EFFECT OF CATECHOLAMINES, SEROTONIN, AND SOME AMINO ACIDS, INJECTED INTO THE AMYGDALA, ON THE CONDITIONED AVOIDANCE REFLEX AND MOTOR ACTIVITY OF RATS

I. V. Komissarov and A. N. Talalaenko

UDC 612.825.1.014.46:615.357.452+615.362.018:547.756.

The effect of catecholamines and serotonin and of some inhibitory or excitatory amino acids, injected into the dorsomedial zones of the amygdala, on the latent period of the conditioned avoidance reflex and on motor activity and muscle tone was investigated in experiments on rats. Dopamine, serotonin, and aspartic acid significantly lengthened the latent period of the avoidance reflex and stimulated the motor activity of the rats. The effects of dopamine were inhibited by haloperidol but not by phentolamine, chlorpromazine, or deseryl. The effects of serotonin were prevented by dihydroergotamine, deseryl, and chlorpromazine, but not by phentolamine, morphine, or haloperidol. Meanwhile antagonists of the biogenic amines were inactive against the effects of aspartic acid. The latent period of the reflex was reduced by noradrenalin but increased by GABA in doses not changing the muscle tone or motor activity of the rats. The effect of noradrenalin was abolished by injection of phentolamine, chlorpromazine, and dihydroergotamine, but not of propranolol, into the amygdala. Monoamine antagonists were ineffective against inhibition of the conditioned avoidance reflex by GABA. The results are interpreted as evidence of the participation of α-adrenergic, D-serotoninergic, or dopamine-sensitive structures of the neurons of the amygdala in the observed effects of the monoamines.

The amygdala plays an important role in the integration of defensive behavioral responses. Electrical stimulation of the amygdala usually induces emotional responses of defensive type and an avoidance reflex [1, 7, 10] or it inhibits conditioned defensive responses in rats and dogs [5, 10]. Destruction of the amygdala interferes with the formation of the conditioned avoidance reflex in cats [4]. During the formation of a passive defensive reflex in rats injection of noradrenalin into the dorsomedial zone of the amygdala reduced the effect of nociceptive stimulation [18] or weakened the inhibitory action of punishment on operant behavior [19]. In other experiments, however, microinjection of noradrenalin into the amygdala was accompanied by a depriming effect on the behavioral responses in rats [14]. The neuropharmacological data are in good agreement with the results of histochemical investigations during which axon endings of noradrenergic, serotoninergic, and dopaminergic neurons, whose bodies lie in the region of the nuclei raphe and interpeduncular nucleus, were found in the amygdala [13]. However, the role of the dopaminergic and serotoninergic structures of the amygdala in the regulation of conditioned-reflex activity has not been investigated. It was shown recently [11] that implantation of serotonin into the amygdala interferes with learning processes in rats, significantly depresses the reproduction of the conditioned-defensive avoidance reflex, and leads to its more rapid extinction.

In the investigation described below the neurochemical mechanisms forming the conditioned avoidance reflex and motor activity of rats at the level of the amygdala were studied.

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. 77, No. 2, pp. 51-55, February, 1974. Original article submitted March 26, 1973.

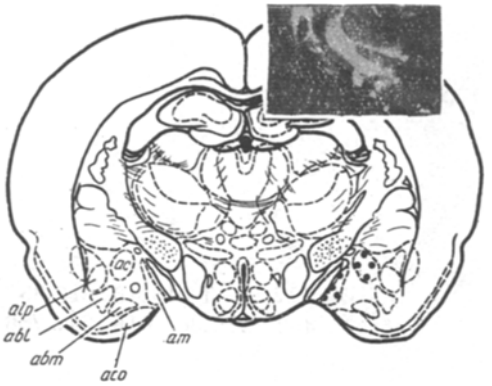

Fig. 1. Scheme of frontal section through rat brain at level of amygdala: ac) central nucleus; am) medial nucleus; aco) cortical nucleus; abm) medial basal nucleus; abl) lateral basal nucleus; alp) postero-lateral nucleus. Black circles represent points of microinjection of amino acids, biogenic amines, and their antagonists. Photomicrograph of frontal section of the same zone shown in top right hand corner. Tip of microinjector needle can be seen in the medial amygdaloid nucleus.

EXPERIMENTAL METHOD

In experiments on 22 male rats weighing 250-360 g the conditioned avoidance reflex was formed as described previously [9]. When the reflex reached a stable level, the rats were anesthetized with ether and, using an atlas of the rat brain [15], a guiding cannula was inserted (by means of the SEZh-2 stereotaxic apparatus) into the dorsomedial region of the right amygdala, through which the microinjections of the solutions of the test drugs were injected. Effects of the monoamines on the conditioned avoidance reflex and also the effects of amino acids producing excitation (aspartic acid) or inhibition (GABA) of unit activity [3, 16], were judged from the length of the latent period of the reflex and on changes in the latent period measured 30 and 60 min after injection of the drugs into the amygdala. To analyze the receptor structures responsible for the effects of the catecholamines, α-adrenergic blocking agents – chlorpromazine, phentolamine, dihydroergotamine – and the β-adrenolytic drug propranolol were injected into the amygdala 15 min before the catecholamines. Deseryl (1-methyl-D-lysergic acid butanolamide; INL-491), chlorpromazine, dihydroergotamine, and haloperidol were used as effective antagonists of serotonin and dopamine. The biogenic amines and their antagonists (2-5 μg of the base) and the neutralized amino acids (10 μg) were injected in a volume of 2-3 μl, using a microinjection system. To examine the selectivity of action of the amines and amino acids on the conditioned avoidance reaction, their muscle-relaxing action and their ability to modify the spontaneous motor activity of the rats were investigated simultaneously by a method described earlier [9]. Animals of the control group received injections of 3 μl bidistilled water. At the end of the experiments the location of the tip of the injection capillary tube was verified morphologically (Fig. 1). In seven rats the test drugs were injected into the structures of the central amygdaloid nucleus and in 15 rats into the medial amygdaloid nucleus.

EXPERIMENTAL RESULTS AND DISCUSSION

Local microinjection of serotonin, dopamine, or aspartic acid caused significant lengthening of the latent period of the conditioned avoidance reflex and at the same time stimulated the motor activity of the rats. Noradrenalin, in a dose of 5 μg, also lengthened the latent period of the reflex but this could be the result of inhibition of the animals' motor activity (Table 1). Injection of 2 μg noradrenalin did not affect the spontaneous motor activity of the rats and facilitated the conditioned avoidance reflex, as reflected in marked shortening of its latent period. Microinjection of GABA (10 μg) into the structures of the amygdala likewise caused no change in the animals' motor activity but it inhibited the conditioned avoidance reflex without significantly lengthening its latent period.

Changes in the conditioned avoidance reflex and motor activity of the rats under the influence of the biogenic amines and amino acids were due to their action on neurons of the amygdala, for special experiments showed that a dye (1.5% methylene blue solution) did not spread beyond the boundaries of the amygdala from 6 to 30 min after microinjection.

The facilitatory effect of noradrenalin could be due to activation of the neocortex, which receives direct efferent impulses from neurons of the amygdala [17]; this effect can also be transmitted through the dorsomedial thalamic nucleus. The presence of direct pathways between the amygdala and the dorsomedial nucleus [20] and the ascending, mainly activating, character of the effects of this associative nucleus of the thalamus on single neurons of the orbitofrontal cortex [6] confirm this conclusion. The effects of serotonin and aspartic acid on the conditioned avoidance reflex can be satisfactorily explained if the presence of direct anatomical connections between neurons of the amygdala and the intralaminar nuclei of the thalamus [20], with an inhibitory effect on the neocortex [8], is remembered. On the other hand, motor activity is evidently stimulated through the participation of the hypothalamo-reticulo-spinal tracts. This point of view

TABLE 1. Effect of Monoamines, Their Antagonists, and Amino Acids Injected into the Amygdala on Conditioned Avoidance Reflex, Motor Activity, and Muscle Tone of Rats (n = 5)

Substance injected	Dose (μg)	Latent period of conditioned avoidance reflex (in sec, meas. 30 min after injection)	Motor activity	Muscle relaxing action (% of animals slipping off the shaft)
		M±m		
Bidistilled water	—	0,94±0,05	51±3,16	0
Noradrenalin	2	0,69±0,03*	53±3,6	0
	5	2,24±0,13*	33±2,49*	0
Dopamine	2	1,21±0,08	64±7,95	0
	5	2,51±0,16*	75±4,19*	0
Serotonin	2	1,2±0,06	63,2±5,54	0
	5	2,38±0,17*	73,4±4,2*	0
Aspartic acid	10	2,47±0,14*	84±4,13*	0
GABA	10	2,46±0,2*	52,4±2,4	0
Phentolamine	2	1,1±0,09	52±4	0
Chlorpromazine	2	1,14±0,05	57,2±8,08	0
Propranolol	2	1,08±0,03	55,6±3,04	0
Haloperidol	3	1,07±0,03	53,8±4,39	0
Deseryl	2	0,81±0,04	60±3,22	0
Dihydroergotamine	2	0,83±0,04	60,4±2,54	0
Morphine	2	1,05±0,06	54,6±3,77	0

*P ≤ 0.05.

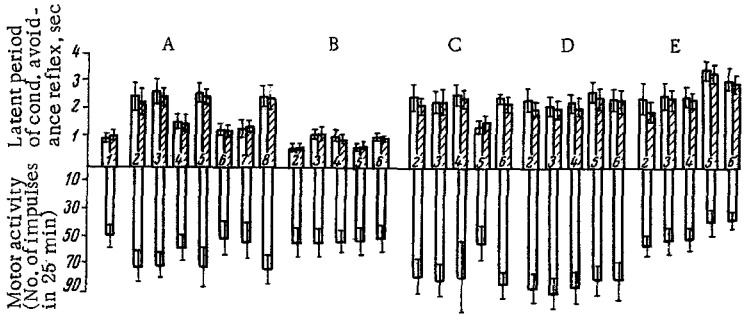

Fig. 2. Effect of monoamine antagonists injected into the amygdala on changes in latent period of conditioned avoidance reflex and motor activity induced by serotonin (A), noradrenalin (B), dopamine (C), aspartic acid (D), and GABA (E). Latent period of conditioned avoidance reflex 30 min (unshaded columns) and 60 min (shaded columns) after microinjection of: 1) bidistilled water; 2) biogenic amines and amino acids; 3 and 4) biogenic amines and amino acids after phentolamine and chlorpromazine; 5) biogenic amines and amino acids after haloperidol (A, C, D, E) or propranolol (B); 6) biogenic amines and amino acids after deseryl (A, B D, E) or dihydroergotamine (B); 7 and 8) serotonin after dihydroergotamine and morphine. Values of M ± m shown.

is supported by morphological evidence of efferent connections of the amygdala with hypothalamus [20]. Electrophysiological investigations have demonstrated that, in principle, spinal reflex activity can be stimulated by electrical excitation of the hypothalamus [12].

Facilitation of the conditioned avoidance reflex by injection of noradrenalin into the amygdala is associated with the effect of the amine on α-adrenergic structures of this formation of the limbic system, for the effect of noradrenalin was substantially facilitated by phentolamine, chlorpromazine, and dihydroergotamine, but not by propranolol. This conclusion is confirmed by experimental results in which weakening of the inhibitory action of punishment on the operant behavior of the rats caused by application of noradrenalin to the amygdala was much less marked if preliminary treatment was given with α-adrenolytics, but not with β-adrenolytics [19]. The effect of serotonin on structures of the amygdala is mediated through D-serotonin receptors, for inhibition of the conditioned avoidance reflex and stimulation of motor activity

were prevented by preliminary injection of deseryl, dihydroergotamine, and chlorpromazine, but not of phentolamine, morphine or haloperidol, into the amygdala. At the same time the effect of serotonin was specific, for neither deseryl nor chlorpromazine had any effect on the behavioral changes induced by microinjection of aspartic acid into the amygdala (Fig. 2).

The results of this neuropharmacological analysis of the effects of dopamine show that inhibition of the conditioned avoidance reflex and stimulation of the motor activity of the rats were mediated through receptors of dorsomedial amygdaloid neurons sensitive to haloperidol only. Chlorpromazine, phentolamine, and deseryl did not prevent the effects of dopamine. Meanwhile the inhibition of the conditioned avoidance reflex and stimulation of motor activity induced by dopamine were specific, for deseryl, which inhibits the analogous effect of serotonin, did not change the dopamine effect, while haloperidol had no action on the similar effects of aspartic acid. Since dopamine is an inhibitory mediator inducing hyperpolarization of CNS neurons [2], the response to its action on neurons of the amygdala is presumably the result of a deficiency of inhibition, leading to excitation of the facilitatory zones of the hypothalamus and to stimulation of the motor activity of the rats [12], and also of the intralaminar nuclei of the thalamus or structures of the lenticular nucleus through which the amygdala achieves its ascending efferent influences [20] that lower the excitability of the cortical neurons [8] and interfere with reproduction of the conditioned-relfex act. GABA acts on the conditioned avoidance reflex similarly to dopamine and serotonin. However, inhibition of the reflex produced by it is explained by its action, not on α-adrenergic, D-serotoninergic, or dopaminergic structures but, evidently, on other parts of the neuron membrane in the dorsomedial amygdala (Fig. 2). This is confirmed by experimental results showing that the character of the inhibitory effect of GABA on CNS neurons and on conditioned-reflex activity is nonspecific [3].

LITERATURE CITED

1. L. Kh. Allikmets and F. P. Belyaev, Zh. Vyssh. Nerv. Deyat., No. 4, 695 (1967).
2. É. B. Arushanyan, Zh. Nevropat. i Psikhiat., No. 4, 595 (1972).
3. A. S. Batuev and I. A. Sytinskii, Uspekhi Sovr. Biol., 59, No. 1, 128 (1965).
4. J. Brady, in: Mechanisms of the Whole Brain [Russian translation], Moscow (1963), p. 138.
5. N. M. Vavilova, Zh. Vyssh. Nerv. Deyat., No. 2, 240 (1967).
6. V. N. Kazakov, Neirofiziologiya, No. 5, 484 (1971).
7. M. M. Mgaloblishvili, Soobshch. Akad. Nauk Gruz. SSR, No. 2, 437 (1971).
8. T. S. Naumova, The Physiology of the Reticular Formation [in Russian], Moscow (1963).
9. A. N. Talalaenko, Byull. Éksperim. Biol. i Med. No. 4, 30 (1973).
10. É. Fonberg, in: Reflexes of the Brain [in Russian], Moscow (1963), p. 44.
11. V. G. Shalyapina and G. Telegdi, Zh. Vyssh. Nerv. Deyat., No. 1, 104 (1972).
12. A. I. Shapovalov and B. A. Arutyunyan, Byull. Éksperim. Biol. i Med., No. 12. 3 (1964).
13. K. Fuxe, T. Hökfelt, and U. Ungerstedt, Internat. Rev. Neurobiol., 13, 93 (1970).
14. Z. P. Horovits, Psychosom. Med., 6, 282 (1965).
15. J. F. R. König and R. A. Klippel, The Rat Brain, Baltimore (1963).
16. K. Krnjevic, Brit. Med. Bull., 21, 10 (1965).
17. W. T. Niemer, E. F. Goodfellow, and J. Speaker, Electroenceph. Clin. Neurophysiol., 15, 827 (1963).
18. D. L. Margules, J. Comp. Physiol. Psychol., 63, 34 (1968).
19. D. L. Margules, Europ. J. Pharmacol., 16, 21 (1971).
20. F. Valverde, Progr. Brain Res., 3, 20 (1963).