ROLE OF MONOAMINERGIC HYPOTHALAMIC MECHANISMS IN DEFENSIVE CONDITIONING IN RATS

A. N. Talalaenko

UDC 612.833.81-06:612.826.4

Experiments were carried out on rats to study the effect of noradrenalin, serotonin, their antagonists, and γ-aminobutyric acid (GABA), injected into the midhypothalamus, on motor activity, muscle tone, and the latent period of defensive conditioning (DC). The bioamines had no effect on motor activity or muscle tone; serotonin, however, shortened while noradrenalin lengthened the latent period of the reflex. The effect of serotonin was abolished by injection of dihydroergotamine, methysergide, and chlorpromaxine into the hypothalamus but was not affected by phentolamine or morphine. Inhibition of the reflex due to noradrenalin was suppressed by dihydroergotamine and phentolamine but not by propranolol and chlorpromazine. Adrenolytics and serotonin antagonists did not affect the inhibition of DC by GABA. The results indicate that the effect of the monoamines on the conditioned defensive reflex are produced through their action on α adrenergic and D serotoninergic structures of the central hypothalamus.

Recent observations have demonstrated the role of the hypothalamus in the formation of conditioned reflexes [3, 6, 16, 21]. However, it is not certain which neurochemical mechanisms of the hypothalamus participate in integrative activity of the brain. Conditioned defensive reflexes are considered to be produced by adrenergic excitation of certain hypothalamic structures [1, 6]. The experimental results correlate with histochemical demonstration of axon endings of dopaminergic and noradrenalinergic neurons in certain nuclei of the hypothalamus [7, 13]. Meanwhile, the discovery of large concentrations of serotonin in the hypothalamus [20], corresponding to the distribution of serotoninergic neurons in the pituitary-adrenal system [7, 13], indicates an important role of this mediator also in the functional connections between the hypothalamus and other brain structures. Nevertheless, there is no information in the literature on the importance of the serotoninergic components of the hypothalamus in defensive conditioning (DC) in animals, although the chemical heterogeneity and functional differentiation of the influences of individual hypothalamic structure on the cerebral cortex are generally accepted [1, 5, 6].

The object of the present investigation was to study the effect of noradrenalin and serotonin, injected into the midhypothalamus, on a defensive conditioned reflex in rats and to demonstrate the concrete neurochemical mechanisms whereby these monoamines act.

EXPERIMENTAL METHOD

Experiments were carried out on 26 male rats weighing 250-290 g. DC was carried out in a special chamber by combining (3 times a day) acoustic stimulation (the ringing of a bell for 15 sec) with application of an electric current (1.5 mA) to the floor of the chamber for 5 sec; the electric shock was applied 11 sec after the beginning of action of the conditioned stimulus. The latent period of the reflex was recorded by means of an electric timer connected simultaneously with the bell and disconnected automatically when the animal jumped from the chamber. In the absolute majority of animals DC was complete by the 6th-7th day of training, but the latent period of the reflex fluctuated considerably. By combining application of the conditioned stimulus followed 1 sec later by the unconditioned stimulus once daily for 5-7 days the latent period

Department of Pharmacology, Donetsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 4, pp. 3-7, April, 1973. Original article submitted February 25, 1972.

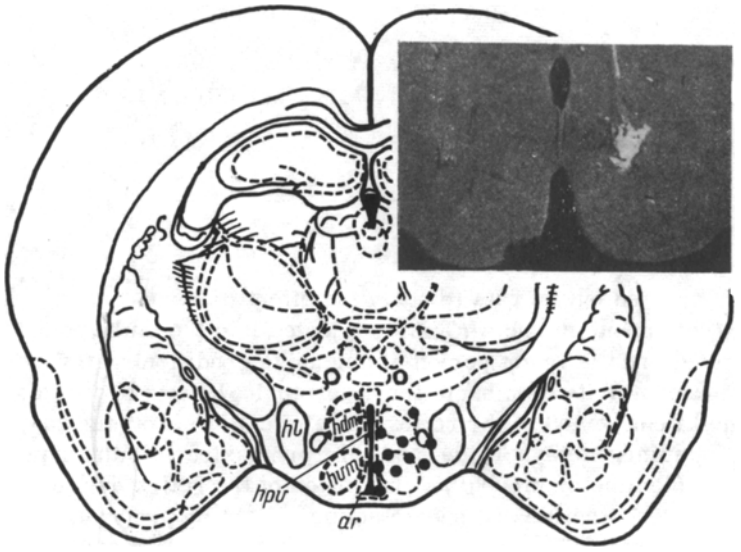

Fig. 1. Diagram of frontal section through the rat brain at the midhypothalamic level: hdm) dorsomedial nucleus; hvm) ventromedial nucleus; hl) lateral hypothalamic nucleus; hpv) periventricular nucleus; ar) arcuate nucleus. Filled circles denote sites of microinjections of GABA, bioamines, and their antagonists. A photomicrograph of a frontal section of the same zone is shown in the top right-hand corner. The location of the tip of the microinjector needle can be seen in the region of the ventromedial hypothalamic nucleus.

of DC was brought down to not more than 1.1 sec. After stabilization of the conditioned reflex the animals were anesthetized with ether, and a conducting cannula was inserted into the region of the right ventromedial hypothalamus nucleus with the aid of an atlas of the rat's brain [9] (Fig. 1). The experiments were resumed 4-5 days later and within a few days the latent period of the conditioned defensive reflex, which had increased after the operation, was reduced to 1-1.1 sec by the method described above. The effect of the monoamines on DC was estimated from the latent period, changes in which were determined in response to injection of noradrenalin and serotonin (5-HT) into the hypothalamus 30 min before the experiment. To analyze the receptors through which the bioamines exerted their effects, the β-adrenergic blocking agent propranolol [10] and the α-adrenolytics dihydroergotamine (DE), phentolamine, and chlorpromazine [19] were injected into the hypothalamus 15 min before the amines. Methysergide (IML-491, Deseril) and DE were used as effective antagonists of the D effects of 5-HT [15]. Morphine, which inhibits effects due to the action of 5-HT on M-serotoninergic structures [17], was also used. The bioamines (2 μg of the base) and their antagonists (1-2 μg of the base) were injected in a volume of 0.002-0.0025 ml by means of an injection system consisting of a capillary tube inserted to the point of injection, a polyethylene tube, and a micromanipulator. To determine the selective action of the amines on DC, their muscle-relaxing action was investigated simultaneously by the "revolving rod" method [12] and their ability to modify spontaneous motor activity was determined. This last effect was tested 30 min after injection of the bioamines into the hypothalamus in a special chamber which enabled horizontal movements of the animals to be recorded by means of 3 photoelectric cells, and expressed as a number of pulses in 25 min. The control group of animals received an injection of 0.003 ml bidistilled water into the hypothalamus. The rats were used in the experiments 5 or 6 times, with intervals of not less than 4-6 days between the individual tests. Altogether 28 series of experiments were carried out, with 5 animals in each. The accuracy of the localization of the injection capillary tube was confirmed histologically.

EXPERIMENTAL RESULTS AND DISCUSSION

Injection of noradrenalin, the adrenolytics (2 μg), and also of 10 μg GABA into the midhypothalamic structures of the rats caused inhibition of DC accompanied by a marked increase in its latent period. Injection of serotonin (2 μg) facilitated DC, as reflected in a significant decrease in the latent periods of the

TABLE 1. Effect of Noradrenalin, 5-HT, Their Antagonists, and GABA Injected into Midhypothalamus on Conditioned Defensive Reflex, Motor Activity, and Muscle Tone of Rats

Drug	Dose (in μg)	Number of experiments	Latent period of DC (in sec; M±m)	Motor activity (M ± m)	Muscle-relaxing action (in percent of animals slipping off the rod)
Bidistilled water	—	5	0,93±0,18	56 ±19,5	0
Noradrenalin	2	5	2,24±0,49 *	61,8±21,3	0
GABA	10	5	1,97±0,44*	58,6±12,5	0
5-HT	2	5	0,63±0,14*	62 ±13,2	0
DE	2	5	1,84±0,34*	51 ±19,6	0
DE	1	5	0,98±0,17	57,4±19,3	0
Chlorpromazine	2	5	2,05±0,24*	57,4±14	0
»	1	5	0,95±0,12	58,2±17,9	0
Phentolamine	2	5	1,78±0,25*	50,2±21,1	0
»	1	5	0,98±0,2	54,8±18,6	0
Propranolol	2	5	1,73±0,23*	51,6±20,1	0
»	1	5	0,98±0,15	57,2±19,9	0
Methysergide	1	5	0,97±0,14	55,6±15,9	0
Morphine	2	5	2,03±0,31*	48,8±22,1	0
»	1	5	1,02±0,16	55,2±26,6	0

* Difference from control statistically significant ($P \leq 0.05$)

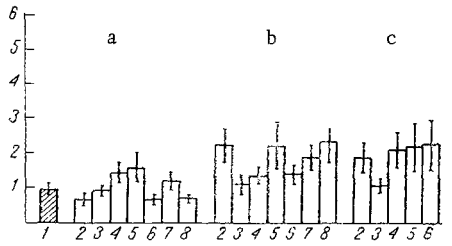

Fig. 2. Effect of 5-HT antagonists and adrenolytics on changes in the latent period of DC due to serotonin (a), noradrenalin (b), and GABA (c). Ordinate, latent period of DC (in sec): 1) latent period of DC; 2, 3) the same 30 and 60 min after injection of bioamines and GABA; 4) the same 30 min after injection of bioamines and GABA preceded by DE; 5) the same 30 min after injection of bioamines and GABA preceded by methysergide; 6) the same 30 min after injection of bioamines and GABA preceded by phentolamine; 7) the same 30 min after injection of bioamines preceded by chlorpromazine; 8) the same 30 min after injection of bioamines preceded by morphine (a) or propranolol (b).

reflex. Conversely, the 5-HT antagonists, used in the same dose, caused inhibition of the conditioned defensive reflex of the animals in the same way as noradrenalin and GABA (Table 1). Meanwhile, injection of bidistilled water in the control series of experiments had no appreciable effect on the latent period of this reflex. The change in DC under the influence of GABA, the monamines, and their antagonists was of short duration: for 60 min after their injection the latent period of the conditioned reflex was the same as originally (Fig. 2). The effects of the bioamines and GABA with respect to DC thus revealed were selective and were unconnected with a decrease in muscle tone or a change in the motor activity of the rats (Table 1), but were probably due to differences in the neurochemical sensitivity of the hypothalamic structures to monoamines. The depriming effect of noradrenalin and GABA on DC can be assumed to be due to the action of these substances on the inhibitory interneurons, revealed by investigation of single unit activity of the ventromedial hypothalamic nucleus [18]. Injection of noradrenalin into the region of this structure of the subcortex in fact induces the development of synchronized slow waves [8], the electroencephalographic equivalent of inhibitory processes in the CNS. Morphological verification of the points of injection did not contradict this conclusion. For example, neurons of the periventricular, ventromedial, and dorsomedial nuclei were found to be exposed to the action of the drugs injected. In some experiments the monoamines were found to have an effect on the lateral hypothalamic and arcuate nuclei (Fig. 1).

The factual evidence obtained in the pharmacological analysis of the observed effects of the bioamines suggests that inhibition of DC by noradrenalin when injected into the hypothalamus is produced through the α-adrenergic biochemical systems of the neurons of this subcortical effector center. This is shown by the fact that the action of this amine in lengthening the latent period of DC was considerably reduced by phentolamine and DE but was unchanged by chlorpromazine and propranolol (1 μg). This conclusion is confirmed

by data in the literature which show that microapplication of α adrenolytics to single hypothalamic neurons abolishes the depression of their spike activity evoked by noradrenalin [17]. The depriming effect of noradrenalin on the conditioned defensive reflex is specific, for the analogous effect of GABA on DC is not affected by DE or phentolamine (1 μg). On the other hand, the effect of serotonin on DC demonstrated by these experiments is connected with the action of the amine on the D-serotoninergic structures of the hypothalamus, for the decrease in latent period of DC produced by 5-HT is prevented by DE, methysergide, and chlorpromazine (1 μg) but not by morphine and phentolamine (Fig. 2). This conclusion is supported by experimental results which showed that methysergide and LSD-25, if injected iontophoretically, block the excitatory responses of brainstem neurons to 5-HT [11], whose increased concentrations in blood flowing from the brain can be determined during electrical stimulation of the medial structures of the anterior and middle hypothalamus [4].

The results indicate that the functioning of the monoaminergic mechanisms of the hypothalamus is essential for the reproduction of conditioned defensive reflexes. Existing indications of functional differentiation and chemical heterogeneity of ascending influences (both inhibitory and activating) which certain hypothalamic structures can exert on the cerebral cortex [2, 5, 6] confirm this view.

LITERATURE CITED

1. P. K. Anokhin, The Biology and Neurophysiology of the Conditioned Reflex [in Russian], Moscow (1968).
2. O. G. Baklavadzhyan, The Autonomic Regulation of Electrical Activity of the Brain [in Russian], Leningrad (1967).
3. M. E. Varga, L. G. Samoilova, and I. N. Tvoritskaya, Abstracts of Proceedings of the 21st Conference on Problems in Higher Nervous Activity [in Russian], Moscow–Leningrad (1966), p. 62.
4. E. A. Gromova, in: Systemic Organization of Physiological Functions [in Russian], Moscow (1969), p. 302.
5. E. A. Gromova, in: The Physiology and Pathophysiology of the Limbico–Reticular System [in Russian], Moscow (1971), p. 47.
6. L. V. Kalyuzhnyi, Uspekhi Sovr. Biol., 68, No. 3, 361 (1969).
7. M. S. Konstantinova, Zh. Évolyuts. Biokhim. i Fiziol., No. 7, 213 (1971).
8. T. N. Oniani, A. A. Ungiadze, and E. V. Abzianidze, Neirofiziologiya, No. 2, 497 (1970).
9. J. Bures, M. Petran, and I. Zachar, Electrophysiological Methods in Biological Research, Academic Press (1967).
10. J. W. Black, W. A. M. Duncan, and P. G. Shanks, Brit. J. Pharmacol., 25, 577 (1965).
11. R. J. Boakes, P. B. Bradley, J. Briggs, et al., Brit. J. Pharmacol., 38, 453 (1970).
12. N. W. Dunham and T. S. Miya, J. Am. Pharm. Assn., Sci. Ed., 46, 208 (1957).
13. K. Fuxe and T. Hökfelt, Acta Physiol. Scand., 66, 245 (1966).
14. J. H. Gaddum and Z. P. Picarelli, Brit. J. Pharmacol., 12, 323 (1957).
15. L. Gyermek, Pharmacol. Rev., 13, 399 (1961).
16. T. Ikeda, Folia Psychiat. Neurol. Jap., 17, 51 (1963).
17. E. Marley and J. D. Stephenson, Brit. J. Pharmacol., 36, 194P (1969).
18. J. P. Murphy and L. P. Ranaud, Brain Res., 9, 385 (1968).
19. M. Nickerson, Pharmacol. Rev., 11, 433 (1959).
20. M. Paasonen, P. MacLean, and N. Giarman, J. Neurochem., 1, 326 (1957).
21. A. Romaniuk, Acta Biol. Exp. (Warsaw), 22, 59 (1962).