EFFECT OF LEU-ENKEPHALIN AND DELTA SLEEP INDUCING PEPTIDE (DSIP) ON ENDOGENOUS NORADRENAL IN RELEASE BY RAT BRAIN SYNAPTOSOMES

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One of the most important mechanisms controling neurotransmitter release in presynaptic modulation, which involves receptors located on nerve endings [12]. Virtually all known mediators and many neuropeptides can act as presynaptic regulators. For instance, opiate peptides can inhibit secretion of substance P, acetylcholine, and dopamine [7-9].

The nonapeptide delta-sleep inducing peptide (DSIP), as several workers have found, causes specific changes in the encephalogram of recipient animals: It prolongs the phase of long-wave or delta sleep [11]. The cellular mechanism of action of DSIP has not yet been explained.

To test the hypothesis that this peptide or its degradation product may be presynaptic regulators of catecholamine release, the action of Leu-enkephalin, DSIP, and amino acids composing DSIP on release of endogenous noradrenalin (NA) from synaptosomes during depolarization was compared.

EXPERIMENTAL METHOD

Subcellular fractions from cerebral hemisphere of noninbred male albino rats were isolated by the method in [1]. Residues of the subcellular fractions were suspended and reprecipitated in the cold in incubation medium: NaCl 115 mM, $NaHCO_3$ 25 mM, glucose 10 mM $MgCl_2$ 1.5 mM, $CaCl_2$ 2.0 mM, parglyine 10 μM, saturated with carbogen at 25°C (pH 7.4). The residues were suspended in the same medium (4 mg protein in 1 ml) and incubated in constant temperature cuvettes for 15 min at 37°C with vigorous mixing. Depolarization was induced by the addition of an equal volume of "stimulation medium," which differed from the incubation medium in its concentrations of KCl (80 mM) and NaCl (35 mM). An equal volume of incubation medium was added to the control cuvette. During depolarization with ouabain the ionic composition of the media in the control and experiments was identical. After the end of incubation the samples were centrifuged at 8000 g for 5 min (Beckman Minifuge B) and the supernatant was used for determination of NA, the residues for measuring enzyme activity. The measurements were made not later than 30 min after addition of ascorbate, on an MPF-2 spectrofluorometer (Hitachi, Japan), at excitation and recording wavelengths of 395 and 510 nm respectively. Lactate dehydrogenase (LDH) activity was determined in the suspension of synaptosomes [6] before and after addition of 0.5% Triton X-100, and succinate dehydrogenase (SDH), Na, K-ATPase, and Mg-ATPase activity were determined by known methods [3, 10]. The protein concentration was measured by a micromodification of the biuret reaction. The Leu-enkephalin and DSIP (Try-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Gly), synthesized in the Institute of Biological Chemistry, Academy of Sciences of the USSR, were generously presented by Corresponding Member of the Academy of Sciences of the USSR V. T. Ivanov. The results were subjected to statistical analysis, using the Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

The ultrastructural and enzymic analysis of fractions of light and heavy synaptosomes (Table 1) showed that the lowest degree of mitochondrial contamination and the best state of preservation of the material were observed in the fraction of light synaptosomes. Incubation for 20 min at 37°C, and exposure to ouabain and to

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TABLE 1. Enzymic Analysis of Subcellular Fractions

Fraction	LDH		B/A	Na, K-ATPase (A)	Mg-ATPase (B)	A/B	SDH
	without Triton X-100 (A)	with Triton X-100 (B)					
Light synaptosomes	0,089	0,758	8,54	5,23	4,70	1.23	5,12
Heavy synaptosomes	0,130	0,350	2,69	6,75	5,75	0,96	10,1
Free mitochondria	0,106	0,115	1,23	—	—	—	11,8

Legend. Mean values of three independent measurements in one (typical) experiment are shown. Mean error of measurements nowhere exceed 10%. Altogether four experiments were done. LDH and ATPase activities expressed in micromoles/min/mg protein at 25 and 37°C respectively, SDH activity in nanomoles/min/mg protein (25°C).

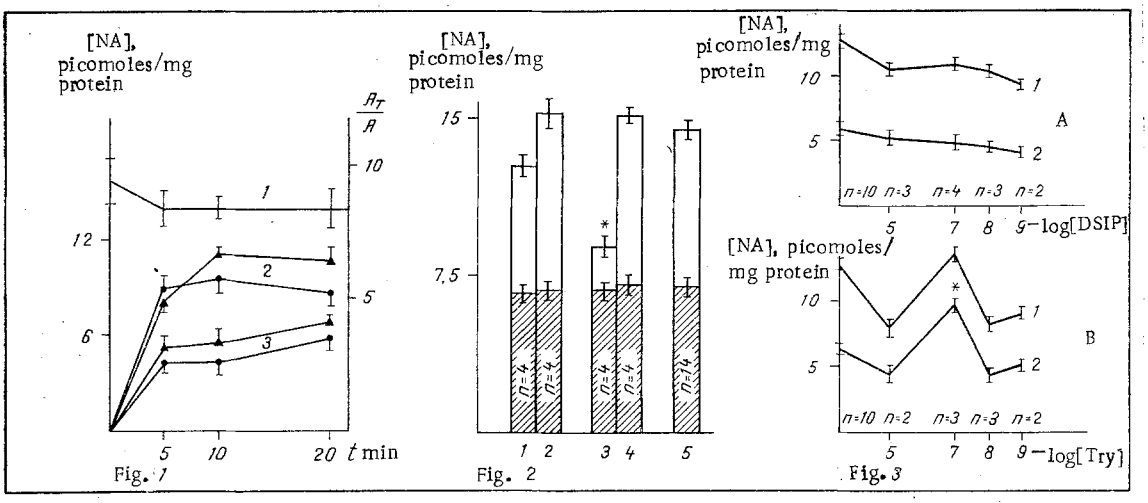

Fig. 1. Effect of duration of incubation and of depolarization procedures on integrity of synaptosomes and secretion of endogenous NA. Abscissa, incubation time (in min); ordinate: on left – NA concentration (in picomoles/mg protein), on right – ratio of activities of LDH measured in presence (A_T) and absence (A) of Triton X-100. 1) A_T/A in presence of 40 mM KCl and 1.5 mM ouabain (data agree fully with control): 2, 3) NA concentration in medium during potassium and ouabain depolarization respectively, 4) NA concentration in medium during spontaneous release. Values expressed in the form $M \pm \sigma$ (four independent experiments, concentration of mediator during depolarization was higher ($P < 0.01$, results of three measurements) in each experiment than in the control.

Fig. 2. Effect of Leu-enkephalin on NA secretion during potassium depolarization of synaptosomes. Abscissa, number of experiments; ordinate, NA concentration in incubation medium (in nanomoles/mg protein). 1) 10^{-6} M enkephalin, 2) the same, in the presence of 10^{-5} M naloxone, 3) 10^{-5} M enkephalin, 4) the same, in the presence of 10^{-5} M naloxone, 5) control. Shaded part of columns – spontaneously released NA, unshaded part – secretion induced by depolarization. *$P \leq 0.05$ compared with control.

Fig. 3. Effect of DSIP (A) and mixture of amino acids (B) on NA secretion during calcium depolarization of synaptosomes. Abscissa, negative logarithm of concentration (in M); ordinate, NA concentration in incubation medium (in nanomoles/mg protein). n) Number of experiments. 1) Depolarization, 2) control. *$P \leq 0.05$ compared with control.

a raised K^+ concentration were shown not to lead to LDH release, i.e., not to disturb the integrity of the synaptosomes (Fig. 1). Meanwhile, after both kinds of depolarization, accumulation of endogenous NA in the incubation medium ended after 10 min. On the basis of these data, depolarization of the suspension of light synaptosomes for 10 min was chosen as the test system with which to study the action of the peptides.

Leu-enkephalin, in concentrations of 10^{-5} and 10^{-6} M inhibited K^+-induced NA secretion (Fig. 2). This effect was completely abolished by naloxone, which proves that presynaptic opiate receptors participate in it. It was shown previously that opiate peptides can inhibit secretion of radioactive material, induced by de-

polarization, from synaptosomes incubated beforehand with ^{3}H-NA, but the nature of the secreted radioactive material in this case was not identified [5]. Our results show that Leu-enkephalin does in fact inhibit secretion of endogenous NA induced by depolarization of synaptosomes.

Unlike Leu-enkephalin, DSIP in concentrations of 10^{-8} to 10^{-5} M affected neither spontaneous nor depolarization-induced secretion of NA (Fig. 3). This is evidence that DSIP has no modulating action on NA secretion. Unlike opiate receptors, specific receptors for DSIP are evidently not present on nerve endings containing NA.

The mixture of amino acids composing DSIP, taken in the same proportions as in this peptide, we used as a control of the action of possible proteolysis products of DSIP. It was found that a mixture of amino acids (such as tryptophan) in concentrations of 10^{-9} to 10^{-5} M did not affect release of the mediator induced by potassium depolarization, but in a concentration of 10^{-7} M it caused the level of spontaneously released NA to rise (Fig. 3). Since no such action was observed with other concentrations of the amino-acid mixture it can be concluded that several independent mechanisms are involved in its realization. It must be pointed out that the amino-acid mixture used contained three hypothetical "mediator" amino acids, whose amides, in the modern view, can play the role of mediators in the CNS: aspartic and glutamic acids and glycine. Accordingly we can explain the action of the mixture of amino acids on spontaneous NA release by the possible effect of mediator amino acids on presynaptic heteroreceptors, controling the secretion of this mediator.

Although, under the experimental conditions chosen, DSIP in a suspension of synaptosomes did not affect NA release, the possibility that in response to central (intraventricular) injection of this peptide the rate of its proteolysis, i.e., of accumulation of free amino acids, may be much greater than in the present experiments, cannot be ignored. A rough calculation shows that if 1 μg DSIP is injected into the III ventricle of the rabbit brain, even if it is accepted that the volume of this ventricle is 1 ml, the concentration of the peptide will be 10^{-6} M. Under these conditions total hydrolysis of 10% of DSIP molecules can maintain a concentration of free amino acids (such as tryptophan) of about 10^{-7} M.

The possibility thus cannot be ruled out that some of the late (latent) effects of DSIP observed after central injection of the peptide [13] may be caused, not so much by the peptide itself as by its proteolysis products, namely free amino acids, which accumulate in a definite order, dictated by the primary structure of the peptide.

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