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Plasma and Cerebrospinal Fluid α-MSH Levels in the Rat After Hypophysectomy and Stimulation of Pituitary α-MSH Secretion[1]

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THODY, A. J., A. A. DE ROTTE AND TJ. B. VAN WIMERSMA GREIDANUS. *Plasma and cerebrospinal fluid α-MSH levels in the rat after hypophysectomy and stimulation of pituitary α-MSH secretion.* BRAIN RES. BULL. 4(2) 213–216, 1979.—Immunoreactive α-MSH was measured in cerebrospinal fluid (CSF) and plasma of rats. While treatment with haloperidol increased α-MSH levels in the plasma concentration of α-MSH in the CSF showed little change. Hypophysectomy also had little effect on the concentration of α-MSH in the CSF despite the fall in plasma α-MSH levels. This lack of correlation between α-MSH levels in the CSF and plasma suggests that the systemic circulation does not deliver α-MSH to the CSF. The apparently normal levels of α-MSH in the hypothalamus after hypophysectomy suggests that this tissue is able to synthesize α-MSH and it is possible that the hypothalamus is a source of the α-MSH in the CSF.

Cerebrospinal fluid α-MSH

CEREBROSPINAL FLUID (CSF) is likely to serve as an important route for many hormones that modulate brain function. A number of different peptide hormones have been found in the CSF [1, 3, 5–7, 9, 12, 24] and while those of hypothalamic origin such as vasopressin, oxytocin and the releasing hormones enter the CSF directly, the way in which pituitary hormones such as ACTH reach the CSF is not yet clear.

Melanocyte-stimulating hormone (MSH) and related peptides are known to affect brain function both in man and experimental animals [2, 11, 28, 29]. While it is generally accepted that these melanotropic peptides are produced by the pituitary gland bioassayable MSH and immunoreactive MSH peptides have been found in different regions of the brain and shown to persist after removal of the pituitary [8, 14, 15, 17, 18, 20–23]. This suggests that the brain, as well as the pituitary, is capable of producing MSH peptides. In this study we report the presence of immunoreactive α-MSH in the CSF of the rat and present data which suggest that at least some of this peptide is of brain rather than pituitary origin.

METHOD

Male Wistar rats were obtained from TNO, Zeist, The Netherlands or bred in the Department of Dermatology, Newcastle, and were used when approximately 180 g in weight. Hypophysectomy was performed by the parapharyngeal approach and confirmed at autopsy by examination of the sella turcia.

Removal of CSF

Rats were anaesthetized with urethane (1.1 g/Kg IP) and polyethylene cannulae inserted into the cisterna magna for the withdrawal of CSF [7]. Approximately 50–100 μl CSF were withdrawn, centrifuged and stored at –20°C until assayed.

Plasma Samples

(1) Polyethylene cannulae were inserted into the jugular vein and advanced to the heart. Approximately 500 μl blood were withdrawn and replaced by an equal volume of 0.9% saline/heparin solution.

(2) In Experiment 2 (see below) the rats were decapitated and trunk blood collected into tubes containing lithium sequestrene.

All blood samples were centrifuged and the plasma stored at –20°C.

Brain Tissue

Rats were decapitated and the brain immediately removed. The hypothalamus was dissected and this and the remainder of the brain minus the olfactory lobes were rinsed

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in ice-cold 0.9% saline, homogenized in 0.1 N HCl and stored at −20°C.

Experiment 1

Plasma and CSF α-MSH levels after release of α-MSH from the pituitary: A sample of CSF was removed and immediately afterwards a sample of blood was withdrawn from the jugular vein.,The rats then received 0.5 mg haloperidol (Janssen Pharmaceuticals) IP which is known to stimulate the release of α-MSH from the pituitary [19,27].Sixty minutes later the second samples of CSF and blood were taken.

Experiment 2

Plasma, CSF and brain levels of α-MSH *after hypophysectomy*: Samples of CSF were removed from normal rats and hypophysectomized rats 9 days after surgery. The rats were then killed by decapitation, trunk blood collected and the brains removed.

Radioimmunoassay of α-MSH

The procedure of Penny and Thody [19] or a modification of this method was used. The modification included the use of an antiserum raised in rabbits after intramuscular injection of synthetic α-MSH (Organon, Oss), coupled to thyroglobulin by means of carbodiimide and emulsified Freunds adjuvant. This antiserum was used at a final dilution of 1:40,000 was specific for the C-terminal Lys-Pro-Val-NH_2 sequence of α-MSH and gave a sensitivity of 2 pg α-MSH/tube. This modified assay was used in Experiment 1 which was carried out in Utrecht and the original method of Penny and Thody [19] was used in Experiment 2 which was carried out in Newcastle.

RESULTS

Plasma and CSF α-MSH Levels in Control Rats

The results are shown in Tables 1 and 2.

The concentration of immunoreactive α-MSH found in the plasma of the rats used in Utrecht was 125.0 ± 6.7 pg/ml (mean ± SEM) and this was similar to that of 156.0 ± 36.9 pg/ml found in the Newcastle rats. Immunoreactive α-MSH was also detected in the CSF but the concentrations were different in the two centres. In the Utrecht rats the CSF immunoreactive α-MSH level was 37.0 ± 10.1 pg/ml and at Newcastle the value was 143.0 ± 33.7 pg/ml, which compared well with the concentration in the plasma. Dilutions of the CSF obtained in Newcastle produced displacement of labelled α-MSH from the antibody and the curves were parallel to those produced with standard α-MSH. Because of the low levels of α-MSH it was impossible to carry out similar immunoreactivity tests with the CSF obtained in Utrecht.

TABLE 1

IMMUNOREACTIVE α-MSH IN PLASMA AND CSF OF MALE RATS BEFORE AND 60 MIN AFTER A SINGLE INJECTION OF HALOPERIDOL

α-MSH pg/ml	Control	After Haloperidol	p
Plasma	125.0 ± 6.7	177.7 ± 10.0	<0.02
CSF	37.0 ± 10.1	21.3 ± 1.3	NS

Nine rats were used. Each value is the Mean ± SEM of 3 pooled samples.
NS = not significant.

Plasma and CSF α-MSH Levels after Haloperidol

This experiment was carried out in Utrecht. The results are shown in Table 1. Sixty minutes after administration of 0.5 mg haloperidol plasma immunoreactive α-MSH had risen from the control level of 125.0 ± 6.7 to 177.7 ± 10.0 pg/ml ($p<0.02$). By contrast CSF immunoreactive α-MSH levels were somewhat reduced 60 min after haloperidol, although the decrease was not significant.

Plasma, CSF and Brain α-MSH Levels after Hypophysectomy

This experiment was carried out in Newcastle. The results are shown in Table 2. After hypophysectomy the immunoreactive α-MSH level in plasma was below the detection limit of the assay (<80 pg/ml). On the other hand, CSF immunoreactive α-MSH levels had increased slightly from 143.0 ± 33.7 pg/ml in the control rats to a level of 209.5 ± 38.4 in the hypophysectomized rats but this increase was not significant. Hypophysectomy had little effect on the immunoreactive α-MSH content of the hypothalamus. The rest of the brain, however, showed a decrease of approximately 55% in immunoreactive α-MSH levels after hypophysectomy, although the change was just short of significance.

DISCUSSION

The present results demonstrate the presence of immunoreactive α-MSH in the CSF of the rat. However, the way in which this peptide enters the CSF is not yet clear. α-MSH is a major melanotrophic peptide of the pars intermedia of the rat [4,26] and is normally released into the systemic circulation [19, 26, 27]. Prolactin and possibly other pituitary hormones are thought to enter the CSF via the circulation by filtration at the choroid plexus [3] but it would seem unlikely that much α-MSH follows this route because no correlation was found between immunoreactive α-MSH levels in plasma and CSF. Therefore, although tritiated α-MSH injected into the systemic circulation enters the brain [10], pituitary α-MSH may well enter the CSF by a more direct route.

It has been suggested that ACTH reaches the CSF by retrograde flow in the portal vessels of the hypophysial stalk [1] and it is possible that α-MSH and other related peptides are also transported in this way [13,16]. In the present study haloperidol which stimulates α-MSH release from the pars intermedia [19,27] produced little change in the concentration of immunoreactive α-MSH in the CSF and it may be that an increased release of α-MSH into the systemic circulation prevents or reduces the amount of α-MSH available for retrograde transport in the portal vessels. Thus when actively releasing α-MSH into the peripheral circulation the pars intermedia may provide only small amounts of α-MSH for circulation in the brain.

We must also consider the possibility that the immunoreactive α-MSH in the CSF is of extra-hypophysial origin. Bioactive and immunoreactive MSH peptides have been found in the brain of both rat and man (see introduction) and their failure to disappear after hypophysectomy suggest that neural tissue is able to synthesize MSH peptides. Although

TABLE 2

IMMUNOREACTIVE α-MSH IN PLASMA, CSF AND BRAIN OF NORMAL AND HYPOPHYSECTOMIZED MALE RATS

α-MSH	Normal	Hypophysectomized	p
Plasma pg/ml	156 ± 37 (8)	ND (5)	—
CSF pg/ml	143 ± 34 (7)	209 ± 38 (10)	NS
Brain* ng	15.5 ± 4.8 (7)	7.0 ± 1.2 (11)	NS
Hypothalamus ng	1.3 ± 0.3 (4)	1.6 ± 0.2 (6)	NS

*Brain = whole brain minus hypothalamus and olfactory lobes.

Each value is the Mean ± SEM for the number of samples indicated in parentheses.

NS = not significant.

the rostral pars intermedia and the pars tuberalis often remain after hypophysectomy and may continue to produce α-MSH it is likely that the hypothalamus is a source of immunoreactive α-MSH in the CSF. The presence of immunoreactive α-MSH in the hypothalamus and its persistence after hypophysectomy suggests that this tissue is able to maintain the apparently normal levels of α-MSH in the CSF after removal of the pituitary. Relatively high concentrations of vasopressin and oxytocin have been found in the CSF after hypophysectomy and it has been suggested that after removal of the pituitary the hypothalmic nuclei responsible for these hormones undergo a compensatory increase in activity [7]. MSH peptides produced in the hypothalamus may be directly release into the 3rd ventricle in the same way as the neurophypophysial peptides and transported via the CSF to various regions of the brain to function as neuromodulators. After hypophysectomy there was, however, a decrease in the rest of the brain. Whether the loss of a pituitary contribution or whether neural MSH peptides undergo a more rapid breakdown after hypophysectomy is not known.

Although α-MSH is the major MSH in the rat pituitary and plasma and has been identified in rat brain [8, 14, 15, 17, 18] other α-MSH like peptides may also be produced by neural tissue [18,25]. In the present study different antisera were used in our two radioimmunoassays and while both assays gave similar levels of immunoreactive α-MSH in plasma different levels were found in the CSF. According to Peng Loh *et al.* [18] the major MSH peptide in the rat brain is similar to α-MSH but lacks some of the N terminal amino acids. This peptide cross reacts with our C terminal α-MSH antiserum [18] and may therefore be present in CSF. Higher levels of immunoreactivity were found in the CSF, however, when the other antiserum was used and it is possible that the CSF also contains N terminal α-MSH like peptides. A number of different MSH peptides may therefore be present in rat CSF, but whether these peptides are identical to those in the brain has yet to be determined. Further characterisation of these peptides will help in the determination of the source of the MSH peptides in the CSF.

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