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## Distribution in Rats Internal Organs of Intraperitoneally Given <sup>125</sup>I-Labeled Heptapeptide [2-8]-Leucopyrokinin ([2-8]-LPK), a Truncated Analog of Insect Neuropeptide Leucopyrokinin\*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

### Abstract

**Background.** It was previously found that synthetic, insect-derived octapeptide leucopyrokinin (LPK) applied directly into the lateral brain ventricle induced a significant antinociceptive effect in rats. Its synthetic truncated analog heptapeptide [2-8]-leucopyrokinin displayed a stronger antinociceptive effect in comparison to native LPK. Moreover it was previously found a high accumulation of these both <sup>125</sup>I-labeled peptides in adrenals, as well as in hypothalamus and in hippocampus of rats brain.

**Objectives.** The aim of the present study was to assess the distribution of <sup>125</sup>I-labeled [2-8]-leucopyrokinin in rats' internal organs and in several parts of the brain after peripheral – intraperitoneal (*i.p.*) administration.

**Material and Methods.** The study was performed on male Wistar rats. A synthetic [2-8]-leucopyrokinin ([2-8]-LPK) was iodinated with Na<sup>125</sup>I. On the day of experiment a solution of <sup>125</sup>I-[2-8]-LPK was *i.p.* injected and the next after 1 and 24 h animals were sacrificed by decapitation. Radioactivity levels in samples of parts of the brain and of internal organs were determined by counter Gamma Auto Count.

**Results.** A uniform, low accumulation <sup>125</sup>I-[2-8]-LPK was found in evaluated samples of the brain and in internal organs.

**Conclusions.** The results of the present study indicate a weak penetration into the brain and internal organs of intraperitoneally applied <sup>125</sup>I-[2-8]-LPK in rats and correspond with previously determined weak biological effects of *i.p.* injected LPK and [2-8]-LPK (*Adv Clin Exp Med* 2015, 24, 4, 579–584).

**Key words:** [2-8]-leucopyrokinin, distribution in rat brain and internal organs, intraperitoneal administration.

Insect octapeptide, leucopyrokinin (LPK) (Glp-Thr-Ser-Phe-Thr-Pro-Arg-LeuNH<sub>2</sub>) present in neurohemal organs, *corpora cardiaca* of Madeira cockroach *Leucophaea maderae* [1–3] exerts a myotropic effect on isolated cockroach hind-gut [1]. A synthetic, truncated analog LPK, without

the first aminoacid (Glp), heptapeptide [2-8]-leucopyrokinin ([2-8]-LPK) displayed a stronger myotropic activity in comparison to the native LPK [1]. Both peptides: LPK and [2-8]-LPK also displayed biological activity in mammals. It has been found that their intracerebroventricular (icv)

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administration induced a significant antinociceptive effect in rats [4, 5]. This effect of [2-8]-LPK was also stronger than that of native LPK [4, 5]. The antinociceptive effect of LPK as well as of [2-8]-LPK was mediated by central opioid receptors and was blocked by naloxone, an opioid receptors antagonist [4–6]. This effect of [2-8]-LPK was stronger than that of native LPK [4, 5]. A slight but significant antinociceptive effect was also observed after peripheral (*i.p.*) administration of high doses [2-8]-LPK in mice [6]. Moreover, it was demonstrated that:  $\mu$  and  $\delta$  central opioid receptors are mainly involved in mechanism of [2-8]-LPK-induced analgesia in rats [7]. Among other biological effects LPK in rats its thermoregulatory effect was reported [8, 9].

Our earlier study on the distribution of  $^{125}\text{I}$ -labeled LPK or [2-8]-LPK, applied directly into the lateral brain ventricle (icv), displayed a high accumulation of both these peptides in the hypothalamus and hippocampus of rats' brain and the highest accumulation in adrenals [10, 11]. On the other hand, *i.p.* administration of  $^{125}\text{I}$ -labeled LPK resulted in a significant accumulation in different internal organs and a poor distribution in different parts of the rat's brain [12].

The aim of the present study was to evaluate the distribution in rats' internal organs and in several regions of the brain of  $^{125}\text{I}$ -[2-8]-LPK injected intraperitoneally, especially to recognize in this experimental model, the degree of [2-8]-LPK crossing the blood-brain barrier in rats.

## Material and Methods

### Animals

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 in Katowice. The rats were kept on 12:12 h 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.

### Experimental Protocol

Two days before experiment, synthetic [2-8]-leucopyrokinin (synthesized in Faculty of Chemistry, University of Wrocław, Wrocław, Poland [5]) was iodinated with  $\text{Na}^{125}\text{I}$  (MBq; RI 58-4, Opidi, Świerk, Poland) using the chloramine procedure connected with gel filtration to separate labeled peptide [13].

On the day of the experiment a solution of  $^{125}\text{I}$ -[2-8]-LPK was *i.p.* injected at the dose of

100 nmols/100 g body weight/0.1 mL 0.9% NaCl. Control animals were treated *i.p.* with  $\text{Na}^{125}\text{I}$  dissolved in the same volume 0.1 mL 0.9% NaCl, at the dose of 0.1 mL/100 g body weight. After 1 and 24 h 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. Parts of the following internal organs were dissected, weighed and taken for investigations: heart, lungs, liver, kidneys, adrenal, testes. Moreover, a sample of the skull bones and approximately 1 mL of the blood were also taken for investigation. 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. The peptide/iodine coefficient, i.e. the relation between the radioactivity determined in either the samples of internal organs or in parts of the brain of  $^{125}\text{I}$ -labeled [2-8]-LPK treated rats and the radioactivity of the same tissue samples of animals treated with  $\text{Na}^{125}\text{I}$  was calculated. The mean radioactivity levels for groups, expressed as CPM/g  $\pm$  standard error mean (SEM) are presented in columns in Fig. 1–2 and 4–5, while the mean peptide/iodine (PI) coefficients ( $\pm$  SEM) are presented in columns in Fig. 3 and 6. The number of rats in the majority of experimental and control groups were within 6–8 animals.

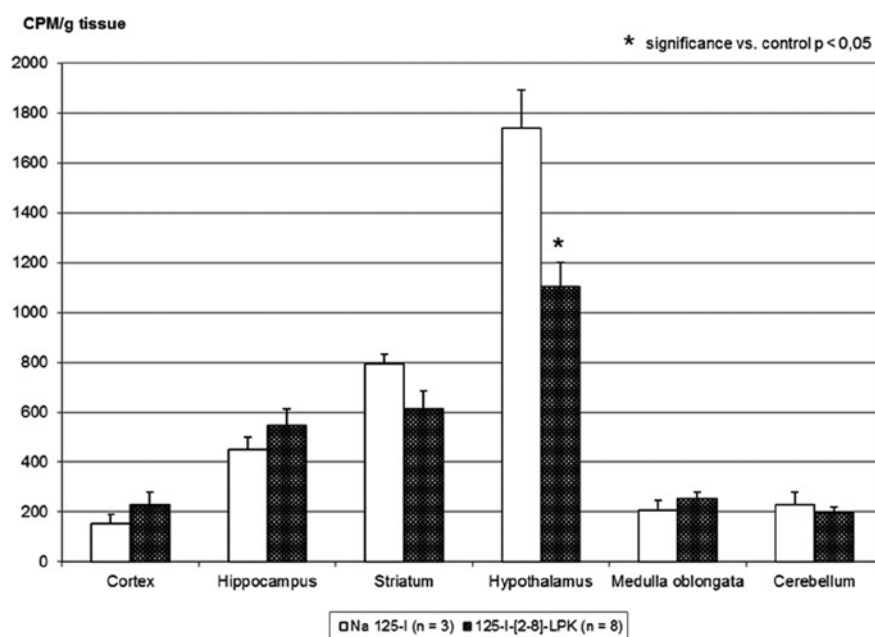
### Statistical Analysis

The results obtained were subjected to statistical analysis by t-test to compare the mean radioactivity levels expressed as CPM/g of the experimental group, i.e.: rats treated with  $^{125}\text{I}$ -labeled [2-8]-LPK with that of control group, which received  $\text{Na}^{125}\text{I}$ . This t-test and computer program for calculations of statistical significance were described by Tallarida and Murray [14]. The statistical significance of differences between experimental and control groups were at  $p \leq 0.05$ .

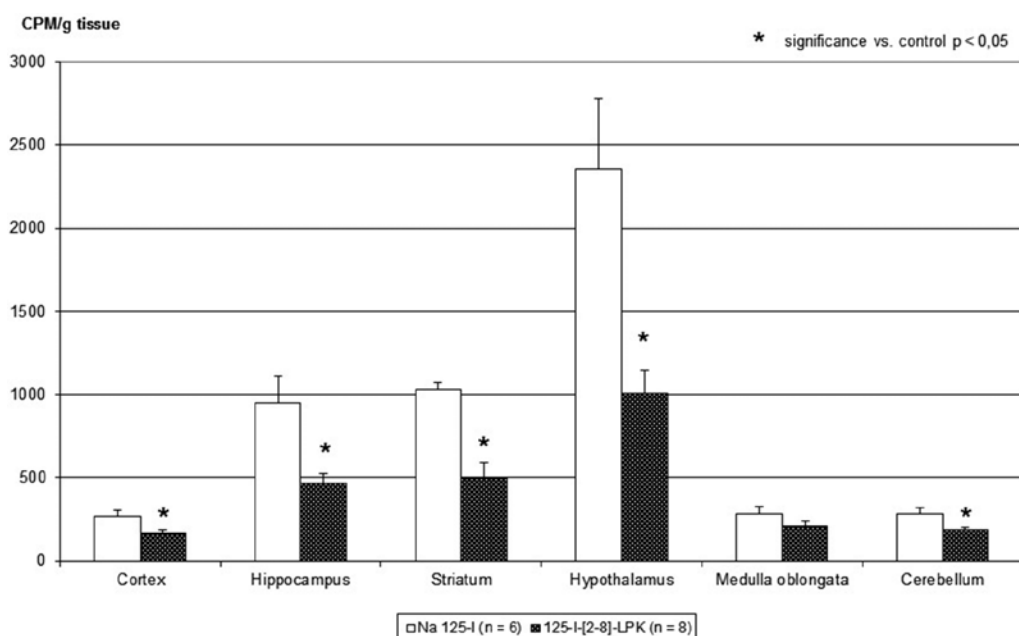
The protocol for this study was approved by the Local Ethical Committee of the Medical University of Silesia (L.dz. NN 0-43-60/99).

## Results

The radioactivity levels recorded after 1 or 24 h after *i.p.* administration of  $^{125}\text{I}$ -[2-8]-LPK were different in various evaluated brain areas and reached the range of 200–1000 cpm/min/g of tissue (Fig. 1, 2). Higher levels of radioactivity were recorded in the hypothalamus, the hippocampus



**Fig. 1.** Distribution of  $^{125}\text{I}$ -labeled [2-8]-leucopyrokinin ([2-8]-LPK) in rat brain 60 min after intraperitoneal injection



**Fig. 2.** Distribution of  $^{125}\text{I}$ -labeled [2-8]-leucopyrokinin ([2-8]-LPK) in rat brain 24 h after intraperitoneal injection

and the striatum (Fig. 1, 2). However, radioactivity levels in almost all parts of the brain of the experimental group ( $^{125}\text{I}$ -[2-8]-LPK treated rats) were similar (Fig. 1) or significantly lower (Fig. 2) than the levels of the control group ( $\text{Na}^{125}\text{I}$  treated rats). Also, the calculated mean values of peptide/iodine (P/I) coefficient in almost all evaluated parts of the brain were about 1.0 or below 1.0 (Fig. 3).

The accumulation of *i.p.* injected  $^{125}\text{I}$ -[2-8]-LPK in several evaluated rats' internal organs is expressed as the level of radioactivity in samples of these internal organs. The mean radioactivity levels in both time intervals 1 and 24 h in the majority

of internal organs of  $^{125}\text{I}$ -[2-8]-LPK treated rats was significantly lower in comparison to the levels in  $\text{Na}^{125}\text{I}$  treated rats (Fig. 4 and 5). Radioactivity levels of samples of all internal organs, except adrenals, of  $^{125}\text{I}$ -[2-8]-LPK as well as of  $\text{Na}^{125}\text{I}$  treated rats were similar (Fig. 4, 5). Radioactivity levels in adrenals were higher in both time intervals of the study (Fig. 4, 5). The values of P/I coefficient in samples of all determined internal organs were below 1.0 (Fig. 6).

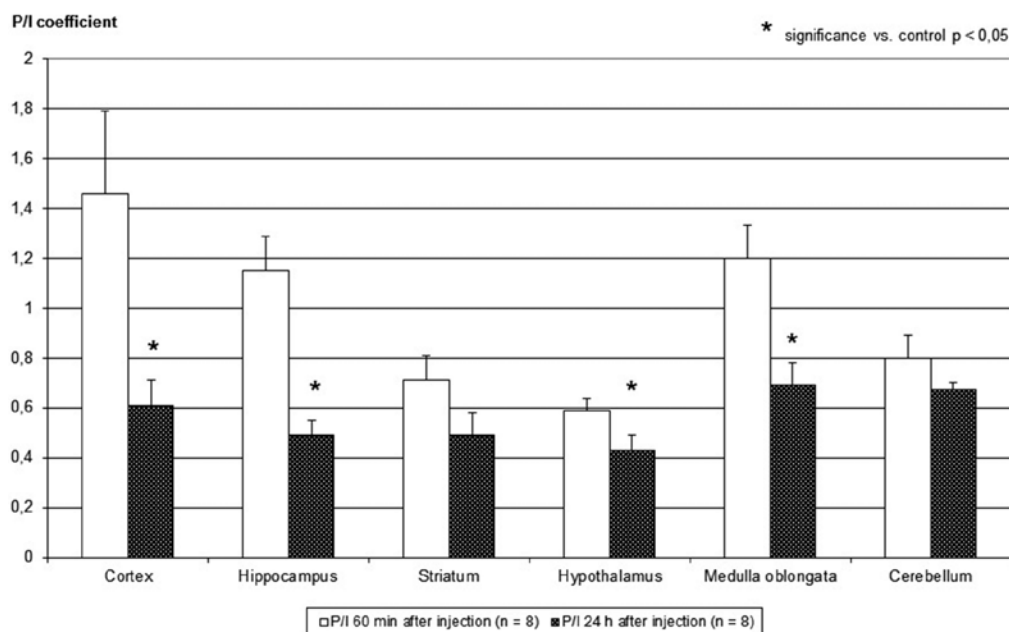


Fig. 3. Distribution of  $^{125}\text{I}$ -labeled [2-8]-leucopyrokinin ([2-8]-LPK) in rat brain after intraperitoneal injection, expressed as peptide/iodine (P/I) coefficient

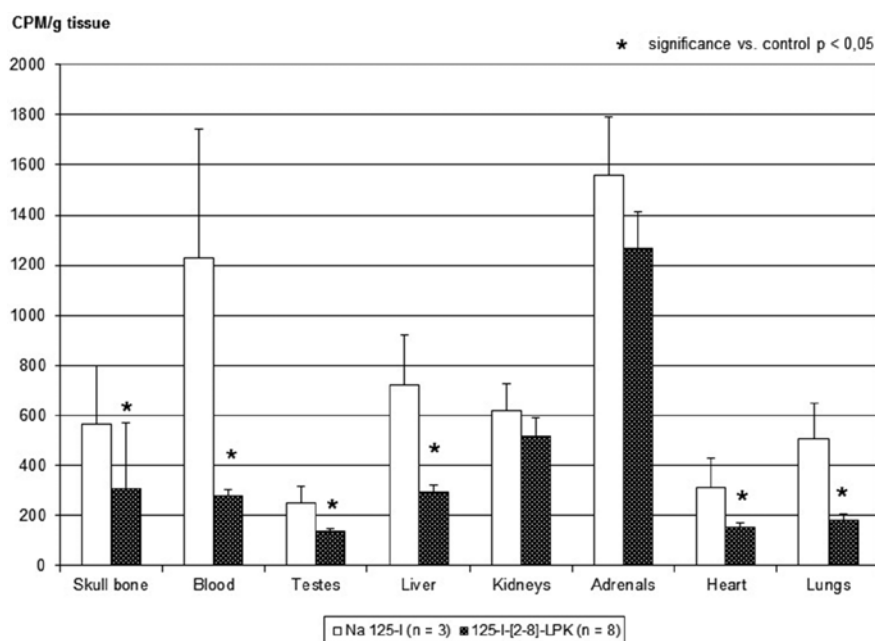
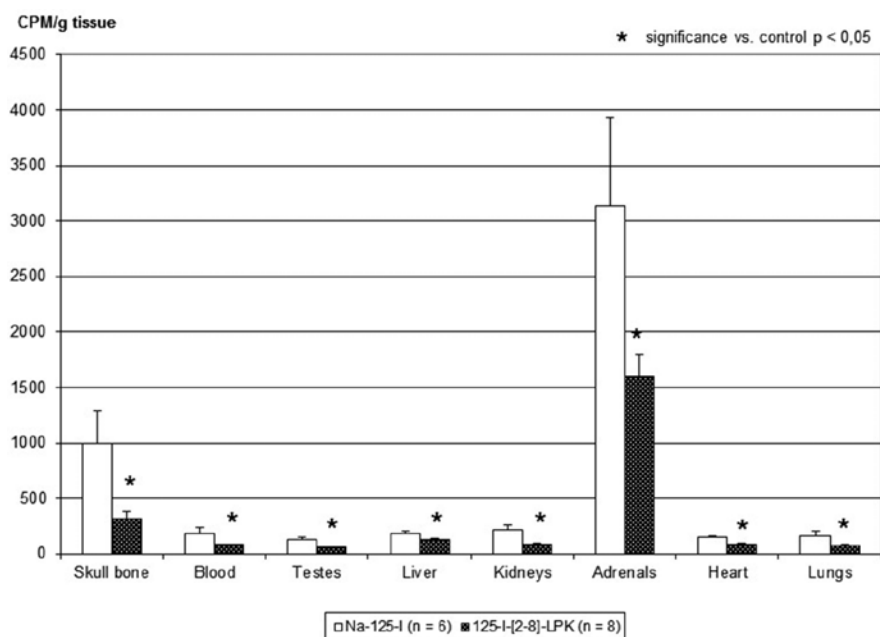


Fig. 4. Distribution of  $^{125}\text{I}$ -labeled [2-8]-leucopyrokinin ([2-8]-LPK) in rat internal organs 60 min after intraperitoneal injection

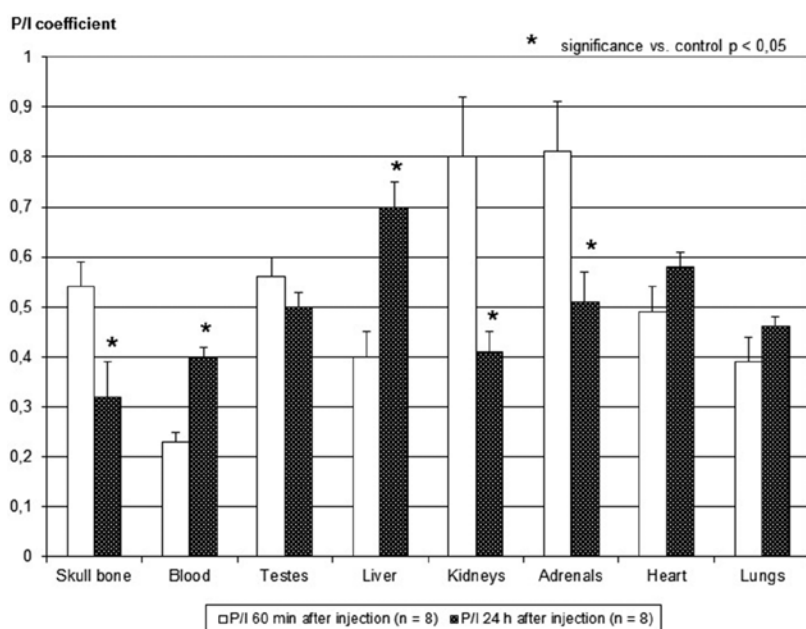
## Discussion

A standard method of iodination of peptides according to Kokot and Stupnicki [13] was used in labeling [2-8]-LPK. The method of labeling peptides with  $^{125}\text{I}$  (iodine) was frequently used in the study of the distribution of labeled peptides in internal organs of experimental animals [15, 16]. It was shown in our previous reports that  $^{125}\text{I}$ -labeled leucopyrokinin (LPK), or  $^{125}\text{I}$ -[2-8]-leucopyrokinin applied directly into the lateral brain ventricle, induced a high accumulation of  $^{125}\text{I}$ -LPK and of  $^{125}\text{I}$ -[2-8]-LPK in rats' hypothalamus, striatum and

hippocampus [10]. The results of the study presented in this report revealed a nearly uniform distribution of *i.p.* applied  $^{125}\text{I}$ -[2-8]-LPK in several internal organs (Fig. 4, 5), and a slight accumulation in adrenals (Fig. 4, 5). *I.p.* administration of  $^{125}\text{I}$ -[2-8]-LPK resulted also in poor accumulation in parts of the brain, expressed as a low radioactivity levels of evaluated samples of the brain (Fig. 1, 2). We regard that *i.p.* administration of  $^{125}\text{I}$ -[2-8]-LPK resulted either in a poor penetration of this peptide from the peritoneal cave *via* blood serum to peripheral organs or in an efficient enzymatic breakdown of this peptide in the blood serum and



**Fig. 5.** Distribution of  $^{125}\text{I}$ -labeled [2-8]-leucopyrokinin ([2-8]-LPK) in rat internal organs 24 h after intraperitoneal injection



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

in peripheral internal organs. Moreover, a poor accumulation of  $^{125}\text{I}$ -[2-8]-LPK in parts of the brain was due to the poor penetration the blood-brain barrier. It is a common opinion that several native peptides weakly cross the blood-brain barrier [17]. The P/I values obtained in the present study in almost all parts of the brain, as well as of internal organs, did not exceed a value of 1 (Fig. 3, 6) and indicated an unselective uptake of  $^{125}\text{I}$ -[2-8]-LPK in tissues of evaluated brain areas and internal organs. The results of our study presented in this report are supported by earlier statements concerning the lack of any significant antinociceptive effect in rats of *i.p.* applied LPK or [2-8]-LPK in rats [4–6] or a slight antinociceptive effect, but determined

after *i.p.* administration of very high doses of [2-8]-LPK[6]. On the other hand, it was shown in our earlier reports that intracerebroventricular administration of LPK or [2-8]-LPK allowed us to overcome the blood-brain barrier, displayed an evident accumulation of these both  $^{125}\text{I}$ -labeled peptides in brain areas [10, 11] and demonstrated their strong biological effect, expressed as an antinociceptive effect in rats [4–6].

Therefore, we conclude that the result of our study proved that intraperitoneal administration of leucopyrokinin analog, synthetic heptapeptide [2-8]-leucopyrokinin results in a weak penetration of this peptide into the brain and to peripheral tissues of the rat.



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