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# Physiological and Biochemical Concomitants of Restraint Stress in Rats

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KEIM, K. L. AND E. B. SIGG. *Physiological and biochemical concomitants of restraint stress in rats.* PHARMAC. BIOCHEM. BEHAV. 4(3) 289–297, 1976. – Restraint stress of 30 min increases plasma CS and lowers hypothalamic NE. Restraints of longer durations are associated with an attenuation of these changes. Daily repetitive restraint enhances the CS response on the second day and progressively diminishes it on subsequent days. Whole brain NE increases on the first day and decreases on Day 2 to 5. The CS response to acute restraint is similar in 5 different normotensive rat strains, but is enhanced in the genetically hypertensive SH rat, its normotensive backbreed WKY, and the DOCA hypertensive Sprague-Dawley rat. Comparison with other stressors (electric foot shock and novel environment) indicate that the responses to restraint are different at least in time course, if not qualitatively.

Restraint stress Plasma corticosterone Brain catecholamines Adrenal catecholamines Stress adaptation Stress physiology

THE restriction of movement in small animals is often required for a variety of physiological and behavioral experiments. Many different methods for restraint are used [16] each causing different degrees of distress to the animal. Procedures which lead to the forced, isolated confinement of an animal in a new environment make restraint a stressor comprised of physiological and psychological factors. The magnitude of the response is not only dependent on the severity of the restraint, but also on many other variables, including duration of the restraint, sex, age, previous housing conditions, handling, past stress experiences, and intrinsic biological factors (light-dark cycle, estrus cycle) on which the stressor is superimposed. Thus, gastric ulceration results only from a combination of restraint with other stressors (e.g., cold or deprivation of food and water) when applied for many hours [4] and its severity is reported to be dependent on rat strain [9].

Recently, controlled experimentation by Mason [17] has revealed that metabolic and endocrine systems respond specifically and in discrete patterns to different stressors. The purpose of this investigation was to examine certain biochemical and physiological consequences of restraint. Emphasis was placed on investigating the relationship between central catecholamines and corticosterone release, particularly in view of the hypothesis that brain catecholamines may play a role in the regulation of ACTH [27]. Different schedules of restraint and several different strains of rats were used to investigate possible differential responses to the stressor. A comparative analysis of grid shock and novel environment with restraint was also made.

## METHOD

### *Animals*

Male rats of the Sprague-Dawley strain and, in one experiment, of several other strains as mentioned in the text were used. All rats were received at 45–55 days of age and individually housed for 2 weeks prior to an experiment. Their body weight at the time of experimentation was approximately 295 g. Food and water were available ad lib, and the animals were kept in an isolated room with regulated temperature (23°C), humidity, and illumination; the light period being from 0600 to 1800 hr.

### *Apparatus*

Restraint stress was effected by placing a rat in a Plexiglas cylinder of 7 cm dia. and variable length to accommodate different sized animals. The space for a 300 g rat was approximately 400 cc and considered a snug fit. Adequate ventilation was provided by means of holes at the sides and head of the tube. Access to water, but not food, was provided when the period of restraint was longer than 4 hr.

Electroshock was delivered to 12 metal rods which constituted the floor of the gridshock cage, a plastic and metal cube (24 x 24 x 24 cm). Six cages were contained in an illuminated, ventilated, sound attenuated chamber. One constant current shock generator supplied 3 cage grids and 2 sec of scrambled shock occurred every 88 sec; intensities and durations of exposure are given in the text.

Novel environmental stress was produced by transferring an animal into a translucent plastic cage (18 x 12 x 28 cm) with a wire mesh top referred to as a shoe box.

### *Blood Pressure, Temperature, and Activity Measurements*

A number of physiological measurements were made within the restraint chamber on animals that were not used for biochemical determinations. Systolic blood pressure, obtained by means of the indirect tailcuff method was

recorded 15, 30, 120, and 240 min after the initiation of restraint. For this purpose, it was necessary to place the rats on a plate which heated the abdominal area to 32° C. Colonic temperatures were taken at the same intervals in a separate group of animals. Body movement within the restrainer was measured by amplifying and integrating the output of a pneumatic pulse transducer coupled to a Grass Model 7P3A integrator; the time constant was 2 sec. The input to the transducer was a sensor which consisted of a short, air-filled, sealed, rubber tube upon which the restrainer was positioned. The activity measurements were carried through 240 min of restraint.

*DOCA-Induced Hypertension*

Two weeks prior to restraint, selected animals were made hypertensive by unilateral nephrectomy, subcutaneous implantation of 25 mg deoxycorticosterone acetate (DOCA) and maintained with drinking water containing 0.9% sodium chloride.

*Experimental Design*

The experimental approach consisted of subjecting rats to a stressor for various lengths of time. Some of the rats were returned to their home cage for 30 min after the stress period to evaluate poststress recovery. Repeated daily stresses were imposed to investigate adaptive mechanisms. The details of the schedules are described in the Results section. All experiments were carried out between 0800 and 1200 hr. During this time resting corticosterone levels did not significantly change. Moreover, the corticosterone response to 30 min of restraint was equipotent when applied at different times within this period. All experimental groups consisted of 6–10 rats. Nonstressed controls were included in all experiments to ascertain basal levels and they were killed at 0800 prior to stressing procedures of the remaining groups.

*Tissue Preparation*

At the end of the experiment the rats were rapidly transferred to a separate laboratory where they were decapitated. This procedure took less than 20 sec. Trunk blood was collected through siliconized funnels into tubes containing heparin (143 USP units) or EDTA (14 mg). Plasma was obtained by centrifugation (3000 rpm for 20 min at 4° C) and then frozen. Following trimming and weighing, tissues designated for catecholamine determinations were homogenized in 0.4 N perchloric acid and allowed to stand at 4° C for one hr prior to freezing.

To evaluate the relative reactivity of the adrenals to ACTH, the glands were rapidly removed, trimmed, weighed, and quartered. They were placed into a small flask containing 2 ml of Krebs Ringer Bicarbonate (KRB) solution and put in a reciprocating water bath maintained at 37° C. Following a 30 min preincubation period, the KRB solution was discarded and a fresh 2 ml solution containing 0.1 U ACTH (Cortrosyn®, Organon) was added. After 2 hr, the KRB solution without the adrenal fragments was transferred to a storage tube along with a 1 ml KRB wash of the incubating vessel and frozen.

In several experiments whole brain (excluding cerebellum and lower brainstem) was used for catecholamine measurements. In other experiments, the brain was dissected into three parts: endbrain, which consisted of the frontal pole anterior to the caudate-putamen complex; hypothalamus, which represented a block extending from behind the chiasma to the mammillary nucleus, the later borders being approximately 1.5 mm from the midline and the dorsal border just above the arcuate nucleus; and brainstem, which included a section between the obex and the anterior level of the pons. In the experiment in which rats were exposed to restraint for 18 hr, the heart was prepared for histological examination with hematoxylin-eosin, trichrom and PAS strains. (The histological analysis was performed by Pharmacon Labs., Scranton, Pa.).

*Biochemical Assays*

The plasma corticosterone assay was structured after the method of Mattingly [18] with minor modifications. Corticosterone was extracted with spectrophotometric grade dichloromethane. The fluorescence of corticosterone was measured with an Aminco-Bowman Spectrophotofluorimeter (excitation 465 mμ, emission 520 mμ) exactly 20 min following addition of the fluorescing reagent (30% absolute ethanol, 70% sulfuric acid). Corticosterone standards were prepared on each day of assay. The alcoholic sulfuric acid-induced corticosterone fluorescence resulted in a linear relationship within the physiological range when plotted in a log-log fashion. Sample values were corrected for blank and recovery factors. Recovery was not less than 80% and precision was greater than 95%. The nonspecific fluorescence which has been reported in adrenalectomized rats [10] was never greater than 1.5 μg/100 ml plasma. The corticosterone released by in vitro administration of 0.1 U ACTH was assayed with the same technique, correcting for dilution factors and tissue weights.

Norepinephrine (NE) and dopamine (DA) eluates were prepared according to the method of Horst *et al.*, [12]. Supernatants of the tissue homogenates were obtained on the day of assay, and the specific catecholamines were differentially eluted from a Dowex AG50W-X4 ($Na^+$ form) column. Appropriate blanks and recovery standards were processed in an identical manner. A standard curve was determined with each assay. Recovery of both catecholamines was consistently between 70 and 80%. Norepinephrine and dopamine were determined fluorometrically according to the method described by Laverty and Sharman [15]. Adrenal norepinephrine and epinephrine (EPI) were determined according to Anton and Sayre [2].

*Statistics*

Statistical differences between the experimental means of the basal control and the stressed groups or between the means of different stressed groups were determined by Student's *t*-test. A difference with $p<0.05$ was regarded as significant.

## RESULTS

*Strain Differences*

The basal plasma CS concentration in 5 different rat strains was found to be remarkably similar during the time of the circadian trough (Table 1). The response to a 30 min restraint also failed to uncover any differences. Plasma CS rose from a basal range between 4.8 and 5.6 μg% to a stress-induced concentration of 54.8 to 61.7 μg%. However,

TABLE 1

TOTAL PLASMA CORTICOSTERONE $\mu$g/100 ml ± SEM IN DIFFERENT RAT STRAINS (N = 6)

| | Fisher* | Sprague-Dawley† | Sprague-Dawley‡ | Hooded§ | Wistar¶ |
|---|---|---|---|---|---|
| Basal level | 4.8±0.3 | 5.4±0.1 | 4.8±0.4 | 5.0±0.3 | 5.6±0.5 |
| 30′ Restraint | 54.8±1.7 | 55.3±1.3 | 58.7±2.5 | 61.7±3.5 | 55.1±2.8 |
| 30′ Recovery | 27.1±2.6 | 40.9±5.3 | 34.8±3.4 | 28.7±4.0 | 43.2±3.8 |
| mbw** | 116±1 | 214±8 | 291±3 | 286±3 | 307±3 |

*Fisher rat (Charles River Laboratories).
†Sprague-Dawley rat (Charles River Laboratories).
‡Sprague-Dawley rat (Carworth Farms).
§Long Evans Hooded rat (Blue Spruce Farms).
¶Wistar rat (Marland Breeding Farms).
**mbw (mean body weight).

TABLE 2

A COMPARISON OF TOTAL PLASMA CORTICOSTERONE $\mu$g/100 ml ± SEM IN SPONTANEOUSLY HYPERTENSIVE (SH) AND SPRAGUE-DAWLEY RATS

| | Sprague-Dawley 70 Day Old | SH Rat 70 Day Old | SH Rat 14 Week Old |
|---|---|---|---|
| Basal level | 5.4±0.3 (10) | 6.1±0.9 (7) | 5.3±0.8 (8) |
| 30′ Restraint | 49.3±3.3 (16) | 70.9±5.8 (6)† | 87.9±3.1 (6)* |
| 60′ Restraint | 59.5±4.8 (11) | 76.5±5.8 (6)† | 83.8±5.2 (6)† |

The number of animals is noted within the parenthesis. Significance in difference of mean from Sprague-Dawley control:
*$p<0.001$;
†$p<0.01$.

the Fisher and the Hooded rat showed a slightly faster post-stress decline in plasma CS than the Wistar and the two Sprague-Dawley strains.

The basal CS concentration in 70 day or 14 week old spontaneously hypertensive (SH) rats was not different from that of the 70 day old Sprague-Dawley control (Table 2). On the other hand, SH rats responded to the same 30 min and 60 min restraint with a significantly greater corticosterone output than the normotensive Sprague-Dawley strain. The response to the stressor in SH rats was greater in older animals.

Sprague-Dawley rats rendered hypertensive with DOCA, unilateral nephrectomy, and maintained with sodium chloride-containing drinking water were not different from their normotensive counterparts in regard to basal corticosterone levels (Table 3), but did respond with a greater stress-induced corticosterone change ($p<0.02$, $df = 10$). On the other hand, WKY rats made hypertensive with the same procedure had elevated resting levels of corticosterone and responded to restraint to a lesser degree than their normotensive partners ($p<0.02$, $df = 10$).

*Duration of a Single Restraint*

When Sprague-Dawley rats were restrained for increasing periods of time, behavioral and biochemical signs of adaptation occurred. Thus, after reaching an approximate peak of 55 $\mu$g% after 60 min of restraint, concentrations of plasma CS declined to 27.1 and 18.5 $\mu$g% after continuous restraint of 2 and 4 hr, respectively (Table 4). In vitro, adrenal reactivity to 0.1 U ACTH increased then decreased in a pattern that closely paralleled that of the plasma CS response (Fig. 1A). If animals were restrained, for only 15 min and then returned to their home cages, a rapid recovery of CS to control levels occurred within one hr.

Catecholamine content in different areas of the brain was also altered during 4 hr of restraint. Thus, while brainstem NE remained unchanged, hypothalamic NE decreased followed by a gradual return to control after 4 hr. Endbrain NE also diminished but did not recover during the 4 hr of restraint. Hypothalamic DA maintained a sustained increase over the 4 hr period (Fig. 1B).

Signs of adaptive mechanisms during continuous restraint were observed in adrenal catecholamines. Adrenal NE showed a peak increase after 30 min of restraint, returned to near control values after 2 hr and rose again to slightly elevated titers at the end of the 4 hr restraint. Adrenal EPI increased rapidly and markedly to a peak concentration after only 15 min and returned to near control concentrations towards the end of the restraint period (Fig. 1C).

The most conspicuous behavioral concomitant of prolonged restraint was a gradual reduction of struggling and turning movements which subsided after 30 to 60 min of

TABLE 3

A COMPARISON OF TOTAL PLASMA CORTICOSTERONE μg/100 ml ± SEM IN DOCA HYPERTENSIVE RATS (N = 6)

| | Sprague-Dawley Normotensive | Sprague-Dawley DOCA, UN, NaCl | WKY Normotensive | WKY DOCA,UN,NaCl |
|---|---|---|---|---|
| Basal level | 5.4±0.3 | 5.9±0.6 | 7.5±0.4 | 16.9±1.5 |
| 30′ Restraint | 55.0±2.2 | 65.4±2.9 | 75.9±1.9 | 41.5±4.2 |
| 30′ Recovery | 25.7±3.3 | 38.8±5.7 | 44.7±3.7 | 27.1±5.6 |

DOCA=25 mg deoxycorticosterone acetate.
UN=Unilateral nephrectomy.
NaCl=0.9% NaCl drinking water.

TABLE 4

THE EFFECT OF RESTRAINT DURATION ON PLASMA CORTICOSTERONE μg/100 ml ± SEM IN SPRAGUE-DAWLEY RATS

| | Restraint Duration, Min | | | | | |
|---|---|---|---|---|---|---|
| | Basal | 15 | 30 | 60 | 120 | 240 |
| # of rats | 190 | 36 | 120 | 40 | 24 | 24 |
| CS Level | 5.4±0.4 | 40.2±2.5 | 49.1±2.5 | 54.7±2.8 | 27.1±3.1 | 18.5±2.1 |
| Minimum | 3.2 | 19.5 | 31.1 | 37.2 | 10.6 | 5.6 |
| Maximum | 11.6 | 56.8 | 68.2 | 88.6 | 29.5 | 18.2 |

restraint, recurring in occasional bursts from time to time (Fig. 2). The indirect systolic blood pressure was not altered during 2 hr of restraint but was significantly decreased ($p<0.025$, $df = 6$) when measured after 4 hr (Table 5). In a few instances the tail pulse could not be obtained after 4 hr of immobilization. The colonic temperature increased 1.7°C within the first 30 min and stayed elevated through 2 hr. After 4 hr of restraint the rectal temperature was near control values.

Since it has been claimed that restraining an animal for durations of 12–24 hr would lead to gastric ulceration and histologically evident cardiac abnormalities, an experiment was designed to investigate the effect of 18 hr of restraint. Following the 18 hr restraint, the plasma CS concentration was elevated to 20.9 μg% (Table 6); heart NE and whole brain NE were slightly increased. When a group of animals was re-exposed to a second 18 hr restraint (following a 6 hr rest interim), plasma CS and brain NE did not differ from the corresponding levels after the single exposure, while heart NE returned to control levels. Histological examination of the heart from both groups did not reveal any abnormalities in the cardiac vasculature or myocardium, and fibrin clots were absent. The gastric mucosa appeared normal in all animals upon gross inspection.

*Multiple Restraints of 30 min Duration*

When rats were restrained once every day for 5 consecutive days, the CS concentration following the 30 min exposure on Day 2 was always higher than that on Day 1 (Fig. 3). However, the recovery to control levels 30 min after the termination of the Day 2 stress session was more rapid than on Day 1. When compared to Day 1 the restraint-induced rise of CS was significantly reduced on Day 5 ($p<0.01$, $df = 8$).

TABLE 5

SYSTOLIC BLOOD PRESSURE AND RECTAL TEMPERATURE DURING RESTRAINT STRESS

| Restraint Duration Min | Systolic Pressure mm Hg ± SEM | Rectal Temperature °C±SEM |
|---|---|---|
| 0 | | 36.3±0.2 |
| 15 | 122±5 | 37.7±0.1 |
| 30 | 122±4 | 38.0±0.1 |
| 60 | 128±9 | 37.9±0.3 |
| 120 | 124±7 | 37.9±0.3 |
| 240 | 98±6 | 36.7±0.2 |

In Sprague-Dawley Rats (n=8).

Concomitant with the changes in plasma CS, whole brain NE, with the exception of the first day, was reduced at the end of each stress period followed by a further decline during the 30 min recovery period in the home cage (Fig. 4). Only on Day 1 did cerebral NE tend to increase, especially during the subsequent half hour home cage period. However, those NE concentrations at the beginning of the restraints following Day 1 (which actually represent 24 hr poststress values) were considerably higher than those of non-stressed controls.

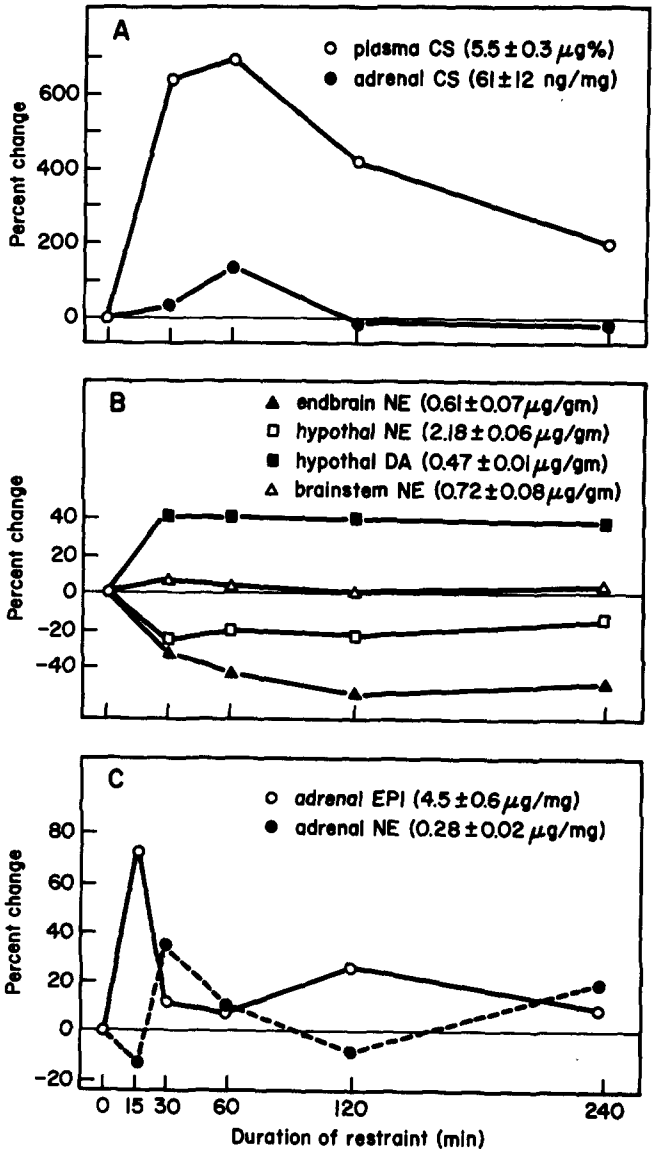


FIG. 1. The effect of restraint duration on: A. plasma corticosterone (CS) and adrenal CS reactivity; B. brain catecholamines; C. adrenal catecholamines. Each point represents a mean of at least five animals. All data is expressed in percent change from control. Control values are given in parentheses and indicated by the open circle on the 0% line. In B, differences in the means were compared to respective, basal controls (each $df = 8$): ■ all $p<0.01$ except 120 min which is not significant (NS) due to a very large error; △ all NS; □ $p<0.001$ at 30 and 120 min, $p<0.05$ at 60 min, NS at 240 min; ▲ $p<0.05$ at 30 min, $p<0.01$ at 60, 120, and 240 min.

*Comparison of Restraint with Foot Shock and Novel Environment*

A quantitative comparison of the time course of the different stressors is summarized in Fig. 5. Preliminary experiments with grid shock indicated that the corticosterone release increased with the intensity of the applied shock, reaching plasma concentrations comparable with those of a 30 min restraint stress between 1.5 and 2 mA.

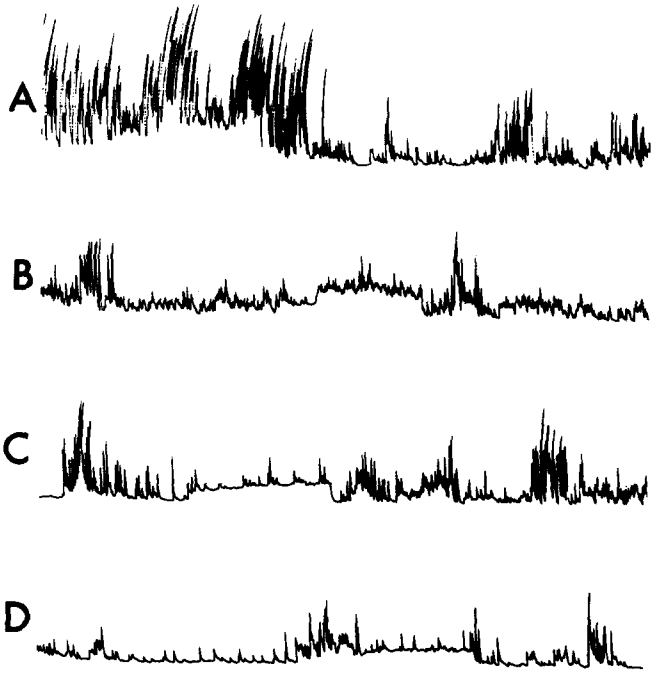


FIG. 2. A typical recording of the effect of continuous restraint on body movement within the restrainer (see text). A: 0–1 hr; B: 1–2 hr; C: 2–3 hr; D: 3–4 hr.

TABLE 6

THE EFFECT OF 18 HR RESTRAINT STRESS ON PLASMA CS, HEART NE AND BRAIN NE

| Group | n | Total Plasma Corticosterone μg/100 ml ±SEM | Heart Norepinephrine μg/g ±SEM | Whole Brain Norepinephrine μg/g ±SEM |
|---|---|---|---|---|
| A | 6 | 4.7±0.3 | 0.94±0.14 | 0.48±0.05 |
| B | 6 | 20.9±7.9 | 1.24±0.08 | 0.53±0.06 |
| C | 6 | 19.9±2.7 | 0.89±0.11 | 0.54±0.02 |

A = Control group.
B = After 18 hr of restraint.
C = After re-exposure to a second 18 hr restraint following a 6 hr home cage period.

Maximal concentrations of corticosterone were reached between 30 and 60 min of restraint or foot shock, whereas placement into a novel environment induced a much slower rise with a peak after 120 min of exposure. Intermittent foot shock applied for 2 to 4 hr caused only a small decline in corticosterone, whereas the decline during prolonged restraint was significantly greater. Regardless of the type of stressor, hypothalamic NE was diminished when rats were stressed for one hr or longer. However, significant differences occurred during the first 30 min exposure to the various stressors. Grid shock raised hypothalamic NE, whereas restraint reduced it significnatly. Acute exposure to a new environment had a minimal diminishing effect.

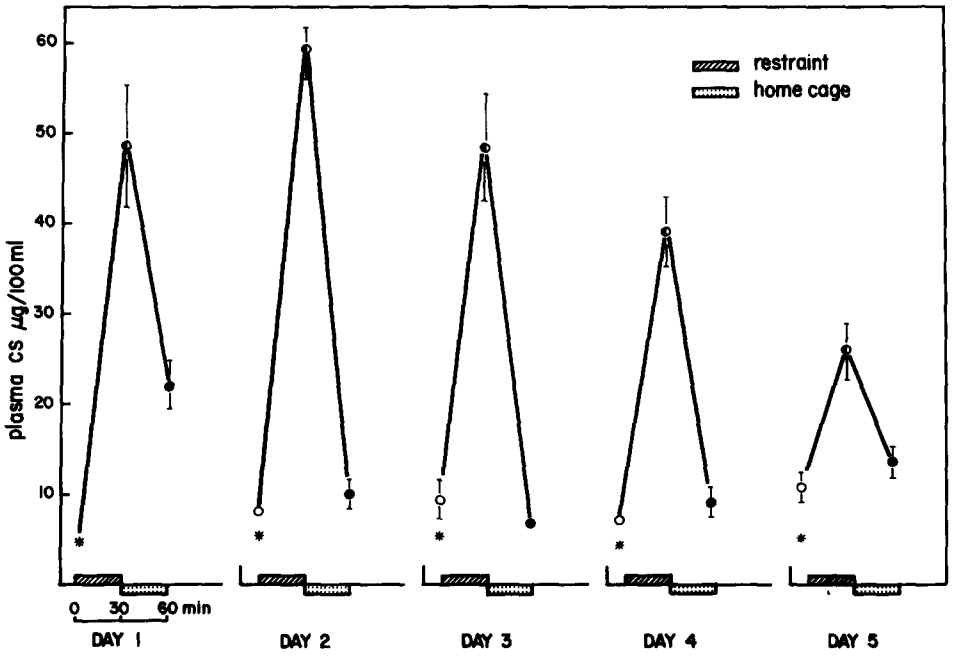


FIG. 3. The effect of 30 min restraint on plasma corticosterone (CS) applied once a day for 5 consecutive days. Each point represents the mean value from 5 rats. Vertical bars represent standard error of the mean; where no vertical bar is shown, the error was <1. *Symbol key:* * basal levels in naive, unstressed rats (daily control): ○ resting level of pre-session, stress experienced group; ◐ CS level immediately after 30 min restraint stress; ● recovery value, post-stress 30 min in home cage.

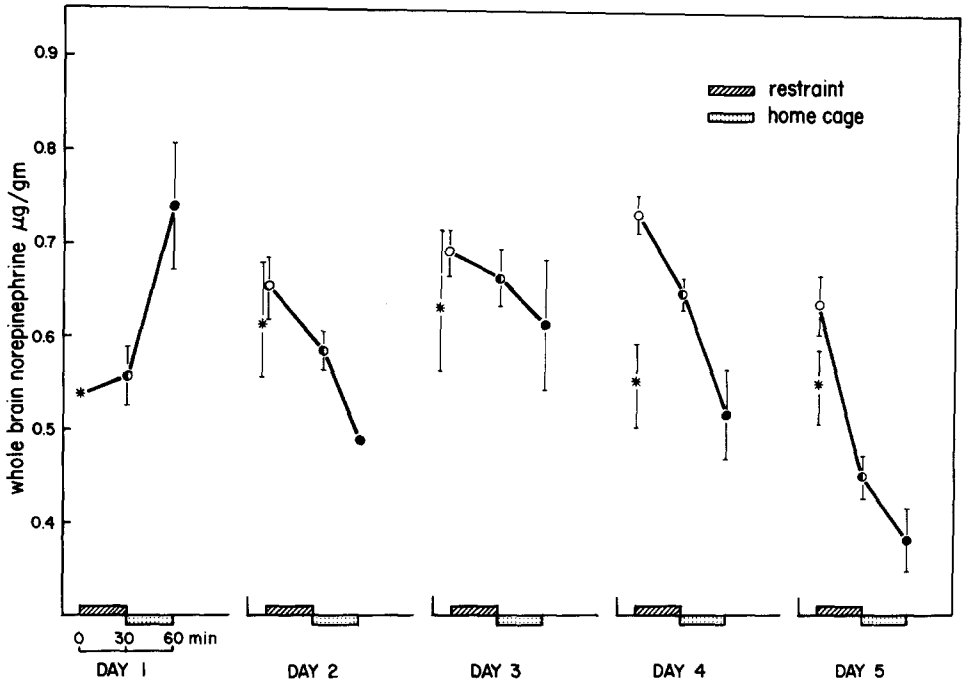


FIG. 4. Changes in whole brain norepinephrine of rats restrained for 30 min once a day for 5 consecutive days. For details see legend of Fig. 3.

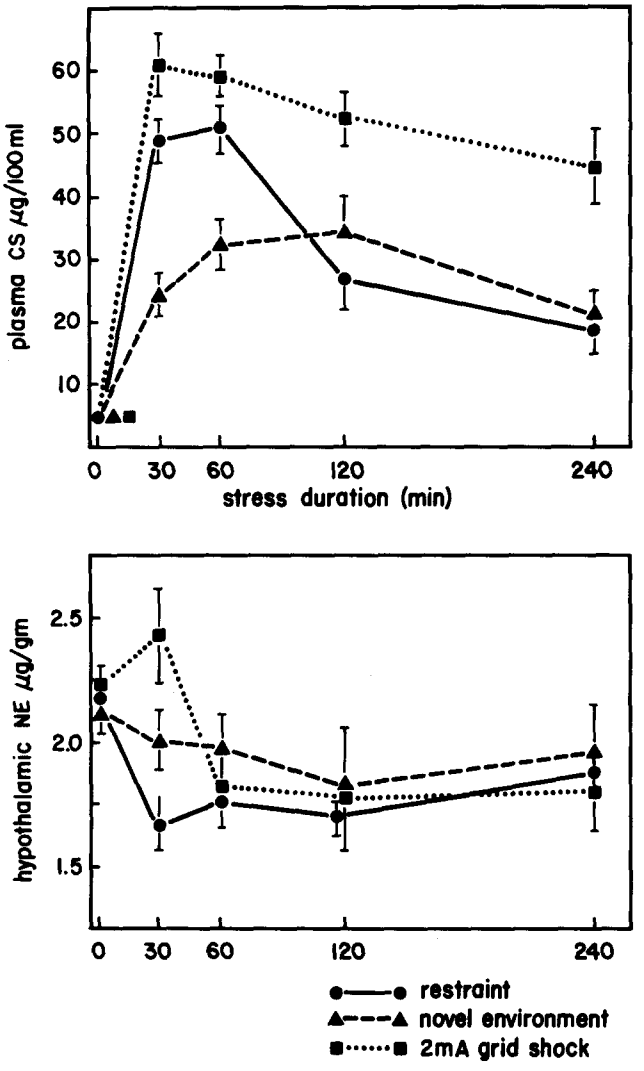


FIG. 5. A comparison of the time course of stress-induced alteration in plasma corticosterone (top) and hypothalamic norepinephrine (bottom) in response to grid shock, restraint, and novel environment. Each point represents a mean of eight animals; vertical bars indicate standard error of the mean.

## DISCUSSION

The basal concentration of plasma CS and its increase in response to restraint stress is similar in the five strains of laboratory rats studied. However, differences in post-stress recovery values were revealed in the present study. Our data indicate that the disposition of the stress-induced CS in Hooded and Fisher rats is somewhat faster than in the Sprague-Dawley and Wistar strains.

The SH rat and its normotensive back breed, the WKY strain, react to restraint with a rise in plasma CS surpassing that of all other strains. This suggests that the stress-induced CS hyperreactivity is inherent in the WKY strain and not directly related to the genetically elevated blood pressure of the SH rat. Hallback and Folkow [11] have demonstrated that the SH rats exhibit exaggerated cardiovascular responses when compared to normotensive control rats after exposure to different levels of psychological stress. Furthermore, Pappas *et al.*, [20] have shown that SH rats are considerably more active than the normotensive Wistar rat in the open field procedure and activity wheels; although emotionality (defined by an open field defecation score) was less in the SH rat.

Sprague-Dawley rats, rendered hypertensive with DOCA, unilateral nephrectomy and salt loading respond to restraint with a greater increase in plasma CS than their normotensive counterparts. On the other hand, WKY rats made hypertensive by the same procedure have an elevated baseline CS level and respond to restraint with a significantly smaller CS release. It appears that the two strains, when submitted to unilateral nephrectomy, DOCA and salt loading, handle basal steroid elaboration and, consequently, stress-induced feedback differently.

Changes in the biochemical responses to increasing durations of restraint reach a peak during the first hour. Hypothalamic and endbrain NE decrease markedly after 30 min of restraint and remain below control levels throughout the 4 hr restraint period. On the other hand, brainstem NE does not change significantly from control throughout the 4 hr restraint. The turnover of whole brain NE or NE in certain cerebral areas, particularly the hypothalamus is increased after a variety of distressing procedures such as muscular exertion [23], environmental temperature changes [22], or electric foot shock [3]. Increases in plasma CS and a diminution of hypothalamic NE have been observed as a consequence of many acute stressful stimuli [8,13]. Thierry [25] has reported decreased central NE levels under the influence of electroshock and immobilization. These authors have suggested that this indicates an increased release of NE which cannot be fully compensated for by increased synthesis. Our findings support the notion that the release of catecholamines from adrenergic terminals in the hypothalamus and endbrain is occurring at a rate faster than their synthesis. On the other hand, the maintenance of the steady state levels of NE in the brainstem is indicative of increased synthesis in the adrenergic cell bodies of this area. Similar conclusions have been reached by Bliss *et al.*, [3].

The stress-induced increase in hypothalamic DA defies a ready physiological interpretation. Several investigators [3,25] have found that DA turnover is increased in electroshock. Chronic restraint of rats has been reported to cause a fall in central DA levels [6]. In contrast, turnover in the nigrostriatal DA neurons is not changed by immobilization stress [5]. While tubero-infundibular DA neurons (which are included in our hypothalamic sample) are believed not to be involved in the regulation of ACTH secretion [7], hypothalamic DA has been shown to play a role in the control of other central endocrine systems [28].

The activation of the adrenal during restraint is reflected by an increase in plasma CS, an immediate alteration in NE and EPI content, and increased stimulation of in vitro CS production by exogenous ACTH. If restraint is continued for periods longer than 60 min, plasma CS, adrenal catecholamines and in vitro adrenal reactivity return toward control levels. We have not observed a decline in adrenal epinephrine during 4 hr of restraint as reported by Kvetnansky and Mikulaj [14], although our findings of essentially unchanged adrenal NE during prolonged immobilization are in close agreement.

The increase in the reactivity of in vitro adrenal glands derived from rats restrained for 60 min is the effect of exogeneously applied ACTH upon an adrenal already

primed by the endogenous ACTH released by the stressor. Adrenal cyclic AMP is activated to over 2000% by 30 min of restraint but is no longer significantly different from controls 150 min after commencement of the stressor [21]. The adrenal cAMP time course during restraint, therefore, parallels that of plasma corticosterone. Since the reactivity of the adrenal cortex to exogenous ACTH is at control values after 2–4 hr of restraint, the near control concentrations of cAMP and plasma corticosterone at this time reflect an adaptive mechanism which may be due to a diminished output of ACTH.

A physiological adaptation during a 4 hr restraint is most visible in regard to motor activity which is pronounced only during the first 30 to 60 min of restraint. Although we have observed an increase in rectal temperature, other investigators [26] have reported a decrease of body temperature in restrained rats. Cardiovascular homeostasis appears to be maintained for two hours. After 4 hr of restraint, however, the blood pressure was diminished.

In experiments in which rats were restrained daily for 5 days, stress-induced plasma CS concentrations progressively decreased following an enhanced response on Day 2. The resting level of the circulating steroid was significantly increased by Day 5, while the stress-induced level was significantly attenuated. During the 5-day restraint, stress-evoked depletion of whole brain NE progressively increased; on Days 4 and 5 the resting brain NE was elevated. This depletion may reflect changes characteristic of a chronic stress situation which has been shown to induce greater activation of NE turnover than exposure to a single stress session [25]. Furthermore, chronic stress has been shown to increase central tyrosine hydroxylase activity [19]; these changes are reflected in prolonged elevations of the total amount of stored catecholamine [24]. However, these findings in whole brain NE do not necessarily refer to hypothalamic aminergic substrates which have been implicated in the regulation of the secretion of ACTH.

A comparison of three different stressors i.e., electric foot shock, restraint and novel environment, indicates that there are differences in regard to the time course and direction of the responses. It is, however, admittedly difficult to establish criteria which permit the study of these three stressors at equal intensity. The peak of the CS response to novel environment is reached later and is less than that of the other type of stressor. The subsequent decline of plasma CS during restraint of longer duration is not nearly as marked with electroshock as stressor. After 30 min of exposure to a stressor there is a divergence of hypothalamic NE: a significant diminution during restraint, an increase during electric foot shock and an insignificant decline during exposure to a novel environment. However, after 60 to 240 min all three stressors lower hypothalamic NE, not differing significantly from each other.

There are two observations from this study which do not sustain the notion that plasma CS levels are inversely related to central catecholamine content. First, the resting post-stress levels of both brain NE and plasma CS increase during the progression of the 5-day stress. Secondly, during restraint episodes, the large increase in plasma CS on Day 1 is accompanied by an insignificant change in whole brain NE, whereas on Day 5 a small rise in plasma CS is accompanied by a large diminution in central NE. While hypothalamic NE responds with a significant reduction concomitant with a rise in plasma CS there is no evidence, at least from these physiological experiments, that plasma CS is causally related to whole brain catecholamine levels. These conclusions are consistent with those of DeSchaepdryver *et. al.* [6].

The possibility that the stress-induced depletion of brain NE, in increasing the transmitter at the receptor site, may be involved in release of ACTH is not evident when using the whole brain preparation. The ratio between the different brain amines, their rates of synthesis and/or release, as well as their roles in different areas of the brain, may better characterize the role of monoamines in the stress response. In this regard, it is interesting to note that selective manipulation of brain amines has been associated with the differential release of the gonadotrophins [1]. Furthermore, the central amines may be selectively involved in various states of adrenocortical function, i.e., the maintenance of resting cyclic patterns, the elaboration of stress induced steroid changes or the control of steroid feedback.

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