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Involvement of Corticosteroids in the Processing of Stressful Life-events. A Possible Implication for the Development of Depression

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In a sub-population of endogenously depressed patients, disturbances of the hypothalamic–pituitary–adrenal axis can be observed. Increased cortisol and CRH levels combined with normal ACTH concentrations have often been reported. Corticosteroids appear to play a role in the mood changes, in depressed subjects. However, their mechanism of action is unknown. In animal experiments, the involvement of corticosteroids in stressor-induced learning was investigated. Three paradigms were used. In the Porsolt swimtest an animal had to learn to adapt to an inescapable situation. In the lithium chloride conditioned taste aversion an animal learned to avoid sugar water. In the amphetamine sensitization a second injection of amphetamine caused a potentiated response, because of conditioning. All three conditions appeared to be stressful because they induced a corticosterone release. When adrenalectomized (ADX) mice were compared to control animals it appeared that, in all three paradigms, their memory function was disturbed. The data indicated that this was a specific glucocorticoid-mediated effect since corticosterone and dexamethasone injections were able to reverse the ADX-induced deficit. The ADX-induced disturbances were only observable at moderate stress levels. More severe stressors (lower water temperature in the Porsolt swimtest, higher lithium chloride and amphetamine doses) also made ADX mice remember their previous experiences. The results suggest that corticosteroids are involved in the consolidation of stressful events and the corresponding coping responses. They play, however, only a role in the case of moderate stressors. In ADX animals no stressor-induced corticosterone increase can occur and therefore these animals only remember severe stressors. In a depressed patient basal steroid levels are increased and consequently very mild stressors, which induce only a small extra steroid release, will be remembered. The remembering of all these negative experiences might be of importance for the development and maintenance of the depression.

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INTRODUCTION

In a sub-population of endogenously depressed patients, disturbances of the hypothalamic–pituitary–adrenal (HPA) axis can be observed. Studies involving the measurement of cortisol levels in urine [1–7], plasma [8, 9], saliva [9–11] and cerebrospinal fluid [8] have shown increased secretion of this hormone in most depressed patients. Subsequent investigations have characterized the peripheral abnormality in more detail. This included evidence of an increase in 24 h plasma cortisol levels, a shortened nocturnal period of quiescence, an earlier nadir of secretion and decreased relative amplitudes in the circadian rhythm [12–15].

ACTH levels appear to be unchanged in depressed patients. Although some studies have shown a hypersecretion [16–18], more often normal or low values have been found [19–23]. CRH levels are altered; higher than normal levels of CRH in the cerebrospinal fluid of depressed patients have been reported [24, 25] but did not relate to plasma cortisol levels before or after dexamethasone. Nemeroff *et al.* [26] have shown a 23% reduction in the number of CRH binding sites

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in the frontal cortex of suicide victims compared with contols. They interpret this as evidence of chronic hypersecretion of CRH in depression. The current hypothesis to account for the above-mentioned biochemical findings in major depression is that there is a disinhibition of the HPA axis. This can clearly be shown by means of the dexamethasone suppression test. Carroll *et al.* [27, 28] developed a diagnostic tool in which 1 mg dexamethasone is given at bedtime and serum cortisol levels are drawn at 0800 and 1600 h the next day. If any of these exceed 5 μg/dl the test is considered to be positive and the patient is termed a non-suppressor, which indicates that his feedback system is disturbed. In depression there is a high proportion of non-suppressors and the response to dexamethasone has been very extensively studied by many investigators using many different cortisol methods and several different regimes of dexamethasone [29–34]. Treatment of depressed patients by means of antidepressants or electroconvulsive therapy resulted in decreased cortisol levels [1, 5]. Also, a normalization of dexamethasone suppression was observed. The drop in dexamethasone-suppressed cortisol levels usually preceded or coincided with good clinical outcome [35].

It is likely that the hypercortisolaemia mediates the effects on mood. Patients suffering from Cushing's syndrome, which is characterized by hypertension, purple striae, truncal obesity, buffalo hump and floried facies, have increased cortisol, normal CRH and increased or normal ACTH levels [36–38], and are in about 70% of the cases depressed [39–45]. This is in contrast to patients suffering from Nelson's syndrome, in which ACTH levels are high and corticostroids absent [46]; in these patients depression is not commonly observed [37]. Lowering plasma cortisol levels, in Cushing's patients, by means of steroid synthesis inhibitors (metyrapone, aminoglutethimide and ketoconazole) reduced the severity of the associated depression [45, 47–49]. A similar action was seen in endogenously depressed patients [50–53] where the compounds proved to be an antidepressent treatment.

Although a great deal of attention has been paid to the hypercortisolaemia in depressed patients and a number of arguments can be listed which suggest that the increased steroid levels play an important role in the observed mood changes, little is known about the mechanism by which the steroids manipulate mood. What do the corticosteroids do that makes the patient depressed? From animal experiments it might be concluded that corticosteroids play a role in learning, performance and retention of behaviour. Learning appears to be attenuated by the corticosteroids. This can clearly be observed using taste aversion and passive avoidance paradigms [54, 55]. Although active avoidance acquisition is not influenced [56, 57] and the learning of appetitive responses is only affected when the motivational level is low [54].

In contrast to the acquisition phase, performance of various learned behaviours is usually improved by corticosteroids. Dexamethasone improves free operant avoidance behaviour [58], differential reinforcement of low rate of operant performance [59] and the reversal of a discriminative appetitive response [54]. Furthermore, the non-relevant intertrial responsiveness of operant behaviour is suppressed by corticosteroids [56].

Retention of the behavioural responses is also influenced. Low doses of steroids facilitate passive avoidance retention [60]. This is in contrast to higher dosages which facilitate the extinction of an active avoidance response [56, 61, 62] of passive avoidance responses [54, 60] and of a conditioned taste aversion [55]. Recently it was shown that a steroid antagonist could disturb the retention of spatial information [63].

The above-mentioned studies do not amalgamate into a clear-cut picture on the role of corticosteroids in the handling of learned behaviours but only suggest an involvement in the processing of events. In the experiments presented here, we tried to investigate this further by studying the role of corticosteroids in simple learning tasks, in which stressor-induced learning can be observed. Three different paradigms were used: the Porsolt swimtest [64], the lithium chloride-induced conditioned taste aversion [65] and the amphetamine sensitization [66]. With every behavioural paradigm four different experiments were performed. In the first experiment the behavioural response of intact animals was investigated. This response was compared to that of ADX animals in experiment 2. Furthermore, the reversibility of the ADX effect, by the glucocorticoid agonists corticosterone and dexamethasone, was investigated. In experiment 3 the stress level of the used paradigm was varied and the consequences on the behaviour of sham-operated and ADX mice were recorded. In experiment 4, it was studied whether the used conditions were capable of inducing a corticosteroid release in intact animals. Finally, all the results were combined and integrated into a hypothetical model which might explain the effects of the corticosteroids on mood.

EXPERIMENTAL

Animals

Male mice [Crl:CD-1(ICR)BR, from Charles River, Germany] weighing 25–35 g were used. They were kept in a temperature-controlled room (21–23°C) under a fixed 12 h light–dark cycle (lights on 0600 h) and were housed in macrolon cages (40 × 23 × 15 cm), 5 animals per cage. Food pellets and drinking solution (tap water for the control and sham-operated mice and saline (0.9%) for the ADX animals) were freely available. During the taste aversion experiments drinking

solutions were only present for 30 min (1600–1630 h) every 24 h.

Adrenalectomy was performed under avertine (tribromoethanol/2-methylbutane-2ol) anaesthesia through a dorsal approach. Sham-operated animals were subjected to the same surgical procedure but the adrenals were not touched. The operations were performed between 0900 h and 1700 h and, after surgery, the mice were left to recover for 2 weeks.

Behavioural procedures

Porsolt swimtest. The procedure followed that described by Porsolt and co-workers [64]. Briefly, mice were placed individually in narrow glass cylinders (height 25 cm, diameter 6.5 cm), containing 6 cm of water, for 15 min (initial test). A retest of 15 min was given 24 h later. During both tests, the animals were observed and the immobility (absence of hindleg movements) was scored via a time-sampling procedure (sampling frequency 1/10 s). After both the initial test and retest sessions, the mice were kept warm and allowed to dry under an electric heater.

Conditioned taste aversion. The procedure followed that described by Peeters and co-workers [65]. Briefly, mice were trained individually (training sessions of 20 min between 0900 and 1200 h) for 4 days in macrolon cages (17 × 11 × 12 cm) equipped with two drinking bottles filled with tap water (or saline in the case of ADX mice). On the fifth day, the bottles were filled with a 5% glucose solution (or 5% glucose in saline) and after a 20 min drinking session the animals were injected with lithium chloride. The mice were left undisturbed for 2 days; tap water or saline were freely available till 1630 h on day 7. On the eighth day the animals were offered the choice between tap water, or saline, and glucose solution (20 min drinking session). The glucose solution intake (in ml) was recorded.

Amphetamine sensitization. The procedure used was modified after Rivet and co-workers [66]. In short, animals were injected with amphetamine on day 1, day 3 and day 5 after which ambulation was measured in macrolon cages (23 × 17 × 14 cm). Three light beams, 2.5 cm above the cage floor, traversed the width of the cage. Two successive interruptions of two adjacent light beams were recorded as an activity score. Scores were registered per 10 min time block and for the total session time of 60 min.

Corticosterone radioimmunoassay

The procedure used was modified after Rodbard and Lewald [67]. Blood samples were obtained after decapitation of the animals. Corticosterone was extracted from the plasma by shaking 25 μl plasma with 475 μl methanol for 3 s. After centrifugation at 8700 g for 3 min a 400 μl aliquot of the methanol fraction was evaporated and the resulting residue was dissolved in 400 μl of 0.2% (v/v) ethylene glycol. Corticosterone was assayed in 50 μl samples at an antibody dilution which yielded 30% binding of [^{3}H]corticosterone. A standard curve of corticosterone (serial dilution at a 1:1 ratio) was made in 0.2% (v/v) ethylene glycol.

RESULTS

Porsolt swimtest

When sham-operated animals were tested, in the paradigm described by Porsolt at a water temperature of 25°C, a gradual increase in the amount of immobility could be observed (see Fig. 1). After about 5 min, the animals were immobile for most of the time. Retesting of the animals on day 2 resulted in immobility almost right from the beginning. Already at min 2 a high immobility score was observed which stayed high till

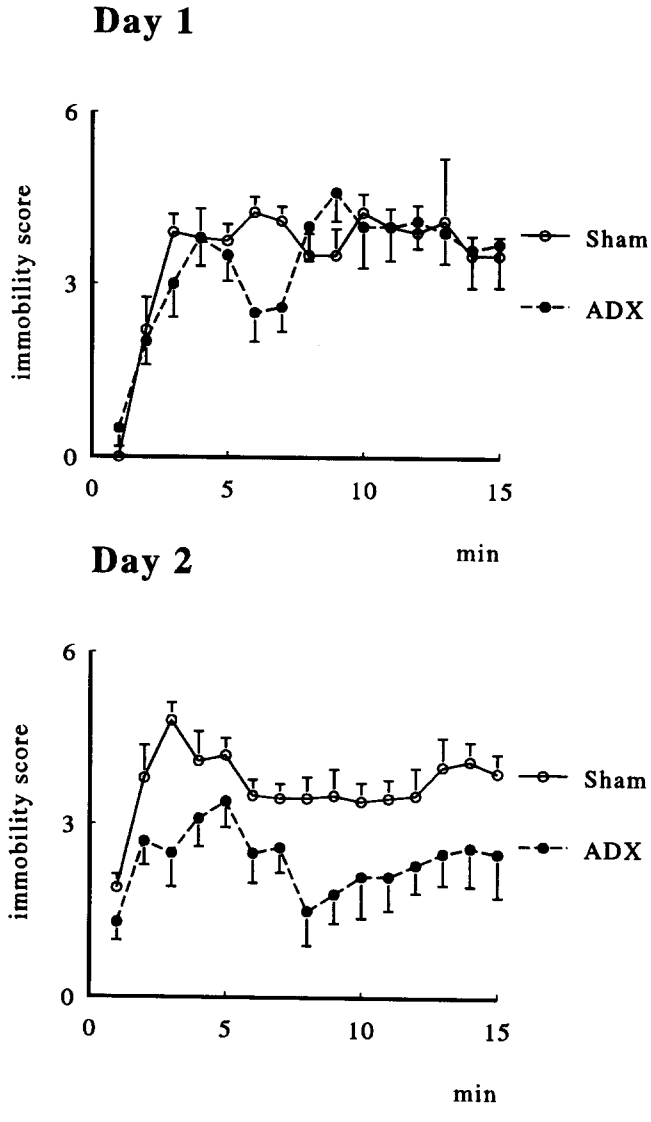

Fig. 1. Comparison between sham-operated (Sham) and adrenalectomized (ADX) mice in the Porsolt swimtest. Two groups of 10 animals were tested on two successive days at a water temperature of 25°C. The immobility score was determined via a time-sampling procedure (1/10 s) during the 15 min test sessions. Mean scores ± SEMs, per min, are shown.

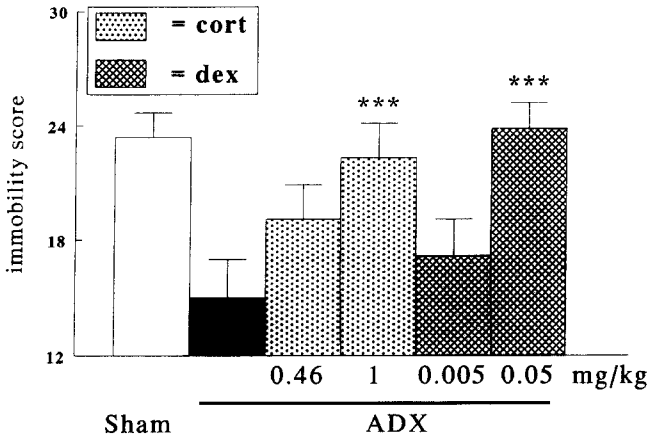

Fig. 2. Total immobiity scores (mean ± SEM), measured during the last 5 min on day 2, for sham-operated mice, ADX animals injected with arachis oil (subcutaneous; 15 min after the swimming session on day 1) and ADX animals injected with corticosterone (0.46 mg/kg, 1 mg/kg) and dexamethasone (0.005 mg/kg, 0.05 mg/kg). The behavioural differences between placebo-treated ADX animals and ADX animals injected with corticosterone or dexamethasone were evaluated by means of Student t-tests (every condition was tested in 10 animals). $^{***}P < 0.001$.

the end of the session. ADX mice showed a similar behaviour, in comparison to shams, on day 1. But on day 2 less immobility was displayed. The total immobility scores, during the first 5 min, for sham-operated animals, were 13.3 ± 1.71 on day 1 and 18.6 ± 1.75 on day 2. For ADX mice 12.4 ± 1.88 and 13.4 ± 1.42 were recorded, respectively. On day 2 there was a significant difference between shams and ADX mice [$T(18) = 2.30$; $P < 0.05$].

In Fig. 2 an alternative representation of the immobility scores of both groups of animals, on day 2, is depicted. In this case, the total immobility score on the last 5 min is shown because more stable differences were observed during this period. This period was consequently used to evaluate the effects of pharmacological and environmental treatments.

Subcutaneous treatment of ADX mice with corticosterone, immediately after the initial swimming test, resulted in increased immobility scores (see Fig. 2) on day 2. Corticosterone 1 mg/kg induced a significantly increased immobility which did not differ any more from the score observed in sham-operated animals. A similar effect was observed after subcutaneous injection of dexamethasone. Dexamethasone 0.05 mg/kg increased ADX immobility scores till sham levels.

The difference described between ADX and sham-operated mice could only be measured when the animals were tested at a water temperature of 25°C. Decreasing the water temperature to 20°C resulted in increased immobility scores in the ADX animals (Fig. 3).

Applying the Porsolt swimtest to intact animals resulted in a corticosteroid release, which could be measured in the blood samples of the mice. Both swimming at a water temperature of 20 and 25 C appeared to be a stressful experience (see Table 1).

Conditioned taste aversion

In Fig. 4 the effects of different doses of lithium chloride on the sugar water intake, on day 8, are depicted. Injection of control animals with lithium chloride, after their first experience with sugar water, resulted in an aversion of the novel taste. Both 10 mg/kg, 22 mg/kg and 46 mg/kg of lithium chloride were capable of inducing a significant avoidance behaviour.

In adrenalectomized animals no aversion could be observed, after applying lithium chloride 22 mg/kg (Fig. 5). The ADX-induced deficit could be decreased by means of the glucocorticoid agonists corticosterone and dexamethasone. Both compounds were injected 5 min after the lithium chloride administration. Corticosterone 50 mg/kg and dexamethasone 0.25 mg/kg decreased the sugar water intake of ADX animals.

In Fig. 6 the effects of different doses of lithium chloride on the sugar water intake of sham-operated and ADX animals are shown. A placebo injection did not induce avoidance behaviour in both sham-operated and ADX animals. A dosage of 22 mg/kg of lithium chloride only induced an aversion in shams, as already shown in Fig. 5. Forty-six mg/kg of lithium chloride induced an even stronger aversion in shams but also induced some avoidance behaviour in ADX animals. This latter effect was, however, not significantly different from that observed after placebo injection.

Injection of lithium chloride 22 mg/kg induced a corticosteroid release in intact animals. Steroid levels in the blood of the injected mice were increased at 30 min after injection, in comparison to placebo-injected animals (Table 1).

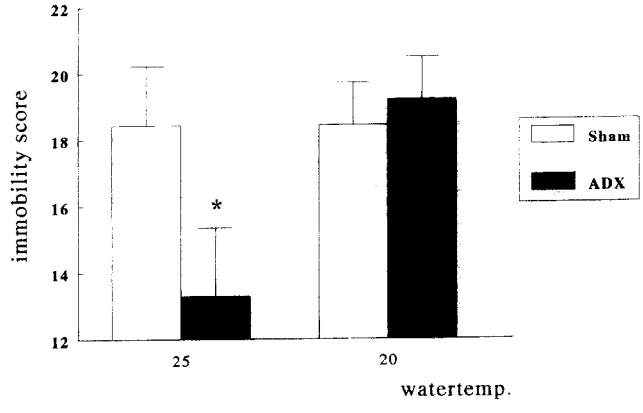

Fig. 3. Total immobility scores (mean ± SEM), measured during the last 5 min on day 2, for sham-operated mice and ADX animals. Animals were tested at water temperatures of 25°C and 20°C. Every condition was evaluated in 10 animals. Statistical comparisons were made by Student t-tests and compared Sham and ADX scores; $^{*}P < 0.05$.

Table 1. Effects of different treatments on corticosteroid levels (μg/100 ml) in plasma of mice

Treatment[a]	T = 0 min	T = 30 min	T = 60 min	T = 120 min
Control[b]	1.2 ± 0.30	0.9 ± 0.13	1.6 ± 0.8	4.9 ± 1.5
Swim 20°C[c]	38.5 ± 2.4***[e]	23.8 ± 2.7***	14.4 ± 3.23***	—
Swim 25°C	28.5 ± 3.6***	30.6 ± 2.7***	11.8 ± 1.2***	—
Plac[d]	4.0 ± 1.7	6.0 ± 1.8	7.5 ± 2.4	6.0 ± 1.9
LiCl 22 mg/kg	8.78 ± 2.18	20.6 ± 2.25***[f]	11.7 ± 2.0	—
Amph 1.0 mg/kg	1.2 ± 0.6	—	8.9 ± 1.4	7.0 ± 2.4
Amph 2.0 mg/kg	2.9 ± 1.2	—	18.0 ± 4.6*	13.0 ± 4.8

[a] Five animals are used per treatment per time point.
[b] Animals are taken from their home cages at various intervals.
[c] Animals are placed in the Porsolt swimtest.
[d] Animals are injected subcutaneously.
[e] Statistical comparison between Control and Swimstress by means of Student-*t* (***$P < 0.001$).
[f] Statistical comparison between Plac and treatment by means of Student-*t* (*$P < 0.05$; ***$P < 0.001$).

Amphetamine sensitization

Amphetamine, in doses ranging from 1 to 2 mg/kg, induced a dose-dependent behavioural activation which can be seen as increases in the activity score (Fig. 7). Repeated administration of amphetamine resulted, furthermore, in a sensitization, which could be observed with the 1.5 and 2.0 mg/kg doses.

In ADX animals amphetamine 1.5 mg/kg did not induce an activation (day 1 score after placebo injection: 257.7 ± 21.1; day 1 score after amphetamine 1.5 mg/kg injection: 211.4 ± 25.3); also no sensitization was observed. In Fig. 8 the sensitization is expressed as the activity score on day 5 divided by the score on day 1. ADX animals had a score of 71% which means that the animals were less active on day 5 than on day 1. The ADX-induced disturbance could be restored by means of corticosterone and dexamethasone injections (from day 1 till 4; every day at 1600 h). Corticosterone 10 mg/kg and dexamethasone 0.5 and 1.0 mg/kg induced a significant sensitization in ADX animals which did not differ from that seen in sham-operated controls.

In Fig. 7 it was already shown that both 1.5 and 2.0 mg/kg of amphetamine induced an activation and a sensitization in intact animals. This is again presented in Fig. 9. In ADX mice amphetamine 1.5 mg/kg was inactive but a dose of 2.0 mg/kg had clear effects. Activity scores were increased till 274.3 ± 35.6 (which is not significantly different from placebo scores) and a clear sensitization could be observed. This sensitization did not differ from that seen in sham-operated animals injected with the same dose.

Injection of amphetamine in intact animals resulted in a corticosterone release (Table 1) which reached

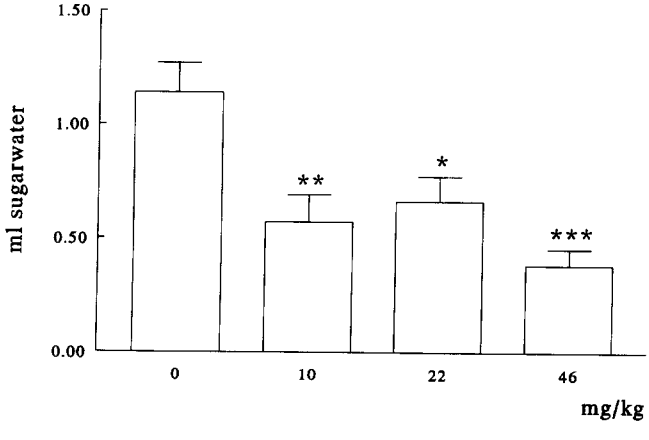

Fig. 4. Sugar water intake (mean ± SEM) on day 8. On day 5 the intact mice were subcutaneously injected, after the drinking of sugar water, with lithium chloride [placebo (0 mg/kg), 10, 22, 46 mg/kg]. Every dosage was tested in 15 animals. By means of Student *t*-tests differences between lithium injections and placebo treatment were evaluated; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

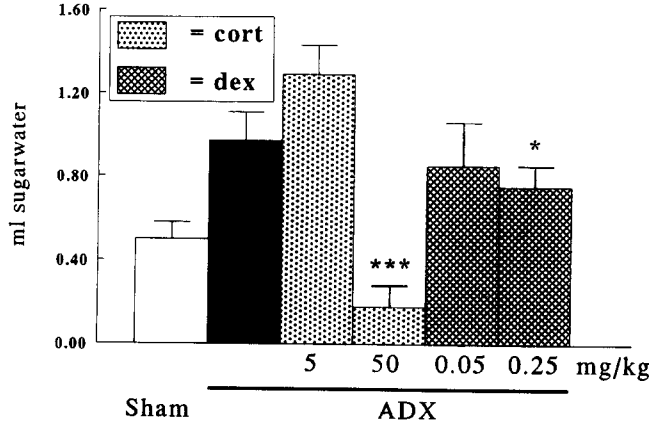

Fig. 5. Sugar water intake (mean ± SEM) on day 8 of sham-operated mice, ADX animals injected with arachis oil (subcutaneous; 5 min after lithium chloride application) and adrenalectomized animals injected with corticosterone (5 mg/kg, 50 mg/kg) and dexamethasone (0.05 mg, 0.25 mg/kg). All animals were injected, on day 5, with lithium chloride 22 mg/kg. Differences between placebo-treated ADX animals and ADX animals injected with corticosterone or dexamethasone were evaluated by means of Student *t*-tests (every condition was tested in 15 animals). *$P < 0.05$; ***$P < 0.001$.

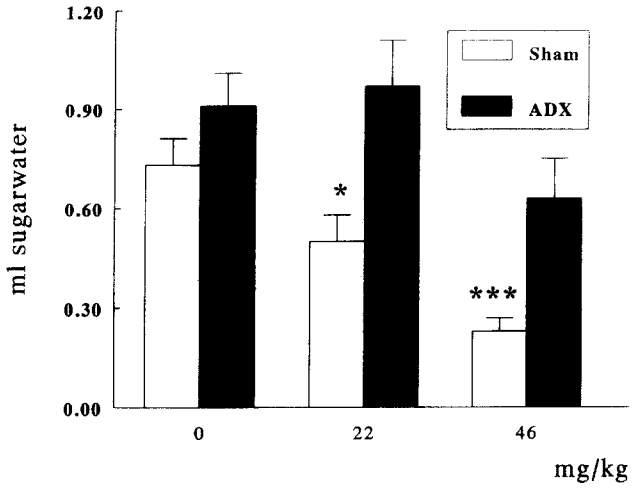

Fig. 6. Sugar water intake (mean ± SEM) on day 8 of sham-operated mice and ADX animals. Animals were injected on day 5 with placebo (0 mg/kg), lithium chloride 22 mg/kg or lithium chloride 46 mg/kg. Every condition was evaluated in 15 mice. Statistical comparisons were made by Student t-tests and compared Sham and ADX scores; $*P < 0.05$; $***P < 0.001$.

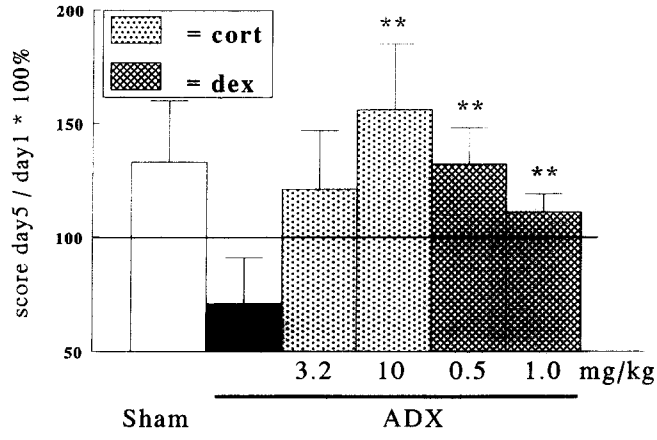

Fig. 8. Recalculated activity scores (score on day 5 divided by score on day 1 × 100%; mean ± SEM) of sham-operated mice, ADX animals injected with mulgophen NaCl (subcutaneous; at 1600 h) and ADX animals injected with corticosterone (3.2 mg/kg, 10 mg/kg) and dexamethasone (0.5 mg/kg, 1.0 mg/kg). All animals were injected, on day 1, day 3 and day 5 with amphetamine 1.5 mg/kg. Differences between placebo-treated ADX animals and ADX animals injected with corticosterone or dexamethasone were evaluated by means of Student t-tests (every condition was tested in 10 animals). $**P < 0.01$.

statistical significance with the 2.0 mg/kg dose an hour after injection.

DISCUSSION

In the Porsolt swimtest, ADX mice showed a clear reduction in the immobility response at 25°C during retesting. This appeared to be influenced by corticosteroids. Corticosterone and dexamethasone increased the immobility score in ADX animals. These findings are in agreement with previous reports. Jefferys and co-workers [68–70] have reported that during a 5 min retest, ADX rats spend significantly less time immobile than do intact controls or ADX rats which were repleted with corticosterone. The same authors also reported that dexamethasone reversed the effect of adrenalectomy if it was present between 15 min and 1 h, but not 4 h, after the initial training swim [69]. Veldhuis [71] described that the steroid antagonist RU486 attenuated the retention of the immobility response when administered briefly before the initial

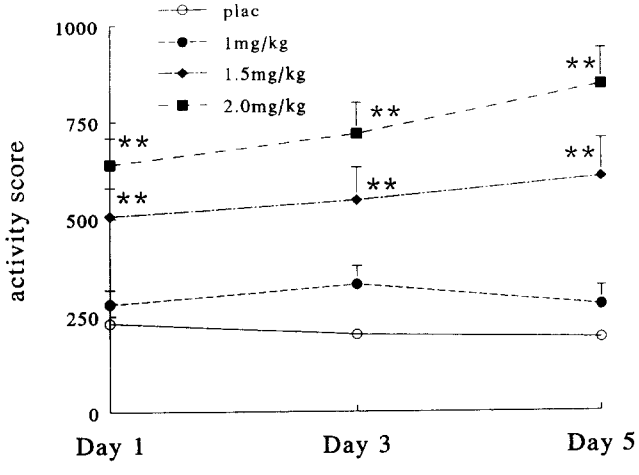

Fig. 7. Effects of subcutaneous amphetamine injections on the activity score (mean ± SEM) of intact animals. Amphetamine was applied in doses of 0 mg/kg (plac; 30 animals), 1 mg/kg (10 animals), 1.5 mg/kg (20 animals) and 2.0 mg/g (40 animals) on day 1, day 3 and day 5. Treatments were compared to placebo injections by means of a repeated measures analysis of variance followed by Tukey protected t-tests ($**P < 0.01$).

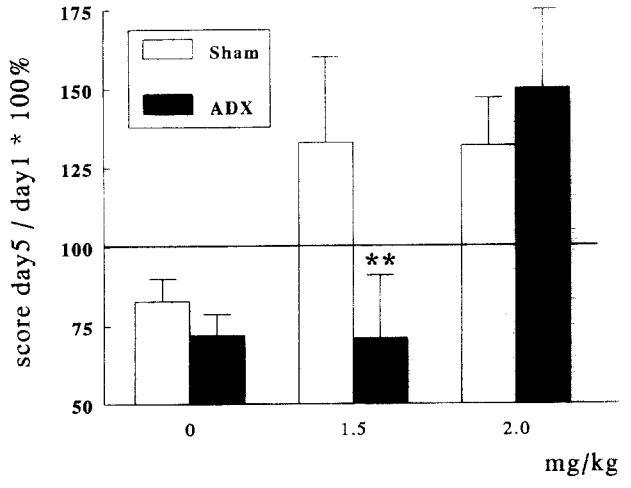

Fig. 9. Recalculated activity scores (score on day 5 divided by score on day 1 × 100%; mean ± SEM) of sham-operated mice and ADX animals. Animals were injected on day 1, day 3 and day 5 with placebo (0 mg/kg), amphetamine 1.5 mg/kg or amphetamine 2.0 mg/kg. Every condition was evaluated in 10 mice. Statistical comparisons were made by Student t-tests and compared Sham and ADX scores; $**P < 0.01$.

test. Local injection studies showed that doses as low as 10 ng of the antagonist intracerebroventricularly and of 1 ng in the dentate gyrus of the hippocampus were behaviourally active [72]. It was hypothesized that the corticosteroids released in response to a stress, such as forced swimming, are necessary for the consolidation or incorporation of a behavioural response to the stress. This conclusion was criticized by Mitchell [73] who showed that for a complete reversal of the effects of adrenalectomy, the presence of corticosterone during both testing and retesting was required which suggests that apart from a role in consolidation, corticosterone also is involved in the retrieval of the immobility response.

Our results do not confirm the findings of Mitchell [73]; both corticosterone and dexamethasone were, when injected just after the first swimming session, capable of completely reversing the ADX-induced deficit. This suggests that a presence of the steroids during the consolidation phase is sufficient. A role for corticosteroids in the retrieval process is also not likely because the retrieval of information occurs during the first minutes of retesting. During this time period steroid levels are still too low and do not act on glucocorticoid receptors.

A surprising finding was the observation that decreasing the water temperature resulted in a normalization of the ADX-induced behaviour. ADX animals displayed on day 2 the same amount of immobility as sham-operated mice. These results suggest that, apart from the corticosteroids, other factors must play a role in the consolidation and retrieval of information. There exists a vast body of literature on the effects of water temperature on stress-induced analgesia [74, 75]. These studies show that stress severity (water temperature) plays an important role in determining the mediator of a stress response. It is suggested that, depending on the severity of the stressor, different stress mediators (corticosteroids, opioids, etc.) are activated. Consequently, in our study, where the stress severity is also manipulated, different stress mediators might be involved. This can explain why there is only a steroid-influenced difference between control and ADX mice at 25°C. At this water temperature, corticosteroids probably play the major role in mediating the stress response, while at other temperatures they are overruled by other stress mediators (for example, opioids [68, 69]).

In the conditioned taste aversion experiments very similar results were obtained. Pairing of sugar water drinking with a lithium chloride injection induced a strong taste aversion in intact animals. This taste aversion was, however, not observed in ADX mice. Again, a role for corticosteroids in consolidation processes was suggested because both corticosterone and dexamethasone, injected just after the application of lithium chloride, were able to restore a taste aversion conditioning in ADX animals. This appears to be a physiological action since in intact animals lithium chloride induces a corticosterone release.

Lithium chloride is a compound which is widely used in taste aversion studies [76–78] and is reported to cause an activation of the HPA axis [79–81]. Inhibition of the axis by means of a pre-exposure to dexamethasone (a mechanism comparable to the dexamethasone suppression test) resulted in an attenuated taste aversion to lithium chloride [79–82]. These results are similar to the effects we observed after adrenalectomy. Both dexamethasone pre-exposure and ADX decrease corticosterone levels and inhibit taste aversion.

Stimulation of the HPA axis by means of ACTH, injected during conditioning, increased the taste aversion [80, 81]. This result might be explained by an ACTH-induced increase in corticosteroid levels. A strange observation was made by Rigter [83] who reported that $ACTH_{4-10}$ delayed the extinction of a learned taste aversion. $ACTH_{4-10}$ is an ACTH analogue which has hardly any effect on the HPA axis. Therefore these results cannot be explained by increases in steroid levels but might indicate that, besides corticosteroids, ACTH itself also has an effect on the learned aversion.

Corticosteroids are not the only factor which mediates the induced taste aversion; this can be concluded from our observations on the taste aversion induced by lithium chloride 46 mg/kg. In contrast to the results, observed with 22 mg/kg, 46 mg/kg of lithium chloride also induced some aversion in ADX mice. This suggests that in ADX mice a factor in addition to corticosteroids is present which is involved in the consolidation process.

Amphetamine, which releases a.o. dopamine, induced, when applied to intact animals, a strong behavioural activation. Repeated administration of the compound resulted in a sensitization or reverse tolerance. In rats a similar sensitization was reported by others [84–86].

Repeated injection of ADX mice with amphetamine caused no sensitization confirming previous findings [66]. Furthermore, it was shown that blockade of the hypothalamic–pituitary–adrenal axis by means of antibodies against CRH also prevented the sensitization to later administration of amphetamine [87].

The ADX-induced deficit could be restored by daily administrations of corticosterone and dexamethasone. These results suggest an involvement of corticosteroids in consolidation processes. Amphetamine causes a corticosterone release, as shown in our experiments and by others [88, 89]. We propose that this steroid increase is important for the sensitization. In the case of the Porsolt swimtest and the conditioned taste aversion, the corticosterone increase had to occur immediately after the acquisition phase to have its effect on memory processes. In the case of the amphetamine sensitization the relationship does not seem to hold so strictly. Injection of corticosterone and dexamethasone at 1600 h, while amphetamine application was between

0900 and 1200 h, is still able to reverse the ADX-induced effects. This observation is not easy to explain since amphetamine only induces a short-lasting steroid release. Rivet and co-workers [66] observed, however, an identical phenomenon in rats, using dexamethasone injections in the evening. Cador *et al.* [90] investigated the influence of chronic modifications of circulating levels of corticosterone on the locomotor response to amphetamine. Different groups of ADX rats were implanted with pellets releasing different amounts of corticosterone. Pellets releasing high amounts, mimicking chronic stress situations, were able to reverse the ADX-induced disturbance. These results show two things: first, the steroid receptor which is important for the amphetamine sensitization is a type II, glucocorticoid receptor. This corresponds to our observations that both corticosterone and dexamethasone can reverse ADX effects. Second, a constant high level of corticosteroids is able to re-induce an amphetamine sensitization. This latter observation also suggests that a close linkage in time, between the acquisition phase and the increased steroid release, is not necessary.

A further complication can be observed when a higher dosage of amphetamine is used; now also a sensitization occurs in ADX animals. This suggests that, like in the Porsolt swimtest and the conditioned taste aversion, additional factors are involved in the consolidation processes. At 2 mg/kg amphetamine, other factors take over and the steroid depletion is not important any more for the observed phenomena.

In summary, our results suggest that corticosteroids play a role in the consolidation of events, and the displayed coping responses, via an action on the glucocorticoid receptor. This role seems to be restricted to moderately stressful events. Severe stressors are also remembered by ADX animals, which indicates that corticosteroids are not the only factor involved.

Several authors have suggested that the intensity of the stressor in aversive learning affects the extent to which the experience is stored into memory [91–93]. There is a general consensus that this effect of stimulus intensity may be mediated by non-specific stress or arousal consequences [91–93]. Gold and McGaugh [91] have suggested that non-specific arousal processes associated with a learning experience may determine whether or not experiences are consolidated and that these processes may involve alterations in the level of hormones from the HPA axis. This view is consistent with the one expressed by Kety [92, 93]. The latter suggests, however, that these arousal effects can also be mediated by catecholamine activity.

Post-training administration of noradrenaline [94], adrenaline [91, 95] and ACTH [96] has been shown to facilitate consolidation of memory following aversive training with a low-intensity training stimulus.

The above results might be interpreted in the following way: arousal changes (mediated by noradrenaline, adrenaline or ACTH release) play their role via intensification of the stressors which are then more easily remembered. The same reasoning might also explain our results. Swim stress, a lithium chloride injection or an amphetamine application induces a corticosteroid release which intensifies the perceived experience and makes the animal remember it. In ADX animals no corticosteroid releases can occur, therefore no intensification takes place and the weak stressor is not stored. More severe stressors might activate more than one stress mediator. They are therefore also intensified via other systems; corticosteroids are consequently not essential for their consolidation.

If corticosteroids play such a crucial role in remembering stressors, one might wonder what will happen in endogenously depressed patients in which steroid levels are chronically elevated. The increased steroids will probably intensify all kinds of negative experiences which will then be remembered. It might well be that the remembering of all these negative events plays an important role in the development and maintenance of the depression.

A number of authors have investigated the perception of negative experiences by endogenously depressed patients. Willner and co-workers [97] observed that depressed patients reported a substantial increase in the severity of hassles they experienced (the Hassles and Uplifts questionnaire has been developed for the measurement of low-grade stressors [98]). Schless *et al.* [99], Lewinsohn and Tarkington [100] and Hammen and Cochran [101] observed very similar results. They reported that depressed patients rated stressful life events as more unpleasant than did non-depressed subjects. Grosscup and Lewinsohn [102] described a decrease in the frequency of pleasant events in depressed patients which was associated with an increase in the unpleasantness of unpleasant events. These studies show that for endogenously depressed patients stressful events are experienced as more unpleasant which might indicate that they are intensified by the increased corticosteroid levels.

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