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Radioautographic Localization of Radioactivity in Rat Brain After Intracarotid Injection of ^{125}I-α-Melanocyte-Stimulating Hormone

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PELLETIER, G., F. LABRIE, A. J. KASTIN AND A. V. SCHALLY. *Radioautographic localization of radioactivity in rat brain after intracarotid injection of ^{125}I-α-melanocyte-stimulating hormone.* PHARMAC. BIOCHEM. BEHAV. 3(4) 671–674, 1975. – ^{125}I-α-melanocyte-stimulating hormone (MSH) was injected into the carotid artery of the rat and the radioactivity localized by radioautography. Radioactivity in the areas surrounding the ventricles and blood vessels after administration of ^{125}I-α-MSH but not ^{125}I-luteinizing hormone indicated passage of labeled material through the blood brain barrier. A specific concentration of radioactivity was found in the striatum and reticular nucleus of the thalamus. This localization, particularly in the thalamus, could be correlated with the previously reported effects of MSH on the brain of animals and man.

MSH Radioautography Hormone Brain Peptide

ALTHOUGH the physiological role of melanocyte-stimulating hormone (MSH) has not yet been elucidated, there is evidence of extrapigmentary functions [5, 6, 7]. In fact, some effects of natural [3] and synthetic [4] MSH as well as its active peptide core (MSH 4–10) [9, 10] have been reported on the central nervous system of man. There is also evidence for a role of MSH in the control of its own secretion at both pituitary [2] and hypothalamic [8] levels.

Our recent studies on the distribution of radioactivity after the intravenous injection of ^{125}I-labeled-α-MSH have shown a high concentration of radioactivity in the pineal gland, posterior pituitary (including intermediate lobe), thyroid, stomach and esophagus [1]. A relatively low uptake of radioactivity was found at that time in brain tissue. The increasing observations of electrical and behavioral effects of MSH on the central nervous system [5, 6, 7] prompted us to use a more sensitive technique, light microscope radioautography, to study the possible uptake of radioactivity in some brain areas after intracarotid injection of ^{125}I-α-MSH.

METHOD

Eighty μCi of ^{125}I-α-MSH dissolved in 0.4 ml of 0.9 percent NaCl were slowly injected into the left carotid artery of the adult male Sprague-Dawley rat. ^{125}I-luteinizing hormone (LH) (80 μCi) was similarly injected in the control animal. Five min after start of the injection, the brain was fixed by intracardiac perfusion or by immersion in a mixture of 1 percent glutaraldehyde – 10 percent formol in 0.1 M cacodylate buffer (pH 7.4). After dehydration in ethanol, the whole brain was embedded in paraffin. Serial frontal sections (7μ) of the whole brain were cut and every tenth section was mounted on glass slides for radioautography. The slides were then coated with NTB-2 Kodak emulsion. After a 7-day exposure time, the sections were processed and poststained in hematoxylin.

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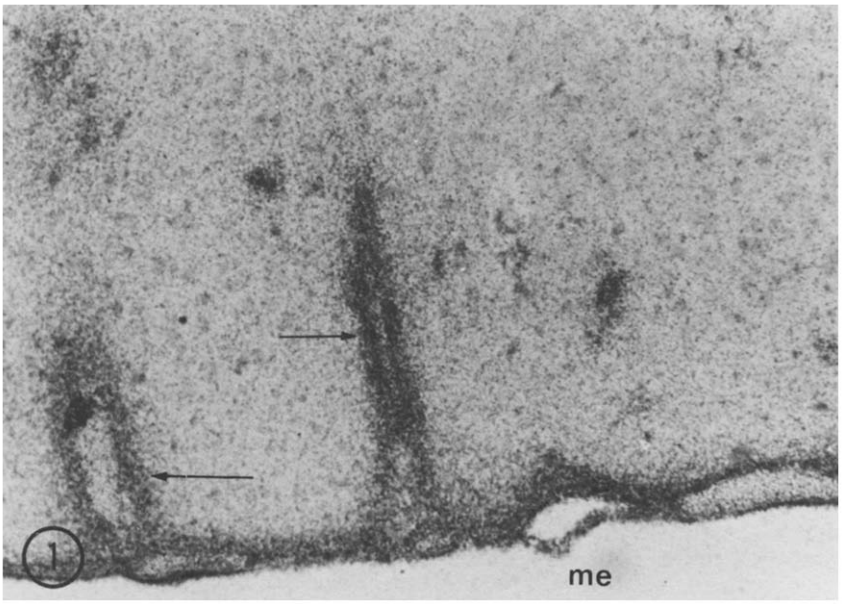

FIG. 1. Low magnification of a portion of the cerebral cortex. A strong reaction is observed in the meninges (me) and around the blood vessels (→). X 300

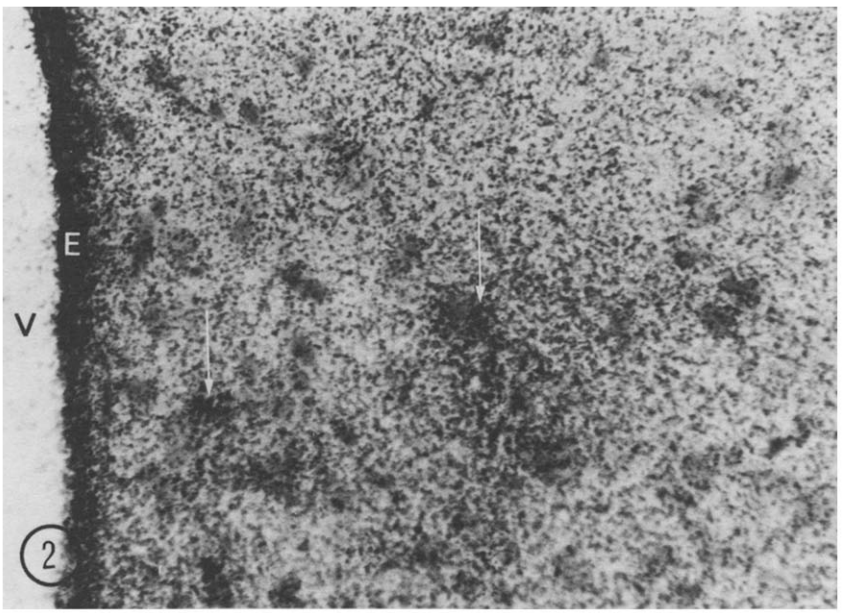

FIG. 2. Portion of the caudate nucleus close to the lateral ventricle (V). A strong reaction is present in the ependymal cells (E) bordering the ventricle. A diffuse reaction is observed in the tissue, whereas a few cells (→) show a strong reaction. X 650

RESULTS

A strong radioautographic reaction was consistently observed in the meninges (Fig. 1), choroid plexus, ependymal cells bordering the lateral ventricles, and in tissue surrounding the blood vessels. In the tissue which was fixed by immersion, a good reaction was also detected in the lumen of the blood vessels. A diffuse reaction was generally present throughout the brain tissue, although a specific concentration of the silver grains was observed over a few cells of the striatum (Figs. 2 and 3) and of the reticular nucleus of the thalamus (Fig. 4). No selective accumulation of radioactivity was observed in the median eminence (Fig. 5) or the subfornical organ.

When ^{125}I-LH was injected instead of ^{125}I-MSH, almost

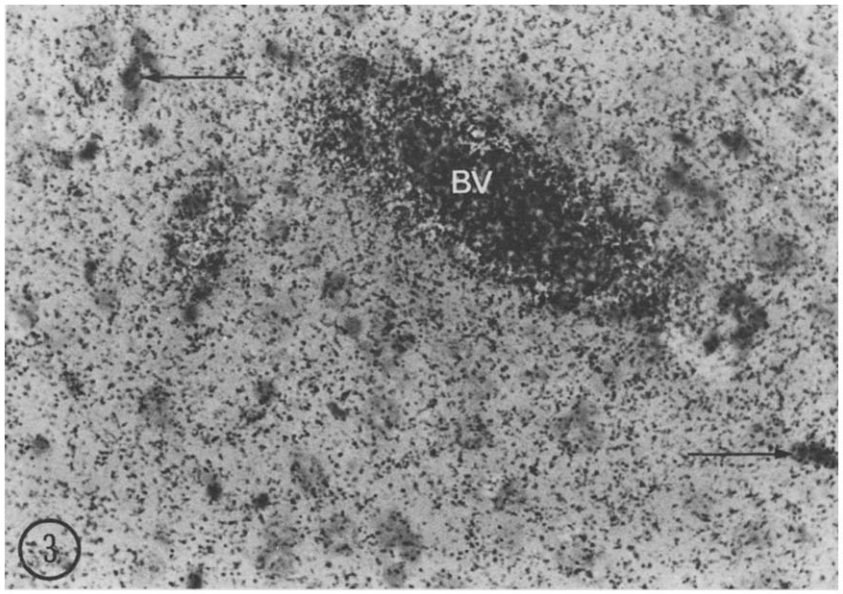

FIG. 3. Caudate nucleus fixed by immersion. A strong accumulation in a blood vessel (BV) is present. Note the accumulation of label over some cells (→). X 700

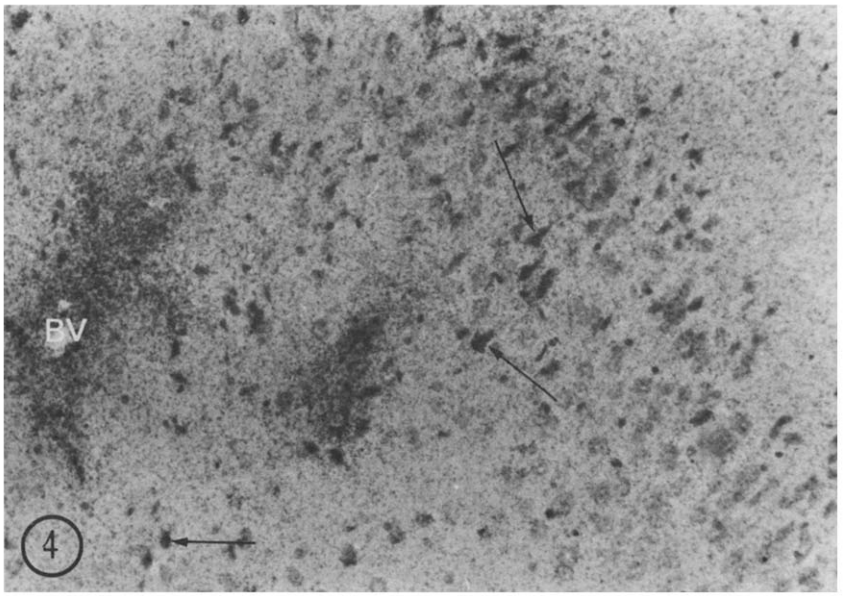

FIG. 4. Nucleus reticularis of the thalamus. Some cell bodies are strongly labeled (→). A strong diffuse reaction around a blood vessel is also present (BV). X 300

no radioactivity was found in the brain tissue even after a longer exposure time (3 weeks).

DISCUSSION

These results show that after injection of ^{125}I-MSH into the carotid artery, radioactive material can diffuse into the brain. Since the choroid plexus, ependymal cells and meninges are strongly labeled, it can be suggested that MSH and/or its metabolites penetrate into the cerebrospinal fluid. Another route of entry of MSH into the brain is probably via the blood vessels as indicated by the high concentration of label occurring around the blood vessels. The diffuse reaction observed in the brain tissue is probably a consequence of radioactivity originating from both the cerebrospinal fluid and blood. These results suggest an absence of a tight blood brain barrier for MSH and/or its metabolites. The lack of penetration of radioactivity into the brain after intraarterial injection of ^{125}I-LH suggests that the observed localization of radioactivity after ^{125}I-MSH is not a general phenomenon.

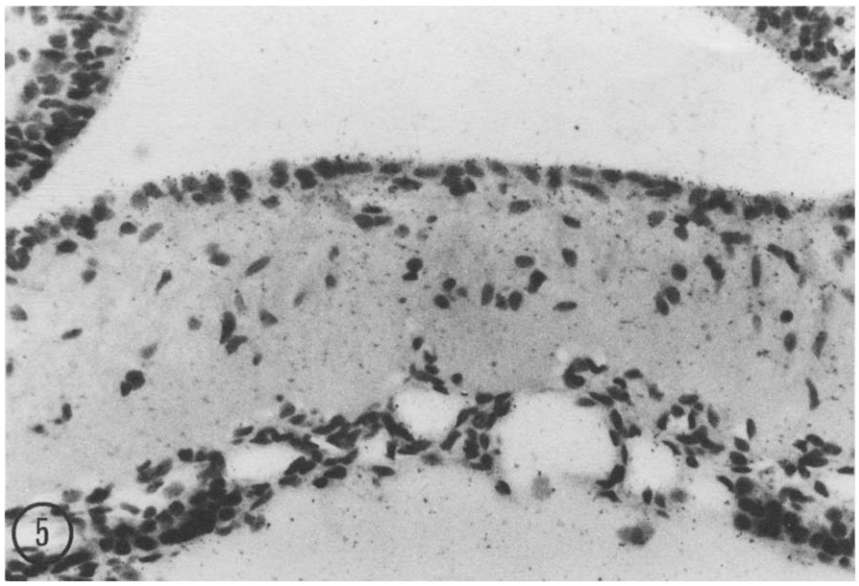

FIG. 5. Median eminence. Only a very weak diffuse reaction can be observed. X 450

The previous observation of a low uptake of radioactivity by brain tissue after intravenous injection of a smaller dose of ^{125}I-MSH into the mouse [1] could probably be explained by the small percentage of radioactivity capable of crossing the blood brain barrier after passage through the general circulation. In the present experiments, the intracarotid route of injection permitted delivery of a larger amount of unmetabolized hormone directly into the brain vessels. Specific uptake of radioactivity by a few cells of the putamen and caudate nucleus as well as the nucleus reticularis of the thalamus was observed. This preferential localization of radioactivity, particularly in the thalamus, is consistent with the modifications of behavior and electrical activity observed in animals and man after injection of MSH.

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