Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 729–736, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00203-7

Corticosterone Influences Forced Swim-Induced Immobility

MARISEL BÁEZ AND MARTA VOLOSIN[1]

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Sucursal 16, C.C. 61, 5016 Córdoba, Argentina

Received 20 November 1993

BÁEZ, M. AND M. VOLOSIN. *Corticosterone influences forced swim-induced immobility.* PHARMACOL BIOCHEM BEHAV **49**(3) 729–736, 1994.—The effect of corticosterone (CS) synthesis inhibition with metyrapone—a blocker of the 11 β-hydroxylase (150 mg/kg IP)—on immobility time during the forced swim test was recorded. Immobility time was measured during a 15-min forced swim (test). Twenty-four hours later rats were subjected to an additional 5 min forced swim (retest). In one experiment, metyrapone or vehicle was administered 3 h before the initial test, while CS (0, 5, 10, or 20 mg/kg SC) was administered 1 h prior to the initial test. Metyrapone significantly reduced immobility time during both test and retest. This effect was reverted in a dose-dependent fashion by CS. In a second experiment, animals exposed to the initial test 24 h before were injected with metyrapone or vehicle 3 h before the retest, while CS (0, 10, or 20 mg/kg SC) was administered 1 h prior the retest. Metyrapone, administered before the retest, reduced immobility time and CS partially reverted metyrapone effect. In another group of animals, serum CS concentrations were evaluated before and after test and retest. In vehicle groups, the high immobility time during test and retest was associated with high CS serum concentrations poststress. In animals receiving metyrapone prior to the initial test, the reduced immobility time was related to low levels of CS after the test and an attenuated secretion following the retest. Moreover, CS (20 mg/kg) and metyrapone + CS groups had high CS levels before the test, which remained high 2 h after the test, although after the retest, both groups showed a pattern of CS secretion similar to that observed in vehicle animals. These findings suggest that CS plays a critical role on the behavioral strategies adopted by rats when they are forced to face an aversive and inescapable stressful situation. Thus, behavioral immobility would require higher CS levels although active behavior would be related to low hormone concentrations.

Forced swim Immobility time Metyrapone Corticosterone Rats

STRESS is considered to be implicated in the ethiology of psychosomatic or psychiatric disorders such as hypertension, gastroduodenitis, immune suppression, neurosis, depression, among others (1,3,15,21,28,30,37,39,45). It is well known that the main neuroendocrine pathways, which are activated in the response of the organism to stress, are the neuronal and adrenomedullary branches of the sympathoadrenal system, as well as the pituitary-adrenocortical axis. Hormones released by both systems appear to play a role in the development of some of these disorders. For instance, adrenal catecholamines may be involved in the development of hypertension (29), cortisol appears to be a critical factor in immune suppression (1,8) and cortisol regulation seems to be abnormal in some subtypes of depression (17,27,42). Accordingly, laboratory investigations performed to study the influence of stress on serum levels of these hormones have proved to be useful in determining the potential role of these hormones in stress-induced illness. Particulary, the behavioral actions of glucocorticoids in animals are of clinical interest, because these hormones can produce striking affective and psychotic disturbances in humans, and elevated levels of cortisol have been reported in certain psychopathological conditions such as depression.

Most animal models of depression are based on the behavioral alterations induced by different inescapable stress paradigms. Although endocrinological outputs have been described in some reports (18,19,43), relatively few works (12,23,44) were done to investigate the functional relationship between hormones released by uncontrollable stressors and the behavioral strategies displayed under these stressful conditions. Therefore, it would be important to investigate whether corticosterone (CS) released during the aversive episode is functionally connected with the behavioral strategies adopted under this stressful situation. Thereby, the aim of the present investigation was to assess the potential CS participation in the behavioral performance during the exposure to an uncon-

[1] To whom requests for reprints should be addressed.

trollable stressor, the forced swim test. During this behavioral paradigm, rats gradually adopt an immobile posture during the initial exposure to this stressful situation (test). In a subsequent exposure to the same situation (retest), they spent most of the time in immobility (34,35). Accordingly, we studied the role of CS on the immobility behavior, by means of the administration of CS synthesis inhibitor, metyrapone, either prior to the test or the retest. An additional purpose was to evaluate if the behavioral effects produced by this pharmacological procedure could be related to different levels of CS secretion. Hence, the time course changes in serum CS concentration were determined in individual rats after their behavioral performance following the test and the retest.

METHOD

Animals and Housing

Male Wistar albino rats from our breeding stock, weighing 230–280 g, were housed in groups of six per cage with a 12 L : 12 D cycle (lights at 0700 h). Animal room temperature was maintained at 22 ± 2°C. The animals were placed in the home cages and spent at least 2 weeks in the animal room before testing. At the moment of test performance they were carried to the testing room with the same characteristics as the previous room as regards temperature, dimensions, and lighting conditions. Water and food were available at all times.

Apparatus

The forced swimming test was performed in a Plexiglas cistern (height 60 cm, diameter 40 cm) filled up with water up to a height of 30 cm., temperature being 23°C. The water level was adjusted in such a way that as the rat floated with the nose above the water its rear paws could not touch the bottom of the container.

Measurement of Immobility

The forced swimming test developed by Porsolt et al. (35) was used. Two swim sessions were conducted in the testing room. An initial 15-min test, in which immobility time was recorded in seconds during three periods, each lasting 5 min. These results are expressed in terms of cumulative periods. After the test, the animals were returned to the animal room and 24 h later, they were placed again in the cistern for 5 min to record immobility time (retest). A rat was judged to be immobile when it remained floating in the water without struggling or swimming and making only those movements necessary to keep its head above water. The water in the cistern was changed after each rat. After the test as well as after retest, rats were dried and put in a heated room (37°C) for 30 min. The person who did behavioral assessment was blind with regard to the treatment condition of each animal. All experiments were performed during the light period, between 0900 and 1500 h.

Experimental Procedure

Experiment 1: Effect of metyrapone and/or CS administration previous initial test on immobility behavior in the forced swimming test. Sixty-four animals were randomly assigned to one of eight groups (n = 8 per group). The eight groups comprised a 2 × 4 × 3 factorial design. Animals were given either metyrapone (150 mg/kg IP) or vehicle (propylene glycol 40% IP) 3 h before test and received either CS (5, 10, or 20 mg/kg SC) or corn oil vehicle (0.1 ml/kg) 1 h before the test. The metyrapone dose employed was 150 mg/kg because in preliminary experiments this dose remarkably attenuated the CS response to 15-min forced swim 3 h after metyrapone injection (Fig. 2a); under these conditions, CS values were comparable to vehicle basal levels. The duration in which animals were immobile during the test was recorded. Animals were subjected to the retest 24 h later, without drug administration.

Experiment 2: Effect of metyrapone and/or CS administration previous retest on immobility behavior in the forced swim test. Forty-eight animals already exposed to the initial test 24 h before were randomly divided into six groups. The six groups comprised a 2 × 3 factorial design. Three hours before retest, 24 animals were given metyrapone and the remaining 24 rats received vehicle. The animals that were injected with metyrapone received corn oil vehicle (n = 7), CS (10 mg/kg, n = 10), or CS (20 mg/kg, n = 7) 1 h before retest. The 24 remaining rats administered with vehicle were injected with corn oil vehicle (n = 8), CS (10 mg/kg, n = 8), or CS (20 mg/kg, n = 8) 1 h before the retest. Immobility time was recorded during the retest.

Experiment 3: Effect of metyrapone and/or CS administration previous initial test on immobility behavior and on serum CS levels. In this study, only one CS dose of 20 mg/kg was used. Rats were equipped surgically with a vinyl catheter (i.d. 0.58 mm; o.d. 0.96 mm) into the entrance of the right atrium (venae cava) via an external jugular venotomy and externalized on the top of the skull according to the techniques described by Steffens (41). Surgery was conducted under complete ether anesthesia. This method allows frequent withdrawal of small amounts of blood without disturbing the rat either behaviorally or physiologically (40,41). After surgery, the rats were housed individually in Plexiglas cages (size: 36 × 24 × 9.5 cm) with woodshavings and allowed to recover for at least 3 days before starting the experiment. During these days, animals were weighed every morning and the cannulas were kept permeable through daily applications of heparinized saline (100 IU/ml). In this way, the animals got used to the sampling procedure. Following the same experimental design as in Experiment 1, 33 heart-cannulated animals were randomly divided into four groups: vehicle (n = 12), metyrapone (n = 7), CS (n = 7), and metyrapone + CS (n = 7). The first blood samples were taken without heparine under baseline conditions in their home cages, in the animal room (−15 min). Afterwards, the rats were carried to the testing room, the immobility time during the test was recorded, and 10, 30, 60, and 120 min after the end of the session blood samples were taken using a balanced repeated measures within-subject design. The animals were subjected to the retest 24 h later, without drug administration, and blood samples were taken following the same timetable of the test. The 0.25 ml blood aliquots were placed in chilled (0°C) dry tubes and centrifugated (4°C) for 10 min at 3000 r.p.m. Serum was separated immediately after and stored at −30°C.

Experiment 4: Immobility time and changes in serum CS concentrations were measured in two 5-min forced swim periods. Eight heart cannulated rats without previous treatment were forced to swim during 5 min on day 1, and 24 h after (day 2) were reexposed to the forced swim episode for 5 min. The immobility time-recording procedures and blood sampling were similar to that described above.

Corticosterone Determination

Serum CS concentrations were determined in duplicate according to a modified protein-binding method (32). Briefly,

25 μl of serum and 75 μl of distilled water were placed in a boiling water recipient for 90 s; after cooling, the samples were incubated with a corticosteroid-binding globuline tracer solution [2% horse serum containing 1,2,6,7-^{3}H-corticosterone (88 Ci/mmol; NEN Chemicals) as tracer]. Unbound steroid was removed using Florisil (Mesh: 60-100-Sigma). Standard CS was supplied by Sigma. The intra- and interassay coefficients of variation were less than 10%, respectively. All the samples that belonged to one same animal were assessed in the same assay. The serum CS concentrations are expressed in terms of μg/dl.

Statistical Analysis

The results were expressed as means ± SEM. Data were analyzed by means of a one-, two-, or three-way analysis of variance (ANOVA), depending on the study. Post hoc group comparisons were conducted using the Fisher's least significant difference test, with an alpha set at 0.05.

RESULTS

Experiment 1

Table 1 shows the effect of metyrapone on immobility time during the test. The animals injected with vehicle displayed a progressive increase in immobility time during the three periods assessed, $F(2, 112) = 958.53$, $p < 0.0001$. Metyrapone administration significantly reduced the immobility time as compared with the vehicle animals in all the times evaluated [metyrapone: $F(1, 56) = 50.26$, $p < 0.0001$; metyrapone × time: $F(2, 112) = 10.59$, $p < 0.0001$]. In metyrapone-pretreated animals CS administration dose dependently blocked the suppression of immobility time (Table 1) [doses: $F(3, 56) = 5.82$, $p < 0.001$; doses × time: $F(6, 112) = 4.379$, $p < 0.0005$]. Thus, although CS per se did not show any significant effect in the three doses, the 5 and 10 mg/kg doses only partially reverted the metyrapone effect during the three periods studied. However, in none of the three periods assessed did the immobility time reach the values of vehicle- and CS-treated animals. Besides, except during the first 5 min, the metyrapone effect was totally reverted by a previous CS injection of 20 mg/kg.

TABLE 2

EFFECT OF METYRAPONE ON IMMOBILITY TIME DURING A 5-MIN FORCED SWIM (RETEST)

Treatment	Immobility Time
Vehicle	256.65 ± 15.63
Metyrapone	159.00 ± 12.53*
CS (5)	233.00 ± 5.48
Metyrapone + CS (5)	200.87 ± 16.63†
CS (10)	253.37 ± 6.82
Metyrapone + CS (10)	252.50 ± 8.22
CS (20)	220.37 ± 13.90
Metyrapone + CS (20)	238.75 ± 7.82

Immobility time was recorded during the retest on the same animals that were subjected to the initial test (Table 1). Results are expressed in seconds. Values represent mean ± SEM ($n = 8$).

*$p < 0.01$ metyrapone vs. vehicle, metyrapone + CS(5), metyrapone + CS(10) and metyrapone + CS(20).

†$p < 0.01$ metyrapone + CS(5) vs. metyrapone + CS(10) and metyrapone + CS(20).

During the retest, vehicle animals remained 80–85% of the time in immobility, whereas the metyrapone treated subjects showed a significant decrease in immobility time, $F(1, 56) = 11.67$, $p < 0.001$, (Table 2). ANOVA revealed a significant effect to the CS doses, $F(3, 56) = 5.70$, $p < 0.001$, and a significant interaction effect of metyrapone × CS doses, $F(3, 56) = 9.57$, $p < 0.001$. Further analyses revealed that the three doses reverted metyrapone effect on immobility time, though only the 10 and 20 mg/kg doses reached levels similar to vehicle animals (Table 2).

Experiment 2

Figure 1 shows the immobility times of animals that were administered with metyrapone 3 h before the retest. All these animals were submitted to an initial 15 min test 24 h before. The vehicle rats as well as the animals that received only CS remained 75–85% of the time in immobility. On the contrary,

TABLE 1

EFFECT OF METYRAPONE ON IMMOBILITY TIME DURING THE INITIAL 15-MIN FORCED SWIM (TEST)

	Immobility time		
Treatment	5 min	10 min	15 min
Vehicle	161.62 ± 12.56	402.75 ± 14.71	632.5 ± 33.18
Metyrapone	50.75 ± 9.18*	179.37 ± 27.34*	368.87 ± 48.12*
CS (5)	160.25 ± 14.19	340.25 ± 19.62	522.75 ± 42.09
Metyrapone + CS (5)	75.12 ± 5.50†	199.37 ± 20.12†	348.00 ± 46.46†
CS (10)	166.25 ± 5.93	409.25 ± 17.16	656.12 ± 33.27
Metyrapone + CS (10)	105.50 ± 10.25‡	275.00 ± 35.08‡	507.00 ± 46.46‡
CS (20)	181.12 ± 13.72	389.00 ± 35.06	626.87 ± 49.22
Metyrapone + CS (20)	125.00 ± 16.46§	326.86 ± 29.68	556.00 ± 53.99

Animals were metyrapone (150 mg/kg IP) and/or CS (5-10-20 mg/kg SC) administered 3 and 1 h before initial test, respectively. Results are expressed in seconds in cumulative periods. Values represent mean ± SEM ($n = 8$).

*$p < 0.01$ metyrapone vs. vehicle (5, 10, and 15 min).

†$p < 0.01$ metyrapone + CS(5) vs. CS(5) (5, 10, and 15 min).

‡$p < 0.01$ metyrapone + CS(10) vs. CS(10) (5, 10, and 15 min).

§$p < 0.01$ metyrapone + CS(20) vs. CS(20) (5 min).

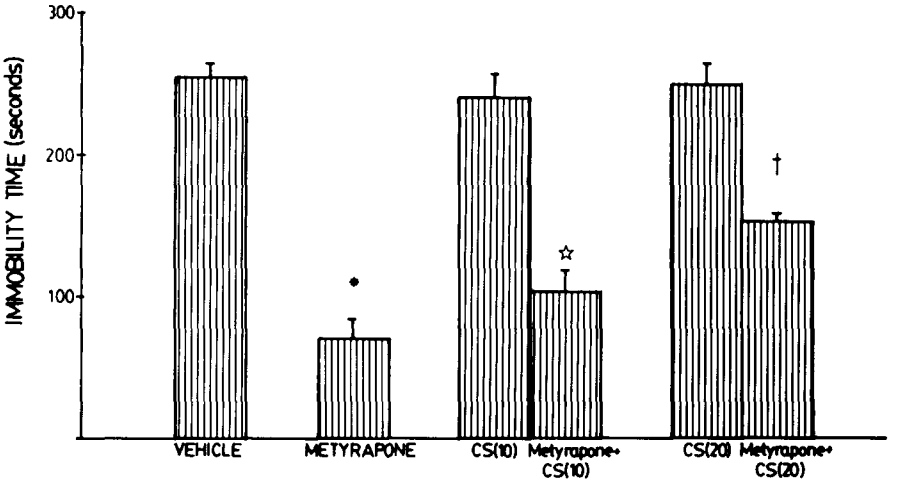

FIG. 1. Effect of metyrapone and/or CS administration before retest on immobility time. All the animals were subjected to test and injected with metyrapone (150 mg/kg IP) and/or CS (10–20 mg/kg SC) 3 and 1 h before retest, respectively. (n = 7–10) *$p < 0.01$ metyrapone vs. vehicle, CS (10) and CS (20). ☆$p < 0.01$ metyrapone + CS(10) vs. CS(20). †$p < 0.01$ metyrapone + CS(20) vs. CS(20) and metyrapone. Values represent mean ± SEM.

metyrapone treated animals showed a significant decrease in immobility time, $F(1, 42) = 197.07, p < 0.0001$. This metyrapone effect was partially reverted by CS administration. [CS: $F(2, 42) = 4.48, p < 0.01$; interaction metyrapone × CS: $F(2, 42) = 6{,}21, p < 0.004$].

Experiment 3

Figure 2a shows the time course changes in serum CS concentrations before (basal) and after test. Three-way ANOVA for repeated measures between blocks showed that CS effect and metyrapone × CS interactions were significant [CS: $F(1, 29) = 82.9, p < 0.001$; interaction metyrapone × CS: $F(1, 29) = 26.97, p < 0.001$]. In addition, within-blocks ANOVA reveled a significative effect of time, $F(4, 116) = 12.24, p < 0.001$, as well as metyrapone × time, $F(4, 116) = 3.92, p < 0.005$, CS × time, $F(4, 116) = 2.64, p < 0.03$, and metyrapone × CS × time, $F(4, 116) = 5.29, p < 0.001$.

Subsequent post hoc analyses revealed that the vehicle group basal CS concentrations increased 10, 30, and 60 min after the end of the test, decreasing to basal values after 120 min. In contrast, previous metyrapone administration did not alter the basal levels as compared with vehicle animals but, as may be expected, induced low and stable steroid concentra-

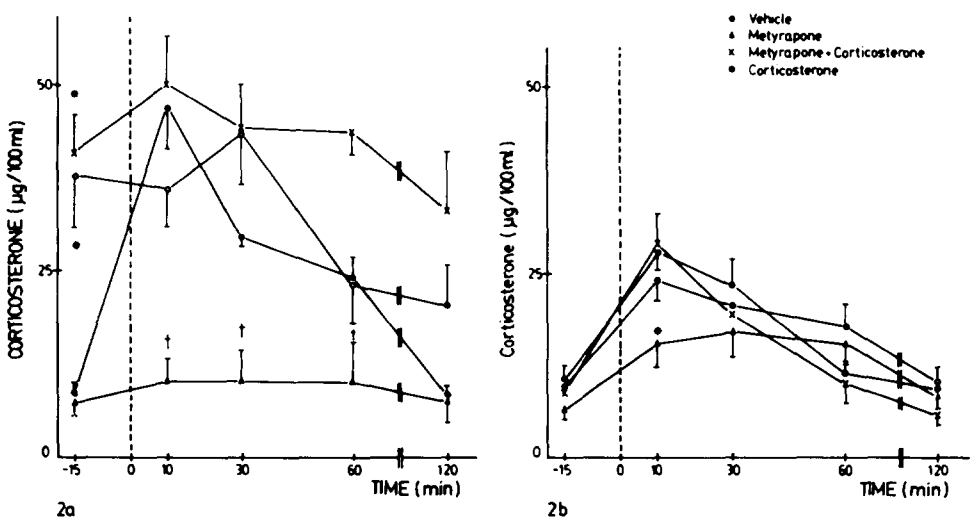

FIG. 2. Time course of changes in serum corticosterone concentrations after test (2a) and retest (2b) in forced swim test (n = 7–12). Zero indicates the start of 15 min test and 5 min retest. Values represent mean ± SEM. (a) t: −15 min *$p < 0.01$ CS and metyrapone + CS vs. vehicle; t: 10, 30, 60 min †$p < 0.01$ metyrapone vs. vehicle. (b) t: 10 min * $p < 0.01$ metyrapone vs. vehicle, CS and metyrapone + CS.

tions throughout the whole period evaluated. CS administered rats as well as metyrapone + CS animals exhibited elevated basal CS levels. Both groups showed similar posttest (t: 10 and 30 min) CS serum concentrations as compared with vehicle animals (t: 10 and 30 min). The decrease in hormonal levels in rats administered with CS alone was quite similar to vehicle animals, whereas in metyrapone + CS groups a 30 min fall delay was observed.

Figure 2b shows CS basal levels and time course changes after retest. ANOVA revealed a significant effect of time, $F(4, 116) = 35.99$, $p < 0.001$, as well as metyrapone × CS × time interaction, $F(1, 116) = 4.92$, $p < 0.0001$. Subsequent analyses showed that vehicle animals as well as CS and metyrapone + CS rats displayed similar patterns of CS responses. Thus, in all three groups, CS peaked 10 min after the end of the retest followed by a gradual declination (t: 30 min), going back to their basal levels between t: 60 and t: 120 min. On the other hand, though, in metyrapone group, basal CS levels showed no significant difference as compared with the remaining groups, the increase in serum CS concentrations postretest was significantly reduced at t: 10 min. Following, such levels remained stable until 60 min postretest, going back to their basal levels at t: 120 min.

The four groups showed immobility times similar to previous experiment ones in both test and retest (data not shown).

Experiment 4

To compare the behavioral and endocrine responses assessed with the ones from animals preexposed to an aversive stimuli of the same nature but minor duration, an experimental design was drawn up in which rats were subjected to two forced swim episodes of 5 min each on days 1 and 2. Immobility time and serum CS concentrations were similar on day 1 and day 2 (Fig. 3a and b).

DISCUSSION

The present article shows that the administration of CS synthesis inhibitor, metyrapone, before the test induced a remarkable reduction in immobility time during the initial exposure to the swim task. This effect was dose dependently reversed by CS, reaching a significant difference following the 20 mg/kg dose. At this dose, steroid levels were similar to those observed in poststress vehicle animals (Fig. 2a). Based on these results, we can suggest that the reduced immobility induced by metyrapone may be due to the inhibition in the increased CS secretion, provoked by this stressful situation.

This evidence contradicts early findings reported by Jefferys et al. (23) and Veldhuis et al. (44), who observed that short-term bilateral adrenalectomy did not alter immobility during the test. However, it can be noted that in the latter study (44), though not significant, the duration of immobility during the initial swimming exposure in adrenalectomized rats was lower than that observed in sham animals. Furthermore, in more recent studies (12), the intracerebroventricular administration of the antiglucocorticoid RU 38486 to intact rats prior to the test did not alter the immobility behavior during the test. However, following central or systemic RU 38486 administration, a disinhibition of the pituitary-adrenal system occurs (12). Thus, the consequent rise in serum CS levels may well counteract the effectiveness of the antagonist in pituitary and brain, and in this way it could explain the reason why the authors were unable to observe an effect of the antiglucocorticoid on immobility behavior.

In agreement with early reports (34,35), in the retest, control rats spent about 70–80% of 5-min period immobile. On the contrary, animals treated with metyrapone prior to the test showed a clear reduction in immobility time during retest, parallel to their performance during the previous exposure to the swim task. The combined administration of CS + metyrapone before the test reversed the decreased immobility observed in the retest in metyrapone-treated rats. This evidence supports the notion that CS is functionally involved in the immobility induced by forced swim. In addition, the behavior displayed during the retest is related to the immobility rate performed in the previous session. Thus, rats injected with metyrapone prior to the test exhibited reduced immobility in the subsequent exposure and, in the same way, CS + metyrapone rats showed similar immobility scores to that observed in control rats. Based on these data, we can claim that CS is critically involved in the immobility induced by the forced swim test, which leads to the behavioral expression observed during retest.

When CS synthesis inhibition took place before the second

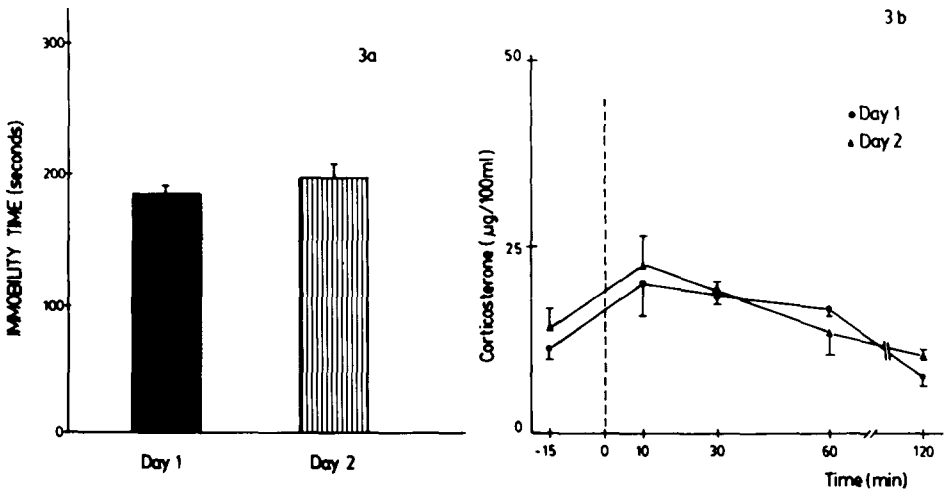

FIG. 3. Immobility time (a) and (b) dynamics of serum CS concentrations in naive animals submitted to two forced swim 5 min periods on day 1 and day 2. ($n = 6$). Values represents mean ± SEM.

exposure to the swim task (retest), in animals without treatment prior to the test, a reduction in their immobility performance was observed. These data confirm that CS is particulary involved in this inactive behavior. However, under these conditions, an additional component (e.g., opioid peptides) may be involved, because the metyrapone effect was only partially reversed by CS (22).

The behavioral effect described following metyrapone was not secondary to a stimulating effect of this drug on motor activity, because, as has been reported, a sedative effect during the first hour postadministration was observed, and no impairment in motor coordination was evident 3 h postadministration (26).

On the other hand, metyrapone increases ACTH secretion owing to lack of glucocorticoids feedback. This peptide hormone, besides its well-known endocrine actions, provokes different behavioral changes (14). In addition, the neurotrophic peptide $ACTH_{4-10}$, a fragment of ACTH, allows the expression of the glucocorticoid effect on immobility behavior of hypophysectomized rats during retest (13). Therefore, the effect observed in our experiments could be related to this peptide hormone influence. However, and although it cannot be fully discarded, this possibility seems unlikely because CS administration reverses metyrapone effect. Moreover, a comparable effect was also observed, employing the same experimental design, following the administration of the synthetic glucocorticoid dexamethasone (unpublished observation).

The evaluation of CS serum concentration changes in response to the forced swim task supports the steroid participation in the behavioral expression of immobility. Thus, the dynamics of serum CS concentrations was related to the behavioral response displayed. That is to say, the posttest serum CS concentrations were elevated in vehicle, CS, and metyrapone + CS-treated animals, which have had high immobility score during the test. The subsequent exposure to the swim task (retest) elicited a similar pattern of CS secretion among vehicle, CS, and metyrapone + CS-pretreated animals. On the contrary, animals that were pretreated with metyrapone exhibited an attenuated endocrine secretion pattern during the retest. Therefore, and in support of a potential relationship between CS secretion and behavioral response, vehicle, CS, and metyrapone + CS rats displayed higher immobility in the retest than metyrapone-pretreated animals. So, under these experimental conditions, behavioral immobility would require higher CS levels while active behavior would be related to low hormone concentrations.

As previously reported, the magnitude of CS secretion as a response to a stressful event depends on the intensity, frequency, and duration of the stimulus, and can be modified by the previous exposure to the same event (2,4,6,10,11,20,24, 25,33). Our paradigm shows that, in vehicle-treated rats, the intensity and duration of CS secretion after the retest (Fig. 2b) were lower than that observed following the test session (Fig. 2a). The attenuation in the secretion of the hormonal response after retest in these rats could be attributed either to an adaptive phenomenon to the homotipic stressor, to the reduced duration of the retest as compared to the previous session, or to both factors. Animals submitted to forced swim sessions for 5 min on 2 consecutive days did not display difference in immobility time, and showed a similar and low CS poststress response between both 5-min periods. Therefore, the higher CS secretion in response to the retest session could be, in fact, related to the previous experience to the 15-min initial test. However, the comparison between the CS secretion in response to the initial exposure to the test (15 min) and to initial 5-min exposure (day 1) showed that the longer duration of the aversive experience also influences the CS secretion. It is also important to point out that rats submitted to initial exposure for longer periods of forced swim display behavioral immobility, which is absent when they were initially exposed to a 5-min period only. Hence, we can conclude that behavioral immobility is produced by longer periods of exposure to an inescapable stimulus which, in turn, would induce a sustained increase in the adrenocortical outflow. Furthermore, the behavioral responses displayed by naive animals in 5 min of forced swim on 2 consecutive days were comparable to that obtained during the 5 min of the retest in animals injected with metyrapone before the test. These findings reinforce the notion that active behaviors are functionally related to lower serum CS concentrations.

In conclusion, the analysis of the behavioral and endocrine results obtained from this paradigm showed that CS plays a critical role on the behavioral strategies adopted by rats when they are forced to face a long-duration inescapable stressful situation. Thus, CS secretion in response to these aversive situations probably facilitates the onset of passive behavior, which has relevance in the behavior performed in a subsequent exposure to this aversive stimuli. In addition, under these conditions, CS also seems to be implicated in this inactive behavior.

It has been postulated that the glucocorticoids mediate some nervous system responses to repeated stress, and that these responses could be involved in the behavioral adaptation to such aversive situations. However, there is evidence showing that even under a repeated stress regime a persistent CS secretion blocks both the development of adaptive behavior and the neurochemical changes that underlie it (26). Besides, rat exposure to a chronic variable stress regime, a model of depression with a high validity degree (46) induces passive behavior through successive exposure to various stressors (16) and also a very remarkable adrenal response after the exposure to a novel stimuli (5). Moreover, preliminary evidence from this laboratory has shown that CS is involved in analogous behavioral expression, such as inactive behavior during long-term inescapable shock, considered as a valid index of experimental depression (31).

These evidences suggest that animal adaptation to certain stressful situations may depend, at least in part, on an adequate balance between CS secretion and other responses to stress; such balance would depend not only on the nature, severity, and duration of stressant stimuli, but also on the strategies displayed by the subject when confronted with the stressor.

A similar relationship could be involved in human disorders precipitated by stress, such as depression. The high cortisol levels in rest of postdexamethasone, which are present in a high percentage of depressive patients, could be associated to some of the core symptoms of clinical depression.

The present experiment outlines the importance of the analysis of neuroendocrine variables, not in an exclusive manner but associating it to the behavioral and neurochemical changes present in this models. This view would provide more validity to these paradigms, and as can be considered as an approach for further understanding of the neural and endocrine mechanisms involved in this behavior and the reversal of these changes following antidepressants.

ACKNOWLEDGEMENTS

This work was supported by grants from Conicor Conicet and A. J. Roemmers Fundation, Argentina. Metyrapone was kindly donated by Laboratorio Ciba-Geigy (Argentina). The authors are grateful to Gabriela Bazán for her assistance with technical English.

REFERENCES

1. Ader, R. (ed.). Psychoneuroimmunology. New York: Academic Press; 1981.
2. Ader, R.; Friedman, S. B. Plasma corticosterone response to environmental stimulation: Effects of duration of stimulation and the 24-hour adrenocortical rhythm. Neuroendocrinology 3:378–386; 1968.
3. Anisman, H.; Zacharko, R. M. Depression: The predisposing influence of stress. Behav. Brain Sci. 5:89–137; 1982.
4. Armario, A.; Montero, J. L.; Balasch, J. Sensitivity of corticosterone and some metabolic variables to graded levels of low intensity stresses in adult male rats. Physiol. Behav. 37:559–561; 1986.
5. Armario, A.; Restrepo, C.; Castellanos, J. M; Balasch, J. Dissociation between adrenocorticotropin and corticosterone responses to restraint stress after previous chronic exposure to stress. Life Sci. 36:2085–2092; 1985.
6. Borrell, J.; Torrellas, A.; Guaza, C.; Borrell, S. Sound stimulation and its effects on the pituitary-adrenocortical function and brain catecholamines in rats. Neuroendocrinology 31:53–59; 1980.
7. Carrol, B. J. Hypothalamic-pituitary function in depresive illness: Insensitivity to hypoglycaemia. Br. Med. J. 3:27–28; 1969.
8. Cunnick, J. E.; Lysle, D. T.; Kuciniski, B. J.; Rabin, B. S. Evidence that shock-induced immune supression is mediated by adrenal hormones and peripheral β-adrenergic receptors. Pharmacol. Biochem. Behav. 36:645–651; 1990.
9. Davies, B.; Caroll, B. J.; Mowbray, R. M. Depresive illness. Springfield, IL: Charles C. Thomas; 1972.
10. Davis, M. Effects of interstimulus interval length and variability on startle-response habituation in the rat. J. Comp. Physiol. Psychol. 72:177–192; 1970.
11. De Boer, S. F.; Slangen, J. L.; Van Der Gugten, J. Adaptation of plasma catcholamine and corticosterone responses to short-term repeated noise stress in rats. Physiol. Behav. 44:273–280; 1988.
12. De Kloet, E. R.; De Kock, S.; Schild, V.; Veldhuis, H. D. Antiglucocorticoid RU 38486 attenuates retention of a behaviour and disinhibits the hypothalamic-pituitary adrenal axis at different brain sites. Neuroendocrinology 47:109–115; 1988.
13. De Kock, S.; De Kloet, E. R. Neurotrophic peptide ACTH (4–10) permits glucocorticoid-facilitated retention of acquired immobility response of hypophysectomized rats. Eur. J. Pharmacol. 141: 461–466; 1987.
14. Dunn, A. J. Effects of ACTH, β-lipotropin, and related peptides on the central nervous system. In: Nemeroff, C. B.; Dunn, A. J., eds. Peptides, hormones and behavior. New York: Spectrum Publications; 1984:273–348.
15. Galosy, R. A.; Clarke, L. K.; Vasko, M. R.; Crawford, I. L. Neurophysiology and neuropharmacology of cardiovascular regulation and stress. Neurosci. Biobehav. Rev. 5:137–175; 1981.
16. García Márquez, C.; Armario, A. Chronic variable stress depresses exploratory activity and behavioral performance in the forced swimming test without altering ACTH response to a novel stressor. Physiol. Behav. 40:33–38; 1987.
17. Gold, P. W.; Loriaux, L.; Roy, A.; Kling, M. A.; Calabrese, J. R.; Kellner, C. H.; Nieman, L. K.; Post, R. M.; Pickar, D.; Galucci, W. Avgerinos, P.; Paul, S.; Oldfield, E. H.; Cutler, G. B.; Chrousos, G. P. Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing disease. N. Engl. J. Med. 314:1329–1334; 1986.
18. Greenberg, L.; Edwards, E.; Henn, F. A. Dexamethasone suppression test in helpless rats. Biol. Psychiatry 26:530–532; 1989.
19. Haracz, J. L.; Minor, T. R.; Wilkins, J. N. Zimmermann, E. M. Learned helplessness: An experimental model DST in rats. Biol. Psychiatry 23:388–396; 1988.
20. Hennessy, M. B.; Heybach, J. P.; Vernikos, J.; Levine, S. Plasma corticosterone concentration sensitively reflect levels of stimulus intensity in the rat. Physiol. Behav. 22:821–825; 1979.
21. Henry, J. P.; Meehan, J. P.; Stephens, P. M. The uses of psychosocial stimuli to induce prolong systalic hypertension in mice. Psychosom. Med. 29:408–432; 1967.
22. Jefferys, D.; Copolov, D.; Funder, J. W. Naloxone inhibits both glucocorticoid and [D-Ala^2, Met^5] enkephalinamide reversal of behavioral effect of adrenalectomy. Eur. J. Pharmacol. 103:205–210; 1984.
23. Jefferys, D.; Copolov, D.; Irby, D.; Funder, J. W. Behavioral effect of adrenalectomy: Reversal by glucocorticoids or [D-Ala^3-Met^5] enkephalinamide. Eur. J. Pharmacol. 92:99–104; 1983.
24. Kant, G. J.; Eggleston, T.; Landman-Roberts, L.; Kenion, C. C.; Driver, G. C.; Meyerhoff, J. L. Habituation to repeated stress in stressor specific. Pharmacol. Biochem. Behav. 22:631–634; 1985.
25. Kant, G. J.; Bunnell, B. N.; Mougey, E. H.; Pennington, L. L.; Meyerhoff, J. L. Effects of repeated stress on pituitary cyclic AMP, and plasma prolactin, corticosterone and growth hormone in male rats. Pharmacol. Biochem. Behav. 18:967–971; 1983.
26. Kennett, G. A.; Dickinson, S. L.; Curzon, G. Central serotonergic responses and adaptation to repeated immobilisation: The effect of the corticosterone synthesis inhibitor metyrapone. Eur. J. Pharmacol. 119:143–152; 1985.
27. Lamberts, S. W. J. Neuro-endocrine aspects of the dexamethasone suppression test in psychiatry. Life Sci. 39:91–95; 1986.
28. Maier, S. F.; Seligman, M. E. P. Learned helplessness: Theory and evidence. J. Exp. Psychol. [Gen.] 105:3–46; 1976.
29. Mc Carty, R.; Kopin, I. J. Alterations in plasma catecholamines and behavior during acute stress in spontaneously hypertensive and Wistar-Kyoto normotensive rats. Life Sci. 22:997–1006; 1978.
30. Murison, R.; Overmier, J. B.; Skoglund, E. J. Serial stressors: Prior exposure to a stressor modulates its later effectiveness on gastric ulcerations and corticosterone release. Behav. Neural Biol. 45:185–195; 1986.
31. Murúa, V. S.; Molina, V. A. Antidepressants reduce inactivity during both inescapable shock administration and shuttle-box testing. Eur. J. Pharmacol. 204:187–192; 1991.
32. Murphy, B. E. P. Some studies of the protein-binding of steroids and their application to the routine micro- and ultramicro measurement of various steroids in body fluids by competitive protein-binding radio assay. J. Clin. Endocrinol. Metab. 27:973–983; 1967.
33. Natelson, B. H.; Creighton, D.; Mac. Carty, R.; Tapp, W. N.; Pitman, D.; Ottenweller, J. E. Adrenal hormones indices of stress in laboratory rats. Physiol. Behav. 39:117–125; 1987.
34. Porsolt, R.; Anton, G.; Blavet, N.; Jalfre, M. Behavioral despair in rats: A new model sensitive to antidepressant treatment. Eur. J. Pharmacol. 47:379–391; 1978.
35. Porsolt, R. D.; Le Pichon, M.; Jalfre, M. Depression: A new animal model sensitive to antidepressant treatments. Nature 266: 730–732; 1977.
36. Rubin, R. T.; Mandell, A. J. Adrenal cortical activity in pathologic emotional state: A review. Am. J. Psychiatry 123:387–400; 1966.
37. Sachar, E. J. Hormonal changes in stress and mental illness. In: Krieger, D. T.; Hughes, J. C., eds. Neuroendocrinology. Sunderland, MA: Sinauer Assoc.; 1980:177–184.
38. Sachar, E. J. Corticosteroids in depressive illness. I. A reevaluation of control issues and the literature.II. A longitudinal psychoendocrine study. Arch. Gen. Psychiatry 17:544–553, 554–567; 1967.
39. Seyle, H. Stress in health and disease. Boston: Butterworths; 1976.
40. Steffens, A. B.; Van der Gugten, J.; Godeke, J.; Luiten, P. G. M.; Strubbe, J. H. Meal-induced increases in parasympathetic and sympathetic activity elicit simultaneous rises in plasma insulin and free fatty acids. Physiol. Behav. 37:119–122; 1986.
41. Steffens, A. B. A method for frequent sampling blood and continuous infusion of fluids in the rat without disturbing the animal. Physiol. Behav. 4:833–836; 1969.
42. Stokes, P. E.; Sikes, C. R. Hypothalamic-pituitary-adrenal axis in affective disorders. In: Meltezer, H. Y., ed. Psychopharmacology: The third generation on progress. New York: Raven Press; 1987:589–607.
43. Swenson, R. M.; Vogel, W. H. Plasma catecholamine and corti-

costerone as well as brain catecholamine changes during coping in rats exposed to stressful foot shock. Pharmacol. Biochem. Behav. 18:689–693; 1983.
44. Veldhuis, H. D.; De Korte, C. C. M. M.; De Kloet, R. Glucocorticoids facilitate the retention of acquired immobility during forced swimming. Eur. J. Pharmacol. 115:211–217; 1985.
45. Weiss, J. M.; Goodman, P. A.; Losito, B. G.; Corrigan, S.; Charry, J. M.; Bailey, W. H. Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine and serotonin levels in various regions of rat brain. Brain Res. Rev. 3:167–205; 1981.
46. Willner, P. The validity of animal models depression. Psychopharmacology (Berlin) 83:1–16; 1984.