EFFECT OF SOME INHIBITORS AND METABOLITES OF CATECHOLAMINE METABOLISM ON THE FIXATION AND REPRODUCTION OF MEMORY TRACES

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When noradrenalin synthesis is blocked by disulfiram, the conditioned-defensive avoidance response (CRAR) is strongly inhibited. This effect can be prevented by preliminary administration of iproniazid, which also has a stimulant action on the formation of CRAR in animals resistant to learning. Prolonged administration of 3,4-dimethoxyphenylethylamine prevents the formation of CRAR and inhibits such a response if already firmly established.

The multicomponent structure of memory, including fixation, storage, and reproduction of information, evidently corresponds to a definite organization of the chemical systems responsible for these processes. Modern views of the key position of nucleic acids and other biopolymers as "storage houses of memory traces" [4, 5, 10] do not rule out a possible role of low-molecular weight biologically-active substances and, in particular, of those connected with the transmission and transformation of nervous impluses [2, 3, 11].

In the investigation described below, by acting on various aspects of metabolism the role of synthesis and conversion of catecholamines (CA) was studied in the formation and reproduction of CRAR, as a widely used experimental model for the study of biomechanisms of behavior.

EXPERIMENTAL METHOD

Experiments were carried out on 80 Wistar albino rats. A CRAR was produced in a two-compartment cage by combining an acoustic stimulus with an electrical stimulus of threshold magnitude (4 V) which was applied at the last (10th) second of action of the conditioned acoustic stimulus. During learning this combination was applied 5 or 6 times daily with intervals of 2 min. The number of responses (running into the other compartment) to the conditioned stimulus was expressed as a percentage of the total number of presentations of the isolated acoustic stimulus, and was defined as the "avoidance index" (AI).

The formation and reproduction of CRAR were studied during the action of various drugs on different aspects of CA metabolism. CA synthesis was inhibited by disulfiram (50 mg/kg, intraperitoneally), which blocks dopamine-β-oxidase. Conversions of CA by the deamination pathway were blocked by iproniazid (100 mg kg, subcutaneously). The compound 3,4-dimethoxyphenylethylamine (DMPEA*; 50 mg/kg, subcutaneously) was used as a CA metabolite, entering into competition with catecholamines possibly in accordance with the "pseudomediator" principle [6, 7]. Reproduction of the CRAR was investigated 10 min and 1-72 h after administration of all these compounds, and CRAR formation was studied during administration of iproniazid and DMPEA. The χ^2 method was used for statistical analysis of the results.

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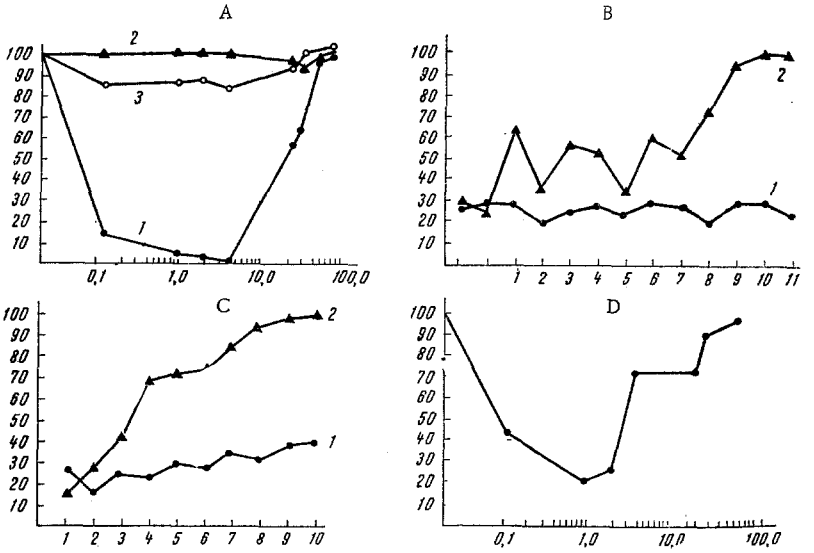

Fig. 1. Effect of inhibitors and metabolites of catecholamine metabolism on formation and reproduction of CRAR in albino rats: A) inhibition of CRAR following injection of disulfiram and prevention of this effect by iproniazid; 1) disulfiram; 2) iproniazid; 3) iproniazid plus disulfiram; B) effect of iproniazid on formation of CRAR in rats resistant to learning; 1) before administration of iproniazid; 2) after injection of iproniazid; C) effect of DMPEA on CRAR formation; 1) DMPEA; 2) control; D) inhibition of CRAR following injection of DMPEA. Abscissa: A and D – time in hours (log); B and C – time (in days); ordinate – avoidance index in per cent (number of responses as a percentage of total number of presentations of conditioned stimulus).

EXPERIMENTAL RESULTS

Investigation of the dynamics of CRAR in the control animals showed that during its formation AI increases progressively and the process is complete in most animals by the 8th-9th day of learning. A firmly established response persists for a long time (several months) with constant and complete reproduction in response to the conditioned stimulus (AI = 100%).

In the animals of the experimental series (in which CA metabolism was acted upon in various ways), definite and varied changes took place both in the formation and in the reproduction of CRAR. DATA for reproduction of CRAR following administration of disulfiram, an inhibitor of dopamine-β-oxidase, are given in Fig. 1A. After injection of this compound, a marked and consistant depression of CRAR was observed within 10 min (AR = 16%, $P < 0.001$); after 2 h the number of responses to the conditioned stimulus continued to diminish, and after 4 h the inhibition was virtually complete. Gradual recovery of the CRAR was then observed and was complete by 48-72 h, and on subsequent days no inhibition of CRAR was observed. It must be emphasized that the disturbances of CRAR produced by inhibition of CA synthesis could be prevented by preliminary inhibition of their monoamine-oxidase conversion. If iproniazid was given to the animals 24 h before disulfiram, reproduction of CRAR remained completely intact in 7 of the 8 animals of this series.

Administration of iproniazid not only prevented the inhibitory action of the inhibitors of CA synthesis on the CRAR, but also facilitated formation of the response in those individual animals which were incapable of learning: either a spontaneous response or one evoked by electrical stimulation (voltage 3-4 times above threshold). The experiments were carried out on animals for which teaching for 4-7 weeks was ineffective. A single injection of iproniazid into these rats overcame their inability to learn (Fig. 1B): a CRAR was formed in the usual 7 to 9 days. It is essential to note that, as a rule, the effect of iproniazid in inhibiting monoamine oxidase persisted during this same period. However, the character of CRAR formation under these conditions was a little unusual and differed from that observed in intact rats by the sharp

fluctuations in AI during the first days after injection of iproniazid. The CRAR developed against the background of iproniazid in animals incapable of learning proved stable in half of the cases (100% reproduction for 20 days), whereas in the remaining animals it continued for only 7-9 days, after which the PI fell again to 20-30%. Repeated injection of iproniazid also was accompanied by a positive effect: recovery of CRAR, which took place much faster and was observed by the 1st or 2nd day after injection of the compound.

Marked disturbances of CRAR formation were observed during the action of DMPEA. In animals taught against the background of administration of this unusual dopamine metabolite for 8 days, AI remained at a low level, whereas in control animals during this period a stable CRAR was formed (Fig. 1C). The difference became significant by the 3rd day of teaching ($P < 0.02$). After the end of DMPEA administration, the CRAR was still formed in most rats, but with considerable delay: on the average only on the 16th day of teaching. Under the influence of DMPEA, not only the formation of CRAR but also the reproduction of an already firmly established response were suppressed. It is clear from Fig. 1D that 10 min after injection of DMPEA, AI was reduced to 40% ($P < 0.01$), after which the rats' conditioned-defensive behavior was inhibited still further, and gradual recovery did not occur until 2 h later. The CRAR was completely restored only after 48-72 h; later no spontaneous disturbances appeared.

Hence, during administration of drugs affecting different aspects of CA metabolism definite changes were found in the formation and reproduction of the CRAR. Among the mechanisms of the inhibitory action of disulfiram on the CRAR, not only blocking of noradrenalin synthesis, but also the consequent accumulation of dopamine must be taken into account. However, this interpretation seems improbable because iproniazid, facilitating the accumulation of dopamine especially in the case of blocking of dopamine-β-oxidase, not only did not strengthen but, on the contrary, prevented the effect of disulfiram. The exceptionally rapid onset of disturbances of CRAR, in the case of inhibition of noradrenalin synthesis occurring before the decrease in its cerebral reserves, can be considered in connection with the view of compartmentalization of CA metabolism in the central nervous system and, in particular, of the existence of a rapidly metabolized (labile) fraction of noradrenalin, only a very small part of the total reserves yet possessing high functional activity [1, 9]. It is important to emphasize that selective liberation of this newly formed noradrenalin has been demonstrated in the peripheral tissues during stimulation of sympathetic nerves [12]. This suggests that the effect now observed of rapid inhibition of CRAR following administration of the dopamine-β-oxidase blocking agent may be due to disturbance of noradrenalin synthesis, and the effect of iproniazid in preventing the disturbance of CRAR may be due to the creation of optimal opportunities for compensation of the defect of synthesis de novo at the expense of the main noradrenalin reserves when their monoamine-oxidase conversion is blocked.

The opposite effects of inhibitors of the synthesis and conversion of CA on the CRAR dynamics, the prevention of changes arising following blocking of CA synthesis by inhibition of their deamination, and other facts are evidence of a possible role of metabolic processes and of CA function in the neurochemical organization of memory traces. Both the direct involvement of catecholamine mechanisms in the formation of conditioned-defensive behavior [13, 14], and the possibility of an indirect effect of CA, with a modulating action on central cholinergic structures [8], must be taken into consideration. It is important to emphasize that changes in metabolism of catecholamines performing a combined mediator and modulator function in the central nervous system exerted their influence on the stages of formation and reproduction of the CRAR, whereas the storage of information was usually undisturbed, as demonstrated by the complete and spontaneous restoration of the CRAR after the procedures applied had been discontinued.

LITERATURE CITED

1. N. B. Vysotskaya and T. M. Shugina, Farmakol. i Toksikol., No. 5, 553 (1967).
2. R. Yu. Il'yuchenok and A. G. Eliseeva, in: The Biological Basis of Memory Traces [in Russian, Moscow (1966), p. 59.
3. P. A. Kometiani, Abstracts of Proceedings of the 4th All-Union Conference on Biochemistry of the Nervous System [in Russian], Tartu (1966), p. 58.
4. O. A. Krylov, V. S. Tongur, and R. A. Danilova, Uspekhi Sovr. Biol., 60, No. 3, 336 (1965).
5. F. Z. Meerson, Plastic Provision for Bodily Functions [in Russian], Moscow (1967).
6. A. M. Utevskii and A. M. Baru, Zh. Vses. Khim. Obshch. im. D. I. Mendeleeva, 9, No. 4, 374 (1964).
7. A. Barbeau, P. Singh, and M. Joubert, Life Sci., 5, 757 (1966).
8. A. Bertler and E. Rosengren, Pharmacol. Rev., 18, Pt. 1, 769 (1966).

9. J. Haggendall and M. Lindqvist, Acta Physiol. Scand., 60, 351 (1964).
10. H. Hyden and E. Egihazy, Proc. Nat. Acad. Sci. (Washington), 48, 13 (1962).
11. H. Hyden and I. Lange, Proc. Nat. Acad. Sci. (Washington), 53, 946 (1965).
12. J. Kopin, G. Breese, K. Kraus, et al., J. Pharmacol. Exp. Ther., 161, 271 (1968).
13. K. C. Moore and R. H. Rech, J. Pharmacol. Exp. Ther., 156, 70 (1967).
14. C. L. Scheckel and E. Boff, Arch. Internat. Pharmacodyn., 152, 479 (1964).