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DISTRIBUTION OF ^{125}I-LABELED [2-8]-LEUCOPYROKININ, ACTIVE ANALOG OF LEUCOPYROKININ IN RATS

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Abstract: The brain and internal organs distribution of ^{125}I-labeled [2-8]-leucopyrokinin ([2-8]-LPK), a truncated analog of leucopyrokinin (LPK), an insect myotropic peptide injected into the lateral brain ventricle was determined in rats. A high accumulation of this analog in adrenals and in the hypothalamus and hippocampus of the brain was found. A lesser but significant [2-8]-LPK accumulation in other internal organs and parts of the brain was also observed. The results of the present study confirm results of our previous study performed on the distribution of labeled native LPK in rats. A possible significance of obtained results for the brain function was discussed.

Keywords: leucopyrokinin, [2-8]-leucopyrokinin, distribution, rat

Octapeptide leucopyrokinin (LPK) (Glp-Thr-Ser-Phe-Thr-Pro-Arg-Leu amide) was isolated from the head and corpora cardiaca of Madeira cockroach *Leucophaea maderae* [1, 2]. It exerted a significant myotropic effect on isolated cockroach hindgut [1]. A truncated LPK analog [2-8]-LPK exhibited a higher myotropic effect than the native LPK [1]. It has also been found in our laboratory that intracerebroventricular (*icv*) injection of LPK induced a significant antinociceptive effect in rats [3, 4]. This effect of [2-8]-LPK was also more pronounced than for the native LPK [3, 4].

These effects of both peptides: LPK and [2-8]-LPK were blocked by naloxone, an opioid receptor antagonist [3, 4, 5]. It was also demonstrated that the antinociceptive effect of [2-8]-LPK is mediated mainly by central μ- and δ-opioid receptors [6].

A high accumulation in the hypothalamus and hippocampus of rats brain, and the highest accumulation in adrenals of *icv* injected ^{125}I-labeled LPK were reported [7]. These observations prompted us to determine the accumulation of ^{125}I-labeled [2-8]-LPK in the brain and internal organs of rats.

MATERIAL AND METHODS

The study was performed on male Wistar rats of 200–250 g body weight, obtained from the Animal Farm of the Medical University of Silesia. The animals were kept on 12:12 light/dark cycle (light on from 6 a.m. to 6 p.m.) with free access to standard food (Murigran, Motycz, Lublin, Poland) and water. One week before the experiment polyethylene cannulas (external diameter 0,7 μm, internal diameter 0,4 μm, length 40 mm) (Tomel, Tomaszów Maz., Poland) were implanted into the right lateral brain ventricle (*icv*) under thiopental anesthesia (Thiopental 40 mg/kg *ip*, United Pharmaceutical Works, Prague, Czech Republic) according to the following coordinates: 4 mm depth from the surface of the skull, 2 mm to the right from the sagittal suture, using the same technique as in our previous reports [4, 8]. Cannulas were fixed to the skull bones with acrylic Deltamed PM 16 glue (Chemical Factory, Oświęcim, Poland).

Two days before the experiment synthetic [2-8]-LPK (synthesized in the Faculty of Chemistry, University of Wrocław) (4) was iodinated with

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$Na^{125}I$ (MBq; RI 58-4, Opidi, Świerk, Poland) using the chloramine procedure connected with gel filtration to separate labeled hormone [9].

On the day of experiment a solution of ^{125}I-[2-8]-LPK in a volume of 10 µl of 0.9% NaCl was injected through implanted cannula into the lateral brain ventricle using a Hamilton microsyringe. Control animals were treated with $Na^{125}I$ dissolved in the same volume of 10 µl of 0.9% NaCl. After 1 and 24 h the animals were sacrificed by decapitation and the brains were immediately removed from the skull. The cortex, hippocampus, striatum, hypothalamus, medulla oblongata and cerebellum were dissected, weighed and taken for investigation. The following parts of internal organs were dissected, weighed and taken for investigation: heart, lungs, liver, kidneys, adrenals, testes, a sample of the skull bones and approximately 1 ml of blood. The radioactivity of samples was determined by the counter Gamma Auto Count (LKB, Uppsala, Sweden). The number of impulses on 1 min and 1 g of the fresh tissue (CPM/g) were calculated.

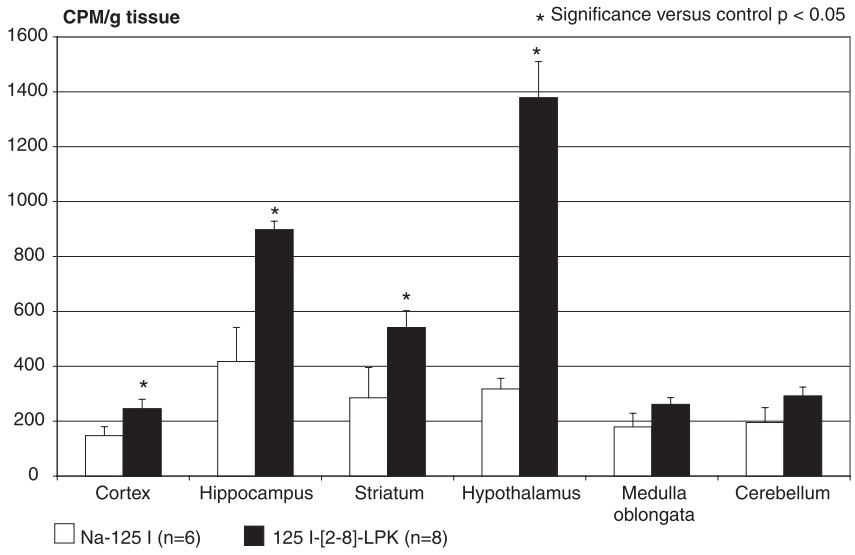

Figure 1. Distribution of ^{125}I-labeled [2-8]-LPK in rat brain 60 min after *icv* injection.

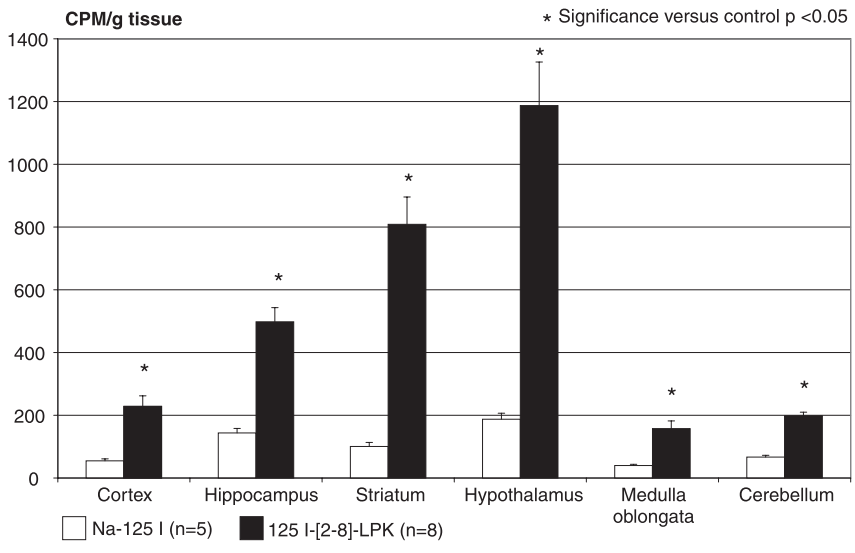

Figure 2. Distribution of ^{125}I-labeled [2-8]-LPK in rat brain 24 h after *icv* injection.

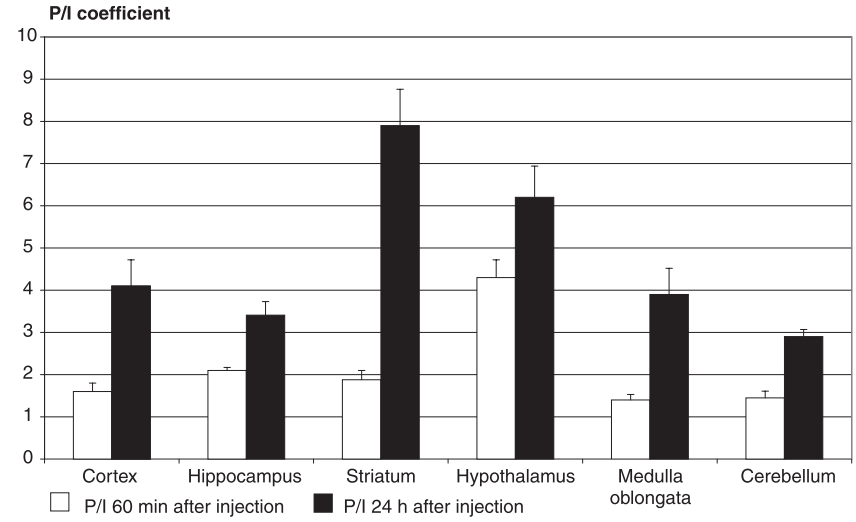

Figure 3. Distribution of ^{125}I-labeled [2-8]-LPK in rat brain after *icv* injection expressed as peptide/iodine (P/I) coefficient.

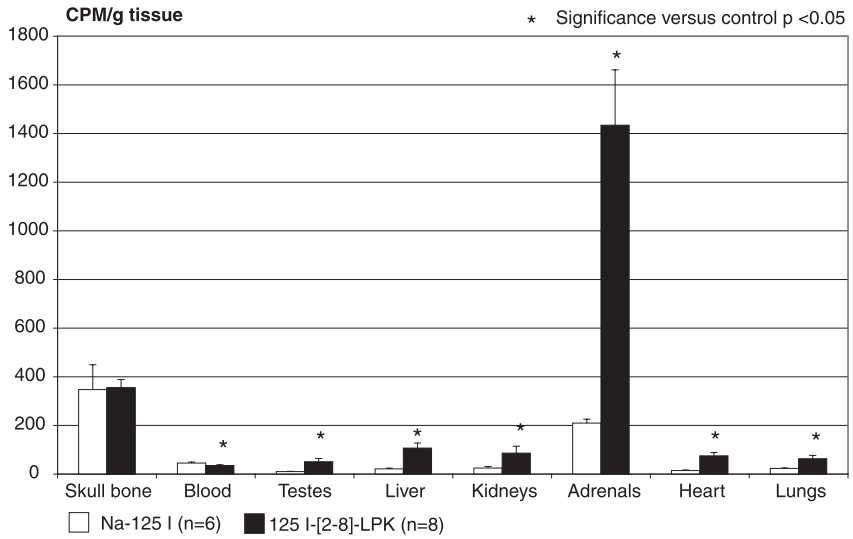

Figure 4. Distribution of ^{125}I-labeled [2-8]-LPK in rat internal organs 60 min after *icv* injection.

The peptide/iodine (P/I) coefficient i.e. the relation between the radioactivity determined in parts of the brain and internal organs of ^{125}I-labeled [2-8]-LPK treated rats and the radioactivity of the same tissue samples of animals treated with Na^{125}I was calculated.

The results obtained were subjected to analysis of variance (ANOVA) and post-ANOVA Dunnett's test to compare the mean radioactivity of ^{125}I-labeled [2-8]-LPK with that of Na^{125}I-treated animals (control group) [10].

The protocol for this study was approved by the local Ethical Commitee of the Medical University of Silesia (L.dz.NN 0-43-60/99).

RESULTS

1 h after *icv* injection of ^{125}I-labeled [2-8]-LPK the highest, significant levels of radioactivity were found in the hypothalamus and hippocampus. In the cortex and striatum significant moderately increased levels of radioactivity were also observed, while in

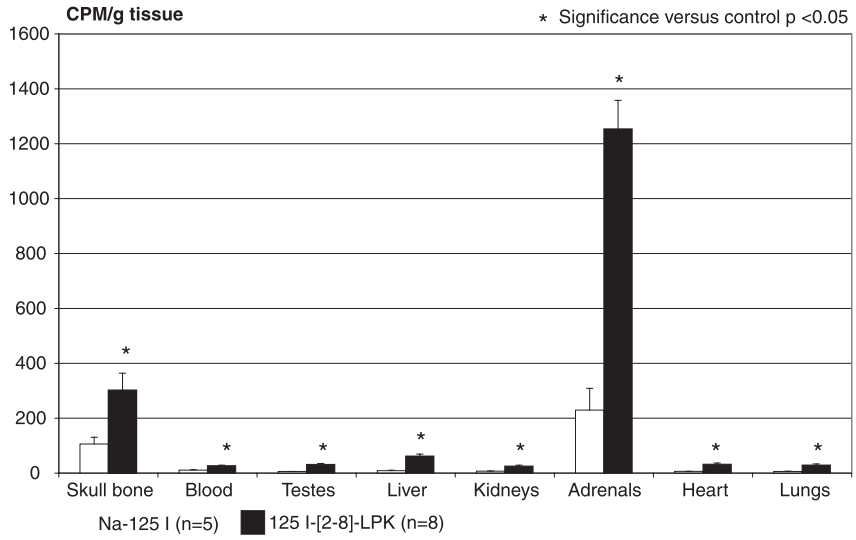

Figure 5. Distribution of ^{125}I-labeled [2-8]-LPK in rat internal organs 24 h after *icv* injection.

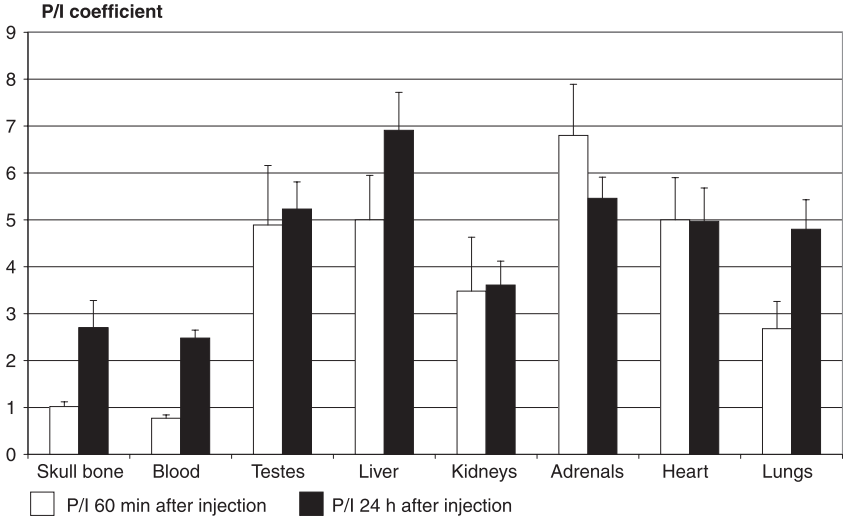

Figure 6. Distribution of ^{125}I-labeled [2-8]-LPK in rat internal organs after *icv* injection expressed as peptide/iodine (P/I) coefficient.

the cerebellum and medulla oblongata any significant changes of radioactivity were recorded (Figure 1). After 24 h there were also significant, very high levels of radioactivity in the hypothalamus, striatum and hippocampus (Figure 2). On the other hand, in other parts of the brain: cortex, medulla oblongata and cerebellum moderately significant elevation of radioactivity was found (Figure 2).

Either 1 or 24 h after *icv* injection of ^{125}I-[2-8]-LPK, a marked and significant increase of radioactivity in the majority of determined samples of internal organs was found, except for adrenals (Figures 4, 5), where very high levels of radioactivity were found.

The values of P/I coefficient in determined parts of the brain and in samples of internal organs were within the range of 2,5 to 8 (Figures 3 and 6).

DISCUSSION

A standard technique of iodination [2-8]-LPK [10], the same as that used in our previous study of

native LPK [7] and prolactin [11, 12], was used in these experiments. *Icv* injection of ^{125}I-[2-8]-LPK was performed to pass through the blood-brain barrier, and obtain good penetration into different parts of the brain because many native peptides displayed poor penetration of the blood-brain barrier [13].

Uptake of iodinated [2-8]-LPK into different parts of the brain and internal organs was expressed as a significant increase of radioactivity in determined samples of tissues of the experimental group in comparison to the controls. We also calculated the P/I coefficient in order to differentiate between peptide and iodine capture. The P/I values obtained in the present study evidently exceeded a value of 1 in the majority of cases (by a range 2,5 to 8) (Figures 3 and 6) and indicated selective uptake of ^{125}I-[2-8]-LPK in evaluated tissues.

The results of the present study seem to be the first demonstration of accumulation of labeled [2-8]-LPK in parts of the rat brain and its internal organs, and completely confirmed our previous study distribution of labeled native LPK in the same rat tissue samples [7]. The highest accumulation of this iodinated, synthetic analog was found in adrenals and in the hypothalamus and hippocampus of rat brains, while in other internal organs and in other parts of the brain it was evidently lower. Such high accumulation of labeled [2-8]-LPK may modulate neuronal function in these parts of the brain, especially the transmission in central opioid receptors. This effect was expressed as evident antinociceptive effect observed after intracerebral or peripheral injection of this analog in rats [3–6], while other behavioural effects were not recorded in rats [8].

Very high accumulation of labeled [2-8]-LPK into the hypothalamus makes also possible the modulation of the function of hypothalamic thermoregulatory centers. It was previously described that either *icv* or *ip* injections of [2-8]-LPK induced biphasic, dose-dependent changes in rat rectal temperature [14, 15]. Moreover, the highest and long-lasting [2-8]-LPK accumulation in adrenals suggests the possibility of modulation of hormonal effects of this peptide.

Acknowledgements

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