ZOOLOGICAL SCIENCE **15**: 567–572 (1998)

Augmentation of Prolactin Release by α-Melanocyte Stimulating Hormone Is Possibly Mediated by Melanocortin 3-Receptors in the Mouse Anterior Pituitary Cells

Yoshiaki Morooka, Souichi Oomizu, Sakae Takeuchi and Sumio Takahashi*

Department of Biology, Faculty of Science, Okayama University, Tsushima, Okayama 700-8530, Japan

ABSTRACT—Suckling- and estrogen-induced prolactin release from the anterior pituitary is mediated by α-melanocyte stimulating hormone (α-MSH) secreted by the intermediate lobe of the pituitary in the rat. Melanocortin 5-receptors are expressed in the anterior pituitary and probably mediate the α-MSH function. In contrast, the mouse anterior pituitary does not express the receptor. To examine whether or not α-MSH regulates prolactin release in mice, we performed cell immunoblot assay using anterior pituitary cells from adult female mice. We found that α-MSH acted on mammotrophs (prolactin-secreting cells) and stimulated prolactin release in a dose dependent manner. A series of RT-PCR using oligonucleotide primer pairs specific for each subtypes of melanocortin receptors revealed that the melanocortin 3-receptor is the sole receptor expressed in the mouse anterior pituitary. These results suggest that α-MSH-induced prolactin release is mediated by melanocortin 3-receptors in female mice.

INTRODUCTION

Melanocyte-stimulating hormone (MSH), secreted from the intermediate lobe of the pituitary, regulates melanin pigmentation in most vertebrates. Besides the effects on pigment cells, mammalian MSH is now widely known to have a number of biological functions through melanocortin (MC) receptors. Five subtypes of MC receptors (MC-1 receptor ~ MC-5 receptor) have been reported, and are distributed in various tissues in mammals (Cone *et al.*, 1996). In the rat, α-MSH acts as a mammotrophic factor to augment the release of prolactin (PRL) release in lactating female rats (Hill *et al.*, 1991, 1993) or estrogen-treated rats (Murai and Ben-Jonathan, 1990; Ellerkmann *et al.*, 1991, 1992). Recent studies on the tissue distribution of the MC receptors demonstrated that the MC-5 receptor is expressed in the rat and ovine anterior pituitaries (Barrett *et al.*, 1994; Griffon *et al.*, 1994). These observations suggest that α-MSH stimulates PRL release through MC-5 receptors in the rat. However, the expression of MC-5 receptor mRNA was not detected in the mouse pituitary (Labbe *et al.*, 1994), and the effects of α-MSH on the release of PRL, to our knowledge, have been not yet reported in the mouse. The species difference with regard to the expression of the MC-5 receptor led us to examine whether α-MSH is involved in the regulation of PRL release in the mouse pituitary.

In the present study, we examined effects of α-MSH on PRL release from single cells of the mouse anterior pituitary by the cell immunoblot assay. To validate our assay system, effect of thyrotropin releasing hormone (TRH) on PRL release was studied, since TRH is known to stimulate PRL release (Tashjian *et al.*, 1971; Rivier and Vale, 1974). In addition, the type of MC receptor expressed in these cells was determined by a series of RT-PCR using oligonucleotide primer pairs specific for each of the MC receptor. Our results indicate that α-MSH acts on mammotrophs to augment the release of PRL, and that the MC-3 receptor is the sole MC receptor expressed in the mouse anterior pituitary, suggesting that α-MSH stimulates the release of PRL from the anterior pituitary via activation of MC-3 receptors in female mice. No function has been yet ascribed to the mouse MC-3 receptor in any tissues, and thus this is the first report on the physiological relevance of the MC-3 receptor.

MATERIALS AND METHODS

Animals

Two to three month-old female mice of the ICR strain (CLEA Japan Inc., Osaka, Japan) were used. They were kept in a temperature-controlled animal room (20-22°C; lights on, 07:00-21:00), and given a commercial diet and tap water *ad libitum*. All animal care and experiments were performed in accordance with the Guidelines of Animal Experimentation, Faculty of Science, Okayama University,

* Corresponding author: Tel. +81-86-251-7866; FAX. +81-86-251-7876.

Japan.

Pituitary cell dissociation and culture

Pituitary glands were removed, and the posterior and intermediate lobes were discarded. The anterior pituitary cells were isolated according to the method of Oomizu and Takahashi (1996) . The dissociated cells were suspended at a density of 5×10^5 cells/ml in an 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (DMEM/F12, Sigma, St. Louis, MO, USA) with 10% fetal bovine serum. These cells were seeded in 24-well culture plates (Becton Dickinson, Uncoln Park, NJ, USA) and incubated at 37°C in water-saturated atmosphere of 5% CO_2 and 95% air for 3 to 4 days, before the cell immunoblot assay, in order to allow the cell damage caused by the enzymatic cell dispersion to heal. The cell viability was usually about 90% when assessed by trypan blue exclusion.

Cell immunoblot assay

Cell immunoblot assay was performed according to the method of Kendall and Hymer (1987) with slight modification. Briefly, the anterior pituitary cells were harvested from culture dishes using 0.02% trypsin-1 mM EDTA in Ca^{2+}-Mg^{2+}-free-Hanks' solution containing 20 mM HEPES and bovine serum albumin (0.3% w/v, BSA) at 37°C for 5 min and were dispersed into single cells (the cell viability was about 90%). These cells were suspended at the cell density of 2×10^5 cells/ml in DMEM/F12 with supplement (hydrocortisone, 100 mg/ml; triiodthyronine, 400 ng/l; transferrin, 10 mg/ml; bovine glucagon, 10 ng/ml; parathyroid hormone 200 ng/l and sodium selenite, 5 mg/ml) and secretagogues (TRH and α-MSH) when needed. The cell suspensions were immediately used for the cell immunoblot assay. TRH was obtained from Peptide Institute (Osaka, Japan), and α-MSH from Sigma.

The polyvinylidene difluoride transfer membranes (21 × 28 mm, Immobilon, Millipore, Bedford, MA, USA) were used for the assay. They were prewetted in methanol for 30 sec, rinsed in distilled water for 5 min and equilibrated with DMEM/F12 for 60 min before cell blotting. The membranes were placed on plastic film with their water-repellent side up. One hundred microliters of the cell suspension was dispersed on the membranes, and a cover slip (18 × 24 mm) was gently placed on the cell suspension. The cell suspension was spread into the cover slip's area. Cells on the membranes were never broken by this operation. The membranes were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After the incubation, the membranes were rinsed gently in 0.01 M phosphate-buffered saline for 15 min, then treated with 1% skim milk at 4°C for overnight to block unoccupied sites.

The PRL absorbed on the transfer membranes was immunostained, using rabbit antibodies generated against mouse PRL (Shikibo, Kusatsu, Japan) by the avidin-biotin-peroxidase complex technique (Hsu *et al.*, 1980). The specificity of antibodies to mouse PRL was ascertained (Takahashi *et al.*, 1994). Immunoreactivity of PRL was finally visualized using 4-chloro-1-naphthol as chromogen. After the color development, the membranes were rinsed twice with distilled water for 30 min and dried in air. The specificity of cell immunoblot was examined by omitting the antibody and by replacing the antibody with normal rabbit serum. In both cases immunoreactive PRL was not detected.

Image analysis

The immunoreactivity detected on the membrane was analyzed with a light microscope equipped with a CCD video camera (SONY XC-009P, Tokyo, Japan) and VIDAS Image Analysis System (Zeiss/Kontron, Germany) as follows. (1) Using a CCD video camera a black-and-white digital image of the membrane was produced at a resolution of 512 × 512 pixels, 8 bits/pixel = 256 gray levels. (2) The background gray values were measured. (3) The immunostained PRL spots were detected, and displayed on a monitor display. Too small objects or the objects touching edge of the image frame were discarded. (4) The non-PRL spots, for example colored scratch or too irregular shaped objects were also discarded. (5) Average value of gray scale and number of pixels of each spot were measured. The amount of PRL secreted was determined as follows: PRL amount = {(average gray value of PRL spot) – (back ground gray value)} × the number of pixels of each spot. PRL amount measured was expressed as percentages by regarding the amount of controls as 100%. In each assay 300 blots (PRL cells) were measured, and three independent assays were performed.

In our preliminary study, serially diluted pituitary extracts were applied to the transfer membrane, and immunostained to visualize PRL absorbed. The amount of PRL contained in the pituitary extracts was analyzed in our image analysis system. The PRL amount estimated was closely correlated with concentrations of pituitary extracts. We concluded that the amount of PRL secreted was able to be estimated using our cell immunoblot assay system.

Statistical analysis

Statistical differences in the average among groups were analyzed with a one-way analysis of variance followed by the multiple range test of Duncan. Statistical differences in the frequency distribution of PRL levels among groups were analyzed with Kolmogorov-Smirnov's two-sample test.

PCR primers

Oligonucleotide primers for specific amplification of each MC receptor were designed based upon nucleotide sequences of mouse MC receptors reported previously (Mountjoy *et al.*, 1992; Desarnaud *et al.*, 1994; Labbe *et al.*, 1994; Kubo *et al.*, 1995). Since the sequence of mouse MC-4 receptor gene was not available in DNA data bases, primers for MC-4 receptors were determined based upon the report by Boston and Cone (1996). Amplification of MC-3 receptor cDNA and MC-5 receptor cDNA shared a common primer pair. The presence of a *Msp*I site in the amplified DNA fragment of the MC-5 receptor enables to distinguish it from that of the MC-3 receptor. The primer sequences were as follows:

MC-1 receptor-5'(TCAGAGCCTTGGTGCCTGTATGTG),
MC-1 receptor-3'(CCAGAAGGATAGTAAGGGTGGCAG),
MC-2 receptor-5'(AACTCCGATTGTCCTGATGTAG),
MC-2 receptor-3'(CTTTTGAATGCATCTCTGAGCTC),
MC-3/5 receptor-5'(AACCTGCACTCWCCCATGTACTTCT),
MC-3/5 receptor-3'(GATSACGGAGTTGCACATGATGAG),
MC-4 receptor-5'(ATACGGATGCCCAGAGCT),
MC-4-receptor-3'(AAGAAGCTTTATTTGGGAG).

The length of amplified DNA fragments of MC-1, MC-2, MC-3, MC-4 and MC-5 receptors should be 652, 815, 681, 554 and 675 bp, respectively.

Reverse transcription and PCR

Genomic DNA from newborn mice and total RNA from anterior pituitaries of adult female mice were prepared following the method described (Sambrook *et al.*, 1989). Approximately 2 μg of the RNA was subjected to reverse transcription using Superscript II Reverse Transcriptase (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer's instructions. One tenth aliquot of the reaction was used in each PCR using primer pairs specific for each of MC receptors. In the case of PCRs using genomic DNA as a template, 0.8 μg of DNA was used in each PCR. The PCRs were carried out using AmpliTaq Gold DNA polymerase (PERKIN ELMER, Forster City, CA, USA) and a thermal cycler (Gene Amp PCR System 9600, PERKIN ELMER). The conditions for the PCR were as follows: after activation of the DNA polymerase by incubating for 9 min at 95°C, 40 cycles of reactions including denaturation for 30 sec at 95°C and extension for 1 min at 60°C were performed, followed by additional extension for 10 min at 60°C. One tenth aliquot of each reaction was electrophoresed on 2.0% agarose gel, stained with ethidium bromide, and photographed under ultraviolet illumination. Each of the amplified DNA frag-

ments were subcloned into pGEM3Zf(+) and subjected to sequencing. Dideoxynucleotide sequencing was performed using fluorescent primers and an automated DNA sequencer (Applied Biosystems 373A).

RESULTS

Effects of TRH and α-MSH on PRL release

The relative amount of PRL released during 5-hr incubation is illustrated in Fig. 1. The PRL amount increased as incubation was prolonged. The rate of PRL secretion decreased between 2 and 3 hr of incubation. Both TRH (10^{-7} M) and α-MSH (10^{-7} M) treatment stimulated PRL secretion compared with controls. In the following experiments, 1 hr-incubation was used for the measurement of PRL secretion.

TRH significantly increased the amount of PRL released in a dose-dependent manner (10^{-11} – 10^{-7} M, Fig. 2). Thus, stimulatory effect of TRH on PRL release was confirmed in our system, indicating that the conditions of cell immunoblot assay in our study are suitable for analyzing PRL release.

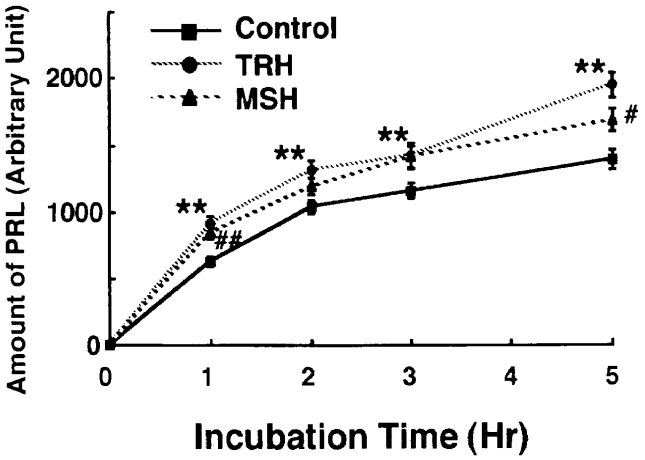

Fig. 1. Effects of TRH (10^{-7} M) and α-MSH (10^{-7} M) treatment on PRL secretion from single cells of adult female mouse pituitaries during incubation. Each point and bars depict the mean and its standard error of the PRL amount released from about 300 PRL cells. TRH increased PRL release compared with corresponding controls (** $p < 0.01$). α-MSH increased PRL release compared with controls (# $p < 0.05$, ## $p < 0.01$).

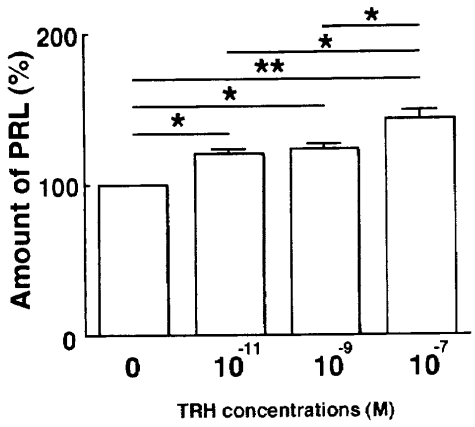

Fig. 2. Effects of TRH on PRL secretion from single cells of adult female mouse pituitaries during 1-hr incubation. Each column shows mean of three independent assays with standard errors. * $p < 0.05$, ** $p < 0.01$.

As in the case of TRH, α-MSH significantly increased the amount of PRL released in a dose-dependent manner (10^{-11} – 10^{-7} M, Fig. 3). Figure 4 shows the typical frequency distribution of the amount of PRL release from single PRL cells in response to α-MSH of various concentrations (10^{-11} – 10^{-7} M). The frequency distribution of the amount of PRL release displayed a unimodal right side-trailing pattern at any doses of α-MSH. Although the mode of the frequency was not affected, the median value of the PRL amount at 10^{-7} M α-MSH was shifted rightward compared with controls.

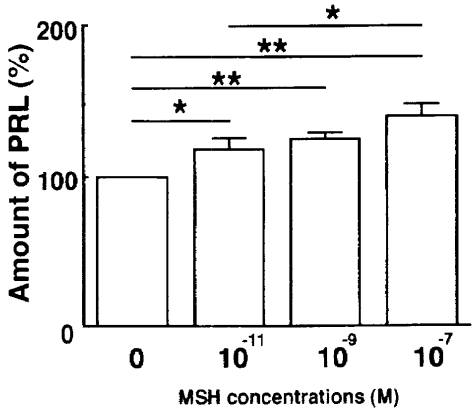

Fig. 3. Effects of α-MSH on PRL secretion from single cells of adult female mouse pituitaries during 1-hr incubation. Each column shows mean of three independent assays with standard errors. * $P < 0.05$, ** $P < 0.01$.

Detection of MC receptors by RT-PCR

Evaluation of the specificity of primers used for amplification of each MC receptor was carried out by a series of PCRs using genomic DNA as a template and sequencing. As shown in Fig. 5A, each primer pairs amplified DNA species with the expected length. Digestion of PCR-products with *Msp*I showed that the primer pair used for both the MC-3 receptor and the MC-5 receptor can amplify both genes with the same quantity (Fig. 5B). Sequence analysis of each PCR-amplified DNA fragment revealed that they were identical to the reported MC receptors (data not shown). The PCR using MC-4 receptor primers generated two species of DNA fragments. The nucleotide and predicted amino acid sequence of the larger DNA fragment (554 bp) shared 90.0 and 95.1%, respectively, identity with those of the human MC-4 receptor. On the other hand, the smaller DNA fragment (263 bp) was observed even in the absence of the template (data not shown), and had no homology with the human MC-4 receptor, suggesting that the former is the mouse MC-4 receptor gene and the latter is an artifact of PCR. The sequence of the mouse MC-4 receptor is available from DDBJ, EMBL, and GenBank Data Libraries under the accession number AB009664.

Using these specific primers for the mouse MC receptors, total RNA prepared from anterior pituitaries of adult female mice were analyzed by RT-PCR. As shown in Fig. 6A, the amplification of DNA fragments were only observed in PCR

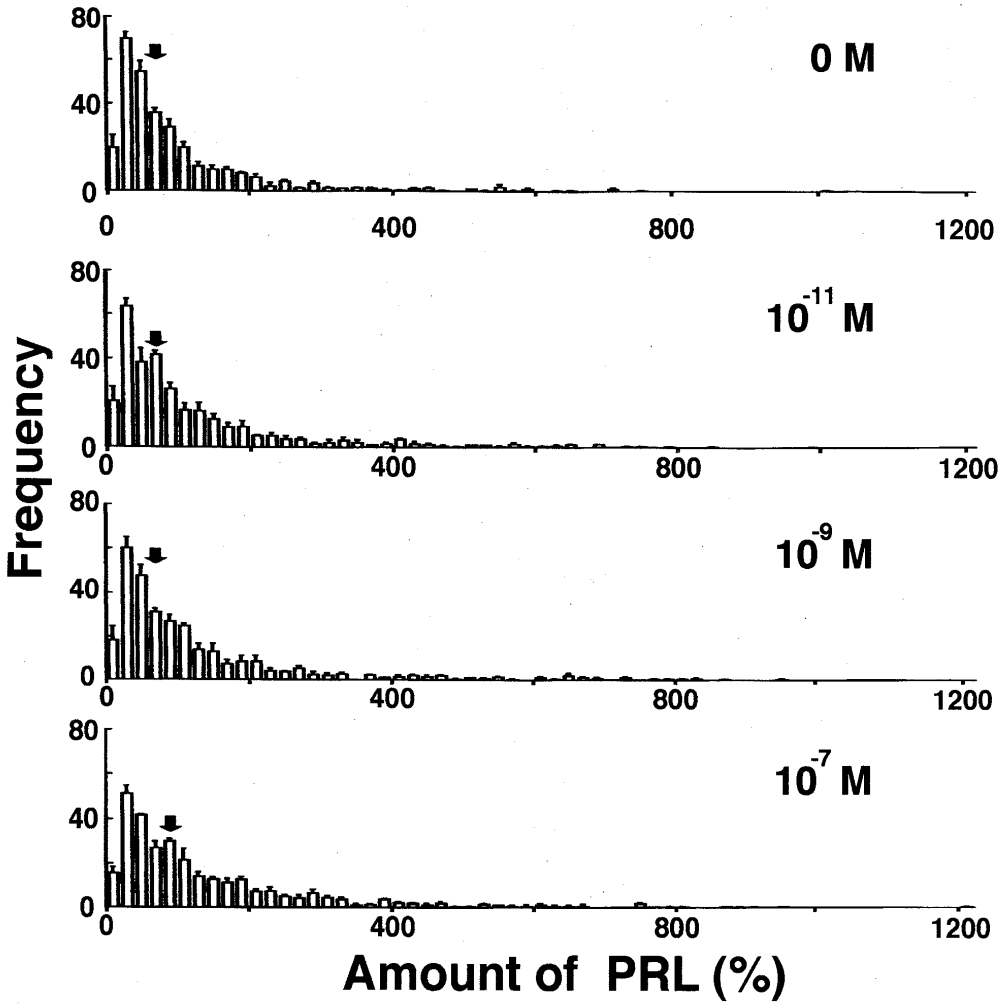

Fig. 4. Typical frequency distribution patterns of PRL secretion from single cells of adult female mouse pituitaries during 1-hr incubation. About 300 blots (PRL cells) were measured in all groups. The arrow indicates the median value of PRL release. The distribution pattern of PRL secretion in α-MSH treatment (10^{-7} M) statistically differed from that in control ($P < 0.01$).

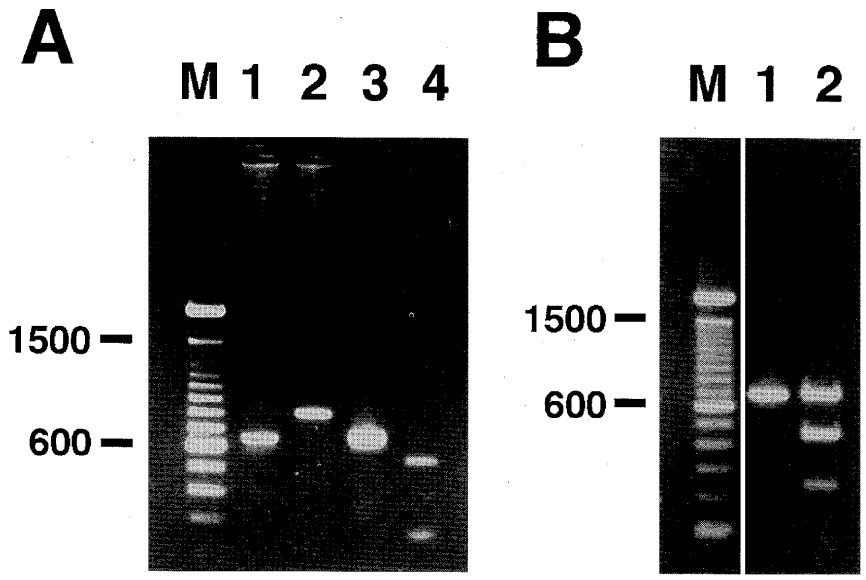

Fig. 5. Evaluation of the specificity of oligonucleotide primers. Primers specific for each MC receptor were designed and tested against genomic DNA. (**A**) Specific amplifications of MC receptor genes. Products of PCRs using primer pairs specific for the MC-1 receptor (lane 1), the MC-2 receptor (lane 2), the MC-3/5 receptors (lane 3) and the MC-4 receptor (lane 4) were electrophoresed. (**B**) Restriction patterns of the DNA fragments obtained by PCR using the MC-3/5 receptors-specific primer pair. The amplified DNA fragments were electrophoresed intact (lane 1) or after digested with *Msp*I (lane 2). In each case, a 100 bp ladder was used as a molecular marker.

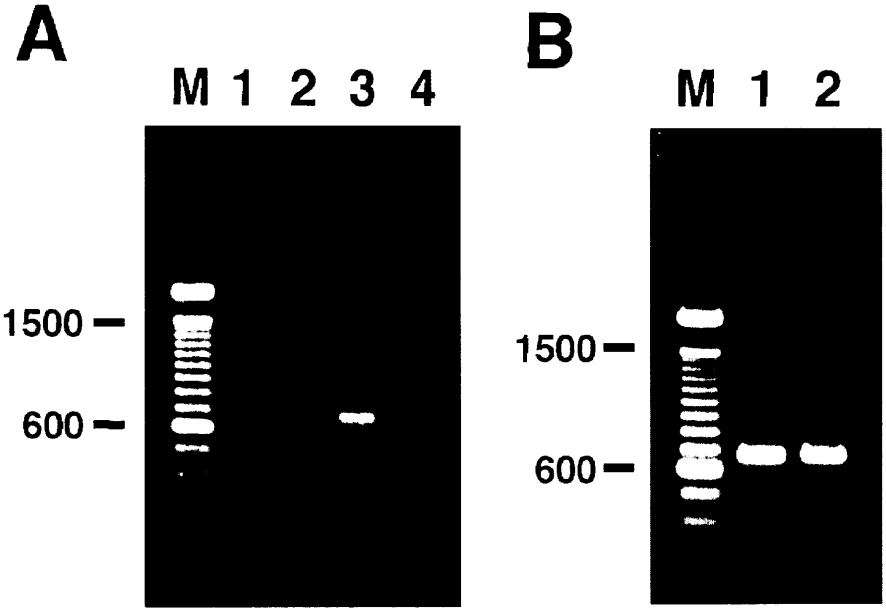

Fig. 6. Identification of the type of MC receptors expressed in anterior pituitaries of adult female mice. (**A**) RT-PCR analysis of MC receptor expression in anterior pituitaries of adult female mice. Reaction mixtures of RT-PCRs using primer pairs specific for the MC-1 receptor (lane 1), the MC-2 receptor (lane 2), the MC-3/5 receptor (lane 3) and the MC-4 receptor (lane 4) were electrophoresed. (**B**) Restriction patterns of the DNA fragments obtained by RT-PCR using MC-3/5 receptor-specific primer pair. The amplified DNA fragments were electrophoresed intact (lane 1) or after digested with *Msp*I (lane 2). In each case, a 100 bp ladder was used as a molecular marker. *Msp*I failed to digest the RT-PCR product, indicating that cDNA for the mouse MC-3 receptors was amplified.

using the MC-3 receptor /MC-5 receptor primers. Inability of the PCR-product to be digested with *Msp*I (Fig. 6B) suggested that only MC-3 receptor cDNA was amplified by the PCR. In order to confirm it, we performed sequencing of the DNA fragment and found that it was indeed a cDNA for the mouse MC-3 receptor (data not shown). These results indicate that the MC-3 receptor is the sole MC receptor expressed in anterior pituitaries of adult female mice.

DISCUSSION

TRH is well known to stimulate PRL secretion *in vivo* (Rivier and Vale, 1974) and *in vitro* (Tashjian *et al.*, 1971). Using the reverse hemolytic plaque assay (Neill and Frawley, 1983) and the cell immunoblot assay (Arita *et al.*, 1992), the stimulatory effect of TRH on PRL release had been confirmed in the rat pituitary. In the mouse pituitary PRL release was stimulated by TRH at concentrations ranging from 10^{-11} to 10^{-7} M. These effective doses of TRH on PRL release agreed with those reported in the rat pituitary cells (Boockfor *et al.*, 1986; Luque *et al.*, 1986; Arita *et al.*, 1992). Thus, the present result indicates that TRH regulates PRL secretion in the mouse pituitary, and the cell immunoblot assay is applicable for the measurement of the amount of PRL which was released from single PRL cells in the mouse pituitary.

Several reports indicated that α-MSH stimulated PRL release in the rat pituitary, and suckling-induced PRL release was mediated by α-MSH (Hill *et al.*, 1991, 1993). We demonstrated using the cell immunoblot assay in the adult female mice that α-MSH stimulated PRL release. Our present result is in agreement with those previous reports. Thus, the present study clearly showed that α-MSH was also a mammotrophic factor in the adult female mice pituitary. This indicates that similar regulatory mechanism in PRL secretion operates in the mouse pituitary.

α-MSH secreted from the intermediate lobe has to diffuse to PRL cells of the anterior lobe as a paracrine factor. PRL cells which are adjacent to the intermediate lobe must be more susceptible to stimuli. In this view, it is noteworthy that in lactating female rats PRL cells in a central area which is proximate to the intermediate lobe become more responsive to stimulatory secretagogues such as TRH (Nagy *et al.*, 1991). PRL cells exposed to α-MSH may increase PRL release as shown in the present study, and also may enhance the responsiveness to another stimuli.

MC-3 receptor mRNA was detected in the adult female mice, indicating that MC-3 receptors expressed in the pituitary. Desarnaud *et al.* (1994) demonstrated that the expression of the mouse MC-3 receptor mRNA was detected in the hypothalamus, thalamus and striatum, and no signal could be detected in the pituitary. The reason for this discrepancy is not clear. It may be due to the difference in the sensitivity of each experimental procedure used. Alternatively, the expression of the MC-3 receptor in anterior pituitary cells may occur only in female mice, since α-MSH-mediated PRL release had been observed in lactating or estrogen treated female rats (Hill *et al.*, 1991, 1993; Murai and Ben-Jonathan, 1990; Ellerkmann *et al.*, 1991, 1992). Our results also provide possible mechanism for regulating PRL release by adrenocorticotropic hormone (ACTH) in a paracrine fashion, because melanocortin peptides, including α-, β-, γ-MSH and ACTH, had been reported to bind and activate cloned mouse MC-3

receptors with similar potency order (Desarnaud *et al.*, 1994). It remains unclear at present whether or not α-MSH is the physiological agonist of the MC-3 receptor in the mouse anterior pituitaries, and that whether or not there may be a species difference between mice and rats in the type of MC receptors expressed in the anterior pituitary. Further analysis is required to elucidate molecular mechanisms underlying the regulation of PRL release by melanocortin peptides.

Recently, Zheng *et al.* (1997) demonstrated that some, but not all, of mammotrophs from lactating rats bind radiolabelled α-MSH. This suggests that α-MSH receptors are expressed in some of mammotrophs. We did not identify the cell types of MC-3 receptor-expressing cells. In our cell immunoblot assay system, all dissociated pituitary cells were seeded in diluted concentration. Therefore, there is little possibility that pituitary cells could interact each other in our system. Apparently, α-MSH acts on mammotrophs directly via α-MSH-specific receptors. Therefore, it is highly possible that MC-3 receptors are expressed in mammotrophs.

In conclusion, we demonstrated here that α-MSH acts directly on the mouse pituitary cells, and stimulates PRL release possibly mediated by MC-3 receptors. This is the first report on the physiological relevance of the mouse MC-3 receptor.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid from Itoh Science Foundation and Ryobi-Teien Foundation to S. Takeuchi, and Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan to S. Takahashi.

REFERENCES

Arita J, Kojima Y, Kimura F (1992) Lactotrophs secreting small amounts of prolactin reveal great responsiveness to thyrotropin-releasing hormone: analysis by the sequential cell immunoblot assay. Endocrinology 130: 3167–3174

Barrett P, MacDonald A, Helliwell R, Davidson G, Morgan P (1994) Cloning and expression of a new member of the melanocyte-stimulating hormone receptor family. J Mol Endocrinol 12: 203–213

Boockfor FR, Hoeffler JP, Frawley LS (1986) Analysis by plaque assays of GH and prolactin release from individual cells in cultures of male pituitaries. Neuroendocrinology 42: 64–70

Boston BA, Cone RD (1996) Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. Endocrinology 137: 2043–2050

Cone RD, Lu D, Koppula S, Vage DI, Klungland H, Boston B, Chen W, Orth DN, Pouton C, Kesteroson R (1996) The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. Recent Prog in Horm Res 51: 287–318

Desarnaud F, Labbe O, Eggerickx D, Vassart G, Parmentier M (1994) Molecular cloning, functional expression and pharmacological characterization of a mouse melanocortin receptor gene. Biochem J 299: 367–373

Ellerkmann E, Nagy GyM, Frawley LS (1991) Rapid augmentation of prolactin cell number and secretory capacity by an estrogen-induced factor released from the neurointermediate lobe. Endocrinology 129: 838–842

Ellerkmann E, Nagy GyM, Frawley LS (1992) α-melanocyte-stimulating hormone is a mammotrophic factor released by neurointermediate lobe cells after estrogen treatment. Endocrinology 130: 133–138

Griffon N, Mignon V, Facchinetti P, Diaz J, Schwartz J-C, Sokoloff P (1994) Molecular cloning and characterization of the rat fifth melanocortin receptor. Biochem Biophys Res Commun 200: 1007–1014

Hill JB, Nagy GyM, Frawley LS (1991) Suckling unmasks the stimulatory effect of dopamine on prolactin release: possible role for α-melanocyte-stimulating hormone as a mammotrope responsiveness factor. Endocrinology 129: 843–847

Hill JB, Lacy ER, Nagy GyM, Görcs TJ, Frawley LS (1993) Does α-melanocyte-stimulating hormone from the pars intermedia regulate suckling-induced prolactin release? Supportive evidence from morphological and functional studies. Endocrinology 133: 2991–2997

Hsu SM, Raine L, Fanger H (1980) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29: 577–580

Kendall ME, Hymer WC (1987) Cell blotting: a new approach to quantify hormone secretion from individual rat pituitary cells. Endocrinology 121: 2260–2262

Kubo M, Ishizuka T, Kojima H, Kakinuma M, Koike T (1995) Cloning of a mouse adrenocorticotropin receptor-encoding gene. Gene 153: 279–280

Labbe O, Desarnaud F, Eggerickx D, Vassart G, Parmentier M (1994) Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. Biochemistry 33: 4543–4549

Luque EH, Munoz de Toro M, Smith PF, Neill JD (1986) Subpopulations of lactotropes detected with the reverse hemolytic plaque assay show differential responsiveness to dopamine. Endocrinology 130: 3167–3174

Mountjoy KG, Robbins LS, Mortrud MT, Cone RD (1992) The cloning of a family of genes that encode the melanocortin receptors. Science 257: 1248–1251

Murai I, Ben-Jonathan N (1990) Acute stimulation of prolactin release by estradiol: mediation by the posterior pituitary. Endocrinology 126: 3179–3184

Nagy GM, Boockfor FR, Frawley LS (1991) The suckling stimulus increases the responsiveness of mammotropes located exclusively within the central region of the adenohypophysis. Endocrinology 128: 761–764

Neill JD, Frawley LS (1983) Detection of hormone release from individual cells in mixed populations using a reverse hemolytic plaque assay. Endocrinology 112: 1135–1137

Oomizu S, Takahashi S (1996) Insulin stimulates the proliferation of mouse anterior pituitary cells *in vitro*. Biomed Res 17: 365–371

Rivier C, Vale W (1974) *In vivo* stimulation of prolactin secretion in the rat by thyrotropin releasing factor, related peptides and hypothalamic extracts. Endocrinology 95: 978–983

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning-A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York

Takahashi S, Oomizu S, Kobayashi Y (1994) Proliferation of pituitary cells in streptozotocin-induced diabetic mice: effect of insulin and estrogen. Zool Sci 11: 445–449

Tashjian Jr AH, Barowsky NJ, Jensen DK (1971) Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. Biochem Biophys Res Commun 43: 516–523

Zheng T, Villalobos C, Nusser KD, Gettys TW, Faught WJ, Castaño JP, Frawley LS (1997) Phenotypic characterization and functional correlation of α-MSH binding to pituitary cells. Am J Physiol 272: E282–E287

(Received February 25, 1998 / Accepted March 30, 1998)