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Radioautographic Localization of Radioactivity in Rat Brain after Intraventricular or Intracarotid Injection of ^{3}H-L-Prolyl-L-Leucyl Glycinamide

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PELLETIER, G., F. LABRIE, A. J. KASTIN, D. COY AND A. V. SCHALLY. *Radioautographic localization of radioactivity in rat brain after intraventricular or intracarotid injection of ^{3}H-L-prolyl-L-leucyl glycinamide.* PHARMAC. BIOCHEM. BEHAV. 3(4) 675–679, 1975. – Tritiated L-prolyl-L-leucyl-glycinamide was injected into the carotid artery or lateral ventricle of rats and the radioactivity localized by radioautography. After intracarotid administration, the radioactivity labeled the meninges, ependymal cells bordering the ventricles, and cells of the choroid plexus, as well as the median eminence and subfornical organ and thus may have reached the cerebrospinal fluid. After intraventricular injection, specific localization of radioactivity was found in cells of the nucleus lateralis septi, nucleus medialis septi, putamen, globus pallidus, indusium griseum, hippocampus, corpus callosum, and meninges. The results after intracarotid injection suggest that L-prolyl-L-leucyl-glycinamide may cross the blood brain barrier and the results after intraventricular injection suggest sites of localization which might be correlated with the previously reported effects of the tripeptide on the brain.

MIF Radioautography Hormone Brain Peptide

WE have recently reported the organ distribution of radioactivity after intravenous injection of tritiated L-prolyl-L-leucyl-glycinamide, α-melanocyte stimulating hormone (MSH)-release inhibiting factor (MIF-I) [2]. The radioactivity was mainly localized in the pineal, anterior and posterior (including the intermediate lobe) pituitary, and epididymal and brown fat, but was not found in significant amounts in the brain.

In animals, MIF-I has been found to potentiate the behavioral effects of L-DOPA [7] and reduce the tremors induced by oxotremorine [8], actions which suggested a possible anti-parkinsonian effect. In the human being, administration of MIF-I was observed to have some beneficial effects in Parkinson's disease [1, 4, 5] and possibly in mental depression [3]. Other CNS effects of the MIF tripeptide have been summarized recently [6]. These extrapituitary effects of L-prolyl-L-leucyl-glycinamide prompted us to use high resolution radioautography to study the distribution of radioactivity in the brain after intraventricular or intracarotid injection of the tritiated peptide.

METHOD

Male Sprague-Dawley rats, weighing 250–300 g, were used in these experiments. By means of a stereotaxic

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instrument, ^{3}H-L-prolyl-L-leucyl-glycinamide (20 μCi) was injected into the left lateral ventricle in a volume of about 20 μl. The animals were fixed by intracardiac perfusion at 5 and 30 min after the intracerebral injection; 200 μCi of the tritiated peptide were also injected into the left carotid, the animal being fixed 5 min after the injection.

The fixation was performed by perfusing 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. For radioautography, serial sections (7 μ) of the whole brain were cut and every tenth section was mounted on glass slides which were subsequently coated with the NTB-2 Kodak emulsion. After appropriate exposure time, the sections were processed and poststained in hematoxylin.

RESULTS

Intracarotid Injection

As observed with light microscopic radioautography, 5

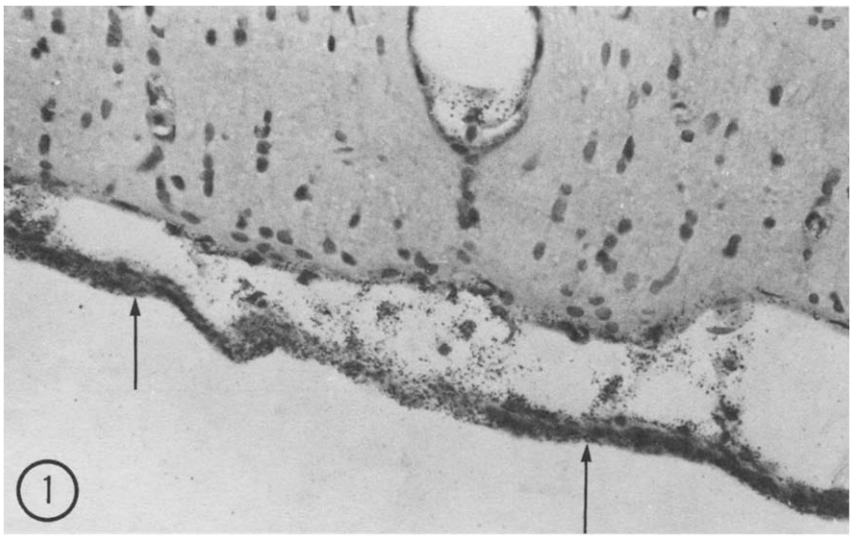

FIG. 1. Portion of the nucleus lateralis of the hypothalamus 5 min after the intracarotid injection of ^{3}H-L-prolyl-L-leucyl-glycinamide. The meninges slightly detached from the brain (arrows) are strongly positive. The rest of the brain tissue is completely negative. X 350

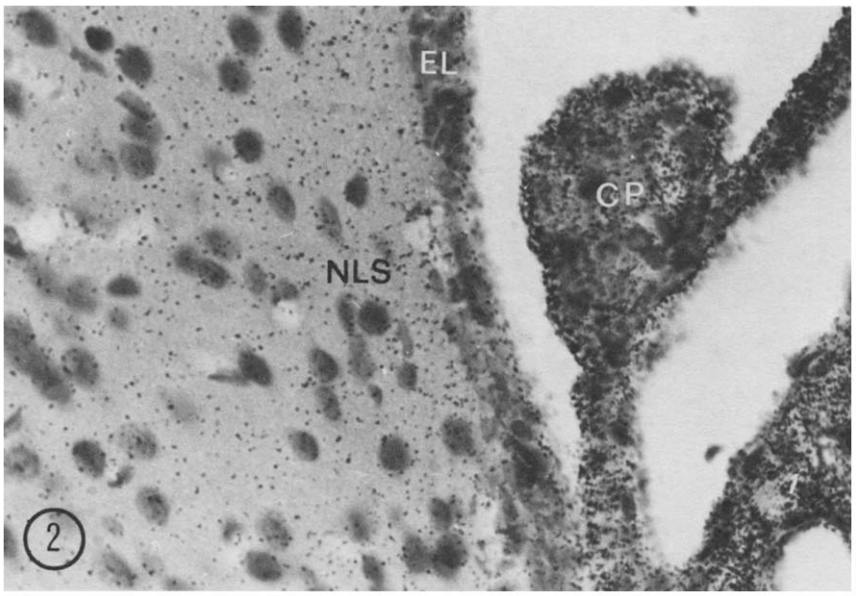

FIG. 2. This radioautograph shows a strong reaction in the choroid plexus (CP) and the ependymal layer (EL) of the lateral ventricle 5 min after the intracarotid injection of ^{3}H-L-prolyl-L-leucyl-glycinamide. A diffuse reaction is also observed in the portion of the nucleus lateralis septi (NLS) close to the ventricle. X 700

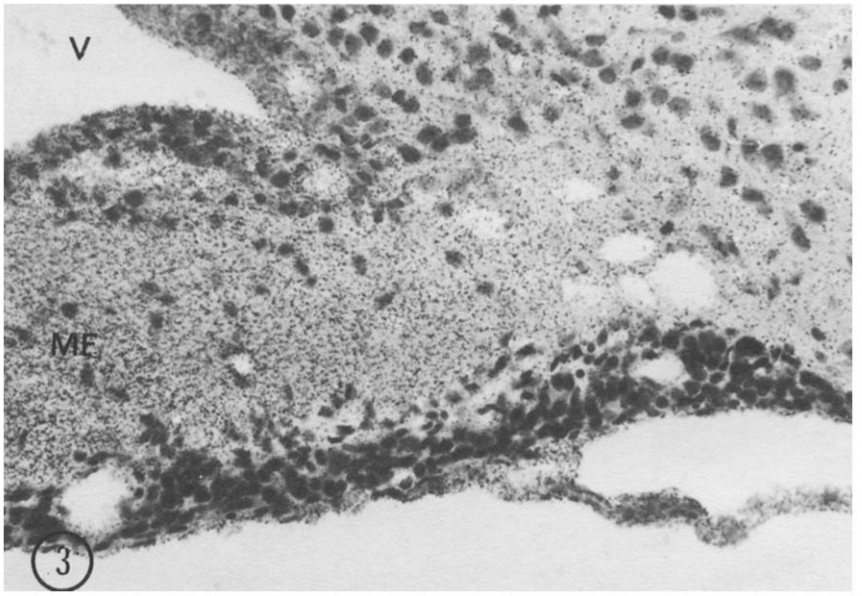

FIG. 3. Portion of the hypothalamus 5 min after the intracarotid injection of ^{3}H-L-prolyl-L-leucyl-glycinamide. A strong diffuse reaction is present in the median eminence (ME). This reaction decreases in intensity at some distance from the median eminence. V = third ventricle. X 400

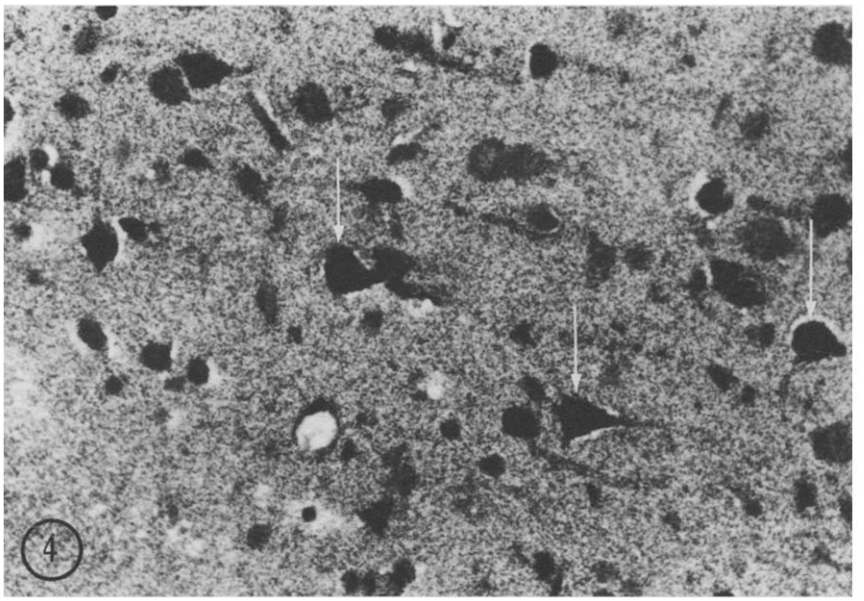

FIG. 4. Portion of the putamen 5 min after the intraventricular injection of ^{3}H-L-prolyl-L-leucyl-glycinamide. A diffuse radioautographic reaction is found whereas many cell bodies are strongly labeled (→). X 300

min after the intracarotid injection of ^{3}H-L-prolyl-L-leucyl-glycinamide, the radioactivity was mainly concentrated in the meninges, choroid plexus, ependymal cells bordering the ventricles, median eminence and subfornical organ (Figs. 1–3). Some degree of diffusion of radioactivity from the ventricles was also noted, but no specific cellular uptake was observed. The other parts of the brain were completely negative at this dose.

Intraventricular Injection

Careful examination of the sections revealed retention of radioactivity in cells of some specific brain areas, similar localization being observed at 5 and 30 min after injection of the tritiated peptide. These areas of increased uptake of radioactivity were found in the nucleus lateralis septi, nucleus medialis septi, putamen, globus pallidus, indusium griseum, hippocampus, corpus callosum, and meninges (Fig.

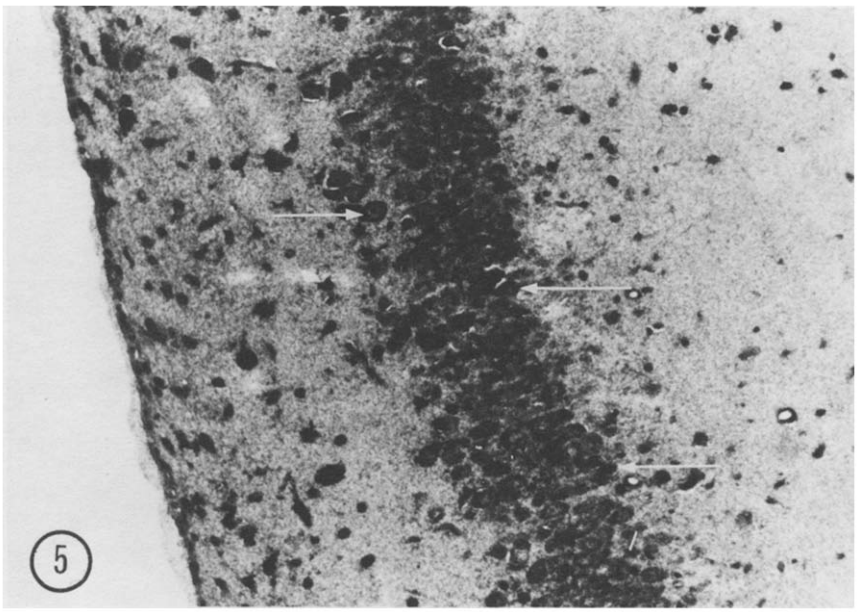

FIG. 5. Portion of the hippocampus 5 min after the intraventricular injection of ^{3}H-L-prolyl-L-leucyl-glycinamide. All the cell bodies (→) are strongly labeled. × 250

4 and 5). The median eminence and the subfornical organ were diffusely but not selectively labeled. Some degree of diffusion of radioactivity from the ventricles to the surrounding tissues was consistently observed. This diffusion was always seen over a short distance from the ventricle and did not necessarily produce a specific uptake by the cells located in the proximity of the ventricles.

DISCUSSION

The present data suggest that after intracarotid injection of ^{3}H-L-prolyl-L-leucyl-glycinamide the radioactivity may reach the cerebrospinal fluid (CSF). This is indicated by labeling of the meninges, ependymal cells bordering the ventricles, and cells of the choroid plexus. The passage of radioactivity from the blood to the CSF probably occurred at the level of the choroid plexus. The specialized areas such as the median eminence and the subfornical organ show a strong accumulation of radioactivity probably because of an absence of the blood brain barrier at these sites [9]. It is likely that the radioactivity originates from both the fenestrated capillaries and the CSF. Our previous observation of a low uptake of radioactivity after the intravenous injection of a smaller dose of labeled MIF–I (4 μCi) into a mouse could be explained by the limited amount of radioactivity which can reach the brain after passage through the general circulation.

When the tritiated peptide is injected directly into the CSF, the high local concentration of radioactivity facilitates the detection of a specific accumulation of significant amounts of the peptide and/or its metabolites. It is possible that administration of large doses of L-prolyl-L-leucyl-glycinamide into the carotid artery could produce a high concentration of the hormone in the CSF and thus lead to detectable uptake in specific brain areas.

Although the approach used does not discriminate between the intact peptide and its metabolites, it is very useful as a first step to identify the different structures capable of concentrating radioactivity. Moreover, it is not known whether the tripeptide itself or its metabolites are responsible for the modifications of behavior observed after parenteral administration. Since radioactivity accumulates in large amounts in the cells of the striatum, it is possible that this highly selective uptake could be related to the reported beneficial effects of MIF-I in Parkinson's disease.

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