Pharmacology Biochemistry & Behavior, Vol. 26, pp. 653–659. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Variations of Norepinephrine Concentrations Following Chronic Stressor Application

HYMIE ANISMAN, JILL IRWIN, WAYNE BOWERS, PARDEEP AHLUWALIA AND ROBERT M. ZACHARKO

Department of Psychology, Carleton University, Ottawa, Ontario K1S 5B6 Canada

Received 5 May 1986

ANISMAN, H., J. IRWIN, W. BOWERS, P. AHLUWALIA AND R. M. ZACHARKO. *Variations of norepinephrine concentrations following chronic stressor application.* PHARMACOL BIOCHEM BEHAV **26**(4) 653–659, 1987.— Exposure to acute uncontrollable footshock increased utilization of central norepinephrine (NE), and in some brain regions, most notably the hypothalamus, a decline in amine concentrations was induced. Utilization of NE was likewise increased in mice exposed to footshock on 14 consecutive days, but the NE reduction was not evident, suggesting that the chronic stressor provoked a compensatory increase of amine synthesis. In mice that were decapitated 24 hr after the chronic shock regimen, NE concentrations exceeded those of nonshocked animals or mice decapitated immediately after the last shock session, possibly reflecting a sustained increase of amine synthesis. The altered NE utilization and concentrations associated with chronic footshock were evident irrespective of whether the stressor was applied on a predictable schedule or on an intermittent basis, although the former treatment was somewhat more effective in increasing concentrations and utilization.

Norepinephrine MHPG Stress Chronic

SEVERAL behavioral disturbances typically associated with acute uncontrollable stressors are not evident in animals that had been repeatedly exposed to the aversive stimuli [19, 22, 25]. Likewise, it has been demonstrated that the effects of acute stressors on central norepinephrine (NE) concentrations may be absent in animals exposed to a repeated stressor regimen [4, 13, 22]. It has been demonstrated that following acute stressor application the utilization and synthesis of NE are increased in several brain regions [4, 13, 21]. If the stressor is sufficiently severe or protracted, then the rate of amine utilization may exceed synthesis, resulting in net reduction of amine concentrations [4, 20, 22, 23]. It is thought that the reduction of NE concentrations may render the animal less well prepared to contend with environmental demands, thus permitting expression of behavioral deficits. With repeated stressor application a compensatory increase of amine synthesis is provoked, thereby assuring adequate amine supplies [4, 10, 13, 17, 22]. Indeed, following repeated stressor application NE concentrations may actually exceed those of nonstressed animals [8,17], and hence the organism may be better prepared to deal with environmental demands.

Although the alterations of NE turnover and concentrations associated with acute stressors of moderate severity are fairly transient [4,23], more protracted NE alterations are associated with a chronic stressor. For instance, it was reported that 24 hr following chronic stressor application NE concentrations exceeded those of nonstressed animals [17]. Such an effect may be due to a sustained increase of NE synthesis [11,21] as well as a reduction in the utilization of NE following stressor termination [17]. Indeed, the enhanced NE synthesis associated with chronic aversive stimulation was at least as pronounced 24 hr after stressor termination as immediately after such treatment [11]. Furthermore, it was reported that 24 hr after exposure to a chronic stressor regimen the disappearance of NE in animals treated with a tyrosine hydroxylase inhibitor was less marked than in nonstressed animals [17]. Thus, the possibility exists that repeated exposure to a stressor results in the rapid mobilization of amines in order to contend with the immediate demands placed upon the organism. However, upon stressor termination amine utilization rates decline, resulting in the conservation of amines in order to contend with impending stressors.

While a great deal is known about the variables which influence NE activity following acute stressor application, considerably less information is available concerning the factors that influence the effects of chronic stressors. For instance, the magnitude of the NE alterations are directly related to acute stressor severity, and it appears that the NE alterations are more readily provoked in some brain regions than in others [16,23]. It remains to be determined to what extent stressor severity influences the NE variations induced by a chronic regimen, and whether the rate of neurochemical adaptation is comparable across brain regions. Behavioral analyses have also indicated that the course of the adaptation may depend on the nature of the stressors employed. Although repeated exposure to shock was shown to result in behavioral adaptation [22,25], such an effect was not evident

TABLE 1

MEAN (±S.E.M.) CONCENTRATIONS OF NE (μg/g TISSUE) AS A FUNCTION OF THE NUMBER OF SHOCKS APPLIED AND THE TIME AFTER SHOCK TREATMENT

Shock Treatment		Time Following Shock (Hr) .01	0.25	1.0	3.0
		Hypothalamus			
No Shock	NE	1.181 ± 0.022			
60 Shocks	NE	0.978 ± 0.051*	1.064 ± 0.059*	1.181 ± 0.070	1.160 ± 0.033
180 Shocks	NE	1.043 ± 0.070*	1.033 ± 0.027*	1.125 ± 0.071	1.006 ± 0.178*
		Hippocampus			
No Shock	NE	0.281 ± 0.007			
60 Shocks	NE	0.241 ± 0.009*	0.268 ± 0.024	0.302 ± 0.019	0.286 ± 0.013
180 Shocks	NE	0.236 ± 0.021*	0.278 ± 0.005	0.294 ± 0.009	0.288 ± 0.015
		Cortex			
No Shock	NE	0.215 ± 0.009			
60 Shocks	NE	0.223 ± 0.018	0.181 ± 0.013	0.227 ± 0.013	0.204 ± 0.007
180 Shocks	NE	0.189 ± 0.005	0.218 ± 0.014	0.207 ± 0.080	0.210 ± 0.012

*$p<0.05$ relative to nonshocked animals.

in animals that had been exposed to a chronic regimen involving different types of stressors [6,8]. In effect, exposure to unpredictable and/or intermittent stressors may not permit adequate adaptation. Accordingly, animals exposed to such a treatment may ultimately be less well prepared to deal with subsequent environmental challenges.

The present investigation was undertaken to assess whether (a) increased NE concentrations associated with an uncontrollable stressor vary with the number of shock trials animals receive during each session, (b) the increased NE concentrations are, in fact, more pronounced 24 hr after stressor termination than immediately afterward, and (c) the course of the neurochemical adaptation is dependent on the intermittence of the stressor regimen.

METHOD

Subjects

Four experiments involved 72, 80, 32 and 77 naive, male, CD-1 mice obtained from Charles River (Canada) Ltd. at 50–55 days of age. Mice were acclimatized to laboratory conditions for 10–14 days before being employed as experimental subjects. For the duration of the experiment mice were individually housed and permitted free access to food and water.

Apparatus

Footshock was administered in four identical black Plexiglas chambers which measured 30×14×15 cm. The floor of each chamber was composed of 0.32 cm stainless steel rods spaced 1.0 cm apart (center to center), connected in series through neon bulbs. In addition, the end walls of each chamber were lined with stainless steel plates which were connected in series with the grid floor. Shock (150 μA, 60 Hz, AC) was delivered to the grid floor through a 3000-V source, thereby providing relatively constant current. Illumination in each chamber was reduced by a 0.63 cm red Plexiglas roof.

Procedure

Experiment 1 was conducted simply to assess the time course of the NE reduction associated with two levels of acute shock (60 vs. 180 shocks). Mice were individually placed in the shock chambers and exposed to either 0, 60 or 180 shocks of 6 sec duration over a 1.1 hr period. For the 60 shock group the stressor was applied at 60 sec intervals, while in the 180 shock condition footshock was applied at 16 sec intervals. Thus mice of each condition spent an equivalent amount of time in the shock chambers. Mice were decapitated either 0.01, 0.25, 1.0 or 3.0 hr after the shock session (n=6/group), brains were removed, dissected and frozen in liquid nitrogen. Tissue was stored at −70°C until subsequent fluorometric determination of norepinephrine.

In the second experiment the effects of chronic exposure to 60 or 180 shocks on NE concentrations were assessed. On each of 15 successive days mice were individually placed in the shock chambers for a 1.1 hr period. Independent groups of mice (n=10/group) received 0, 1, 3, or 15 shock sessions over a 15 day period. For groups that received 1 or 3 shock sessions the shock was applied only on the last day(s), and on the preceding days mice were exposed to the apparatus but not shocked. Half the mice received 60 shocks of 6 sec duration (150 μA) at intervals of 60 sec, whereas the remaining mice received 180 shocks of 6 sec duration at 16 sec intervals. The possibility existed that the adaptation would be most apparent when animals received a relatively large number of shocks throughout the chronic regimen, but challenged on the day of decapitation with a smaller number of shock presentations. Accordingly, two additional groups were included in the experiment which were exposed to 3 and 15 days of shock, respectively. These groups received 180 shocks on each day, except for the last day when only 60 shocks were delivered. Immediately after the last shock session mice were decapitated, brains removed, dissected and frozen for subsequent NE determination.

Experiment 3 was conducted to assess NE concentrations

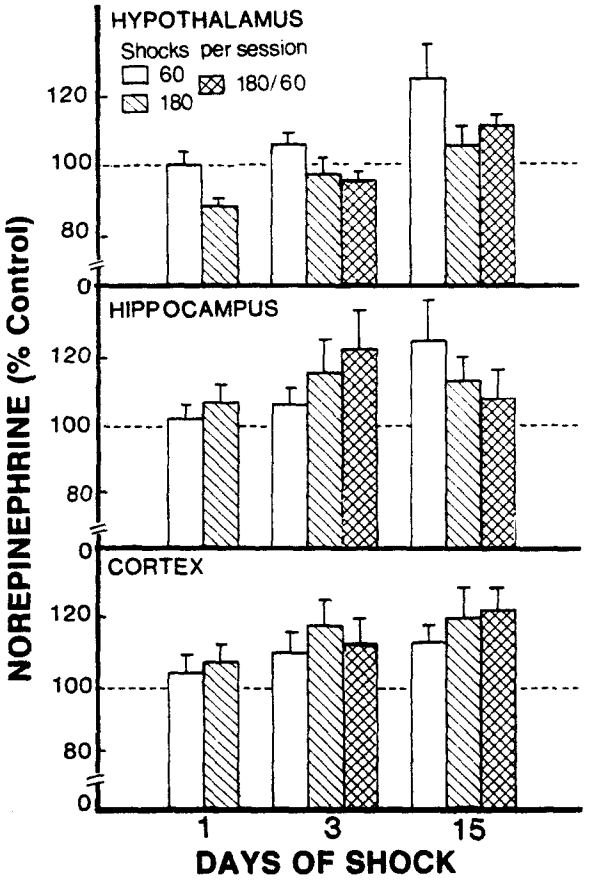

FIG. 1. Concentrations of hypothalamic, hippocampal and cortical NE as percentage of control values (±S.E.M.) in mice exposed to inescapable footshock on either 1, 3 or 15 days. Mice received either 60 (solid bars) or 180 (hatched bars) shocks during each shock session (150 μA, 6 sec duration). Two additional groups (cross hatched bars) received 180 shocks throughout the course of the shock regimen, except for the last day when only 60 shocks were delivered.

immediately and 24 hr following a chronic shock regimen. Mice were randomly assigned to four treatment conditions (n=8/group). One group of mice received 180 shocks (6 sec duration, 150 μA at 16 sec intervals) on each of 14 successive days. Twenty-four hours following the last stress session mice were decapitated and brains removed and dissected as in Experiment 1. A second group received the same shock treatment; however, prior to decapitation on the fifteenth day mice received a session of 60 shocks (6 sec duration, 150 μA at 60 sec intervals). The third group received handling on each of 14 days, and on the fifteenth day exposed to a single session of 60 shocks (150 μA) at 60 sec intervals. The fourth group served as a handling control and was not exposed to shock. Immediately thereafter mice were decapitated and tissue dissected for subsequent amine determination. As in Experiment 1 tissue was stored at −70°C until subsequent fluorometric determination of NE concentrations.

Experiment 4 was conducted to evaluate the effects of chronic intermittent shock on NE concentrations and on the accumulation of the NE metabolite, MHPG. Earlier studies had indicated that the use of long duration shocks (6 sec) such as those used in the preceding studies favored the development of a learned immobility response [7], which could ostensibly influence the perceived controllability of the stressor, and thus influence NE turnover. In order to minimize the probability of learned responses being established each shock session involved 360 shocks of 2 sec duration [3,7]. Earlier studies conducted in this laboratory had confirmed that a single shock session involving these parameters led to a reduction of central NE concentrations, while adaptation resulted from repeated predictable shock [8]. As in the preceding experiments mice were individually housed and assigned to 4 treatment conditions. Unlike the preceding experiments the treatment procedures were applied over a 60, rather than a 15 day period. Mice of one group were exposed to the shock treatment (360 shocks, 2 sec duration, 150 μA) only on the last day of the experiment. Mice of the second group were exposed to the shock on the last 15 days, otherwise these animals, like those of the first group, were left undisturbed in their cages, except to change the cage bedding at 7 day intervals. Mice of the third group likewise received 15 shock sessions; however, this procedure was not applied on successive days. Rather, shock sessions were applied on a variable schedule averaging every fourth day (range=1–7 days). In each of the preceding groups placement in the apparatus always occurred at the same time of day (i.e., between 1000 and 1200 hr). Mice of the fourth group were treated like animals in the second group, except that placement in the apparatus occurred at various times of the day on a random schedule (0900–1800 hr). Four additional groups were handled and placed in the apparatus just as the shock groups had been. However, the shock treatment was not delivered. The final session for all groups was applied on Day 60 between 1000 and 1200 hr. Thus, differences between groups could not be attributed to the time of day at which the last treatment was applied. Immediately after the last session mice were decapitated, brains dissected and frozen. Tissue was stored at −70°C for subsequent determination of NE and MHPG using the HPLC procedure of Lasley *et al.* [14].

Amine Determinations

In Experiments 1–3 tissue was homogenized in acidified butanol, and NE extracted according to the procedure of Maickel *et al.* [15]. A fluorescent derivative was then prepared using a minor modification of the method of Richardson and Jacobowitz [17], and the resulting fluorescence was measured using an Aminco-Bowman spectrofluorometer.

In Experiment 4 high performance liquid chromatography using a slight modification of the method of Lasley *et al.* [14] was used to determined levels of NE and nonsulphated MHPG. Brain tissue was homogenized by sonification in 0.2 M perchloric acid containing an appropriate amount of 4-hydroxy-3-methoxyphenylethanol (MOPET) as an internal standard. After centrifugation to remove particulate matter, the supernatant was filtered using a 0.2 μm regenerated cellulose filter. Aliquots of the filtrate were injected onto a Brownlee RP-18 Spheri-5 column (22×0.45 cm) protected by a guard column of the same material (3×0.45 cm). The mobile phase was comprised of 0.14 M monochloracetic acid with 0.1 mM disodium EDTA, 1.2 mM sodium octyl sulphate and 5% acetonitrile (apparent pH 3.0). The water used in the mobile phase was double distilled in glass and the solution was degassed prior to use. The mobile phase flow was maintained at 1.8 ml/min using a Waters 6000A pump, and sample injections into the HPLC system were controlled by a Waters WISP 710B autoinjector. Amperometric detection was

performed using an LC-4B detector and a TL-5 glassy carbon cell (Bioanalytic Systems), with an applied potential maintained at +0.80V against Ag/AgCl reference electrode. Output from the detector was plotted using a Hewlett-Packard 3390A integrator.

RESULTS AND DISCUSSION

Acute Shock

The concentrations of NE in each of the brain regions at various intervals following a single shock session are depicted in Table 1. Several tissue samples were lost during the course of the biochemical determinations, and hence the degrees of freedom for the analyses varied with the brain region examined. Control NE concentrations represent the pooled values of mice that were not shocked, and Dunnett's tests (α=0.05) were used to compare the effects of the shock treatments to those of nonshocked mice. Although hypothalamic NE concentrations of mice that received the 60 and 180 shock treatments did not differ from one another, both these treatments reduced NE concentrations at the 0.01 and 0.25 hr intervals relative to nonshocked animals. Within 1 hr NE concentrations of mice that received 60 or 180 shocks were comparable to those of nonshocked animals, but at the 3 hr interval NE concentrations were again found to be significantly reduced in the 180 shock group. The absence of the depletion at the 1 hr interval in the 180 shock group was likely a spurious result, since we typically have observed the NE reduction following 180 shocks to persist beyond this interval [2]. However, it will be noted that at the 3 hr interval in the 180 shock group the variability in NE concentrations is appreciably greater than at other intervals, possibly reflecting differential NE recovery rates across animals. The hippocampal NE reductions induced by the stressor were fairly transient, with NE concentrations reaching control levels within 0.25 hr of shock termination (see Table 1). Finally, systematic variations of cortical NE were not provoked by the shock treatment.

Chronic Shock

Since the chronic study was conducted in several replications, the NE concentrations were converted to percent of nonshocked animals. Analysis of variance of the percent change of hypothalamic NE indicated that the 60 and 180 shock treatments did not differentially influence amine concentrations, $F(1,41)=0.88$, $p>0.10$. Likewise, the interactions between the Number of Days of Shock and the Number of Shocks applied during each session did not approach statistical significance, $F(2,41)<1$. However, NE levels were influenced by the Number of Days of Shock mice received, $F(2,41)=5.55$, $p<0.05$. Newman-Keuls multiple comparisons (α=0.05) revealed that hypothalamic NE concentrations were higher in mice that received 15 sessions of footshock than in animals that received a single shock session (see Fig. 1). Dunnett's tests (α=0.05) also revealed that hypothalamic NE concentrations in mice that received 15 shock sessions exceeded those of nonshocked mice. Concentrations of hypothalamic NE in mice that received 180 shocks on a single occasion appeared to be somewhat lower than that of nonshocked mice; however, Dunnett's tests revealed that this difference did not reach statistical significance.

Concentrations of NE in hippocampus and cortex were not found to differ between mice that received 60 or 180 shocks per session, nor did the amine levels differ as a function of whether mice received 1, 3 or 15 shock sessions. Dunnett's tests (α=0.05), however, confirmed that in hippocampus 15 sessions of 60 shocks significantly increased NE concentrations relative to nonshocked animals. Likewise, 60 or 180 shocks applied on 15 consecutive days were found to increase cortical NE concentrations relative to nonshocked mice. Clearly, repeated exposure to a stressor effectively increased NE concentrations, and such an effect was evident even if the shock treatment, when acutely applied, did not provoke a significant decline of NE concentrations. Furthermore, it appeared that 60 and 180 shocks administered daily were equally effective in producing the neurochemical adaptation.

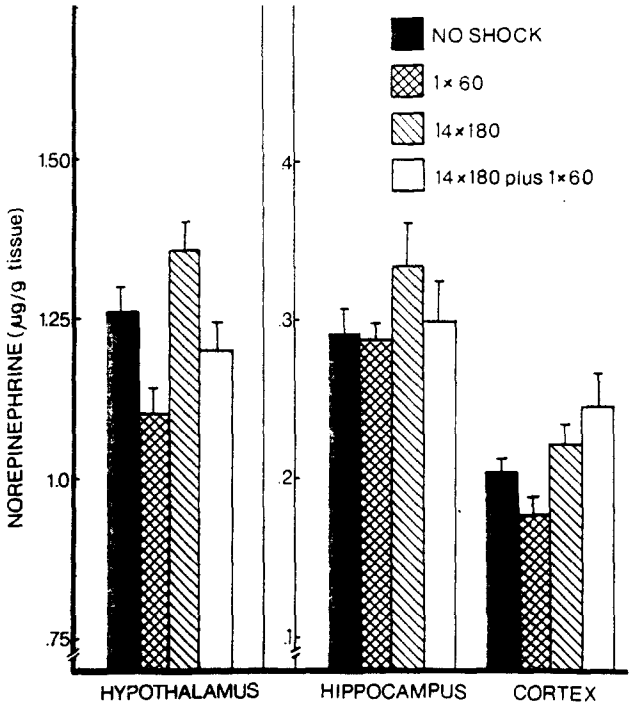

FIG. 2. Concentrations of NE (μg/g tissue±S.E.M.) in hypothalamus, hippocampus and cortex of mice exposed to various shock regimens. Mice were decapitated either immediately after exposure to the apparatus without being shocked (black bars), a single session of 60 shocks of 6 sec duration, 150 μA (cross hatched bars), or 14 sessions of 180 shocks on successive days followed by a session of 60 shocks (white bars). An additional group of mice that received 14 sessions of 180 shocks on successive days was decapitated 24 hr after the last shock session (hatched bars).

Chronic Shock: Immediate and 24 Hr Variations

Figure 2 displays the concentrations of NE following acute shock, immediately after repeated shock, and 24 hr following the repeated shock regimen (Experiment 3). Analysis of variance confirmed that hypothalamic NE levels were reduced in mice that received the shock treatment immediately prior to decapitation, $F(1,23)=5.28$, and increased in animals that had been exposed to the chronic shock regimen, $F(1,23)=8.08$, $p<0.01$. Since a priori predictions had been made concerning the effects of the two chronic shock conditions, Newman-Keuls multiple comparisons of the simple effects were conducted. These comparisons con-

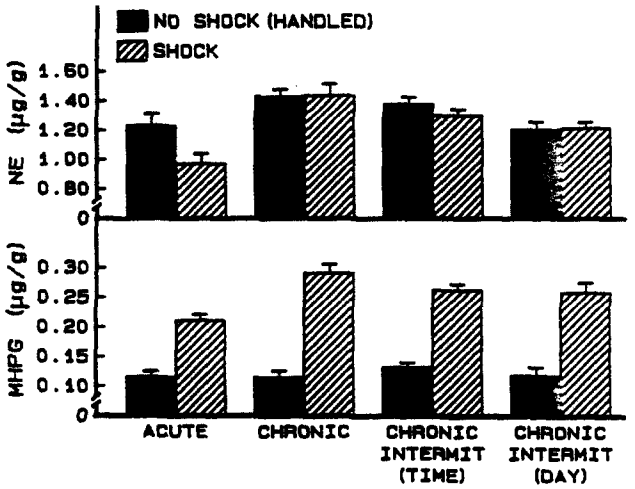

FIG. 3. Concentrations of hypothalamic NE (±S.E.M.) in mice that received either a single session of inescapable shock (acute), 15 sessions of inescapable shock on consecutive days between 1000 and 1200 hr (chronic), 15 sessions of inescapable shock at varying times of the day (chronic intermit—time), or on 15 occasions randomly applied over 1 60 day period (chronic intermit—day). Control groups (solid bars) were placed in the apparatus at equivalent times, but shock was withheld.

firmed that hypothalamic NE concentrations were higher in mice exposed to repeated shock and decapitated 24 hr later relative to nonshocked animals or animals that were decapitated immediately after the chronic shock session. As in the case of the hypothalamic NE variations, concentrations of hippocampal NE were increased in mice that had been exposed to repeated shock, $F(1,23)=5.72$, $p<0.05$. Finally, the analysis of cortical NE concentrations revealed that the Repeated Shock treatment significantly elevated NE concentrations, $F(1,23)=5.76$, $p<0.05$. In addition, the interaction between the Chronic Shock treatment and Acute Shock exposure approached statistical significance, $F(1,23)=3.59$, $p=0.072$. Pairwise comparisons revealed that acute shock produced a marginal reduction of NE concentrations ($p<0.10$), and such an effect was not evident in mice that had received the Repeated Shock treatment. Indeed, as seen in Fig. 2, in mice that received repeated shock NE concentrations exceeded those of animals that had only received a single session of inescapable shock.

Intermittent Shock

The concentrations of hypothalamic NE in the intermittent shock study are shown in Fig. 3. Analysis of variance revealed that NE concentrations in hypothalamus varied as a function of whether animals were exposed to footshock, $F(1,69)=3.94$, $p=0.051$, and the Schedule of treatment application, $F(3,69)=11.88$, $p<0.01$. The Shock treatment × Schedule interaction approached, but did not reach, statistical significance, $F(3,69)=2.51$, $p=0.066$. Newman-Keuls multiple comparisons were nevertheless conducted between the means of the simple main effects, since a priori predictions had been made concerning this interaction. These comparisons ($\alpha=0.05$) confirmed that a single shock session reduced NE concentrations relative to similarly treated nonshocked animals. Moreover, levels of NE in mice that received a single shock session were significantly lower than those of mice that received 15 sesssions of shock exposure irrespective of whether the shocks were applied over successive days or on an intermittent schedule. Indeed, in mice that received the chronic schedule of inescapable shock NE concentrations were not reduced relative to animals that had only been exposed to the apparatus.

Analysis of variance of the MHPG concentrations revealed a significant Shock treatment × Schedule interaction, $F(3,69)=3.64$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha=0.05$) confirmed that relative to the nonshock condition a single session of inescapable shock increased accumulation of the metabolite, as did exposure to 15 shock sesssions. Moreover, the accumulation of MHPG was significantly greater following repeated shock sessions than after a single session. As in the case of the NE concentrations, this occurred irrespective of the schedule of shock delivery, although predictable shock was somewhat more effective in this respect. Indeed, comparisons between the chronic shock regimens revealed that shock applied at the same time of day over 15 successive days resulted in a somewhat greater increase of MHPG concentrations relative to those seen in mice that had been exposed to the stressor on the intermittent schedules ($0.05<p<0.10$). These data suggest that upon acute exposure to a stressor the utilization of NE is increased, and the depletion of NE under these conditions results from synthesis not keeping pace with demand. With repeated exposure to a stressor further increases of NE utilization are incurred possibly reflecting an adaptive change to meet environmental demands. Inasmuch as NE concentrations are not reduced under these conditions it is likely that the increased utilization was accompanied by a compensatory increase of amine synthesis.

Analysis of the hippocampal NE concentrations revealed only a significant main effect of the Treatment schedule, $F(3,59)=4.72$, $p<0.05$. Newman-Keuls multiple comparisons revealed that mice that received the chronic shock treatment at the same time of day or at different times of the day had higher NE concentrations than acutely shocked mice or mice that had received the shock treatment on the intermittent schedule. Although the Schedule × Shock treatment interactions did not reach statistical significance, it is important to indicate that the treatment schedule hardly affected NE concentrations in nonshocked mice, and most of the variance associated with the main effect of the Treatment schedule was attributable to the variations of NE concentrations in the acutely shocked and intermittently shocked animals.

GENERAL DISCUSSION

In accordance with earlier investigations [15, 20, 22], acute exposure to inescapable shock enhanced the utilization of NE, and provoked a transient reduction in the concentration of this amine. As reported by Nakagawa *et al.* [15], the effectiveness of the stressor in reducing NE concentrations varied across brain regions, being more readily apparent in the hypothalamus than in the hippocampus and cortex. Additionally, it appeared that the time course for the NE variations was dependent on the number of shocks mice received, as well as the brain region examined. The reduction of NE induced by 60 shocks persisted for only a brief period, returning to control values within 1 hr in the hypothalamus, and within 15 min in the hippocampus. Following 180 shocks, the reduction of hypothalamic NE was still present 3 hr after the shock session. These data are consistent with studies show-

ing that the NE reductions were more persistent as stressor intensity increased [16]. Using more severe footshock than that employed in the present investigation, Weiss *et al.* [22] likewise noted regional specificity with respect to the recovery of NE concentrations. In particular, it was observed that NE concentrations within the locus coeruleus did not return to control levels until 72 hr after stressor termination, while in hypothalamus control levels of the amine were detected 48 hr after shock termination. Inasmuch as some of the behavioral disturbances associated with uncontrollable stressors are relatively transient, while others are fairly persistent [1, 5, 7, 24] it may be essential not only to consider the magnitude of the amine variations provoked by aversive stimuli, but also the persistence of these alterations [23].

In contrast to the effects of acute inescapable shock, reductions of NE were not evident following repeated application of the stressor. Following administration of inescapable shock over 15 consecutive days concentrations of NE equalled or exceeded those of nonshocked animals. This was the case irrespective of whether mice received 60 or 180 shocks during each session. Since the utilization of NE was greater after chronic than after acute shock, it is likely that the increased concentration of NE associated with the chronic regimen was attributable to a compensatory increase of amine synthesis. The finding that NE concentrations were higher 24 hr after a chronic schedule than immediately after such a treatment may be attributable to the increased synthesis persisting for some time after stressor termination [11,21], whereas utilization rates rapidly decline towards or below those of nonstressed animals [17].

The NE reduction ordinarily associated with acute shock was absent in mice that had received repeated exposure to the stressor. This was observed regardless of whether shock was presented on a predictable or an intermittent schedule. Thus, it appears that the adaptation was not entirely dependent on the stressor being presented on a systematic schedule. Yet, it will be recalled that NE levels and the accumulation of MHPG were somewhat greater in mice that had received the shock treatment on a predictable schedule than in mice that received the shock on an intermittent basis. While the magnitude of the NE and MHPG variations as a function of the predictable and intermittent shock treatments was not sufficiently large to accept unequivocally that this variable is fundamental in determining the course of the adaptation, this possibility certainly warrants consideration. Indeed, it has been reported that although behavioral adaptation occurs following repeated exposure to a predictable stressor, the magnitude of behavioral alterations are more pronounced when animals are exposed to stressors on an intermittent, unpredictable schedule (cf. [6, 9, 19, 22]).

ACKNOWLEDGEMENTS

Supported by Grants from the Ontario Mental Health Foundation and from the Medical Research Council of Canada (MT-6486 and MA-8130). J.I. is a Natural Sciences and Engineering Research Council Fellow, and P.A. is an Ontario Mental Health Fellow.

REFERENCES

1. Anisman, H. Vulnerability to depression. In: *Neurobiology of Mood Disorders*, edited by R. M. Post and J. C. Ballenger. Baltimore: Williams & Wilkins, 1984.
2. Anisman, H. and R. M. Zacharko. Behavioral and neurochemical consequences associated with stressors. In: *Stress-Induced Analgesia*, edited by D. D. Kelly. *Annals of the New York Academy of Sciences, vol 467*, 1986.
3. Anisman, H., D. de Catanzaro and G. Remington. Escape performance following exposure to inescapable shock: Deficits in motor response maintenance. *J Exp Psychol [Anim Behav]* **4**: 197–218, 1978.
4. Anisman, H., L. Kokkinidis and L. S. Sklar. Contribution of neurochemical change to stress-induced behavioral deficits. In: *Theory in Psychopharmacology, Vol 1*, edited by S. J. Cooper. London: Academic Press, 1981.
5. Bruto, V. and H. Anisman. Alterations of exploratory patterns induced by uncontrollable shock. *Behav Neural Biol* **37**: 302–316, 1983.
6. Burchfield, S. R. Stress: An integrative framework. In: *Stress: Psychological and Physiological Interactions*, edited by S. R. Burchfield. *Stress: Psychological and Physiological Interactions*, New York: Hemisphere, 1985.
7. Glazer, H. I. and J. M. Weiss. Long-term interference effect: An alternative to "learned helplessness." *J Exp Psychol [Anim Behav]* **2**: 202–213, 1976.
8. Irwin, J., P. Ahluwalia and H. Anisman. Sensitization of norepinephrine activity following acute and chronic footshock. *Brain Res* **379**: 98–103, 1986.
9. Katz, R. J. Animal models of depression: Pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav* **16**: 965–968, 1982.
10. Kobayashi, R. M., M. Palkovits, J. S. Kizer, D. M. Jacobowitz and I. J. Kopin. Selective alterations of catecholamines and tyrosine hydroxylase activity in the hypothalamus following acute and chronic stress. In: *Catecholamines and Stress*, edited by E. Usdin, R. Kvetnansky and I. J. Kopin. New York: Elsevier, 1976.
11. Kramarcy, N. R., R. L. Delanoy and A. J. Dunn. Footshock treatment activates catecholamine synthesis in slices of mouse brain regions. *Brain Res* **290**: 311–319, 1984.
12. Kvetnansky, R. Recent progress in catecholamines under stress. In: *Catecholamines and Stress: Recent Advances*, edited by E. Usdin, R. Kvetnansky and I. J. Kopin. New York: Elsevier, 1980.
13. Kvetnansky, R., A. Mitro, M. Palkovits, M. Brownstein, T. Torda, M. Vigas and L. Mikulaj. Catecholamines in individual hypothalamic nuclei in stressed rats. In: *Catecholamines and Stress*, edited by E. Usdin, R. Kvetnansky and I. J. Kopin. New York: Elsevier, 1976.
14. Lasley, S. M., A. Michaelson, R. D. Greenland and P. M. McGinnis. Simultaneous measurement of tyrosine, tryptophan and related monoamines for determination of neurotransmitter turnover in discrete rat brain regions by liquid chromatography with electrochemical detection. *J Chromatogr* **305**: 27–42, 1984.
15. Maickel, R. P., R. H. Cox, J. Saillant and F. P. Miller. A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int J Neurochem* **7**: 275–281, 1968.
16. Nakagawa, R., M. Tanaka, Y. Kohno, Y. Noda and N. Nagasaki. Regional responses of rat brain noradrenergic neurones to acute intense stress. *Pharmacol Biochem Behav* **14**: 729–732, 1981.
17. Roth, K. A., I. V. Mefford and J. D. Barchas. Epinephrine, norepinephrine, dopamine and serotonin: differential effects of acute and chronic stress on regional brain amines. *Brain Res* **239**: 417–424, 1982.
18. Richardson, J. S. and D. M. Jacobowitz. Depletion of norepinephrine by intraventricular injection of 6-hydroxydopamine: A biochemical, histochemical and behavioral study in rats. *Brain Res* **58**: 117–133, 1973.
19. Stone, E. A. and J. E. Platt. Brain adrenergic receptors and resistance to stress. *Brain Res* **237**: 405–414, 1982.

20. Thierry, A. M. Effects of stress on the metabolism of serotonin and norepinephrine in the central nervous system of the rat. In: *Hormones, Metabolism and Stress: Recent Progress and Perspectives*, edited by S. Nemeth. Bratislava: Publishing House of the Slovak Academy of Sciences, 1973.
21. Thierry, A. M., F. Javoy, J. Glowinski and S. S. Kety. Effects of stress on the metabolism of norepinephrine, dopamine, and serotonin in the central nervous system of the rat. I. Modifications of norepinephrine turnover. *J Pharmacol Exp Ther* **163:** 163–171, 1968.
22. Weiss, J. M., H. I. Glazer and L. A. Pohorecky. Coping behavior and neurochemical changes: An alternative explanation for the original "learned helplessness" experiments. In: *Animal Models in Human Psychobiology*, edited by G. Serban and A. Kling. New York: Plenum, 1976.
23. Weiss, J. M., P. A. Goodman, B. G. Losito, S. Corrigan, J. M. Charry and W. H. Bailey. Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine and serotonin levels in various brain regions of rat brain. *Brain Res Rev* **3:** 167–205, 1981.
24. Zacharko, R. M., W. J. Bowers, L. Kokkinidis and H. Anisman. Region-specific reductions of intracranial self-stimulation after uncontrollable stress: Possible effects on reward processes. *Behav Brain Res* **9:** 129–141, 1983.
25. Zacharko, R. M., W. J. Bowers and H. Anisman. Responding for brain stimulation: stress and desmethylimipramine. *Prog Neuropsychopharmacol Biol Psychiatry* **8:** 601–606, 1984.