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(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: KARLSEN, Allan, Ertmann [DK/DK]; Vibevang 9, DK-3450 Allerød (DK).

(74) Agent: THALSØ-MADSEN, Birgit; Corporate Patents, Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd (DK).

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(54) Title: A DNA SEQUENCE ENCODING NITRIC OXIDE SYNTHASE

(57) Abstract

A DNA sequence encoding a pancreatic islet cell inducible nitric oxide synthase (iNOS) is used to prepare recombinant pancreatic islet iNOS. The iNOS may be used in an assay of identifying inhibitors of pancreatic iNOS in the presence of a substrate.

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A DNA SEQUENCE ENCODING NITRIC OXIDE SYNTHASE

FIELD OF INVENTION

The present invention relates to a DNA construct comprising a DNA sequence encoding a nitric oxide synthase, a method of producing the nitric oxide synthase, a method of using the nitric oxide synthase to screen for inhibitors of nitric oxide synthase, and a test kit for use in the method.

BACKGROUND OF THE INVENTION

Insulin dependent diabetes mellitus (IDDM) is caused by immune-mediated destruction of β-cells in the islets of Langerhans. Morphological studies of islets at the time of diagnosis reveal infiltration by chronic inflammatory cells (W. Gepts, Diabetes 14, 1965, pp. 619-633) and β-cell destruction (insulitis). Chronic inflammatory cells and endothelial cells are the major producers of cytokines. The cytokines interleukin-1 (IL-1) α and β affect β-cell function and morphology in rat islets in organ culture in a time and dose dependent manner, stimulation being followed by inhibition of insulin release (T. Mandrup-Poulsen et al., Diabetologica 29, 1986, pp. 63-67; G.A. Spinas et al., Acta Endocrinol. (Copenhagen) 113, 1986, pp. 551-558; W.S. Zawalich and V.A. Diaz, Diabetes 35, 1986, pp. 1119-1123; P.G. Comens et al., Diabetes 36, 1987, pp. 963-970; S. Sandler et al., Endocrinology 121, 1987, pp. 1424-1431) with associated loss of islet DNA and selective β-cell cytotoxicity (T. Mandrup-Poulsen et al., Acta Path. Microbiol. Immunol. Scand. 95, 1987, pp. 55-63; S. Sandler et al., Endocrinology 124, 1989, pp. 1492-1501; D.L. Eizirik et al., Endocrinology 128, 1991, pp. 1611-1616; S. Helquist et al., Autoimmunity 10, 1991, pp. 311-318). IL-1β acts on β-cells via IL-1 receptors, but the signal transduction mechanism is unknown. Important post-receptor events associated with the inhibitory action of IL-1β on β-cells include a rapid increase in cytosolic Na^+, protease activation, de novo protein synthesis, impaired mithochondrial glucose oxidation and the induction of intracellular free oxygen and nitric oxide radicals (T. Mandrup-Poulsen et al., Autoimmunity 4, 1989, pp. 191-218; C. Southern et al., FEBS Lett. 276, 1990, pp. 42-44).

Nitric oxide (NO) is synthesized by the enzyme nitric oxide synthase (NOS) which converts L-arginine to citrulline and NO. Initial chracterization of NO synthases from different cell types suggests that two distinct forms exist: a constitutively expressed Ca^{2+}/calmodulin-dependent form and a cytokine-inducible, calmodulin-independent form. Constitutive production of nanomolar amounts of NO by endothelial cells appears to be vital to the regulation of homeostasis. Additionally, constitutive production of NO is critical for signal transduction in the central nervous system. The inducible form of NOS (iNOS) found in macrophages, monocytes, liver etc., produces micromolar amounts of NO which are likely to contribute to local tissue damage and systemic hypertension which accompanies septic shock as well as other inflammatory disorders. These two forms exhibit differences in regulation of expression, cofactor dependence, tissue distribution and subcellular localization.

More specifically, synthesis of NO has been found to be induced by IL-1 in islet β-cells purified by fluorescence activated cell sorting, but not in non-β-cells (J.A. Corbett et al., J. Clin. Invest. 90, 1992, pp. 2384-2391), probably by activation of the cytokine-inducible form of NO synthase. NO is toxic to the β-cell either in itself or through the hydroxyl radical from peroxynitrite formed by reaction with O_2.

Recent data suggest that the constitutive and inducible forms of NOS exist in a number of isoforms. Furthermore, studies indicate that NO production by macrophages and endothelial cells may also be distinguished on the basis of the specificity of the arginine binding site for different inhibitors. The combination of enzyme diversity and substrate specificity suggests the possibility of developing specific inhibitors of NOS for therapeutic purposes.

SUMMARY OF THE INVENTION

The observation that pancreatic islet cells can be induced by cytokines to produce nitric oxide synthase suggest that a specific form of nitric oxide synthase involved in β-cell destruction is found in islets. The inhibition of this nitric oxide synthase may therefore be desirable to prevent or at least delay the onset of IDDM. It is the object of the present invention to prepare a pancreatic islet cell cytokine inducible nitric oxide synthase to be used in the screening for substances which act as inhibitors of the nitric oxide synthase.

The present invention relates to a DNA construct comprising a DNA sequence encoding a pancreatic islet cell inducible nitric oxide synthase (iNOS).

In another aspect, the present invention relates to a method of isolating inhibitors of pancreatic islet cell inducible nitric oxide synthase, the method comprising incubating the iNOS encoded by said DNA sequence with a substance suspected of being an iNOS inhibitor in the presence of a suitable substrate for iNOS, and detecting any effect of said substance on the interaction of the iNOS with said substrate.

In the present context, the term "inhibitor" is intended to indicate a substance which inhibits the catalytic activity of the enzyme to convert L-arginine to citrulline and nitric oxide. It is the object of the present invention to isolate an inhibitor which is capable of specifically inhibiting the iNOS produced by pancreatic islets so that on administration, it will not interfere with the various essential functions of NO synthase elsewhere in the body.

In a further aspect, the present invention relates to a test kit for isolating inhibitors of pancreatic islet cell inducible nitric oxide synthase, the kit comprising in separate containers

(a) iNOS encoded by the DNA sequence according to any of claims 1-6, and

(b) a suitable substrate therefor.

DETAILED DESCRIPTION OF THE INVENTION

The DNA construct of the invention encoding the iNOS may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the iNOS by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989). For the present purpose, the DNA sequence encoding the iNOS is preferably of mammalian origin, i.e. derived from a mammalian pancreatic islet genomic DNA or cDNA library. In particular, the DNA sequence may be of rodent origin, e.g. rat or mouse origin, or of human origin.

The DNA construct of the invention encoding the iNOS may also be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by Beaucage and Caruthers, Tetrahedron Letters 22 (1981), 1859 - 1869, or the method described by Matthes et al., EMBO Journal 3 (1984), 801 - 805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

Finally, the DNA construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire DNA construct, in accordance with standard techniques. The DNA construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., Science 239 (1988), 487 - 491.

In a currently preferred embodiment, the DNA construct of the invention comprises the following partial DNA sequence

```
TTTCCAAGCT TGCCGCCACC ATGGCTTGCC CTGGAAGTTT CTCTTCAGAG
TCAAATCCTA CCAAGGTGAC CTGAAAGAGG AAAAGGACAT TAACAACAAC
GTGGAGAAAA CCCCAGGTGC TATTCCCAGC CCAACAACAC AGGATGACCC
TAAGAGTCAC AAGCATCAAA ATGGTTTCCC CCAGTTCTCA CTGGGACTGC
ACAGAATGTC CAGAGATCCC TGGACAAGTC TGCATGTGAC TCCATCGACC
CGCCCACAGC ACGTGAGGAT CAAAAACTGG GGCAATGGAG AGATTTTTCA
CGACACCCTT CACCACAAGG CCACCTCGGA TATCTCTTGC AAGTCCAAAT
TATGCATGGG GTGCATCATG AACTCCAAGA GTTTGACCAG AGGACCCAGA
GACAAGCCCA CCCCAGTGAG GAGCTTCTGT GCCTCAAGCC AATTGAATTC
ATTAACCAGT ATTATGGCTC CTTCAAAGAG GCAAAAATAG AGGAACATCT
GGCCAGGCTG GAAGCCCGTA ACAAAGGAAA TAGAAACAAC AGGAACCTAC
CAGCTCACTC TGGATGAGCT CATCTTTGCC ACCAAGATGG CCTGGAGGAA
actGCCCCTC GCTGCATCGG CAGGATTCAG TGGTCCAACC TGCAGGTCTT
CGATGCCCGG AGCTGTAGCA CTGCATCAGA AATGTTCCAG CATATCTGCA
GACACATACT TTACCGACTA ACAGTGGCAA CATCAGGTCG GCCATTACTG
TGTTCCCCCA GCGGAGCGAT GGGAAGCATG ACTTCCGGAT CTGGAATTCC
CAGCTCATCC GGTACGCTGG CTACCAGATG CCCGATGGCA CCATCAGAGG
GGATCCTGCC ACCTTGGAGT TCACCCAGTT GTGCATCGAC CTGCTGGAAG
CCCCGCTACG GCCGCTTCGA TGTGCTGCCT CTGGTCCTGC AGGCTCACGG
TCAAGATCCA GAGGTCTTTG AAATCCCTCC TGATCTTGTG CTGGAGGTGA
CCATGGAGCA CCCAAAGTAC GAATGGTTCC AAA (SEQ ID NO:1)
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or a homologue thereof encoding a protein with iNOS activity.

The partial DNA sequence shown above has been isolated from a rat islet cDNA library as described below. The sequence has been found to be significantly homologous to the previously published (C.R. Lyons et al., J. Biol. Chem. 267, 1992, pp. 6370-6374) mouse macrophage sequence (cf. Fig. 1A and 1B). However, the gene encoding mouse macrophage iNOS is inducible by lipopolysaccharide and interferon-γ, whereas the iNOS produced in pancreatic islets is inducible by interleukin-1 (IL-1) (rat) or a mixture of IL-1, tumour necrosis factor α (TNF-α) and interferon-γ (IFN-γ) (human), indicating that significant differences may exist between the mouse macrophage iNOS and the rat islet iNOS. In the present context, the term "homologue" is intended to indicate a natural variant of the DNA sequence encoding rat islet iNOS, such as a variant produced in pancreatic islets of another species, in particular in human pancreatic islets, or a variant produced by modification of the DNA sequence shown above. Examples of suitable modifications of the DNA sequence are nucleotide substitutions which do not give rise to another amino acid sequence of the iNOS but which may correspond to the codon usage of the host organism into which the DNA construct is introduced or nucleotide substitutions which do give rise to a different amino acid sequence and therefore, possibly, a different protein structure. Other examples of possible modifications are insertion of one or several nucleotides into the sequence, addition of one or several nucleotides at either end of the sequence, or deletion of one or several nucleotides at either end or within the sequence. However, any protein produced from such a homologous DNA sequence should exhibit an iNOS activity (e.g. with respect to substrate specificity) similar to that of the native iNOS.

In a particularly preferred embodiment, the invention relates to the full-length rat islet iNOS shown in the Sequence Listing as SEQ ID NO:6, or a suitable modification thereof, as defined above.

In another preferred embodiment, the present invention relates to a DNA sequence encoding human islet iNOS, comprising the partial DNA sequences shown in the Sequence Listing as SEQ ID

NO:9 and SEQ ID NO:10, or a suitable modification thereof, as defined above.

In a further aspect, the present invention relates to a recombinant expression vector comprising a DNA construct of the invention. The recombinant expression vector into which the DNA construct of the invention is inserted may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding the iNOS should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the iNOS in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell Biol. 1 (1981), 854 -864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809 - 814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasuvedan et al., FEBS Lett. 311, (1992) 7 - 11). Suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US

4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter.

The DNA sequence encoding the iNOS may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) terminators. The vector may further comprise elements such as polyadenylation signals (e.g. from SV40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

The recombinant expression vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hygromycin or methotrexate.

The procedures used to ligate the DNA sequences coding for the iNOS, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

The host cell into which the expression vector of the invention is introduced may be any cell which is capable of producing the iNOS and is preferably a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. *Xenopus laevis* oocytes or mammalian cells, in particular insect and mammalian cells.

Examples of suitable mammalian cell lines are the COS (ATCC CRL 1650), BHK (ATCC CRL 1632, ATCC CCL 10), CHL (ATCC CCL39) or CHO (ATCC CCL 61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601 - 621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327 - 341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422 - 426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603, Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841 - 845.

Alternatively, fungal cells (including yeast cells) may be used as host cells of the invention. Examples of suitable yeasts cells include cells of *Saccharomyces* spp. or *Schizosaccharomyces* spp., in particular strains of *Saccharomyces cerevisiae*. Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus* spp. or *Neurospora* spp., in particular strains of *Aspergillus oryzae* or *Aspergillus niger*. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277.

The iNOS may then be produced by a method which comprises culturing a cell as described above in a suitable nutrient medium under conditions which are conducive to the expression of the iNOS and recovering the resulting iNOS from the culture.

The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The iNOS produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, affinity chromatography, or the like.

In the method of the invention of isolating inhibitors of iNOS, the preferred substrate is L-arginine, though other substrates for the enzyme may also be used. Using L-arginine as the substrate is preferred because it is attempted to achieve the closest possible approximation to the native system. When using L-arginine as the substrate, the inhibitory activity of a suspected iNOS inhibitor may be determined by measuring the amount of L-arginine or citrulline after incubation, compared to a control which does not contain any suspected iNOS inhibitor. A substatially unchanged amount of L-arginine in the incubation mixture is indicative of the presence of an inhibitor, as is a decreased amount or absence of citrulline.

Alternatively, the formation of nitric oxide (NO) resulting from the incubation may be determined, decreased NO formation indicating the presence of an iNOS inhibitor. In a preferred embodiment, the formation of NO may be measured indirectly by adding an indicator. Such an indicator may, for instance, be guanylate cyclase which is strongly activated by NO to produce cyclic GMP from GTP. In this system, the formation of NO is determined by measuring the amount of cyclic GMP formed on incubation (formation of cyclic GMP may for instance be measured as described in J.A. Corbett et al., Biochem. J. 287, 1992, pp. 229-235). NO formation may also be determined by measuring the amount of nitrite formed on incubation (NO is converted to nitrite in the presence of free oxygen) (e.g. as described by L.C. Green et al., Anal. Biochem. 126, 1982, pp. 131-138).

The present invention is further illustrated in the following Example which should not be regarded as limiting, in any way, the scope of the invention as claimed.

EXAMPLE 1

Cloning of rat islet iNOS

Rat islets were isolated from newborn rats after collagenase digestion of rat pancreases (as described by J. Brunstedt et al., in Methods in Diabetes Research, vol. 1 (Laboratory Methods, Part C), J. Larner and S.J. Pohl (eds.), Wiley & Sons, New York, 1984, pp. 254-288). After isolation, the islets were precultured for 3-7 days in RPMI 1640 + 10% fetal calf serum at 37°C. The islets were then incubated with 150 U/ml recombinant IL-1 β (prepared by Novo Nordisk A/S) for 48 hours in a medium containing 150 islets/300 μl of RPMI 1640 + 0.5% normal human serum. Control islets were cultured similarly in a medium which did not contain any IL-1. The culture media were collected and analysed for nitrite production by mixing 150 ml of medium with an equal volume of Griess reagent (1 part of 0.1% naphthylethylene diamine dihydrochloride, 1 part of 1% sulfanilamide in 5% H_3PO_4) and incubated for 10 minutes at room temperature (L.C. Green et al., Anal. Biochem. 126, 1982, pp. 131-138). The absorbance at 550 nm was determined on an immuno reader (NIPPON InterMed KK, Tokyo, Japan) against a sodium nitrite standard curve.

The islet were harvested, and total RNA from IL-1 induced and non-induced islets was prepared by CsCl gradient centrifugation (as described by J.M. Chrigwin et al., Biochemistry 18, 1979, 5294-5299). 3 μg of the isolated RNA was subjected to RT-PCR in accordance with the manufacturer's instructions (RT-PCR kit available from Invitrogen, San Diego, CA). Briefly, the RNA was reverse transcribed and the resulting single-stranded DNA was used directly for the PCR reaction. In the PCR reaction, the following oligonucleotides based on the previously published mouse macrophage iNOS sequence (Lyons et al., supra) were used as primers

#290: 5'-TCC AAG CTT GCC GCC ACC ATG GCT TGC CCC TGG-3' (SEQ ID NO:2)

#294: 5'-TG (GA)AA CCA (CT)TC (GA)TA (CT)TT (AGCT)GG (GA)TG (CT)TC CAT-3'

A standard PCR reaction was run, and a 1 kb fragment was detected in the PCR reaction based on RNA from IL-1 induced islets, whereas no such fragment appeared from the PCR reaction based on RNA from non-induced islets.

The amplified transcript was purified on a 1% agarose gel and subsequently cut out of the gel and purified by centrifugation through a filter. The purified transcript was then re-amplified with the same primers to obtain enough material for cloning. The DNA resulting from this re-amplification was then cloned into the TA vector (Invitrogen) in accordance with the manufacturer's instructions.

The resulting clones were sequenced by the method described by Tabor and Richardson, Proc. Natl. Acad. Sci. USA 84, 1987, pp. 4767-4771 by means of the Sequenase kit (available from US Biochemical Corp.). The resulting sequence showed a high degree of homology to the previously published mouse macrophage iNOS sequence (cf. Fig. 1A and 1B, wherein RATBCL indicates the partial rat islet iNOS sequence of the invention, and MUSMAC indicates the corresponding mouse macrophage iNOS sequence).

EXAMPLE 2

Cloning of full-length rat islet iNOS

Islets were isolated from 3-6 days old Wistar rats (Møllegaard, Lille Skensved, Denmark) following collagenase digestion of the pancreas as described by Brunstedt J, Nielsen JH, Lernmark Å, and The Hagedorn Study Group (1984) Isolation of islets from mice and rats. In: Larner J, Pohl SL, ed. Methods in diabetes research, (Laboratory methods, part C).

New York: Wiley & Sons, 254-288. vol 1). Following isolation the islets were kept in culture for 3-7 days at 37° C in RPMI 1640 (Gibco, Paisley, Scotland) + 10% fetal calf serum (FCS) 100,000 IU/l penicillin, 100 mg/l streptomycin and 20 mM HEPES buffer. Exposure to cytokines for 20-48 hours was performed in the same buffer, however, with 0.5% normal human serum added in stead of FCS. Rat insulinoma (RIN-5AH-T 2B) cells (Karlsen AE, Fujimoto WY, Rabinovitch P, Dube S, Lernmark Å (1991) Effects of sodium butyrate on proliferation-dependent insulin gene expression and insulin release in glucose-sensitive RIN-5AH cells. J. Biol. Chem. 266:7542-7548) and MSL cells (Madsen OD, Andersen LC, Michelsen B, Owerbach D, Larsson L-I, Lernmark Å, Steiner DF (1988) Tissue-specific expression of transfected human insulin genes in pluripotent clonal rat lines induced during passage in vivo. Proc. Natl. Acad. Sci. (USA). 85:6652-6656) were cultured as previously described and exposed to cytokines for different lengths of time (1 or 3 days). Every day cells were collected for mRNA isolation, the proliferation rate determined by counting and media collected for insulin measurement. The cytokine used was human recombinant IL-1b with a specific activity of 400 U/ng (produced at Novo-Nordisk A/S, Bagsvaerd, Denmark), TNFα obtained from Sigma and rat IFNγ is from Sigma. The IL-1 used for the RIN cell experiments was obtained from Immunex. Insulin accumulated in the culture media was measured by RIA as previously described (Karlsen, 1991, supra).

RNA was extracted from the islets and cells by a modification of the 8M guanidine method as previously described (Karlsen AE, Hagopian WA, Grubin CE, Dube S, Disteshe CM, Adler DA, Barmeier H, Mathewes S, Grant FJ, Foster D, Lernmark A (1991) Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc. Natl. Acad. Sci. USA. 88:8337-8341) and cDNA was prepared (Invitrogen) according the manufacturer's description using oligo-(dT) as primer. A partial (1 kb) rat islet cDNA was first cloned by standard RT-PCR using the primer 5'CCAAGCTTGCCGCCACCATGGCTTGCCCCTGG (SEQ ID

NO:3) in conjunction with degenerate primers 3'TG(GA)AACCA(CT)TC(GA)TA(CT)T(TG)(GT)GG(GA)TG(CT)TCCAT spanning the bases 256-1254 of the mouse macrophage iNOS cDNA (Xie Q, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Ding A, Troso T, Nathan C (1992) Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science. 256:225-228). A cDNA clone (3.5 kb) spanning the entire coding region was later isolated using the primers 5'AGAAGCACAAAGTCACAGA (SEQ ID NO:4) and 3'ACTTCTGTCTCTCCAAACCC (SEQ ID NO:5) spanning the bases 1-3530 of the subsequently published rat smooth muscle iNOS cDNA sequence (Nunokawa Y, Ishida N, Tanaka S (1993) Cloning of inducible nitric oxide synthase in rat vascular smooth muscle cells. Biochem. Biophys. Res. Com. 191(1):89-94). The RT-PCR fragments were ligated into the pCRII vector (Invitrogen) following separation by agarose electrophoresis. The product was transfected into E.coli (One-shot, Invitrogen). To diminish potential sequencing artefacts resulting from AmpliTaq-polymerase (Cetus, Perkin Elmer) misincoorporated nucleotides, the full length rat islet iNOS cDNA was also cloned and sequenced following pfu polymerase RT-PCR. Despite the exonuclease activity of this polymerase, the amplified products were also cloned into the pCRII vector, however with lower efficiency than following AmpliTaq PCR. The cloned cDNA were sequenced using the automatic laser fluorescence DNA sequencer (ALF ##) and the sequences were analyzed using the software package from the University of Wisconsin Genetic Computer Group (Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res. 12:387-396).

For expression of the cloned iNOS the coding region was subcloned into the HindIII and Not1 sites of the pcDNA3 vector (Invitrogen) and expressed under control of the CMV promoter in the human embryonic kidney cell line 293 (publicly available from the American Type Culture collection as ATCC CRL 1573) following standard calciumphosphate transfection. Expression of the iNOS enzyme and resulting NO production of the transfectants after 3 days of culture as well as from the cytokine exposed β-cell- and islet cultures were estimated from accumulated nitrite in the culture media as described by Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitritye and [12N]nitrate in biological fluids. Anal. Biochem. 126:131-138. Analysis of iNOS mRNA expression in the rat insulinoma cells was done by Northern hybridization as previously described (Karlsen et al., 1991, supra) using the cloned iNOS cDNA as a probe. The same amount of mRNA (5 μg) was loaded to each lane and the integrity and amount was verified by ethidium bromide staining before blotting to nitrocellulose filter. iNOS mRNA expression in islets was determined by RT-PCR using the above described primers (and PCR cycle-conditions).

The IL-1 induced iNOS mRNA expression was reflected by an induced NO production, measured as nitrite accumulation into the media (14.3 pmol/islet) in contrast to the non-exposed islets where no nitrite above the detection limit of 2 pmol/islet could be detected. Analysis of rat islet β-cell lines, the rat insulinoma (RIN) cells and an insulin producing pluripotent (MSL) cell-line revealed a similar induced NO production following exposure to IL-1 alone or in combination with other cytokines which could not be detected in the non-exposed cells.

The full sequence of the cloned rat islet iNOS was found to be

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1   AGAAGCACAA AGTCACAGAC ATGGCTTGCC CCTGGAAGTT TCTCTTCAGA
51  GTCAAATCCT ACCAAGGTGA CCTGAAAGAG GAAAAGGACA TTAACAACAA
101 CGTGGAGAAA ACCCCAGGTG CTATTCCCAG CCCAACAACA CAGGATGACC
151 CTAAGAGTCA CAAGCATCAA AATGGTTTCC CCCAGTTCCT CACTGGGACT
201 GCACAGAATG TTCCAGAATC CCTGGACAAG CTGCATGTGA CTCCATCGAC
251 CCGCCCACAG CACGTGAGGA TCAAAAACTG GGGCAATGGA GAGATTTTtC
301 ACGACACCCT TCACCACAAG GCCACCTCGG ATATCTCTTG CAAGTCCAAA
351 TTATGCATGG GGTCCATCAT GAACTCCAAG AGTTTGACCA GAGGACCCAG
401 AGACAAGCCC ACCCCAGTGG AGGAGCTTCT GCCTCAAGCC ATTGAATTCA
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451 TTAACCAGTA TTATGGCTCC TTCAAAGAGG CAAAAATAGA GGAACATCTG
501 GCCAGGCTGG AAGCcGTAAC AAAGGAAATA GAAACAACAG GAACCTACCA
551 GCTCACTCTG GATGAGCTCA TCTTTGCCAC CAAGATGGCC TGGAGGAACG
601 CCCCTCGCTG CATCGGCAGG ATTCAGTGGT CCAACCTGCA GGTCTTCGAT
651 GCCCGGaGCT GTAGCACTGC ATCAGAAATG TTCCAGCATA TCTGCAGACA
701 CATACTTTAC GCCACTAACA GTGgcAACAT CAGGTCGGCC ATTACTGTGT
751 TCCCCCAGCG GAgcgATGGG AAGCATGACT TCCGGATCTG GAATTCCCAG
801 CTCATCCGGT ACGCTGGCTA CCAGATGCCC GATGGCACCA TCAGAGGgGA
851 TCCTGCCACC TTGGAGTTCA CCCAGTTGTG CATCGACCTG GGCTGGAAgc
901 CccGCTATGG CCGCTTCGAT GTGCTGCCTC TGGTCCTGCA GGCTCACGGT
951 CAAGATCCAG AGGTCTTTGA AATCCCTCCT GATCTTGTGC TGGAGGtGAC
1001 CATGGAGCAT CCCAAGTACG AGTGGTTCCA GGAGCTCGGG CTGAAGTGGT
1051 ATGCGCTGCC TGCCGTGGCC AACATGCTCC TGGAGGTGGG TGGCCTCGAG
1101 TTCCCAGCCT GCCCCTTCAA TGGTTGGTAC ATGGGCACCG AGATTGGAGT
1151 CCGAGACCTC TGTGACACAC AGCGCTACAA CATCCTGGAG GAAGTgGGcA
1201 GGAGGATGGG CCTGGAGACC CACACACTGG cCTCCctctg gAAAGACCGG
1251 GCTGTCACCG AGATCAATGC AGCTGTGCTC CATAGTTTTC AGAAGCAGAA
1301 TGTGACCATC ATGGACCACC ACACAGCCTC AGAGTCCTTC ATGAAGCACA
1351 TGCAGAATGA GTACCGgGCC CGAGGAGGCT GCCCTGCAGA CTGGATTTGG
1401 CTGGTCCCTC CGGTGTCCGG GAGCATCACC CCTGTGTTCC ACCAGGAGAT
1451 GTTGAACTAC GTCCTATCTC CATTCTACTA CTACCAGATC GAGCCCTGGA
1501 AGACCCACAT CTGGCAGGAT GAGAAGCTGA GGCCCAGGAG GAGAGAGATC
1551 CGGTTCACAG TCTTGGTGAA AGCGGTGTTC TTTGCTTCTG TGCTAATGCG
1601 GAAGGTCATG GCTTCCCGCG TCAGAGCCAC AGTCCTCTTT GCTACTGAGA
1651 CAGGAAAGTC GGAAGCGCTA gCCAGGGACC TGGCTgCCTT GTTCAGCTAC
1701 GCCTTCAACA CCAAGGTTGT CTGCATGGAA CAGTATAAGG CAAACACCTT
1751 GGAAGAGGAA CAACTACTGC TGGTGGtGAC AAGCACATTT GGCAATGGAG
1801 ACTGCCCCAG CAATGGGCAG ACTCTGAAGA AATCTCTGTT CATGATGAAA
1851 GAACTCGGGC ATACCTTCAG GTATCgGGTA TTTGGCCTGG GCTCCAGCAT
1901 GTACCCTCAG TTCTGTGCCT TTGCTCATGA CATCGACCAG AAACTGTCTC
1951 ACCTGGGaGC CTCCCAGCTT GcCCCAACCG GAGAAGGGGA CGAACTCAGC
2001 GGGCAGGAGG ACGCCTTCCG CAGCTGGGcT GTGCAAaCCT TCCGGGCAGC
2051 CTGTGAGACG TTCGAtgttc gaaGCAAACA TTGCATTCAG ATCCCGAAAC
2101 GCTACACTTC CAACGCAACA TGGGAGCCAG AGCAGTACaA GCTCaCCCAG
2151 AGCCCAGAGC CTCTAGACCT CAAcAAAGCT CTCAGCAGCA TCCACGCCAA
2201 GAACGTGTTC ACCATGAGGC TGAAATCCCT CCAGAATCTG CAGAGTGAGA
2251 AGTCCAGCCG CACCACCCTC CTTGTtcAAC TCACCTTCGA GGGCAGCCGA

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2301 GGcCCCAGCT ACCTACCTGG GGaACaCCTG GGGATTTTCC CAGGCAACCA
2351 GACGGCCCTG GTGCAAGGGA TCTTggagcg aGTTGTGGAT TGTTCTTCGC
2401 CAGACCAAAC TGTGTGCCTG GAGGTTCTAG ATGAGAGTGG CAGCTACTGG
2451 GTCAAAGACA AGAGGCTTCC CCCCTGCTCA CTCAGGCAAG CCCTCACCTA
2501 CTTCCTGGAC ATCACTACCC CTCCCACCCA gCTGCAGCTC CACAAGCTGG
2551 CCCGCTTTGC CACGGAAGAG ACGCACAGGC AGAGGTTGGA GGCCTTGTGT
2601 CAGCCCTCAG AGTACAACGA TTGGAAGTTC AGCAACAACC CCACGTTCCT
2651 GGAGGTGCTG GAAGAGTTCC CATCATTGCG TGTGCCTGCT GCCTTCCTGC
2701 TGTCGCAGCT CCCCATTCTG AAGCCCCGCT ACTACTCCAT CAGCTCCTCC
2751 CAGGACCACA CCCCCTCGGA GGTCCACCTC ACTGTGGCTG TGGTCACCTA
2801 TCGCACCCGA GATGGTCAGG GTCCCCTGCA CCATGGCGTC TGCAGCACTT
2851 GGATCAATAA CCTGAAGCCC GAAGACCCAG TGCCCTGCTT TGTGCGGAGT
2901 GTCAGTGGCT TCCAGCTCCC TGAGGACCCC TCCCAGCCCT GCATCCTCAT
2951 TGGGCCCGGT ACAGGCATTG cCCCCTTCCG AAGTTTCTGG CAGCAGCGGC
3001 TCCATGACTC TCAGCGCAGA GGGCTCAAAG GAGGCCGCAT GACCTTGGTG
3051 TTTGGGTGCA GGCACCCAGA GGAGGACCAC CTCTATCAGG AAGAAATGCA
3101 GGAGATGGTC CGCAAGGGAG TGTTGTTCCA GGTGCACACA GGCTACTCCC
3151 GGCTGCCCGG AAAACCCAAG GTCTACGTTC AAGACATCCT GCAGAAAGAG
3201 CTGGCCGACG AGGTGTTCAG CGTGCTCCAC GGGGAGCAGG GCCACCTCTA
3251 TGTTTGTGGC GATGTGCGCA TGGCTCGGGA TGTGGCTACC ACTTTGAAGA
3301 AGCTGGTGGC CGCCAAGCTG AACTTGAGTG AGGAGCAGGT TGAGGATTAC
3351 TTCTTCCAGC TCAAGAGCCA GAAACgTTAT CATGAGGATA TCTTCGGTGC
3401 GGTCTTTTCC TATGGAGTGA AAAAGGGCAA CGCTTTGGAG GAGCCCAAAG
3451 GCACAAGACT CTGACACCCA GAAGAGTTAC AGCATCTGGC CCTAAATAAA
3501 ATGACAGTGA gGGTTTGGAG AGACAGAAGT (SEQ ID NO:6)
```

Sequence analyses revealed identity among the different rat islet iNOS clones obtained and demonstrated more than 99% identity at both nucleotide and amino acid level to the published rat hepatocyte and smooth muscle iNOS sequences, 93% identity to the mouse macrophage iNOS, and 80% identity to the published human iNOS'es. That the cloned rat islet iNOS transcript was indeed expressed in β-cells was envisaged by cloning an identical 1 Kb cDNA from IL-1 stimulated RIN cells that was not amplified/detectable from the non-stimulated cells (data not shown). Furthermore Northern blot analysis with the cloned iNOS cDNA as probe, detected a 4.5kb transcript exclusively in the IL-1 exposed RIN cells. Exposure of the RIN cells to a low (0,2ng/ml) or high (2ng/ml) IL-1 concentration for one or three days illustrated a dose- and time dependent expression of the iNOS transcript.

Transient expression of the cloned iNOS gene under the CMV promoter in the mammalian embryonic kidney cell line 293 demonstrated enzymatic activity of the recombinant iNOS which was dose-dependently inhibited by 1.1 and 2.2 mM of the arginine analogue NAME and aminoguanidine, whereas 25mM of nicotinamide, a concentration previously shown to reduce IL-1 induced nitrite production in rat islets by 50% did not influence the enzymatic activity. The results appear from Table 1.

Table 1

Control	3.3
iNOS	45.87
iNOS + 1.1 mM NAME	25.23
iNOS + 2.2 mM NAME	16.8
iNOS + 1.1 mM aminoguanidine	8.4
iNOS + 25 mM nicotine amide	44.97

EXAMPLE 3

Cloning of human islet iNOS

Human islets were incubated in a mixture of IL-1, TNF-α and IFN-γ for 6 hours before they were harvested and mRNA was isolated as described in Example 2. Expression of iNOS was detected as described in Example 2. iNOS expression could not be detected in unstimulated human islets. The human islet iNOS was cloned by RT-PCR as described in Example 2 on the isolated mRNA with primers based on the human hepatocyte sequence

(Geller et al. Proc. Natl. Acad. Sci. USA 90, 1993, pp. 3491-3495). The sequence of the primers was as follows:

#12 5'-AGT TCT CAA GGC ACA GGT CTC-3' (SEQ ID NO:7)
#2696 5'-GCT CCA TCC TTA AGT TCT-3' (SEQ ID NO:8)

The cloned human islet iNOS was sequenced as described in Example 2. The sequence of 5' untranslated and translated human islet iNOS was determined to be

1 AGTTCTCAAG GCACAGGTCT CTTCCTGGTT TGACTGTCCT TACCCCGGGG
51 AGGCAGTGCA GCCAGCTGCA ASCCCACAGT GAAGAACATC TGAGCTCAAA
101 TCCAGATAAG TGACATAAGT GACCTGCTTT GTAAAGCCAT AGAG**ATG**GCC
151 TGTCCTTGAA AATTTCTGTT CAAGACCAAA TTCCACCAGT ATGCAATGAA
201 TGGGGRAAAA GACATCAACA ACAATGTGGA GAAAGCCCCC TGTGCCACCT
251 CCAGTCCAGT GACACAGGAT GACCTTCAGT ATCACAACCT CAGCAAGCAG
301 CAGAATGAGT CCCCGCAGCC CCTCGTGGAG ACgGGAAAGA AGTCTCCAGA
351 ATCTCTGGTC AAGCTGGATG CAACCCCcATT GTCCTCCCCA CCGCATGTGA
401 GGATCaAAAA CTGGGGCAGC GGGATGACTT TCCAAGACAC ACCTCACCAT
451 AAGGCCAAAG GGATTTTAAC TTGCAGGTCC AAAWYTTGCC TGGGGTCCAT
501 T (SEQ ID NO:9)

(initiation codon at position 145 shown bold and underlined)

The 3'-end of human islet iNOS has the following sequence

1 AGACGACTCA CTATAGGGCG AATTGGGCCC TCTAGATGCA TGCTCGAGCG
51 GCCGCCAGTG TGATGGATAT CTGCAGAATT CGGCTTTATC CCGGGCTCCA
101 TCCTTAAGTT CTGTGCCGGC AGCTTTAACC CCTCCTGTAG GCCCTCAGAG
151 CGCTGACATC TCCAGGCTGC TGGGCTGCAC CGCCACCCTG TCCTTCTTCG
201 CCTCGTAAGG AAATACAGCA CCAAAGATAT CTTCGTGATA GCGCTTCTGG
251 CTCTTGAGCT GAAAGAAATA GTCCTCGACC TGCTCCTCAT TCAATTTCAG
301 CTTGGCAGCC ACCAGCTGCT TCAGGGTGTG GGCCACGTCC CGGGCCATGC
351 GCACATCCCC GCAAACATAG AGGTGGCCTG GCTCCTTGTG GAGCACACSG
401 AGCACCTCGC TGGCCAGCTG CTGCCSCAGG ATGTCCTGAA CATAGACCTT
451 GG (SEQ ID NO:10)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: Bagsvaerd
(E) COUNTRY: Denmark
(F) POSTAL CODE (ZIP): 2880
(G) TELEPHONE: +45 4444 8888
(H) TELEFAX: +45 4449 3256

(ii) TITLE OF INVENTION: A DNA Sequence Encoding Nitric Oxide Synthase

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1033 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTTCCAAGCT TGCCGCCACC ATGGCTTGCC CTGGAAGTTT CTCTTCAGAG TCAAATCCTA 60

CCAAGGTGAC CTGAAAGAGG AAAAGGACAT TAACAACAAC GTGGAGAAAA CCCCAGGTGC 120

TATTCCCAGC CCAACAACAC AGGATGACCC TAAGAGTCAC AAGCATCAAA ATGGTTTCCC 180

CCAGTTCTCA CTGGGACTGC ACAGAATGTC CAGAGATCCC TGGACAAGTC TGCATGTGAC 240

TCCATCGACC CGCCCACAGC ACGTGAGGAT CAAAAACTGG GGCAATGGAG AGATTTTTCA 300

CGACACCCTT CACCACAAGG CCACCTCGGA TATCTCTTGC AAGTCCAAAT TATGCATGGG 360

GTGCATCATG AACTCCAAGA GTTTGACCAG AGGACCCAGA GACAAGCCCA CCCCAGTGAG 420

GAGCTTCTGT GCCTCAAGCC AATTGAATTC ATTAACCAGT ATTATGGCTC CTTCAAAGAG 480

GCAAAAATAG AGGAACATCT GGCCAGGCTG GAAGCCCGTA ACAAAGGAAA TAGAAACAAC 540

AGGAACCTAC CAGCTCACTC TGGATGAGCT CATCTTTGCC ACCAAGATGG CCTGGAGGAA 600

ACTGCCCCTC GCTGCATCGG CAGGATTCAG TGGTCCAACC TGCAGGTCTT CGATGCCCGG 660

AGCTGTAGCA CTGCATCAGA AATGTTCCAG CATATCTGCA GACACATACT TTACCGACTA 720

ACAGTGGCAA CATCAGGTCG GCCATTACTG TGTTCCCCCA GCGGAGCGAT GGGAAGCATG 780

ACTTCCGGAT CTGGAATTCC CAGCTCATCC GGTACGCTGG CTACCAGATG CCCGATGGCA 840

CCATCAGAGG GGATCCTGCC ACCTTGGAGT TCACCCAGTT GTGCATCGAC CTGCTGGAAG 900

CCCCGCTACG GCCGCTTCGA TGTGCTGCCT CTGGTCCTGC AGGCTCACGG TCAAGATCCA 960

GAGGTCTTTG AAATCCCTCC TGATCTTGTG CTGGAGGTGA CCATGGAGCA CCCAAAGTAC 1020

GAATGGTTCC AAA 1033

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TCCAAGCTTG CCGCCACCAT GGCTTGCCCC TGG 33

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCAAGCTTGC CGCCACCATG GCTTGCCCCT GG 32

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

AGAAGCACAA AGTCACAGA 19

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACTTCTGTCT CTCCAAACCC 20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3530 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGAAGCACAA AGTCACAGAC ATGGCTTGCC CCTGGAAGTT TCTCTTCAGA GTCAAATCCT 60
ACCAAGGTGA CCTGAAAGAG GAAAAGGACA TTAACAACAA CGTGGAGAAA ACCCCAGGTG 120
CTATTCCCAG CCCAACAACA CAGGATGACC CTAAGAGTCA CAAGCATCAA AATGGTTTCC 180
CCCAGTTCCT CACTGGGACT GCACAGAATG TTCCAGAATC CCTGGACAAG CTGCATGTGA 240
CTCCATCGAC CCGCCCACAG CACGTGAGGA TCAAAAACTG GGGCAATGGA GAGATTTTTC 300
ACGACACCCT TCACCACAAG GCCACCTCGG ATATCTCTTG CAAGTCCAAA TTATGCATGG 360
GGTCCATCAT GAACTCCAAG AGTTTGACCA GAGGACCCAG AGACAAGCCC ACCCCAGTGG 420
AGGAGCTTCT GCCTCAAGCC ATTGAATTCA TTAACCAGTA TTATGGCTCC TTCAAAGAGG 480
CAAAAATAGA GGAACATCTG GCCAGGCTGG AAGCCGTAAC AAAGGAAATA GAAACAACAG 540
GAACCTACCA GCTCACTCTG GATGAGCTCA TCTTTGCCAC CAAGATGGCC TGGAGGAACG 600
CCCCTCGCTG CATCGGCAGG ATTCAGTGGT CCAACCTGCA GGTCTTCGAT GCCCGGAGCT 660
GTAGCACTGC ATCAGAAATG TTCCAGCATA TCTGCAGACA CATACTTTAC GCCACTAACA 720
GTGGCAACAT CAGGTCGGCC ATTACTGTGT TCCCCCAGCG GAGCGATGGG AAGCATGACT 780
TCCGGATCTG GAATTCCCAG CTCATCCGGT ACGCTGGCTA CCAGATGCCC GATGGCACCA 840
TCAGAGGGGA TCCTGCCACC TTGGAGTTCA CCCAGTTGTG CATCGACCTG GGCTGGAAGC 900
CCCGCTATGG CCGCTTCGAT GTGCTGCCTC TGGTCCTGCA GGCTCACGGT CAAGATCCAG 960
AGGTCTTTGA AATCCCTCCT GATCTTGTGC TGGAGGTGAC CATGGAGCAT CCCAAGTACG 1020
AGTGGTTCCA GGAGCTCGGG CTGAAGTGGT ATGCGCTGCC TGCCGTGGCC AACATGCTCC 1080
TGGAGGTGGG TGGCCTCGAG TTCCCAGCCT GCCCCTTCAA TGGTTGGTAC ATGGGCACCG 1140
AGATTGGAGT CCGAGACCTC TGTGACACAC AGCGCTACAA CATCCTGGAG GAAGTGGGCA 1200
GGAGGATGGG CCTGGAGACC CACACACTGG CCTCCCTCTG GAAAGACCGG GCTGTCACCG 1260
AGATCAATGC AGCTGTGCTC CATAGTTTTC AGAAGCAGAA TGTGACCATC ATGGACCACC 1320
ACACAGCCTC AGAGTCCTTC ATGAAGCACA TGCAGAATGA GTACCGGGCC CGAGGAGGCT 1380
GCCCTGCAGA CTGGATTTGG CTGGTCCCTC CGGTGTCCGG GAGCATCACC CCTGTGTTCC 1440
ACCAGGAGAT GTTGAACTAC GTCCTATCTC CATTCTACTA CTACCAGATC GAGCCCTGGA 1500
AGACCCACAT CTGGCAGGAT GAGAAGCTGA GGCCCAGGAG GAGAGAGATC CGGTTCACAG 1560
TCTTGGTGAA AGCGGTGTTC TTTGCTTCTG TGCTAATGCG GAAGGTCATG GCTTCCCGCG 1620

TCAGAGCCAC AGTCCTCTTT GCTACTGAGA CAGGAAAGTC GGAAGCGCTA GCCAGGGACC 1680

TGGCTGCCTT GTTCAGCTAC GCCTTCAACA CCAAGGTTGT CTGCATGGAA CAGTATAAGG 1740

CAAACACCTT GGAAGAGGAA CAACTACTGC TGGTGGTGAC AAGCACATTT GGCAATGGAG 1800

ACTGCCCCAG CAATGGGCAG ACTCTGAAGA AATCTCTGTT CATGATGAAA GAACTCGGGC 1860

ATACCTTCAG GTATCGGGTA TTTGGCCTGG GCTCCAGCAT GTACCCTCAG TTCTGTGCCT 1920

TTGCTCATGA CATCGACCAG AAACTGTCTC ACCTGGGAGC CTCCCAGCTT GCCCCAACCG 1980

GAGAAGGGGA CGAACTCAGC GGGCAGGAGG ACGCCTTCCG CAGCTGGGCT GTGCAAACCT 2040

TCCGGGCAGC CTGTGAGACG TTCGATGTTC GAAGCAAACA TTGCATTCAG ATCCCGAAAC 2100

GCTACACTTC CAACGCAACA TGGGAGCCAG AGCAGTACAA GCTCACCCAG AGCCCAGAGC 2160

CTCTAGACCT CAACAAAGCT CTCAGCAGCA TCCACGCCAA GAACGTGTTC ACCATGAGGC 2220

TGAAATCCCT CCAGAATCTG CAGAGTGAGA AGTCCAGCCG CACCACCCTC CTTGTTCAAC 2280

TCACCTTCGA GGGCAGCCGA GGCCCCAGCT ACCTACCTGG GGAACACCTG GGGATTTTCC 2340

CAGGCAACCA GACGGCCCTG GTGCAAGGGA TCTTGGAGCG AGTTGTGGAT TGTTCTTCGC 2400

CAGACCAAAC TGTGTGCCTG GAGGTTCTAG ATGAGAGTGG CAGCTACTGG GTCAAAGACA 2460

AGAGGCTTCC CCCCTGCTCA CTCAGGCAAG CCCTCACCTA CTTCCTGGAC ATCACTACCC 2520

CTCCCACCCA GCTGCAGCTC CACAAGCTGG CCCGCTTTGC CACGGAAGAG ACGCACAGGC 2580

AGAGGTTGGA GGCCTTGTGT CAGCCCTCAG AGTACAACGA TTGGAAGTTC AGCAACAACC 2640

CCACGTTCCT GGAGGTGCTG GAAGAGTTCC CATCATTGCG TGTGCCTGCT GCCTTCCTGC 2700

TGTCGCAGCT CCCCATTCTG AAGCCCCGCT ACTACTCCAT CAGCTCCTCC CAGGACCACA 2760

CCCCCTCGGA GGTCCACCTC ACTGTGGCTG TGGTCACCTA TCGCACCCGA GATGGTCAGG 2820

GTCCCCTGCA CCATGGCGTC TGCAGCACTT GGATCAATAA CCTGAAGCCC GAAGACCCAG 2880

TGCCCTGCTT TGTGCGGAGT GTCAGTGGCT TCCAGCTCCC TGAGGACCCC TCCCAGCCCT 2940

GCATCCTCAT TGGGCCCGGT ACAGGCATTG CCCCCTTCCG AAGTTTCTGG CAGCAGCGGC 3000

TCCATGACTC TCAGCGCAGA GGGCTCAAAG GAGGCCGCAT GACCTTGGTG TTTGGGTGCA 3060

GGCACCCAGA GGAGGACCAC CTCTATCAGG AAGAAATGCA GGAGATGGTC CGCAAGGGAG 3120

TGTTGTTCCA GGTGCACACA GGCTACTCCC GGCTGCCCGG AAAACCCAAG GTCTACGTTC 3180

AAGACATCCT GCAGAAAGAG CTGGCCGACG AGGTGTTCAG CGTGCTCCAC GGGGAGCAGG 3240

GCCACCTCTA TGTTTGTGGC GATGTGCGCA TGGCTCGGGA TGTGGCTACC ACTTTGAAGA 3300

AGCTGGTGGC CGCCAAGCTG AACTTGAGTG AGGAGCAGGT TGAGGATTAC TTCTTCCAGC 3360

TCAAGAGCCA GAAACGTTAT CATGAGGATA TCTTCGGTGC GGTCTTTTCC TATGGAGTGA 3420

AAAAGGGCAA CGCTTTGGAG GAGCCCAAAG GCACAAGACT CTGACACCCA GAAGAGTTAC 3480

AGCATCTGGC CCTAAATAAA ATGACAGTGA GGGTTTGGAG AGACAGAAGT 3530

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGTTCTCAAG GCACAGGTCT C 21

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCTCCATCCT TAAGTTCT 18

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 497 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
AGTTCTCAAG GCACAGGTCT CTTCCTGGTT TGACTGTCCT TACCCCGGGG AGGCAGTGCA    60
GCCAGCTGCA ACCCACAGTG AAGAACATCT GAGCTCAAAT CCAGATAAGT GACATAAGTG   120
ACCTGCTTTG TAAAGCCATA GAGATGGCCT GTCCTTGAAA ATTTCTGTTC AAGACCAAAT   180
TCCACCAGTA TGCAATGAAT GGGGAAAAGA CATCAACAAC AATGTGGAGA AAGCCCCCTG   240
TGCCACCTCC AGTCCAGTGA CACAGGATGA CCTTCAGTAT CACAACCTCA GCAAGCAGCA   300
GAATGAGTCC CCGCAGCCCC TCGTGGAGAC GGGAAAGAAG TCTCCAGAAT CTCTGGTCAA   360
GCTGGATGCA ACCCCATTGT CCTCCCCACC GCATGTGAGG ATCAAAAACT GGGGCAGCGG   420
GATGACTTTC CAAGACACAC CTCACCATAA GGCCAAAGGG ATTTTAACTT GCAGGTCCAA   480
ATTGCCTGGG GTCCATT                                                  497
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 450 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
AGACGACTCA CTATAGGGCG AATTGGGCCC TCTAGATGCA TGCTCGAGCG GCCGCCAGTG    60
TGATGGATAT CTGCAGAATT CGGCTTTATC CCGGGCTCCA TCCTTAAGTT CTGTGCCGGC   120
AGCTTTAACC CCTCCTGTAG GCCCTCAGAG CGCTGACATC TCCAGGCTGC TGGGCTGCAC   180
CGCCACCCTG TCCTTCTTCG CCTCGTAAGG AAATACAGCA CCAAAGATAT CTTCGTGATA   240
GCGCTTCTGG CTCTTGAGCT GAAAGAAATA GTCCTCGACC TGCTCCTCAT TCAATTTCAG   300
```

CTTGGCAGCC ACCAGCTGCT TCAGGGTGTG GGCCACGTCC CGGGCCATGC GCACATCCCC 360

GCAAACATAG AGGTGGCCTG GCTCCTTGTG GAGCACACGA GCACCTCGCT GGCCAGCTGC 420

TGCCCAGGAT GTCCTGAACA TAGACCTTGG 450

CLAIMS

1. A DNA construct comprising a DNA sequence encoding a pancreatic islet cell inducible nitric oxide synthase (iNOS).

2. A DNA construct according to claim 1, wherein the DNA sequence is of mammalian origin.

3. A DNA construct according to claim 2, wherein the DNA sequence is of rodent origin.

4. A DNA construct according to claim 3, wherein the DNA sequence is of rat or mouse origin.

5. A DNA construct according to claim 2, wherein the DNA sequence is of human origin.

6. A DNA construct according to any of claims 1-5, which comprises the following partial DNA sequence

TTTCCAAGCT TGCCGCCACC ATGGCTTGCC CTGGAAGTTT CTCTTCAGAG
TCAAATCCTA CCAAGGTGAC CTGAAAGAGG AAAAGGACAT TAACAACAAC
GTGGAGAAAA CCCCAGGTGC TATTCCCAGC CCAACAACAC AGGATGACCC
TAAGAGTCAC AAGCATCAAA ATGGTTTCCC CCAGTTCTCA CTGGGACTGC
ACAGAATGTC CAGAGATCCC TGGACAAGTC TGCATGTGAC TCCATCGACC
CGCCCACAGC ACGTGAGGAT CAAAAACTGG GGCAATGGAG AGATTTTTCA
CGACACCCTT CACCACAAGG CCACCTCGGA TATCTCTTGC AAGTCCAAAT
TATGCATGGG GTGCATCATG AACTCCAAGA GTTTGACCAG AGGACCCAGA
GACAAGCCCA CCCCAGTGAG GAGCTTCTGT GCCTCAAGCC AATTGAATTC
ATTAACCAGT ATTATGGCTC CTTCAAAGAG GCAAAAATAG AGGAACATCT
GGCCAGGCTG GAAGCCCGTA ACAAAGGAAA TAGAAACAAC AGGAACCTAC
CAGCTCACTC TGGATGAGCT CATCTTTGCC ACCAAGATGG CCTGGAGGAA
actGCCCCTC GCTGCATCGG CAGGATTCAG TGGTCCAACC TGCAGGTCTT
CGATGCCCGG AGCTGTAGCA CTGCATCAGA AATGTTCCAG CATATCTGCA
GACACATACT TTACCGACTA ACAGTGGCAA CATCAGGTCG GCCATTACTG
TGTTCCCCCA GCGGAGCGAT GGGAAGCATG ACTTCCGGAT CTGGAATTCC

CAGCTCATCC GGTACGCTGG CTACCAGATG CCCGATGGCA CCATCAGAGG
GGATCCTGCC ACCTTGGAGT TCACCCAGTT GTGCATCGAC CTGCTGGAAG
CCCCGCTACG GCCGCTTCGA TGTGCTGCCT CTGGTCCTGC AGGCTCACGG
TCAAGATCCA GAGGTCTTTG AAATCCCTCC TGATCTTGTG CTGGAGGTGA
CCATGGAGCA CCCAAAGTAC GAATGGTTCC AAA (SEQ ID NO:1)

or a homologue thereof encoding a protein with iNOS activity.

7. A DNA construct according to any of claims 1-5, which comprises the DNA sequence shown in the Sequence Listing as SEQ ID NO:6, or a suitable modification thereof.

8. A DNA construct according to any of claims 1-5, which comprises either or both of the DNA sequences shown in the Sequence Listing as SEQ ID NO:9 and SEQ ID NO:10, or a suitable modification thereof.

9. A recombinant expression vector comprising a DNA construct according to any of claims 1-8.

10. A cell comprising a DNA construct according to any of claims 1-8 or a vector according to claim 9.

11. A cell according to claim 10, which is a eukaryotic cell.

12. A method of producing a pancreatic islet cell inducible nitric oxide synthase, the method comprising culturing a cell according to claim 10 or 11 under conditions permitting the production of the iNOS and recovering the resulting iNOS from the culture.

13. A method of isolating inhibitors of pancreatic islet cell inducible nitric oxide synthase, the method comprising incubating iNOS encoded by the DNA sequence according to any of claims 1-8 with a substance suspected of being an iNOS inhibitor in the presence of a suitable substrate for iNOS, and

detecting any effect of said substance on the interaction of the iNOS with said substrate.

14. A method according to claim 13, wherein the substrate is L-arginine.

15. A method according to claim 14, wherein the formation of citrulline resulting from said incubation is determined, decreased citrulline formation indicating that said substance is an iNOS inhibitor.

16. A method according to claim 13, wherein the formation of nitric oxide (NO) resulting from said incubation is determined, decreased NO formation indicating that said substance is an iNOS inhibitor.

17. A method according to claim 16, wherein an indicator of the presence of nitric oxide is added.

18. A method according to claim 17, wherein the indicator is guanylate cyclase, and wherein the amount of cyclic GMP formed from GTP is indicative of the presence of NO.

19. A test kit for isolating inhibitors of pancreatic islet cell inducible nitric oxide synthase, the kit comprising in separate containers

(a) iNOS encoded by the DNA sequence according to any of claims 1-8, and

(b) a suitable substrate therefor.

20. A test kit according to claim 19, wherein the substrate is L-arginine.

21. A test kit according to claim 19, which further comprises, in a separate container, an indicator of the presence of nitric oxide.

22. A test kit according to claim 21, which comprises, in separate containers, GTP and guanylate cyclase.

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RATBCL- ------TGGCTTGCCCT-GGAAGTTTCTCTTCAGAGTCAAATCCTACCAA -64
              ::::::::::  ::::::::::::::: ::::::::::::::::
MUSMAC- CAGACATGGCTTGCCCCTGGAAGTTTCTCTTCAAAGTCAAATCCTACCAA -300
           MetAlaCysProTrpLys......

RATBCL- GGTGACCTGAAAGAGGAAAAGGACATTAACAACAACGTGGAGAAAACCCC -114
         :::::::::::::::::::::::::::::::::::::: ::::::::::
MUSMAC- AGTGACCTGAAAGAGGAAAAGGACATTAACAACAACGTGAAGAAAACCCC -350

RATBCL- AGGTGCTATTCCCAGCCCAACAACACAGGATGACCCTAAGAGTCACAAGC -164
          ::::: ::: ::::::::::: ::: ::::::::::::::::::
MUSMAC- TTGTGCTGTTCTCAGCCCAACAATACAAGATGACCCTAAGAGTCAC---- -396

RATBCL- ATCAAAATGGTTTCCCCCAGTTCT-CACTGGGACTGCACAGAATGTC-CA -212
          :::::::: : ::: ::: ::  ::::::::: :::::::::::  ::
MUSMAC- --CAAAATGGCTCCCCGCAGCTCCTCACTGGGACAGCACAGAATGTTCCA -444

RATBCL- GAGATCCCTGGACAAGTCTGCATGTGACTCCATCGACCCGCCCACAGCAC -262
        :: ::::::::::::: :::::::::::   ::::::::: :::::: :
MUSMAC- GA-ATCCCTGGACAAG-CTGCATGTGAC---ATCGACCCGTCCACAGTAT -489

RATBCL- GTGAGGATCAAAAACTGGGGCAATGGAGAGATTTTTCACGACACCCTTCA -312
        :::::::::::::::::::::: :::::::::::: :: ::::: :::::
MUSMAC- GTGAGGATCAAAAACTGGGGCAGTGGAGAGATTTTGCATGACACTCTTCA -539

RATBCL- CCACAAGGCCACCTCGGATATCTCTTGCAAGTCCAAATTATGCATGGGGT -362
        :::::::::::: :::::: :: ::::::::::::: :  ::: ::::::
MUSMAC- CCACAAGGCCACATCGGATTTCACTTGCAAGTCCAAGTCTTGCTTGGGGT -589

RATBCL- GCATCATGAACTCCAAGAGTTTGACCAGAGGACCCAGAGACAAGCCCACC -412
         :::::::::: :::::::::::::::::::::::::::::::::: :::
MUSMAC- CCATCATGAACCCCAAGAGTTTGACCAGAGGACCCAGAGACAAGCCTACC -639

RATBCL- CCAGTGA-GGAGCTTCTGTGCCTCAAGCCAATTGAATTCATTAACCAGTA -461
        ::  ::  :::::: :::  ::::: :::: :::: ::::: ::::::::
MUSMAC- CCTCTGGAGGAGCTCCTG--CCTCATGCCA-TTGAGTTCATCAACCAGTA -686

RATBCL- TTATGGCTCCTTCAAAGAGGCAAAAATAGAGGAACATCTGGCCAGGCTGG -511
        :::::::::::: :::::::::::::::::::::::::::::::::::::
MUSMAC- TTATGGCTCCTTTAAAGAGGCAAAAATAGAGGAACATCTGGCCAGGCTGG -736

RATBCL- AAGCCCGTAACAAAGGAAATAGAAACAACAGGAACCTACCAGCTCACTCT -561
        ::::  ::::::::::::::::::::::::::::::::::::::::::::
MUSMAC- AAGCT-GTAACAAAGGAAATAGAAACAACAGGAACCTACCAGCTCACTCT -785

RATBCL- GGATGAGCTCATCTTTGCCACCAAGATGGCCTGGAGGAAACTGCCCCTCG -611
        :::::::::::::::::::::::::::::::::::::::  :::::::::
MUSMAC- GGATGAGCTCATCTTTGCCACCAAGATGGCCTGGAGGAA--TGCCCCTCG -833

RATBCL- CTGCATCGGCAGGATTCAGTGGTCCAACCTGCAGGTCTTCGATGCCCGGA -661
        ::::::::::::::: ::::::::::::::::::::::: :: :: ::::
MUSMAC- CTGCATCGGCAGGATCCAGTGGTCCAACCTGCAGGTCTTTGACGCTCGGA -883
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Fig. 1a

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RATBCL- GCTGTAGCACTGCATCAGAAATGTTCCAGCATATCTGCAGACACATACTT -711
         ::::::::: :::   :::::::: ::::: ::::::::::::::::::
MUSMAC- ACTGTAGCACAGCACAGGAAATGTTTCAGCACATCTGCAGACACATACTT -933

RATBCL- TACC-GACTAACAGTGGCAACATCAGGTCGGCCATTACTGTGTTCCCCCA -760
        ::    :: ::::  ::::::::::::::::::::: ::::::::::::::
MUSMAC- TATGCCACCAACAATGGCAACATCAGGTCGGCCATCACTGTGTTCCCCCA -983

RATBCL- GCGGAGCGATGGGAAGCATGACTTCCGGATCTGGAATTCCCAGCTCATCC -810
        :::::: :: :: :: ::::::::: :: :::::::::: ::::::::::
MUSMAC- GCGGAGTGACGGCAAACATGACTTCAGGCTCTGGAATTCACAGCTCATCC -1033

RATBCL- GGTACGCTGGCTACCAGATGCCCGATGGCACCATCAGAGGGGATCCTGCC -860
        :::::::::::::::::::::::::::::::::::::::::::: :::::
MUSMAC- GGTACGCTGGCTACCAGATGCCCGATGGCACCATCAGAGGGGATGCTGCC -1083

RATBCL- ACCTTGGAGTTCACCCAGTTGTGCATCGACCTG--CTGGAAGCCCCGCTA -908
        ::::::::::::::::::::::::::::::::   :::::::::::::::
MUSMAC- ACCTTGGAGTTCACCCAGTTGTGCATCGACCTAGGCTGGAAGCCCCGCTA -1133

RATBCL- CGGCCGCTTCGATGTGCTGCCTCTGGTCCTGCAGGCTCACGGTCAAGATC -958
         :::::::: :::::::::::::::::: :::: ::: : ::::::::::
MUSMAC- TGGCCGCTTTGATGTGCTGCCTCTGGTCTTGCAAGCTGATGGTCAAGATC -1183

RATBCL- CAGAGGTCTTTGAAATCCCTCCTGATCTTGTGCTGGAGGTGACCATGGAG -1008
        :::::::::::::::::::::::::::::::: :::::::::::::::::
MUSMAC- CAGAGGTCTTTGAAATCCCTCCTGATCTTGTGTTGGAGGTGACCATGGAG -1233

RATBCL- CACCCAAAGTACGAATGGTTCCAAA                          -1033
        :: :: :::::::: ::::::::
MUSMAC- CATCCCAAGTACGAGTGGTTCCAGGAGCTCGGGTTGAAGTGGTATGCACT -1283
```

Fig. 1b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00146

A. CLASSIFICATION OF SUBJECT MATTER

IPC : C12N 9/02, C12N 15/53
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)

IPC : C12N

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

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, CA, GENBANK

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Services, file 154, Dialog accession no. 08193824, Corbett JA et al: "Does nitric oxide mediate autoimmune destruction of beta-cells? Possible therapeutic interventions in IDDM", & Diabetes (UNITED STATES) Aug 1992, 41 (8) p897-903 --	1-22
X	Dialog Information Services, file 154, Medline, Dialog accession no. 08072618, Medline accession no. 92210618, Lyons CR et al: "Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line", & J Biol Chem (UNITED STATES) Mar 25 1992, 267 (9) p6370-4	1-12
Y	 --	13-22

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"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
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1994	21 -07- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Jack Hedlund Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00146

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 154, Medline, Dialog accession no. 08481721, Medline accession no. 93191721, Nunokawa Y et al: "Cloning of inducible nitric oxide synthase in rat vascular smooth muscle cells", & Biochem Biophys Res Commun (UNITED STATES) Feb 26 1993, 191 (1) p 89-94	1-12
Y		13-22
	--	
A	SCIENCE, Volume 255, 1992, Harald H. H. W. Schmidt et al, "Insulin Secretion from Pancreatic B Cells Caused by L-Arginine-Derived Nitrogen Oxides" page 721 - page 723	1-12
Y		13-22
	--	
Y	PROC. NATL. ACAD. SCI., Volume 86, July 1989, Richard G. Knowles et al, "Formation of nitric oxide from L-arginine in the central nervous system: A transduction mechanism for stimulation of the soluble guanylate cyclase" page 5159 - page 5162	13-22
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