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Effects of Receptor Blockers on ACTH-Induced Changes in Extinction of Active Avoidance Reflex in Rat

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BALÁZS, M. AND G. TELEGDY. *Effects of receptor blockers on ACTH-induced changes in extinction of active avoidance reflex in rat.* PHARMACOL BIOCHEM BEHAV 31(3) 515–518, 1988.—The actions of blockers of dopaminergic receptors (haloperidol), alpha-receptors (phenoxybenzamine), beta-receptors (propranolol) and muscarinic cholinergic receptor (atropine) on the ACTH-induced delay of the extinction of active avoidance behavior were studied in rats. In the doses used, none of the receptor blockers modified the extinction of active avoidance behavior. ACTH delayed the extinction. However, the dopamine receptor blocker (haloperidol) and the muscarinic cholinergic receptor blocker (atropine) did prevent the action of ACTH in delaying the extinction of active avoidance behavior, whereas the alpha- (phenoxybenzamine) and beta- (propranolol) receptor blockers were ineffective. The results suggest that mainly dopaminergic and cholinergic mediations are involved in the delaying action of ACTH on the extinction of active avoidance behavior.

ACTH Haloperidol Phenoxybenzamine Propranolol Atropine Extinction of active avoidance behavior

A great number of data indicate that ACTH can influence learned behavior. In intact rats ACTH delays the extinction of shuttle-box avoidance behavior (3,19), facilitates passive avoidance behavior (1,18), delays the extinction of food-motivated behavior in hungry rats (11,20), etc. It has proved that the smallest fragment of $ACTH_{1-39}$ which is still active in behavioral paradigms is $ACTH_{4-7}$ (7,14). As concerns the mechanism of action, a number of possibilities have been proposed. It has been shown that ACTH can alter the central catecholaminergic and serotonergic systems. Changes in the activity of the dopaminergic (8, 9, 22, 23), noradrenergic and serotonergic systems have been reported following ACTH administration (22,23).

In our previous experiments, evidence was presented demonstrating that the levels of dopamine in the hypothalamus and striatum change following ACTH administration, which cannot be abolished by adrenalectomy (21,22). The changes observed in norepinephrine and serotonin were absent in adrenalectomized animals (21,22). These findings suggested to us that the dopaminergic system might play an essential role in mediating the behavioral action of ACTH. Our previous experiments with other neuropeptides (21) suggested that the use of receptor blockers, even in a small dose which itself would not influence a given behavioral paradigm, could block or facilitate the action of certain neuropeptides, if the peptide exerts its action via neurotransmitters.

In the present experiments, further evidence is presented concerning the action of ACTH on brain transmitters related to the extinction of active avoidance behavior.

In these experiments, blockers of dopamine receptors (haloperidol) alpha-receptors (phenoxybenzamine), beta-receptors (propranolol) and muscarinic cholinergic receptors (atropine) were used alone or in combination with ACTH.

METHOD

Animals

For the experiments, CFY male rats weighing 150–200 g were used. The animals were kept at constant temperature in artificial light (schedule: 12 hr light and 12 hr dark). The light period started at 6.00 a.m.

Behavioral Method

Active avoidance behavior. Active avoidance behavior was studied in a platform jumping conditioning apparatus described earlier (24), with a modification (27). Briefly, the conditional stimulus (CS) was the light of a 40 W electric bulb. The unconditional stimulus (US) was an electric shock of 0.2 mA delivered through the grid floor of the apparatus to

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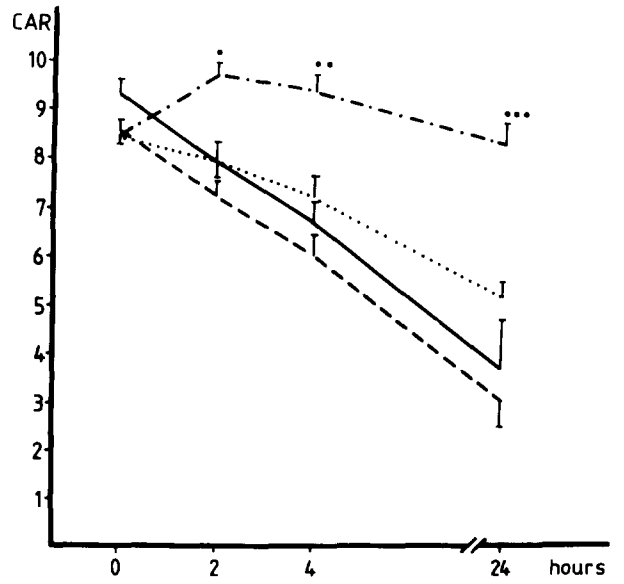

FIG. 1. Effect of ACTH and haloperidol on extinction of active avoidance behavior. Solid line: Control, dashed-dotted line: ACTH (2 I.U./rat), dashed line: haloperidol (20 μg/kg IP), dotted line: haloperidol + ACTH. Significance versus saline=●$p<0.05$, ●●$p<0.01$, ●●●$p<0.001$. Vertical lines are standard error of the mean.

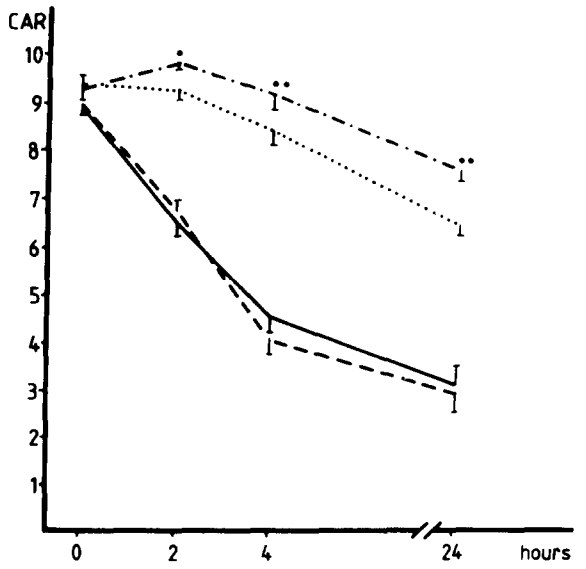

FIG. 2. Effect of ACTH and phenoxybenzamine on extinction of active avoidance behavior. Solid line: Control, dashed-dotted line: ACTH (2 I.U./rat), dashed line: phenoxybenzamine (2 mg/kg IP), dotted line: phenoxybenzamine + ACTH. Significance versus saline=●$p<0.05$, ●●$p<0.001$. Vertical lines are standard error of the mean.

the paws of the rat. In order to avoid the electric shock, the animals could jump onto a platform placed on the side of the apparatus. Each day for three consecutive days, 10 trials were performed, with a mean intertrial interval of 60 sec. The CS was presented for a maximum of 10 sec, or it was terminated as soon as the animal had made the responses. On the fourth day, extinction trials were run and the US was no longer applied. Animals which made at least 8 conditional responses out of 10 trials were selected for further experimentation. Immediately after the first extinction trial, the animals were treated with haloperidol (G. Richter, Budapest) (20 μg/kg IP) or phenoxybenzamine (Smith Kline French, Herts) (2 mg/kg IP) or propranolol (Imperial Chemical Industries, Macclesfield, England) (10 mg/kg IP) or atropine (EGIS, Budapest) (2 mg/kg IP). Thirty minutes following this receptor blocker administration, ACTH (corticotropine, Organon, Oss, 2 I.U./animal) was given subcutaneously alone or in combination with the receptor blocker. The control animals received 0.9 percent saline.

The animals were subjected to extinction trials 2, 4, and 24 hr after the treatment. Each group consisted of 10 animals, selected out of 14–16 animals.

Statistical Analysis

For statistical analysis, the Kruskal-Wallis test was used.

RESULTS

ACTH delayed extinction. Haloperidol in the dose applied (20 μg/kg IP) had no action on extinction as compared with the saline-treated controls. However, when administered before ACTH treatment, it prevented the action of ACTH ($p<0.05$ at 2 hr, $p<0.01$ at 4 hr and $p<0.001$ at 24 hr) (Fig. 1).

Phenoxybenzamine in a dose of 2 mg/kg IP had no action on extinction per se. Pretreatment with phenoxybenzamine did not modify the action of ACTH (Fig. 2).

Propranolol in a dose of 10 mg/kg IP did not modify the extinction, and was also ineffective in modifying the action of ACTH. The difference between the results for the ACTH groups and the ACTH plus propranolol-treated group was not significantly statistically (Fig. 3).

Atropine in a dose of 2 mg/kg IP had no action on extinction as compared to the saline-treated controls. It completely blocked the action of ACTH (Fig. 4).

DISCUSSION

The presented experimental evidence suggested that mainly the dopaminergic and cholinergic transmitter mechanisms are involved as mediators in the ACTH-caused delay in the extinction of active avoidance behavior.

During the last few years, considerable evidence has been reported concerning the action of ACTH on brain neurotransmitters.

ACTH administration increased the noradrenaline concentration in the locus coeruleus, but decreased levels were found in the hypothalamic nuclei (in the supraoptic nucleus, the arcuate nucleus and the ventromedial nucleus) (8,9). The α-MPT-induced disappearance of norepinephrine was increased by the hormonally active $ACTH_{1-24}$ (16) and the hormonally inactive, but behaviorally active $ACTH_{4-10}$ (17,28). This approach has been criticized, since α-MPT has also a stressor effect, and the changes observed under these conditions could be the sum of the effects of ACTH and stress (13).

$ACTH_{4-10}$ increased the ^{3}H-tyrosine conversion to ^{3}H-catecholamine (29) in the whole brain. $ACTH_{1-24}$ or $ACTH_{4-10}$ increased the rates of disappearance of ^{3}H-noradrenaline from the hypothalamus, hippocampus and

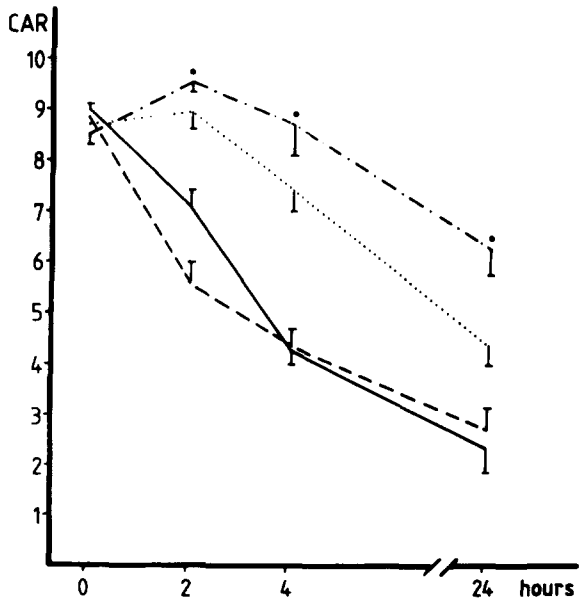

FIG. 3. Effect of ACTH and propranolol on extinction of active avoidance behavior. Solid line: Control, dashed-dotted line: ACTH (2 I.U./rat), dashed line: propranolol (10 mg/kg IP), dotted line: propranolol + ACTH. Significance versus control=●$p<0.05$. Vertical lines are standard error of the mean.

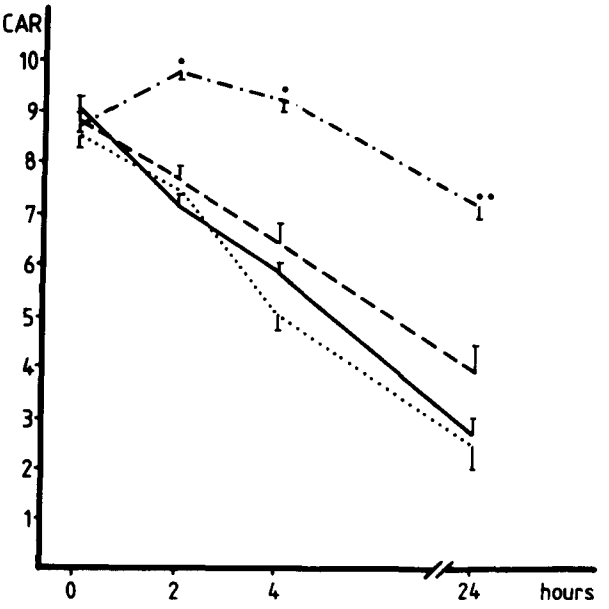

FIG. 4. Effect of ACTH and atropine on extinction of active avoidance behavior. Solid line: Control, dashed-dotted line: ACTH (2 I.U./rat), dashed line: atropine (2 mg/kg IP), dotted line: atropine + ACTH. Significance versus saline=●$p<0.05$, ●●$p<0.01$. Vertical lines are standard error of the mean.

cortex of adrenalectomized rats (6). Hormonally inactive $ACTH_{4-10}$ injected into the locus coeruleus increased the noradrenaline turnover in the hippocampus and cortex (4,5). In our previous experiments, ACTH decreased the steady-state levels of norepinephrine in the septal area and the hippocampus (22,23), but this effect was absent in adrenalectomized rats.

From the evidence presented above, it seems that ACTH, which is hormonally active, might have a dual action. It stimulates adrenal secretion, and hence the corticoids can change the brain norepinephrine metabolism in the central nervous system. However, it might also have a direct action on the noradrenergic system. The direct action may or may not be related to the action on certain behavioral reactions. The lack of any effect of the alpha-receptor blocker on the ACTH-caused delay in extinction suggests that the noradrenergic mechanism is not important in mediating this action in our hands, in the same experimental paradigm, the same amount of phenoxybenzamine blocked the action of somatostatin on extinction (26). Furthermore, the same dose of phenoxybenzamine can block the central adrenoceptor (15). These results emphasize the fact that the lack of any effects of phenoxybenzamine on ACTH action is not due to the relatively low dose of phenoxybenzamine used. Since phenoxybenzamine equally binds to $alpha_1$/$alpha_2$-adrenoceptors in the brain (15), the negative data strengthen the conclusion that neither $alpha_1$- nor $alpha_2$-adrenoreceptors are involved in mediating the action of ACTH in the present experiments.

Since propranolol—nonselective beta-adrenergic receptor antagonist—in a conventionally used dose, which per se had no effect on the behavioral paradigm used, was ineffective in modifying the ACTH action, the involvement of beta-adrenergic receptors can also be ruled out.

A number of data demonstrate the effects of ACTH on brain dopamine [for a review, see (28)]. The steady-state levels of dopamine were increased by the systemic injection of hormonally-active ACTH (22,23). Some of the effects were also present in adrenalectomized animals (22,23). The grooming activity induced by ACTH can be blocked by systemic injection of the dopamine receptor antagonist haloperidol (30). Our data also showed that the ACTH-caused delay in the extinction of active avoidance behavior can be blocked by haloperidol, and thus the dopaminergic system might play a role in mediating this action. However, one has to realize that haloperidol is not an exclusive dopamine receptor blocker.

Very few data are available concerning the action of ACTH on the brain cholinergic system. Torda and Wolff (25) reported that ACTH increases acetylcholine synthesis in the neuromuscular junction in intact and hypophysectomized rats.

$ACTH_{1-24}$ administration induced an increase in hippocampal acetylcholine turnover (31). $ACTH_{1-24}$ causes stretching and yawning, which is preceded by grooming [for references, see (12,31)]. This action can be prevented with an anticholinergic drug (10). Our presented data suggest that the muscarinic cholinergic system is also involved in mediating the action of cholinergic receptors on the extinction of active avoidance behavior. Since the cholinergic system may be regulated transsynaptically by dopamine, the change in the activity of the dopaminergic system might also be the cause of the alteration of the cholinergic system.

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