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(54) **ASSAY FOR AND USES OF PEPTIDE HORMONE RECEPTOR AGONISTS**

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(57) **ABSTRACT**

The invention features a method for determining whether a candidate compound is a non-peptide agonist of a peptide hormone receptor. In this method, a candidate compound is exposed to a form of the peptide hormone receptor, or to a protein that interacts with a peptide hormone receptor, which has an enhanced ability to amplify the intrinsic activity of a non-peptide agonist. The second messenger signaling activity of the enhanced receptor is measured in the presence of the candidate compound, and compared to the second messenger signaling activity of the wildtype receptor measured in the absence of the candidate compound. A change in second messenger signaling activity indicates that the candidate compound is an agonist. An increase in second messenger signaling activity indicates that the compound is either a full or partial positive agonist; a decrease in second messenger signaling activity indicates that the compound is an inverse (also termed a 'negative') agonist. The invention further embraces a method of using a peptide hormone receptor agonist for the treatment or prevention of a physiological disease, as well as particular enhanced receptors and the nucleic acid sequences which code for them.

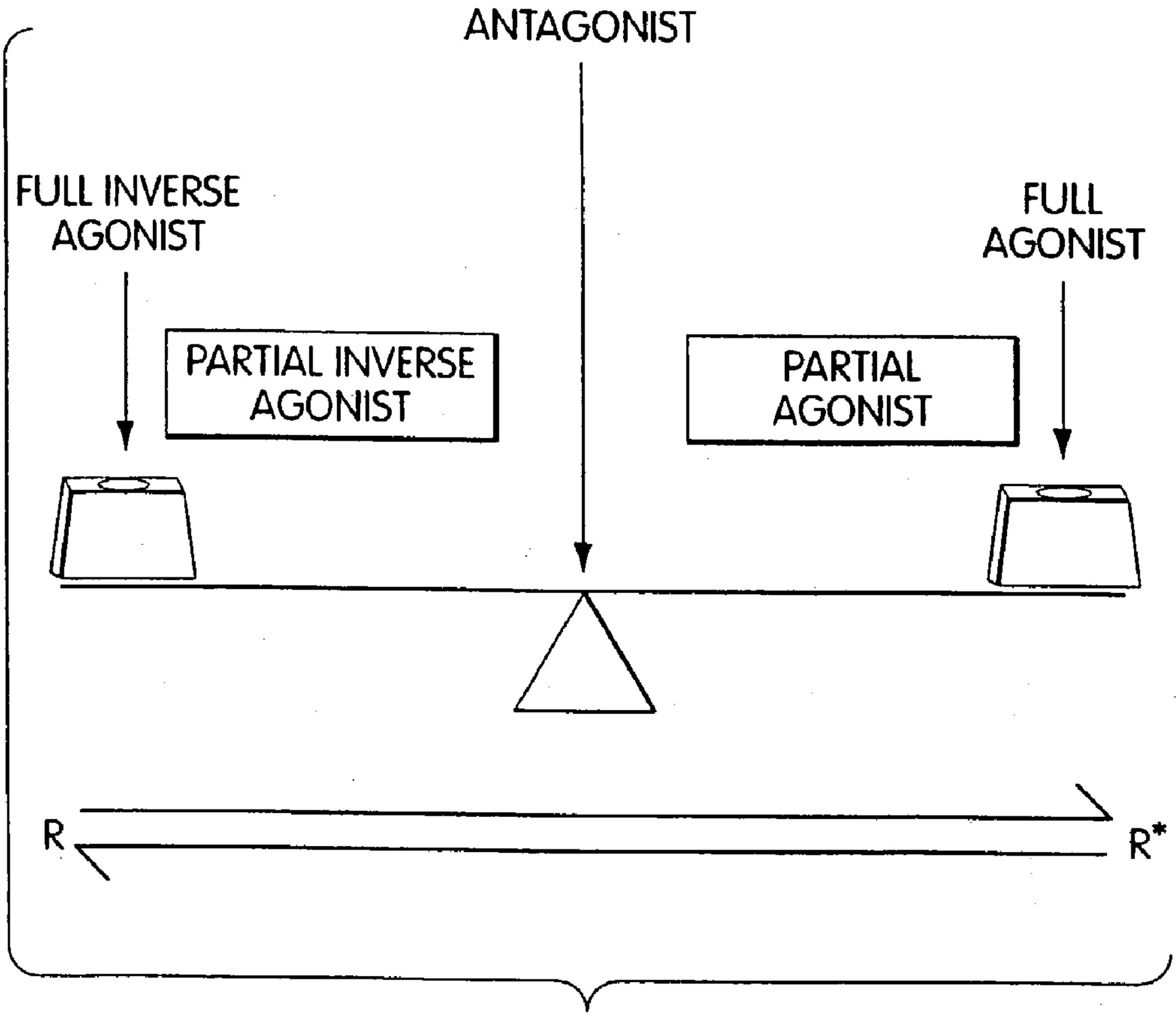

ANTAGONIST
FULL INVERSE AGONIST
PARTIAL INVERSE AGONIST
FULL AGONIST
PARTIAL AGONIST
R
R*

Fig. 1

```
                    1                                                  50
Mastomys CCK-B      .......... .........ME LLXLNSSVQG PGPGSGSSLC HPGVSLLNSS
Rat CCK-B           .......... .........ME LLKLNRSVQG PGPGSGSSLC RPGVSLLNSS
Human CCK-B         .......... .........ME LLKLNRSVQG TGPGPGASLC RPGAPLLNSS
Canine CCK-B        .......... .........ME LLKLNRSAQG SGAGPGASLC RAGGALLNSS
Human CCK-A         .......... .....MDVVD SLLVNGSNIT PPCELGLEN. ..........
Rat CCK-A           MSHSPARQHL VESSRMDVVD SLLMNGSNIT PPCELGLEN. ..........
Xenopus CCK-XL      ......MESL RSLSNISALH ELLCRYSNLS GTLTWNLSST NGTHNLTTAN

                    51                                                 100
Mastomys CCK-B      SAGNLSCEPP RI..RGTGTR ELELAIRITL YAVIFLMSIG GNMLIIVVLG
Rat CCK-B           SAGNLSCDPP RI..RGTGTR ELEMAIRITL YAVIFLMSVG GNVLIIVVLG
Human CCK-B         SVGNLSCEPP RI..RGAGTR ELELAIRITL YAVIFLMSVG GNMLIIVVLG
Canine CCK-B        GAGNLSCEPP RL..RGAGTR ELELAIRVTL YAVIFLMSVG GNVLIIVVLG
Human CCK-A         ......ETLF CLDQPRPS.K EWQPAVQILL YSLIFLLSVL GNTLVITVLI
Rat CCK-A           ......ETLF CLDQPQPS.K EWQSALQILL YSIIFLLSVL GNTLVITVLI
Xenopus CCK-XL      WPPWNLNCTP ILDRKKPSPS DLNLWVRIVM YSVIFLLSVF GNTLIIIVLV

                    101                                                150
Mastomys CCK-B      LSRRLRTVTN AFLLSLAVSD LLLAVACMPF TLLPNLMGTF IFGTVICKAV
Rat CCK-B           LSRRLRTVTN AFLLSLAVSD LLLAVACMPF TLLPNLMGTF IFGTVICKAI
Human CCK-B         LSRRLRTVTN AFLLSLAVSD LLLAVACMPF TLLPNLMGTF IFGTVICKAV
Canine CCK-B        LSRRLRTVTN AFLLSLAVSD LLLAVACMPF TLLPNLMGTF IFGTVVCKAV
Human CCK-A         RNKRMRTVTN IFLLSLAVSD LMLCLFCMPF NLIPNLLKDF IFGSAVCKTT
Rat CCK-A           RNKRMRTVTN IFLLSLAVSD LMLCLFCMPF NLIPNLLKDF IFGSAVCKTT
Xenopus CCK-XL      MNKRLRTITN SFLLSLALSD LMVAVLCMPF TLIPNLMENF IFGEVICRAA

                    151                 (A)                            200
Mastomys CCK-B      SYLMGVSVSV STLNLVAIAL ERYSAICRPL QARVWQTRSH AARVILATWL
Rat CCK-B           SYLMGVSVSV STLNLVAIAL ERYSAICRPL QARVWQTRSH AARVILATWL
Human CCK-B         SYLMGVSVSV STLSLVAIAL ERYSAICRPL QARVWQTRSH AARVIVATWL
Canine CCK-B        SYLMGVSVSV STLSLVAIAL ERYSAICRPL QARVWQTRSH AARVIIATWM
Human CCK-A         TYFMGTSVSV STFNLVAISL ERYGAICKPL QSRVWQTKSH ALKVIAATWC
Rat CCK-A           TYFMGTSVSV STFNLVAISL ERYGAICRPL QSRVWQTKSH ALKVIAATWC
Xenopus CCK-XL      AYFMGLSVSV STFNLVAISI ERYSAICNPL KSRVWQTRSH AYRVIAATWV

                    201                                                250
Mastomys CCK-B      LSGLLMVPYP VYTVVQP.V. ..GPRVLQCM HRWPSARVRQ TWSVLLLMLL
Rat CCK-B           LSGLLMVPYP VYTMVQP.V. ..GPRVLQCM HRWPSARVQQ TWSVLLLLLL
Human CCK-B         LSGLLMVPYP VYTVVQP.V. ..GPRVLQCV HRWPSARVRQ TWSVLLLLLL
Canine CCK-B        LSGLLMVPYP VYTAVQPAG. ..GARALQCV HRWPSARVRQ TWSVLLLLLL
Human CCK-A         LSFTIMTPYP IYSNLVPFTK NNNQTANMCR FLLPNDVMQQ SWHTFLLLLL
Rat CCK-A           LSFTIMTPYP IYSNLVPFTK NNNQTANMCR FLLPSDAMQQ SWQTFLLLIL
Xenopus CCK-XL      LSSIIMIPYL VYNKTVTFPM KDRRVGHQCR LVWPSKQVQQ AWYVLLLTIL

                    251                                                300
Mastomys CCK-B      FFIPGVVMAV AYGLISRELY LGLRFDGDND SDTQSRVRNQ GGLPGG..TA
Rat CCK-B           FFIPGVVIAV AYGLISRELY LGLHFDGEND SETQSRARNQ GGLPGG..AA
Human CCK-B         FFIPGVVMAV AYGLISRELY LGLRFDGDSD SDSQSRVRNQ GGL.......
Canine CCK-B        FFVPGVVMAV AYGLISRELY LGLRFDEDSD SE..SRVRSQ GGLRGG..AG
Human CCK-A         FLIPGIVMMV AYGLISLELY QGIKFEASQK KSAKER.... ..........
Rat CCK-A           FLLPGIVMVV AYGLISLELY QGIKFDASQK KSAKEK.... ..........
Xenopus CCK-XL      FFIPGVVMIV AYGLISRELY RGIQFEMDLN KEAKAH.... ..........
```

Fig. 2A

```
                    301                                                  350
Mastomys CCK-B      PGPVHQNGGC RHVT.VAGED NDGCYVQLPR SR..LEMTTL TTPTPGPGLA
Rat CCK-B           PGPVHQNGGC RPVTSVAGED SDGCCVQLPR SR..LEMTTL TTPTPGPVPG
Human CCK-B         PGAVHQNGRC RPETGAVGED SDGCYVQLPR SRPALELTAL TA..PGPGSG
Canine CCK-B        PGPAPPNGSC RPEGGLAGED GDGCYVQLPR SRQTLELSAL TAPTPGPGGG
Human CCK-A         .....KPSTT SSGKY...ED SDGCYLQKTR PPRKLELRQL STGS.SSRAN
Rat CCK-A           .....KPSTG SSTRY...ED SDGCYLQKSR PPRKLELQQL SSGSGGSRLN
Xenopus CCK-XL      .....KSGVS TPTTIPSGDE GDGCYIQVTK RRNTMEMSTL .TPSVCTKMD

                    351          (B)      (C)                        400
Mastomys CCK-B      ...S.ANQAK LLAKKRVVRM LLVIVLLFFL CWLPIYSANT WCAFDGPGAH
Rat CCK-B           ...PRPNQAK LLAKKRVVRM LLVIVLLFFL CWLPVYSVNT WRAFDGPGAQ
Human CCK-B         ...SRPTQAK LLAKKRVVRM LLVIVVLFFL CWLPVYSANT WRAFDGPGAH
Canine CCK-B        ...PRPYQAK LLAKKRVVRM LLVIVVLFFL CWLPLYSANT WRAFDSSGAH
Human CCK-A         RIRSNSSAAN LMAKKRVIRM LIVIVVLFFL CWKPIFSANA WRAYDTASAE
Rat CCK-A           RIRSSSSAAN LIAKKRVIRM LIVIVVLFFL CWMPIFSANA WRAYDTVSAE
Xenopus CCK-XL      RARINNSEAK LMAKKRVIRM LIVIVAMFFI CWMPIFVANT WKAFDELSAF

                    401                                                  450
Mastomys CCK-B      RALSGAPISF IHLLSYASAC VNPLVYCFMH RRFRQACLDT CARCCPRPPR
Rat CCK-B           RALSGAPISF IHLLSYVSAC VNPLVYCFMH RRFRQACLDT CARCCPRPPR
Human CCK-B         RALSGAPISF IHLLSYASAC VNPLVYCFMH RRFRQACLET CARCCPRPPR
Canine CCK-B        RALSGAPISF IHLLSYASAC VNPLVYCFMH RRFRQACLET CARCCPRPPR
Human CCK-A         RRLSGTPISF ILLLSYTSSC VNPIIYCFMN KRFRLGFMAT FPCCPNPGPP
Rat CCK-A           KHLSGTPISF ILLLSYTSSC VEPIIYCFMN KRFRLGFMAT FPCCPNPGPP
Xenopus CCK-XL      NTLTGAPISF IHLLSYTSAC VNPLIYCFMN KRFRKAFLGT FSSCIKP...

                    451                                  485
Mastomys CCK-B      ARFRPLPDED PPTPSIASLS RLSYTTISTL GPG..
Rat CCK-B           ARPQPLPDED PPTPSIASLS RLSYTTISTL GPG*.
Human CCK-B         ARPRALPDED PPTPSIASLS RLSYTTISTL GPG*.
Canine CCK-B        ARPRPLPDED PPTPSIASLS RLSYTTISTL GPG*.
Human CCK-A         GARGEVGEEE EGGTTGASLS RFSYSHMSAS VPPQ*
Rat CCK-A           GVRGEVGEEE DGRTIRALLS RYSYSHMSTS APPP*
Xenopus CCK-XL      .CRNFRDTDE DIAATGASLS KFSYTTVSSL GPA*.
```

Fig. 2B

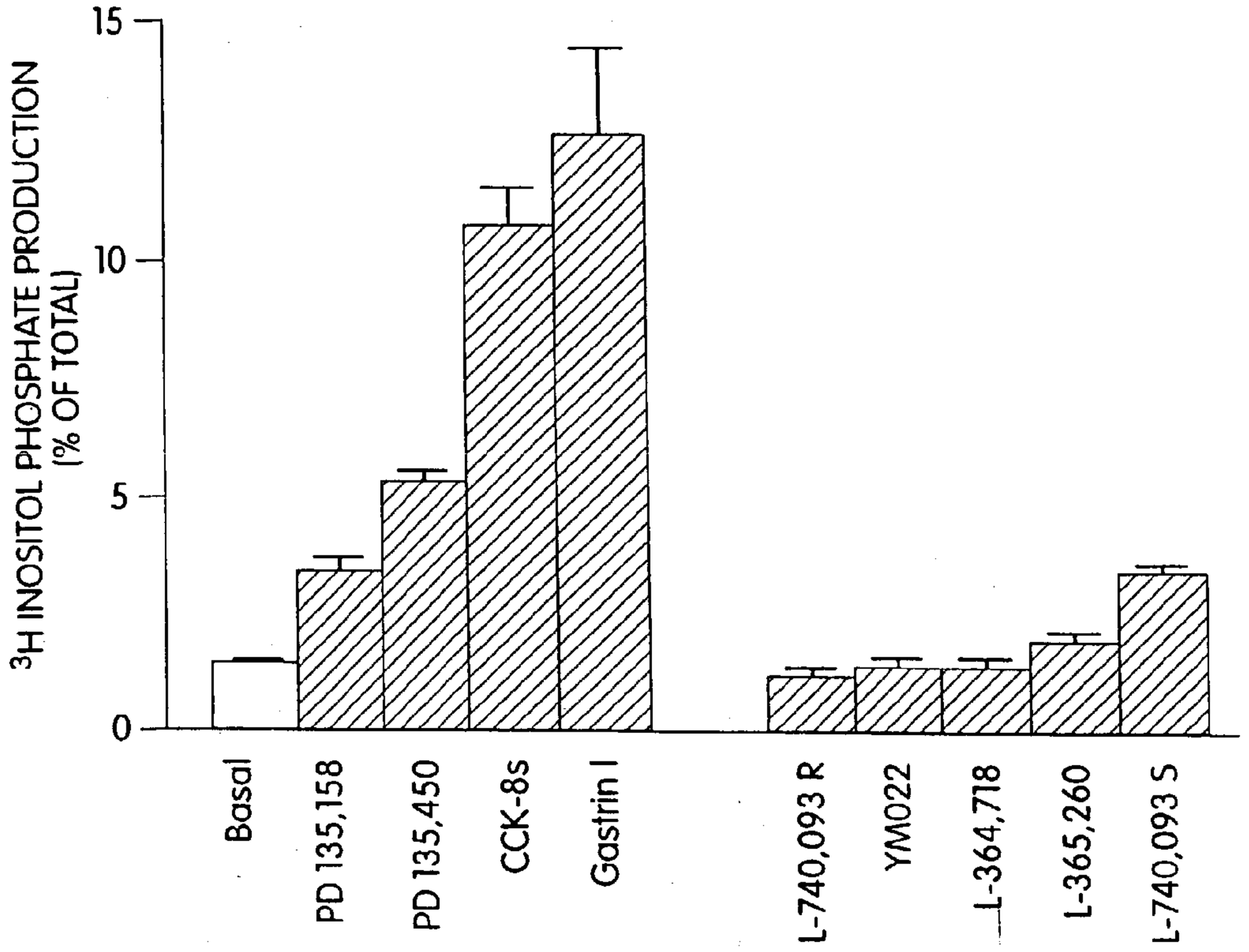

15
10
5
0
^{3}H INOSITOL PHOSPHATE PRODUCTION
(% OF TOTAL)
Basal
PD 135,158
PD 135,450
CCK-8s
Gastrin I
L-740,093 R
YM022
L-364,718
L-365,260
L-740,093 S

Fig. 3A

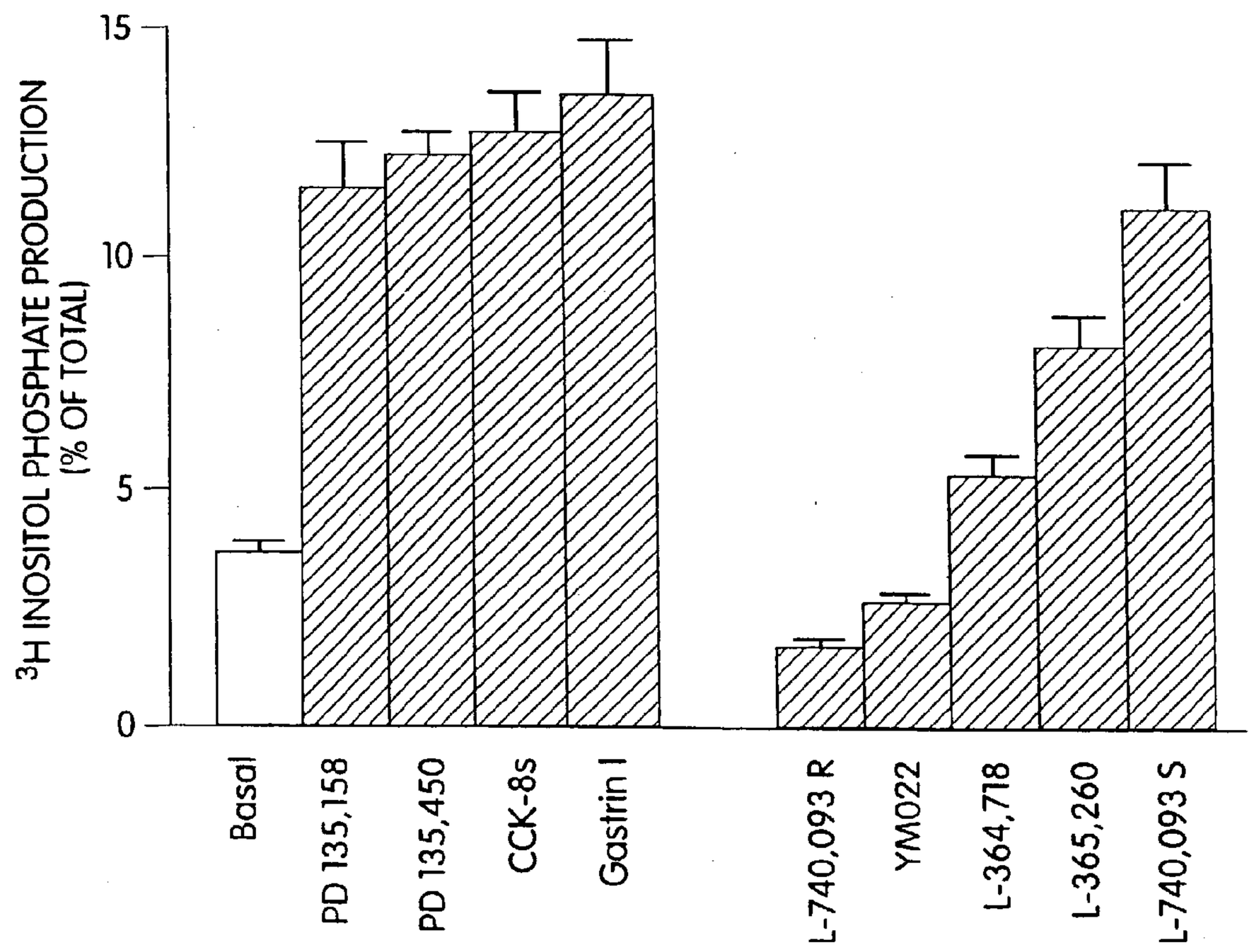

15
10
5
0
^{3}H INOSITOL PHOSPHATE PRODUCTION
(% OF TOTAL)
Basal
PD 135,158
PD 135,450
CCK-8s
Gastrin I
L-740,093 R
YM022
L-364,718
L-365,260
L-740,093 S

Fig. 3B

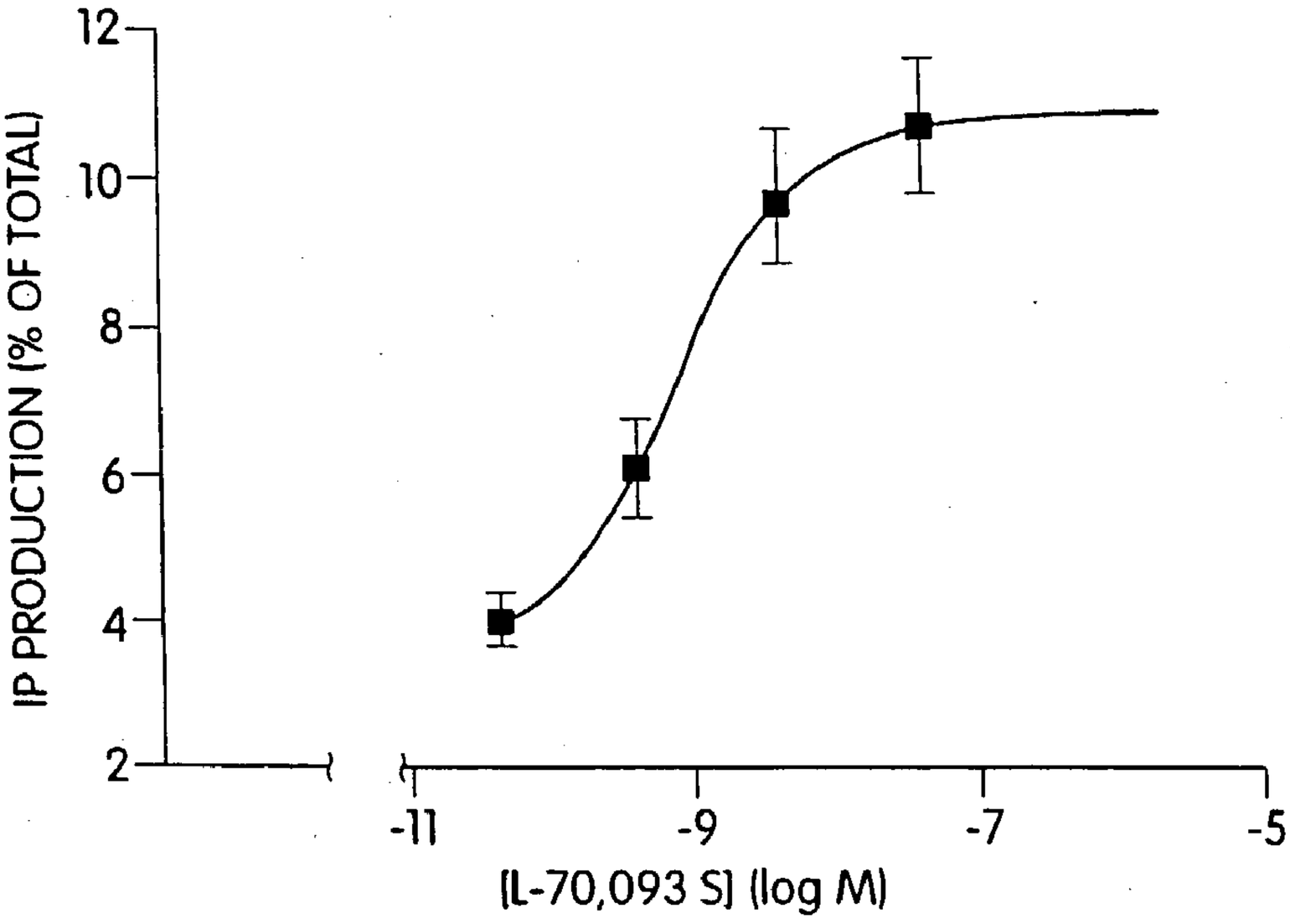

IP PRODUCTION (% OF TOTAL)
12
10
8
6
4
2
-11
-9
-7
-5
[L-70,093 S] (log M)

Fig. 4A

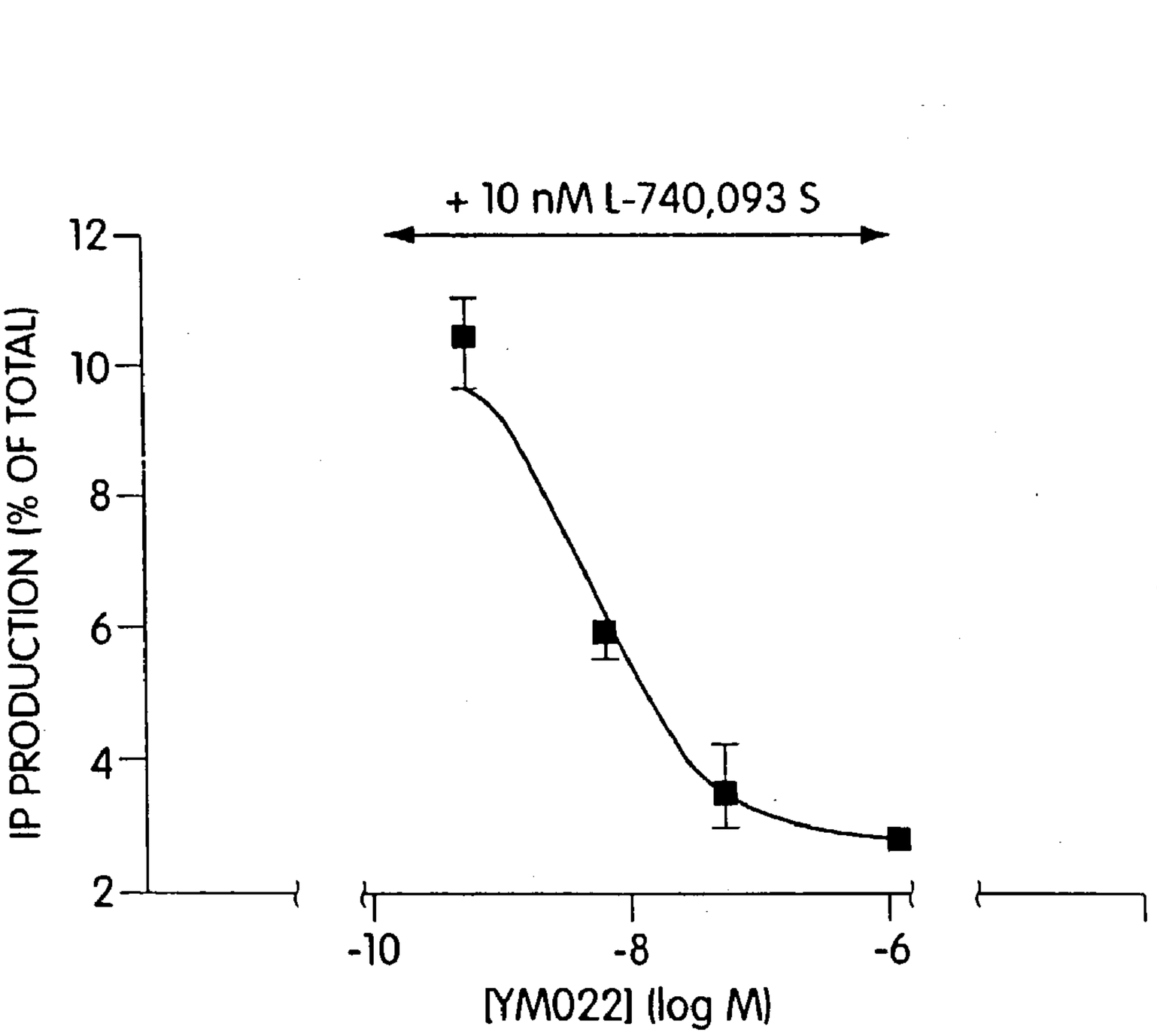

+ 10 nM L-740,093 S
IP PRODUCTION (% OF TOTAL)
12
10
8
6
4
2
-10
-8
-6
[YM022] (log M)

Fig. 4B

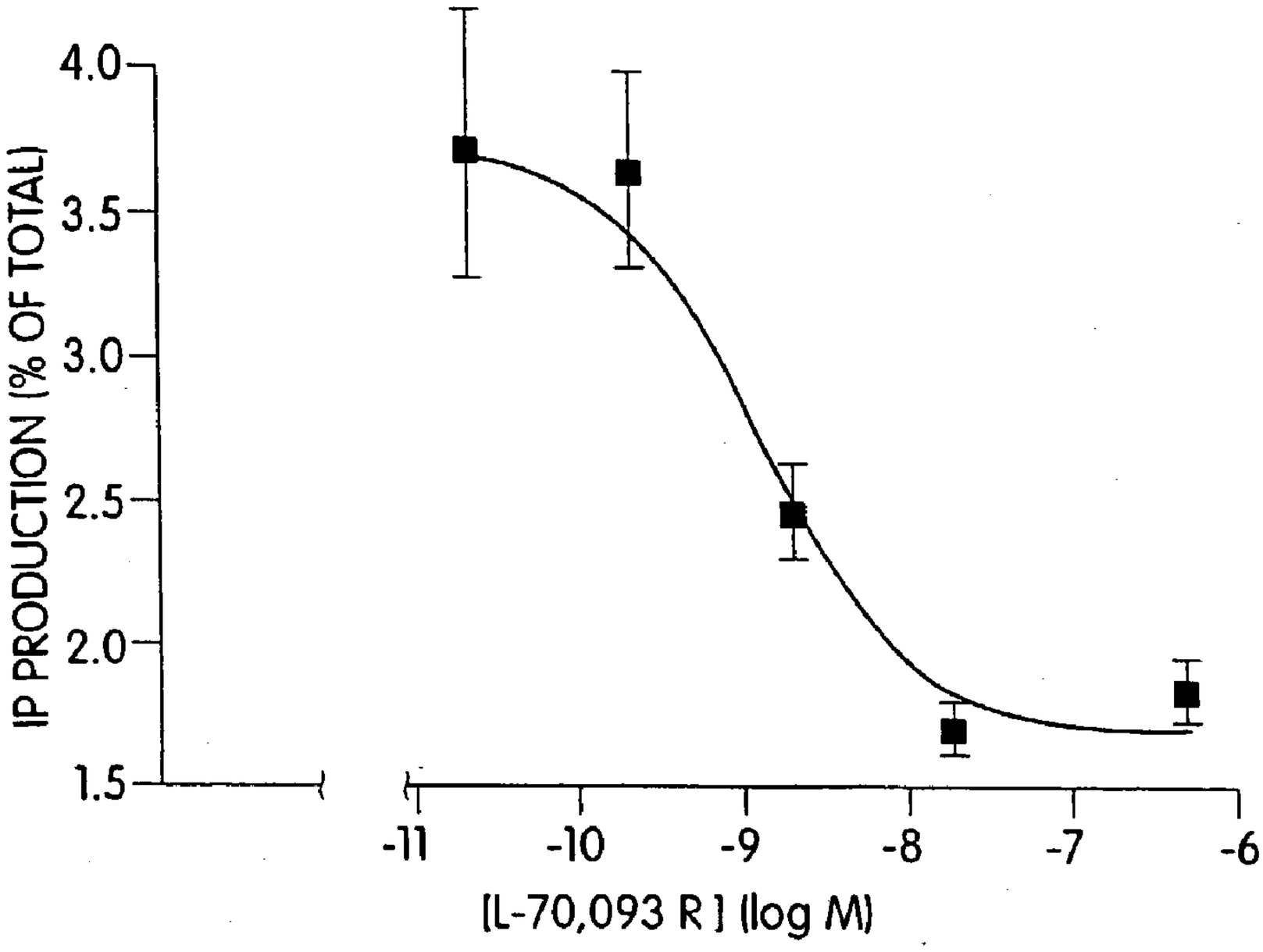

IP PRODUCTION (% OF TOTAL)
4.0
3.5
3.0
2.5
2.0
1.5
-11
-10
-9
-8
-7
-6
[L-70,093 R] (log M)

Fig. 5A

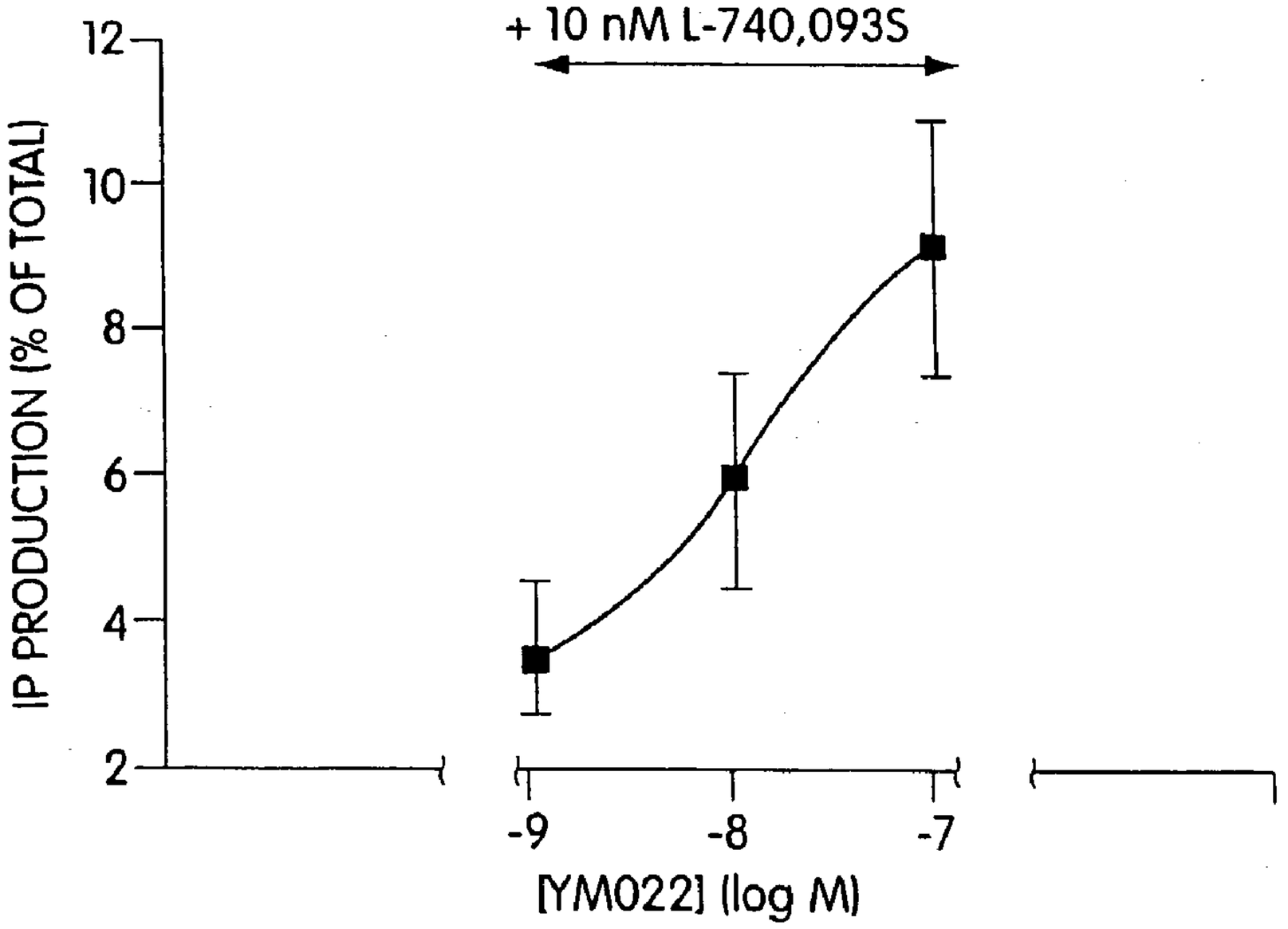

+ 10 nM L-740,093S
IP PRODUCTION (% OF TOTAL)
12
10
8
6
4
2
-9
-8
-7
[YM022] (log M)

Fig. 5B

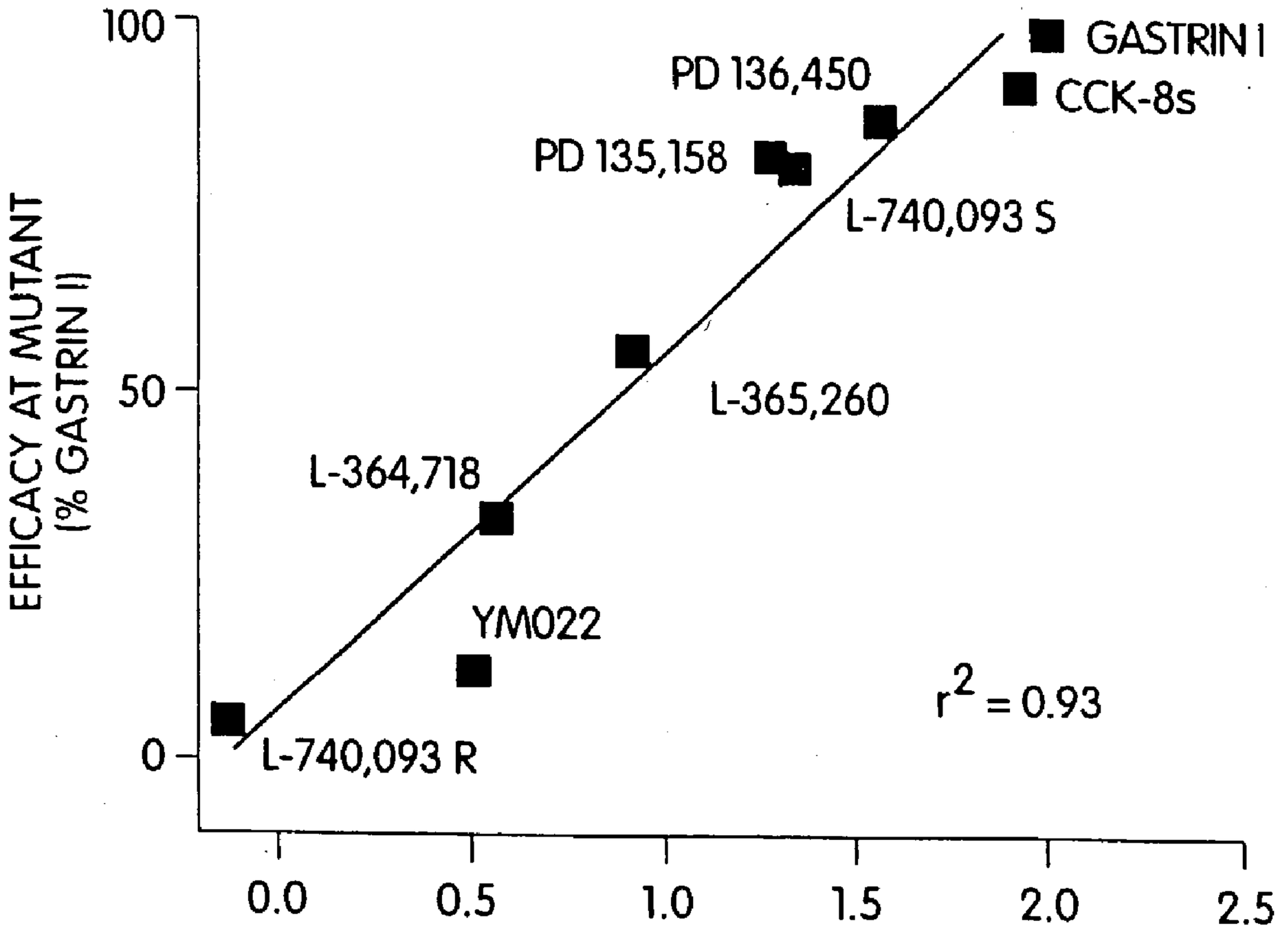

100
50
0
EFFICACY AT MUTANT
(% GASTRIN II)
GASTRIN I
CCK-8s
PD 136,450
PD 135,158
L-740,093 S
L-365,260
L-364,718
YM022
L-740,093 R
$r^2 = 0.93$
0.0
0.5
1.0
1.5
2.0
2.5
LOG EFFICACY AT WILD TYPE
(% GASTRIN I)

Fig. 6

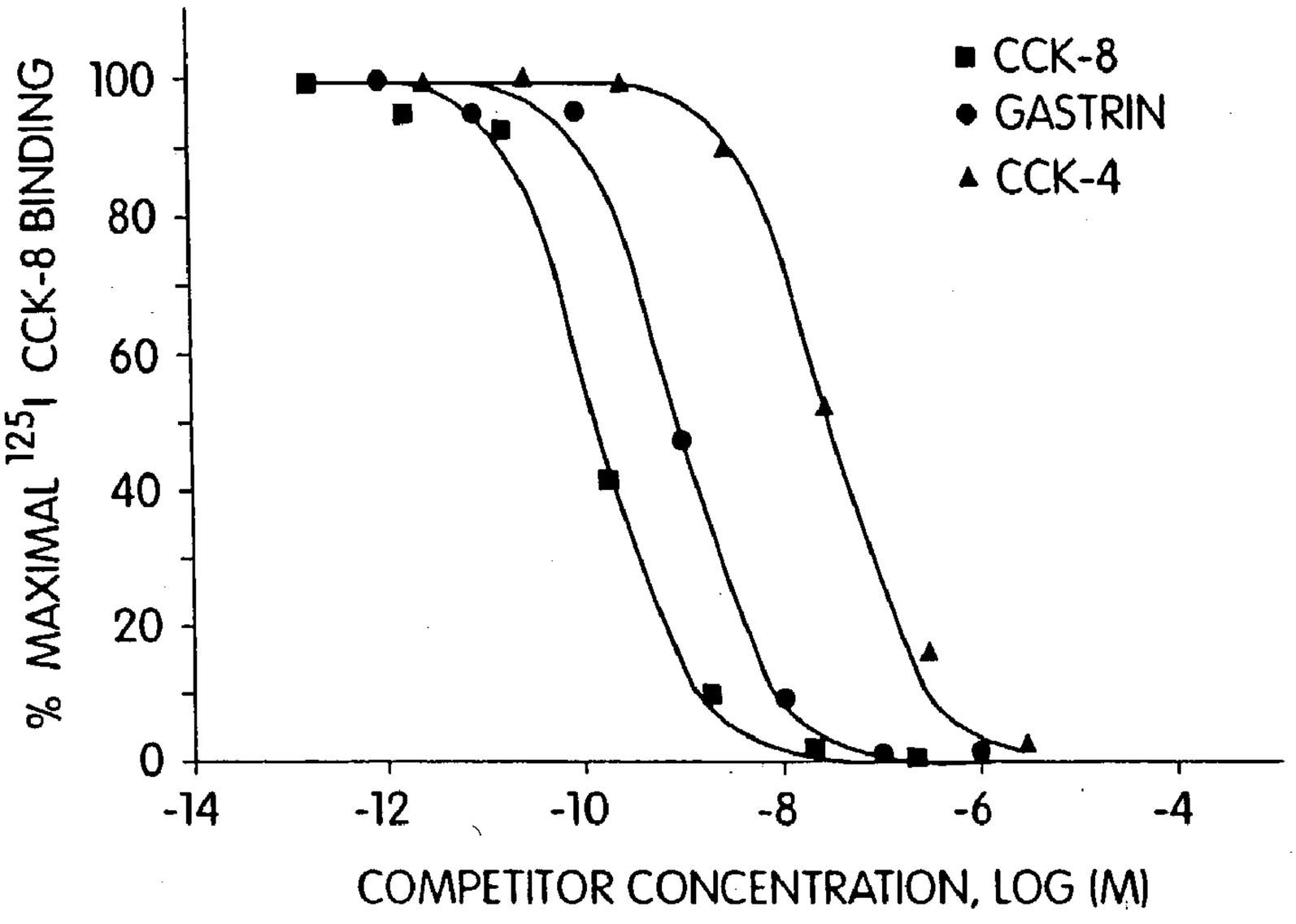

% MAXIMAL ^{125}I CCK-8 BINDING
100
80
60
40
20
0
-14
-12
-10
-8
-6
-4
COMPETITOR CONCENTRATION, LOG (M)
CCK-8
GASTRIN
CCK-4

Fig. 7A

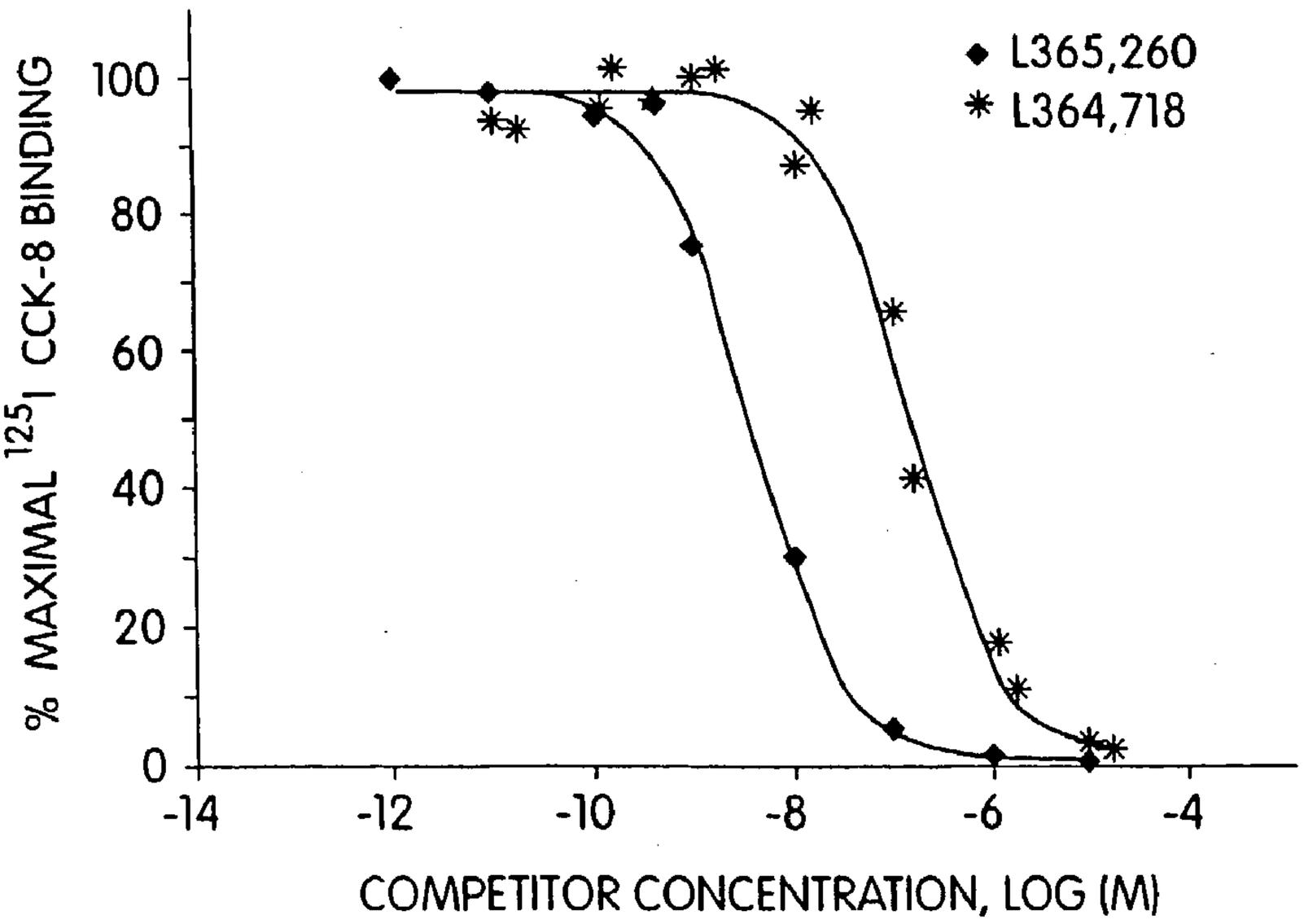

% MAXIMAL ^{125}I CCK-8 BINDING
100
80
60
40
20
0
-14
-12
-10
-8
-6
-4
COMPETITOR CONCENTRATION, LOG (M)
L365,260
L364,718

Fig. 7B

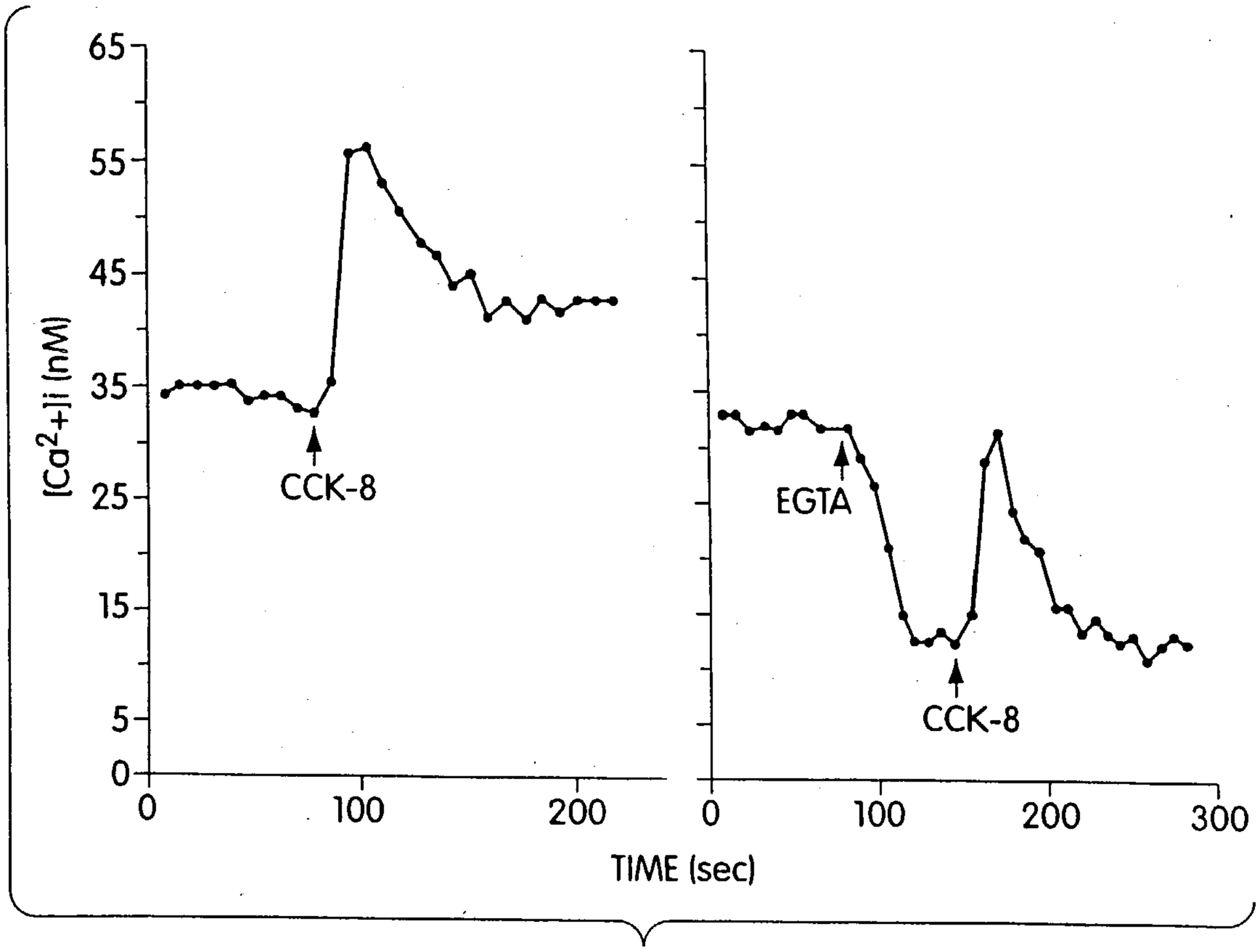

65
55
45
35
25
15
5
0
[Ca2+]i (nM)
CCK-8
EGTA
CCK-8
0
100
200
0
100
200
300
TIME (sec)

Fig. 8A

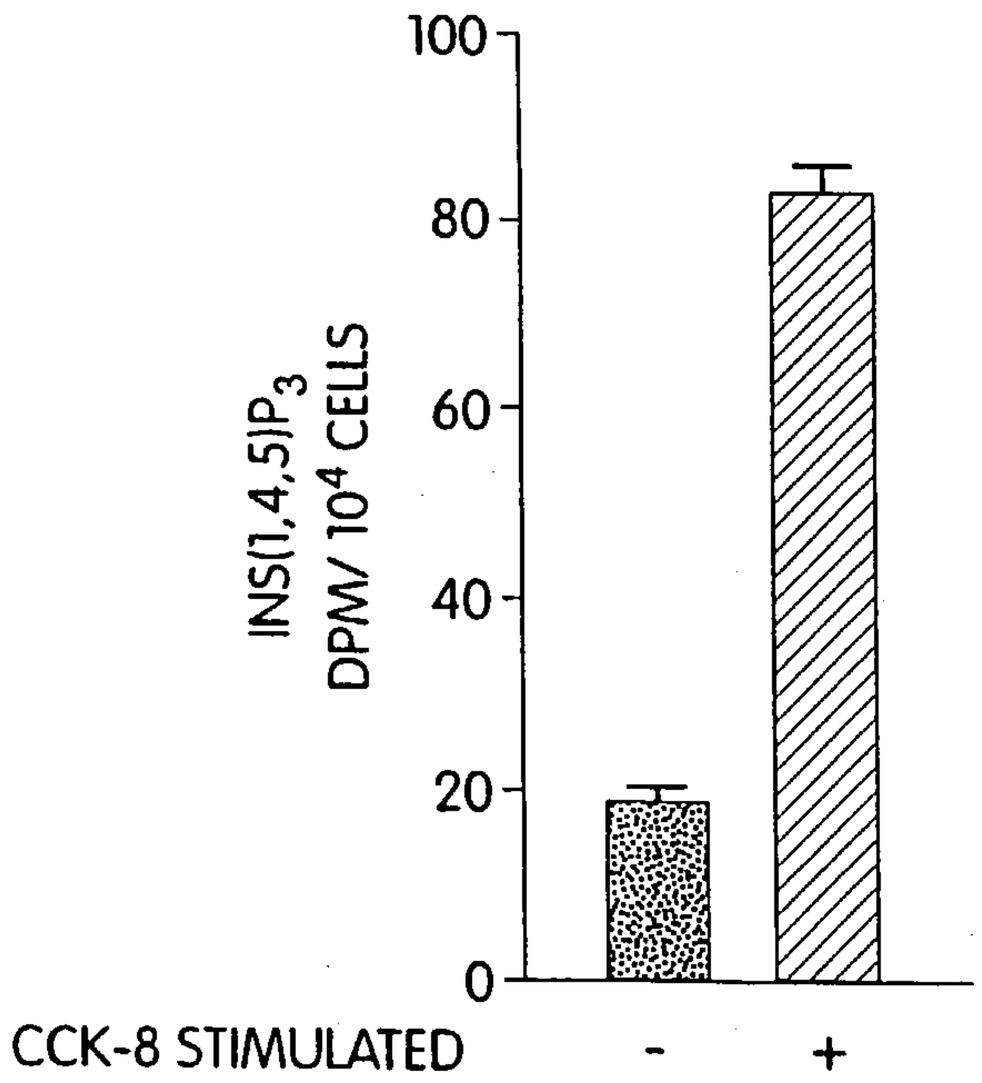

100
80
60
40
20
0
INS(1,4,5)P3
DPM/ 10^4 CELLS
CCK-8 STIMULATED
-
+

Fig. 8B

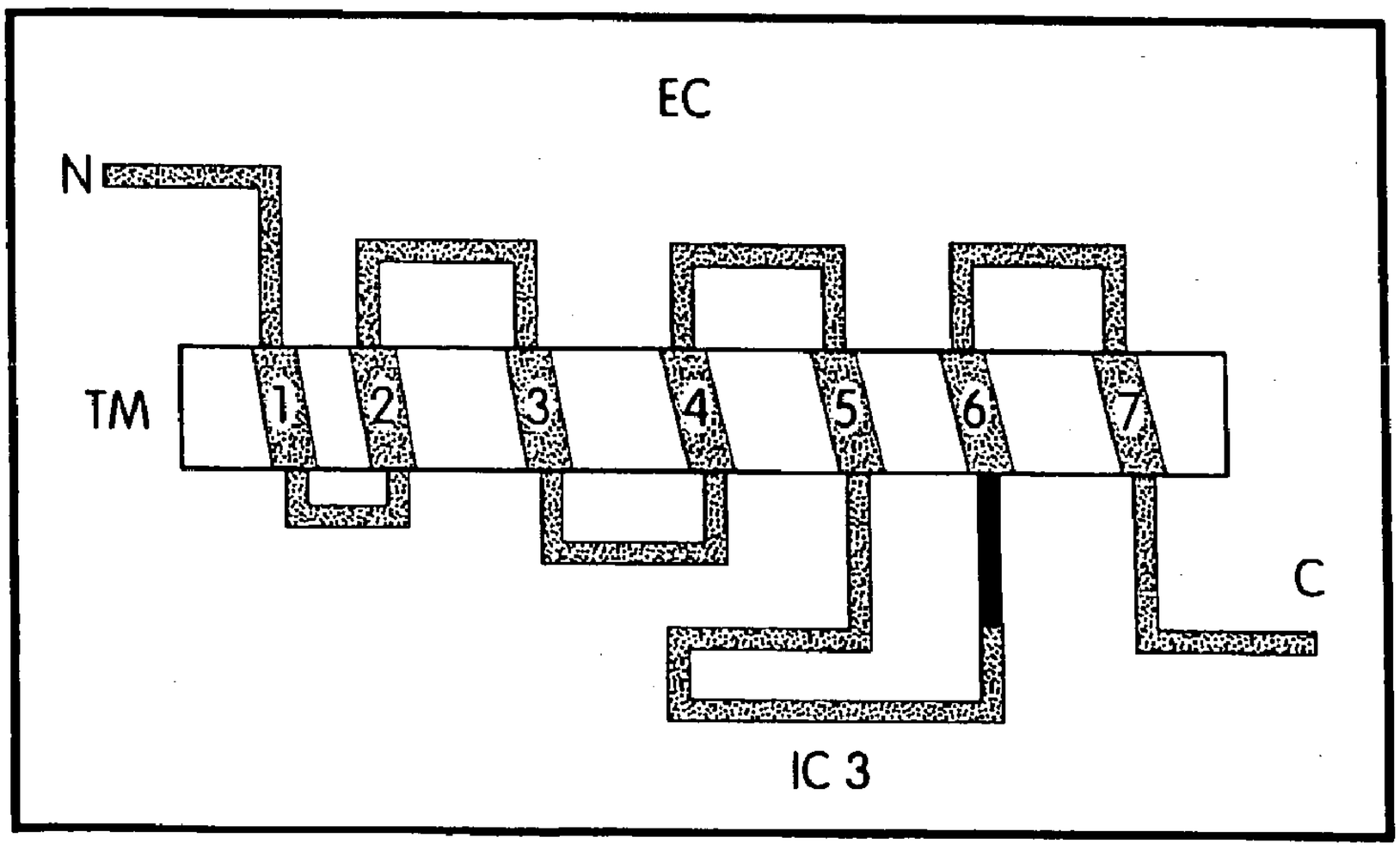

EC
N
TM
1
2
3
4
5
6
7
C
IC 3

Fig. 9

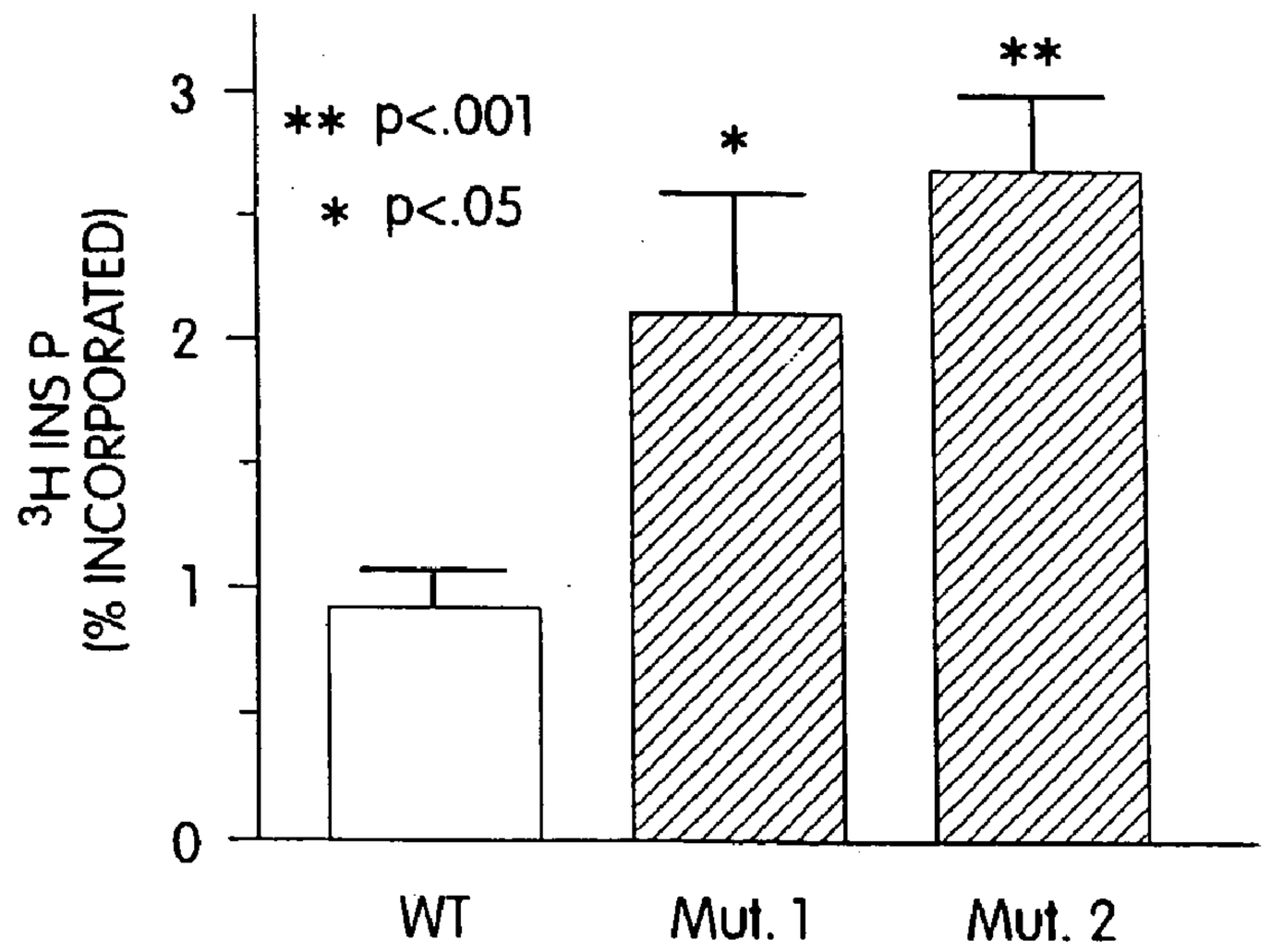

** p<.001
* p<.05
^{3}H INS P
(% INCORPORATED)
3
2
1
0
*
**
WT
Mut. 1
Mut. 2

Fig. 10

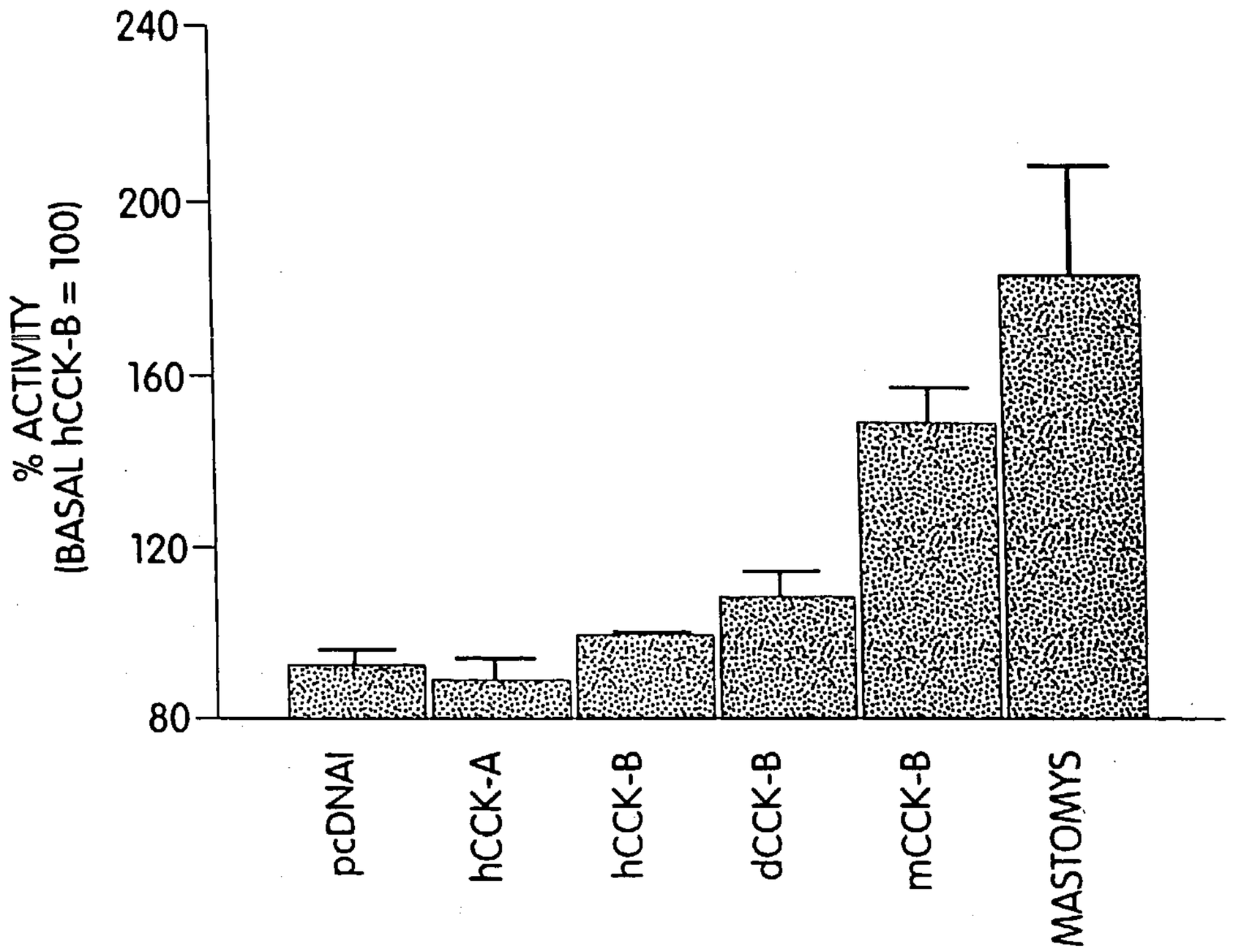


Fig. 11A

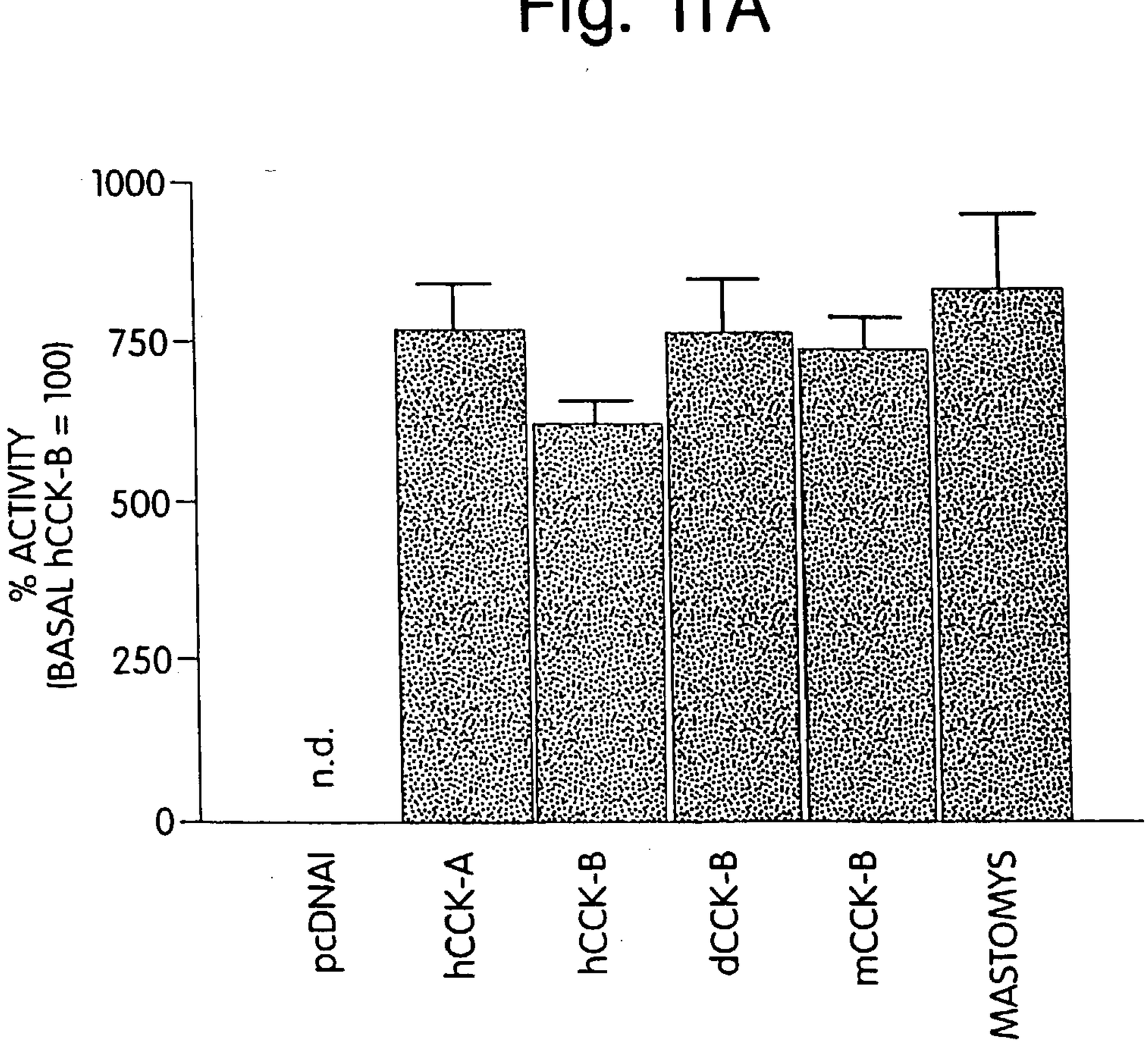


Fig. 11B

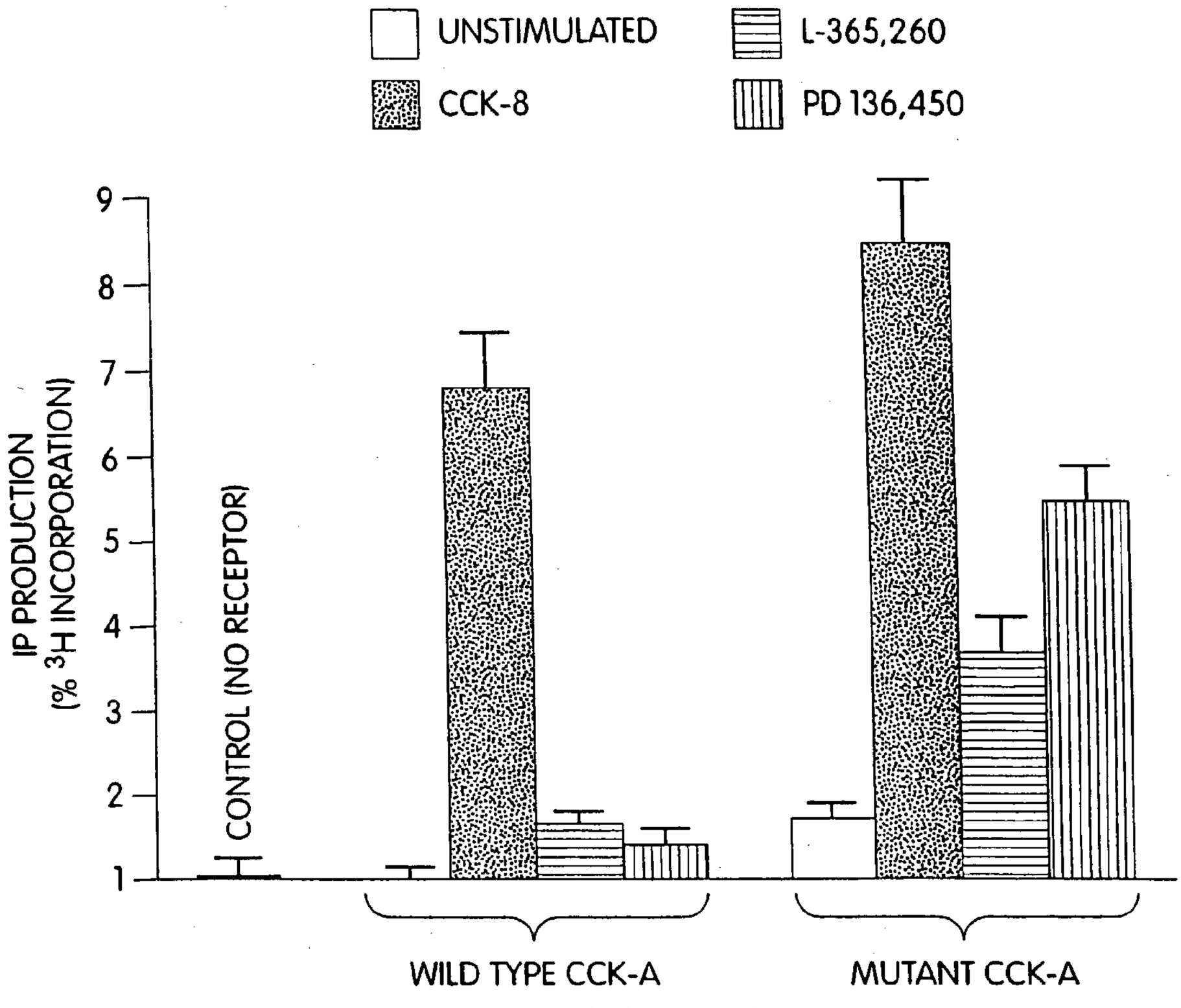

UNSTIMULATED
L-365,260
CCK-8
PD 136,450
IP PRODUCTION
(% 3H INCORPORATION)
9
8
7
6
5
4
3
2
1
CONTROL (NO RECEPTOR)
WILD TYPE CCK-A
MUTANT CCK-A

Fig. 12

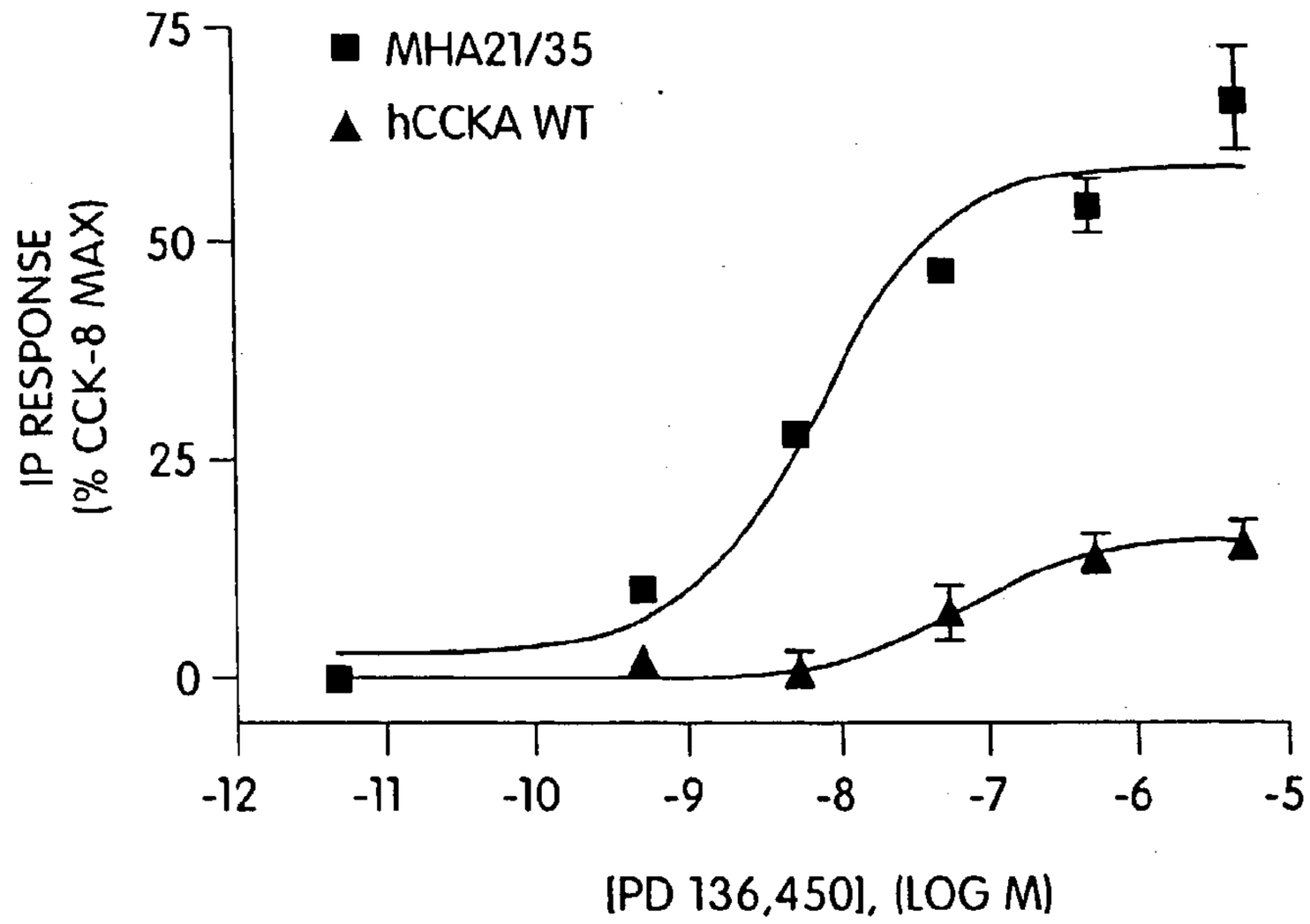

MHA21/35
hCCKA WT
IP RESPONSE
(% CCK-8 MAX)
75
50
25
0
-12
-11
-10
-9
-8
-7
-6
-5
[PD 136,450], (LOG M)

Fig. 13

ASSAY FOR AND USES OF PEPTIDE HORMONE RECEPTOR AGONISTS

BACKGROUND OF THE INVENTION

[0001] This application is a divisional of U.S. application Ser. No. 09/004,349, filed Jan. 8, 1998 (now U.S. Pat. No. 6,566,080) which is a continuation of U.S. application Ser. No. 08/718,047, filed Sep. 3, 1996 (now abandoned) which is a continuation-in-part of U.S. application Ser. No. 08/570,157, filed Dec. 11, 1995 (now U.S. Pat. No. 5,750,353).

[0002] This invention was made in part with Government funding under National Institute of Health grant #DK46767, and the Government therefore has certain rights in the invention.

[0003] The invention relates to methods of identifying and using a compound that is an agonist to a peptide hormone receptor.

[0004] Peptide hormone receptors are important targets for drug research because a considerable number of diseases and other adverse effects result from abnormal receptor activity. One peptide hormone of interest, cholecystokinin (CCK), is a neuropeptide with two distinct receptors: CCK-A and CCK-B/gastrin (Vanderhaeghen et al., *Nature*, 257:604-605, 1975; Dockray, *Nature*, 264:568-570, 1976; Rehfeld, *J. Biol. Chem.*, 253:4022-4030, 1978; Hill et al., *Brain Res.*, 526:276-283, 1990; Hill et al., *J. Neurosci.*, 10:1070-1081, 1990; Woodruff et al., *Neuropeptides*, (Suppl.) 19:57-64, 1991). The peripheral type receptor, the CCK-A receptor, is located in discrete brain nuclei and, in certain species, the spinal cord, and is also involved in gallbladder contraction and pancreatic enzyme secretion. The CCK-B/gastrin receptor is most abundant in the cerebral cortex, cerebellum, basal ganglia, and amygdala of the brain, in parietal cells of the gastrointestinal tract, in ECL cells, as well as in kidney cells. CCK-B receptor antagonists have been postulated to modulate anxiety, panic attacks, analgesia, and satiety (Ravard et al., *Trends Pharmacol. Sci.*, 11:271-273, 1990; Singh et al., *Proc. Natl. Acad. Sci. U.S.A.*, 88:1130-1133, 1991; Faris et al., *Science*, 219:310-312, 1983; Dourish et al., *Eur. J. Pharmacol.*, 176:35-44, 1990; Wiertelak et al., *Science*, 256:830-833, 1992; Dourish et al., *Science*, 245:1509-1511, 1989).

SUMMARY OF THE INVENTION

[0005] Applicants have developed a systematic screening assay for identifying an agonist specific for a peptide hormone receptor, e.g., a peptide, peptoid, or non-peptide agonist. The assay is based on applicants' recognition that a peptide hormone receptor able to amplify the intrinsic activity of a ligand, e.g., a constitutively active peptide hormone receptor, is useful as a screening vehicle for identifying a receptor-specific agonist. A receptor with a signaling activity higher than the corresponding human wild-type basal level of signaling activity is further useful for detecting the reduction in signaling activity induced by an inverse agonist. In both cases, the receptor amplifies the signal generated when the ligand interacts with its receptor, relative to the signal generated when the ligand interacts with a human wild-type receptor. Thus, forms of a receptor with the ability to amplify receptor signaling are useful for efficiently screening positive and inverse agonists to the corresponding human wild-type form of the receptor.

[0006] Accordingly, the invention features a method for determining whether a candidate compound is an agonist of a peptide hormone receptor. In this method, a candidate compound is exposed to a form of the peptide hormone receptor which has a greater, or an enhanced, ability to amplify the intrinsic activity of an agonist (hereafter,an 'enhanced receptor'). The second messenger signaling activity of the enhanced receptor is measured in the presence of the candidate compound, and compared to the second messenger signaling activity of the enhanced receptor measured in the absence of the candidate compound. A change in second messenger signaling activity indicates that the candidate compound is an agonist. For example, an increase in second messenger signaling activity indicates that the compound is either a full or partial positive agonist; a decrease in second messenger signaling activity indicates that the compound is an inverse (also termed a 'negative') agonist. The method can further comprise using the agonist to treat or to prevent a physiological disorder involving a peptide hormone receptor by administering to a mammal the identified agonist in an agonist-effective amount.

[0007] By "intrinsic activity" is meant the ability of a ligand to activate a receptor, i.e., to act as an agonist. By 'amplify' is meant that the signal generated when the ligand interacts with the enhanced receptor is either higher for a positive agonist, or lower for an inverse agonist, than the signal produced when the same ligand interacts with a corresponding non-enhanced receptor, e.g., a wild-type human receptor. A 'non-enhanced receptor,' for the purposes of this invention, is a wild-type human receptor for the peptide hormone of interest. By "corresponding" is meant the same type of peptide hormone receptor albeit in another form, e.g., a constitutively active mutant receptor. By way of example, the corresponding wild-type form of a constitutively active mutant CCK-B/gastrin receptor would be a wild-type CCK-B/gastrin receptor; the human CCK-B/gastrin receptor is the corresponding human form of the rat CCK-B/gastrin receptor.

[0008] An "agonist," as used herein, includes a positive agonist, e.g., a full or a partial positive agonist, or a negative agonist, i.e., an inverse agonist. An agonist is a chemical substance that combines with a receptor so as to initiate an activity of the receptor; for a peptide hormone receptor, the agonist preferably alters a second messenger signaling activity. A positive agonist is a compound that enhances or increases an activity, e.g., a second messenger signaling activity, of a receptor. A "full agonist" refers to an agonist capable of activating the receptor to the maximum level of activity, e.g., a level of activity which is induced by a natural, i.e., an endogenous, peptide hormone. A "partial agonist" refers to a positive agonist with reduced intrinsic activity relative to a full agonist. As used herein, a "peptoid" is a peptide-derived partial or full agonist (Horwell et al., *Eur. J. Med. Chem.*, 30 Suppl.:537S-550S, 1995; Horwell et al., *J. Med. Chem.*, 34:404-14, 1991).

[0009] An "inverse agonist," as used herein, has a negative intrinsic activity, and reduces the receptor's signaling activity relative to the signaling activity of the wild-type receptor measured in the absence of the inverse agonist. In contrast, an "antagonist," as used herein, refers to a chemical substance that inhibits the ability of an agonist to increase or decrease receptor activity. A 'full,' or 'perfect' antagonist has no intrinsic activity, and no effect on the receptor's basal

activity (**FIG. 1**). Peptide-derived antagonists are, for the purposes herein, considered to be non-peptide ligands.

[**0010**] A diagram explaining the difference between full and partial agonists, inverse agonists, and antagonists is shown in **FIG. 1** (see also Milligan et al., *TIPS*, 16:10-13, 1995). In **FIG. 1**, the position of the equilibrium between an inactive state R and an active state R* varies with individual receptors and is altered by the presence of receptor ligands. Agonists function by stabilizing R* while inverse agonists preferentially stabilize R. A continuum of ligands between full agonists (at the extreme right-hand side of the see-saw as these move the equilibrium furthest to the right) and full inverse agonists (at the extreme left-hand side of the see-saw) is expected to exist. Antagonists, which do not alter the position of the equilibrium, define the position of the fulcrum. An antagonist is, e.g., a competitive or a non-competitive inhibitor.

[**0011**] Examples of peptide hormone receptor specific peptide and non-peptide agonists useful in the screening assay of the invention are described below. Non-peptide ligands include, but are not limited to, benzodiazepines and derivatives thereof, e.g., azabicyclo[3.2.2]nonane benzodiazepine ("L-740,093"; see Castro Pineiro et al., WO 94/03437; Castro Pineiro et al., U.S. Pat. No. 5,521,175). L-740,093 S and L-740,093 R refer to the S-enantiomer and the R-enantiomer of L-740,093, respectively. Where the peptide hormone receptor is a CCK-A receptor or a CCK-B/gastrin receptor, useful peptide agonists include, but are not limited to, gastrin (e.g., sulphated ("gastrin II") or unsulphated ("gastrin I") forms of gastrin-17, or sulphated or unsulphated forms of gastrin-34), or cholecystokinin (CCK) (e.g., sulfated CCK-8 (CCK-8s), unsulphated CCK-8 (CCK-8d), CCK-4, or pentagastrin). Full agonists of the CCK-B/gastrin receptor include, but are not limited to, CCK-8s, and more preferably gastrin (gastrin I).

[**0012**] An enhanced receptor may, but does not always, have a higher basal activity than the basal activity of a corresponding human wild-type receptor. Methods for measuring the activity of an enhanced receptor relative to the activity of a corresponding wild-type receptor are described and demonstrated below. Examples of enhanced receptors include synthetic mutant receptors, e.g., constitutively active mutant receptors; other mutant receptors with normal basal activity which amplify the intrinsic activity of a compound; naturally-occurring mutant receptors, e.g., those which cause a disease phenotype by virtue of their enhanced receptor activity, e.g., a naturally-occurring constitutively active receptor; and either constitutively active or wild-type non-human receptors, e.g., rat, mouse, mastomys, Xenopus, or canine receptors or hybrid variants thereof, which amplify an agonist signal to a greater extent than does the corresponding wild-type human receptor.

[**0013**] Further examples of peptide hormone receptors useful in the screening assay of the invention include, but are not limited to, receptors specific for the following peptide hormones: amylin, angiotensin, bombesin, bradykinin, C5a anaphylatoxin, calcitonin, calcitonin-gene related peptide (CGRP), corticotropin releasing hormone (CRH), chemokines, cholecystokinin (CCK), endothelin, erythropoietin (EPO), follicle stimulating hormone (FSH), formyl-methionyl peptides, galanin, gastrin, gastrin releasing peptide, glucagon, glucagon-like peptide 1, glycoprotein hormones, gonadotrophin-releasing hormone, leptin, luteinizing hormone (LH), melanocortins, neuropeptide Y, neurotensin, opioid, oxytocin, parathyroid hormone, secretin, somatostatin, tachykinins, thrombin, thyrotrophin, thyrotrophin releasing hormone, vasoactive intestinal polypeptide (VIP), and vasopressin. An enhanced receptor can further embrace a single transmembrane domain peptide hormone receptor, e.g., an insulin receptor.

[**0014**] The invention also features a method of isolating a form, e.g., a mutant form, of a peptide hormone receptor that is suitable for detecting agonist activity in a ligand, e.g., a peptide, peptoid, or non-peptide ligand. The method involves (a) exchanging a region of a functional domain of a first peptide hormone receptor with a corresponding region of a functional domain of a second peptide hormone receptor; and (b) measuring the ability of the first peptide hormone receptor to amplify an agonist signal relative to a corresponding wild-type human receptor. The functional domain can be an intracellular loop, parts of a transmembrane domain adjacent to an intracellular loop, a transmembrane domain, a region of a transmembrane domain distal to an intracellular loop, or an extracellular loop. A level of amplification by the first peptide hormone receptor that is greater than the level of amplification by the wild-type human receptor indicates that the first peptide hormone receptor is suitable for detecting agonist activity in a non-peptide ligand. The corresponding region can be between one and ten amino acids, e.g., a block of five to ten amino acids, or up to thirty or a hundred amino acids in length. The first and second peptide hormone receptors are preferably linked to different second messenger pathways. Those skilled in the art know which particular amino acids of the peptide hormone receptors are considered to be within extracellular, intracellular (cytoplasmic), or transmembrane regions of the receptor. For example, extracellular, intracellular, and transmembrane regions of the CCK-B/gastrin receptor are determined by sequence alignment with other receptors (**FIG. 2**), or by hydropathy analysis (Baldwin, *EMBO J.*, 12:1693-1703, 1993). Conformation receptor modelling is described further below.

[**0015**] Another method of isolating a form of a peptide hormone receptor suitable for detecting agonist activity in a non-peptide ligand involves (a) constructing a series of mutant forms of the receptor by replacing an original amino acid with another amino acid, i.e., a replacement amino acid; and (b) measuring the ability of the resulting peptide hormone receptor mutant form to amplify an agonist signal relative to the level of amplification by a corresponding wild-type human receptor. An amplification in the peptide hormone receptor mutant form that is greater than the level of amplification of the corresponding wild-type human receptor indicates that the mutant form is suitable for detecting agonist activity in a non-peptide ligand. The replaced amino acid can lie in an intracellular domain of the receptor or in a region of a transmembrane domain flanking an intracellular portion of the receptor, e.g., the intracellular domain-proximal half of the transmembrane domain, or within, e.g., 8 or 10 amino acids of the intracellular domain. The replacement amino acid can be of the same type in each of the mutant constructs; alternatively, various types of

amino acids can be substituted at random. The replacement amino acid can be of the same charge, or of a different charge, than the original amino acid, e.g., a negative amino acid can be exchanged for a positive amino acid, a positive amino acid can be exchanged for a negative amino acid, or a positive or negative amino acid can be exchanged for a neutral amino acid. Preferably, the replacement amino acid is glutamine, glutamic acid, aspartic acid, or serine.

[**0016**] Also embraced are the various mutant peptide hormone receptors disclosed herein, and their respective nucleic acid coding sequences. Mutant peptide hormone receptors of the invention include, but are not limited to, the CCK-A receptor MHA21/35, and the mutant CCK-B/gastrin receptors MH40, MH128, MH156, MH162, MH31, MH131, MH13, MH130, MH129, and MH72. Plasmid manipulation, storage, and cell transformation can be performed by methods known to those of ordinary skill in the art. See, e.g., Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., NY, 1988, 1995.

[**0017**] With an efficient and rapid assay for identifying an agonist specific for a given peptide hormone receptor, those skilled in the art can identify agonists to serve as lead compounds for further pharmaceutical research. In particular, systematic chemical modifications can be made, and their effects can be further assessed using an enhanced receptors according to the method of the invention. By following such a development strategy the intrinsic activity of new agonists can be optimized so as to be useful therapeutically against a disease involving a peptide hormone receptor.

[**0018**] Knowing that a particular ligand functions as a positive or inverse agonist, as opposed to an antagonist, facilitates identifying which ligand species is most likely to achieve a given physiological effect, or to achieve a physiological effect absent an unwanted side effect. Thus, the invention further features a method for the treatment or prevention of a physiological disorder involving a peptide hormone receptor that includes administering to a mammal, e.g., a human, a ligand which acts as an agonist on a peptide hormone receptor. The ligand is administered in an agonist-effective amount, i.e., in an amount that has a full or a partial inverse, or a full or a partial positive, agonist effect on a peptide hormone receptor. The peptide hormone receptor can be, but need not be limited to, a receptor specific for one of the following peptide hormones: amylin, angiotensin, bombesin, bradykinin, C5a anaphylatoxin, calcitonin, calcitonin-gene related peptide (CGRP), corticotropin releasing hormone (CRH), chemokines, cholecystokinin (CCK) (e.g., the CCK-A or the CCK-B/gastrin receptor), endothelin, erythropoietin (EPO), follicle stimulating hormone (FSH), formyl-methionyl peptides, galanin, gastrin, gastrin releasing peptide, glucagon, glucagon-like peptide 1, glycoprotein hormones, gonadotrophin-releasing hormone, insulin, leptin, luteinizing hormone (LH), melanocortins, neuropeptide Y, neurotensin, opioid, oxytocin, parathyroid hormone, secretin, somatostatin, tachykinins, thrombin, thyrotrophin, thyrotrophin releasing hormone, vasoactive intestinal polypeptide (VIP), and vasopressin.

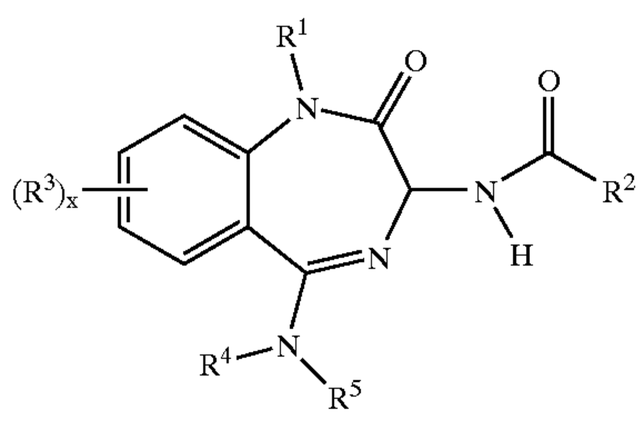
[**0019**] An inverse agonist is particularly useful for treating or preventing a physiological disorder that results from enhanced basal activity of a peptide hormone receptor, e.g., a constitutively active receptor. For example, an inverse agonist is useful for treating a neoplasm that results from, or is sustained or aggravated by, enhanced activity of a peptide hormone receptor, e.g., a naturally-occurring peptide hormone receptor. Examples include, but are not limited to, a CCK-B/gastrin related tumor, e.g., a neuroendocrine tumor, Zollinger Ellinger Syndrome, or a gastric carcinoid tumor; a TSH-related tumor; multiple endocrine neoplasia type I; a lung tumor, e.g., a small-cell carcinoma; a brain tumor, e.g., a brain tumor involving CCK; a kidney tumor, e.g., hypernephroma or renal cell carcinoma. Agonists are particularly useful for treating a primary tumor, e.g., a tumor in a tissue that expresses a peptide hormone receptor, e.g., a tumor in the pancreas, the pituitary, or the adrenal gland.

[**0020**] In other embodiments of the invention, an inverse agonist of the LH receptor can be a useful compound for treating or preventing precocious puberty; an inverse agonist of the FSH receptor can be a useful compound for treating or preventing infertility; an inverse agonist of the TSH receptor can be a useful compound for treating or preventing thyroid adenomas. An inverse or positive agonist of the G-LP1 receptor can be a useful compound for treating or preventing obesity or diabetes, e.g., type-I or type-II diabetes.

[**0021**] Non-peptide agonists are further useful for treating or preventing a disorder involving the gastrointestinal tract, or a disorder involving, e.g., sleep, anxiety, panic, appetite regulation, stress, or pain, or a disorder discussed below.

[**0022**] A candidate compound useful in a method of treatment or prevention of a physiological disorder of the invention is a ligand that binds with specificity to a peptide hormone receptor, e.g., a peptide, peptoid, or non-peptide ligand, preferably a non-peptide ligand. A candidate compound is shown to be a positive or inverse agonist by a screening assay disclosed herein. Exemplified candidate compounds include the peptoid compounds ((see, e.g., Horwell et al., *Eur. J. Med. Chem.*, 30 Suppl.:537S-550S, 1995; Horwell et al., *J. Med. Chem.*, 34:404-14, 1991); the dipeptoid analogues of CCK (see, e.g., Horwell et al. *J. Med. Chem.*, 34:404-14, 1991); cyclic nucleotides and modified amino acids (see, e.g., Dethloff et al., *Drug Metab.*, 24:267-93, 1992), the benzodiazepine derivatives, e.g.., the compounds described in Bock et al., *J. Med. Chem.*, 33:450-55, 1990, or a derivative thereof. Additional benzodiazepine derivatives having a peptide hormone receptor agonist activity are described in the following patents and patent applications, each of which is hereby incorporated by reference: EPA 167919, EPA 284256, EPA 434360, EPA 434364, EPA 434369, EPA 514125, EPA 51426, EPA 514133, EPA 508796, EPA 508797, EPA 508798, EPA 508799, EPA 523845, EPA 523846, EPA 559170, EPA 549039, EPA 667,334, WO 9211246, WO 93032078, WO 9308175, WO 9307131, WO 9317011, WO 9319053, WO 9308175, WO 9413648, WO 9403437, WO 9611689, and U.S. Pat. No. 5,521,175. Also encompassed are those compounds described in Henke et al., *J. Med. Chem.*, 39:2655-58, 1996; or in Willson et al., *J. Med. Chem.*, 39:3030-34, 1996 (both hereby incorporated by reference) which are shown to be inverse agonists.

[0023] As a further example, a benzodiazepine compound useful in a method of treatment or prevention of a physiological disorder resulting from a peptide hormone receptor is a benzodiazepine compound of formula (I):



[0024] wherein:

[0025] R^1 represents H, C_{1-6} alkyl optionally substituted by one or more halo, C_{3-7} cycloalkyl, cyclopropylmethyl, $(CH_2)_r$imidazolyl, $(CH_2)_r$triazolyl, $CH_2))_r$tetrazolyl (where r is 1, 2 or 3), $CH_2CO_2R^{11}$ (where R^{11} is C_{1-4}alkyl) or $CH^2CONR^6R^7$ (where R^6 and R^7 each independently represents H or C_{1-4}alkyl, or R^6 and R^7 together form a chain CH_2p where p is 4 or 5;

[0026] R^2 represents NHR^{12} or $(CH_2)_qR^{13}$ (where $_s$ is 0, 1, 2, or 3);

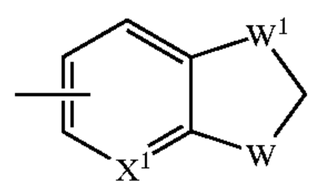
[0027] R^3 represents C_{1-6}alkyl, halo or NR^6R^7, where R^6 and R^7 are as previously defined;

[0028] R^4 and R^5 each independently represents H, C_{1-12}alkyl optionally substituted by NR^9R^9, (R^9 and R^9, are as previously defined) or an azacyclic or azabicyclic group, C_{4-9}cycloalkyl optionally substituted by one or more C_{1-4}alkyl groups, C_{4-9}cycloalkylC_{1-4}alkyl optionally substituted in the cycloalkyl ring by one or more C_{1-4}alkyl groups, optionally substituted aryl, optionally substituted arylC_{1-6}alkyl or azacyclic or azabicyclic groups, or R^4 and R^5 together form the residue of an optionally substituted azacyclic or azabicyclic ring system;

[0029] x is 0, 1, 2, or 3;

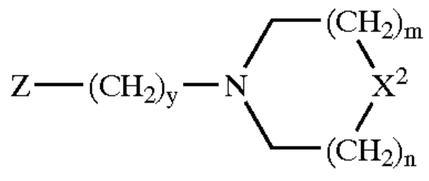
[0030] R^{12} represent a phenyl or pyridyl group optionally substituted by one or more substituents selected form C_{1-6}alkyl, halo, hydroxy, C_{1-4}alkoxy, (CH_2)q-tetrazolyl optionally substituted in the tetrazole ring by C_{1-4}alkyl, (CH_2)q-imidazolyl, (CH_2)q-triazolyl (qhere q is 0, 1, 2, or 3), $_9$ 5-hydroxy-4-pyrone, NR^6R^7, NR^9COR^{11}, $NR^9CONR^{9'}R^{11}$ (where R^9 and R^9, are each independently H or C_{1-4}alkyl and R^{11} is as previously defined), $SO(C_{1-6}alkyl)$, $SO_2(C_{1-6}alkyl)$, trifluoromethyl, $CONHSO_2R^8$, SO_2NHCOR^8 (where R^8 is C_{1-6}alkyl, optionally substituted aryl, 2,2-difluorocyclopropane or trifluoromethyl), SO^2NHR^{10} (where R^{10} is a nitrogen containing heterocycle), $B(OH)_2$, $(CH_2)qCO_2H$, where q is as previously defined; or

[0031] R^{12} represents a group:



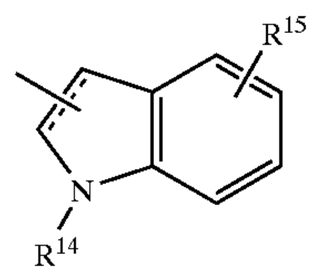
[0032] where X^1 represents CH or N; W represents CH_2 or NR^9, where R^9 is as previously defined, and W^1 represents CH_2, or W and W^1 each represent O; or

[0033] R^{12} represents phenyl substituted by a group:

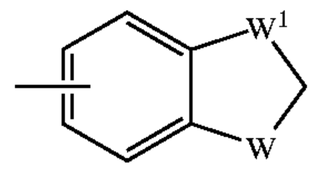


[0034] wherein X^2 is O, S or NR^9, where R^9 is as previously defined; z is a bond, O or S; m is 1, 2 or 3; n is 1, 2, or 3; and y is 0, 1, 2, or 3;

[0035] R^{13} represents a group:



[0036] where R^{14} represents H or C_{1-6}alkyl, R^{15} represents H, C_{1-6}alkyl, halo or NR^6R^7, where R^6 and R^7 are as previously defined; and the dotted line represents an optional covalent bond; and pharmaceutically acceptable salts or prodrugs thereof, with the provision that, when NR^4R^5 represents an unsubstituted azacyclic ring system, R^2 does not represent NHR^{12} where R^{12} is optionally substituted phenyl or:



[0037] Compounds of formula (I) are intended to embrace all possible racemers and isomers, including optical isomers, and mixtures thereof. Each expression, where occurring more than once in any structure, intended to be independent of its definition elsewhere in the same structure. The present invention includes within its scope prodrugs of the compounds of formula (I) above. In general, such prodrugs will be functional derivatives of the compounds of formula (I) which are readily convertible in vivo into the required compound of formula (I). Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs" ed. H. Bungaard, Elsevier, 1985.

[0038] As used herein, unless otherwise indicated, alkyl means straight or branched chain saturated hydrocarbon;; halo includes fluoro, chloro, bromo, and iodo; as used herein, unless otherwise indicated, alkyl means straight or branched chain saturated hydrocarbon; azacyclic means non-aromatic nitrogen-containing monocyclic, and azabicyclic means non-aromatic nitrogen-containing bicyclic; aryl means optionally substituted carbocyclic or heterocyclic aromatic groups, especially phenyl; heteroaryl means aromatic rings preferably having r or 6 ring atoms and containing at least one atom selected from O, S, and N. Compounds of formula (I) are prepared according to the methods of WO 94/03437, hereby incorporated by reference.

[0039] By using the screening assay of the invention, those skilled in the art can easily identify which candidate ligand is optimal for therapeutic or preventive use. For example, preferred agonists and their respective derivatives include, but are not limited to, L-740,093 [3(R,S)-Amino-1,3-dihydro-5-((1S,4S)-5-methyl-2,5-diazabicyclo[2,2,1] heptan-2-vl)-2H-1-propyl-1,4-benzodiazepin-2-one]; L-740,093 R [(−)-N-[5-(3-azabicyclo[3.2.2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1H-1,4-benzodiazepin-3-yl]-N'-[3-methylphenyl]urea]; L-740.093 S [(+)-N-[5-(3-azabicyclo[3.2.2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1H-1,4-benzodiazepin-3-yl]-N'-[3-methylphenyl]urea]; L-365,260 [3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-N'-(3-methylphenylurea)]; and L-364,718.

[0040] Other terms used in the various embodiments of the invention will be understood from the following definitions. For example, by a "peptide hormone" is meant a polypeptide that interacts with a target cell by contacting an extracellular receptor, i.e., a "peptide hormone receptor." A "peptide" is used loosely herein to refer to a molecule comprised, at least in part, of amino acid residues that are connected to each other by peptide bonds. A "mutant receptor" is understood to be a form of the receptor in which one or more amino acid residues in the corresponding receptor which predominates in nature, e.g., in a naturally-occurring wild-type receptor, have been either deleted or replaced with a different type of amino acid residue. By a "constitutively active receptor" is meant a receptor with a higher basal activity level than the corresponding wild-type receptor, where "activity" refers to the spontaneous ability of a receptor to signal in the absence of further activation by a positive agonist. The basal activity of a constitutively active receptor can also be decreased by an inverse agonist. A "naturally-occurring" receptor refers to a form or sequence of the receptor as it exists in an animal, or to a form of the receptor that is synonymous with the sequence known to those skilled in the art as the "wild-type" sequence. Those skilled in the art will understand a "wild-type" receptor to refer to the conventionally accepted "wild-type" amino acid consensus sequence of the receptor, or to a "naturally-occurring" receptor with normal physiological patterns of ligand binding and signaling. A "second messenger signaling activity" refers to production of an intracellular stimulus (including, but not limited to, cAMP, cGMP, ppGpp, inositol phosphate, or calcium ion) in response to activation of the receptor, or to activation of a protein in response to receptor activation, including but not limited to a kinase, a phosphatase, or to activation or inhibition of a membrane channel.

[0041] "Sequence identity," as used herein, refers to the subunit sequence similarity between two nucleic acid or polypeptide molecules. When a given position in both of the two molecules is occupied by the same nucleotide or amino acid residue, e.g., if a given position (as determined by conventionally known methods of sequence alignment) in each of two polypeptides is occupied by serine, then they are identical at that position. The identity between two sequences is a direct function of the number of matching or identical positions, e.g., if 90% of the positions in two polypeptide sequences are identical, e.g., 9 of 10, are matched, the two sequences share 90% sequence identity. Methods of sequence analysis and alignment for the purpose of comparing the sequence identity of two comparison sequences are well known by those skilled in the art. "Biological activity," as used herein, refers to the ability of a peptide hormone receptor to bind to a ligand, e.g., an agonist or an antagonist, and to induce signaling.

[0042] Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

DETAILED DESCRIPTION

[0043] We first briefly describe the drawings.

DRAWINGS

[0044] **FIG. 1** is a schematic diagram showing the relationship between a full or partial agonist, an inverse agonist, and an antagonist.

[0045] **FIG. 2** is an illustration showing a multiple alignment of cloned CCK receptor deduced amino acid sequences: mastomys CCK-B (SEQ ID NO: 1), rat CCK-B (SEQ ID NO: 2), human CCK-B (SEQ ID NO: 3), canine CCK-B (SEQ ID NO: 4), human CCK-A (SEQ ID NO: 5), rat CCK-A (SEQ ID NO: 6), and xenopus CCK-XL (SEQ ID NO: 7). 'A' marks the position in the hCCK-A receptor where an E to Q substitution results in an increase in PD 135,158 intrinsic activity without increasing basal receptor activity. 'B' marks the position in the hCCK-B receptor where an L to either S or E substitution results in an increase in basal activity. The corresponding L to S in the hCCK-A receptor does not result in an increase in basal activity. 'C' marks the position in the hCCK-B receptor where a V to E substitution results in an increase in basal activity. The corresponding I to E substitution in the human CCK-A receptor does not result in an increase in basal activity. The numbering shown is generic; each receptor is different based on deletions or insertions.

[0046] **FIG. 3** is a bar graph showing that the intrinsic activity of peptide, peptide-derived and non-peptide ligands at the wild-type CCK-B/gastrin receptor (top panel) is amplified in a constitutively active receptor mutant (bottom panel).

[0047] **FIG. 4** is an illustration of inositol phosphate production by the non-peptide agonist L-740,093. Top panel: L-740,093 S stimulated inositol phosphate production in COS-7 cells expressing a constitutively active human CCK-B/gastrin receptor. Bottom panel: YM022 antagonizes the partial agonist activity induced by 10 nM L-740,093-S.

[0048] **FIG. 5** is an illustration of the inhibition of inositol phosphate production of the non-peptide inverse agonist

L-740,093R. Top panel: L-740,093 R inhibits basal inositol phosphate production in COS-7 cells expressing a constitutively active human CCK-B/gastrin receptor. Bottom panel: The inverse agonist activity induced by 10 nM L-740,093 R is partially abolished by YM022 in a concentration-dependent fashion.

[**0049**] **FIG. 6** is a comparison of intrinsic activities of CCK-B/gastrin receptor ligands utilizing the wild-type and the constitutively active receptors. Values for all compounds follow a logarithmic-linear correlation (r2=0.93).

[**0050**] **FIG. 7** is a competition binding curve showing the extent of ^{125}I-CCK-8 receptor binding. Binding of ^{125}I-CCK-8 to COS-7 cells, transiently transfected with hCCK-B-pcDNAI is shown in the presence of increasing concentrations of CCK-8, gastrin I, and CCK-4 (part A) and L-364,718 and L-365,260 (part B).

[**0051**] **FIG. 8** is a graph showing second messenger signaling (i.e., mobilization of intracellular calcium) in COS-7 cells that express the recombinant human brain CCK-B receptor with (part A, left panel) and without (part A, right panel) the addition of the calcium chelator, EGTA. This is paralleled by an increased production of inositol phosphate (part B).

[**0052**] **FIG. 9** is a schematic representation of the seven transmembrane (TM) domain structure of the human CCK-B/gastrin receptor. The C-terminal domain of the third intracellular loop is highlighted in black.

[**0053**] **FIG. 10** is a bar graph of basal inositol phosphate accumulation in COS-7 cells transfected with wild-type CCK-B/gastrin receptor (WT), or with one of two constitutively active mutants (Mut.1, Mut.2).

[**0054**] **FIG. 11** is a bar graph showing a functional comparison of the CCK receptors human CCK-A (hCCK-A), human CCK-B (hCCK-B), dog CCK-B (dCCK-B), mouse CCK-B (mCCK-B), and the mastomys CCK receptor.

[**0055**] **FIG. 12** is a bar graph showing a comparison of the efficiency by which the agonists CCK-8, L-365,260, and PD136,450 stimulate inositol phosphate (IP) production when contacting a wild-type CCK-A receptor or a constitutively active CCK-A receptor (MHA21/35).

[**0056**] **FIG. 13** is a graph showing the level of inositol phosphate (IP) response at varying concentrations of the peptoid PD 136,450, a partial positive agonist. ■: mutant CCK-A receptor MHA21/35; ▼: wild-type human CCK-A receptor.

[**0057**] Recent drug development efforts have yielded small molecules which competitively block G-protein coupled peptide hormone receptors by acting as antagonists. In contrast, very few non-peptide ligands have been identified which activate this family of receptors. Here, applicants demonstrate that chemical modifications of non-peptide ligands, known to act as antagonists for the CCK-B/gastrin receptor, can result in converting the ligands from antagonists to either positive agonists or to inverse agonists.

[**0058**] Changes in the intrinsic activity of the ligand resulting from such modifications were detectable because applicants designed a screening assay which employed a constitutively active mutant of the human CCK-B/gastrin receptor (^{325}L→E, MH162). Several peptide, peptoid and benzodiazepine-based nonpeptide ligands were tested in this assay, and evaluated for the ability to activate the recombinant wild-type or constitutively active mutant receptor, respectively. Full positive agonists had similar signaling efficacy in both receptors when compared to the intrinsic activity of the peptide agonist CCK-8s. The signalling efficacy of ligands with lower intrinsic activity was logarithmically amplified when a constitutively active mutant receptor was used in the assay. The prototype benzodiazepine-derived non-peptide antagonist L-365,260 barely increased basal activity of the human wild-type CCK-B/gastrin receptor, but was identified as a partial agonist using the ^{325}L→E mutant MH162. Minor chemical modification of L-365,260 resulted in compounds which were pure antagonists (YM022), partial agonists (L-740,093 S) or inverse agonists (L-740,093 R). Thus, the process of discovering novel non-peptide agonists, e.g., those with positive inverse or intrinsic activity, should be expedited by using enhanced receptors, e.g., constitutively active mutant receptors, in the screening assay.

[**0059**] I. Working Examples:

[**0060**] A. CCK-B/gastrin receptor: The following example demonstrates the use of an enhanced peptide hormone receptor, the constitutive CCK-B/gastrin receptor MH162 (^{325}L→E), to screen for an agonist.

[**0061**] Using a constitutively active mutant of the human CCK-B/gastrin receptor it was discovered that several benzodiazepine-based putative non-peptide 'antagonists' had detectable intrinsic activity as agonists when binding to this receptor.

[**0062**] The constitutively active CCK-B/gastrin receptor mutant MH162 (^{325}L→E) was transiently overexpressed in COS-7 cells. The fact that it was constitutively active was evident from ligand-independent production of inositol phosphate; the wild-type receptor, in contrast, exhibits only ligand-dependent inositol phosphate production. Both mutant and wild-type receptors induced similar inositol phosphate production when maximally stimulated with the peptide agonists CCK-8s or gastrin I (**FIG. 3**). In contrast, the different intrinsic activities of three benzodiazepine-derived compounds, L-740,093 R, YM022 and L-365,260 were only detected by using the constitutive CCK-B receptor mutant MH162 in the assay. Each of these compounds was previously considered a prototype non-peptide antagonist of the wild-type CCK-B/gastrin receptor (Castro Pineiro et al., WO 94/03437; Lotti et al., *Eur. J. Pharmacol.*, 162:273-280, 1989; Nashida et al., *J. Pharmacol. Exp. Ther.*, 270:1256-61, 1994; Nashida et al., *J. Pharmacol. Exp. Ther.*, 269:725-31, 1994). The intrinsic activity (percent maximal stimulation of inositol phosphate formation) of all compounds was tested at concentrations that were at least 100-fold higher than the corresponding receptor affinities.

[**0063**] The benzodiazepine L-365,260 had 62% efficacy when compared to the full agonist CCK-8s, and was on that basis identified as a partial agonist in the ^{325}L→E constitutively active mutant receptor MH162 (**FIG. 3**, right section). In fact, close re-examination of the function of L-365,260 with the wild-type CCK-B/gastrin receptor also revealed a barely detectable, yet significant, increase in inositol phosphate production that had not been seen with other non-peptide compounds.

[0064] From the above results it was concluded that minor changes in the chemical groups attached to the benzodiazepine backbone can result in marked alterations in intrinsic activity of small non-peptide compounds. The stereochemistry of benzodiazepine-derived CCK receptor ligands is another feature which can alter binding affinity as well as receptor selectivity (Showell et al. *J. Med. Chem.* 37:719-721, 1994).

[0065] The following additional observations confirmed that differences in ligand stereochemistry determine the functional properties of the CCK-B/gastrin receptor specific compounds. For example, it was noted that L-740,093 S was almost a full agonist in the ^{325}L→E CCK-B/gastrin receptor mutant MH162 (**FIG. 3**). When tested with the human wild-type CCK-B/gastrin receptor, L-740,093 S functions as a partial agonist (25% efficacy compared with CCK-8s). As such, L-740,093 S is the first non-peptide agonist known to be specific to the CCK-B/gastrin receptor. The mirror image of L-740,093 S, L-740,093 R, has properties opposite to those of the S enantiomer. L-740,093 R reduces the basal activity of the constitutively active receptor almost to wild-type levels, and is therefore acting as an inverse agonist.

[0066] One advantage of the instant screening assay is its ability to distinguish between an agonist, e.g., an inverse agonist, and an antagonist. When testing an agonist, the level of signaling activity observed with an enhanced form of a peptide hormone receptor (e.g., a constitutive receptor) differs from the activity observed with a human wild-type receptor. In contrast, an antagonist shows similar signalling activities with both enhanced and human wild-type receptor. A pure antagonist is further expected to attenuate the effects of both positive and inverse agonists.

[0067] Of the compounds tested, YM022 came closest to being a 'perfect' antagonist, having almost no intrinsic activity on either the wild-type or the constitutively active CCK-B/gastrin receptor. In both the wild-type and the constitutively active receptors, YM022 blocked CCK-8s induced inositol phosphate production with almost identical affinity, reflected by similar pA2 values (9.78 and 9.37, respectively). Consistent with the functional classification of L-740,093 S as a non-peptide agonist, the inositol phosphate production induced by this compound could be blocked by YM022 (pA2=9.54; **FIG. 4**). YM022 was also able to attenuate the inverse agonist activity of L-740,093 R on the constitutively active CCK-B/gastrin receptor (**FIG. 5**). In a concentration-dependent manner, YM022 partially restored basal activity to the constitutively active receptor which had been inhibited by 20 nM L-740,093 R. The fact that basal activity was not restored completely is explained by the fact that YM022 itself is a weak inverse agonist in this mutant rather than a pure receptor antagonist.

[0068] The pA2 value measures the functional affinity of a competitive antagonist. In contrast to IC_{50} values (50% inhibitory concentration), pA_2 values are independent of which agonist concentrations are used to measure antagonist affinities. Ideally, pA_2 values should also be independent of what specific agonist compounds are tested to assess antagonist affinities. The pA2 value is defined as the negative logarithm of the specific antagonist concentration which shifts the agonist concentration-response curve by a factor of two to the right. In other words, in the presence of a given antagonist concentration, one would need twice as much agonist as would be required in the absence of antagonist to induce the same effect. pA_2 values of competitive antagonists are typically assessed by Schift plots, but can also be measured by simplified 'null' methods (Lazareno et al., *Trends in Pharmacol. Sci.*, 14:237-239, 1993).

[0069] In addition to non-peptide ligands, the constitutively active mutant CCK-B receptor MH162 (^{325}L→E) amplified the intrinsic activity of peptide-derived partial agonists, called peptoids (Horwell et al., *Eur. J. Med. Chem.*, 30 Suppl.::537S-550S, 1995; Horwell et al., *J. Med. Chem.*, 34:404-14, 1991). The peptoids used in the following experiments were obtained by sequentially modifying CCK-4, which is a tetrapeptide comprising the four carboxyterminal amino acids of CCK. Two prototype peptoid compounds, PD 135,158 and PD 136,450, were converted from partial agonists when tested in the presence of wild-type CCK-B/gastin receptor to almost full agonists in the presence of constitutively active CCK-B/gastrin receptor. Thus, peptide-derived as well as non-peptide compounds can exhibit an increased efficacy when tested with the constitutively active versus the wild-type CCK-B/gastrin receptor. Despite these marked alterations in efficacy, the ratio of wild-type versus mutant receptor affinities, as determined by ^{125}I-CCK-8 competition binding experiments, fell within a two-fold range (Table 1A). There was no apparent correlation between the intrinsic activity of CCK-B/gastrin receptor ligands and potency shifts between the wild-type and the constitutively active receptors (Table 1B).

[0070] The intrinsic activity of L-740,093 S was comparable to that observed for the 'peptoid' ligand PD 135,158, a compound that has been recently demonstrated to be a partial agonist in vivo (Ding et al., *Gastroenterology*, 109:1181-87, 1995).

[0071] Precedent with the constitutively active CCK-B/gastrin receptor illustrates a new strategy using mutant receptors as a 'magnifying glass' to screen for non-peptide lead compounds with some degree of intrinsic activity. The constitutively active ^{325}L→E mutant MH162 reliably enhanced detection of the intrinsic activity a compound possesses for stimulating the wild-type receptor (**FIG. 6**). This was true over the spectrum of peptide, 'peptoid,' and non-peptide ligands tested.

TABLE 1

A) ^{125}I CCK-8 binding affinities of tested ligands

Compound	Wild-type receptor Ki (nM)	MH162 Mutant Ki (nM)	Ratio (Wild-type/ Mutant)
Gastrin I	1.35 ± 0.28	0.80 ± 0.16	1.69
CCK-8s	0.12 ± 0.01	0.07 ± 0.01	1.71
PD 135, 158	2.25 ± 0.61	1.01 ± 0.19	2.23
PD 136, 450	0.99 ± 0.1	0.59 ± 0.12	1.68
L-740, 093 R	0.19 ± 0.02	0.18 ± 0.04	1.06
YM022	0.07 ± 0.01	0.08 ± 0.01	0.88
L-364, 718	150 ± 42	170 ± 34	0.88
L-365, 158	7.16 ± 0.87	7.83 ± 1.51	0.91
L-740, 093 S	19.5 ± 1.5	16 ± 1.4	1.22

TABLE 1-continued

	B) Signaling potencies of tested ligands		
Compound	Wild-type receptor IC50 (nM) 95% C.I.	MH162 Mutant IC50 (nM) 95% C.I.	Ratio (Wild-type/ Mutant)
Gastrin I	0.24 (0.12–0.48)	0.20 (0.06–0.69)	1.20
CCK-8s	0.14 (0.11–0.19)	0.15 (0.08–0.30)	0.93
PD 135, 158	1.05 (0.29–3.83)	1.01 (0.21–4.80)	1.04
PD 136, 450	0.36 (0.04–3.35)	0.58 (0.23–1.46)	0.62

[**0072**] B. CCK-A receptor: In another example of using an enhanced receptor to identify an agonist, a CCK-A receptor mutant, MHA21/35 (^{138}E→Q and ^{303}ANLM→HVSA modifications of SEQ ID NO: 5), was used to test the peptoid compound PD 136,450. **FIG. 12** illustrates the ability of the MHA 21/35 CCK-A mutant receptor to enhance the intrinsic agonist activity of the peptide CCK-8, the non-peptide L-365, 260, and the peptoid PD 136,450 relative to human wild-type CCK-A receptor. **FIG. 13** illustrates the amplification of partial agonist efficacy by the constitutive CCK-A receptor MHA21/35 as a function of the concentration of peptoid agonist PD 136,450.

[**0073**] II. Receptor Binding and Activity Assays:

[**0074**] A. Receptor Binding Assays: The binding of a ligand to a CCK receptor, e.g., the CCK-A or the CCK-B/gastrin receptor, can be measured according to the following example. In this example, the binding affinity of a ligand to the human CCK-B/gastrin receptor is measured.

[**0075**] COS-7 cells (1.5×10^6) were plated in 10-cm culture dishes (Nunc) and grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in a 5% CO_2, 95% air incubator at 37° C. After an overnight incubation, cells were transfected (Pacholczyk et al., *Nature* 350:350-354, 1991) with 5-7 μg of a pcDNA I expression vector containing hCCKB (HCCKB-pcDNA I). Twenty-four hours after transfection cells were split into 24-well dishes (2×10^4 cells/well) (Costar). After an additional 24 hours, competition binding experiments were performed in Hank's buffer supplemented with 25 mM phenylmethylsulfonyl fluoride (PMSF). Twenty pM of ^{125}I CCK-8 (DuPont-New England Nuclear) was used as radioligand. Equilibrium binding occurred after incubation for 80 min. at 37° C. Cell monolayers were then washed three times, hydrolyzed in 1 N NaOH, and the amount of radioactivity to the receptor was quantified. Unlabeled agonists (e.g., CCK-8s, unsulphated CCK-8 (CCK-8us), gastrin I, CCK-4 (Peninsula)) and antagonists (L364,718 and L365,260 (Merck)) were tested over the concentration range of 0.1 pM to 10 μM. All binding experiments were repeated three to five times.

[**0076**] The competition data were analyzed using computer software which is specifically designed for the purpose of radioligand binding assays (Inplot 4.0, GraphPad, San Diego, Calif.). Analyses of competition and saturation binding data can also be performed using computerized non-linear curve fitting (McPherson, G. A., *J Pharmacol Methods*, 14:213-28, 1985).

[**0077**] The affinities of all agonists and antagonists were confirmed by repeating the above assay using Chinese hamster ovary (CHO) cells stably transfected with human CCK-B/gastrin receptor cDNA. This CHO cell line was established by transfecting a hCCKB-pcDNAI Neo expression vector (Invitrogen) into CHO cells using a standard lipofection protocol (Bethesda Research Laboratories) followed by G418 selection.

[**0078**] Where binding parameters are determined in isolated plasma membranes, binding can be performed, e.g., for 60 min. at 22° C. (Kopin et al., *Proc. Natl. Acad. Sci. USA*, 89:3605-09, 1992). Separation of bound and free radioligand can be achieved by receptor-binding filtermat filtration (Klueppelberg, U. G., et al., 1989, *Biochemistry* 28:3463-8).

[**0079**] In order to compare the binding specificity of CCK-B/gastrin mutant receptors of the invention with the binding specificity typical of wild-type CCK-B/gastrin receptors see Matsumoto et al. (*Am J Physiol.*, 252:G143-G147, 1987) and Lee et al. (*J. Biol. Chem.*, 268(11):8164-69, 1993).

[**0080**] Comparison of binding affinity to that of a wild-type human CCK-B receptor: A base line value for binding of a radiolabelled ligand to a human wild-type receptor, e.g., the human CCK-B/gastrin receptor was determined (see Lee et al., *J. Biol. Chem.*, 268(11):8164-69, 1993). Agonist affinities of the human brain CCK-B/gastrin receptor expressed in COS-7 cells were characterized (**FIG. 7**). The structurally related agonists CCK-8s, gastrin I, and CCK-4 all competed in a concentration-dependent manner for binding of ^{125}I-CCK-8 to COS-7 cells expressing the recombinant receptor. The calculated IC_{50} values for CCK-8s, gastrin I, and CCK-4 are 0.14, 0.94, and 32 nM respectively (**FIG. 7**, part A). Similar ^{125}I-CCK-8 competition curves were assessed with L-364,718 and L-365,260 (**FIG. 7**, part B), and revealed IC_{50} values of 145 and 3.8 nM, respectively. Untransfected cells showed no displaceable binding.

[**0081**] B. Receptor Signaling Activity Assays:

[**0082**] Binding of an agonist to a CCK receptor elicits an increase in the intracellular calcium concentration and in phosphatidylinositol hydrolysis.

[**0083**] Measurement of $[Ca^{2+}]$: Forty-eight hours after transfection with hCCKB-pcDNAI, COS-7 cells were loaded with the Ca^{2+} fluorophore fura-2 in modified Krebs-Ringer bicarbonate buffer. Changes in the fluorescence emission ratios (340:380 nm) after stimulation of cells with 10^{-7}M CCK-8s or 10^{-6}M gastrin I were measured as previously described (Rajan et al., *Diabetes*, 38:874-80, 1989). Extracellular calcium can be chelated with EGTA (2.5 mM) to confirm that a gastrin-induced increase in $[Ca^{2+}]$ originates primarily from intracellular $[Ca^{2+}]$ pools.

[**0084**] Measurement of Inositol phosphate Metabolites: COS-7 cells transfected with hCCKB-pcDNAI were cultured in inositol-free Dulbecco's modified Eagle's medium (DMEM, GIBCO) which was supplemented with 10 μCi/ml [^{3}H] myo-inositol (ARC) for 24 hours prior to analysis. After 1 hour of equilibration in modified Krebs-Ringer bicarbonate, the cells were stimulated with 10^{-7}M CCK-8s for 10 seconds and harvested in methanol-HCl. The aqueous phase was extracted with chloroform, lyophilized, and analyzed for inositol 1,4,5-triphosphate (Ins-1,3,4,5-P_3) and inositol 1,3,4,5-tetrakisphosphate (Ins-1,3,4,5-P_4) by strong anion-exchange high performance liquid chromatography (Auger et al., *Cell*, 57:167-75, 1989).

[0085] Comparison of signaling activity to that of a wild-type human CCK-B receptor: A baseline level of human wild-type CCK-B receptor second messenger signaling activity was measured in response to CCK-8s stimulation of COS-7 cells expressing the receptor (**FIG. 8**; see Lee et al., *J. Biol. Chem.*, 268(11):8164-69, 1993). CCK-8s (10^{-7}M) triggered a marked increase in free cytosolic calcium, $[Ca^{2+}]_i$ (**FIG. 8**, part A, left panel). There was no change in free cytosolic calcium in cells transfected with the empty expression vector, pcDNAI. After chelation of extracellular calcium (1.5 mM Ca^{2+} in the buffer) by 2.5 mM EGTA, addition of CCK-8s (10^{-7} M) still transiently increased $[Ca^{2+}]_i$ (**FIG. 8**, part A right panel), suggesting that the initial peak of the CCK-induced increase in $[Ca^{2+}]_i$ originated primarily from intracellular Ca^{2+} pools. The arrows indicate the addition of CCK-8s (0.1 μM) or EGTA (2.5 mM). The pattern of $[Ca^{2+}]_i$ response suggests that the binding of CCK-8s to the recombinant receptor triggers intracellular signaling through activation of phospholipase C. This was confirmed by measurement of inositol phosphate metabolites in hCCKB-pcDNA I-transfected COS-7 cells 10 seconds after CCK-8s stimulation (**FIG. 8**, part B). This time point was chosen because it immediately precedes the CCK-8-induced $[Ca^{2+}]_i$ peak. CCK-8s (10^{-7}M) increased the level of Ins-1,4,5-P_3 by 453% over control, unstimulated hCCKB-pcDNA I-transfected COS-7 cells (n=3, $p<0.001$). The level of Ins-1,3,4,5-P_4, an immediate metabolite of Ins-1,4,5,-P_3, also increased by 186% over control (n=3, $p<0.01$).

[0086] A simplified method for measuring total inositol phosphate content: While the above method specifically assesses Ins(1,4,5)P_3 content, a simplified screening method can be used to test for the total concentration of inositol phosphate; the simplified method does not distinguish between specific isoforms. (This method was used to measure inositol phosphate generation for the experiments shown in **FIGS. 3, 4, 5, 6**, and **10**.)

[0087] COS-7 cells transfected with receptor cDNA-pcDNAI were cultured in inositol-free, serum-free Dulbecco's modified Eagle's medium (DMEM, GIBCO), supplemented with 3 μCi/ml ^{3}H-myo-inositol (NEN, 45-80 Ci/mmol), for 18 hours prior to analysis. The cells were then washed twice with DMEM/10 mM $LiCl_2$ and twice with phosphate-buffered saline/10 mM $LiCl_2$. After stimulation with putative agonists in phosphate-buffered saline 10/mM $LiCl_2$ for 30 minutes at 37° C., cells were scraped in ice-cold methanol. Lipids were extracted with chloroform (Pfeiffer et al., *FEBS Lett.*, 204:352-356, 1986). The upper phase was analyzed for inositol phosphates by strong anion exchange chromatography, using Dowex 1-X8 columns (BIORAD) and differential elution with water/60 mM ammonium fornate/2 M ammonium fornate. Eluted radioactivity was measured by liquid scintillation counting, and inositol phosphate content was expressed as a percentage of total ^{3}H-radioactivity applied to the columns.

[0088] Further information on the second messenger pathways linked to the native parietal cell gastrin receptor can be obtained in the following references: Muallem, S. et al., 1984, *Biochim Biophys Acta* 805:181-5; Chew, C. S. et al., 1986, *Biochim Biophys Acta* 888:116-25; Roche,S. et al., 1991, *FEBS Letts.*, 282:147-51.

[0089] In addition to inositol phosphate production, second messenger signaling activity can be measured according to, e.g., cAMP, cGMP, ppGpp, or calcium ion production, or using as indicators, e.g., intracellular pH, pH-sensitive dyes, or expression of a reporter gene, e.g., a luciferase gene, or measuring channel activity or cell depolarization or hyperpolarization by electrophysiological techniques.

[0090] III. Suitable Peptide Hormone Receptors with the Ability to Amplify the Intrinsic Activity of a Non-peptide Agonist:

[0091] The screening assay of the invention can be performed using peptide hormone receptors that have a higher activity than the corresponding human wild-type receptor. An enhanced basal activity amplifies the intrinsic activity of ligands, and is useful for detecting either activation of the receptor by a partial agonist, or inhibition by an inverse agonist. Receptors that do not have an enhanced basal activity relative to the corresponding wild-type receptor, but still amplify the intrinsic activity of a partial agonist, are also useful.

[0092] Examples of peptide hormone receptors that are useful for screening non-peptide agonists include various forms of the receptors that interact with the following peptide hormones (along with references for their respective wild-type amino acid sequences): amylin, angiotensin, bombesin, bradykinin, C5a anaphylatoxin, calcitonin, calcitonin-gene related peptide (CGRP), chemokines, cholecystokinin (CCK), endothelin, follicle stimulating hormone (FSH), formyl-methionyl peptides, galanin, gastrin, gastrin releasing peptide, glucagon, glucagon-like peptide 1, glycoprotein hormones, gonadotrophin-releasing hormone, leptin, luteinizing hormone (LH), melanocortins, neuropeptide Y, neurotensin, opioid, oxytocin, parathyroid hormone, secretin, somatostatin, tachykinins, thrombin, thyrotrophin, thyrotrophin releasing hormone, vasoactive intestinal polypeptide (VIP), and vasopressin. An enhanced receptor. can further embrace a single transmembrane domain peptide hormone receptor, e.g., an insulin receptor. The wild-type amino acid sequences of the above peptide hormone receptors is available in, and/or referenced in, Watson and Arkinstall, *The G-Protein Linked Receptor*, Academic Press, NY., 1994.

[0093] Forms of a peptide hormone receptor that are capable of amplifying the intrinsic activity of an agonist include, but are not limited to, the following forms of receptors:

[0094] 1. Mutant peptide hormone receptors that are capable of amplifying the intrinsic activity of partial. agonists.

[0095] An example is given of a mutant human CCK-A receptor that enhances the intrinsic activity of the partial 'peptoid' agonist PD 135,158, yet causes no apparent increase in agonist-independent basal receptor activity, is the mutant CCK-A receptor MHA35. MHA35 was made by replacing amino acids 138-ERY-140 of the human wild-type CCK-A receptor with QRY in the vector pcDNAI. (See **FIG. 2** for an illustration of the wild-type CCK-A receptor amino acid sequence.)

[0096] 2. CCK-A receptors in which one or more of residues ^{138}E, ^{305}L, and ^{312}I are replaced with any other amino acid residue, e.g., a serine, aspartic acid, glutamine, or glutamic acid residue. For example, a constitutively active CCK-A receptor mutant was constructed by replacing

five amino acids (^{138}E→Q and ^{303}ANLM→HVSA modifications of SEQ ID NO: 5); the resulting modified receptor is called MHA 21/35.

[0097] 3. CCK-B/gastrin receptors in which one or more of residues ^{151}E, ^{325}L, and ^{332}V are replaced with any other amino acid residue, e.g., a serine, aspartic acid, glutamine, or glutamic acid residue.

[0098] 4. Naturally-occurring Mutant Receptors, including but not limited to naturally-occurring constitutively active mutant receptors, that are associated with a disease or other adverse phenotype, e.g., a phenotype that results from a constitutively active naturally-occurring mutant receptor. Examples include, but are not limited to, the following peptide hormone receptors:

[0099] a) Point mutations in the luteinizing hormone (LH) receptor gene are responsible for some incidences of precocious puberty. Mutant receptors of the invention can be constructed by altering the following amino acid residues of the LH receptor: the alanine residue at position 568 to another amino acid, e.g., to a valine (Latronico et al., *J. Clin. Endo. & Meta.*, 80(8):2490-94, 1995); the asparagine residue at position 578 to another amino acid, e.g., to a glycine or a tyrosine (Kosugi et al., *Human Mol. Genet.*, 4(2):183-88, 1995; Laue et al., *Proc. Natl. Acad. Sci. USA*, 92(6):1906-10, 1995); the Met residue at position 571 to another amino acid, e.g., to an Ile, or the Thr residue at 577 to another amino acid residue, e.g., to an Ile (Kosugi et al., supra; Laue et al. supra); the Ile residue at position 542 to another amino acid, the Asp residue at position 564 to another amino acid, the Cys residue at position 581 to another amino acid, or the Asp residue at position 578 to another amino acid (Laue et al., supra); amino acid residues within transmembrane helices 5 or 6, e.g., in the intracellular domain-proximal portion of transmembrane helix 6, or in intracellular loop 3 (Laue et al. supra).

[0100] Also embraced are mutations at the corresponding residues of the follicle stimulating hormone (FSH) receptor and the thyroid stimulating hormone (TSH) receptor (Latronico et al., supra).

[0101] b) A naturally-occurring constitutively active parathyroid (PTH) receptor can result from a His to Arg substitution at conserved position 223 (Schipani et al., *Science*, 268:98-100, 1995). A constitutively active mutant G-LP1 receptor can be constructed by substituting alternative amino acids at the corresponding residues in related receptors, e.g., substituting another amino acid for the homologue His in the glucagon-like peptide 1 (G-LP1) receptor. A similar change in any of the receptors related to PTH or G-LP1 by amino acid homology including, but not limited to, secretin, vasoactive intestinal polypeptide, glucagon, G-LP1, and calcitonin.

[0102] Non-peptide positive or inverse agonists identified in a screening assay employing any of the above-listed naturally occurring mutant receptors can be therapeutically useful against a corresponding adverse pnenotype.

[0103] 5. Strategies to identify synthetic mutant receptors.

[0104] Deletional analysis defines intracellular receptor domains important in second messenger signaling: Recombinant CCK-A and CCK-B/gastrin receptors are both coupled to phospholipase-C activation. Applicants hypothesized that the third intracellular loop of the CCK-B/gastrin receptor would include residues that are important in second messenger signaling. To test this hypothesis, a series of deletion mutants was made, each lacking between six and 55 amino acids located in the third intracellular loop. Each mutant receptor was expressed in COS-7 cells and was tested for [^{125}I]CCK-8s binding and ^{3}H inositol phosphate formation. Deletion of a twelve amino acid segment in the carboxy-terminal end of the third intracellular loop resulted in normal affinity and capacity for CCK-8s binding, but caused a 90% reduction of maximal CCK-8s induced inositol phosphate formation; all other receptors in this series signaled normally. The region containing the twelve amino acids that proved to functionally important was then screened for constitutively active point mutations, as described below.

[0105] Strategy 1: Domain swapping with cAMP generating receptors results in constitutive receptor activity: A method for rapidly identifying constitutively active mutant receptors relies on exchanging functional domains between two receptors, the domains being, e.g., approximately 5-10 amino acids in length. One of the two receptors is a form of the receptor which is the main template of the desired mutant receptor, e.g., a wild-type receptor; the second receptor is a different peptide hormone receptor from the first. Candidate receptors are coupled to different signal transduction pathways, e.g., a signal via a same or different second messenger pathways, yet are closely related in their amino acid sequence. These criteria are based on the idea that stretches of amino acids which function normally in their native context can confer agonist-independent signaling when transplanted into a closely related receptor which is linked to a different second-messenger signaling pathway.

[0106] The domain swapping strategy was used to identify constitutively active mutants of the CCK-B/gastrin receptor. A series of short segments in the third intracellular loop were sequentially replaced with homologous amino acid sequence from the vasopressin 2 receptor, which is the receptor most nearly identical in sequence to hCCK-B. Vasopressin 2 is also a good candidate for swapping domains with the CCK-B/gastrin receptor because it is, different from the latter, linked to the adenylate cyclase signaling pathway.

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309 transmembrane domain VI 359

LT APGPGSGSRP TQAKLLAKKR VVRMLLVIVV LFFLCWLPVY SANTWR AFD

AHVSA [MH40]

SA [MH128]

-continued

S	[MH156]
E	[MH162]

[**0107**] When tested, a five amino acid substitution (QAKLL (SEQ ID NO: 13) to AHVSA (SEQ ID NO: 14)) into the homologous position of the CCK-B/gastrin receptor resulted in constitutive activity of the CCK-B/gastrin receptor. The QAKLL (SEQ ID NO: 13) to AHVSA (SEQ ID NO: 14) substitution caused an increased level of basal inositol phosphate formation to 290% of the wild-type CCK-B/gastrin receptor (**FIG. 9**, Mutant 2). In addition, mutations causing constitutive activity include replacement of LL to SA, L to S, and L to E.

[**0108**] Strategy 2: Glutamic acid scanning mutagenesis identifies constitutively active receptors: In addition to, or as a substitute for, deletion analysis or domain swapping, mutant receptors can be made using a process applicants have named 'amino acid scanning mutagenesis.' Amino acid scanning mutagenesis involves sequentially replacing each amino acid found in either an intracellular loop or in the half of the transmembrane domain flanking the intracellular portion of the receptor. An experimental option is to change the charge of the amino acid, e.g., to exchange a negative for a positive amino acid, a positive for a negative amino acid, or a positive or negative amino acid for a neutral amino acid. Another option would be to exchange each amino acid, e.g., each neutral amino acid, with another neutral amino acid.

[**0109**] In the case of the CCK-B/gastrin receptor, deletion analysis was initially used to define a functionally important twelve amino acid segment within the third intracellular loop which was important for second messenger signaling. Subsequently, each of the neutral amino acids within the 12 of this segment was replaced sequentially with another amino acid, preferably with glutamic acid. The scanning analysis technique revealed that one of the glutamic acid substitutions caused a 228% increase in the basal-level of inositol phosphate accumulation, relative to the wild-type value, in transiently transfected COS-7 cells (**FIG. 9**, Mutant 1).

[**0110**] In this example, applicants focused on the region limited to the carboxy end of the third intracellular (IC) loop and the portion of the sixth transmembrane domain which flanks the third IC loop. Glutamic acid residues (E) were introduced in place of neutral amino acid residues.

```
   309                                                    359
25 LT APGPGSGSRP TQAKLLAKKR VVRMLLVIVV LFFLCWLPVY SANTWR AFD
                    E E E    EE
```

[**0111**] Constitutively active receptors include an amino acid replacement at ^{325}L→E (MH162; SEQ ID NO: 19), and at ^{332}V→E (MH129; SEQ ID NO: 22). Each mutant was constructed in a pcDNAI vector as described above.

[**0112**] **FIG. 9** is a schematic representation of the seven transmembrane (TM) domain structure of the human CCK-B/gastrin receptor. The C-terminal domain of the third intracellular loop, which is crucial for intracellular signaling, is highlighted in black. Within this segment, two mutations were found to confer constitutive activity on the receptor. One of the mutations was constructed by glutamic acid substitution scanning (Mutant 1; MH129); a second mutation was constructed by domain swapping (Mutant 2; MH162). A bar graph showing the basal inositol phosphate accumulation in COS-7 cells, which had been transfected with the wild-type CCK-B/gastrin receptor or with two different constitutively active mutants, is shown in **FIG. 10**.

[**0113**] Strategy 3: A third method for making a mutant receptor is to align the receptor of interest with a known constitutively active mutant receptor, including, but not limited to, peptide hormone, biogenic amine, rhodopsin, or other G-protein coupled receptors. An example of such an alignment is shown in **FIG. 2**. Generally, mutations which result in constitutive activity in the known mutant can be introduced into the corresponding position of the receptor of interest. Examples of known constitutively active mutant receptors include, but are not limited to, the follicle stimulating hormone (FSH) receptor, the thyroid stimulating hormone (TSH) receptor, and the luteinizing hormone receptor, e.g., a 568 Ala to Val mutation in the LH receptor (Latronico et al., *J. Clin. Endo. & Meta.*, 80(8):2490-94, 1995).

[**0114**] This method, based on alignment, was employed to construct a CCK-A mutant receptor. A multiple alignment map was made which included the human and rat CCK-A sequences, the mastomys, rat, human, and canine CCK-B/gastrin receptor, and a Xenopus CCK-A/CCK-B intermediate receptor (CCK-XL; **FIG. 2**). Based on this map, conserved amino acids 138-ERY-140 of the CCK-A receptor were replaced with amino acids QRY, based on a known constitutively active rhodopsin mutant with enhanced transducing activation (Arnis et al., *J. Biol. Chem.*, 269:23879-81, 1994). The altered amino acid residues are positioned in transmembrane domain III and flank the second intracellular loop. Although the basal level of signaling was not increased, the intrinsic activity of the non-peptide ligand PD 135,158 was significantly increased.

[**0115**] Strategy 4: Additional mutant receptors can be made by sequentially deleting intracellular portions of the receptor, and looking for an increase in basal activity, or for overactivity of a partial agonist, relative to the wild-type receptor.

[**0116**] Wild-type Receptors with Enhanced Basal Activity:

[**0117**] Peptide hormone receptors useful in the method of the invention can include non-human receptors which have the ability to amplify the intrinsic activity of non-peptide

agonist as compared to the corresponding human wild-type receptor, or which have a higher basal level of activity than does the human wild-type receptor.

[**0118**] In **FIG. 11**, basal levels of inositol phosphate production were measured for human CCK-A (hCCK-A), human CCK-B (hCCK-B), dog CCK-B (dCCK-B), mouse CCK-B (mCCK-B), and the mastomys CCK receptor (**FIG. 11**, part A), and expressed relative to the basal level of hCCK-B.

[**0119**] Single experiments were also performed for the rat CCK-B/gastrin receptor and for the related Xenopus CCK receptor (Table 2). The human ^{325}L to E mutant served as a positive control (n=14).

TABLE 2

receptor	basal (% of human basal)	CCK-8s stimulated (% human basal)
rat CCK-A	77	684
Xenopus CCK	74	442
MH162	231 ± 7	771 ± 36

[**0120**] The wild-type human CCK-A and CCK-B/gastrin receptors induced only insignificant changes of basal inositol phosphate production in COS-7 cells (as compared to control cells transfected with the empty plasmid vector, pcDNAI). Similarly, the wild-type rat CCK-A and canine CCK-B/gastrin receptors, as well as the closely related Xenopus CCK receptor all appeared more or less functionally silent in the basal state. In contrast, the wild type mouse CCK-B/gastrin receptor and its homologue from mastomys natalensis significantly increased basal inositol phosphate production in COS-7 cells over pcDNAI controls. When compared with the slight basal activity of the wild type human CCK-B/gastrin receptor, it was estimated that the basal activities of the wild type mouse and mastomys homologues were 7- and 11-fold higher, respectively. For comparison, the ^{325}L-E mutant of the human CCK-B/gastrin receptor appeared to be at least 16-fold more active than the human wild type receptor in its basal state. It should be noted that the described species differences in basal activities were clearly not related to different degrees of receptor expression, since the maximal response to stimulation with CCK-8s was comparable for all tested receptors (positive control).

[**0121**] IV. Therapeutic Use.

[**0122**] The ability to pharmacologically modulate wild-type or constitutively active receptor activity opens the door for a new class of clinically useful drugs. Enhanced receptors enable the discovery of drugs with the ability to act as agonists, thereby having advantages for treatment or prevention of a broad spectrum of diseases. Constitutively active mutants of the thyrotropin, luteinizing hormone, and parathyroid hormone receptors are already known to occur in nature (see above) and might provide a starting point for non-peptide agonist/inverse agonist screening. For example, drugs which silence constitutively active thyroid stimulating hormone receptors, which are implicated in the etiology of thyroid adenomas, could be used to inhibit tumor growth. Similarly, in patients with constitutively active luteinizing hormone receptors, inverse agonists could delay the onset of precocious puberty.

[**0123**] Non-peptide agonists, including but not limited to the compounds of formula (I), are useful as agonists for treating and preventing central nervous system disorders wherein CCK and/or gastrin receptors are involved. Examples of such disease states include gastrointestinal diseases, including gastrointestinal ulcers such as peptic and duodenal ulcers, irritable bowel syndrome, gastroesophagenal reflux disease or excess pancreatic or gastrin secretion, acute pancreatitis, or motility disorders; central nervous system disorders, including central nervous system disorders caused by CCK interaction with dopamine, serotonin and other monoamine neurotransmitters, such as neuroleptic disorders, tardive dyskinesia, Parkinson's disease, psychosis, or Gilles de la Tourette syndrome; depression; schizophrenia; disorders of appetite regulatory systems; Zollinger-Ellison syndrome, antral and cell hyperplasia, or pain.

[**0124**] Non-peptide agonists are further useful for:

[**0125**] 1) the treatment or prevention of neurological disorders involving anxiety or panic, wherein a CCK and/or gastrin receptor is involved;

[**0126**] 2) directly inducing analgesia, opiate or non-opiate mediated, as well as anesthesia or loss of the sensation of pain;

[**0127**] 3) preventing or treating the withdrawal response produced by chronic treatment or abuse of drugs or alcohol. Such drugs include, but are not limited to, benzodiazepines, cocaine, alcohol, and nicotine;

[**0128**] 4) the treatment of stress and its relationship with drug abuse;

[**0129**] 5) the treating oncologic disorders wherein a CCK receptor may be involved. Examples of such oncologic disorders include small cell adenocarcinomas and primary tumors of the central nervous system glial and neuronal cells. Examples include, but are not limited to, tumors of the lower esophagus, stomach, intestine, colon, and lung, including small cell lung carcinoma;

[**0130**] 6) the treating or preventing of neurodegenerative disorders arising as a consequence of a pathological condition, e.g., stroke, hypoglycemia, cerebral palsy, transient cerebral ischemic attack, cerebral ischaemia during cardiac pulmonary surgery or cardiac arrest, perinatal asphyxia, epilepsy, Huntington's chorea, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, Olivo-ponto-cerebellar atrophy, anoxia such as from drowning, spinal cord and head injury, and poisoning by neurotoxins, including environmental neurotoxins.

[**0131**] The compounds of formula (I) may further be used to induce miosis for therapeutic purposes after certain types of examination and intraocular surgery. An example of intraocular surgery would include cataract surgery with implantation of an artificial lens. The CCK inverse agonist compounds can be used to prevent miosis occurring in association with, e.g., iritis, uveitis and trauma. Conversely, agonist derivatives can be used to induce miosis, e.g., for the treatment of glaucoma. The present invention therefore provides a compound of formula (I) or a salt or prodrug thereof for use in the preparation of a medicament.

[0132] The present invention also provides a compound of formula (I) for use in therapy.

[0133] In a further or alternative embodiment the present invention provides a method for the treatment or prevention of a physiological disorder involving CCK and/or gastrin receptor, which method comprises administration to a patient in need thereof of a CCK and/or gastrin receptor agonist or inverse agonist amount of a compound of formula (I).

[0134] When a compound is used as an agonist or inverse agonist of a peptide hormone receptor in a human subject, the daily dosage will normally be determined by the prescribing physician. The dosage generally will vary with the age, weight, and response of the individual patient, as well with as the severity of the patient's symptoms. However, in most instances, an effective daily dosage will be in the range of from about 0.001 mg/kg to about 2 μg/kg of body weight; preferably, of from about 0.01 mg/kg to about 200 mg/kg, e.g., from about 0.1 mg/kg to about 100 mg/kg of body weight, administered in single or divided doses. One skilled in the art knows that in some cases it will be necessary to use dosages outside these limits.

[0135] Further guidance from in vitro tests are instructive; e.g., agonists of the invention are effective in the subnanomolar range (e.g., L-740,093 R) and in the 20 nM range (e.g., L-740,093 S). An agonist of the invention can be administered orally in single or divided doses, or systemically, or by other means known to one skilled in the art.

[0136] Other Embodiments

[0137] The invention can further embrace additional methods of amplifying the activity of, so as to detect, an agonist or inverse agonist which affects the activity of a peptide hormone receptor. For example, an amplification scheme can involve modifying an intracellular factor which is involved neutralizing the expression of a peptide hormone receptor regulator, e.g., either a G-protein or a molecule which acts as a downstream messenger. An amplification scheme can alternatively involve optimizing the kinetic conditions of a signal transduction assay which is used for detecting receptor signaling. For example, the incubation time can be prolonged so as to detect agonist activity with a particular assay, e.g., a β-galactosidase assay, or shortened so as to avoid desensitization.

[0138] Further information on peptide hormone receptor amino acid sequences, receptor-specific agonists and antagonists, receptor conformation, pharmacology, receptor-encoding genes, animal models for subsequent follow-up studies, and database accession numbers can be obtained from: Watson and Arkinstall, *The G-Protein Linked Receptor*, Academic Press, NY., 1994; see also, Kolakowski, L. F., "The G Protein-Coupled Receptor Database," World-Wide-Web Site, GCRDB-WWW.

[0139] From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

[0140] Other embodiments are within the following claims.

SEQUENCE LISTING

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Ser Gly Ser Ser Leu Cys His Pro Gly Val Ser Leu Leu Asn Ser Ser
            20                  25                  30

Ala Gly Asn Leu Ser Cys Glu Pro Pro Arg Ile Arg Gly Thr Gly Thr
        35                  40                  45

Arg Glu Leu Glu Leu Ala Ile Arg Ile Thr Leu Tyr Ala Val Ile Phe
    50                  55                  60

Leu Met Ser Ile Gly Gly Asn Met Leu Ile Ile Val Val Leu Gly Leu
65                  70                  75                  80

Ser Arg Arg Leu Arg Thr Val Thr Asn Ala Phe Leu Leu Ser Leu Ala
                85                  90                  95

Val Ser Asp Leu Leu Leu Ala Val Ala Cys Met Pro Phe Thr Leu Leu
            100                 105                 110

Pro Asn Leu Met Gly Thr Phe Ile Phe Gly Thr Val Ile Cys Lys Ala
        115                 120                 125
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Val Ser Tyr Leu Met Gly Val Ser Val Ser Val Ser Thr Leu Asn Leu
    130                 135                 140

Val Ala Ile Ala Leu Glu Arg Tyr Ser Ala Ile Cys Arg Pro Leu Gln
145                 150                 155                 160

Ala Arg Val Trp Gln Thr Arg Ser His Ala Ala Arg Val Ile Leu Ala
                165                 170                 175

Thr Trp Leu Leu Ser Gly Leu Leu Met Val Pro Tyr Pro Val Tyr Thr
            180                 185                 190

Val Val Gln Pro Val Gly Pro Arg Val Leu Gln Cys Met His Arg Trp
        195                 200                 205

Pro Ser Ala Arg Val Arg Gln Thr Trp Ser Val Leu Leu Leu Met Leu
    210                 215                 220

Leu Phe Phe Ile Pro Gly Val Val Met Ala Val Ala Tyr Gly Leu Ile
225                 230                 235                 240

Ser Arg Glu Leu Tyr Leu Gly Leu Arg Phe Asp Gly Asp Asn Asp Ser
                245                 250                 255

Asp Thr Gln Ser Arg Val Arg Asn Gln Gly Gly Leu Pro Gly Gly Thr
            260                 265                 270

Ala Pro Gly Pro Val His Gln Asn Gly Gly Cys Arg His Val Thr Val
        275                 280                 285

Ala Gly Glu Asp Asn Asp Gly Cys Tyr Val Gln Leu Pro Arg Ser Arg
    290                 295                 300

Leu Glu Met Thr Thr Leu Thr Thr Pro Thr Pro Gly Pro Gly Leu Ala
305                 310                 315                 320

Ser Ala Asn Gln Ala Lys Leu Leu Ala Lys Lys Arg Val Val Arg Met
                325                 330                 335

Leu Leu Val Ile Val Leu Leu Phe Phe Leu Cys Trp Leu Pro Ile Tyr
            340                 345                 350

Ser Ala Asn Thr Trp Cys Ala Phe Asp Gly Pro Gly Ala Met Arg Ala
        355                 360                 365

Leu Ser Gly Ala Pro Ile Ser Phe Ile His Leu Leu Ser Tyr Ala Ser
    370                 375                 380

Ala Cys Val Asn Pro Leu Val Tyr Cys Phe Met His Arg Arg Phe Arg
385                 390                 395                 400

Gln Ala Cys Leu Asp Thr Cys Ala Arg Cys Cys Pro Arg Pro Pro Arg
                405                 410                 415

Ala Arg Pro Arg Pro Leu Pro Asp Glu Asp Pro Pro Thr Pro Ser Ile
            420                 425                 430

Ala Ser Leu Ser Arg Leu Ser Tyr Thr Thr Ile Ser Thr Leu Gly Pro
        435                 440                 445

Gly
```

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<400> SEQUENCE: 2

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Met Glu Leu Leu Lys Leu Asn Arg Ser Val Gln Gly Pro Gly Pro Gly
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Ser Gly Ser Ser Leu Cys Arg Pro Gly Val Ser Leu Leu Asn Ser Ser
            20                  25                  30
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```
Ser Ala Gly Asn Leu Ser Cys Asp Pro Pro Arg Ile Arg Gly Thr Gly
        35                  40                  45

Thr Arg Glu Leu Glu Met Ala Ile Arg Ile Thr Leu Tyr Ala Val Ile
    50                  55                  60

Phe Leu Met Ser Val Gly Gly Asn Val Leu Ile Ile Val Val Leu Gly
65                  70                  75                  80

Leu Ser Arg Arg Leu Arg Thr Val Thr Asn Ala Phe Leu Leu Ser Leu
                85                  90                  95

Ala Val Ser Asp Leu Leu Leu Ala Val Ala Cys Met Pro Phe Thr Leu
            100                 105                 110

Leu Pro Asn Leu Met Gly Thr Phe Ile Phe Gly Thr Val Ile Cys Lys
        115                 120                 125

Ala Ile Ser Tyr Leu Met Gly Val Ser Val Ser Val Ser Thr Leu Asn
    130                 135                 140

Leu Val Ala Ile Ala Leu Glu Arg Tyr Ser Ala Ile Cys Arg Pro Leu
145                 150                 155                 160

Gln Ala Arg Val Trp Gln Thr Arg Ser His Ala Ala Arg Val Ile Leu
                165                 170                 175

Ala Thr Trp Leu Leu Ser Gly Leu Leu Met Val Pro Tyr Pro Val Tyr
            180                 185                 190

Thr Met Val Gln Pro Val Gly Pro Arg Val Leu Gln Cys Met His Arg
        195                 200                 205

Trp Pro Ser Ala Arg Val Gln Gln Thr Trp Ser Val Leu Leu Leu Leu
    210                 215                 220

Leu Leu Phe Phe Ile Pro Gly Val Val Ile Ala Val Ala Tyr Gly Leu
225                 230                 235                 240

Ile Ser Arg Glu Leu Tyr Leu Gly Leu His Phe Asp Gly Glu Asn Asp
                245                 250                 255

Ser Glu Thr Gln Ser Arg Ala Arg Asn Gln Gly Gly Leu Pro Gly Gly
            260                 265                 270

Ala Ala Pro Gly Pro Val His Gln Asn Gly Gly Cys Arg Pro Val Thr
        275                 280                 285

Ser Val Ala Gly Glu Asp Ser Asp Gly Cys Cys Val Gln Leu Pro Arg
    290                 295                 300

Ser Arg Leu Glu Met Thr Thr Leu Thr Thr Pro Thr Gly Pro Val Pro
305                 310                 315                 320

Gly Pro Arg Pro Asn Gln Ala Lys Leu Leu Ala Lys Lys Arg Val Val
                325                 330                 335

Arg Met Leu Leu Val Ile Val Leu Leu Phe Phe Leu Cys Trp Leu Pro
            340                 345                 350

Val Tyr Ser Val Asn Thr Trp Arg Ala Phe Asp Gly Pro Gly Ala Gln
        355                 360                 365

Arg Ala Leu Ser Gly Ala Pro Ile Ser Phe Ile His Leu Leu Ser Tyr
    370                 375                 380

Val Ser Ala Cys Val Asn Pro Leu Val Tyr Cys Phe Met His Arg Arg
385                 390                 395                 400

Phe Arg Gln Ala Cys Leu Asp Thr Cys Ala Arg Cys Cys Pro Arg Pro
                405                 410                 415

Pro Arg Ala Arg Pro Gln Pro Leu Pro Asp Glu Asp Pro Pro Thr Pro
            420                 425                 430
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Ser Ile Ala Ser Leu Ser Arg Leu Ser Tyr Thr Thr Ile Ser Thr Leu
        435                 440                 445

Gly Pro Gly
    450


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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Glu Leu Leu Lys Leu Asn Arg Ser Val Gln Gly Thr Gly Pro Gly
 1               5                  10                  15

Pro Gly Ala Ser Leu Cys Arg Pro Gly Ala Pro Leu Leu Asn Ser Ser
            20                  25                  30

Ser Val Gly Asn Leu Ser Cys Glu Pro Pro Arg Ile Arg Gly Ala Gly
        35                  40                  45

Thr Arg Glu Leu Glu Leu Ala Ile Arg Ile Thr Leu Tyr Ala Val Ile
    50                  55                  60

Phe Leu Met Ser Val Gly Gly Asn Met Leu Ile Ile Val Val Leu Gly
65                  70                  75                  80

Leu Ser Arg Arg Leu Arg Thr Val Thr Asn Ala Phe Leu Leu Ser Leu
                85                  90                  95

Ala Val Ser Asp Leu Leu Leu Ala Val Ala Cys Met Pro Phe Thr Leu
            100                 105                 110

Leu Pro Asn Leu Met Gly Thr Phe Ile Phe Gly Thr Val Ile Cys Lys
        115                 120                 125

Ala Val Ser Tyr Leu Met Gly Val Ser Val Ser Val Ser Thr Leu Ser
    130                 135                 140

Leu Val Ala Ile Ala Leu Glu Arg Tyr Ser Ala Ile Cys Arg Pro Leu
145                 150                 155                 160

Gln Ala Arg Val Trp Gln Thr Arg Ser His Ala Ala Arg Val Ile Val
                165                 170                 175

Ala Thr Trp Leu Leu Ser Gly Leu Leu Met Val Pro Tyr Pro Val Tyr
            180                 185                 190

Thr Val Val Gln Pro Val Gly Pro Arg Val Leu Gln Cys Val His Arg
        195                 200                 205

Trp Pro Ser Ala Arg Val Arg Gln Thr Trp Ser Val Leu Leu Leu Leu
    210                 215                 220

Leu Leu Phe Phe Ile Pro Gly Val Val Met Ala Val Ala Tyr Gly Leu
225                 230                 235                 240

Ile Ser Arg Glu Leu Tyr Leu Gly Leu Arg Phe Asp Gly Asp Ser Asp
                245                 250                 255

Ser Asp Ser Gln Ser Arg Val Arg Asn Gln Gly Gly Leu Pro Gly Ala
            260                 265                 270

Val His Gln Asn Gly Arg Cys Arg Pro Glu Thr Gly Ala Val Gly Glu
        275                 280                 285

Asp Ser Asp Gly Cys Tyr Val Gln Leu Pro Arg Ser Arg Pro Ala Leu
    290                 295                 300

Glu Leu Thr Ala Leu Thr Ala Pro Gly Pro Gly Gly Ser Gly Ser Arg
305                 310                 315                 320

Pro Thr Gln Ala Lys Leu Leu Ala Lys Lys Arg Val Val Arg Met Leu
                325                 330                 335
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Leu Val Ile Val Val Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser
            340                 345                 350

Ala Asn Thr Trp Arg Ala Phe Asp Gly Pro Gly Ala His Arg Ala Leu
        355                 360                 365

Ser Gly Ala Pro Ile Ser Phe Ile His Leu Leu Ser Tyr Ala Ser Ala
    370                 375                 380

Cys Val Asn Pro Leu Val Tyr Cys Phe Met His Arg Arg Phe Arg Gln
385                 390                 395                 400

Ala Cys Leu Glu Thr Cys Ala Arg Cys Cys Pro Arg Pro Pro Arg Ala
                405                 410                 415

Arg Pro Arg Ala Leu Pro Asp Glu Asp Pro Pro Thr Pro Ser Ile Ala
            420                 425                 430

Ser Leu Ser Arg Leu Ser Tyr Thr Thr Ile Ser Thr Leu Gly Pro Gly
        435                 440                 445


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Pro Gly Ala Ser Leu Cys Arg Ala Gly Gly Ala Leu Leu Asn Ser Ser
            20                  25                  30

Gly Ala Gly Asn Leu Ser Cys Glu Pro Pro Arg Leu Arg Gly Ala Gly
        35                  40                  45

Thr Arg Glu Leu Glu Leu Ala Ile Arg Val Thr Leu Tyr Ala Val Ile
    50                  55                  60

Phe Leu Met Ser Val Gly Gly Asn Val Leu Ile Ile Val Val Leu Gly
65                  70                  75                  80

Leu Ser Arg Arg Leu Arg Thr Val Thr Asn Ala Phe Leu Leu Ser Leu
                85                  90                  95

Ala Val Ser Asp Leu Leu Leu Ala Val Ala Cys Met Pro Phe Thr Leu
            100                 105                 110

Leu Pro Asn Leu Met Gly Thr Phe Ile Phe Gly Thr Val Val Cys Lys
        115                 120                 125

Ala Val Ser Tyr Leu Met Gly Val Ser Val Ser Val Ser Thr Leu Ser
    130                 135                 140

Leu Val Ala Ile Ala Leu Glu Arg Tyr Ser Ala Ile Cys Arg Pro Leu
145                 150                 155                 160

Gln Ala Arg Val Trp Gln Thr Arg Ser His Ala Ala Arg Val Ile Ile
                165                 170                 175

Ala Thr Trp Met Leu Ser Gly Leu Leu Met Val Pro Tyr Pro Val Tyr
            180                 185                 190

Thr Ala Val Gln Pro Ala Gly Gly Ala Arg Ala Leu Gln Cys Val His
        195                 200                 205

Arg Trp Pro Ser Ala Arg Val Arg Gln Thr Trp Ser Val Leu Leu Leu
    210                 215                 220

Leu Leu Leu Phe Phe Val Pro Gly Val Val Met Ala Val Ala Tyr Gly
225                 230                 235                 240

Leu Ile Ser Arg Glu Leu Tyr Leu Gly Leu Arg Phe Asp Glu Asp Ser
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                245                 250                 255

Asp Ser Glu Ser Arg Val Arg Ser Gln Gly Gly Leu Arg Gly Gly Ala
            260                 265                 270

Gly Pro Gly Pro Ala Pro Pro Asn Gly Ser Cys Arg Pro Glu Gly Gly
        275                 280                 285

Leu Ala Gly Glu Asp Gly Asp Gly Cys Tyr Val Gln Leu Pro Arg Ser
    290                 295                 300

Arg Gln Thr Leu Glu Leu Ser Ala Leu Thr Ala Pro Thr Pro Gly Pro
305                 310                 315                 320

Gly Gly Gly Pro Arg Pro Tyr Gln Ala Lys Leu Leu Ala Lys Lys Arg
                325                 330                 335

Val Val Arg Met Leu Leu Val Ile Val Val Leu Phe Phe Leu Cys Trp
            340                 345                 350

Leu Pro Leu Tyr Ser Ala Asn Thr Trp Arg Ala Phe Asp Ser Ser Gly
        355                 360                 365

Ala His Arg Ala Leu Ser Gly Ala Pro Ile Ser Phe Ile His Leu Leu
    370                 375                 380

Ser Tyr Ala Ser Ala Cys Val Asn Pro Leu Val Tyr Cys Phe Met His
385                 390                 395                 400

Arg Arg Phe Arg Gln Ala Cys Leu Glu Thr Cys Ala Arg Cys Cys Pro
                405                 410                 415

Arg Pro Pro Arg Ala Arg Pro Arg Pro Leu Pro Asp Glu Asp Pro Pro
            420                 425                 430

Thr Pro Ser Ile Ala Ser Leu Ser Arg Leu Ser Tyr Thr Thr Ile Ser
        435                 440                 445

Thr Leu Gly Pro Gly
    450


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 1               5                  10                  15

Pro Cys Glu Leu Gly Leu Glu Asn Glu Thr Leu Phe Cys Leu Asp Gln
            20                  25                  30

Pro Arg Pro Ser Lys Glu Trp Gln Pro Ala Val Gln Ile Leu Leu Tyr
        35                  40                  45

Ser Leu Ile Phe Leu Leu Ser Val Leu Gly Asn Thr Leu Val Ile Thr
    50                  55                  60

Val Leu Ile Arg Asn Lys Arg Met Arg Thr Val Thr Asn Ile Phe Leu
65                  70                  75                  80

Leu Ser Leu Ala Val Ser Asp Leu Met Leu Cys Leu Phe Cys Met Pro
                85                  90                  95

Phe Asn Leu Ile Pro Asn Leu Leu Lys Asp Phe Ile Phe Gly Ser Ala
            100                 105                 110

Val Cys Lys Thr Thr Thr Tyr Phe Met Gly Thr Ser Val Ser Val Ser
        115                 120                 125

Thr Phe Asn Leu Val Ala Ile Ser Leu Glu Arg Tyr Gly Ala Ile Cys
    130                 135                 140
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-continued

```
Lys Pro Leu Gln Ser Arg Val Trp Gln Thr Lys Ser His Ala Leu Lys
145                 150                 155                 160

Val Ile Ala Ala Thr Trp Cys Leu Ser Phe Thr Ile Met Thr Pro Tyr
                165                 170                 175

Pro Ile Tyr Ser Asn Leu Val Pro Phe Thr Lys Asn Asn Asn Gln Thr
            180                 185                 190

Ala Asn Met Cys Arg Phe Leu Leu Pro Asn Asp Val Met Gln Gln Ser
        195                 200                 205

Trp His Thr Phe Leu Leu Leu Ile Leu Phe Leu Ile Pro Gly Ile Val
    210                 215                 220

Met Met Val Ala Tyr Gly Leu Ile Ser Leu Glu Leu Tyr Gln Gly Ile
225                 230                 235                 240

Lys Phe Glu Ala Ser Gln Lys Lys Ser Ala Lys Glu Arg Lys Pro Ser
                245                 250                 255

Thr Thr Ser Ser Gly Lys Tyr Glu Asp Ser Asp Gly Cys Tyr Leu Gln
            260                 265                 270

Lys Thr Arg Pro Pro Arg Lys Leu Glu Leu Arg Gln Leu Ser Thr Gly
        275                 280                 285

Ser Ser Ser Arg Ala Asn Arg Ile Arg Ser Asn Ser Ser Ala Ala Asn
    290                 295                 300

Leu Met Ala Lys Lys Arg Val Ile Arg Met Leu Ile Val Ile Val Val
305                 310                 315                 320

Leu Phe Phe Leu Cys Trp Met Pro Ile Phe Ser Ala Asn Ala Trp Arg
                325                 330                 335

Ala Tyr Asp Thr Ala Ser Ala Glu Arg Arg Leu Ser Gly Thr Pro Ile
            340                 345                 350

Ser Phe Ile Leu Leu Leu Ser Tyr Thr Ser Ser Cys Val Asn Pro Ile
        355                 360                 365

Ile Tyr Cys Phe Met Asn Lys Arg Phe Arg Leu Gly Phe Met Ala Thr
    370                 375                 380

Phe Pro Cys Cys Pro Asn Pro Gly Pro Pro Gly Ala Arg Gly Glu Val
385                 390                 395                 400

Gly Glu Glu Glu Glu Gly Gly Thr Thr Gly Ala Ser Leu Ser Arg Phe
                405                 410                 415

Ser Tyr Ser His Met Ser Ala Ser Val Pro Pro Gln
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Met Ser His Ser Pro Ala Arg Gln His Leu Val Glu Ser Ser Arg Met
 1               5                  10                  15

Asp Val Val Asp Ser Leu Leu Met Asn Gly Ser Asn Ile Thr Pro Pro
            20                  25                  30

Cys Glu Leu Gly Leu Glu Asn Glu Thr Leu Phe Cys Leu Asp Gln Pro
        35                  40                  45

Gln Pro Ser Lys Glu Trp Gln Ser Ala Leu Gln Ile Leu Leu Tyr Ser
    50                  55                  60

Ile Ile Phe Leu Leu Ser Val Leu Gly Asn Thr Leu Val Ile Thr Val
65                  70                  75                  80
```

-continued

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Leu Ile Arg Asn Lys Arg Met Arg Thr Val Thr Asn Ile Phe Leu Leu
                85                  90                  95

Ser Leu Ala Val Ser Asp Leu Met Leu Cys Phe Cys Met Pro Phe Asn
            100                 105                 110

Leu Ile Pro Asn Leu Leu Lys Asp Phe Ile Phe Gly Ser Ala Val Cys
        115                 120                 125

Lys Thr Thr Thr Tyr Phe Met Gly Thr Ser Val Ser Val Ser Thr Phe
    130                 135                 140

Asn Leu Val Ala Ile Ser Leu Glu Arg Tyr Gly Ala Ile Cys Arg Pro
145                 150                 155                 160

Leu Gln Ser Arg Val Trp Gln Thr Lys Ser His Ala Leu Lys Val Ile
                165                 170                 175

Ala Ala Thr Trp Cys Leu Ser Phe Thr Ile Met Thr Pro Tyr Pro Ile
            180                 185                 190

Tyr Ser Asn Leu Val Pro Phe Thr Lys Asn Asn Asn Gln Thr Ala Asn
        195                 200                 205

Met Cys Arg Phe Leu Leu Pro Ser Asp Ala Met Gln Gln Ser Trp Gln
    210                 215                 220

Thr Phe Leu Leu Leu Ile Leu Phe Leu Leu Pro Gly Ile Val Met Val
225                 230                 235                 240

Val Ala Tyr Gly Leu Ile Ser Leu Glu Leu Tyr Gln Gly Ile Lys Phe
                245                 250                 255

Asp Ala Ser Gln Lys Lys Ser Ala Lys Glu Lys Lys Pro Ser Thr Gly
            260                 265                 270

Ser Ser Thr Arg Tyr Glu Asp Ser Asp Gly Cys Tyr Leu Gln Lys Ser
        275                 280                 285

Arg Pro Pro Arg Lys Leu Glu Leu Gln Gln Leu Ser Ser Gly Ser Gly
    290                 295                 300

Gly Ser Arg Leu Asn Arg Ile Arg Ser Ser Ser Ser Ala Ala Asn Leu
305                 310                 315                 320

Ile Ala Lys Lys Arg Val Ile Arg Met Leu Ile Val Ile Val Val Leu
                325                 330                 335

Phe Phe Leu Cys Trp Met Pro Ile Phe Ser Ala Asn Ala Trp Arg Ala
            340                 345                 350

Tyr Asp Thr Val Ser Ala Glu Lys His Leu Ser Gly Thr Pro Ile Ser
        355                 360                 365

Phe Ile Leu Leu Leu Ser Tyr Thr Ser Ser Cys Val Asn Pro Ile Ile
    370                 375                 380

Tyr Cys Phe Met Asn Lys Arg Phe Arg Leu Gly Phe Met Ala Thr Phe
385                 390                 395                 400

Pro Cys Cys Pro Asn Pro Gly Pro Pro Gly Val Arg Gly Glu Val Gly
                405                 410                 415

Glu Glu Glu Asp Gly Arg Thr Ile Arg Ala Leu Leu Ser Arg Tyr Ser
            420                 425                 430

Tyr Ser His Met Ser Thr Ser Ala Pro Pro Pro
        435                 440
```

<210> SEQ ID NO 7
<211> LENGTH: 453
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

-continued

<400> SEQUENCE: 7

```
Met Glu Ser Leu Arg Ser Leu Ser Asn Ile Ser Ala Leu His Glu Leu
 1               5                  10                  15

Leu Cys Arg Tyr Ser Asn Leu Ser Gly Thr Leu Thr Trp Asn Leu Ser
            20                  25                  30

Ser Thr Asn Gly Thr His Asn Leu Thr Thr Ala Asn Trp Pro Pro Trp
        35                  40                  45

Asn Leu Asn Cys Thr Pro Ile Leu Asp Arg Lys Lys Pro Ser Pro Ser
    50                  55                  60

Asp Leu Asn Leu Trp Val Arg Ile Val Met Tyr Ser Val Ile Phe Leu
65                  70                  75                  80

Leu Ser Val Phe Gly Asn Thr Leu Ile Ile Ile Val Leu Val Met Asn
                85                  90                  95

Lys Arg Leu Arg Thr Ile Thr Asn Ser Phe Leu Leu Ser Leu Ala Leu
            100                 105                 110

Ser Asp Leu Met Val Ala Val Leu Cys Met Pro Phe Thr Leu Ile Pro
        115                 120                 125

Asn Leu Met Glu Asn Phe Ile Phe Gly Glu Val Ile Cys Arg Ala Ala
    130                 135                 140

Ala Tyr Phe Met Gly Leu Ser Val Ser Val Ser Thr Phe Asn Leu Val
145                 150                 155                 160

Ala Ile Ser Ile Glu Arg Tyr Ser Ala Ile Cys Asn Pro Leu Xaa Ser
                165                 170                 175

Arg Val Trp Gln Thr Arg Ser His Ala Tyr Arg Val Ile Ala Ala Thr
            180                 185                 190

Trp Val Leu Ser Ser Ile Ile Met Ile Pro Tyr Leu Val Tyr Asn Lys
        195                 200                 205

Thr Val Thr Phe Pro Met Lys Asp Arg Arg Val Gly His Gln Cys Arg
    210                 215                 220

Leu Val Trp Pro Ser Lys Gln Val Gln Gln Ala Trp Tyr Val Leu Leu
225                 230                 235                 240

Leu Thr Ile Leu Phe Phe Ile Pro Gly Val Val Met Ile Val Ala Tyr
                245                 250                 255

Gly Leu Ile Ser Arg Glu Leu Tyr Arg Gly Ile Gln Phe Glu Met Asp
            260                 265                 270

Leu Asn Lys Glu Ala Lys Ala His Lys Asn Gly Val Ser Thr Pro Thr
        275                 280                 285

Thr Ile Pro Ser Gly Asp Glu Gly Asp Gly Cys Tyr Ile Gln Val Thr
    290                 295                 300

Lys Arg Arg Asn Thr Met Glu Met Ser Thr Leu Thr Pro Ser Val Cys
305                 310                 315                 320

Thr Lys Met Asp Arg Ala Arg Ile Asn Asn Ser Glu Ala Lys Leu Met
                325                 330                 335

Ala Lys Lys Arg Val Ile Arg Met Leu Ile Val Ile Val Ala Met Phe
            340                 345                 350

Phe Ile Cys Trp Met Pro Ile Phe Val Ala Asn Thr Trp Lys Ala Phe
        355                 360                 365

Asp Glu Leu Ser Ala Phe Asn Thr Leu Thr Gly Ala Pro Ile Ser Phe
    370                 375                 380

Ile His Leu Leu Ser Tyr Thr Ser Ala Cys Val Asn Pro Leu Ile Tyr
385                 390                 395                 400
```

-continued

```
Cys Phe Met Asn Lys Arg Phe Arg Lys Ala Phe Leu Gly Thr Phe Ser
                405                 410                 415

Ser Cys Ile Lys Pro Cys Arg Asn Phe Arg Asp Thr Asp Glu Asp Ile
            420                 425                 430

Ala Ala Thr Gly Ala Ser Leu Ser Lys Phe Ser Tyr Thr Thr Val Ser
        435                 440                 445

Ser Leu Gly Pro Ala
    450
```

<210> SEQ ID NO 8
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

```
Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
 1               5                  10                  15

Leu Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
            20                  25                  30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35                  40                  45

Ala Phe Asp
    50
```

<210> SEQ ID NO 9
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

```
Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Ala His Val
 1               5                  10                  15

Ser Ala Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
            20                  25                  30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35                  40                  45

Ala Phe Asp
    50
```

<210> SEQ ID NO 10
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

```
Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
 1               5                  10                  15

Ser Ala Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
            20                  25                  30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35                  40                  45

Ala Phe Asp
    50
```

<210> SEQ ID NO 11
<211> LENGTH: 51

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
 1 5 10 15

Ser Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
            20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35 40 45

Ala Phe Asp
    50

<210> SEQ ID NO 12
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
 1 5 10 15

Glu Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
            20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35 40 45

Ala Phe Asp
    50

<210> SEQ ID NO 13
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Gln Ala Lys Leu Leu
 1 5

<210> SEQ ID NO 14
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Ala His Val Ser Ala
 1 5

<210> SEQ ID NO 15
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
 1 5 10 15

Leu Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
            20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35 40 45

-continued

Ala Phe Asp
50

<210> SEQ ID NO 16
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Glu Lys
1 5 10 15

Glu Leu Glu Lys Lys Arg Glu Glu Arg Met Leu Leu Val Ile Val Val
20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 17
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Glu Lys
1 5 10 15

Leu Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 18
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Glu
1 5 10 15

Leu Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 19
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
1 5 10 15

Glu Leu Ala Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
20 25 30

-continued

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 20
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
1 5 10 15

Leu Leu Glu Lys Lys Arg Val Val Arg Met Leu Leu Val Ile Val Val
20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 21
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
1 5 10 15

Leu Leu Ala Lys Lys Arg Glu Val Arg Met Leu Leu Val Ile Val Val
20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 22
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys
1 5 10 15

Leu Leu Ala Lys Lys Arg Val Glu Arg Met Leu Leu Val Ile Val Val
20 25 30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
35 40 45

Ala Phe Asp
50

<210> SEQ ID NO 23
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Leu Thr Ala Pro Gly Pro Gly Ser Gly Ser Arg Pro Thr Gln Ala Lys

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 1               5                   10                  15

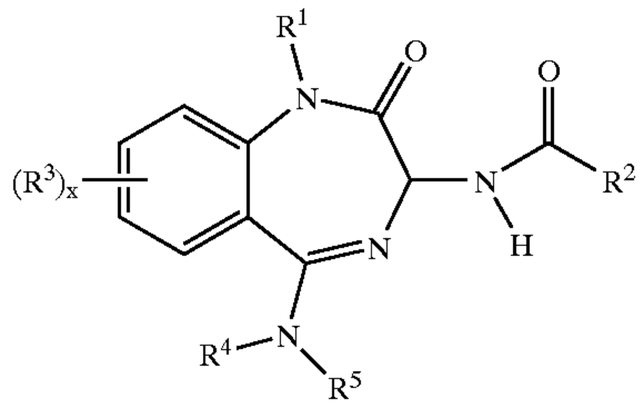
Leu Leu Ala Lys Lys Arg Glu Glu Arg Met Leu Leu Val Ile Val Val
            20                  25                  30

Leu Phe Phe Leu Cys Trp Leu Pro Val Tyr Ser Ala Asn Thr Trp Arg
        35                  40                  45

Ala Phe Asp
    50
```

What is claimed is:

1. A method for treating or preventing a physiological disorder involving a CCK or gastrin peptide hormone receptor, said method comprising administering to a mammal an agonist-effective amount of a compound of formula (I):



wherein:

R^1 represents H, C_{1-6} alkyl optionally substituted by one or more halo groups, C_{3-7} cycloalkyl, cyclopropylmethyl, $(CH_2)_r$imidazolyl, $(CH_2)_r$triazolyl, $(CH_2)_r$tetrazolyl, where r is 1, 2 or 3, $CH_2CO_2R^{11}$, where R^{11} is C_{1-4}alkyl, or $CH_2CONR^6R^7$, where R^6 and R^7 each independently represent H or C_{1-4}alkyl, or R^6 and R^7 together form a chain $CH_{2(p)}$ where p is 4 or 5;

R^2 represents NHR^{12} or $(CH_2)_sR^{13}$, where s is 0, 1, 2, or 3;

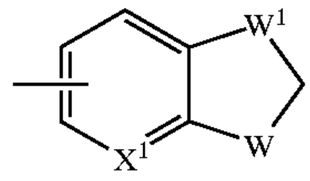
R^3 represents C_{1-6}alkyl, halo, or NR^6R^7, where R^6 and R^7 are as previously defined;

R^4 and R^5 each independently represent H, C_{1-12}alkyl optionally substituted by $NR^9R^{9'}$, where R^9 and R^9, are each independently H or C_{1-4}alkyl, an azacyclic or azabicyclic group, C_{4-9}cycloalkyl optionally substituted by one or more C_{1-4}alkyl groups, C_{4-9}cycloalkyl, C_{1-4}alkyl optionally substituted in the cycloalkyl ring by one or more C_{1-4}alkyl groups, optionally substituted aryl, optionally substituted arylC_{1-6}alkyl or azacyclic or azabicyclic groups, or R^4 and R^5 together form the residue of an optionally substituted azacyclic or azabicyclic ring system;

x is 0, 1, 2, or 3;

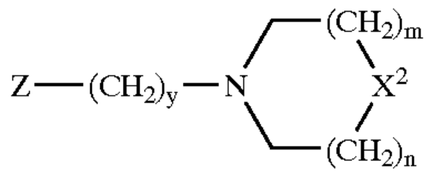
R^{12} represents a phenyl or pyridyl group optionally substituted by one or more substituents selected form C_{1-6}alkyl, halo, hydroxy, C_{1-4}alkoxy, $(CH_2)_q$-tetrazolyl optionally substituted in the tetrazole ring by C_{1-4}alkyl, $(CH_2)_q$-imidazolyl, $(CH_2)_q$-triazolyl, where q is 0, 1, 2, or 3, 5-hydroxy-4-pyrone, NR^6R^7, NR^9COR^{11}, $NR^9CONR^{9'}R^{11}$, where R^6, R^7, R^9, $R^{9'}$, and R^{11} are each as previously defined, $SO(C_{1-6}alkyl)$, $SO_2(C_{1-6}alkyl)$, trifluoromethyl, $CONHSO^2R^8$ or SO_2NHCOR^8, where R^8 is C_{1-6}alkyl, optionally substituted aryl, 2,2-difluorocyclopropane or trifluoromethyl, SO_2NHR^{10}, where R^{10} is a nitrogen containing heterocycle, $B(OH)_2$, $(CH_2)_qCO_2H$, where q is as previously defined; or

R^{12} represents a group:



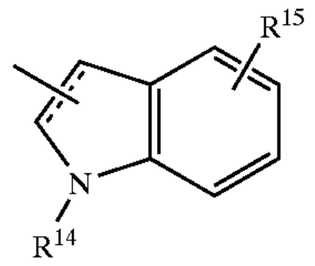
where X^1 represents CH or N; W represents CH_2 or NR^9, where R^9 is as previously defined, and W^1 represents CH_2, or W and W^1 each represent O; or

R^{12} represents phenyl substituted by a group:



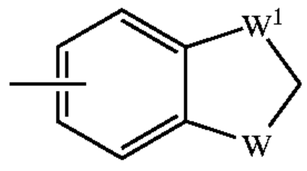
wherein X^2 is O, S, or NR^9, where R^9 is as previously defined; z is a bond, O, or S; m is 1, 2 or 3; n is 1, 2, or 3; and y is 0, 1, 2, or 3;

R^{13} represents a group:



where R^{14} represents H or C_{1-6}alkyl; R^{15} represents H, C_{1-6}alkyl, halo or NR^6R^7, where R^6 and R^7 are as previously defined; and the dotted line represents an optional covalent bond; and pharmaceutically acceptable salts or prodrugs thereof,

with the provision that, when NR^4R^5 represents an unsubstituted azacyclic ring system, R^2 does not represent NHR^{12} where R^{12} is optionally substituted phenyl or:



where W and W^1 are as previously defined.

2. The method of claim 1, wherein said compound is 3(R,S)-Amino-1,3-dihydro-5-((1S,4S)-5-methyl-2,5-diazabicyclo[2,2,1]heptan-2-vl)-2H-1-propyl-1,4-benzodiazepin-2-one.

3. The method of claim 1, wherein said compound is (−)-N-[5-(3-azabicyclo[3,2,2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1H-1,4-benzodiazepin-3-yl]-N'-[3-methylphenyl]-urea.

4. The method of claim 1, wherein said compound is (+)-N-[5-(3-azabicyclo[3,2,2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1H-1,4-benzodiazepin-3-yl]-N'-[3-methylphenyl]-urea.

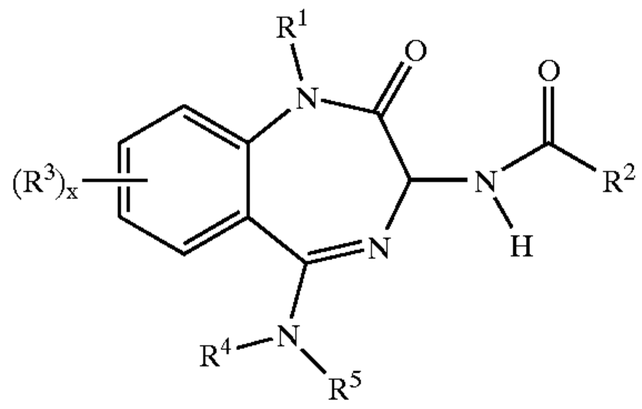
5. The method of claim 1, wherein said peptide hormone receptor is the CCK-B/gastrin receptor.

6. The method of claim 1, wherein said peptide hormone receptor is the CCK-A receptor.

7. The method of claim 1, wherein said physiological disorder is a neoplasm.

8. The method of claim 1, wherein said neoplasm is a primary tumor.

9. A compound of formula (I):



wherein:

R^1 represents H, C_{1-6} alkyl optionally substituted by one or more halo groups, C_{3-7} cycloalkyl, cyclopropylmethyl, $(CH_2)_r$imidazolyl, $(CH_2)_r$triazolyl, $(CH_2)_r$tetrazolyl, where r is 1, 2 or 3, $CH_2CO_2R^{11}$, where R^{11} is C_{1-4}alkyl, or $CH_2CONR^6R^7$, where R^6 and R^7 each independently represent H or C_{1-4}alkyl, or R^6 and R^7 together form a chain $CH_{2(p)}$ where p is 4 or 5;

R^2 represents NHR^{12} or $(CH_2)_sR^{13}$, where s is 0, 1, 2, or 3;

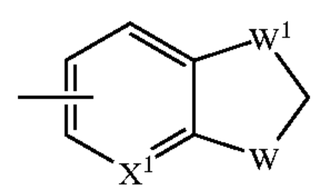
R^3 represents C_{1-6}alkyl, halo, or NR^6R^7, where R^6 and R^7 are as previously defined;

R^4 and R^5 each independently represent H, C_{1-12}alkyl optionally substituted by $NR^9R^{9'}$, where R^9 and R^9, are each independently H or C_{1-4}alkyl, an azacyclic or azabicyclic group, C_{4-9}cycloalkyl optionally substituted by one or more C_{1-4}alkyl groups, C_{4-9}cycloalkyl, C_{1-4}alkyl optionally substituted in the cycloalkyl ring by one or more C_{1-4}alkyl groups, optionally substituted aryl, optionally substituted arylC_{1-6}alkyl or azacyclic or azabicyclic groups, or R^4 and R^5 together form the residue of an optionally substituted azacyclic or azabicyclic ring system;

x is 0, 1, 2, or 3;

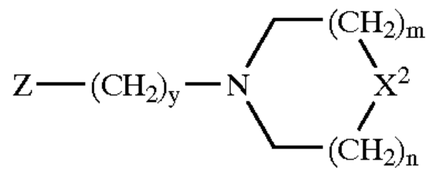
R^{12} represents a phenyl or pyridyl group optionally substituted by one or more substituents selected form C_{1-6}alkyl, halo, hydroxy, C_{1-4}alkoxy, $(CH_2)_q$-tetrazolyl optionally substituted in the tetrazole ring by C_{1-4}alkyl, $(CH_2)_q$-imidazolyl, $(CH_2)_q$-triazolyl, where q is 0, 1, 2, or 3, 5-hydroxy-4-pyrone, NR^6R^7, NR^9COR^{11}, $NR^9CONR^{9'}R^{11}$, where R^6, R^7, R^9, $R^{9'}$, and R^{11} are each as previously defined, $SO(C_{1-6}$alkyl), $SO_2(C_{1-6}$alkyl), trifluoromethyl, $CONHSO^2R^8$ or SO_2NHCOR^8, where R^8 is C_{1-6}alkyl, optionally substituted aryl, 2,2-difluorocyclopropane or trifluoromethyl, SO_2NHR^{10}, where R^{10} is a nitrogen containing heterocycle, $B(OH)_2$, $(CH_2)_qCO_2H$, where q is as previously defined; or

R^{12} represents a group:



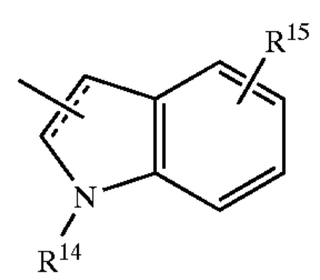
where X^1 represents CH or N; W represents CH_2 or NR^9, where R^9 is as previously defined, and W^1 represents CH_2, or W and W^1 each represent O; or

R^{12} represents phenyl substituted by a group:



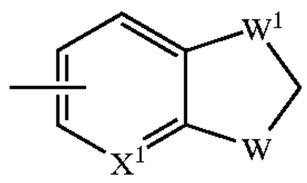
wherein X^2 is O, S, or NR^9, where R^9 is as previously defined; z is a bond, O, or S; m is 1, 2 or 3; n is 1, 2, or 3; and y is 0, 1, 2, or 3;

R^{13} represents a group:



where R^{14} represents H or C_{1-6}alkyl; R^{15} represents H, C_{1-6}alkyl, halo or NR^6R^7, where R^6 and R^7 are as previously defined; and the dotted line represents an optional covalent bond; and pharmaceutically acceptable salts or prodrugs thereof,

with the provision that, when NR^4R^5 represents an unsubstituted azacyclic ring system, R^2 does not represent NHR^{12} where R^{12} is optionally substituted phenyl or:



where W and W^1 are as previously defined.

10. The method of claim 9, wherein said compound is 3(R,S)-Amino-1,3-dihydro-5-((1S,4S)-5-methyl-2,5-diazabicyclo[2,2,1]heptan-2-vl)-2H-1-propyl-1,4-benzodiazepin-2-one.

11. The method of claim 9, wherein said compound is (−)-N-[5-(3-azabicyclo[3,2,2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1H-1,4-benzodiazepin-3-yl]-N'-[3-methylphenyl]-urea.

12. The method of claim 9, wherein said compound is (+)-N-[5-(3-azabicyclo[3,2,2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1H-1,4-benzodiazepin-3-yl]-N'-[3-methylphenyl]-urea.

* * * * *