Molecular Cloning and Functional Characterisation of MCH$_2$, a Novel Human MCH Receptor

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running title: MCH$_2$, a novel MCH receptor.
ABSTRACT

Melanin-concentrating hormone (MCH) is involved in the regulation of feeding and energy homeostasis. Recently, a 353 amino-acid splice variant form of the human orphan receptor SLC-1 (1) (hereafter referred to as MCH₁) was identified as an MCH receptor. This report describes the cloning and functional characterisation of a novel, second, human MCH receptor, which we designate MCH₂, initially identified in a genomic survey sequence as being homologous to MCH₁ receptors. Using this sequence, a full length cDNA was generated with an open reading frame of 1023 bp, encoding a polypeptide of 340 amino acids, with 38% identity to MCH₁ and with many of the structural features conserved in G protein coupled receptors. This newly discovered receptor belongs to class 1 (rhodopsin – like) of the G protein coupled receptor superfamily. HEK293 cells transfected with MCH₂ receptors responded to nanomolar concentrations of MCH with an increase in intracellular Ca²⁺ levels and increased cellular extrusion of protons. In addition, fluorescently labelled MCH bound with nanomolar affinity to these cells. The tissue localisation of MCH₂ receptor mRNA, as determined by quantitative RT-PCR, was similar to that of MCH₁ in that both receptors are expressed predominantly in the brain. The discovery of a novel MCH receptor represents a new potential drug target and will allow the further elucidation of MCH-mediated responses.
INTRODUCTION

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide that was first discovered in teleost fish, where it acts as a skin colour-regulating hormone (2). In rodents its tissue distribution in the perikarya of the lateral hypothalmus and the zona incerta suggests that MCH may be involved in a variety of behavioural responses (3). Similar tissue distributions have been reported in both bird (4) and monkey (5). Reports implicating MCH in the regulation of feeding behaviour show that increased food intake occurs after direct administration of MCH into the brain (6) and that MCH is up-regulated after fasting and in obese, leptin-deficient mice (7). There are also reports that suggest MCH may be involved in aggressive behaviour, anxiety and reproductive function (8,9).

Recently, several groups independently identified a 353 amino-acid splice variant of the orphan G-protein coupled receptor (GPCR) SLC-1 (1) as an MCH receptor (10, 11, 12, 13 &14). In view of the findings of the current study we propose that this form of SLC-1 be hereafter referred to as MCH1. Southern blot and related studies have indicated the absence of additional MCH receptor subtypes that closely resemble MCH1 at the DNA level (3, 10 &15). However, since degeneracy in receptor ligand pairings throughout the GPCR superfamily is common, we reasoned that other MCH receptors with low homology to the MCH1 receptor may exist. This suggestion is supported by reports of pharmacological differences between the MCH1 receptor and MCH binding sites in various cell lines and tissues (10).
Sequencing of the human genome resulted in the deposition of vast amounts of unannotated sequence in public databases in recent years. In the present study, we firstly describe how we identified a sequence with low but significant homology to the MCH$_1$ receptor from one of these databases, and cloned a full length cDNA from this. We then demonstrate that the predicted 340 amino acid polypeptide product of this sequence, which we term MCH$_2$, exhibits many of the structural features of the GPCR superfamily, is a member of the class 1 (rhodopsin-like) sub-family and, after heterologous expression in HEK293 cells, is selectively activated by nanomolar concentrations of MCH. Lastly, to investigate the biological significance of this finding we compare the tissue distribution of MCH$_1$ and MCH$_2$ receptors by RT-PCR analysis.
EXPERIMENTAL PROCEDURES

Receptor Cloning, Transient Expression and Generation of Stable Cell lines -

A 195bp genomic survey sequence (GSS) (AQ311725) was identified which when translated exhibited 42% identity at the amino acid level to transmembrane four region of MCH1. Primers were designed to perform 5’ ( 5’ - CAGAGTACATCGTCAGGGGATGTCAAAT CAAAA- 3’) and 3’ (5’ – TACTTTGCCCTCGTCCAACCATTT- 3’) rapid amplification of cDNA ends (RACE) on a Marathon human foetal brain cDNA template (Clontech). Extension of the known sequence at both the 5’ and 3’ ends revealed a coding sequence of 1023 bp with an in-frame upstream stop codon at position –24 (Fig.1). The full-length gene was amplified from the foetal brain cDNA template using the following primers (forward 5’- ACAATGAATCCATTTCATGCATCTTGT- 3’ and reverse 5’ – TGCTGCTAAGAGTCACAAGTAGCACAAGAAG-3’). The cDNA was cloned into pcDNA3.1/V5/His-TOPO (Invitrogen) and both strands were sequenced on an ABI sequencer. HEK293 cells were transfected with the recombinant plasmid using lipofectAMINE plus reagent (Life Technologies) following the manufacturer’s instructions. Stable cell lines were generated by selection in geneticin (16) and clones were screened by MCH induced calcium mobilisation on a fluorometric imaging plate reader (as described below).

Calcium Mobilisation Assays – Intracellular calcium assays were carried out essentially as described previously (17). The maximum change in fluorescence above baseline, measured on a fluorometric imaging plate reader (FLIPR- Molecular Devices), was used to determine agonist response. For cross-screening studies,
HEK293 cells were screened against a large library of over 1500 known and putative GPCR agonists, including all known mammalian neuropeptides, as described previously (17). Peptides in this library were tested at a final concentration of > 100 nM and other potential agonists > 1 µM. For antagonist studies, test substances were added 30 minutes before addition of an EC₅₀ concentration of agonist. Data was analysed using GraFit (Erithacus Software). Peptides were purchased and synthesized as described previously (10).

**Microphysiometry** - Changes in extracellular pH of HEK293 cells stably transfected with MCH₂ receptors were monitored using the Cytosensor microphysiometer (Molecular Devices) (18). Cells were seeded into ploy-L-lysine coated cytosensor capsules (0.2 million cells/capsule) and cultured overnight. The capsules were placed on the cytosensor and equilibrated for 2 hours with modified RPMI 1640 medium (Gibco), pH7.4. MCH was applied to the cells for 20 seconds prior to the "get rate", and were then washed with running medium to remove ligand. Extracellular acidification rates were determined as the rate of change of sensor output during the periodic interruption of media flow. Media was allowed to flow for 80 seconds, and stopped for 38 seconds. Rates were measured over 30 seconds, starting 8 seconds after flow was stopped.

**Laser Scanning Cytometry Binding Assays** – HEK293 cells transiently expressing MCH₂ receptors were seeded into 16 well chambers (Lab-Tek, Nalge Nunc Int.). The cells were grown in EMEM medium (including L-glutamine, 10% foetal calf serum, 1% non-essential amino acids and 400 µg / l G418) for 24 hours, and then incubated at 37°C for 30 minutes with Cy5 labelled MCH (Pharmacia Amersham) at concentrations ranging from 0 to 40 nM in HEPES buffered saline (including 2.5 mM MgCl₂, 1.5 mM CaCl₂ and 0.5% BSA). The cells were then...
washed in HEPES buffered saline minus the BSA and fixed with 4% paraformaldehyde. Analysis was performed using a laser scanning cytometer (CompuCyte). Excitation of the Cy5 label by a 5 mW HeNe laser resulted in the emission of fluorescence which was collected through a 650 nm long path filter and was measured by monitoring the red fluorescence maximal pixel intensity. Non-specific binding was determined in the presence of 40 μM unlabelled MCH, and specific binding derived fluorescence determined by subtraction of this from total binding. The data was analysed using Sigma Plot (SPSS Inc.)

**TaqMan RT-PCR Tissue Localisation** – Quantitative RT-PCR was performed using gene specific primers to MCH$_2$ and MCH$_1$ receptors on mRNA from twenty body tissues and 19 brain regions as previously described (19). The mRNA from each tissue and brain region analysed by TaqMan quantitative RT-PCR was derived from four individuals, two male and two female (except prostate). The MCH$_2$ receptor primers were forward 5'-TTGCCTGTAAGTCATCATGA-3', TaqMan probe 5'-ACGAGGGCAAAGTACCTGTCCACACTCATGTA-3' CCACACTCATT-3' and reverse 5'-AACGTGTCAGTGCAATGTTG-3'. The MCH$_1$ receptor primers were forward 5'-GCCACCATGGAATGCTGG-3', TaqMan probe 5'-CAATGCCAGCAACCTCTGAGGC-3' and reverse 5'-GGTGATCCTGCCGAAGTGA-3'. Primers designed to the house keeping gene GAPDH forward 5'-CAAGGTCATCCATGCAACACTTGG-3', TaqMan probe 5'-ACCAGATCCATCCATGCAACACTTGG-3' and reverse 5'-GGCCATCCACAGTCTTCTGG-3' were used to produce reference mRNA profiles.
RESULTS

The MCH₂ receptor was cloned from human foetal brain tissue cDNA. The 1023 bp ORF encodes a 340 amino acid protein that structurally resembles members of the GPCR superfamily (fig.1). The receptor contains a short N-terminus, 7 distinct hydrophobic membrane-spanning domains and the highly conserved DRY motif located at the interface between the third transmembrane helix and the cytoplasm (20). The receptor has only one initiator methionine in the open reading frame, which contrasts with three such putative initiator methionines in MCH₁. There are two putative N-linked glycosylation sites and there is no characteristic signal peptide. BLAST analysis of public databases revealed MCH₁ to be its most homologous relative. The two GPCRs are 57% identical at the nucleotide level, 59% similar and 38% identical at the amino acid level (fig. 2). Somatostatin receptors were the most similar receptors to these two putative paralogues, with approximately 26% identity at the amino acid level.

We transiently transfected HEK293 cells with MCH₂ receptor cDNA and tested these cells for responsiveness to MCH. MCH (100nM) induced a clear, robust, transient increase in intracellular Ca²⁺ in MCH₂ receptor transfected cells, but not in control cells transfected with the same vector containing a mu opiate receptor (fig. 3a). The dose dependency of this response was investigated and an EC₅₀ value (± S.E., n = 3) of 8.57 ± 0.62 nM determined. To confirm the specificity of MCH₂ receptor activation by MCH we also screened these cells against a large library of known and putative GPCR ligands, including all known mammalian neuropeptides, at final concentrations greater than 100 nM. MCH was the only substance in this library observed to elicit MCH₂ receptor mediated Ca²⁺ responses in these cells.
An HEK293 cell line stably expressing the MCH2 receptor was established and used in all further functional studies. The concentration dependence of MCH2 receptor activation by MCH and related peptides was investigated in the intracellular Ca\(^{2+}\) assay (fig.3b). MCH, salmon-MCH and [Phe\(^{13}\), Tyr\(^{19}\)]-MCH all behaved as agonists with similar potencies (EC\(_{50}\) values [± S.E., n=3] of 5.65 ± 1.78, 7.14 ± 3.13, 4.29 ± 0.48 nM, respectively). Variant-MCH, the putative product of a second, variant form of the MCH gene (21), was a weak agonist (EC\(_{50}\) > 3000 nM). To determine the type of G-protein mediating this response, we pretreated cells with pertussis toxin (100 ng /ml, for 16 hours). The toxin treatment had no effect on calcium mobilisation by MCH in these cells (data not shown), suggesting that the MCH2 receptor is coupled to G proteins of the G\(_{q/11}\) subfamily.

In a variety of native receptor studies, a number of peptides have been reported to exhibit either functional antagonism of MCH responses or inhibit binding to MCH binding sites (22, 23 & 24). To investigate whether these effects are mediated via MCH2 receptors, we tested the following peptides in agonist and antagonist modes over a range of concentrations up to 10 µM in an intracellular Ca\(^{2+}\) assay; rat atrial natriuretic peptide (ANP)(1-28), rat ANP (3-28), human C-type natriuretic peptide-22, human brain natriuretic peptide-32, gamma-endorphin, and alpha-MSH. We also tested somatostatin-14, somatostatin-28 and cortistatin-14 because of the similarity of MCH\(_1\) and MCH\(_2\) receptors to somatostatin receptors. We also tested a number of putative products of the authentic MCH precursor and of a second, variant form of the MCH gene; neuropeptide-EI (NEI), neuropeptide-GE (NGE), MCH-gene-overprinted peptide–14 (MGOP-14), and variant neuropeptide-EI (vNEI). None of these peptides were active as agonists or antagonists at concentrations up to 10 µM.
To further confirm that the MCH$_2$ receptor responded specifically to MCH we monitored extracellular acidification rates in cells stably expressing the receptor using the technique of microphysiometry. 1 nM and 10 nM MCH caused robust increases in the rate of proton extrusion in transfected cells, but not in non-transfected HEK293 cells (fig. 4). Control studies demonstrated that 100 µM muscarine elicited similarly robust acidification responses in both MCH$_2$ receptor-transfected and non-transfected cells (data not shown), indicating that both cell lines are capable of responding in this assay, and thus confirming the specificity of the response to MCH. The dose dependency of the acidification response was also investigated and an EC$_{50}$ value (± S.E., n=4) of 1.43 ± 0.44 nM determined.

We used laser scanning cytometry to measure the binding parameters of fluorescently labelled MCH to MCH$_2$ receptors. Saturation analysis of Cy5-labelled MCH binding to HEK293 cells transiently transfected with MCH2 receptors showed specific, high affinity binding with a Kd (± S.E., n=3) of 6.02 +/- 0.46nM (Fig.5), although expression levels were apparently low, with the fluorescence signal (specific binding) arising from approximately 5% of the population. This value was comparable to the potencies observed for non-labelled forms of MCH obtained in the two functional assays.

We compared the tissue distributions of MCH$_1$ and MCH$_2$ receptors using quantitative TaqMan RT-PCR. The mRNA profiles of the two receptors were similar, showing predominant expression in the brain (Fig.6). However, a major difference was the much higher relative levels of MCH$_1$ compared with the MCH$_2$ mRNA in pituitary. The distribution of the two receptors within individual regions of the brain is similar but there are subtle differences. For example the hypothalamus, locus
coeruleus, medulla oblongata and cerebellum appear to express higher relative levels of MCH₁ compared with MCH₂ mRNA.

DISCUSSION

This report describes the cloning and functional characterisation of a second, novel MCH receptor. Several lines of evidence suggest that MCH is involved in the regulation of food intake and energy balance. Mice have been generated that carry a targeted deletion of the MCH gene (25). These knock-out mice show reduced food intake, have reduced body weight and are leaner. The localization of MCH in the lateral hypothalamus and the zona incerta, areas which are involved in the regulation of ingestive behaviour, is consistent with this role in feeding. However, perikarya from these areas project widely throughout the central nervous system, suggesting an involvement of MCH in a wide range of behaviours. Thus MCH has also been shown to interact within the hypothalamo-pituitary-adrenal axis or stress axis (26 & 27) and to be anxiogenic (28). Hyperactivity of the stress-axis is known to occur in individuals suffering from depression. Thus MCH may also have a significant role to play in psychiatric disorders.

Despite the large amount of data concerning the physiological actions of MCH, a receptor had not until recently been identified. Within a short period of time several groups (10,11, 12, 13 & 14) discovered that a 353 amino acid splice variant form of SLC-1 (MCH₁) is an MCH receptor. Within the GPCR superfamily, there are numerous examples of natural ligands which can activate more than one molecular species of GPCR. We therefore reasoned that additional MCH receptors may exist. However, low stringency southern blot studies (10), and related studies (3, 15),
suggest that additional receptors with high sequence identity to MCH1 are unlikely to exist. We therefore interrogated public databases to identify sequences with low but significant levels of homology to MCH1. A short sequence was identified in a GSS with homology to MCH1. Extension of this sequence revealed a full length cDNA with many of the motifs characteristic of a GPCR, which we designated MCH2 in view of its sequence similarity to MCH1 and its subsequent characterisation.

MCH2, like MCH1, shares low but significant homology to somatostatin receptors. We tested three naturally occurring ligands for somatostatin receptors against MCH2 receptors and did not observe any agonism or antagonism of MCH responses. Similar studies have demonstrated that somatostatin does not interact with MCH1 receptors (10,11 &15).

Recent studies have investigated the molecular mechanism by which MCH interacts with MCH1 (29), and demonstrate that Asp\textsuperscript{123} in transmembrane 3 (TM3) plays a key role in the formation of a complex between receptor and MCH, possibly by direct interaction with the Arg\textsuperscript{11} of MCH. Interestingly, this TM3 aspartate is conserved within MCH2 (Asp\textsuperscript{113}) and may therefore play a similar role in both paralogues. An equivalent aspartate residue with functional significance for receptor activation has also been identified in the related families of opiate (30) and somatostatin (31) receptors, and the same residue has for many years been recognised to be conserved in all biogenic amine GPCRs, where it acts as a counter-ion for the protonated amine moiety of the ligand (32).

Both functional and binding assays were used to confirm MCH as the cognate ligand for this receptor. Further characterisation using an intracellular Ca\textsuperscript{2+} assay demonstrated that salmon MCH and the synthetic analogue [Phe\textsuperscript{13}, Tyr\textsuperscript{19}]-MCH were equipotent with MCH and were full agonists at MCH2. However, variant-MCH, the
putative product of a second, variant form of the MCH gene (21), was approximately 500 fold less potent than MCH. This profile of agonist activity is essentially similar to that observed at MCH₁ (10). These data also demonstrate that variant-MCH is unlikely to act as a natural ligand for either MCH₁ or MCH₂ receptors.

To further characterise the MCH₂ receptor we tested, as both agonists and antagonists, a number of peptides that have been reported to bind with low affinity to MCH binding sites in various cell lines and tissues. Thus, MCH binding sites in mouse melanoma cells (22), human keratinocytes (23), and human brain (24) are weakly displaced by a number of natriuretic peptides. Since these peptides do not bind to MCH₁ receptors it has been proposed that they may bind to novel MCH receptors (10). In the present study we demonstrate that the MCH₂ receptor is not activated or antagonised by these peptides. This may therefore imply the existence of still further subtypes of MCH receptor. However, recent studies (33) have demonstrated that the specific binding to some of these cell lines is unlikely to be due to the presence of a receptor involved in signal transduction, since binding is largely localized to microsomal, and not plasma membranes, and the internalization kinetics are not typical of a receptor-mediated event.

We also tested a number of peptides that have been reported to either functionally antagonize MCH responses, or are putative products of either the authentic or variant MCH gene. Thus α-MSH and MCH have mutually antagonistic effects on a number of different physiological functions including feeding behaviour (34 & 35). The lack of activity of α-MSH at MCH₂ receptors parallels similar findings with the MCH₁ receptor (10, 11 & 12), and confirm that both peptides exert their effects via separate receptor families. Likewise, we observed that none of the additional putative products of the authentic and variant MCH precursor (NGE, NEI,
vNEI, MGOP-14) appear to interact with MCH$_2$ receptors. Previous studies have also demonstrated that these peptides do not interact with MCH$_1$ receptors (10 & 11). Taken together, these data indicate that any biological effects of these peptides are most likely to be mediated by receptors other than the two known MCH receptors.

Both MCH$_1$ and MCH$_2$ receptors are expressed predominantly in the brain. Within the brain, the pattern of expression of the two paralogues is similar. Both paralogues are widely distributed, but with mRNA profiles showing notably higher contributions from limbic areas such as amygdala, hippocampus, and parahippocampal gyrus, and in a number of cortical regions (cingulate-, medial frontal-, and superior frontal-gyri). The widespread distribution of MCH$_1$ is in agreement with that observed previously in the rat brain (11 & 36). Since the patterns of expression of these paralogues are so similar, these data suggest that neither paralogue can yet be selectively implicated in mediating any specific effect of MCH. The lower relative levels of MCH$_2$ mRNA in pituitary and hypothalamus compared with MCH$_1$ suggests that this newly discovered MCH receptor may be involved in physiological processes other than feeding or neuroendocrine modulation.

The identification of a second MCH receptor will help to further elucidate the role of MCH in energy homeostasis and feeding behaviour, as well as in disorders such as obesity and social anxiety disorder.
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FIGURE LEGENDS.

Fig. 1. **Nucleotide and the deduced peptide sequence of the MCH2 receptor.** The seven hydrophobic transmembrane spanning domains are indicated in *bold*; two cysteine residues available for disulphide bridge formation are marked with *asterisks* and two potential N-linked glycosylation sites are indicated in *bold italics* and *underlined*. The nucleotide sequence reported here has been submitted to GenBank and assigned the accession number AF347063.

Fig. 2. **Protein alignment of MCH1 and MCH2 receptors.** The amino acid sequences were aligned with CLUSTAL W (37) using the BLOSUM series substitution matrix. The alignment is viewed using GENEDOC (38). Identical amino acids are highlighted in black. Amino acid residues are numbered in the right hand margin.

Fig. 3. **MCH2 receptor-mediated intracellular Ca^{2+} responses to MCH in HEK293 cells.** A) Cells transiently transfected with MCH2 DNA responded to 100 nM MCH with a clear, robust, transient increase in intracellular Ca^{2+} (filled circles). However, cells transiently transfected with the same vector containing a mu-opiate receptor did not respond to MCH (open circles). B) Cells stably expressing MCH2 receptors responded in a dose-dependent fashion to MCH and two related peptides with robust increases in intracellular Ca^{2+}. Data shown are from a single representative experiment and values are given as means of triplicate determinations.
(± S.E.). MCH (filled circles), salmon MCH (open squares), [Phe^{13}, Tyr^{19}]-MCH (open triangles), variant-MCH (filled squares).

Fig. 4. **MCH increases extracellular acidification rates in HEK293 cells stably transfected with MCH₂ receptor (filled symbols), but not in non-transfected cells (open symbols).** Cells were exposed to either 10 nM (squares or diamonds) or 1 nM (triangles) MCH over the time course indicated by the bar. Data are taken from a single experiment representative of a total of three such experiments.

Fig. 5. **Saturation binding analysis of a fluorescently labelled analogue of MCH (Cy5-MCH) to HEK293 cells transiently transfected with MCH₂ receptors.** Values are means (± S.E.M.) of three normalized data points, each from three separate experiments.

Fig. 6. **Tissue localisation of MCH₁ and MCH₂ receptors using TaqMan (RT-PCR) methodology.** The expression profile of the two receptors over a range of 20 different human tissues and 19 different brain regions is shown. Data are presented as the mean (±S.E.M.) mRNA copies detected of four non-diseased individuals (2 males, 2 females except prostate) for each tissue from 1ng of Poly A⁺ RNA. Each of the 4 individuals intestines samples represent a 50:50 mix of one persons small intestine and another persons colon (same sex). Each brain sample represents an equal part of the RNA pool of the 19 key brain regions used. The GAPDH distribution profiles for the same samples are shown for comparison.
Fig. 1

```
-110  CGGCCGCCGGCAGGGTTCGCGAGGCACCCACGCTCCTAAAAAGAGCACGACGCACCCGAT   -51
  CGGCCGCCGGCAGGGTTCGCGAGGCACCCACGCTCCTAAAAAGAGCACGACGCACCCGAT
   M N P

  TTTTCGTACCTCTGTTGAAACACCTCTGCGGAACCTTATAAACAAACTCTGGAATAAAGAG
    F H A S C W N T S A E L L N K S W N K E

  TTTGCTTATCAAAACTGCGGCTGTGCTGATTACATGGTACATCTCATTCTAAAACCTCAATTT
  T A S V V D T V I L P S M I G I

  ATCTGTTCAACAGGGGCTGTGGAACACATCTACATGGTACATCTAATCTAATAAAAGAGCAG
  I C S T G L N I L I V F T I R S R

  AAAAAACAGTCCCTCTGAAACATCTATATCTCGCGTCAACCTGGCTGTGGCTGATTTGGTCA
  K K T V P D I Y I C N L A V A D L V H I

  GTTGGAAATGCTTACCTTATGTTGAAACACCTCTGCGGAACCTTATAAACAAACTCTGGAATAAAGAG
  V G M P F L I H Q W A R G G E W V F P G G

  CCTCTCCTCGGAAACATCTACATGGTACATCTAATCTAATAAAAGAGCAG
  L A L P V W V S K V I K F D G V E S

  ACAAATTTTTTTTTTCTCTACCTTATGTTGAAACCTTATAAACAAACTCTGGAATAAAGAG
  C* A F D L T S P D D V L W Y T L Y L T I

  ACCTGTCCTCCTACCTTCTGCGGAACCTTATAAACAAACTCTGGAATAAAGAG
  T T F F F F P L I L V C Y I L I L C Y

  ACCTGTCCTCCTACCTTCTGCGGAACCTTATAAACAAACTCTGGAATAAAGAG
  T W E M Y Q Q N K D A R C C N P S V P K

  CAGARAGTGTAAGATGCTGACCATCGGAAACATCTACATGGTACATCTAATCTAATAAAAGAGCAG
  Q X V M K L T K M V L V L V V F I L S

  GCTGCCCTTATCCCTATGGAACACATCTACATGGTACATCTAATCTAATAAAAGAGCAG
  A A P Y H V I Q L V N L Q M E Q P T L A

  TTTTATGCTGAACTACCTTCTCCTGCGTATCGATGGAACAGCCCACACTGGCC
  F Y V G Y L S I C L S Y A S S S N P

  TTTTATGCTGAACTACCTTCTCCTGCGTATCGATGGAACAGCCCACACTGGCC
  F L Y I L L S G N F Q K R L P Q I Q R R

  GCGACTGAGAAGGAATATCAACTACATGGAACACATCTACATGGTACATCTAATAAAAGAGCAG
  A T E K E I N N M G N T L K S H F *

  ACAAATTTTTTTTTTCTCTACCTTATGTTGAAACCTTATAAACAAACTCTGGAATAAAGAG
  T T T T A T G C T G A T G G A A A A T T T C C A A A C A G T C G G C C T C A A A A T C C A A A A A G A A A G A A G A A

  ACAAATTTTTTTTTTCTCTACCTTATGTTGAAACCTTATAAACAAACTCTGGAATAAAGAG
  F L Y I L L S G N F Q K R L P Q I Q R R

  GCGACTGAGAAGGAATATCAACTACATGGAACACATCTACATGGTACATCTAATAAAAGAGCAG
  A T E K E I N N M G N T L K S H F *

  TTTTATGCTGAACTACCTTCTCCTGCGTATCGATGGAACAGCCCACACTGGCC
  A T E K E I N N M G N T L K S H F *
Fig. 2

```
  20  *  40  *
MCH-1: MDLEASLLEFGPAS...DGDPGLTSAGSPRTGSISYINIBSVFTE...: 50
MCH-2: --------MNPGHACWTTGAEIKNSWNKEFAYQATASVVDVTLPSM...: 44

  60  *  80  *
MCH-1: LLLKEGTVIPAVVKKKLHWCPN...PLPLPLLLLPLFLLE...: 100
MCH-2: TSTKVILIVTIPIR...K-K-K-K-K-K-K-K-K-K-K-K-K-K: 90

  120  *  140  *
MCH-1: LLLMGHVLVSTMILAMANRIKSTVLLAPAIQVTVL...: 150
MCH-2: HOWARQRTKPGDLTSLTCHCRNACSAAKTYEVDOFLKIZIFPR: 140

  160  *  180  *
MCH-1: S.KPCKPSVATLVCILQYESTVGFSTHAVRARLPGLAVGAVGHH: 200
MCH-2: LDKRTTYKIRINLGLQAASFLAIPVWVSKETKDKVESAPDLS: 190

  220  *  240  *
MCH-1: TPEGLYAPVTQFFLAGALPVFVTVTAAYVSGQRMQSSVAPAS...Q: 244
MCH-2: TEPVLDPNLTTTFFLELEVCTILTYCWEMYQQNKDARCCNP: 239

  260  *  280  *
MCH-1: RSIKETKRYRATAIAICLPSFCWAVYVTVLQCSISRTTVAVL...: 294
MCH-2: SVPKOQWMLKTVMLVTVVQGILAAYARGLLDNLVQMEAGLSLVNYV: 289

  320  *  340  *
MCH-1: AAAGQSYTLSTLTVFAKLSYETPKFVLSVPAQQQLRAVSAQTAQD: 344
MCH-2: LRTLYLIVLIVTFILSNLAQSLQPIQREGLEKETKINMSTFLKSH: 339

MCH-1: EERTESKGT: 353
MCH-2: F---------------: 340
```
Fig. 3

A

![Graph A](image)

Change in fluorescence intensity units (x10,000)

B

![Graph B](image)

Change in fluorescence intensity units (x10,000)

[peptide] (M)

10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}

Time (s)

0 20 40 60 80

Change in fluorescence intensity units (x1000)

0 2 4

[peptide] (M)

10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
Fig. 4

![Acidification rate (%) vs. Time (min)](chart)

- Acidification rate (%) on the y-axis.
- Time (min) on the x-axis.

The graph shows the acidification rate (%) over time, with peaks and troughs indicating changes in the process.
Fig. 5

[Graph showing specific binding vs. [Cy5-MCH] (nM)]
Molecular cloning and functional characterisation of MCH2, a novel human MCH receptor
Jeffrey Hill, Malcolm Duckworth, Paul Murdock, Gillian Rennie, Cibele Sabido-David, Robert S. Ames, Philip Szekeres, Shelagh Wilson, Derk J. Bergsma, Israel S. Gloger, Dana S. Levy, Jon K. Chambers and Alison I. Muir

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