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Situation Specific Effects of Stressor Controllability on Plasma Corticosterone Changes in Mice

CHRISTOPHER R. PRINCE AND HYMIE ANISMAN[1]

Department of Psychology, Carleton University, Ottawa, Ontario, Canada

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PRINCE, C. R. AND H. ANISMAN. *Situation specific effects of stressor controllability on plasma corticosterone changes in mice.* PHARMACOL BIOCHEM BEHAV **37**(4) 613–621, 1990.—The immediate and proactive effects of controllable and uncontrollable stressors on plasma corticosterone were assessed in CD-1 mice. A progressive increase of plasma corticosterone concentrations was associated with graded increases in stressor severity. When a footshock stressor was employed, however, the magnitude of the glucocorticoid response, as well as the decay of plasma corticosterone concentrations, was independent of stressor controllability. This was the case regardless of the number of escapable vs. yoked inescapable shock trials mice received, the spacing of shock trials (i.e., applied within a single session or spaced over days), or the degree to which the escape response had been established. In contrast, in a swim task stressor controllability influenced plasma corticosterone concentrations provided that the escape response required of the animal was a highly prepared one (i.e., swim to an illuminated region). When mice were required to emit a contraprepared response (swim to dark) corticosterone concentrations did not differ between escapable and inescapable swim. It is suggested that glucocorticoid secretion is a fundamental response to stressors, and the differential effects of controllable and uncontrollable stressors will be most apparent when the response required of the animal is a highly prepared one.

Stressor Controllability Preparedness Corticosterone

CONTROLLABLE and uncontrollable stressors differentially influence subsequent performance in a wide range of behavioral paradigms (26, 39, 40), and differentially influence the turnover and levels of central norepinephrine (NE) and serotonin (5-HT) (39,40), as well as some components of the immune response (9). While stressors have also been reported to engender marked increases of plasma corticosterone concentrations in several species (32), the contribution of stressor controllability is less clear. Uncontrollable stressors were shown to produce a greater increase of plasma corticosterone than controllable stressors in rats (26, 35, 38), dogs (12) and rhesus monkeys (15). Moreover, in avoidance/escape paradigms, the extent of the corticosterone rise was reduced once rats mastered the response-outcome contingencies (7,10).

In contrast to these earlier reports, however, it was reported (25) that parameters of controllable and uncontrollable tail shock which differentially influence later performance in a shock escape task, produced comparable alterations of plasma corticosterone and ACTH in rats. In accordance with these findings, it has been observed (37) that the corticosterone rise in rats trained in a Sidman avoidance task was comparable to that of yoked rats. This was the case irrespective of whether animals received 3, 6 or 21 h of training, despite the fact that after 21 h of training the response was well established and permitted the expression of differences in NE utilization between controllably and uncontrollably shocked animals. Interestingly, if rats received 5 days of prior avoidance training, then subsequent exposure to 3 h of controllable shock resulted in a smaller increase of plasma corticosterone relative to the yoked condition.

Taken together, it appears that there are conditions wherein controllable and uncontrollable shock may differentially influence plasma corticosterone concentrations. Yet, the data reported by Maier et al. (25) and Tsuda and Tanaka (37) indicate that those parameters of stressor controllability which differentially influence behavior and central catecholamine activity are distinct from those which affect corticosteroid concentrations. As such, these data suggest that the corticosterone alterations may be unrelated to many of the behavioral, as well as the NE changes induced by stressors.

Unlike the relatively large number of investigations that have assessed the role of stressor controllability on plasma corticosterone in rats, scant information is available concerning the effects of this variable in mice, although stressors reliably increase corticosterone in this species (33). One purpose of the present investigation was to determine the effects of controllable and uncontrollable shock on plasma corticosterone in mice, employing parameters which differentially affect shock escape performance and central catecholamine activity. Inasmuch as corticosterone secretion may

[1]Requests for reprints should be addressed to Hymie Anisman.

be dependent on the stressor parameters employed, as well as the degree to which a response was established, the glucocorticoid response was assessed in mice that received either limited or extensive training. Furthermore, it was considered that the effectiveness of a controllable stressor in eliciting a corticosterone response might be dependent upon the preparedness of the response required of the animal. Accordingly, plasma corticosterone concentrations were also assessed under conditions where the response required of the animal was a highly prepared or contraprepared one.

EXPERIMENTS 1–4

The initial series of experiments was conducted to establish whether various amounts of escapable and yoked inescapable footshock would differentially influence plasma corticosterone concentrations, and whether stressor controllability would proactively influence the response to a subsequently applied stressor. Since both the magnitude and decay of the corticosterone response may be influenced by stressors (33,35), in most of the studies of the present investigation plasma corticosterone concentrations were determined at various times following stressor termination. Experiments 1–3 assessed the effects of controllable and uncontrollable footshock on plasma corticosterone concentrations as a function of the amount and regimen of training mice received. Experiment 4 evaluated whether controllable and uncontrollable footshock in mice would differentially influence the glucocorticoid response elicited by a subsequently applied stressor, although earlier studies had revealed that this was not the case in rats (12,25).

METHOD

Subjects

Experiments 1–4 involved 90, 105, 50, and 155 naive, male CD-1 mice, respectively. Mice were procured from Charles River (Canada), St. Constant, Quebec at 55–60 days of age. Upon arrival in the laboratory, mice were housed in groups of five in standard opaque polypropylene cages and maintained on a 12-h day-night cycle (lights on from 0800–2000) with ad lib access to food and water. Mice served as experimental subjects following a 7–14-day acclimatization period to the laboratory environment. All stress procedures, testing, and the sacrificing of animals were conducted between 0830 and 1430 h.

Apparatus

Escape training was conducted in three identical Plexiglas shuttle boxes, $26.4 \times 9.0 \times 15.5$ cm. The floor of each box was composed of 0.32 cm stainless steel rods spaced 1.0 cm apart (centre to centre). The rods, as well as stainless steel plates that lined the end walls of each chamber, were connected in series with neon bulbs. The roofs of the boxes consisted of 0.63 cm red Plexiglas which reduced the amount of light entering the chamber. Shock was delivered to the grid floor from a 3,000 V source, thereby providing relatively constant current.

Each shuttle box was divided into two compartments by a stainless steel gate. When the gate was open a 1.27 cm hurdle separated the two compartments, and a 7.0×7.7 cm space permitted access to the adjacent compartment. Situated 1.1 cm on either side of the partition were two infrared photodetector units, 1.0 and 4.0 cm above the grid floor. The photodetectors were wired such that if the beams on both sides were crossed simultaneously, which might occur when the mouse was halfway across the partition, the cells would not trigger. Only when the mouse crossed the beam in the shock compartment and broke the beam on the safe side, did the cells trigger. The shuttle boxes, which were maintained in sound-attenuating chambers, were operated by a microcomputer system constructed at the Carleton University Science Workshops.

Procedure

In Experiment 1 mice were assigned to one of three conditions. Mice of one group were placed individually in the shuttle boxes and received 360 escape trials. On each trial, shock was presented for 1.5 s (150 μA, 60 Hz, AC) after which the gate separating the two compartments opened, thus permitting escape. This prodecure was employed to ensure that mice received approximately 2 s of shock on each trial, a procedure which has previously been shown to produce reliable behavior deficits in several paradigms, as well as alterations of NE levels and turnover (40). The maximum amount of shock presented on any given trial was 24 s, and the interval between each trial was 9 s. Mice of the second group were placed individually in the shuttle boxes and exposed to 360 shocks over which behavioral control was not possible (i.e., yoked condition). For these animals shock onset occurred at exactly the same time as it did for mice in the escapable group. Shock offset, however, occurred when their respective partners successfully escaped from shock. Thus, both escapably and inescapably shocked mice received identical amounts of shock at exactly the same time, but only mice in the escapable condition could terminate shock by making the appropriate response. Mice in the third group served as the nonshocked control condition. Mice were placed individually in the shuttle boxes for periods that matched the escapable and inescapable shock groups, but shock was not delivered.

Following the shock treatment mice were returned to their cages and then decapitated either immediately, 15, 30, 60, or 180 min later ($n = 6$/group). Trunk blood was collected, centrifuged, and the plasma samples stored at $-70°C$ until plasma corticosterone concentrations were assessed fluorometrically using a modification of the method of Givner and Rochefort (14). Owing to the possibility that circadian variability might influence corticosterone concentrations, in all experiments decapitation and trunk blood collection was undertaken between 1230–1430 h. For each triad (escapable, yoked inescapable and no shock mice) the procedure was conducted at the same time by different experimenters.

In the second experiment we assessed the effects of a smaller number of shock trials (60 shocks), such that the escape response was not well established. The procedure of Experiment 2 was identical to that of Experiment 1, except that only 60 escape or yoked shock trials were delivered. Likewise, trunk blood was collected either immediately, 15, 30, 60, or 180 min later ($n = 7$/group).

The third experiment of this series assessed the effects of repeated exposure to the 60 shock trials on each of 1, 3 or 5 days. Preliminary studies revealed that 60 shocks/day resulted in a submaximal glucocorticoid response, but repeated training permitted escape performance to be well established. The procedure of Experiment 3 was essentially identical to that of Experiment 2, except that independent groups of mice were exposed either to 60 escapable shocks, 60 yoked inescapable shocks, or no shock on each of either 1, 3, or 5 days ($n = 5$/group). An additional group of mice ($n = 5$) was placed individually in home cages and left undisturbed for 5 days until the time of decapitation. Since peak plasma corticosterone concentrations were reached approximately 15 min after stressor termination in Experiment 2, and graded stressor effects could be determined at this time (cf. Experiments 1 and 2), mice were sacrificed 15 min after the last shock re-

ceived. As in the preceding experiments trunk blood was collected and centrifuged, and frozen plasma samples stored at −70°C for subsequent corticosterone determinations.

Finally, the fourth experiment was conducted to determine whether stressor controllability would proactively influence the glucocorticoid response engendered by limited stressor exposure. Mice of Experiment 4 received either a single session of 360 escapable shocks, yoked inescapable shock or were placed in the apparatus but not shocked as described in Experiment 1. Immediately following shock treatment, mice were housed individually and provided with food and water. Twenty-four h afterward, mice in each group were subdivided and exposed either to 30 inescapable shocks of 2-s duration or were handled and placed in the apparatus but not shocked. Mice were then returned to their cages and at one of 5 intervals afterward (immediately, 15, 30, 60, or 180 min) they were decapitated and trunk blood was collected (n = 5/group). A separate group of mice (n = 5/group) was housed individually at the outset of the study and left undisturbed in their home cages. Trunk blood was collected from these animals at the same time as it was for animals that were sacrificed immediately after the reexposure session, and served as a nonhandled, nonapparatus-exposed control. As in the preceding experiments, frozen plasma samples were stored for subsequent corticosterone determinations.

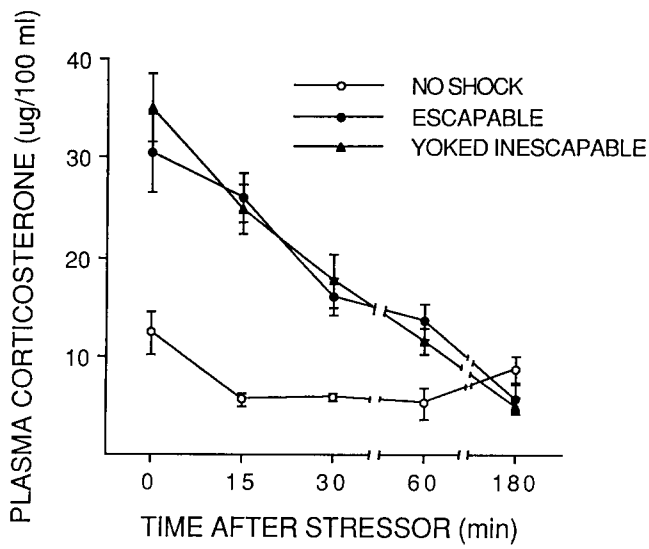

FIG. 1. Mean (±S.E.M.) plasma corticosterone concentrations (μg/100 ml) in mice at various times after exposure to either 360 escapable shocks, yoked inescapable shock, or no shock.

RESULTS AND DISCUSSION

The mean amount of shock mice received on each trial of Experiment 1 was 2.65 ± 0.23 s. Figure 1 shows the mean plasma corticosterone concentrations at various times after exposure to escapable shock, yoked inescapable shock, or no shock treatment. Analysis of variance revealed that plasma corticosterone concentrations varied as a function of the Shock Treatment × Time interaction, $F(8,68) = 6.51$, $p<0.001$. Newman-Keuls multiple comparisons of the main effects comprising the interaction ($\alpha = 0.05$) confirmed that among nonshocked mice plasma corticosterone levels did not vary significantly over time following the initial treatment, although immediately after apparatus exposure corticosterone levels were somewhat higher, although not significantly so, than at the 15–60-min intervals ($0.05<p<0.10$). In contrast, in both shocked groups, plasma corticosterone concentrations decayed with time, such that levels were significantly lower 30–180 min after shock than immediately after stressor exposure. Between group comparisons at each time interval revealed that at the 0, 15, 30, and 60-min intervals, mice that received either escapable or yoked inescapable shock exhibited significantly higher plasma corticosterone concentrations than their nonshocked counterparts. The two shock conditions, however, yielded comparable elevations of corticosterone levels at each of the time points.

In Experiment 2 (60 shock trials) mice received an average of 5.45 ± 0.67 s of shock per trial. Figure 2 shows the time-dependent changes in mean plasma corticosterone concentrations following exposure to escapable, yoked inescapable, and no shock. As seen in Fig. 2, exposure to 60 shock trials produced a less dramatic rise in plasma corticosterone levels compared to those observed following more protracted training in Experiment 1 (cf. Figs. 1 and 2). Analysis of variance revealed that plasma corticosterone concentrations varied as a function of Shock Treatment × Time interaction, $F(8,85) = 2.09$, $p<0.05$. Subsequent Newman-Keuls multiple comparisons of the simple effects comprising the interaction ($\alpha = 0.05$) indicated that in nonshocked animals plasma corticosterone levels tended to decline over time following apparatus exposure (see Fig. 2). Indeed, whereas in Experiment 1 this decline approached but did not reach statistical significance, in Experiment 2, plasma corticosterone levels immediately after exposure to the apparatus exceeded those observed 60 and 180 min following this experience. In both shocked groups, plasma corticosterone concentrations were increased by the stressor treatment, but this effect was evident only at the 15-, 30-, and 60-min time periods. Indeed, glucocorticoid concentrations immediately after shock were comparable to those of the nonshocked animals. Interestingly, in Experiment 2, plasma corticosterone levels did not reach the magnitude they did in the preceding study, but the elevated corticosterone levels persisted as long as in Experiment 1. Even though the maximal corticosterone response induced by the stressor was approximately half that of Experiment 1, the escapable and inescapable treatments did not differentially influence glucocorticoid concentrations. Thus, the failure to detect effects of stressor controllability cannot be ascribed to the treatment being too intense, thereby precluding potential treatment effects from being detected.

It is likely that the delay in the peak corticosterone increase observed after 60 shock trials may not only be due to the fewer number of shocks received, but also to the time over which the shock was delivered. In Experiment 1, where mice received 360 shocks over 1.1 h, peak plasma corticosterone concentrations were evident immediately after stressor termination. In contrast, in Experiment 2, the smaller number of shocks was administered over 11 min and peak plasma corticosterone concentrations were evident between 15 and 30 min after stressor termination. Thus, it is possible that the delay of peak values in Experiment 2 may be attributable to the time required for the expression of the plasma corticosterone response after stressor exposure, or may be related to the time of initial stressor inception, rather than the time since stressor termination. Other investigations have similarly shown a delay in peak corticosterone values following stressors of brief duration [see (11,31)].

Although a nonhandled control group was not included in this study, the time-dependent decay of plasma corticosterone concentrations suggests that the experience of being handled and placed in the apparatus was responsible for the elevated plasma corticosterone levels seen immediately after treatment. Moreover, on the assumption that handling, coupled with apparatus exposure, reflects a mild stressor relative to that of footshock, the time-depen-

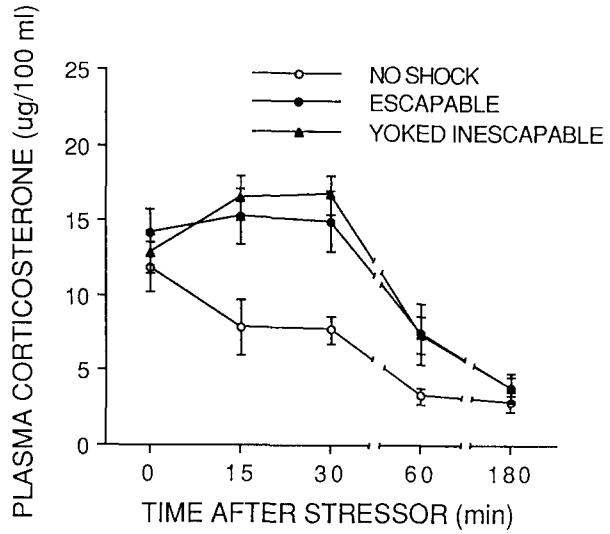

FIG. 2. Mean (±S.E.M.) plasma corticosterone concentrations (μg/100 ml) at various intervals after exposure to either 60 escapable shocks, yoked inescapable shock or no shock.

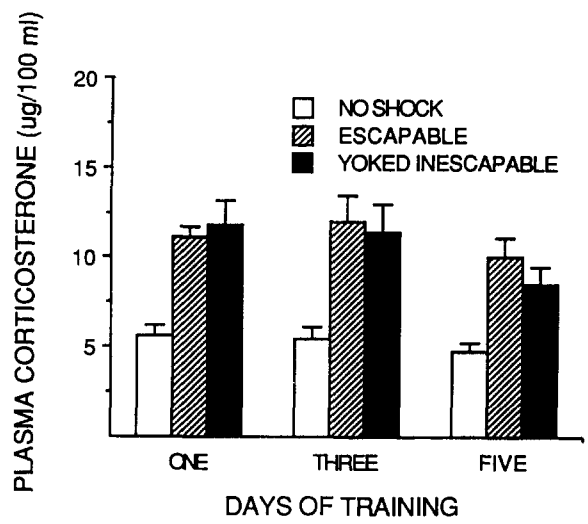

FIG. 3. Mean (±S.E.M.) plasma corticosterone concentrations (100 μg/ml) in mice 15 min after exposure to either 1, 3 or 5 days of escape training (60 trials/day), yoked inescapable shock, or no shock. In mice not exposed to any treatment whatsoever the mean (±S.E.M.) plasma corticosterone value was 3.39±0.43.

dent variations of plasma corticosterone levels suggest that (a) at the lower range, increments of stressor severity engender graded plasma corticosterone changes, and (b) the decay of the corticosterone response following stressor termination varies as a function of mild vs. relatively severe stressors.

Mice of Experiment 3 received an average of 4.73 (±0.83), 3.77 (±0.45), and 2.37 (±0.19) s of shock per trial on the first, third, and fifth day of escape training, respectively. Analysis of variance revealed that the shock treatment influenced plasma corticosterone concentrations, $F(2,34)=24.89$, $p<0.01$. Once again, Newman-Keuls multiple comparisons revealed that shocked animals exhibited comparable increases of plasma corticosterone regardless of whether or not control over shock offset was possible (see Fig. 3). Likewise, there were no differences in corticosterone concentrations as a function of the number of training sessions mice received. Thus, it appears that even though the response was fairly well established after 5 days of escape training, corticosterone values did not differentiate between animals that received controllable or uncontrollable shock.

The mean amount of shock received per trial during the training session of Experiment 4 was 2.23±0.17 s. Unlike the proactive influence of aversive stimulation on central neurochemical changes (3, 6, 18), prior stressor exposure failed to alter the corticosterone response upon subsequent reexposure to limited amounts of shock. Plasma samples of 6 animals were lost during the course of the corticosterone assay, thus the analysis of variance was performed with unequal number of subjects in each group. The analysis revealed that the initial treatment mice received did not influence the concentrations of plasma corticosterone. The glucocorticoid values were found to vary as a function of the Shock Reexposure × Time interaction, $F(4,114)=3.75$, $p<0.01$. Subsequent Newman-Keuls multiple comparisons ($\alpha=0.05$) confirmed that mice exposed to 30 shocks 24 h after initial treatment exhibited higher plasma corticosterone concentrations at 15 and 30 min after stressor termination than mice that had not been shocked 24 h after the initial treatment. As in Experiment 2, peak corticosterone values in shocked animals were reached early after stressor termination and returned to baseline levels at approximately 60 minutes. Moreover, as observed in Experiments 1 and 2, relative to a severe stressor, 30 shocks did not induce as marked an increase of plasma corticosterone concentration, and the rate of decay was more gradual. Pairwise comparisons between escapably and yoked inescapably shocked mice failed to reveal differences at any of the intervals. Moreover, planned comparisons between handled (i.e., placement in apparatus only) and nonhandled mice revealed differences at the 0- and 15-min sampling intervals after footshock termination (see Fig. 4), suggesting that the glucocorticoid response was sensitive to the mild stress of being handled.

EXPERIMENT 5

Little attention has been devoted to the proposition that the impact of stressor controllability on plasma corticosterone concentrations may be related to the characteristics of the response required of the animal. For instance, it is conceivable that the rise of plasma corticosterone may be limited when the response required of the animal is a highly prepared one. As such, differences between a controllable and an uncontrollable stressor would be most readily detected. Under conditions where animals are required to emit a contraprepared response it might similarly be expected that the availability of an escape response would lead to less profound corticosterone changes relative to that elicited by a yoked stressor. Alternatively, the proposition might be entertained that when animals are required to emit a contraprepared response, having control over the stressor may actually be more aversive than not having control. In effect, the conflict generated by requiring an animal to emit a response contrary to that which it is prepared to emit may result in more profound neurochemical or hormonal changes than an uncontrollable stressor. Thus, it is possible that the effects of controllability on central catecholamine levels and plasma corticosterone secretion may vary depending not only upon the nature of the task, but also upon the specific response required of the animal.

It has been reported that in a brightness discrimination task involving water-escape, mice exhibited a tendency to approach an illuminated arm of a water-filled Y-maze, and tended to avoid the

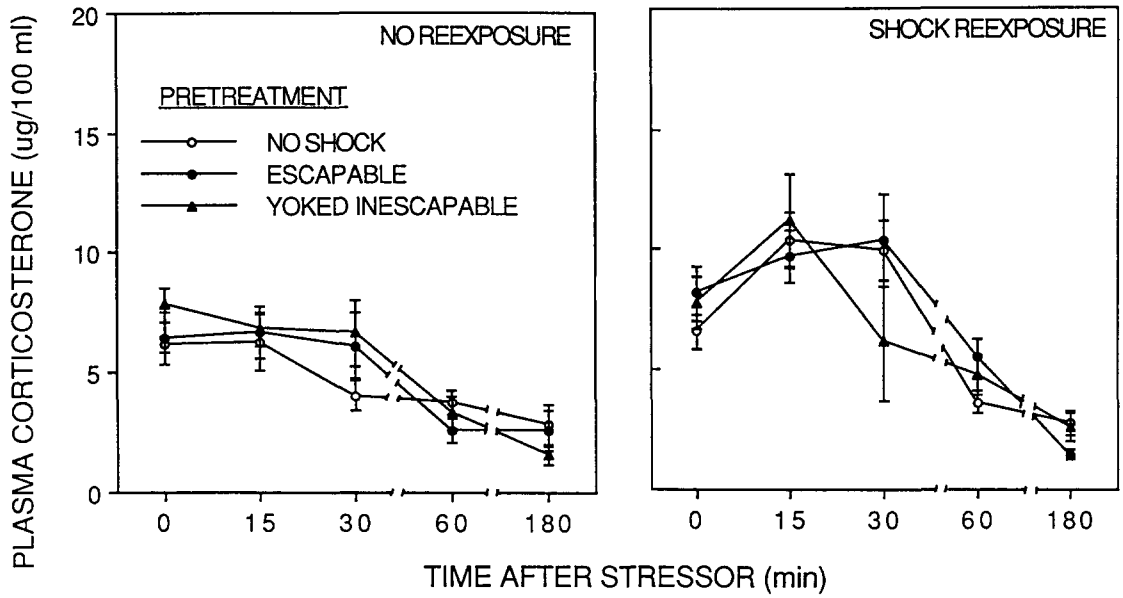

FIG. 4. Mean (±S.E.M.) plasma corticosterone concentrations (μg/100 ml) in mice exposed to either 360 escape trials, yoked inescapable shock, or no shock and 24 h later reexposed to either 30 shocks of 2-s duration or no shock. In untreated mice that had been left in their home cages a mean (±S.E.M.) plasma corticosterone value of 1.66±0.23 was obtained.

nonilluminated area (8). Indeed, the response of swimming to the illuminated arm of the maze is acquired readily, whereas numerous test sessions are necessary for animals to acquire the response of swimming to the nonilluminated arm (36). Accordingly, this paradigm not only permits evaluation of the neurochemical and endocrine response to an escapable stressor, but also permits assessment of the biochemical response under conditions where animals are required to emit either a highly prepared (i.e., swim to light) or a contraprepared (i.e., swim to dark) response. Experiment 5 was conducted to evaluate the corticosterone changes elicited when mice were required to swim either to an illuminated or to a nonilluminated portion of a maze, and to determine whether stressor controllability under these conditions influenced the magnitude of the corticoid response.

METHOD

Subjects and Apparatus

A total of 120 naive, male CD-1 mice served as subjects for Experiment 5. The subject characteristics were the same as those described in Experiment 1.

Water Y-maze. Water-escape training was conducted in a 30 cm high × 11.5 cm wide Y-maze constructed of 0.4 cm thick dark gray polyvinylchloride panels. The start arm of the maze was 15 cm in length and the two goal arms, which were separated from each other by a 30° angle, were 60.5 cm in length. The outer edge of each goal arm measured 44 cm, whereas the inside edge measured 38 cm. A movable ramp, placed at an angle of 26° from the floor of either of the goal arms, permitted mice to escape from the water. The maze was filled with water (20°C) to a height of 15 cm. A guillotine door, situated at the choice point of the maze, was lowered once the mouse entered the choice area. This prevented the mouse from reentering the start arm. Each goal arm of the maze was illuminated by a light bulb (14 V) 6 cm from the end of each arm, and located on the ventral surface of the apparatus roof. A 0.4 cm thick red translucent Plexiglas roof, which permitted observation of the mouse, covered the V-shaped portion of the maze. The location of the ramp and the illuminated arm of the maze could be manually altered.

Procedure

Mice were assigned to one of the following treatment conditions and tested between 1230 and 1430 h (n=12/group). Mice in one condition received 10 trials in which they were required to swim to the illuminated arm to escape from the water Y-maze, while in a second condition mice were required to swim to the nonilluminated arm of the maze to escape. On each trial, mice were placed individually in the start arm and required to swim the length of that arm, turn in a predetermined direction, and climb onto the ramp before being removed from the water. If a mouse failed to find the escape ramp within 30 s, it was removed from the maze and placed in a dry holding cage for an intertrial interval of 90 s. The position of the correct arm was varied from trial to trial according to a predetermined random sequence. A third group of mice was matched (yoked) with those mice that were required to swim to light. For these animals, however, escape was not possible from the water Y-maze. The guillotine was kept in place, thus separating the start arm from the rest of the maze. Mice were placed at the choice point (i.e., base) of the V-shaped segment of the maze and were permitted to swim freely. For this group of mice, the frequency and duration of light and dark presentations on each trial was dependent upon the pattern of responding of those mice that were required to emit the prepared response. A fourth group of mice was also exposed to this yoked procedure, however, these animals were exposed to the same pattern of light presentations as mice required to emit the contraprepared response (i.e., swim to the nonilluminated arm). The two yoked groups were not tested simultaneously with the escapable animals, but rather received their inescapable trial during the intertrial interval of their respective escapable partners. Mice in

TABLE 1

MEAN (±S.E.M.) LATENCIES (S) TO REACH THE CHOICE POINT AND EXIT RAMP, NUMBER OF DISCRIMINATION ERRORS COMMITTED, AND THE NUMBER OF ERRORLESS TRIALS IN MICE REQUIRED TO SWIM TO THE ILLUMINATED OR NONILLUMINATED ARM OF A WATER-FILLED Y-MAZE FOR EITHER 1 OR 5 SESSIONS

	Days of Training	
	1	5
Latency to Choice Point		
Illuminated Arm	4.96 (0.32)	4.03 (0.39)
Nonilluminated Arm	9.30 (1.12)	6.33 (1.03)
Latency to Exit Ramp		
Illuminated Arm	7.94 (0.69)	5.43 (0.44)
Nonilluminated Arm	16.77 (1.20)	11.92 (2.24)
Discrimination Errors		
Illuminated Arm	3.00 (0.55)	1.00 (0.41)
Nonilluminated Arm	7.92 (0.66)	4.50 (0.99)
Errorless Trials		
Illuminated Arm	7.42 (0.42)	9.00 (0.41)
Nonilluminated Arm	4.17 (0.46)	6.50 (0.86)

each group were subdivided and received either 1 or 5 days of water-escape training (10 trials/day). Two additional groups were neither exposed to the swim test nor handled, but were maintained in their home cages for either one day or for 5 days. One minute after the final test session trunk blood was collected for subsequent corticosterone determinations.

RESULTS AND DISCUSSION

The mean latencies to reach the choice point and the escape ramp of the maze, as well as the total number of errors committed (where more than a single error may be committed on a single trial) and the number of errorless trials are shown in Table 1. Analysis of variance of the latency to reach the choice point and exit ramp revealed longer latencies in mice required to swim to the dark than in mice required to swim to light, F's(1,44) = 17.15, $p<0.01$ and 32.95, $p<0.01$, respectively. Not surprisingly, shorter latencies to reach the choice point and exit ramp were evident on the fifth test day than on the first test day, F(1,44) = 5.94, $p<0.05$ and 7.62, $p<0.01$, respectively.

The measures of discrimination performance paralleled the escape latency data. Analysis of variance of the total number of discrimination errors indicated that fewer errors were made by mice required to swim to light than by mice required to swim to dark, F(1,44) = 37.73, $p<0.01$. In addition, the total number of errors committed decreased over the course of the study, F(1,44) = 15.62, $p<0.01$. Similarly, analysis of variance of the number of errorless trials revealed that in mice required to swim to light a greater number of errorless trials occurred relative to mice required to swim to dark, F(1,44) = 25.74, $p<0.01$. After 5 days of testing an overall improvement in discrimination performance was observed in both swim conditions, F(1,44) = 11.94, $p<0.01$.

Figure 5 shows the mean (±S.E.M.) plasma corticosterone values in mice exposed to either escapable or yoked inescapable swim after 1 and 5 days of testing. A completely randomized factorial design (2 × 2 × 2) was employed to analyze plasma corticosterone values in swim treated animals, and Dunnett's t-tests ($\alpha = 0.05$) were used to compare the effects of swim treatment to the nonstressed control groups. Analysis of variance of the mean plasma corticosterone levels yielded a significant Controllability × Days interaction, F(1,85) = 4.38, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) of the simple effects comprising this interaction indicated that after a single training session animals exposed to yoked inescapable swim exhibited significantly higher plasma corticosterone levels than animals that could escape. After 5 days of training, however, these differences were not evident. In addition, Dunnett's t-statistic confirmed that greater elevations in plasma corticosterone concentration occurred in mice exposed to any of the swim conditions than in nonstressed animals.

Although the Swim Condition × Controllability × Days interaction failed to reach significance ($p<0.10$), Newman-Keuls multiple comparisons of the simple effects comprising this interaction were conducted since a priori predictions had been made concerning specific groups. These comparisons revealed that among mice required to swim to dark, corticosterone secretion did not vary as a function of stressor controllability. In contrast, following a single training session, mice required to swim to light exhibited significantly lower plasma corticosterone levels relative to their yoked counterparts. However, after 5 days of testing this difference was absent. It appears that the secretion of plasma corticosterone in the water-escape task may be differentially influenced by stressor controllability. Such an effect was dependent on the response required of the animal (i.e., swim to light vs. swim

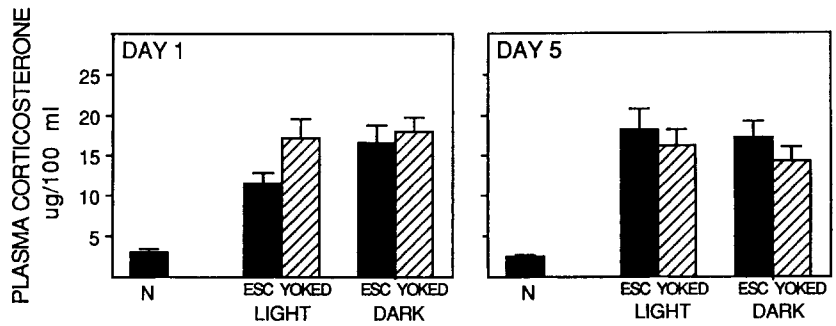

FIG. 5. Mean (±S.E.M.) plasma corticosterone concentrations (μg/100 ml) in mice permitted to escape in a swim task (ESC) or exposed to an identical amount of swim which was not escapable (YOKED). The escape response could be achieved in one set of mice by swimming to an illuminated arm of a maze (LIGHT) and in another group by swimming to a nonilluminated arm (DARK). Additional animals were not exposed to the water (N). Mice were exposed to the treatments for either 1 (left hand panel) or 5 sessions (right hand panel) on consecutive days.

to dark) and also as a function of the number of swim sessions mice received.

GENERAL DISCUSSION

The results of the present investigation confirmed that exposure to a stressor resulted in a marked increase in plasma corticosterone concentrations, which persisted for approximately 1 h. Although some investigators have demonstrated that the plasma corticosterone response does not distinguish between moderate and severe stressor conditions (23, 30, 31), the magnitude of this response in the present investigation varied with the stressor regimen employed. In particular, plasma corticosterone elevations were marked upon exposure to a relatively strong stressor (360 shocks), but were less pronounced after fewer footshock trials (60 shocks). Additionally, the corticosterone response was exquisitely sensitive to mild stressors, such as handling and exposure to a novel environment. These manipulations provoked small, but reliable, increases in plasma glucocorticoid levels, which persisted for brief intervals (15–30 min). Taken together, the present results, like those of previous investigations (4, 17, 20), indicate that the magnitude and the duration of the corticosterone response may be sensitive to graded levels of environmental change.

Despite the apparent sensitivity of the plasma corticosterone response, neither the magnitude of the corticosterone increase, the time of peak plasma corticosterone concentrations, nor the rate of decay was differentially influenced by controllable and uncontrollable shock. This was the case regardless of whether mice received limited or relatively extensive training (60 vs. 360 trials). Moreover, the magnitude of the glucocorticoid response was not diminished even when animals had established mastery over the response contingencies (i.e., after several days of escape training). While it is conceivable that uncontrollable shock is perceived as being more stressful than controllable shock, such a differentiation was not evident in terms of plasma corticosterone secretion. These findings extend the previous results of Maier et al. (25), which revealed that the ability to control stressor termination does not influence the secretion of plasma corticosterone, although similar manipulations affected performance in a shuttle escape task and in tests of analgesia. In fact, using the same parameters of controllable and uncontrollable footshock, applied under essentially identical conditions in the same strain of mouse, it was previously shown in this laboratory that differential changes were evident with respect to hypothalamic and hippocampal NE turnover and levels (2,21), shuttle-escape performance, as well as responding for ICSS (1,40). Thus, it is not likely that the failure to detect glucocorticoid variations as a function of stressor controllability stems from the specific stressor or task parameters employed.

As in the case of the shock treatment, the stress associated with the swim task enhanced plasma glucocorticoid secretion. Of particular interest, however, was the finding that in the water-escape task plasma corticosterone concentrations varied as a function of controllability, but such an effect was dependent upon the specific response required of the animal. In mice required to emit the contraprepared response of swimming to dark, the increase of plasma corticosterone was comparable to that of yoked mice. In contrast, in animals required to perform the highly prepared response of swimming to light, lower plasma corticosterone concentrations were evident relative to their yoked inescapable partners. Such an effect, however, was evident only on the first day of swim testing, despite the fact that after 5 days of testing the escape response was relatively well established. It is conceivable that the cumulative impact of swim on the secretion of plasma corticosterone may have obscured the differential effects of stressor controllability after repeated testing.

The fact that the corticosterone response was not affected by factors such as shock controllability is inconsistent with several earlier reports using rats, dogs and monkeys (5, 16, 28), and was, indeed, surprising given the apparent sensitivity of this response. It is tempting to speculate that the failure to detect an effect of shock controllability in the present investigation may have been species related. However, the fact that stressor controllability influenced the corticosterone response in the swim escape study makes this possibility an unlikely one. Indeed, if it is assumed that corticosterone secretion is a fundamental response to stressful events and thus essential for the organism's well being, it might be disadvantageous for such a response to be determined by factors such as the psychological dimension of controllability. After all, when an organism is initially exposed to a stressor it is not immediately apparent to the animal whether the stressor is controllable or uncontrollable. Only with repeated trials will animals learn whether offset of the aversive event is dependent upon its responses. Indeed, if one function of the corticosterone response is to prevent excessive activation of other systems, hence obviating physiological damage [e.g., preventing an excessive immune response; see (27)]; or alternatively to prevent the occurrence of β-NE receptor downregulation (34), then it would be expected that the glucocorticoid response would be enhanced by stressor application regardless of factors such as controllability. Of course, if an avoidance response is exceedingly well established (7, 10, 17), or if animals are required to emit a highly prepared response, then the plasma corticosterone secretion may be diminished.

In contrast to reports which suggested that glucocorticoid activity is attenuated following the establishment of an escape/avoidance response (7, 10, 12), in the present investigation repeated escape training over 5 days in either the shock or swim paradigm did not limit the extent of the plasma corticosterone increase. Of course, it is possible that with further stressor exposure the contribution of stressor controllability on the plasma corticosterone response might have emerged (19). For instance, using active avoidance and escape paradigms, respectively, Coover et al. (7) and Davis et al. (10) showed that following 15 training sessions plasma corticosterone concentrations decreased considerably from peak values. However, in these studies, animals were successful in acquiring the response contingencies by the fifth session, but the secretion of plasma corticosterone did not reflect the accompanying behavioral changes until much later in training (10). Indeed, Herrmann et al. (19) reported that in relatively well trained animals corticosterone concentrations were higher prior to an avoidance session than after, while the converse was true of yoked subjects. It was thus suggested that the plasma corticosterone response in well trained animals may be part of a preparatory response which enables the organism to engage in adaptive coping behaviors at an appropriate time. In the present investigation the repeated training schedule involved only 5 escape sessions. Likewise, the corticosterone changes induced by reexposure to cues associated with shock were determined in animals that had previously received limited training. It is conceivable that differences in corticosterone values as a function of stressor controllability would have emerged following a more extended training regimen.

Finally, in contrast to the central NE and DA changes associated with reexposure to a stressor or to stressor related cues (3, 6, 18), in the present investigation stressor application did not influence the plasma corticosterone response to a subsequently applied stressor or to cues that had been associated with the stressor. These results are consistent with other reports which failed to demonstrate proactive effects of stressor exposure on the subsequent corticosterone response to an environmental insult (13,

16, 22). In contrast to these findings, however, other investigators demonstrated that reexposure to cues associated with the initial stressor or subsequent exposure to a novel environment influenced plasma corticosterone concentrations (5, 24, 28). Moreover, as already indicated, in relatively well-trained animals, presession concentrations of corticoids may be elevated in comparison to postsession levels or to that of animals in which the response contingencies had not been as well established (19). Thus, it seems that there are conditions wherein a stressor may proactively influence the corticosterone response. However, the conditions which lend themselves to such an outcome may be different from those which are effective in eliciting proactive changes in central catecholamine activity.

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