ANALYSIS OF ADRENERGIC AND SEROTONINERGIC STRUCTURES OF CENTRAL COMPONENTS OF THE SHAKING AND FLEXOR REFLEXES IN RABBITS

A. N. Talalaenko

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Experiments were carried out on rabbits to investigate the effect of intraperitoneal injection of dioxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HT) on the duration of the latent period of the shaking and flexor reflexes. The results showed that DOPA significantly increases, while 5-HT shortens the latent period of both reflexes. An increase in the latent period of these reflexes was also produced by rausedil and DOPA, given against the background of disulfiram. The effect of DOPA was abolished by tropaphen, and the effect of 5-HT by dihydroergotoxin, indicating the participation of α-adrenergic and D-serotoninergic structures in the changes in the functional state of the central components of these two motor reflexes.

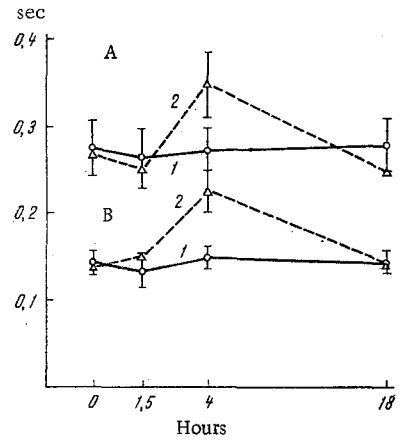

Fig. 1. Latent period of shaking (A) and flexor (B) reflexes before (1) and after (2) injection of rausedil. Abscissa, time of observation (in h); ordinate, latent period of reflexes (in sec).

The role of adrenergic and serotoninergic mechanisms in spinal cord function has been demonstrated experimentally [2, 4, 17, 26]. It is assumed that the biogenic amines perform an inhibitory function in physiological responses of the spinal cord [14, 17, 24].

Precursors of noradrenalin and serotonin in fact block the conduction of impulses from afferent pathways of the flexor reflex to motoneurons [12], while microinjections of adrenalin, noradrenalin, and serotonin cause suppression of spontaneous electrical activity of interneurons [17] or spinal motoneurons [24]. However, this hypothesis is contradicted by investigations which demonstrated the ability of serotonin and 5-hydroxytryptophan (5-HT) to facilitate the flexor reflex in cats [2, 25] and to increase the amplitude of primary evoked potentials of the spinal cord [14, 22]. These results are confirmed by observations showing the ability of monoamine oxidase inhibitors to raise the serotonin level in the tissues of the spinal cord, correlating with increased amplitude of the monosynaptic spike [13].

The object of the present investigation was to determine the nature of the central adrenergic and serotoninergic structures of

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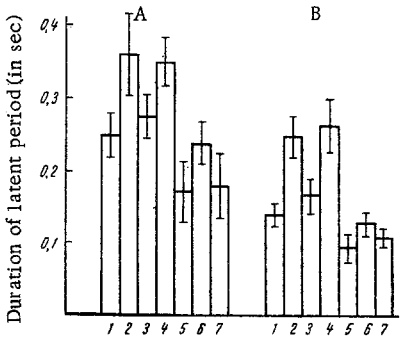

Fig. 2

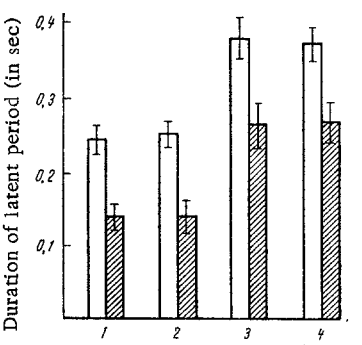

Fig. 3

Fig. 2. Effect of tropaphen, propranolol, dihydroergotoxin, and morphine on changes in the latent period of the shaking (A) and flexor (B) reflexes produced by DOPA and 5-HT: 1) latent period of reflexes studied; 2) the same after injection of DOPA; 3) the same after injection of DOPA followed by tropaphen; 4) the same after injection of DOPA followed by propranolol; 5) the same after injection of 5-HT; 6) the same after injection of 5-HT followed by dihydroergotoxin; 7) the same after injection of 5-HT followed by morphine.

Fig. 3. Changes in latent period of shaking and flexor reflexors under the influence of disulfiram with or without subsequent injection of DOPA: 1) latent period of shaking (unshaded columns) and flexor (shaded columns) reflexes; 2) the same 18 h after administration of disulfiram; 3) the same 4 h after injection of DOPA following administration of disulfiram; 4) the same 18 h after injection of DOPA.

the shaking and flexor reflexes, the central components of which lie in the medulla and the lumbar division of the spinal cord [6, 7, 22].

EXPERIMENTAL METHOD

Experiments were carried out on 35 rabbits weighing 2-3 kg. The experiments constituted 26 series, in each of which 5 or 6 aminals were used. The animals were used not more than once a week in the experiments. Shaking and flexor reflexes were evoked by regular square pulses (20/sec, 0.5 msec) which were applied through needle electrodes inserted beneath the skin of the upper third of the concha auriculae [7] or beneath the skin of a phalanx of the rabbit's hind limb [6], and they were recorded on four occasions at intervals of 5 min by means of the PV-53L reflexometer, at double the threshold strength. Some workers consider that much of the latent period of the motor responses is taken up with the transmission of excitation in the central part of the corresponding reflex arcs [1, 3, 6]. The actual conduction time along the nerve fibers (afferent and efferent) is only a few milliseconds [1]; synaptic delay at the neuromuscular synapse occupies 0.5 msec [11], while the time taken for contractions of the rabbit's plantar flexor muscles is 23 msec [5]. In the present experiments the latent period of the shaking and flexor reflexes was 0.253 ± 0.038 and 0.139 ± 0.019 sec, respectively, so that a substantial part of the latent period of both these reflexes was taken up by the summation time in the central components of the reflex system. With this fact in mind, the functional state of the central components of the shaking and flexor reflexes was judged from the length of their latent period, with evaluation of the changes in its duration after intravenous injection (4 h before the experiment) of dioxyphenylalanine (DOPA) and 5-HT in doses of 50 mg/kg body weight. In 2 series of experiments the animals were injected with rausedil (5 mg/kg, intraperitoneally) and the latent period of the two reflexes was measured 1.5, 4, and 18 h after the injection. The effect of a combination of disulfiram and DOPA on the latent period of the reflex responses also was investigated. Disulfiram (300 mg/kg) was made up in 2% starch mucilage and administered by gastric tube on two occasions at an interval of 18 h. DOPA was given to the animals 2 h after the second dose of disulfiram. To analyze the receptor structures of the central components of the shaking and flexor reflexes, through which the specific effects of the catecholamines and serotonin are exerted, the following drugs were injected 30 min before the experiment into the marginal vein of the ear: morphine (2.5 mg/kg), which inhibits effects due

to 5-HT on muscarine-like serotoninergic structures [18], the β-receptor blocking agent [15] propranolol (5 mg/kg), the α-adrenolytic [9] tropaphen (2.5 mg/kg), and dihydroergotoxin (2 mg/kg), used as an effective D-antagonist of serotonin [20].

EXPERIMENTAL RESULTS AND DISCUSSION

Preliminary experiments showed that the latent period of the shaking and flexor reflexes remained substantially unchanged for 1.5, 4, and 18 h of the experiment. The latent period of both reflexes was unchanged 1.5 h after injection of rausedil into the animals, but 4 h after its injection, i.e., in the phase of exhaustion of the functionally active fraction of the monoamines [21] and an increase in the content of their free forms in the brain tissue [10], a significant increase in the latent period of both reflexes was observed (Fig. 1). Since 18 h after the injection of rausedil, when the labile reserves of bioamines in the brain tissue were restored [21], the latent period of both reflexes was the same as initially, it can be concluded that the effect of rausedil was due to its action on central monoaminergic mechanisms and was brought about by an increase in the content of either serotonin or catecholamines. However, participation of serotoninergic mechanisms in the rausedil effect is unlikely because injection of 5-HT into the rabbits caused a marked shortening of the latent period of both reflexes studied (Fig. 2). The second suggestion is more likely because injection of DOPA into the rabbits, increasing the noradrenalin and dopamine levels in the brain tissue [8], was followed, as in the experiments with rausedil, by an increase in the latent period of the reflexes. Further evidence in support of the view that adrenergic mechanisms are responsible for the increase in latent period of the shaking and flexor reflexes was given by experiments in which DOPA was injected after preliminary preparation of the animals with disulfiram. It was found that disulfiram has no effect on the latent period of either reflex, but the subsequent administration of DOPA caused a marked increase in the latent period of both reflexes, not only 4 h, but also 18 h after the injection of DOPA (Fig. 3). Disulfiram, which blocks β-hydroxylase, is known to increase the dopamine level in the brain tissue and, at the same time, to cause a progressive decrease in the noradrenalin concentration [19]. Comparison of these observations with the results of the present experiments shows that the increase in latent period of the two reflexes under the influence of DOPA took place through the participation of dopaminergic mechanisms. This conclusion is supported by other investigations which showed that the dopamine concentration is significantly higher than the noradrenalin concentration in the rabbit spinal cord [8] and of experiments demonstrating inhibition of the dopamine effect on monosynaptic reflexes in cats [23].

It thus follows from the results of these experiments that the difference between the effects of the precursors of the biogenic amines on motor reflexes depends on differences in neurochemical sensitivity of the central components of the reflex arcs to monoamines. It is known that neither DOPA nor 5-HT disturbs neuromuscular transmission or changes the responses of skeletal muscles to direct stimulation [2, 16, 27]. Meanwhile, the increase in latent period of the shaking and flexor reflexes produced by DOPA was diminished by tropaphen, but not by propranolol. The effect of 5-HT was unchanged by morphine, but if 5-HT was given after preliminary administration of dihydroergotoxin, the latent period of the two reflexes was not shortened (Fig. 2).

The results described above show that the effect of the catecholamines formed from DOPA on the central components of the shaking and flexor reflexes is due to the action of the amines on α-adrenergic biochemical systems. Conversely, the action of serotonin, formed from 5-HT, on the central components of the reflex arcs of both these reflexes is effected through D-serotoninergic structures. This conclusion correlates with the results of investigations showing that the facilitatory effect of 5-HT and tryptamine on the flexor reflex in cats is suppressed by BOL and methysergide, specific blocking agents of serotonin receptors of the D-type [13, 22, 27].

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