TRYPTOPHAN-5-HYDROXYLASE ACTIVITY IN RABBIT BRAIN SYNAPTOSOMES AT DIFFERENT TIMES AFTER A SINGLE INJECTION OF THE OPIOID PEPTIDE Tyr-D-Ala-Gly-Phe-NH_2

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KEY WORDS: tryptophan-5-hydroxylases; opioid peptide; synaptosomes; single injection; late effect.

It has recently been shown that the different effects of morphine and opioid peptides, such as lowering of pain sensitivity, changes in motor activity, activation of prolactin secretion, and so on, are mediated through the monoaminergic systems of the brain and, in particular, the serotoninergic system [8, 12, 13]. A fall in the serotonin concentration or blocking of serotoninergic receptors has been shown to abolish these effects of the substances mentioned above. However, the precise mechanisms of interaction of opioid peptides and morphine with the serotoninergic system of the brain have not yet been elucidated.

The writer previously [5] found a late effect of the tetrapeptide amide Tyr-D-Ala-Gly-Phe-NH_2 (TPA) on the serotoninergic system of the rabbit brain, manifested as a change in the serotonin concentration in the synaptosomes of some brain formations 5 days after a single injection.

According to data in the literature, TPA not only has marked analgesic activity, exhibited immediately after administration [2, 10], but also has the ability to depress the motor activity of animals, and this effect develops to its maximal degree 3-5 days after a single injection [3].

To discover the possible mechanism of the change in the state of the serotoninergic system at different stages of the action of TPA, the writer studied the activity of the key enzyme of serotonin biosynthesis, tryptophan-5-hydroxylase, in synaptosomes of the motor cortex and caudate nucleus of the rabbit brain 30 min and 5 days after a single injection. Some results of this investigation were published previously [6].

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing about 2.0 kg. TPA was injected subcutaneously into the experimental animals in a dose of 500 μg/kg in 0.9% NaCl solution, and control rabbits received an injection of the same volume of NaCl solution alone. The animals were decapitated (in the morning before 9 and 10 a.m.) 30 min and 5 days after the single injection, the brain was removed, and the motor cortex and caudate nucleus were isolated. All operations with the brain were carried out at 0°C. Synaptosomes were isolated [9] and sedimented by centrifugation at 100,000g for 60 min. The residue of synaptosomes was homogenized in 0.05 M Tris-HCl, pH 7.6, containing 2 mM dithiothreitol and 0.5% Triton X-100, and centrifuged for 20 min at 50,000g. Activity of tryptophan-5-hydroxylase was determined in the supernatant as described previously [4], using 6,7-dimethyl-5,6,7,8-tetrahydropterine as the coenzyme. Protein was determined by Lowry's method [11]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Tryptophan-5-hydroxylase activity in synaptosomes of the caudate nucleus of the control rabbits was found to be twice as high as that in synaptosomes of the motor cortex, namely

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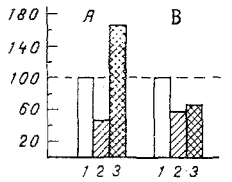

Fig. 1. Tryptophan-5-hydroxylase activity in synaptosomes of motor cortex (A) and caudate nucleus (B) of rabbit brain 30 min and 5 days after a single injection of TPA (in % of control). 1) Control (nine experiments), 2) 30 min after injection (4-5 experiments), 3) 5 days after injection (4-5 experiments).

159.67 ± 14.99 and 80.84 ± 8.69 pmoles of 5-hydroxytryptophan formed per milligram protein per hour. This confirms data [4, 7] obtained by the writer previously at the tissue level, evidence that the activity of this enzyme in the mammalian cerebral cortex is significantly lower than in the basal ganglia.

A significant decrease in tryptophan-5-hydroxylase activity in synaptosomes of the motor cortex and caudate nucleus by 64 and 43%, respectively ($P < 0.01$ in both cases; Fig. 1) was observed 30 min after a single injection of TPA in a dose of 500 μg/kg.

Differences in its action on activity of the test enzyme appeared 5 days after a single injection of TPA. For instance, tryptophan-5-hydroxylase activity in synaptosomes of the motor cortex increased by 68.4% compared with the control ($P < 0.01$), whereas in synaptosomes of the caudate nucleus it remained 35.6% below the control ($P < 0.01$; Fig. 1). Under these same experimental conditions, this effect was accompanied (as the writer showed previously [5]) by a significant fall in the serotonin concentration in synaptosomes of the caudate nucleus, whereas in synaptosomes of the motor cortex its level showed a tendency to rise.

This investigation thus showed that TPA has a marked effect on activity of the key enzyme of serotonin biosynthesis. Whereas in the caudate nucleus tryptophan-5-hydroxylase activity was significantly depressed at both times after a single injection, in the motor cortex the response of the enzyme to TPA was phasic: its activity was depressed 30 min and increased 5 days after the injection.

It is difficult as yet to explain what causes this change in enzyme activity: whether TPA acts directly on tryptophan-5-hydroxylase or whether its effect is mediated through a different mechanism. It is evident that both these hypotheses could apply to the present case, because preliminary experiments showed that TPA (45 nM) *in vitro* lowers activity of the enzyme isolated from the motor cortex (by 51%), but not from the caudate nucleus. During the action of TPA *in vivo*, however, it changed activity of the enzyme in both brain structures (Fig. 1).

In the present investigation a late effect of TPA on tryptophan-5-hydroxylase also was discovered, in the form of a change in its activity in synaptosomes in the different brain formations 5 days after a single injection (Fig. 1). It must be particularly emphasized that the late effect of TPA was quite specific, for experiments with morphine (7 mg/kg, subcutaneously) showed that it does not affect tryptophan-5-hydroxylase activity 5 days after a single injection [6]. The data on the late effect of TPA also indicate regional differences in the action of the opioid TPA on the serotoninergic system of the rabbit brain. In the writers' opinion this must be taken into consideration when the different effects of opioid peptides are analyzed with a view to their possible clinical application [1].

The writer postulated previously [5] that the late effect of a single injection of opioid TPA on the brain serotoninergic system may be connected with long-term changes in regulatory mechanisms responsible for the storage, utlization, and release of serotonin from nerve endings. The results of the present investigation suggest that, besides the mechanisms mentioned above, another point of the action of opioid peptides on the serotoninergic system may be the key enzyme of serotonin biosynthesis, tryptophan-5-hydroxylase.

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FUNCTIONAL STATE OF THE SEROTONINERGIC SYSTEM OF THE THYROTOXIC THYROID GLAND

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In thyrotoxicosis (diffuse toxic goiter) "escape" of the thyroid gland (TG) from thyrotrophic regulatory influences is observed. Under these conditions the role of activators of thyroid function is played primarily by thyroid-stimulating immunoglobulins [13, 15]. The problem of the role of other stimulators of thyroid activity, above all biogenic amines, under these circumstances remains unsolved. Yet the solution to this problem may be directly relevant to the elucidation of certain aspects of the pathogenesis of thyrotoxicosis.

In the investigation described below the functional state of the serotoninergic system of the thyrotoxic TG was studied. The mechanisms of the activating effect of serotonin on the follicular apparatus of TG are quite complicated [4, 8, 10-12]. It has been observed that the stimulating effect of serotonin on thyroid function is most clearly manifested during blocking of thyrotrophic influences [9].

EXPERIMENTAL METHOD

Concentrations of serotonin [2] and of its precursor 5-hydroxytryptophan (5-HTP) [1] and monoamine oxidase (MAO) activity [6], using serotonin as the substrate, were determined in thyroid tissue obtained during operations on 26 patients with manifest thyrotoxicosis and in the unchanged paranodal tissue of TG obtained from 14 patients with nodular euthyroid goiter (control group). The rate of incorporation and efficiency of the reaction of uptake of ^{3}H-5-HTP (specific activity 5 Ci/mmole, from Amersham Corporation, England), by the gland also

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