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CORTICOSTERONE DECREASES THE EFFICACY OF ADRENALINE TO AFFECT PASSIVE AVOIDANCE RETENTION OF ADRENALECTOMIZED RATS

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Summary

Short-term (48h) adrenalectomy (ADX) resulted in a deficit in the retention of a passive avoidance response. An inverted U-shaped dose-response relationship was found following immediate post-learning administration of adrenaline (A). A in a dose range of 0.005 - 5 µg/kg s.c. facilitated later retention. While corticosterone (CS) replacement alone had no effect, pretreatment with CS (300 µg/kg) was followed by a shift in the dose-response curve of A in ADX rats. Ten thousand times higher doses of A were required to improve retention behavior. Administration of the potent synthetic glucocorticoid dexamethasone failed to affect the responsiveness to A. It is concluded that corticosterone decreases the efficacy by which adrenaline affects later retention behavior of ADX rats. The specificity of corticosterone in this interaction suggests the involvement of the corticosterone receptor system which has its predominant localization in hippocampal neurons.

Introduction

Ever since the recognitions by W.B.Cannon (1) and H.Selye (2) that the adrenal gland, both the medulla and the cortex, is of primary importance in the maintenance of homeostasis and adaptation to environmental challenges, a possible interaction between adrenomedullary and cortical hormones has been in the focus of interest. Studies in the fifties suggested that adrenaline may enhance adrenal steroid output by increasing ACTH release (3). Subsequent studies have been focussed on the interactions at target organ level in the periphery. A number of observations suggest that adrenocortical hormones potentiate the cardiovascular (pressor) responses to endogenously released or exogenously administered catecholamines (4, 5, 6).

Behavioral adaptation to environmental changes represents an important physiological mechanism in maintaining homeostasis. Observations during the last two decades indicate that steroid hormones may profoundly affect adaptive behaviors (see for review 7,8) presumably via specific receptor systems in the brain (8). The involvement of adrenal catecholamines in brain processes such

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as learning and memory has been re-emphasized more recently (9, 10). Our observations also point to this direction. It was found that bilateral removal of the adrenals up to five days prior to the single learning trial of a passive avoidance response induces a severe deficit in the retention of this behavior (11). Replacement therapy with corticosterone (CS) failed to normalize, while adrenaline (A) treatment in a dose range of 0.005 to 5.0 µg/kg b.w. resulted in a dose-dependent recovery of the behavioral deficit of ADX rats. Adrenomedullectomy (ADMX) resulted in a similar behavioral deficit as ADX. However, higher doses of A were required to correct the retention behavior or ADMX rats than that of the ADX ones (11). Accordingly, one aspect of these findings was that ADMX rats appeared to be less sensitive to A than ADX ones.

The major difference between ADX and ADMX rats is the absence or presence of circulating adrenal steroids. It may therefore be that corticosteroids were responsible for the differential behavioral sensitivity of ADX and ADMX rats towards A. Accordingly, the present experiments were aimed to investigate whether replacement therapy with CS in ADX rats could modify the action of various doses of A on the retention of a passive (inhibitory) avoidance response. The rat brain contains a receptor system for CS with a predominant localization in hippocampal, septal and amygdaloid neurons (12, 13, 14). This receptor system displays a remarkable specificity in binding CS. Dexamethasone (DEX), a potent synthetic glucocorticoid is poorly retained by these brain receptors (15, 16). In peripheral target tissues including the anterior pituitary DEX has a much higher affinity for glucocorticoid receptors than CS (15). In order to study the specificity of steroid-catecholamine interaction in affecting behavior, experiments were performed with CS and with DEX too. Therefore, short-term (48h) ADX rats received A and CS or DEX alone or in combination immediately after learning of a passive avoidance response and their retention behavior was studied a day later.

Methods

Male Wistar rats weighing between 160-180 g were obtained from own breeding stock at the Rudolf Magnus Institute. They were housed in groups of 4-5 rats per cage and kept under a controlled light-dark schedule (light on between 6:00 and 20:00 h).

Bilateral adrenalectomy (ADX) was performed by a dorsal approach under light ether anaesthesia. ADX rats received physiological saline as drinking solution. Sham-adrenalectomy (SHAM) was performed through the same surgical procedure as adrenalectomy but the adrenals were left intact. These animals received tap water as drinking solution.

Passive (inhibitory) avoidance behavior was studied in a one-trial learning step-through situation (17). This behavioral test employs the innate preference of rodents for dark above light environments. Briefly, on day one the rats were adapted to a dark, large compartment of the apparatus. Subsequently, they were exposed to an elevated lit platform and allowed to enter the dark compartment. Three more of such trials were given on day 2. Upon entering the dark after the third trial, the rats received inescapable electric footshock (0.5 mA, a.c. for 2 sec). The latency to re-enter the dark compartment (up to a maximum of 300 sec) was measured 24 h later and served as the index for the retention of the passive avoidance behavior. Animals were ADX or sham-operated 48 h prior to the learning (shock) trial. The behavioral observations were carried out between 12:00 and 15:00 h. The medial latencies were calculated and the data were analyzed by means of Mann-Whitney's non-parametric ranking test.

Adrenaline (Epinephrine bitartarate, Sigma) was freshly prepared for treatment by dissolving in saline to yield the required doses (expressed as free base) in a constant injection volume of 0.2 ml. Treatment was given subcutaneously (sc) immediately after the acquisition trial. CS and DEX (generously donated by Organon International B.V., Oss, The Netherlands) were also freshly prepared by dissolving in ethanol and then saline was added to yield the required doses in a constant volume of 0.5 ml (1% ethanol/saline). CS or DEX was injected sc 60 min prior to the acquisition trial in a dose of 300 µg/kg body weight. Control animals received the corresponding injections of saline and/or vehicle (1% ethanol/saline) at the corresponding time intervals.

Results

Effect of adrenalectomy and post-training adrenaline administration

Short-term ADX (48h) resulted in a significant decrease of avoidance latencies as compared to the median value obtained in sham-operated rats (fig. 1).

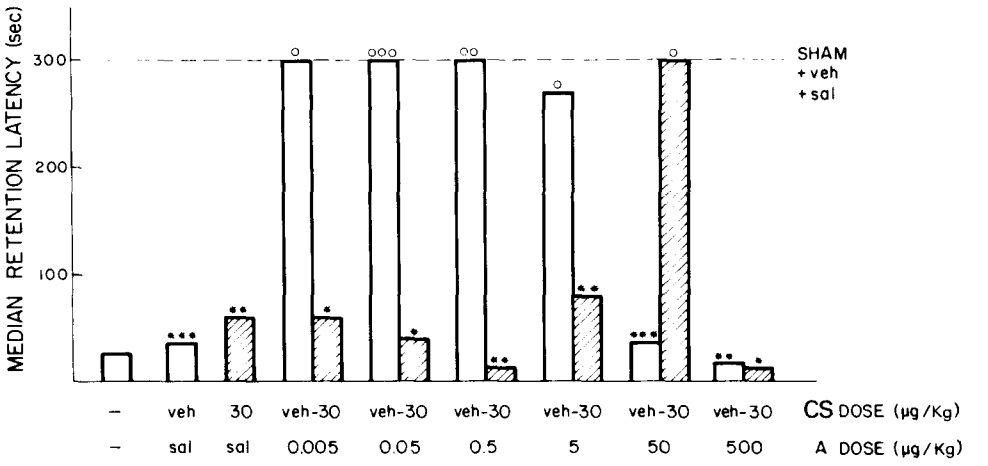

Fig. 1. Retention of a passive avoidance response in adrenalectomized (ADX) rats treated with corticosterone (CS) and/or adrenaline (A). Rats were ADX 48h prior to the acquisition session and subjected to the specified doses of CS 60 min prior and/or A immediately after the acquisition trial. Each bar represents the medial retention latency from 7 to 12 animals. Significance of the difference vs.ADX+veh+sal: $^{o}p < 0.05$; $^{oo}p < 0.01$; $^{ooo}p < 0.001$. Significance of the difference vs. sham+veh+sal: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Facilitation of retention behavior was observed in ADX rats receiving A in a dose range of 0.005, 0.05, 0.5 and 5.0 µg/kg immediately after the acquisition trial. In these experimental groups, median retention latencies were in the same range as those found in the corresponding control group. Higher doses of A (50-500 µg/kg) were ineffective on retention behavior. Median avoidance latencies of ADX rats treated with these high doses of the amine were similar to those displayed by the corresponding control-ADX group.

Effect of pre-training corticosterone and post-training adrenaline administration

Figure 1 also shows that s.c. administration of CS (300 µg/kg b.w.) one hour prior to the learning trial had no effect on retention behavior. ADX rats that received CS (300 µg/kg b.w.) and 0.005, 0.05, 0.5 or 5.0 µg/kg of A showed retention latencies that were not significantly different from those measured in ADX rats receiving only the vehicle of the amine. Thus, pre-training CS treatment abolished the improving effects of low and medium doses of A. However, ADX rats treated with CS and a dose of 50.0 µg/kg of A showed retention latencies in the same order of magnitude as those measured in the sham-operated group. The avoidance latencies were significantly longer than that observed in ADX rats treated with this dose of A only. Pre-training administration of CS failed to influence the retention latency of ADX rats treated with a dose of 500 µg/kg of A.

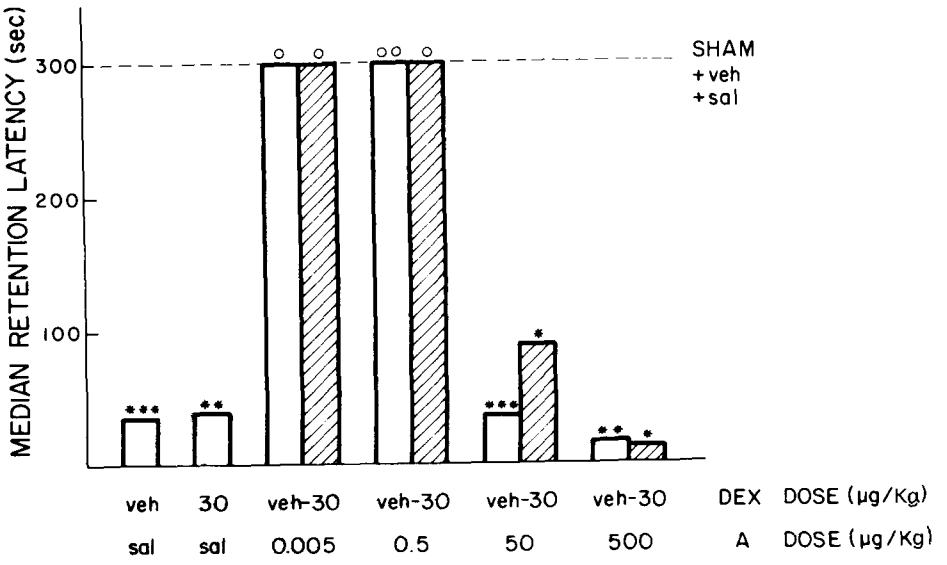

Fig. 2. Retention of a passive avoidance response in adrenalectomized (ADX) rats treated with dexamethasone (DEX) and/or adrenaline (A). Rats were ADX 48h prior to the acquisition session subjected to the specified doses of DEX 60 min prior and/or A immediately after the acquisition trial. Each bar represents the median retention latency from 7 to 12 animals.
Significance of the difference vs. ADX+veh+sal: $^{o}p < 0.05$; $^{oo}p < 0.01$
Significance of the difference vs. sham+veh+sal: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Effects of pre-training dexamethasone and post-training adrenaline administration

Figure 2 shows that ADX rats treated with DEX only (300 µg/kg) showed retention latencies in the same order of magnitude as those found in the corresponding control ADX group. ADX rats treated with DEX plus 0.005 or 0.5 µg/kg of A displayed retention latencies similar to those measured in ADX rats only treated with the corresponding doses of the amine. Similarly, ADX rats treated with DEX plus the higher doses of A (50.0 or 500.0 µg/kg) displayed retention la-

tencies not significantly different from those measured in ADX rats only treated with the corresponding doses of A. These results indicate that DEX pretreatment did not interfere the effects of A on passive avoidance behavior.

Discussion

Behavioral actions of adrenal cortical and medullary hormones represent a major adaptive mechanism that serves coping with physical and/or psychosocial environmental challenge. Adrenomedullary hormones are important modulators of post-learning events such as consolidation of memory thereby affecting later behavior (9, 10, 11). Corticosteroids, on the other hand, have been implicated in affecting the elimination of no more relevant behavioral responses without influencing memory per se (8, 11).

The present experiments showed that corticosterone pretreatment of adrenalectomized rats decreased the efficacy of adrenaline to affect the impaired inhibitory avoidance behavior of ADX rats. The shift in the efficacy of A was about ten thousend fold. Corticosteroid pretreatment alone had no effect on later retention behavior of ADX rats. Accordingly, the effect of A on memory processes had been modified by CS, but the steroid had no own effect in this case. These findings while reinforcing the general notion on the involvement of adrenal hormones in behavioral adaptation suggest a novel aspect of interaction between adrenal catecholamines and corticosteroids.

The described interaction between adrenomedullary and glucocorticoid hormones showed a remarkable specificity for CS. Dexamethasone, the highly potent glucocorticoid in an equimolar dose failed to modify the behavioral responsiveness to A. This specificity provides a good argument to postulate that regulation of behavioral sensitivity to A involves limbic (hippocampal) corticosterone sensitive mechanisms. As mentioned in the Introduction, a brain steroid receptor system with predominant localization in hippocampal neurons displays high binding specificity for CS over DEX (12-16). If the interaction would have involved peripheral corticosteroid sensitive mechanisms a comparable effect of CS and DEX would have been expected. The effectiveness of the used dose of CS (300 µg/kg) may also be used as an indirect evidence for the involvement of hippocampal CS-sensitive neuronal receptor system. It was found earlier that this dose of CS normalizes the occupancy of hippocampal cytoplasmic CS receptors and the CS-dependent behavioral deficits of ADX rats 60 min following peripheral administration (18). It is worth mentioning that behavioral specificity for CS vs. DEX was found in forced extinction (18), exploratory behavior (19) and extinction of food-rewarded runway response of ADX rats (20).

The mechanisms by which A affects inhibitory avoidance behavior and interacts with CS are not yet known. That CS decreases the behavioral efficacy of A, while the peripheral actions of A are potentiated by corticosteroids (4-6) suggests the unlikelyness of the involvement of circulatory action of A in behavioral regulation. Neuroendocrine mechanism(s) may, however, be involved. It has been shown that catecholamines facilitate the release of α-MSH and β-endorphin from the intermediate lobe of the pituitary gland and the amines may also be involved in the physiological release of these peptide hormones as the consequence of stressors (21). Since an opiomelanocortin system in the brain is rather similar in endproducts of intracellular biosynthesis to that of the intermediate lobe (see 22), it may well be that A action on behavior involves opiomelanocortin system in the brain. ADX exerted a transient depletory effect on brain (hippocampal) immunoreactive ACTH which was not affected by steroid replacement (23). It would be of interest to investigate whether A and CS would affect central opiomelanocortin system in a way that is similar to the described behavioral interaction in ADX rats.

The adrenal medullary and cortical functions are mainly interpreted in terms of stress mechanisms. The interactions between catecholamines and corticosteroids in relation to behavior may be explained as mechanisms which increase the resistance to stressful events. Stressors lead to adrenal catecholamine and corticosteroid release. It is postulated that neural processes are protected by corticosterone against deleterious effects of high catecholamine levels. This protective action seems to be mediated by specific limbic (hippocampal) corticosterone receptor systems. Alternatively, the temporal relationship between adrenal catecholamine and corticosteroid release due to stressful events (rapid release of A followed by a slower one of CS) cannot exclude the possibility that CS serves to "switch off" stress-mechanisms by decreasing the efficacy of adrenaline action.

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