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DOSE-DEPENDENT SUPPRESSION OF ADRENOCORTICAL ACTIVITY WITH METYRAPONE: EFFECTS ON EMOTION AND MEMORY

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SUMMARY

Different levels of circulating corticosterone are considered to produce different emotional states and effects on learning and memory. The purpose of the present study was to use different doses of the 11-beta-hydroxylase inhibitor metyrapone to produce dose-dependent inhibition of the synthesis of corticosterone and examine the consequences of that on several cognitive and emotional parameters. Systemic (SC) injections of metyrapone (25 or 50 mg/kg) dose-dependently suppressed increases in plasma concentrations of corticosterone induced by spatial training in a water maze, but did not affect plasma corticosterone levels in non-stressed rats. Treatment with the higher and lower dose of metyrapone also differentially affected behavioral measures of emotion and memory. Administration of 50 mg/kg, but not 25 mg/kg, of metyrapone impaired acquisition performance in the spatial water maze task. Both doses of metyrapone impaired retention. The impairment in retention was attenuated by dexamethasone (0.3 mg/kg) given systemically immediately after training, but not by corticosterone (0.3 mg/kg). During the exposure to a conditioned stressor of inescapable footshock, the higher, but not the lower dose of metyrapone attenuated fear-induced immobility. In contrast, the lower, but not the higher dose attenuated the anxiety state in an elevated plus-maze in a novel environment immediately after exposure to the conditioned stressor. It is suggested that emotion, learning, and memory are differentially affected by the different doses of metyrapone due to interference with different types of adrenal steroid receptors and consequent induction of various corticosterone receptor states. © 1997 Elsevier Science Ltd.

Keywords—Plasma corticosterone; Glucocorticoid receptor; Mineralocorticoid receptor; Anxiety; Fear; Water maze; Elevated plus-maze; Immobility.

INTRODUCTION

Adrenal glucocorticoids have complex and often opposing actions on fear, anxiety, learning, and memory (for reviews see: de Kloet, 1991; Bohus, 1994). Removal of endogenous glucocorticoids by adrenalectomy (ADX) produces anxiogenic effects (Weiss et

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al., 1970; File et al., 1979), whereas administration of corticosterone induces either anxiogenic or anxiolytic effects as a function of the dose of corticosterone (File et al., 1979). In contrast, fear-induced immobility is blocked by ADX and restored by corticosterone replacement (Bohus, 1987). Conditioning processes related to emotion such as extinction of inhibitory avoidance learning is impaired by ADX, and restored with corticosterone (Bohus & de Kloet, 1981). Adrenalectomy also impairs spatial orientation learning (Oitzl & de Kloet, 1992). In adrenally intact animals, peripheral administration of corticosterone and potent glucocorticoids usually impair learning and memory (see: Bohus, 1994), but a low dose of corticosterone has been found to enhance memory consolidation (Kovacs et al., 1977).

The findings indicating that there are different types of adrenal steroid receptor systems in the brain suggest that these complex effects of glucocorticoids on emotion and memory may be mediated by different receptors. Experiments using radioligand binding, immunocytochemistry and in situ hybridization have identified two distinct intracellular receptor systems for corticosterone in the rat brain: Mineralocorticoid receptors (MRs or Type I) and glucocorticoid receptors (GRs or Type II) (Chao et al., 1989; Fuxe et al., 1987; Herman et al., 1989; Reul & de Kloet, 1985; van Eekelen et al., 1988). A critical difference between MRs and GRs is their affinity for corticosterone and synthetic ligands. GRs show the highest affinity to potent synthetic glucocorticoids such as dexamethasone, RU 26988 and RU 28362. In contrast, MRs have the highest affinity for the natural steroids aldosterone and corticosterone, and bind dexamethasone with only a very low affinity (Reul & de Kloet, 1985; Reul et al., 1989; Sutanto & de Kloet, 1987): MRs' affinity for corticosterone is about one magnitude of order higher than that of GRs. Low (basal) circulating levels of corticosterone predominantly occupy MRs, and GRs become substantially occupied by corticosterone during stress and at the circadian peak. These binding affinities for corticosterone, together with differences in neuroanatomical distribution may underlie the distinct functional properties of the two receptor types. Neurons in the hippocampus, septum and amygdala express MRs in high densities (Arriza et al., 1988; Herman et al., 1989; van Eekelen et al., 1988). In contrast, GRs are widely distributed and occur in high densities in many brain regions involved in regulation of the stress response (Agnati et al., 1987; Fuxe et al., 1987). Considered together, these findings suggest that the extent of the changes in endogenous (i.e. severity of stress experience) or experimentally induced peripheral corticosterone levels via different receptor involvement may determine the differential effects on memory and emotion.

The present study examined the effects of metyrapone, an 11-beta-hydroxylase inhibitor, that is a powerful and selective blocker of corticosteroid synthesis in animal and human (Haynes, 1990). It blocks the conversion of deoxycorticosterone, the corticosterone precursor, in the rat adrenal cortex and thus prevents the synthesis and subsequent release of corticosterone into the bloodstream (Strashimirov & Bohus, 1966). Although metyrapone has for many years been used clinically to decrease glucocorticoid levels in humans in order to investigate pituitary reserve of adrenocorticotropin (see Haynes, 1990), it has not been used extensively as a tool in research on stress in animals. Importantly, a single peripherally administered injection of metyrapone induces a temporary, dose-dependent inhibition of glucocorticoid synthesis. The purpose of the present experiments was to use metyrapone to produce dose-dependent inhibition of synthesis of corticosterone which would result in different levels of circulating corticosterone. Another advantage of metyrapone treatment over that of ADX is that metyrapone does not disrupt the synthesis and release of the

adrenomedullary hormone epinephrine (S.F. de Boer, personal communication). This is important because several corticosterone effects are mediated by influences involving peripheral catecholamines (Borrell et al., 1984).

The present study used different doses of peripherally administered metyrapone in order to induce different circulating stress levels of corticosterone, and examine the effects on emotion and memory. A growing body of evidence suggests that different behavioral tests measure different aspects of fear and anxiety and that these processes may involve different brain systems, both in terms of neuroanatomy and neurochemistry. Therefore, animals were tested in several acute and conditioned emotional test situations. The experiments also examined the effects on memory of posttraining injections of corticosterone and dexamethasone, to examine whether glucocorticoid administration can restore the behavioral effects of metyrapone treatment.

MATERIAL AND METHODS

Subjects

Male Sprague–Dawley rats ($n = 179$; weighing 270–300 g) from Charles River Laboratories were used. They were housed separately in a temperature-controlled colony room (22°C) and maintained on a 12-h/12-h light–dark regime (lights on between 0700h and 1900h) with free access to food and water. The experiments were carried out between 0900h and 1500h.

Drug Treatment

Two doses of the 11-beta-hydroxylase inhibitor metyrapone [2-methyl-1,2-di-3-pyridyl-1-propanone (Sigma)] 25 or 50 mg/kg (in a volume of 2.0 ml/kg) were injected SC 90 min before the exposure of the animals to the test situation. The drug was dissolved in polyethylene glycol and diluted with a 0.9% saline solution to reach the appropriate concentration. The final concentration of polyethylene glycol was 40%. The vehicle control contained the same polyethylene glycol concentration. Each animal received only one injection of metyrapone or the vehicle control solution.

Corticosterone or the synthetic glucocorticoid dexamethasone was administered at a dose of 0.3 mg/kg SC (in a volume of 2.0 ml/kg) immediately after completion of five training trials in a water maze task (see below). These drugs were dissolved in 100% ethanol and subsequently diluted in 0.9% saline to reach the appropriate concentration. The final concentration of ethanol was 2%.

Water Maze Task

The water maze was a circular galvanized steel tank 1.83 m in diameter and 0.58 m in height. The tank was filled with water (27°C) to a depth of 20 cm. The maze was located in a room containing several extramaze cues. Four starting positions were equally spaced around the perimeter of the pool. A Perspex platform (20 × 25 cm) was placed 25 cm away from the edge of the pool. The platform was submerged 2.5 cm below the water surface. On the training day, each rat received an SC injection of either vehicle or metyrapone (25 or 50 mg/kg) 90 min before training. Before the first training trial the rat was directly placed on the submerged platform for 30 s. On each of five trials (i.e. swims), the rat was placed into the tank randomly at one of the four designated starting points, facing the wall, and allowed to escape onto the submerged platform. The platform was located in a fixed position during the

entire procedure. If an animal did not escape within 60 s starting from the release into the tank, it was manually guided to the platform. Latency to mount the platform was recorded on all trials. After mounting the platform the animal was allowed to stay there for 20 s, and was subsequently placed into a holding cage for 30 s until the start of the next trial. Immediately after completion of these five trials, the rats received an SC injection of either vehicle or 0.3 mg/kg corticosterone or dexamethasone. Retention was tested 48 h after training. Three retention trials were given, with the platform in the same location as during the training trials. The latency to escape on each of these three trials was recorded. The average latency on these three trials was used as the index of retention.

Fear-induced Immobility

The experiment was designed on the basis of previous evidence (Roozendaal et al., 1990) indicating that rats display immobility when tested in an apparatus where they previously received a footshock. The rats were trained in a step-through inhibitory avoidance apparatus (McGaugh et al., 1988) located in a sound-attenuated room. The apparatus consisted of a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top, 6.4 cm wide at the floor) divided into two compartments separated by a sliding door that opened by retracting into the floor. The starting compartment (31 cm long) was illuminated and the shock compartment (60 cm long) was dark. On the training day, the rat was placed in the starting compartment of the apparatus with an open door. As the rat stepped into the dark compartment, the sliding door was closed and an inescapable footshock (0.45 mA for 3 s) was given immediately in half of the animals (stress group). The rat was removed from the dark alley 30 s after termination of the footshock. In the remaining animals, no footshock was administered and the rat was retained in the dark compartment for 30 s (control group) and then returned to its home cage. Forty-eight hours later, each animal received an SC injection of either vehicle or metyrapone (25 or 50 mg/kg) 90 min before testing. The animal was placed directly in the dark compartment (with closed sliding door) in which no further footshock was given. The effect of drug treatment on the relative time spent on immobility (i.e. when the animal was completely motionless) was measured for a period of 5 min.

Elevated Plus-maze

Immediately after the 5-min period exposure to the compartment where they had previously received a footshock, the animals were transported to another room and placed in an elevated plus-maze. The elevated plus-maze consisted of two open (50 × 10 cm), and two closed arms (50 × 10 × 50 cm), with an open roof, arranged such that the two arms of each type were opposite to each other (Pellow et al., 1985). The maze was elevated to a height of 50 cm above the floor. Each rat was tested for 5 min on the elevated plus-maze. The maze was carefully cleaned with 20% ethanol after each animal. Time spent on open arms relative to open+closed arms is used as an index of the anxiolytic or anxiogenic effects (Pellow et al., 1985). Furthermore, the number of entries in the closed and entries in the open arms in the elevated plus-maze was measured. Previous studies indicated that conditioned emotional stress of fear of inescapable footshock enhances the anxiogenic response of the elevated maze (Korte et al., 1995).

Corticosterone Assay

A separate group of 60 animals was injected with vehicle or metyrapone (25 or 50 mg/kg) 90 min before half of them were trained in a water maze. The experimental design was

Table I. Effects of metyrapone on plasma corticosterone levels

	Drug	Plasma corticosterone μg/dl (± SEM)
Control	Vehicle	5.6 (1.2) (n = 9)
	Metyrapone (25 mg/kg)	7.5 (2.0) (n = 9)
	Metyrapone (50 mg/kg)	4.1 (1.1) (n = 10)
Swim stress	Vehicle	45.7 (2.8) (n = 10)*
	Metyrapone (25 mg/kg)	26.8 (2.7) (n = 10)†
	Metyrapone (50 mg/kg)	13.9 (1.4) (n = 10)†‡

* $p < .01$ compared to control/vehicle group; † $p < .01$ compared to stress/vehicle group; ‡ $p < .01$ compared to stress/metyrapone (25 mg/kg) group. The number of animals per group is presented in parentheses.

similar to that described above. The other half of animals did not receive any training and served as non-stressed controls. Fifteen minutes after onset of training, the animals were decapitated and trunk blood was collected in heparinized tubes and stored on ice. After centrifugation at 5000 r.p.m. for 10 min, the supernatant was stored at −50°C until assay. Corticosterone was assayed by radioimmunoassay using a commercial kit (ICN Biomedicals, Costa Mesa, CA, USA). Samples were measured in duplicate with an intraassay variability of 6%. The absolute detection level of corticosterone was 0.1 μg/dl.

Statistics

Behavioral results of the water maze were analyzed during training with a two-way analysis of variance (ANOVA) with metyrapone treatment (three levels) as the between-subject variable, and trial number (five levels) as the within-subject variable. Retention of the water maze was analyzed with a two-way ANOVA with metyrapone (three levels) and steroid administration (three levels) both as between-subject factors. Data of fear-induced immobility and the elevated plus-maze were subjected to a two-way ANOVA with stress (two levels) and metyrapone (three levels) as the between-subject variables. Plasma corticosterone levels were analyzed using a two-way ANOVA with stress (two levels) and metyrapone treatment (three levels) as the between subject variables. Further analysis was done with post hoc t-tests to determine the source of the detected significances in the ANOVAs. A probability level of $< .05$ was taken as statistical significance.

RESULTS

Effects of Metyrapone on Plasma Corticosterone Levels

Two-way ANOVA of data indicated a significant interaction between stress and metyrapone treatment on plasma corticosterone levels ($F(2,52) = 30.21$; $p < .0001$). As shown in Table I, the stress of spatial training in a water maze induced a significant increase in plasma levels of corticosterone in vehicle-treated rats ($p < .01$). Metyrapone treatment dose-dependently decreased the stress-induced increase in plasma corticosterone levels. Both the lower and higher dose of metyrapone suppressed plasma corticosterone concentrations in stressed rats (both $p < .01$). However, plasma concentrations of rats

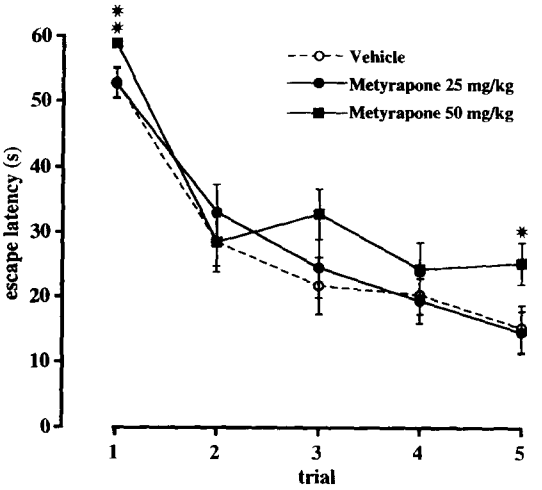

Fig. 1. Escape latencies in seconds for rats receiving vehicle or metyrapone (25 or 50 mg/kg) SC injections 90 min before the training of five trials of water maze. Data represent the mean (± SEM) of 18 vehicle, and 21 (25 mg/kg) and 25 (50 mg/kg) metyrapone injected rats $*p < .05$; $**p < .01$ compared with the corresponding vehicle animals.

treated with the higher dose were significantly lower than those of rats treated with the lower dose of metyrapone ($p < .01$). Metyrapone did not affect plasma corticosterone concentrations of control rats that did not receive any training in the water maze ($F(2,27) = 1.38$; NS).

The Effects of Metyrapone on Acquisition Performance in a Water Maze

As can be seen in Fig. 1, latencies to find the escape platform decreased with training ($F(4,264) = 58.86$; $p < .0001$). More importantly, a significant drug effect was observed ($F(2,264) = 4.57$; $p < .05$). On the first and fifth training trial the performance of animals given the higher dose of metyrapone (50 mg/kg) was significantly impaired compared to that of animals treated with either vehicle or the lower dose of metyrapone (25 mg/kg) (first trial, $p < .01$; fifth trial, $p < .05$). The lower dose of metyrapone (25 mg/kg) did not affect escape latencies.

The Effects of Metyrapone, Corticosterone and Dexamethasone on Retention Performance in a Water Maze

Latencies to escape from the water on the retention test trials 48 h after training, calculated as the average of the three retention trials, are shown in Fig. 2. Pretreatment with either the lower or higher dose of metyrapone prior to training impaired retention performance as indicated by longer escape latencies ($F(2,60) = 5.78$; $p < .01$). Immediate posttraining injections of dexamethasone did not affect retention of animals given vehicle injections or of the higher dose of metyrapone prior to training, but reversed the retention impairment induced by the lower dose of metyrapone ($p < .05$). In contrast with the effects of dexamethasone, posttraining injections of corticosterone impaired retention performance in vehicle pretreated animals ($p < .01$). However, corticosterone was ineffective in

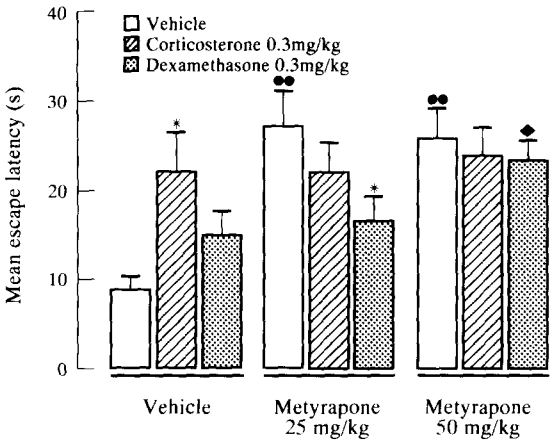

Fig. 2. Average escape latencies in seconds (± SEM) on the retention test for rats receiving SC injections of vehicle or metyrapone (25 or 50 mg/kg) 90 min before training, and immediate posttraining injections of vehicle, corticosterone or dexamethasone (0.3 mg/kg). ●●$p < .01$ compared with the corresponding vehicle pretreated group; *$p < .05$ compared with the posttraining vehicle group receiving the same metyrapone pretreatment; ◆$p < .05$ compared with the corresponding vehicle pretreated group. The number of animals per group is between six and nine.

metyrapone-pretreated animals displaying impaired retention behavior. In fact, the vehicle group and both metyrapone groups given posttraining injections of corticosterone did not differ in retention performance.

Effects of Metyrapone on Fear-induced Immobility

Two-way ANOVA of data indicated a significant effect of a previously received footshock on immobility ($F(1,38) = 21.06$; $p < .0001$) and a significant stress×drug

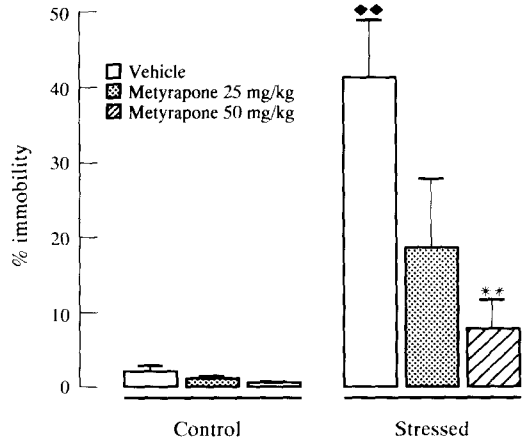

Fig. 3. Effect of metyrapone injections on the time spent in immobility in percentage (± SEM) during re-exposure to the former shock compartment in stressed and control animals. **$p < .01$ compared with the corresponding vehicle group; ◆◆$p < .01$ compared with the corresponding control group. The number of animals per group is between seven and eight.

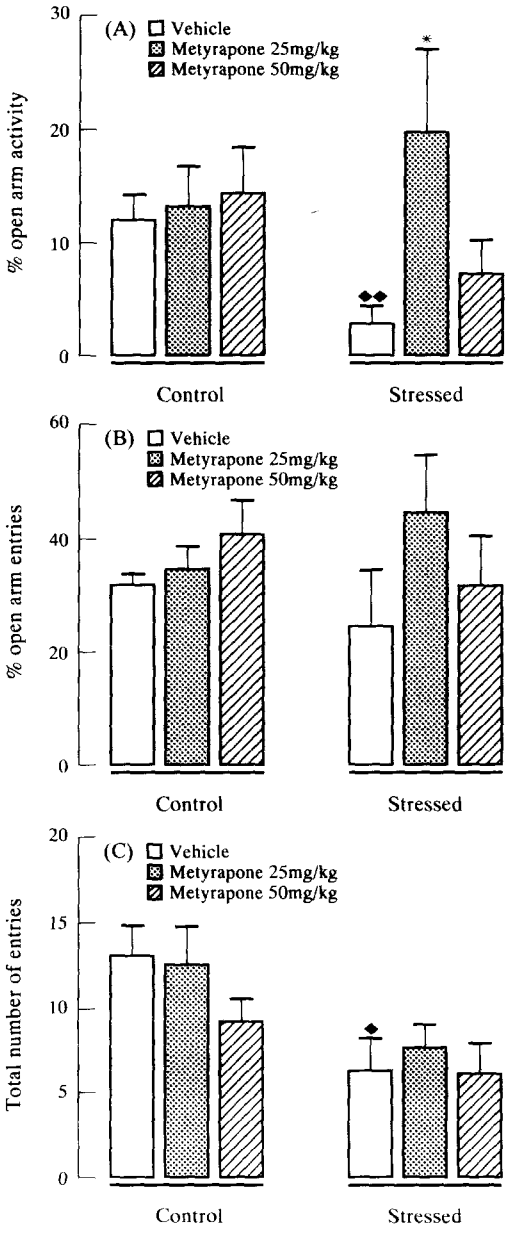

Fig. 4. Effect of metyrapone injections on open arm activity in percentage (± SEM) (A), relative number of open arm entries (± SEM) (B) and the sum of entries in open and closed arms (± SEM) (C) in the elevated plus-maze in stressed and control animals. *$p < .05$ compared with corresponding vehicle group; ◆ $P < .05$; ◆◆$p < .01$ compared with the corresponding control group. The number of animals per group is between seven and eight.

interaction ($F(2,38) = 3.32$; $p < .05$). As shown in Fig. 3, the higher (50 mg/kg), but not the lower (25 mg/kg) dose of metyrapone significantly decreased the percentage of time spent in immobility in stressed (i.e. formerly footshocked) rats in comparison with stressed vehicle-treated animals ($p < .01$). The level of immobility was low in control (i.e. non-footshocked)

Table II. Summary of the behavioral data

	Metyrapone 25 mg/kg	Metyrapone 50 mg/kg	Receptor type involved*
Corticosterone level	↓	↓↓	
Water maze acquisition	—	↓	MR
Water maze retention	↓	↓	GR
Fear-induced immobility	—	↓	MR
Fear-potentiated anxiety	↓	—	GR or GR/MR

* As determined with specific GR and MR agonists and antagonists (Korte et al., 1995; Oitzl & de Kloet, 1992).
— Indicates no change.

animals, and thus metyrapone did not significantly affect this immobile behavior ($F(2,21) = 1.32$; NS).

Effects of Metyrapone in the Elevated Plus-maze

The effects of prior footshock and metyrapone on plus-maze behaviors were not significant as analyzed by a two-way ANOVA. However, a one-way ANOVA revealed a significant metyrapone effect in the stressed animals ($F(2,21) = 4.1$; $p < .05$) (Fig. 4A). The relative time in the open arms was increased following injections of 25 mg/kg ($p < .05$), but not of 50 mg/kg of metyrapone. In control animals, metyrapone did not alter the percentage of open arm activity ($F(2,21) = 0.13$; NS). The stressed and control animals did not differ in the relative number of open arm entries ($F(1,38) = 0.07$; NS). Further, metyrapone did not affect total open arm entries ($F(2,38) = 1.25$; NS) (Fig. 4B). The total number of entries (i.e. in open+closed arms) was significantly decreased in stressed compared to control animals ($F(1,38) = 10.70$; $p < .005$). Metyrapone treatment did not significantly alter the total number of entries ($F(2,38) = 1.11$; NS) (Fig. 4C).

DISCUSSION

The findings of the present experiments indicate that, in rats, systemic administration of metyrapone dose-dependently suppressed stress-induced rises in plasma corticosterone in the water maze test. Although metyrapone-induced changes in corticosterone levels were not determined during fear-induced immobility, it is likely that similar dose-dependent effects exist. The two doses of metyrapone also differentially affected behavioral indices of emotion and memory. Some behavioral effects were induced by injections of the lower dose of metyrapone, whereas other effects were only produced by the higher dose. The higher dose but not the lower dose of metyrapone significantly impaired acquisition performance in the water maze, whereas both doses were effective in impairing retention. Only the higher and not the lower dose of metyrapone decreased fear-induced immobility. In the test of fear-potentiated anxiety in the elevated plus-maze, the lower dose of metyrapone increased open arm activity (see Table II).

The pattern of dose-dependent effects of metyrapone on different behavioral measures suggests that the effects may be due to the different levels of circulating corticosterone. It is

interesting to compare the distinct behavioral effects produced by the two doses of metyrapone with results from experiments using specific MR and GR antagonists. As is discussed below, these comparisons suggest that the lower dose of metyrapone predominantly blocked GR-mediated responses, whereas the higher dose impaired both GR- and MR-mediated processes (see Table II). As was noted above, basal levels of corticosterone predominantly occupy MRs, which have a high affinity for corticosterone. In addition, higher corticosterone levels elicited during and after stress result in activation of GRs (Reul & de Kloet, 1985; Reul et al., 1989; Sutanto & de Kloet, 1987). Because the test situations used in these experiments were aversive, they induced the release of high levels of corticosterone (Biro et al., 1993; de Boer et al., 1990; Roozendaal et al., 1992; Selden et al., 1990). Thus, in vehicle-treated animals both GRs and MRs were probably occupied. Small decreases in plasma corticosterone levels would be expected to reduce the occupancy rate of GRs, whereas large decreases in corticosterone level would reduce the occupancy of both GRs and MRs. In the water maze task the higher dose of metyrapone impaired acquisition performance. Previous evidence indicated that MRs are involved in acquiring information that lead to appropriate behavioral responses in a water-maze paradigm (de Kloet, 1991; Oitzl & de Kloet, 1992).

Both doses of pretraining metyrapone impaired water maze retention performance. These findings are consistent with those of similar experiments using adrenalectomized animals (Oitzl & de Kloet, 1992). Both in the present and latter study, the memory impairment was attenuated by dexamethasone, but not corticosterone, administered immediately after the training session. Previous findings indicated that, in adrenally intact rats, posttrial central administration of the selective GR antagonist RU 38486 impaired water maze retention (Oitzl & de Kloet, 1992). That is, the blockade of central GRs impaired consolidation of spatial information. In contrast, intraventricular administration of anti-mineralocorticoids did not impair consolidation. This result suggests that consolidation depends upon poststress activation of glucocorticoid sensitive central pathways. This argumentation is based thus in part on the assumption that the synthetic glucocorticoid dexamethasone is in vivo a GR agonist. Indeed, previous in vivo studies of both behavioral and neurochemical nature support this view (Bohus et al., 1982). However, posttraining injections of dexamethasone were only effective following pretreatment with the lower, and not the higher, dose of metyrapone.

One possible explanation of the results is that a substantial occupation of MRs may be essential to reverse deficits at GRs caused by the inhibition of corticosterone synthesis by metyrapone. MRs remain occupied after administration of the lower dose of metyrapone, whereas they may be deprived of corticosterone following injection of the higher dose of the drug. Blockade of corticosterone synthesis by metyrapone induces the release of deoxycorticosterone, the corticosterone precursor, into the circulation (Strashimirov & Bohus, 1966). As indicated by in vivo studies, deoxycorticosterone may bind to corticosteroid receptors in the brain (Bohus et al., 1982), block the receptor functions, and thus prevent the action of dexamethasone. This alternative explanation is supported by the finding that deoxycorticosterone pretreatment abolishes the effects of corticosterone in attenuating impaired extinction behavior usually seen in adrenalectomized rats (Bohus & de Kloet, 1981). Finally, a simple behavioral interpretation is that the retention deficit is due to an impairment of acquisition produced by the higher dose of metyrapone. Dexamethasone given posttraining is then unable to reverse the retrograde learning deficit. This interpretation is, however, not supported by the finding that the lower dose of metyrapone also causes a

retention deficit without affecting acquisition behavior. The reversibility of this metyrapone effect with dexamethasone again points to a complex interaction between available corticosterone and MR and GR function. Acquisition behavior is dependent on MR and GR function, whereas posttraining consolidation is a GR dependent process. This conclusion is then consonant with previous studies by Oitzl & de Kloet (1992).

The higher dose of metyrapone decreased fear-induced immobility during re-exposure to the former shock compartment, whereas the lower dose was ineffective. Fear-induced immobility is also blocked after ADX and this effect is reversed by corticosterone, but not by the more specific GR agonist dexamethasone (Bohus, 1987). Such findings suggest that MRs are selectively involved in the expression of fear-induced immobility, and are consistent with the findings of studies using intraventricular injections of selective MR and GR antagonists (Bohus et al., 1990; Korte et al., 1995). One should, however, emphasize that this conclusion is valid for fear-induced immobility resembling freezing behavior. For example, immobility in a forced swimming paradigm (floating behavior) is dependent on GR function (Veldhuis et al., 1985; Jefferys & Funder, 1987; Mitchell & Meaney, 1991; Korte et al., 1997). Further, some evidence indicates that immobile behavior to unconditioned stimuli in preweanling rats (i.e. behavioral inhibition) is not affected by ADX or corticosterone treatment when performed after the development of the behavioral inhibition response (Takahashi, 1994).

In the elevated plus-maze, the lower dose of metyrapone increased open arm activity when the animals were tested immediately after exposure to the conditioned stressor. This finding suggests that fear-potentiated anxiety behavior in the plus-maze is differentially affected by GRs. The present results are consistent with findings of a recent study examining the effects of intraventricularly administered selective GR and MR antagonists using the same experimental paradigm (Korte et al., 1995). In that experiment, the GR antagonist RU 38486 attenuated fear-potentiated anxiety behavior in the elevated plus-maze without affecting fear-induced immobility. Furthermore, an unpublished study (Oitzl & de Kloet, personal communication) found that GR blockade did not affect plus-maze behavior in animals that had not received prior fear potentiation. Thus, the present findings are consonant with previous evidence suggesting that the higher and lower doses of metyrapone may differentially affect MR- and GR-mediated processes.

The effects of the lower dose of metyrapone are thus similar to those produced by GR antagonism. The effects of the higher dose of metyrapone are more complex. Whereas the effects of the higher dose in the immobility test are similar to those produced by MR blockade, the higher dose did not reduce potentiated anxiety in the elevated plus-maze. Reduced immobility and subsequently abrogated enhanced anxiety behavior was found following intracerebroventricular administration of an MR antagonist. Interestingly, combined administration of MR and GR antagonists fails to influence fear-potentiated anxiety behavior, but it also fails to affect immobility behavior (Korte et al., 1995). It is likely that a different receptor state is produced by the MR antagonist and by the higher dose of metyrapone. Whereas GR occupation is unaffected by the MR antagonist, the higher dose of metyrapone probably deprives both MRs and GRs of the endogenous ligand corticosterone. Binding of deoxycorticosterone to both receptor types may be another reason to have produced the unexpected effect of the higher drug dose in the elevated plus-maze.

In sum, the findings of the present studies clearly indicated that lower and higher doses of metyrapone differentially affected learning, memory and emotional expressions. The findings are consistent with the assumption that metyrapone has a dose-dependent effect on

MR- and GR-mediated processes. This suggestion is also supported by the effects induced by posttraining injections of corticosterone and dexamethasone on retention of the water maze task. MRs and GRs are involved in different aspects of anxiety and fear-motivated behaviors. MRs are involved in the expression of fear in situations where the animal reacts with freezing behavior. GRs seem to be more involved in mechanisms underlying the generalization of anxiety. In the water maze, MRs have been shown to be involved in developing a behavioral strategy to search for the platform, whereas GRs are involved in the regulation of memory consolidation.

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