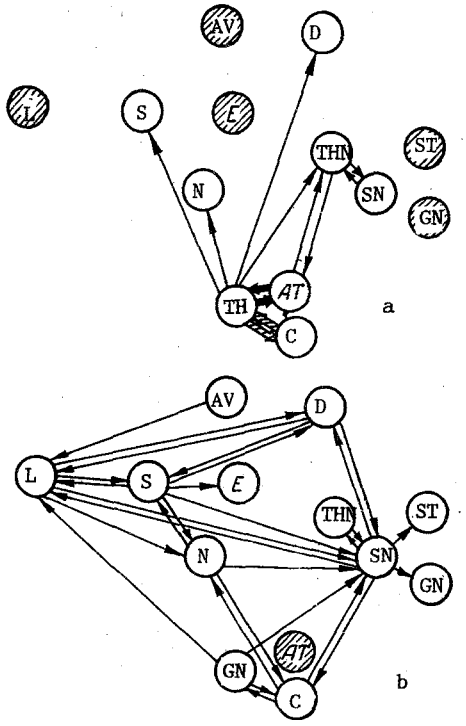

Fig. 1. Graphs of diadic transitions of behavioral acts in experiments with physiological saline (a) and after buprenorphine (0.1 mg/kg) (b). Empty circles – highly probable forms of behavior, obliquely shaded circles – improbable forms. AT) Attack, TH) threat, D) defense, C) circulation, AV) avoidance, N) nuzzling male, SN) sitting in nest in contrast with offspring, G) grooming in nest, ST) standing up on hind limbs in nest, THN) threatening in nest, L) locomotion, S) sitting, E) eating. Symbols used to indicate statistical probabilities of transitions: thin arrow $p < 0.01$, broad broken arrow $0.01 < p < 0.03$; broad solid arrow $p > 0.03$.

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EFFECT OF TUFTSIN ON LEUCYL AMINOPEPTIDASE ACTIVITY OF SUBCELLULAR COMPONENTS OF THE CEREBRAL CORTEX

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KEY WORDS: tuftsin; synaptosomes; brain mitochondria; leucyl aminopeptidase.

The neurotransmitter and neuromodulator role of different peptides is responsible for interest in the enzymes of their metabolism, which participate in the synthesis and breakdown of these peptides. The investigation described below was conducted on leucyl aminopeptidase (LAP), an aminopeptidase of the arylamidase class which degrades enkephalins; it is present in the tissues of many mammals and changes its activity in a number of pathological processes (encephalitis, cataracts, brain tumors) [12, 15].

The tetrapeptide tuftsin is a natural endogenous factor which incorporates four amino acids (Thr-Lys-Pro-Arg), it passes readily through the blood-brain barrier, and a fragment of it (Lys-Pro-Arg) is a component of enkephalins (substance P, neurotension), which regulate

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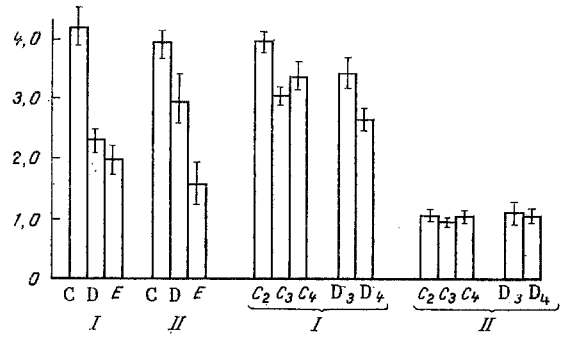

Fig. 1

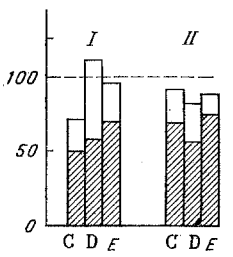

Fig. 2

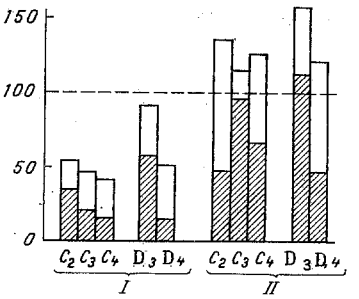

Fig. 3

Fig. 1. Specific activity of LAP (μmoles β-naphthylamine/mg protein/min) in subcellular fractions of sensomotor and visual cortex of normal rabbits. I) Sensomotor and II) visual cortex. C_2) Upper, C_3) middle, and C_4) lower subfractions of membranes of light synaptosomes; D_3) middle and D_4) lower subfractions of membranes of heavy synaptosomes; E) mitochrondria.

Fig. 2. Change (in percent of control) in specific LAP activity in synaptosomal and mitochondrial fractions under the influence of tuftsin. C) Light, D) heavy synaptosomes. Shaded columns indicate exposure to tuftsin for 15 min, unshaded – for 75 min. Remainder of legend as to Fig. 1.

Fig. 3. Change (in percent of control) in specific LAP activity in subfractions of synaptic membranes under the influence of tuftsin. Legend as to Figs. 1 and 2.

central functions in various ways. By stimulating negative motivational responses and interferring in catecholaminergic processes [1, 2, 4], tuftsin selectively influences the activity of several membrane enzymes [3].

Considering the dependence of the direction and effect of tuftsin on the dose and time of its administration, it was decided to study LAP activity in subcellular fractions and synaptic membranes isolated from the visual and sensomotor cortex of rabbits 15 and 75 min after injection of tuftsin (the experiments were conducted jointly with E. I. Orlova.)

EXPERIMENTAL METHOD

Chinchilla rabbits were used. Tuftsin was injected intraperitoneally in a dose of 300 μg/kg body weight in physiological saline, whereas control rabbits received physiological saline alone. After the tuftsin had acted for 15 and 75 min the rabbits were decapitated and LAP activity was determined spectrofluorometrically by the method in [11] in isolated mitochondria from cell bodies (E), and subfractions of synaptosomes (C – light, D – heavy) and of synaptosomal membranes (C_2, D_2 – upper, C_3, D_3 – middle, C_4, D_4 – lower), obtained from these synaptosomes after hypoosmotic shock in an interrupted sucrose density gradient [5]. The results were expressed in micromoles of β-naphthylamine forms per milligram protein per minute (emission 360 nm, excitation 450 nm). The standard was β-naphthylamine obtained from "Lawson." The reaction medium contained: leucyl-β-naphthylamide $1.2 \cdot 10^{-4}$ M as the substrate, 0.05 M succinate buffer, pH 6.5, 10^{-3} M dithiothreitol, the enzyme preparation (subfraction) with a protein concentration of 50-100 μg in a volume of 3 ml. The reaction was recorded in a thermostatically controlled chamber at 37°C for 15 min in "Hitachi" MPF-4 spectrofluorometer (Japan).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the highest activity of the enzyme in the control animals was found in light synaptosomes, the lowest in mitochondria.

Specific activity of the enzyme depended on the brain region from which the subfractions were obtained. For instance, in mitochondria isolated from the sensomotor cortex LAP activity was 29% higher than in mitochondria from the visual cortex. Differences between light and heavy synaptosomes in the sensomotor cortex were more marked than in the visual cortex (183 and 123%, respectively). Specific LAP activity in subfractions of synaptic membranes (upper, middle, lower) was identical, but it was higher in the sensomotor than in the visual cortex. After exposure to tuftsin for 15 min LAP activity fell in both regions of the cortex, but

more so in synaptosomes than in the intracellular mitochondria; however, differences in the reactions to the peptides between heavy and light synaptosomes could not be detected (Fig. 2). Under the influence of tuftsin, activity of the enzyme fell sharply (by 40-80%) in all membrane fractions of the sensomotor cortex. In the visual cortex, in lower (C_4, D_4) and upper (C_2) subfractions of synaptosomal membranes of light and heavy synaptosomes, LAP activity was reduced by between 36 and 54%; in the middle membrane subfractions of light (C_3) and heavy (D_3) synaptosomes, it remained at the control level (Fig. 3).

An increase in the duration of exposure to tuftsin to 75 min restored activity of the enzyme almost to the control level, but only for the intracellular mitochondria in both regions of the cortex. LAP activity in the synaptosomes (especially light) of the sensomotor cortex, although increased, still remained below the control level. LAP activity of all membrane subfractions from the sensomotor cortex remained considerably lower than in the control, whereas in the visual cortex, especially in subfractions D_2 and D_3, it actually exceeded the control value.

Differences in the level of LAP activity were thus found in the control animals both in the homonymous cellular components of the brain regions studied and between subcellular components of the same region. This situation also is confirmed by a number of investigations [8-10, 13, 14] aimed at studying the regional distribution of arylamidases both in parts of the brain (neocortex, paleocortex, basal ganglia, nuclei of the brain stem, etc.) and in subcellular organelles isolated from one particular region. Complementary to data in the literature, the results of the present investigation revealed some particular features of the comparative distribution of LAP in subcellular organelles and their membranes, isolated from the two regions of the cerebral cortex. Under normal conditions activity of this enzyme in synaptosomes and their membranes from the sensomotor cortex is higher than that in the visual cortex.

Analysis of the experimental results indicates that during the selective action of tuftsin on LAP activity, synaptosomes and their membranes are predominantly involved in this process. The reaction develops with time, and is reflected more in cellular organelles of of the sensomotor than of the visual cortex. With an increase in the duration of action of tuftsin, activity of the enzyme in synaptosomes of both regions of the cortex studied was restored almost to its initial level, but in contrast with this, LAP activity in all subfractions of the synaptic membranes of the sensomotor cortex remained almost 50% below the control level, whereas in the visual cortex it actually exceeded that level.

The results are in agreement with data [6, 7] obtained by quantitative cytochemical methods and by autoradiography, revealing differences in the response of individual morphological and functional types of neurons (projection, association) in the sensomotor and visual cortex, with respect to parameters such as protein metabolism. Comparison of our own data with the results of cytochemical studies conducted in our laboratory suggests that exposure of the CNS to the action of tuftsin is reflected in protein metabolism at not only the cellular, but also the subcellular level.

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