DELAYED EFFECT OF TETRAPEPTIDE TYR-D-ALA-GLY-PHE-NH_2 ON SEROTONIN CONTENT IN RABBIT BRAIN SYNAPTOSOMES

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The endogenous opiate peptides — methionine- and leucine-enkephalins and their synthetic analogs, in which the glycine in position 2 is replaced by D-alanine — give morphine-like pharmacologic effects such as depression of pain sensitivity, changes in motor activity, and so on [1, 15]. It has been suggested that the action of these peptides is mediated by monoaminergic and, in particular, serotoninergic, brain systems. For instance, a fall in the serotonin level brought about in various ways partially blocks morphine analgesia, whereas elevation of its level potentiates it [10]. Moreover, if serotoninergic neurons in the CNS are inactivated, morphine does not exhibit its complete clinical effect [15]. However, the fine mechanisms of interaction of morphine and opiate peptides with the serotoninergic system of the brain have not yet been elucidated. It is generally considered that morphine, if given as a single dose, causes the serotonin concentration in brain tissue to rise [7], but this was found in experiments with inhibition of monoamine oxidase activity. Meanwhile there is evidence that morphine and synthetic analogs of enkephalins do not raise the serotonin level as such in brain tissue, but the concentration of its metabolite 5-hydroxyindoleacetic acid [6, 7, 15]. In other words, it can be tentatively suggested that morphine and opiate peptides increase the degree of serotonin utilization.

The tetrapeptide Tyr-Gly-Gly-Phe or its synthetic analog Tyr-D-Ala-Gly-Phe-NH_2, is known to be the minimal structural unit capable of binding with opiate receptors and exerting an analgesic action [9, 13]. The tetrapeptidamide Tyr-D-Ala-Gly-Phe-NH_2 exhibits marked analgesic activity, although weaker than morphine [9].

In the overwhelming majority of investigations the state of parameters of the serotoninergic system was determined either within a few hours after a single injection of morphine or opiate peptides or after their repeated administration for a period of several weeks [5, 7].

The object of this study was to investigate the serotonin concentration in synaptosomes of certain formations of the rabbit brain 5 days after a single injection of the tetrapeptide-amide Tyr-D-Ala-Gly-Phe-NH_2 (TPA). The reason was that changes in a number of electrophysiological parameters of the rat brain lasted several days after a single injection of TPA.

EXPERIMENTAL METHOD

Male rabbits weighing 2.0-2.2 kg were used. The animals were given a subcutaneous injection of TPA in 0.9% NaCl solution in a dose of 500 μg/kg body weight. Rabbits receiving a subcutaneous injection of the same volume of 0.9% NaCl solution served as the control. The animals were decapitated 5 days after the injection, the brain was removed, and the motor cortex and caudate nucleus were isolated. All operations with the brain were performed at 0°C. Synaptosomes were isolated as in [8]. The synaptosomes (light and heavy together) were sedimented by centrifugation at 100,000g for 60 min. The residue of synaptosomes was suspended in 0.5 ml of 0.1 N HCl and frozen at —20°C. To determine the serotonin concentration in the synaptosomes they were thawed, 0.1 N HCl saturated with NaCl was added to a final volume of 1.0 ml, and the mixture was homogenized. All subsequent procedures were carried out as described in our modification of the spectrofluorometric o-phthalic dialdehyde method [2].

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TABLE 1. Effect of a Single Injection of the Tetrapeptideamide Tyr-D-Ala-Gly-Phe-NH_2 on Serotonin Concentration (in μg/mg protein) in Synaptosomes of Various Structures of Rabbit Brain (M ± m, n = 5)

Experimental conditions	Motor cortex	Caudate nucleus
Control	5,82±0,43	45,79±3,57
Injection of TPA	7,71±0,76	22,10±2,57

Protein was determined by Lowry's method [12]. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results are given in Table 1. The serotonin concentration in synaptosomes from the motor cortex of the control rabbits was about one-seventh of that in synaptosomes from the caudate nucleus. This is further confirmation of results obtained at the tissue level [4], which show that the serotonin concentration in subcortical structures of the mammalian brain is several times higher than in the cortex.

The serotonin concentration in synaptosomes of the rabbit caudate nucleus was reduced by 51.7% ($P < 0.01$) 5 days after a single injection of TPA in a dose of 500 μg/kg ($P < 0.01$). Changes in its concentration in synaptosomes from the motor cortex were not significant. These experiments thus revealed a delayed effect of opiate TPA on the serotoninergic system of the brain, manifested 5 days after a single injection. The absence of changes in the serotonin concentration in synaptosomes of the motor cortex can be explained by regional differences in the metabolism of this monoamine in the brain [3] and the effect of opiate peptides on it [6, 15].

Under the influence of morphine or the synthetic analog met-enkephalin, release of labeled serotonin into perfusate from sections consisting of a combination of gray matter of the aqueduct of Sylvius and dorsal raphe nucleus (these sections were preincubated with labeled serotonin) is considerably activated. The same effect of morphine also was found in experiments with synaptosomes [10]. The investigation cited showed that morphine, *in vitro*, on the one hand stimulated serotonin release from synaptosomes, but on the other hand inhibited binding of serotonin with serotonin-binding protein of the soluble fraction of synaptosomes, which, it is suggested, is concerned in the storing and (or) transport of this monoamine. Consequently, it can be postulated that serotonin release *in vivo* from its storage sites under the influence of opiate peptides, on the one hand, causes its more rapid secretion from terminal nerve endings and, on the other hand, increases its accessibility for the metabolite action of monoamine oxidase. This last hypothesis is confirmed by data showing [6, 7, 15] that the 5-hydroxyindoleacetic acid concentration in brain tissue is increased by the action of morphine and opiate peptides. Stimulation of serotonin release from nerve endings is probably an essential factor for manifestation of the analgesic effect of morphine and the enkephalins, for preliminary emptying of the serotonin depots with reserpine lowered the analgesic activity of the above-mentioned compounds [11]. The authors cited suggested that morphine and enkephalins evidently modulate serotonin release from nerve endings or activate its turnover in them. It is difficult as yet to explain what causes this prolonged effect of a single dose of TPA on the serotoninergic system. It is undoubtedly based on a long-term change in the regulatory mechanisms responsible for the storing, utilization, and release of serotonin from nerve endings.

Such delayed biochemical effects of single injections of opiate peptides, not previously reported, are evidently of great biological importance. It can be postulated that effects of neuropeptides similar to that we have established are linked with memory processes at different levels of duration [1]. It is also important to note the relevance of this effect to applied studies of opiate peptides: Such delayed manifestations of the action of these compounds must be taken into account before some of them are subjected to clinical trials.

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ENDOGENOUS ACETYLCHOLINE-INDUCED Na,K-ATPase ACTIVATORS AND INHIBITORS

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It was shown previously that the level of microsomal Na,K-ATPase activity in nerve cells is controlled by acetylcholine (ACh) [3, 4] or its pharmacologic analogs [2, 6]. Since ACh in a cell-free system activates the enzyme and since this effect is abolished by actinomycin D (a transcription inhibitor) and puromycin (a translation inhibitor), it was logical to suggest that ACh induces synthesis either of the enzyme itself or of a protein-activating factor [3, 4]. The investigation described below was undertaken to determine the correct alternative.

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. The animals were quickly decapitated and the whole brain (without the cerebellum and caudal part of the medulla) was homogenized in a cold solution of 0.3 M sucrose, 0.05 M Tris-HCl, pH 8.0 (5 ml per brain). Aliquots of homogenates (3.8 ml each) in the experimental tests were treated with 0.2 ml of an aqueous solution of either ACh (to a final concentration of 10^{-5}-10^{-3} M) or eserine (to a concentration of 10^{-6}-10^{-5} M), and in the control tests with 0.2 ml water, and incubated for 45 min at 37°C with periodic shaking. The reaction was stopped by the addition of 10 ml of cold 0.3 M sucrose containing 5 mM EDTA. The mixture was centrifuged for 15 min at 12,000g. The supernatant was drawn off and centrifuged for 60 min at 30,000g. The residue of microsomes was suspended in 2.4 ml of 0.05 M Tris-HCl, pH 7.55, and their Na,K-ATPase activity was determined by the method described previously [3]. All samples (1 ml) contained 0.5 mM EDTA. The control lev-

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