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The Effect of Acute, Chronic and Chronic Intermittent Stress on the Central Noradrenergic System

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HELLRIEGEL E. T. AND A. P. D'MELLO. *The effect of acute, chronic, and chronic intermittent stress on the central noradrenergic system.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 207–214. 1997.—The objective of this investigation was to examine the immediate and long term effects of acute, chronic and chronic intermittent stress on the central noradrenergic system of rats. Male Sprague–Dawley rats were subjected to one hour of physical immobilization stress either as a single exposure, or as 14 exposures applied either on consecutive days, or randomly over 60 days. Animals were sacrificed immediately, 6 h and 24 h following the last stressor. Levels of norepinephrine (NE) and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-sulfate) were measured in the hypothalamus, hippocampus, cerebral cortex and locus coeruleus region and β-adrenergic receptor (BAR) density was determined in the cortex. Immediately after acute stress, a significant reduction in hypothalamic NE levels and marked increases in MHPG-sulfate levels in all four brain regions were observed. In contrast, immediately after the last stressor of a chronic or chronic intermittent stress regimen, no change in NE concentration was observed while levels of MHPG-sulfate in the four brain regions showed a smaller increase than that observed after an acute stressor. Acute stress induced changes normalized within 6 h while chronic and chronic intermittently stressed animals had altered NE or MHPG-sulfate levels in certain brain regions for up to 6–24 h. Cortical BAR binding parameters remained unchanged after all stress paradigms. © 1997 Elsevier Science Inc.

Norepinephrine 3-methoxy-4-hydroxyphenylethyleneglycol sulfate β-adrenergic receptors Acute stress Chronic stress Stress intermittency

STRESS has been shown to be a predisposing factor to certain types of human depression (26). A number of behavioral parallels have been observed between humans diagnosed as clinically depressed and laboratory animals that have been exposed to uncontrollable stressors (47). In view of these similarities, the neurochemical alterations associated with stress-induced behavioral depression have been the subject of considerable interest. Evidence has accumulated to suggest that brain monoamines and their receptors play a role in mediating an organism's response to stress (1,14) and predisposing it to stress pathology and consequent behavioral depression (3). We are interested in examining the effect of different stress paradigms on central norepinephrine (NE) turnover, as measured by changes in the levels of NE and its major neuronal metabolite 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-sulfate), and on the status of cortical β-adrenergic receptors (BAR's).

Exposure of animals to a variety of acute uncontrollable stressors results in a depletion of brain NE and an increase in the levels of MHPG-sulfate (13,30,32,44). These stress induced alterations are associated with transient behavioral and functional deficits in animals (4,5,48). In contrast, animals subjected to a chronic regimen of stress do not demonstrate a depletion of brain NE (20,24), and also, interestingly, show a progressive reduction in behavioral deficits (43,49). In fact, at longer time intervals following chronic stress exposure NE concentrations may actually exceed those observed in non-stressed control animals (40). This increase in NE concentrations has been attributed to a chronic stress induced increase in the activity of tyrosine hydroxylase (TH), the rate limiting enzyme in the biosynthesis of NE. However, the increase in NE concentrations could also be due to a chronic stress induced decrease in its utilization. However, this hypothesis has not been well examined, since very few studies have measured changes in MHPG-sulfate levels after chronic stress.

It has been shown that stressors administered to animals

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chronically, but on an unpredictable and intermittent basis result in long term functional deficits (22,23). Furthermore, intermittency in the application of stimuli has often been associated with sensitization of a number of pharmacologic and neurophysiologic responses (37). However, to the best of our knowledge, the effects of chronic intermittent stress on the central noradrenergic system have not been examined in detail. It is conceivable that intermittent application of stress might result in sustained alterations in NE and MHPG-sulfate levels.

The majority of studies exploring the effects of acute and chronic stress paradigms have only investigated biochemical changes immediately after stressor termination. However, it is changes in steady-state levels of biochemical parameters that probably reflect dysfunction of homeostatic mechanisms. It is these changes that may be responsible for long term stress-induced behavioral deficits. Surprisingly, the effects of stress on changes in steady-state levels of NE and MHPG-sulfate have not been comprehensively examined. Therefore, the objective of this investigation was to determine and compare both the immediate and long term effects of acute, chronic and chronic intermittent regimens of stress on the status of cortical BAR's, and on the levels of NE and MHPG-sulfate, in the hypothalamus, LC-region, hippocampus and cerebral cortex of the rat.

MATERIALS AND METHODS

Chemicals

NE bitartrate, MHPG-sulfate potassium salt, 3,4-dihydroxybenzylamine hydrobromide (DHBA), vanillic acid and (−)-isoproterenol were obtained from Sigma Co. (St. Louis, MO). [125I]-(−) Iodopindolol (2200 Ci/mmol) was obtained from New England Nuclear (Boston, MA). All other chemicals were of analytical grade and purchased from commercial sources (Fisher Scientific, Pittsburgh, PA, Baxter Healthcare Corp., Edison, NJ).

Animals

All experiments were conducted in male, Sprague–Dawley rats (Ace Animal Inc., Boyertown, PA) weighing 400–450 g. Animals were housed two per cage (240 × 400 × 200 mm standard plastic cage containing wood shavings) under a 12 L:12D cycle (lights on 0600, off 1800) and allowed free access to food and water. All animals were handled on each day to normalize for possible neurochemical changes associated with handling.

Stress Procedure

Immobilization stress was applied as previously described (15).

Experimental Procedure

Examination of the time course of noradrenergic neurochemical changes following exposure of rats to acute, chronic and chronic intermittent physical immobilization stress. Groups of 18 to 24 rats were randomly assigned to control, acute, chronic or chronic intermittent stress groups. Acutely stressed animals were subjected to a single session of 1 h physical immobilization stress. Chronically stressed animals were administered 14 sessions of 1 h physical immobilization stress on 14 consecutive days. Chronic intermittently stressed animals were administered 14 sessions of 1 h immobilization stress on a variable schedule over a period of 60 days. The inter-stress interval ranged from 1–7 days with a mean interval of 3.5 days. On the final day of the experiment a subset of rats from each group was sacrificed by decapitation immediately after exposure to the last stressor. The remaining rats were released from immobilization, returned to their home cages and decapitated 6 h or 24 h later. Control rats were decapitated along with the treatment rats at each of the three time points.

In all experiments, immediately after animals were sacrificed their brains were removed and rapidly dissected on ice into the following regions: hypothalamus, hippocampus and cerebral cortex according to the method of Gispen et al. (12). The LC-region was dissected according to the method of Reis and Ross (38). Brain tissues were immediately frozen on solid CO_2 and stored at −70°C pending analysis of NE, MHPG-sulfate and cortical BAR's.

Biochemical Determinations

NE and MHPG-sulfate. Regional brain concentrations of NE and MHPG-sulfate were determined using HPLC with electrochemical detection as previously described (15).

Cortical β-adrenergic receptors. BAR binding in cortical homogenates was determined using [^{125}I]-(−) Iodopindolol (IPIN) as previously described (7). The dissociation constant (K_d) and receptor density (B_{max}) were determined from Scatchard plots of the binding data. B_{max} and K_d values are expressed in units of fmol/mg protein and pM, respectively. Protein was measured by the method of Lowry et al. (27).

Statistical Analysis

Results are expressed as the mean ± SEM of 6–10 rats. Due to logistical considerations, the study was conducted in two phases. In the first phase we examined the time course of changes in rats following chronic and chronic intermittent stress, and the effects of acute stress were examined in the second phase.

The levels of NE and MHPG in control animals in the two phases of the study showed small but statistically significant differences. Consequently, actual concentration values could not be used to evaluate differences between the three treatments. Therefore, using actual concentration values, we initially evaluated the main effects of each of the three treatments versus the appropriate control at the three time points (0, 6, 24 h) using a one-way ANOVA (chronic and chronic intermittent stress versus control) or a Student-*t* test (acute stress versus control). We then expressed NE and MHPG-sulfate levels and BAR binding parameters for each of the three stress paradigms at the three time points as a percent of their respective control values and analyzed the data using a two-way ANOVA (factors of treatment and time). Wherever the two-way ANOVA indicated a significant treatment effect, time effect or treatment × time interaction a subsequent one-way ANOVA was carried out. Multiple comparisons were conducted using a Student–Newman–Keuls' procedure. All statistical tests were conducted at a 0.05 level of significance.

RESULTS

Hypothalamus

Figure 1A shows hypothalamic NE levels of rats immediately, 6 and 24 h following acute, chronic and chronic intermittent exposure to physical immobilization stress. Immediately

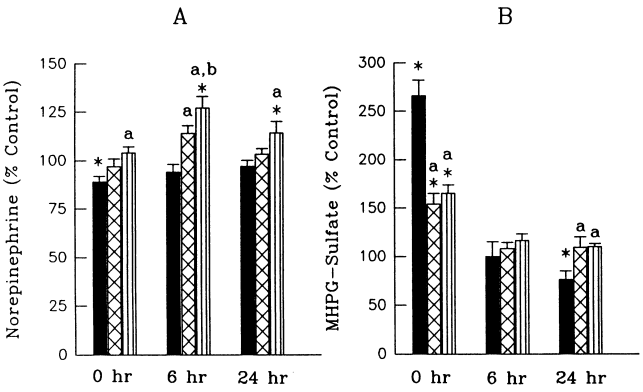

FIG. 1. Changes in the levels of hypothalamic NE (A) and MHPG-sulfate (B) immediately, 6 and 24 h following acute, chronic and chronic intermittent stress (solid bars: acute stressed; crosshatch bars: chronic intermittent stressed; vertical bars: chronic stressed). Data are presented as percent of controls, and represent mean ± SEM of 8 rats in each treatment group at each time point. NE levels in control animals, expressed as ng/g wet weight of tissue, are 3913 ± 91 (mean ± SEM) at 0 and 24 h and 3454 ± 163 at 6 h. MHPG-sulfate levels in control animals, expressed as ng/g wet weight of tissue, are 135 ± 7 (mean ± SEM) at 0 and 24 h and 126 ± 8 at 6 h. *Significantly different from respective non-stressed control group. [a]Significantly different from acute stress group. [b]Significantly different from respective treatment group at $t = 0$.

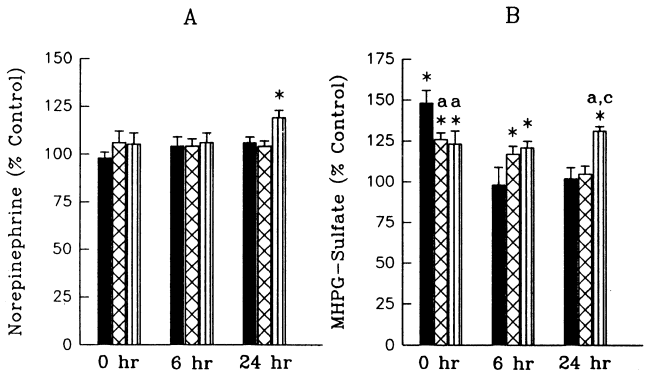

FIG. 2. Changes in the levels of LC-region NE (A) and MHPG-sulfate (B) immediately, 6 and 24 h following acute, chronic and chronic intermittent stress (solid bars: acute stressed; crosshatch bars: chronic intermittent stressed; vertical bars: chronic stressed). Data are presented as percent of controls, and represent mean ± SEM of 6–8 rats in each treatment group at each time point. NE levels in control animals, expressed as ng/g wet weight of tissue, are 1653 ± 46 (mean ± SEM) at 0 and 24 h and 1699 ± 67 at 6 h. MHPG-sulfate levels in control animals, expressed as ng/g wet weight of tissue, are 178 ± 9 (mean ± SEM) at 0 and 24 h and 187 ± 9 at 6 h. *Significantly different from respective non-stressed control group. [a]Significantly different from acute stress group. [c]Significantly different from chronic intermittent stress group.

after acute stress exposure, NE levels were significantly decreased compared to controls ($t = 2.63$; $p < 0.05$). However, within 6 h after the stressor, levels of NE had returned to control and no further change was observed at 24 h. Rats exposed to chronic stress showed no change in NE levels immediately following stress exposure. However, in this paradigm, levels of NE were significantly greater than control animals at 6 h (ANOVA, $F_{2,21} = 7.45$; $p < 0.01$) and 24 h (ANOVA, $F_{2,23} = 3.81$; $p < 0.05$) after the last stressor. At all times after chronic intermittent stress NE levels were not different from control.

Two-way analysis of variance of hypothalamic NE concentrations (expressed as percent of control) demonstrated a significant effect of treatment (ANOVA, $F_{2,63} = 19.92$; $p < 0.0001$) and time (ANOVA, $F_{2,63} = 9.54$; $p < 0.001$). Subsequent one-way analysis of variance for treatment effects showed that NE levels in acutely stressed rats were significantly lower than those of chronically stressed rats immediately after the stressor (ANOVA, $F_{2,21} = 4.41$; $p < 0.05$). At 6 h, significantly higher NE levels were observed in chronic (ANOVA, $F_{2,21} = 12.42$; $p < 0.001$) and chronic intermittent (ANOVA, $F_{2,21} = 12.42$; $p < 0.001$) stressed animals as compared to those seen in acutely stressed animals. Twenty-four h after the last stressor NE levels in chronic intermittently stressed animals were not different from acutely stressed animals. Interestingly, at this time, levels of NE in chronically stressed animals were still higher than those in acutely stressed animals (ANOVA, $F_{2,21} = 4.10$; $p < 0.05$). One-way analysis of variance for the effect of time showed that NE levels in chronically stressed rats at 6 h were not significantly different from those at 24 h, but were significantly greater than the levels observed immediately after stress exposure (ANOVA, $F_{2,21} = 5.03$; $p < 0.05$).

Figure 1B shows changes in the levels of hypothalamic MHPG-sulfate of rats immediately, 6 and 24 h following acute, chronic and chronic intermittent stress. MHPG-sulfate levels were significantly increased immediately after exposure of rats to acute stress ($t = -9.64$; $p < 0.001$), or immediately after the last stress of a chronic or chronic intermittent (ANOVA, $F_{2,23} = 17.81$; $p < 0.0001$) stress paradigm when compared to the corresponding control group. In acutely stressed rats, metabolite levels returned to control within 6 h post-stress, and actually fell below levels of non-stressed control animals 24 h after stress exposure ($t = 2.15$; $p < 0.05$). MHPG-sulfate levels of chronic and chronic intermittently stressed rats returned to control within 6 h, and no further change was observed at 24 h.

Two-way analysis of variance of hypothalamic MHPG-sulfate levels (expressed as percent of control) showed a significant effect of treatment (ANOVA, $F_{2,63} = 8.75$; $p < 0.001$), time (ANOVA, $F_{2,63} = 81.93$; $p < 0.0001$) and treatment × time interaction (ANOVA, $F_{4,63} = 16.35$; $p < 0.0001$). Subsequent one-way analysis of variance for treatment effects revealed that immediately after the stressor MHPG-sulfate levels in acutely stressed rats were significantly higher than those in chronic and chronic intermittently stressed rats (ANOVA, $F_{2,21} = 24.71$; $p < 0.0001$). However, 24 h after stress exposure, metabolite levels in acutely stressed animals were significantly lower than in chronic and chronic intermittent stressed animals (ANOVA, $F_{2,21} = 5.44$; $p < 0.05$). One-way analysis of variance for time effects revealed that MHPG-sulfate levels were significantly higher immediately after exposure of rats to acute stress (ANOVA, $F_{2,21} = 59.15$; $p < 0.0001$), or immediately after the last stress of a chronic (ANOVA, $F_{2,21} = 18.66$; $p < 0.0001$) or chronic intermittent stress paradigm (ANOVA, $F_{2,21} = 7.29$; $p < 0.01$) when compared to their respective treatment groups at 6 and 24 h post-stress.

LC-Region

Changes in the levels of NE and MHPG-sulfate in the LC-region of rats immediately, 6 and 24 h following acute, chronic and chronic intermittent stress are illustrated in Fig. 2. At all

times after the acute and chronic intermittent stress paradigms, NE concentrations were not significantly different from control. Rats exposed to chronic stress showed NE levels that were not significantly different from control immediately and 6 h after stress, but were significantly greater than control animals at 24 h after the last stressor (ANOVA, $F_{2,23} = 9.74$; $p < 0.001$).

As shown in Fig. 2B, MHPG-sulfate levels were significantly increased immediately after exposure of rats to acute stress ($t = -4.89$; $p < 0.0001$) and immediately after the last stress of a chronic and chronic intermittent stress paradigm (ANOVA, $F_{2,23} = 6.41$; $p < 0.01$) when compared to the corresponding control group. However, within 6 h after acute stress, levels of MHPG-sulfate had returned to control and no further change was observed at 24 h. In contrast, chronic intermittently stressed rats showed sustained increases of metabolite levels for up to 6 h post-stress (ANOVA, $F_{2,19} = 5.00$; $p < 0.05$) when compared to control, but levels were not different from control at 24 h post-stress. Chronically stressed rats showed significant increases both at 6 (ANOVA, $F_{2,19} = 5.00$; $p < 0.05$) and 24 h post-stress (ANOVA, $F_{2,23} = 12.17$; $p < 0.001$) when compared to control animals.

Two-way analysis of variance of MHPG-sulfate levels (expressed as percent of control) indicated a significant time effect (ANOVA, $F_{4,61} = 8.89$; $p < 0.001$) and treatment × time interaction (ANOVA, $F_{4,61} = 6.03$; $p < 0.001$). One-way analysis of variance for treatment effects showed that the MHPG-sulfate levels in acutely stressed rats were significantly higher than those of chronic and chronic intermittently stressed rats immediately after the stressor (ANOVA, $F_{2,21} = 4.28$; $p < 0.05$). However, at 6 h post-stress, MHPG-sulfate levels were not significantly different between the three stress paradigms. Surprisingly, at 24 h, chronically stressed animals showed significantly higher MHPG-sulfate levels as compared to those seen in acutely and chronic intermittently stressed animals (ANOVA, $F_{2,21} = 9.24$; $p < 0.01$). One-way analysis of variance for time effects showed that MHPG-sulfate levels were significantly higher immediately after exposure of rats to acute stress as compared to levels in that group at 6 and 24 h post-stress (ANOVA, $F_{2,21} = 10.20$; $p < 0.001$). Also, in chronic intermittently stressed rats, MHPG-sulfate levels were significantly higher immediately after stress exposure than at 24 h post-stress (ANOVA, $F_{2,21} = 4.25$; $p < 0.05$).

Hippocampus

As demonstrated in Fig. 3A, stress had no effect on hippocampal NE levels. These levels were similar to controls at all times after the three stress paradigms.

As shown in Fig. 3B, hippocampal MHPG-sulfate concentrations were significantly increased immediately after exposure of rats to acute stress ($t = -4.69$; $p < 0.0001$), and immediately after the last stress of a chronic or chronic intermittent stress paradigm (ANOVA, $F_{2,23} = 8.77$; $p < 0.01$) as compared to controls. However, in all three stress paradigms, MHPG-sulfate levels returned to control within 6 h, and no further change was observed at 24 h post-stress.

Two factor analysis of variance of hippocampal MHPG-sulfate levels (expressed as percent of control) demonstrated a significant effect of time (ANOVA, $F_{2,62} = 28.91$; $p < 0.0001$) and treatment × time interaction (ANOVA, $F_{4,62} = 5.80$; $p < 0.001$). Subsequent one-way analysis of variance of treatment effects revealed that MHPG-sulfate levels immediately after exposure of rats to acute stress were significantly greater than those observed immediately after chronic and chronic intermittent stress (ANOVA, $F_{2,21} = 8.51$; $p < 0.01$). One-way analysis of variance for time effects revealed that MHPG-sulfate levels were significantly higher immediately after exposure of rats to acute stress (ANOVA, $F_{2,21} = 19.98$; $p < 0.0001$), or immediately after the last stress of a chronic (ANOVA, $F_{2,21} = 7.12$; $p < 0.01$) or chronic intermittent stress paradigm (ANOVA, $F_{2,20} = 4.75$; $p < 0.05$) when compared to their respective treatment groups at 6 and 24 h post-stress.

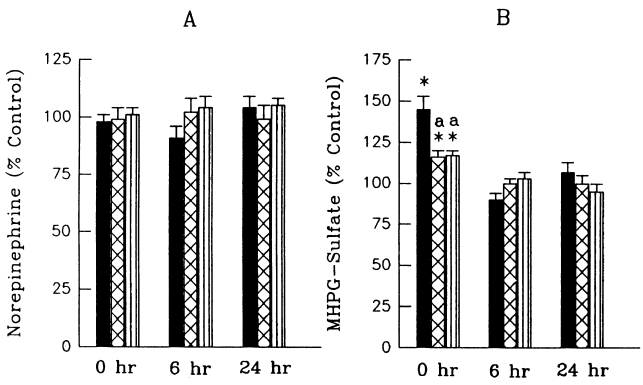

FIG. 3. Changes in the levels of hippocampal NE (A) and MHPG-sulfate (B) immediately, 6 and 24 h following acute, chronic and chronic intermittent stress (solid bars: acute stressed; crosshatch bars: chronic intermittent stressed; vertical bars: chronic stressed). Data are presented as percent of controls, and represent mean ± SEM of 7–8 rats in each treatment group at each time point. NE levels in control animals, expressed as ng/g wet weight of tissue, are 733 ± 29 (mean ± SEM) at 0 and 24 h and 719 ± 33 at 6 h. MHPG-sulfate levels in control animals, expressed as ng/g wet weight of tissue, are 100 ± 3 (mean ± SEM) at 0 and 24 h and 100 ± 5 at 6 h. *Significantly different from respective non-stressed control group. [a]Significantly different from acute stress group.

Cerebral Cortex

As shown in Fig. 4A, stress had no effect on cortical NE concentrations. These levels were similar to controls at all times after the three stress paradigms.

Figure 4B shows changes in the levels of cortical MHPG-sulfate immediately, 6 and 24 h after acute, chronic and chronic intermittent stress. MHPG-sulfate levels were significantly increased immediately after exposure of rats to acute stress ($t = -3.13$; $p < 0.01$), and immediately after the last stress of a chronic intermittent stress paradigm (ANOVA, $F_{2,23} = 5.08$; $p < 0.05$) as compared to the corresponding control group. However, within 6 h after the acute and chronic intermittent stress paradigms, metabolite levels returned to control and no further change was observed at 24 h. At all times after chronic stress MHPG-sulfate levels were not different from control.

Two-way analysis of variance of cortical MHPG-sulfate levels (expressed as percent of control) resulted in a significant time effect only (ANOVA, $F_{2,63} = 11.77$; $p < 0.0001$). Subsequent one-way analysis of variance for time effects showed that MHPG-sulfate levels were significantly higher immediately after exposure of rats to acute stress (ANOVA, $F_{2,21} = 5.89$; $p < 0.01$), or immediately after the last stress of a chronic intermittent stress paradigm (ANOVA, $F_{2,21} = 6.81$; $p < 0.01$) when compared to their respective treatment groups at 6 and 24 h post-stress.

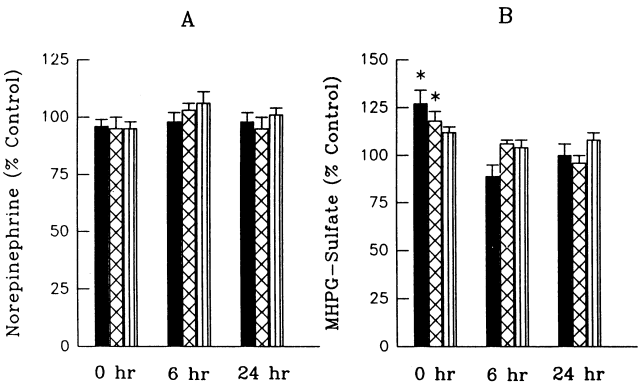

FIG. 4. Changes in the levels of cortical NE (A) and MHPG-sulfate (B) immediately, 6 and 24 h following acute, chronic and chronic intermittent stress (solid bars: acute stressed; crosshatch bars: chronic intermittent stressed; vertical bars: chronic stressed). Data are presented as percent of controls, and represent mean ± SEM of 8 rats in each treatment group at each time point. NE levels in control animals, expressed as ng/g wet weight of tissue, are 607 ± 23 (mean ± SEM) at 0 and 24 h and 562 ± 23 at 6 h. MHPG-sulfate levels in control animals, expressed as ng/g wet weight of tissue, are 106 ± 4 (mean ± SEM) at 0 and 24 h and 105 ± 5 at 6 h. *Significantly different from respective non-stressed control group.

Regional MHPG-Sulfate/NE Ratios

Changes in the regional MHPG-sulfate/NE concentration ratios immediately following exposure of rats to acute, chronic and chronic intermittent stress are shown in Fig. 5. Immediately after acute, chronic and chronic intermittent stress, MHPG-sulfate/NE concentration ratios in all four brain regions were significantly increased when compared to the corresponding control group. Furthermore, the increases in the MHPG-sulfate/NE ratios observed in acute stressed animals were significantly greater than those observed in chronic and chronic intermittently stressed animals in the hypothalamus (ANOVA, $F_{2,21} = 26.35$; $p < 0.0001$), LC-region (ANOVA $F_{2,21} = 5.18$; $p < 0.05$)and hippocampus (ANOVA, $F_{2,21} = 14.27$; $p < 0.0001$). In the cerebral cortex there was no difference in the MHPG-sulfate/NE concentration ratio between the three stress paradigms. In the three stress paradigms, MHPG-sulfate/NE concentration ratios in all four brain regions were similar to the corresponding control group at the 6 and 24 h time points (data not shown).

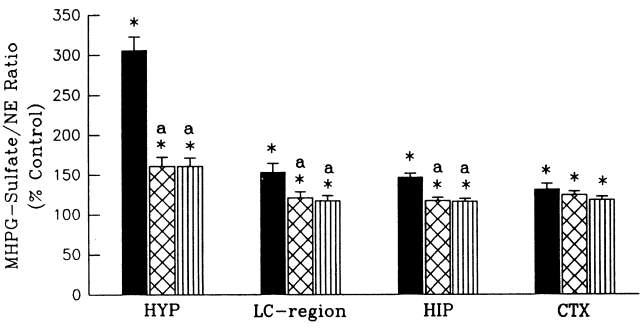

FIG. 5. Changes in the MHPG-sulfate/NE concentration ratio immediately following acute, chronic and chronic intermittent stress (solid bars: acute stressed; crosshatch bars: chronic intermittent stressed; vertical bars: chronic stressed). Data are presented as percent of controls, and represent mean ± SEM of 8 rats in each treatment group. *Significantly different from respective non-stressed control group. [a]Significantly different from acute stress group.

TABLE 1

RAT CORTICAL β-ADRENOCEPTOR BINDING PARAMETERS IMMEDIATELY AND 24 H FOLLOWING ACUTE, CHRONIC AND INTERMITTENT STRESS

Treatment	Time	B_{max} (% Control)	K_d (% Control)
Acute	0	98.0 ± 5	98.0 ± 2
	24	94.8 ± 2	97.0 ± 2
Chronic	0	97.2 ± 3	103 ± 3
	24	101 ± 2	96.5 ± 2
Chronic	0	99.0 ± 4	98.3 ± 1
intermittent	24	106 ± 3	103 ± 5

Data are presented as percent of controls, and represent mean ± SEM of 8 rats in each treatment group at each time point.

B_{max} values in control animals at 0 and 24 h, expressed as fmol/mg protein, are 46 ± 2 (mean ± SEM). K_d values in control animals at 0 and 24 h, expressed at pM, are 136 ± 4 (mean ± SEM).

Cortical BAR's

Table 1 shows cortical BAR binding parameters following exposure of rats to acute, chronic and chronic intermittent stress. Binding parameters were only determined immediately and 24 h after exposure to the last stressor and are expressed as a percent of control values. Immediately and 24 h after exposure of rats to acute, chronic and chronic intermittent stress the receptor density (B_{max}) and affinity constant (K_d) of rat cortical BAR's were not different from the corresponding control values. Furthermore, no significant difference was observed in B_{max} and K_d values of rat cortical BAR's between the three stress paradigms both immediately and 24 h after the last stressor.

DISCUSSION

In the present investigation we examined the immediate and long term effects of acute, chronic and chronic intermittent stress on the status of cortical BAR's and on the levels of NE and MHPG-sulfate in the hypothalamus, hippocampus, LC-region and cerebral cortex of the rat. Immediately after an acute physical immobilization stress, we observed decreases in hypothalamic NE levels, and significant increases in MHPG-sulfate levels in all four brain regions examined. These findings are consistent with a previous report from our laboratory (15), and with the findings of other investigators (13,17,30). The selective depletion of hypothalamic NE might be due to the preferential and higher utilization of NE from this brain region during the stressor (45) or might be due to the different source of NE in this region (11,28). NE levels in the hypothalamus returned to control values within 6 h after the acute stressor; a finding in agreement with the results of previous studies (18,40). However, some investigators have shown more sustained decreases in hypothalamic and LC region levels of NE probably due to the greater severity and longer duration of stressor used in their studies (48).

Hypothalamic, MHPG-sulfate levels were significantly decreased for up to 24 h after the acute stressor. Few studies

have examined the time course of changes in MHPG-sulfate content in the rat brain following acute stress. Our findings are in agreement with the results of another study which has reported decreases in MHPG-sulfate levels in the hippocampus and mid-brain for 6–24 h after an immobilization stressor (18). These results suggest that a single immobilization stressor resulted in sustained neurochemical activity in certain brain regions. Importantly, such activity would not be revealed by the measurement of NE levels alone.

Exposure of rats to chronic immobilization stress caused significant elevations in hypothalamic, hippocampal and LC-region MHPG-sulfate levels immediately after the last stressor, while NE concentrations in the four brain regions were similar to those observed in non-stressed control animals. These findings are consistent with the results of previous studies (2,20,21,24,36). The lack of change in brain NE levels is probably due, in part, to the reported induction of NE biosynthesis in these animals (42). We observed chronic stress induced increases in hypothalamic NE levels at 6 and 24 h after the last stressor and increases in LC region NE levels at 24 h after the last stressor. This increase in the LC region NE levels could be due to the sustained increases in TH activity induced by chronic stress (42). It is conceivable that 6 h after the last stressor of a chronic stress paradigm, synthesis of NE is enhanced in the LC-region. However, a substantial fraction of the synthesized NE is preferentially transported to terminal fields, rather than stored within the cell bodies themselves and could account for the lack of change in LC region NE levels at this time. Presumably, at 24 h after the last stress of a chronic stress regimen, a larger fraction of the NE synthesized in the LC-region is stored within its cell bodies, rather than axoplasmically transported to terminal fields. Therefore, the concentration of NE in the LC-region is increased at this time.

In the present investigation, chronic stress resulted in persistent increases in LC-region MHPG-sulfate content for up to 24 h post-stress, whereas levels of the metabolite returned to control values within 6 h in the hypothalamus and hippocampus. To the best of our knowledge, a time course of the changes in MHPG-sulfate content in these three brain regions after chronic stress has not been previously examined. The reason for the sustained increases in LC-region MHPG-sulfate content in chronically stressed rats is not clear. However, evidence exists which suggests that in rats the synthesis of neurotransmitters is tightly coupled to their release (9,10). Therefore, it is conceivable that the sustained increase in LC-region MHPG-sulfate content is related to the sustained increase in NE biosynthesis which occurs in the LC-region of chronically stressed rats (42).

Results of studies have shown that in animals exposed to stressful stimuli, re-exposure to a subthreshold intensity of stimuli causes marked changes in NE and MHPG-sulfate concentrations (6,8,19). A recent study has shown that the intermittent application of stress leads to greater changes in NE turnover than continous application of stress (41). Based on these findings we hypothesized that exposure of rats to stress on a chronic but intermittent basis would lead to sensitization of the NE and MHPG-sulfate responses to stress which would be reflected in sustained alterations in the levels of NE and MHPG-sulfate. However, such sustained effects were not observed. Instead, at all times after chronic intermittent stress NE levels were not different from control in any of the brain regions examined. In fact, contrary to our expectations, hypothalamic NE levels even showed a tendency toward increases at 6 h post-stress. In addition, significant increases in regional MHPG-sulfate levels were produced only immediately following chronic intermittent stress, except in the LC-region where metabolite levels were increased at 6 h after the last stressor. The reason for the inconsistency in findings between the present investigation and the aforementioned studies is not clearly understood. In our experimental design, the duration of individual stressors and cumulative period of stress in the chronic and intermittent paradigms is identical. However, the inter-stress interval and the different time periods over which the treatments were administered are covariates. Consequently distinct effects of each of these two variables could affect the interpretation of results. Such difficulties are inherent in the conduct of intermittent stress studies. The other study that examined the effect of intermittent stress (41), also maintained the cumulative period of stress a constant. However, the duration of the individual stressors, the inter-stress interval, the period over which the stressors were administered, and the number of stressors were all covariates. In an intermittent stress paradigm it is difficult to vary the inter-stress interval while maintaining the total duration of the treatments a constant.

The increase in brain NE levels after chronic stress have been attributed to a stress induced increase in the biosynthesis of NE which exceeds utilization. In support of this hypothesis, a number of investigators have shown greater increases in TH activity after chronic than after acute stress (25,31). In our studies, detailed measurements of MHPG-sulfate levels after the three stress paradigms have led us to propose an additional hypothesis to explain the difference in NE profiles after acute and chronic stress. The increases in hypothalamic, hippocampal and LC-region MHPG-sulfate levels immediately after acute stress were significantly greater than those observed in these three brain regions immediately after a chronic regimen of stress. Also, hypothalamic, hippocampal and LC-region MHPG-sulfate/NE concentration ratios were significantly greater in acute stressed animals than in chronic stressed animals. It is conceivable that the attenuated MHPG-sulfate responses observed in chronically stressed animals may reflect an additional mechanism in their response to stress, namely a decreased utilization of NE. In support of this hypothesis Pacak et al., using in vivo microdialysis, showed that the NE/dihydroxyphenylglycol (DHPG) concentration ratios in the paraventricular nucleus of the hypothalamus were greater in rats exposed to a single immobilization stress than in rats which were exposed to immobilization stress on seven consecutive days (35). In a subsequent report by the same group of investigators, significantly greater levels of microdialysate NE, MHPG and DHPG in the central nucleus of the amygdala were observed following acute stress than were observed following chronic stress (34). However in contrast to these findings, another group has shown higher hippocampal dialysate concentrations of NE in chronically stressed rats compared to acutely stressed rats (31). The discrepancy in findings could be due to differences in the nature of the stress paradigm used or due to the different brain regions sampled in the study.

As previously documented (33,43), rat cortical BAR binding parameters were unchanged immediately and 24 h after acute stress. In the present study, we had expected to see sustained decreases in the levels of brain NE in chronic intermittently stressed animals, and a consequent upregulation of cortical BAR's in these animals. However, as stated earlier, NE levels remained unchanged in all brain regions after a chronic intermittent regimen of stress. Therefore, it was not surprising that cortical BAR binding parameters were unchanged immediately and 24 h following chronic intermittent

stress. Exposure of rats to chronic stress had no effect on rat cortical BAR binding parameters. In contrast to our observations, previous reports have demonstrated small but significant reductions (33,43) and elevations (29) in the density of BAR's in a number of brain regions of chronically stressed rats. The discrepancy in findings might be due to differences in stress procedures. Alternatively, the inconsistency might be due to the lack of specificity of [^{3}H]dihydroalprenolol (16,39), the radioligand used to characterize BAR binding in these studies. In support of this latter suggestion, a recent study which used a stress paradigm similar to that used in the aforementioned studies, but used [^{125}I] IPIN (a more specific radioligand) to measure BAR density, did not detect a change in BAR number (46). It is therefore probable that chronic stress does not affect cortical BAR binding.

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