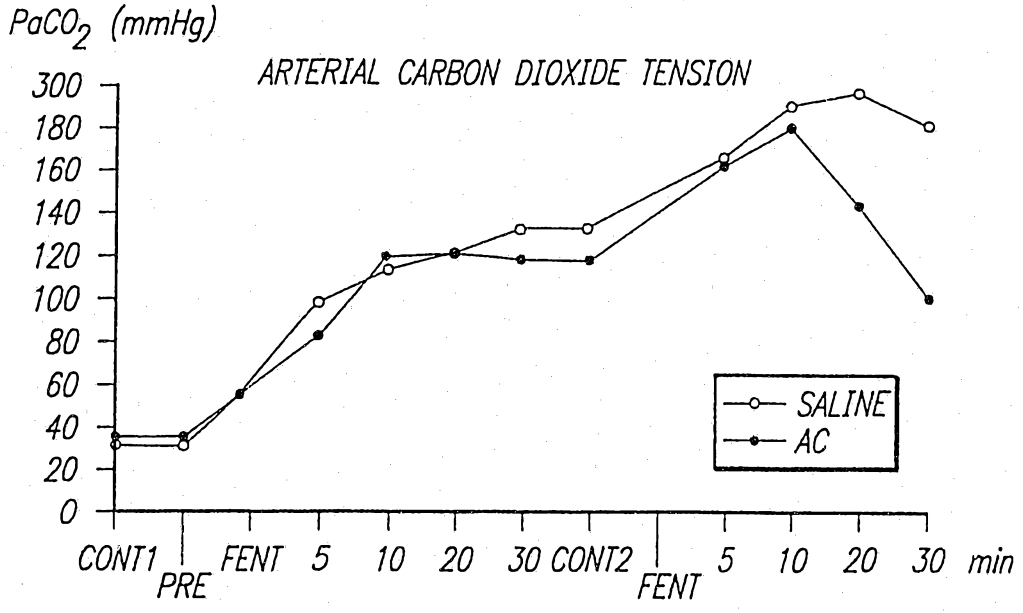

$PaCO_2$ (mmHg)
ARTERIAL CARBON DIOXIDE TENSION
300
180
160
140
120
100
80
60
40
20
0
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(d)

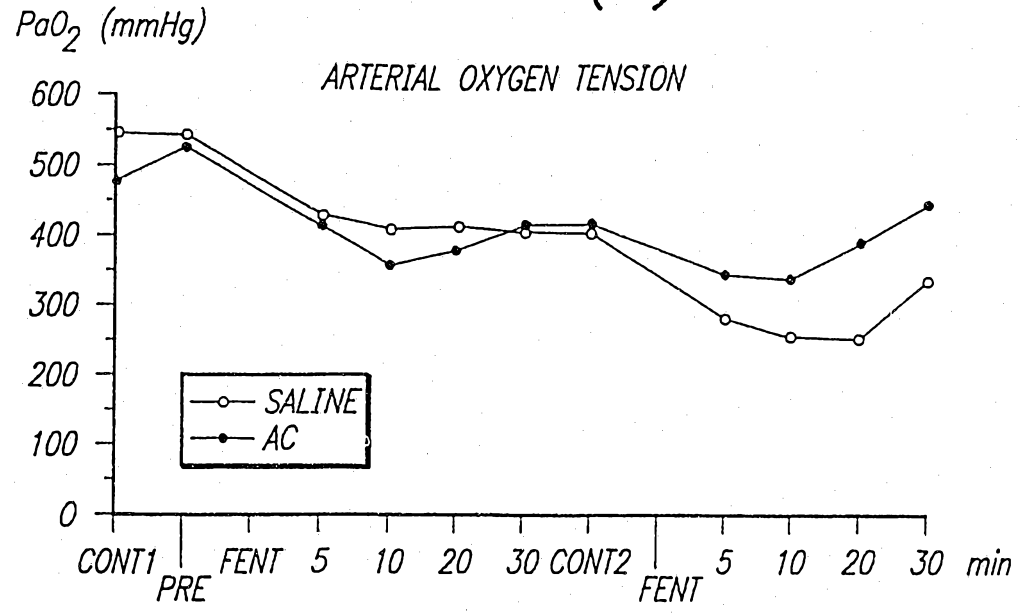

PaO_2 (mmHg)
ARTERIAL OXYGEN TENSION
600
500
400
300
200
100
0
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(e)

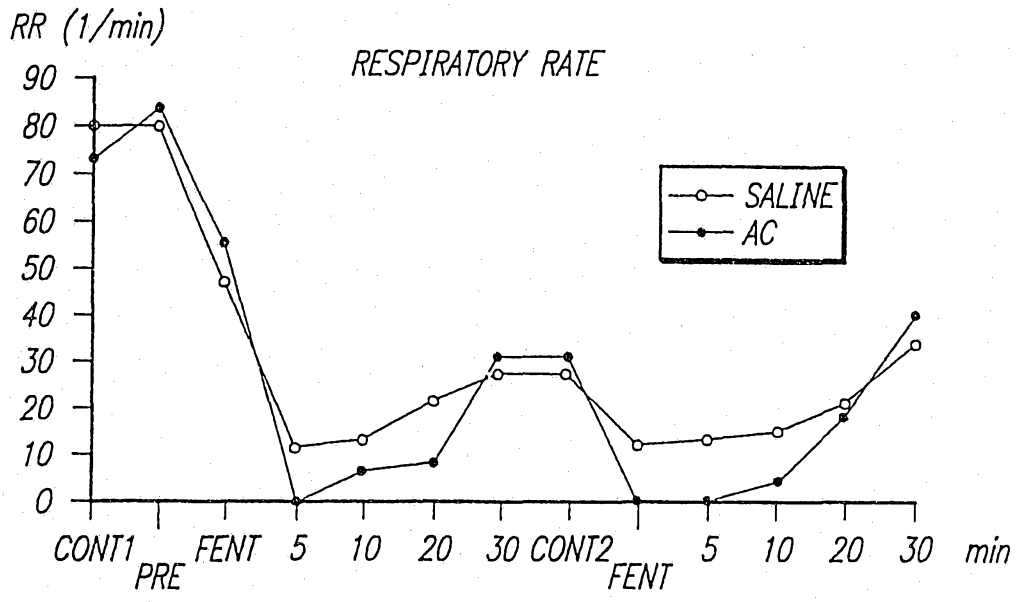

RR (1/min)
RESPIRATORY RATE
90
80
70
60
50
40
30
20
10
0
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(f)

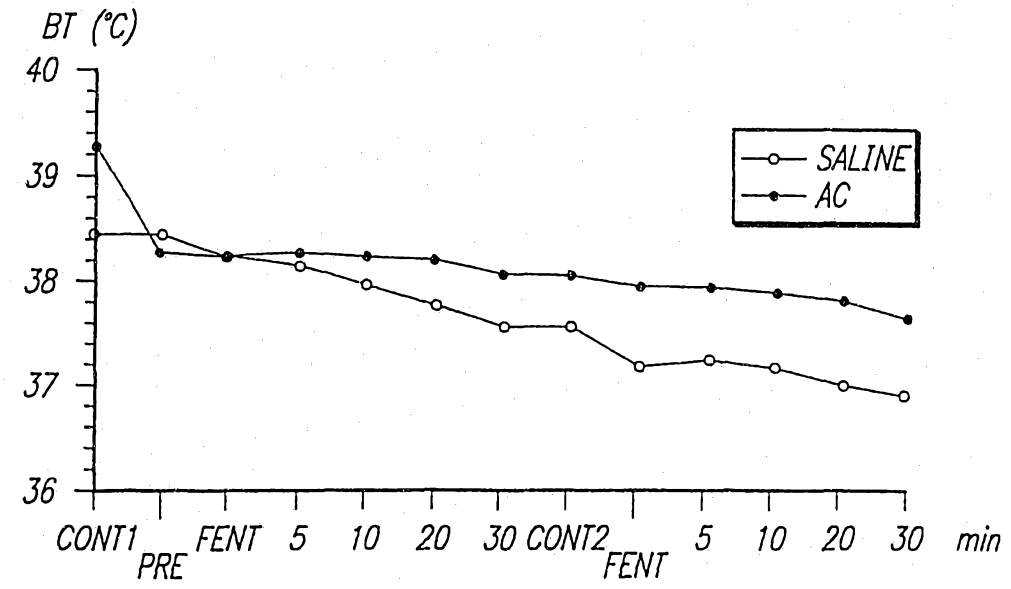

BT (°C)
40
39
38
37
36
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(g)

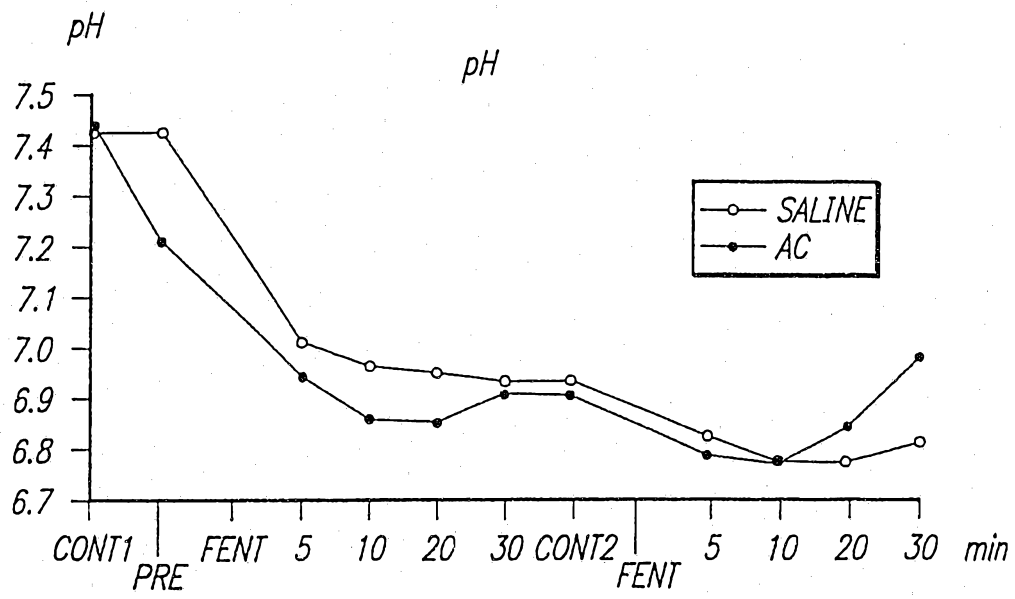

pH
pH
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(h)

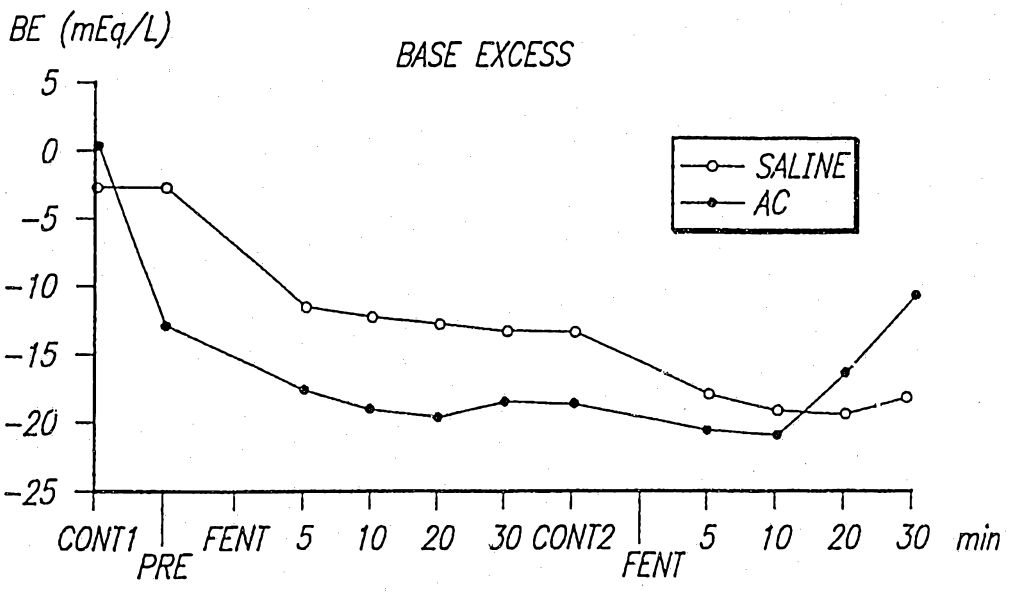

BE (mEq/L)
BASE EXCESS
5
0
-5
-10
-15
-20
-25
SALINE
AC
CONT1
PRE
FENT
5
10
20
30
CONT2
FENT
5
10
20
30
min

FIG. 6(i)

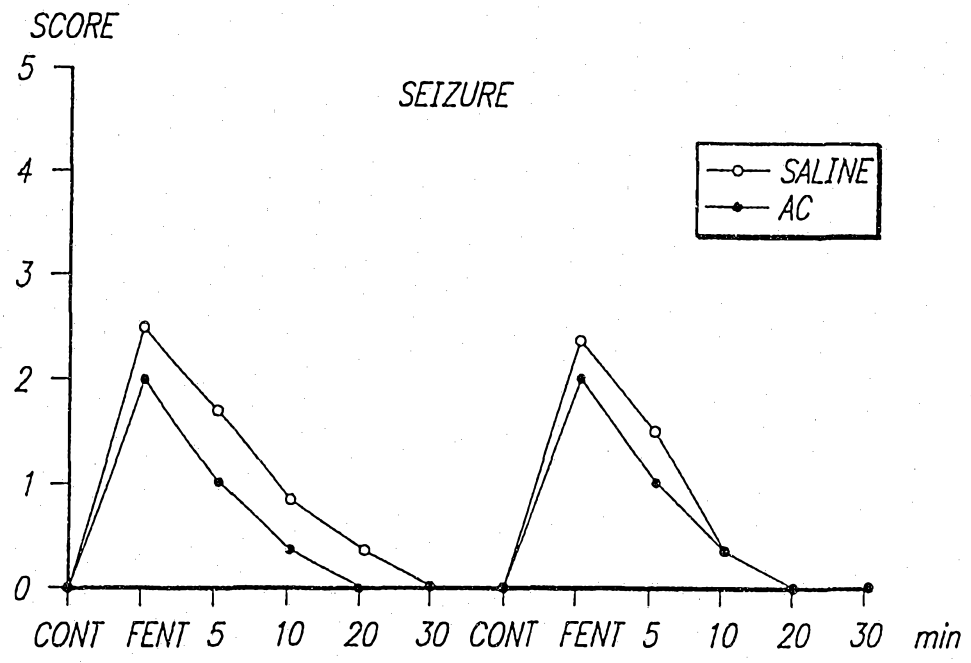

SCORE
SEIZURE
SALINE
AC
5
4
3
2
1
0
CONT FENT 5 10 20 30 CONT FENT 5 10 20 30 min

FIG. 6(j)

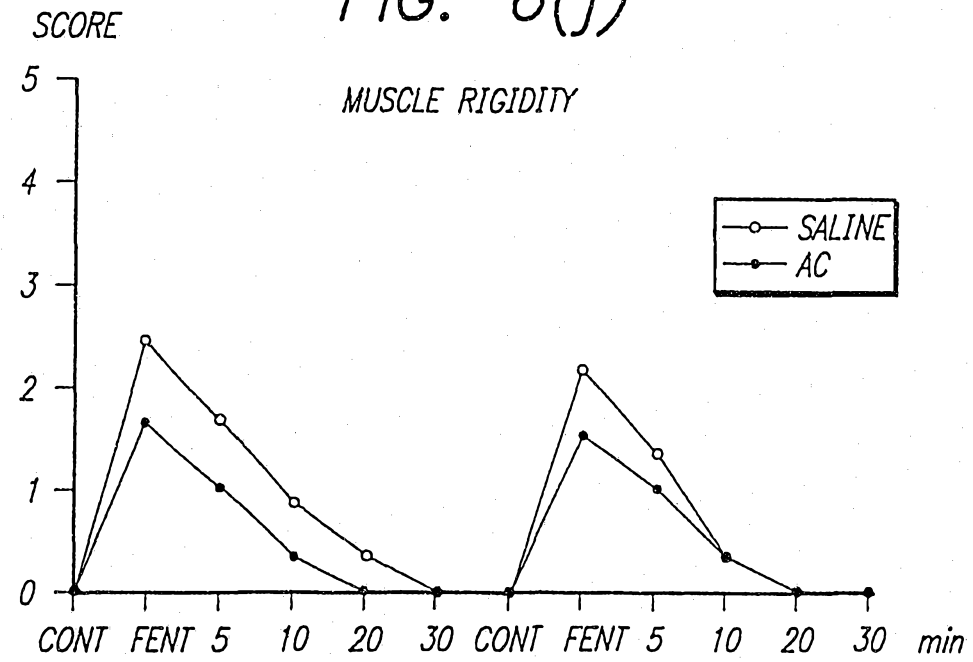

SCORE
MUSCLE RIGIDITY
SALINE
AC
5
4
3
2
1
0
CONT FENT 5 10 20 30 CONT FENT 5 10 20 30 min

FIG. 7(a)

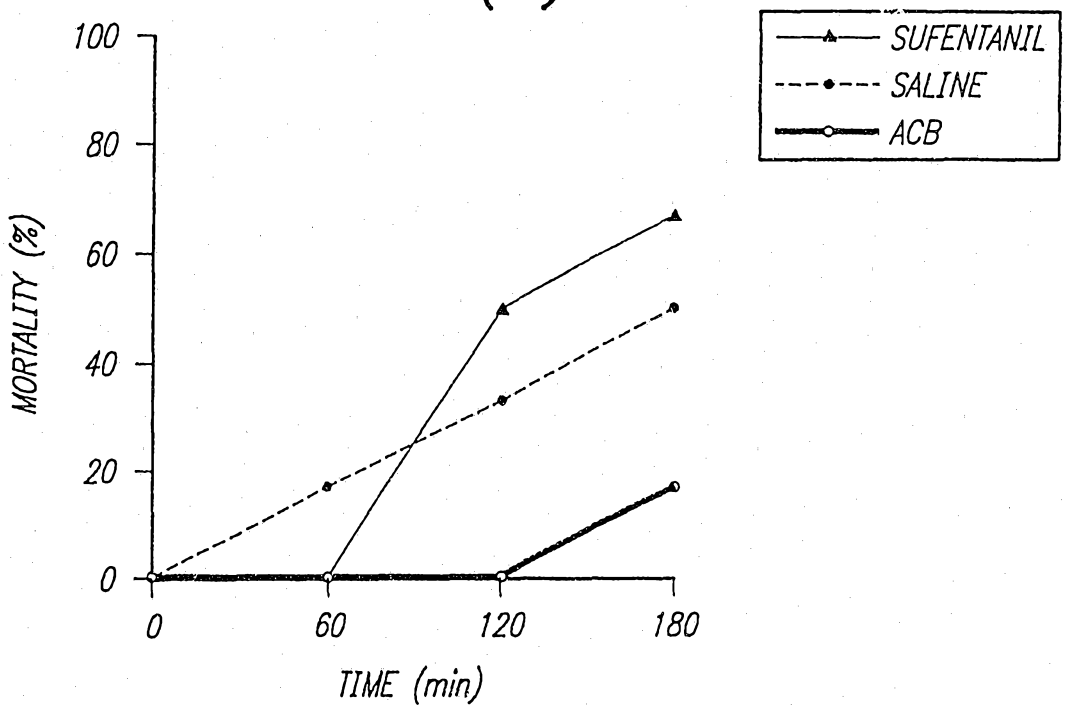

SUFENTANIL
SALINE
ACB
MORTALITY (%)
100
80
60
40
20
0
0
60
120
180
TIME (min)

FIG. 7(b)

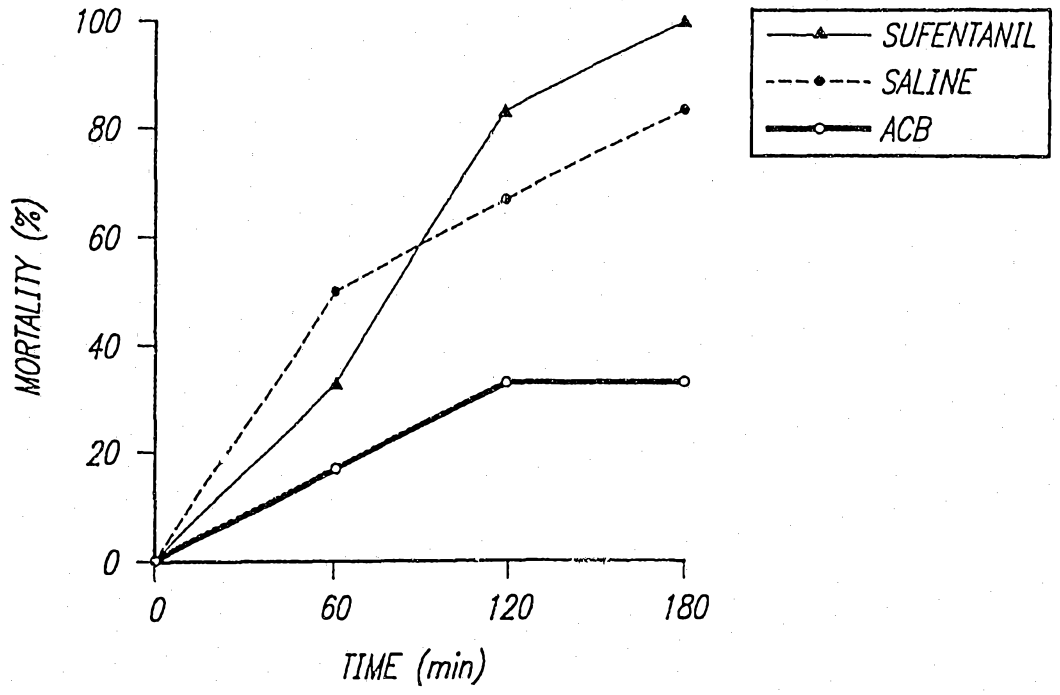

SUFENTANIL
SALINE
ACB
MORTALITY (%)
100
80
60
40
20
0
0
60
120
180
TIME (min)

FIG. 7(c)

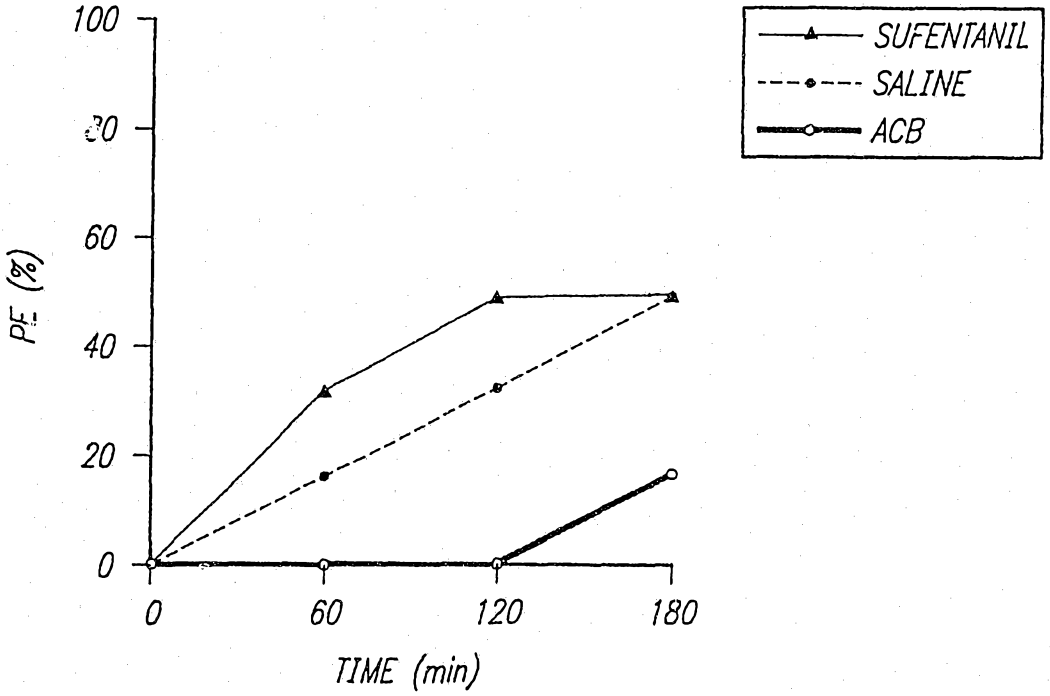

100
80
60
40
20
0
PE (%)
0
60
120
180
TIME (min)
SUFENTANIL
SALINE
ACB

FIG. 7(d)

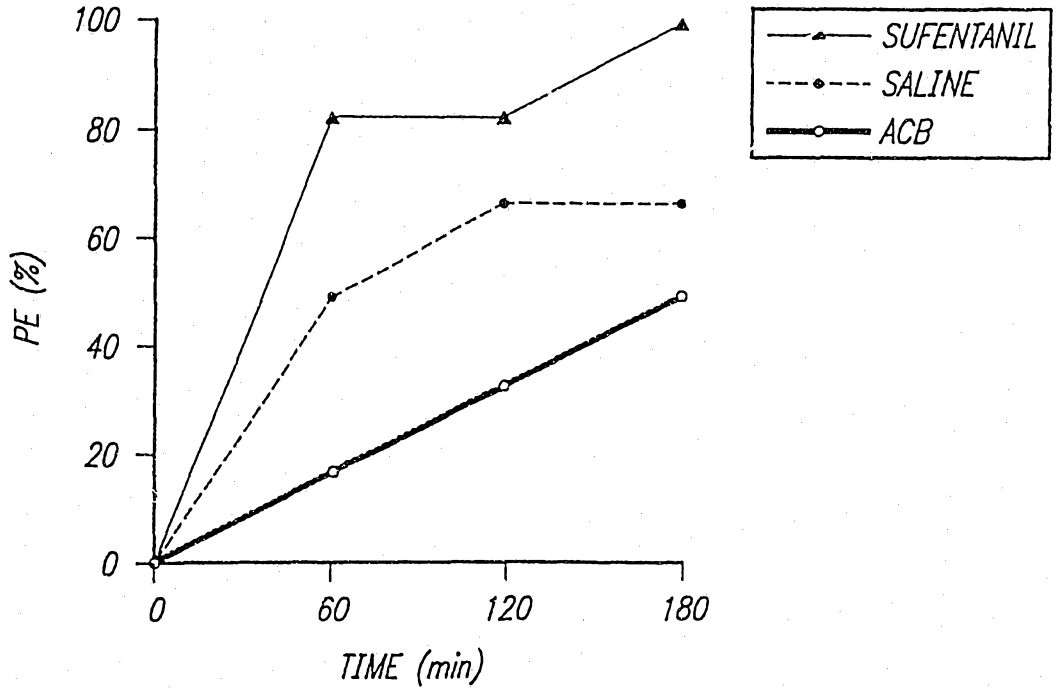

100
80
60
40
20
0
PE (%)
0
60
120
180
TIME (min)
SUFENTANIL
SALINE
ACB

FIG. 8(a)

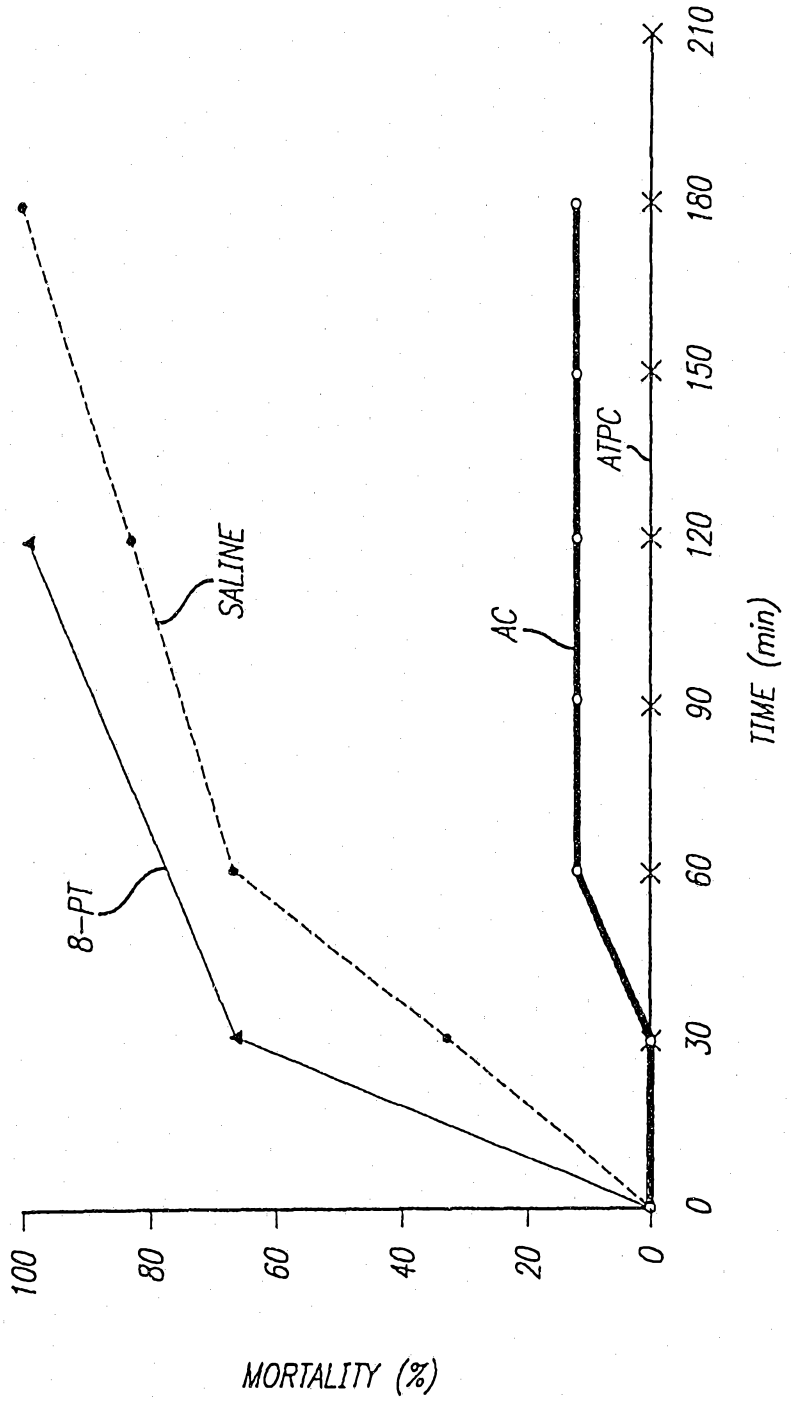
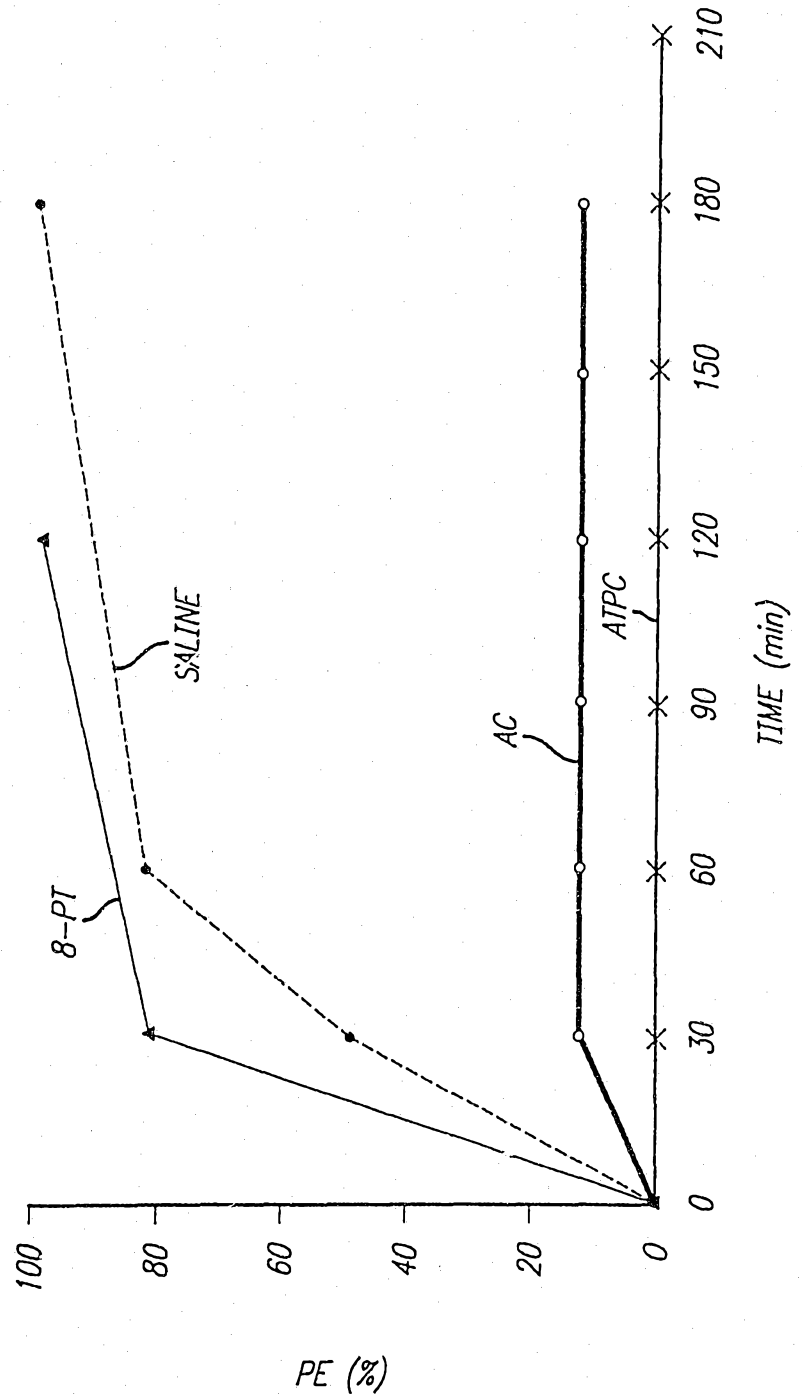

100
80
60
40
20
0
MORTALITY (%)
0
30
60
90
120
150
180
210
TIME (min)
8-PT
SALINE
AC
ATPC

FIG. 8(b)


8-PT
SALINE
AC
ATPC
PE (%)
100
80
60
40
20
0
0
30
60
90
120
150
180
210
TIME (min)

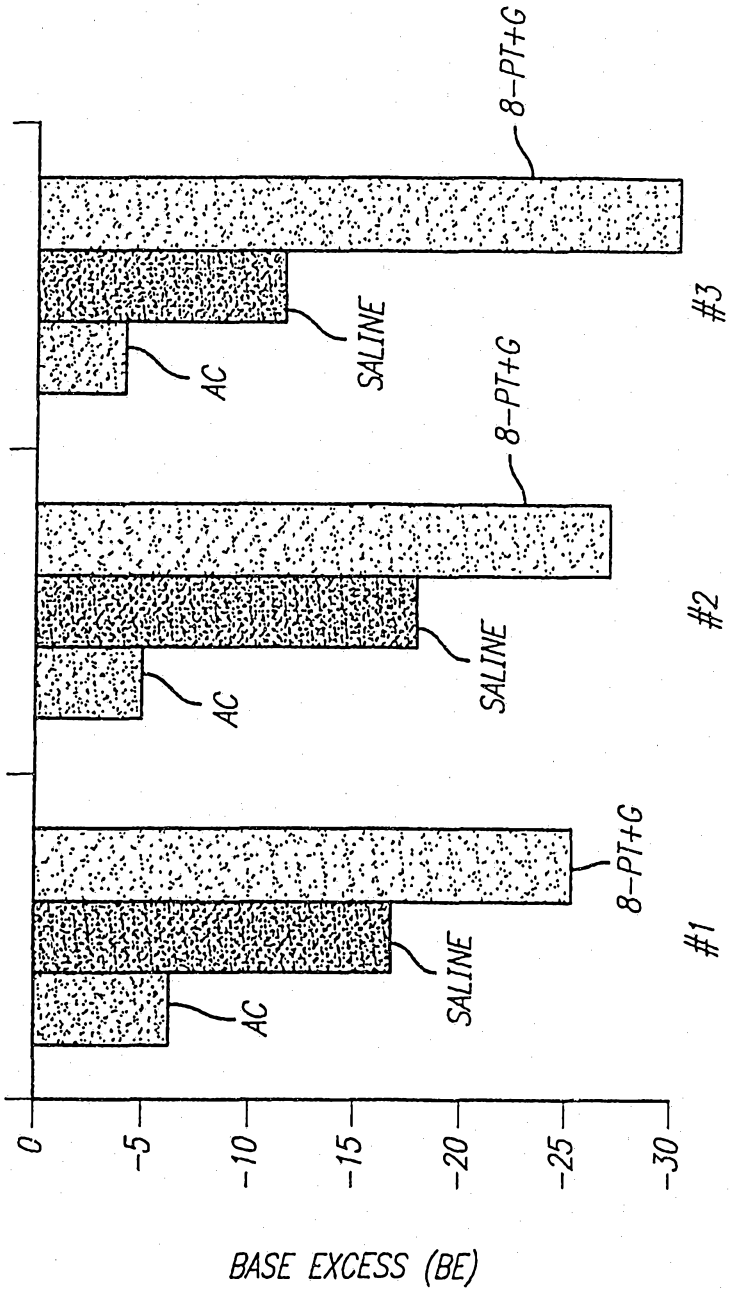

8-PT+G
SALINE
AC
#3
8-PT+G
SALINE
AC
#2
8-PT+G
SALINE
AC
#1
0
-5
-10
-15
-20
-25
-30
BASE EXCESS (BE)

FIG. 9

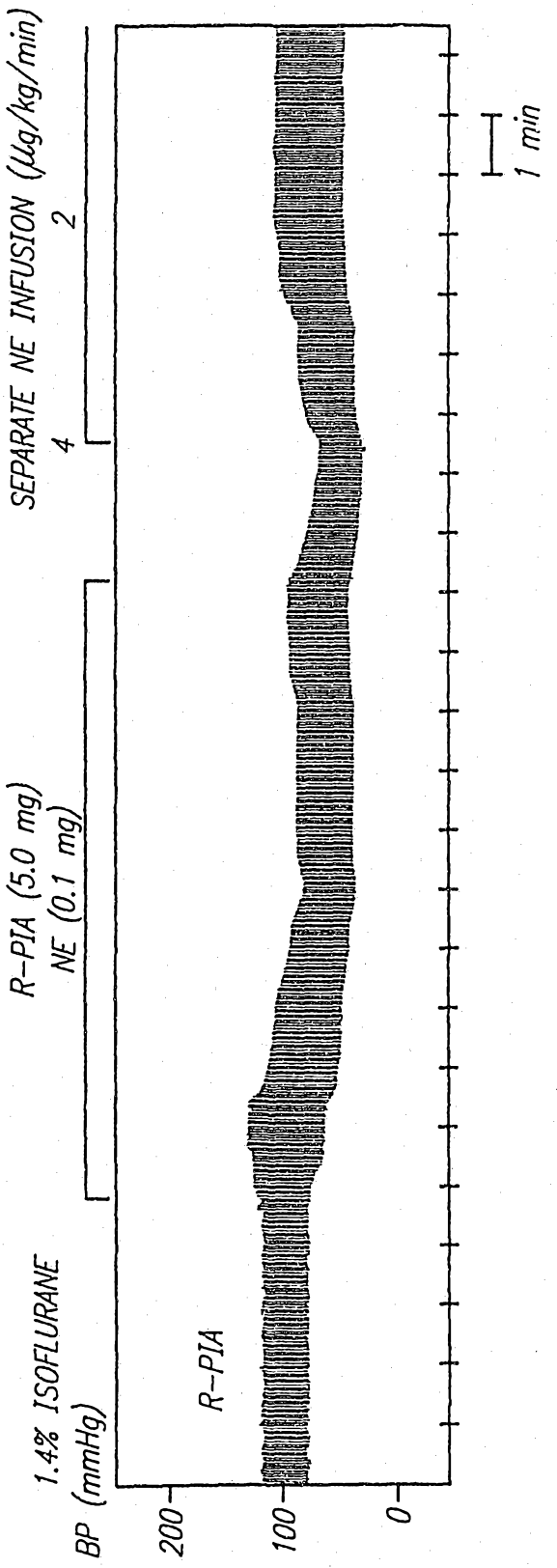

1.4% ISOFLURANE
R-PIA (5.0 mg)
NE (0.1 mg)
SEPARATE NE INFUSION (μg/kg/min)
2
4
BP (mmHg)
200
100
0
R-PIA
1 min

FIG. 10

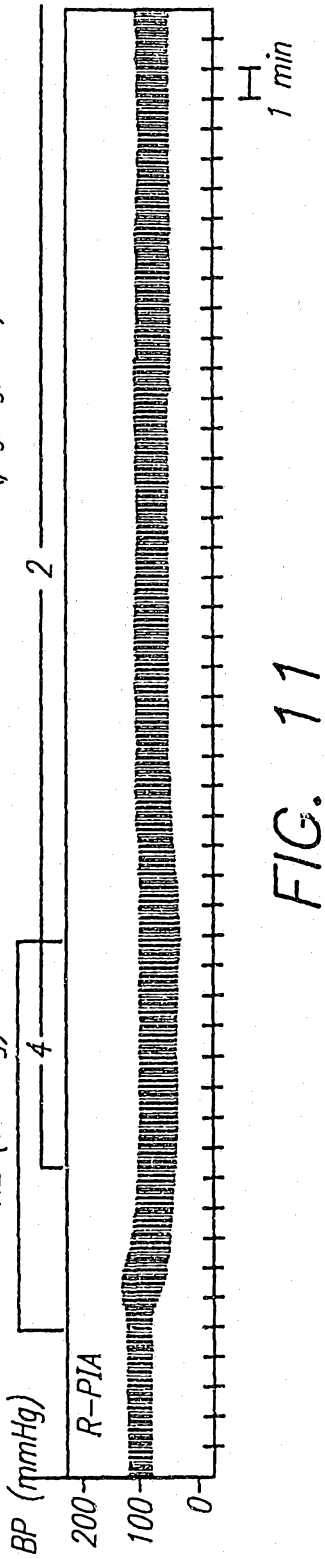

R-PIA (5.0 mg)
NE (0.1 mg)
4
SEPARATE NE INFUSION (µg/kg/min)
2
BP (mmHg)
200
100
0
R-PIA
1 min

FIG. 11

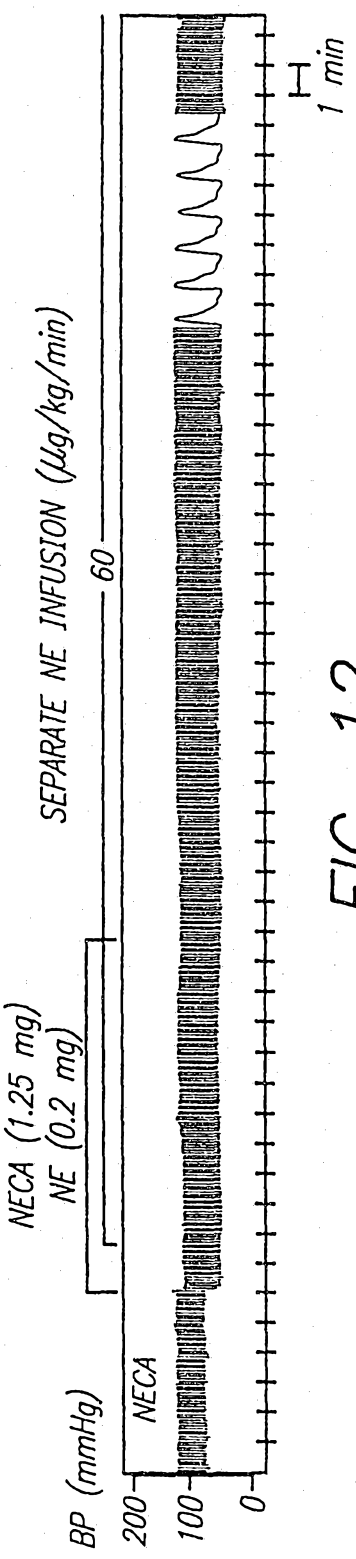

NECA (1.25 mg)
NE (0.2 mg)
SEPARATE NE INFUSION (µg/kg/min)
60
BP (mmHg)
200
100
0
NECA
1 min

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13412

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 31/70, 31/55, 31/135
US CL : 514/46, 221, 653
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/46, 221, 653

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS search terms: combination,coadminist?,concurrent w? therapy drug w/ adenosine, epinephrine and benzodiazepine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,088,756 (VOORHEES) 09 MAY 1978, see claims 1-2.	1-34
Y	US, A, 4,693,996 (STEFFEN) 15 SEPTEMBER 1987, column 1, lines 17-40.	1-34
Y	US, A, 4,605,644 (FOKER) 12 AUGUST 1986, column 1, lines 13-68, column 2, lines 1-68.	1-34
Y	US, A, 4,880,783 (MENTZER, JR. ET AL) 14 NOVEMBER 1989, column 2, lines 5-68.	1-34
Y	US, A, 4,980,379 (BELARDINELLI ET AL) 25 DECEMBER 1990, see claims 1-35.	1-34
Y	US, A, 5,137,712 (KASK ET AL) 11 AUGUST 1992, see claims 1-13.	1-34

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
24 JANUARY 1995	FEB 1 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 308-3230	Authorized officer DEBORAH LAMBKIN Telephone No. (703) 305-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13412

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,206,222 (FORMAN ET AL) 27 APRIL 1993, see claims 1-8.	1-34
Y	US, A, 5,236,908 (GRUBER ET AL) 17 AUGUST 1993, see claims 1-31.	1-34