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Effect of Stress on Norepinephrine-Stimulated Cyclic AMP formation in Brain Slices

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STONE, E. A. *Effect of stress on norepinephrine-stimulated cyclic AMP formation in brain slices.* PHARMAC. BIOCHEM. BEHAV. 8(5) 583–591, 1978. – Experiments were conducted to determine if stressful procedures, which increase brain norepinephrine (NE) release in rats, lower the responsiveness of central noradrenergic receptors as measured by the catecholamine (CA)–induced cAMP accumulation in hypothalamic and cerebral cortical slices. No conclusive evidence of subsensitivity was found after either acute or chronic electric footshock or continuous restraint. Failure to find a significant reduction after stress may have resulted from several methodological problems. These include (a) the inhibition of phosphodiesterase activity with isobutylmethylxanthine, which may have obscured possible adaptive changes in cAMP degradation and/or adenosine-dependent adrenergic receptors; (b) a low initial responsiveness to NE in these animals as suggested by the greater ease in inducing supersensitivity with reserpine than subsensitivity with amphetamine; and (c) the use as a test agent of exogenous NE which may stimulate a far broader population of receptive sites in brain slices than are activated during stress by the local release of endogenous NE.

Stress Catecholamines cAMP Brain slice Receptor Subsensitivity Supersensitivity Amphetamine Reserpine

NOREPINEPHRINE (NE) stimulates the formation of cAMP in brain slices via activation of postsynaptic adrenergic receptors [5]. A number of studies have now shown that the responsiveness of the NE-sensitive cAMP generating system in various regions of the rat brain can be modified by altering the exposure of receptors to NE. Reducing the levels of NE in the brain by a variety of pharmacological and surgical techniques has been found to enhance the responsiveness of brain slices to NE [6, 9, 13, 23, 24]. Conversely, increasing the content or release of the brain amine is accompanied by a reduction in responsiveness [1, 29, 37, 38]. Changes in responsiveness appear to vary with different brain regions and are found to occur most consistently in the cerebral cortex and limbic forebrain areas, less consistently in the hypothalamus and lower brainstem, and least or not at all in the cerebellum and midbrain [7, 23, 24, 38].

The above phenomena, although found primarily after drugs or brain lesions, may occur with changes in environmental stimulation [15] and could be of considerable psychological importance. This is particularly relevant to studies of emotional stress. Virtually all procedures that induce emotional stress thus far studied have been found to increase the release of NE throughout the central nervous system of the rat [35]. It would be expected therefore that emotional stress should reduce the responsiveness of the NE-sensitive cAMP system in the rat brain. In partial support of this notion, it has been found that repeated electroconvulsive shock (ECS) treatments significantly reduce the cAMP response of rat limbic forebrain slices to NE [37]. ECS, which releases brain NE [16], can be considered an emotional stress in that it is an aversive procedure that is actively avoided by rats [20]. However, since it involves direct manipulation of brain tissue, the effects of ECS may not be generalizable to other types of stress that act through normal sensory modalities. In the present studies we have therefore used the procedure of electric footshock to measure the effects of emotional stress on the responsiveness of rat brain slices to NE. Footshock is a highly aversive stimulus that acts by eliciting pain and fear and is thus a more appropriate means for inducing emotional stress. In addition we have compared the effects of stress with those of pharmacological agents known to alter responsiveness so as to assess the relative potency of stress.

METHOD

Animals

Male Sprague Dawley rats (Charles River Inc.) weighing 200–350 g were used in all experiments. The animals were housed in groups of 2–4 per cage and were maintained on an ad lib feeding schedule. Lights were on between 0700 and 1900 hr.

Stress Experiments

Footshock was applied by placing the rat in a 15 × 24 × 24 cm chamber containing a grid floor connected to a constant current shock source with a shock scrambler. In acute footshock studies, the rats received shocks of 2 mA intensity and 1 sec duration at a variable interval (VI)

schedule with a mean interval of 10 sec. Total duration of acute footshock was 75 min. Animals were killed for assay immediately thereafter. For chronic footshock, rats were treated as above except for the following modifications: the shock period was 1 hr per day for nine consecutive days; the VI schedule was 20 sec; and the intensity of the shock was 2 mA for Days 1–3, 3.5 mA for Days 4–6 and 5 mA for Days 7–9. Chronically shocked rats were killed 24 hr after the last shock session. All sessions were conducted between 0900 and 1030 hr.

Rats subjected to chronic restraint stress were confined continuously for 96 hr in wire mesh cylinders, 5 cm dia. and 20 cm length. Rats weighing 320–340 g fit snugly in these cylinders and were unable to turn end for end or upside down. A water dipper and food hopper were inserted through the front of the cylinder. Feeding was unimpaired under these conditions. Rats were sacrificed immediately after release from restraint.

Control rats used in the stress experiments were left undisturbed in their home cages except for daily weighing. These animals were killed at the same time as the corresponding experimental rats. Food intake (24 hr) in the chronic footshock experiments was measured by weighing the remains plus spillage of a known amount of lab chow placed in the home cage.

Pharmacological Experiments

d-Amphetamine HCl was administered for seven days in the animals' drinking water (tap) at a concentration of 10 mg/100 ml. The average daily intake for eight rats over the seven day period was 7.1 mg/kg (range, 5.9–7.9 mg/kg). Treated and nontreated control animals were killed at the end of the seventh day. Reserpine (Serpasil – Ciba Geigy) was injected IP, daily for seven consecutive days at a dose of 1 mg/kg. To retard weight loss, the reserpine treated rats were provided powdered lab chow in shallow dishes within their cages. Reserpinized rats along with noninjected controls were killed 24 hr after the last injection. A preliminary experiment indicated that chronic injection of the reserpine vehicle (provided by Ciba Geigy) did not affect the cAMP response as measured below.

In experiments where reserpine was given along with footshock the following protocol was used: two groups of rats were given reserpine (0.5 mg/kg, IP) daily for 10 days and then once every other day for an additional seven days. Starting on the 11th and lasting through the 17th day, the rats of one group received chronic footshock as above while the rats of the second group were not shocked. The shock period preceded the reserpine injection by 3 hr. A third group of rats who received neither shock nor reserpine served as the control group. All animals were killed 24 hr after the last shock period.

Incubation and Assay Procedures

All analyses were carried out in individual rat brains. Rats were killed by decapitation and their brains rapidly removed. A block of hypothalamic tissue (4–5 mm^3) was dissected as described previously [33] and divided at the midline into two slabs. Two circular areas of posterior cerebral cortex, one from each hemisphere, were punched out with a 0.5 cm dia. steel tube and dissected free of subcortical tissue. Tissues were immediately placed in ice cold oxygenated Krebs Ringer bicarbonate buffer, pH 7.4 and treated according to the procedure of Kreuger *et al.* [17]. Briefly, tissues were chopped twice, the second cut being 90° to the first, with a McIlwain tissue chopper set at 260 μm. After transfer to test tubes containing 5 ml of buffer, the resulting tissue prisms were separated by gentle vortexing and allowed to settle. The supernatant was removed and the slices were resuspended in fresh buffer (20 mg/ml), transferred to flasks and preincubated for 60 min at 37°C with shaking under an atmosphere of 95% O_2 –5% CO_2. Total time elapsed between decapitation and the start of preincubation was 16 min. For stimulation, 200 μl aliquots (approximately 4 mg of tissue) of the slice suspensions were added to tubes containing isobutylmethylxanthine (a phosphodiesterase inhibitor, final concentration, 1 mM) and various concentrations of catecholamines in 100 μl of buffer such that the final volume was 300 μl. Duplicate samples were used for each concentration of the catecholamine. After 15 min of further incubation the tubes containing the slices were placed in a boiling water bath for 9 min, briefly chilled on ice and centrifuged at 2000 g for 15 min. Duplicate 50 or 20 μl aliquots of the supernatants were assayed for cAMP by the method of Brown *et al.* [3]. The remaining pellet was dissolved in 1 N NaOH and analyzed for protein with the method of Lowry *et al.* [18]. Slices that were subjected only to boiling were found to have 93.0 ± 3.4% (mean and SD of 3 determinations) of the cAMP content of those that were first boiled and then homogenized in a teflon and glass tissue grinder, indicating that boiling alone was sufficient to liberate virtually all of the tissue cAMP into the medium. Endogenous NE in nonsliced hypothalamic and cortical tissue was isolated by alumina adsorption and assayed fluorimetrically as described previously [10].

Data Analysis and Presentation

The response to NE was defined as the NE–induced increase in cAMP accumulation above basal (nonstimulated) values. Responses for tissues of individual rats were therefore computed by subtracting each animal's basal level from his NE–stimulated level (or levels in the case of dose response curves). None of the treatments used significantly altered basal levels. The values in Table 1, 2 and 3 are the means and standard errors of these measures for groups of control and experimental animals where N is the number of animals per group. The maximum response was defined as that occurring to 10^{-4} M NE since the latter concentration was found in preliminary experiments to yield the greatest increase in cAMP in brain slices. The EC_{50} value, defined as that concentration of NE which elicits one half the maximum response, was computed graphically from concentration-response curves for individual tissues [14]. Previous studies have shown that alterations in the responsiveness of brain cAMP systems usually involve changes in the maximum response and occasionally the EC_{50} [8]. Therefore, these two values as well as basal levels were chosen for statistical comparison in the present study. Unless otherwise indicated all comparisons were made using the two-tailed *t*-test. The null hypothesis was rejected for all p values <0.05.

RESULTS

Acute Footshock

In agreement with previous results [11] a sigmoidal

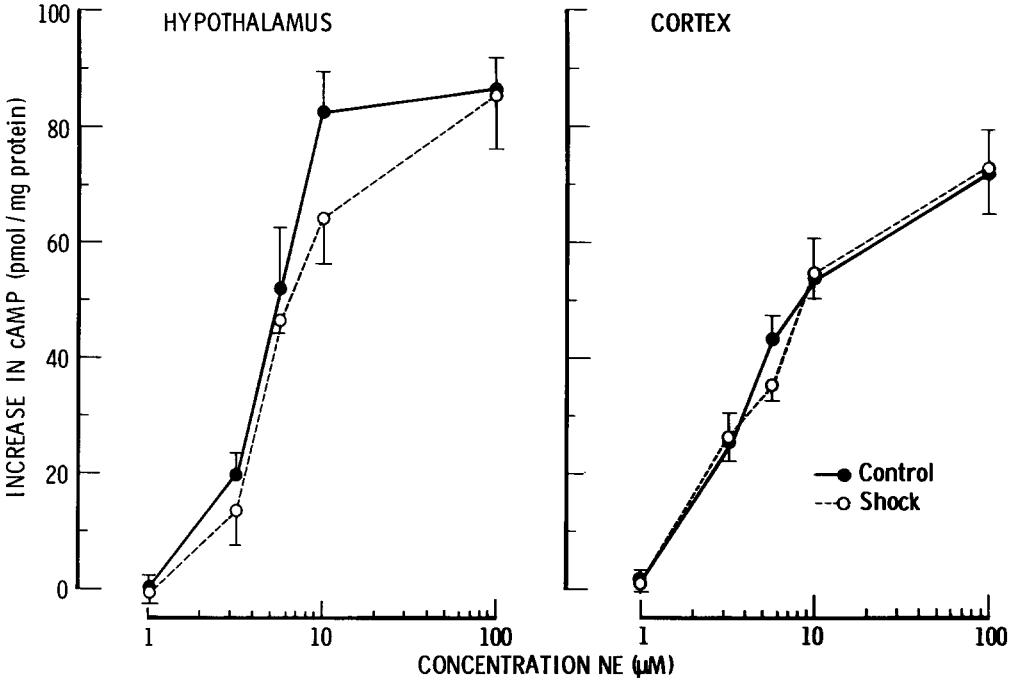

FIG. 1. Effect of acute footshock stress on cAMP responses to NE in hypothalamic and cortical slices. Each point and vertical line is the mean and SEM of eight rats. EC_{50} values for NE in μM (mean ± SEM): Hypothalamus, control, 5.2 ± 0.5, shock, 6.7 ± 1.8; Cortex, control, 4.6 ± 0.3, shock, 5.4 ± 0.3. Basal levels in pmol/mg protein: Hypothalamus, control, 33.3 ± 2.9, shock, 27.0 ± 3.0;Cortex, control, 21.4 ± 0.7, shock, 23.0 ± 0.5.

curve was obtained between the log concentration of NE and cAMP accumulation for both shocked and control groups and in both brain regions (Fig. 1). The maximum response, at 10^{-4} M NE, represented an increase above basal levels of 70 and 85 pmol/mg protein, respectively, for the cortex and hypothalamus of control rats. Control and shocked rats did not differ significantly in either the basal cAMP level or in the maximum response of either tissue. EC_{50} values were not significantly different between the two groups in either tissue (see legend of Fig. 1).

Chronic Footshock

Experiment 1. In the first chronic footshock experiment basal cAMP levels were not affected by the stress but NE–stimulated increases were less in the shocked than control rats at all doses tested and for both tissues (Fig. 2). The absolute difference between the two groups became progressively greater as the concentration of NE was increased. At 10^{-4} M NE, the response of shocked rats was 29.5% less in the hypothalamus and 27.3% less in the cortex. Owing to the high variability within each group, the latter differences were only of borderline statistical significance (hypothalamus, $p = 0.12$; cortex, $p = 0.05$). No differences between the groups were found for their respective EC_{50} values (legend, Fig. 2).

Experiment 2. In order to determine whether the latter results were reliable, a second chronic footshock experiment was run identical to the first except that the tissues were stimulated with only maximum concentrations of NE (10^{-4} M) as well as isoproterenol (ISO) (Table 1). Stress had no effect on basal cAMP levels in either tissue. A small, nonsignificant reduction (9%) in NE–stimulated cAMP formation was found in both tissues of the shocked rats. Shcok did not have consistent or significant effects on ISO–stimulated cAMP formation (hypothalamus, +21.6%; cortex, −2.3%).

Body weight and food intake. In both the first and second chronic footshock experiments the shocked rats showed a marked decrease in weight gain compared to controls over the nine-day period (Fig. 3; Experiment 1, −67.2%, $p<0.001$; Experiment 2, −76.7%, $p<0.001$). Daily food intake, when averaged over Days 2–6 in the first experiment and on Day 7 in the second experiment, was significantly lower in the shocked animals (Experiment 1, −23.3%, $p<0.01$; Experiment 2, −26.5%, $p<0.05$).

Chronic Restraint

Four days of continuous restraint stress produced no change in basal cAMP levels but slightly reduced the formation of cAMP in the hypothalamus and cortex in response to submaximum and maximum concentrations of NE (Table 2). The restraint-induced decreases ranged from 8.3 to 18.3% and were not statistically significant.

Amphetamine and Reserpine

Statistical comparisons were made using each drug treated group and its respective control group. In Fig. 4 the two control groups have been combined for the sake of clarity since they did not differ significantly at any dose. Neither drug affected basal cAMP levels in the hypothalamus or cortex. Amphetamine reduced the NE–elicited increase of cAMP in both tissues. This effect was most marked at the maximum NE concentration where there was a reduction of 24.0% ($p<0.05$) and 21.6% ($p<0.05$) in the hypothalamus and cortex, respectively. Amphetamine

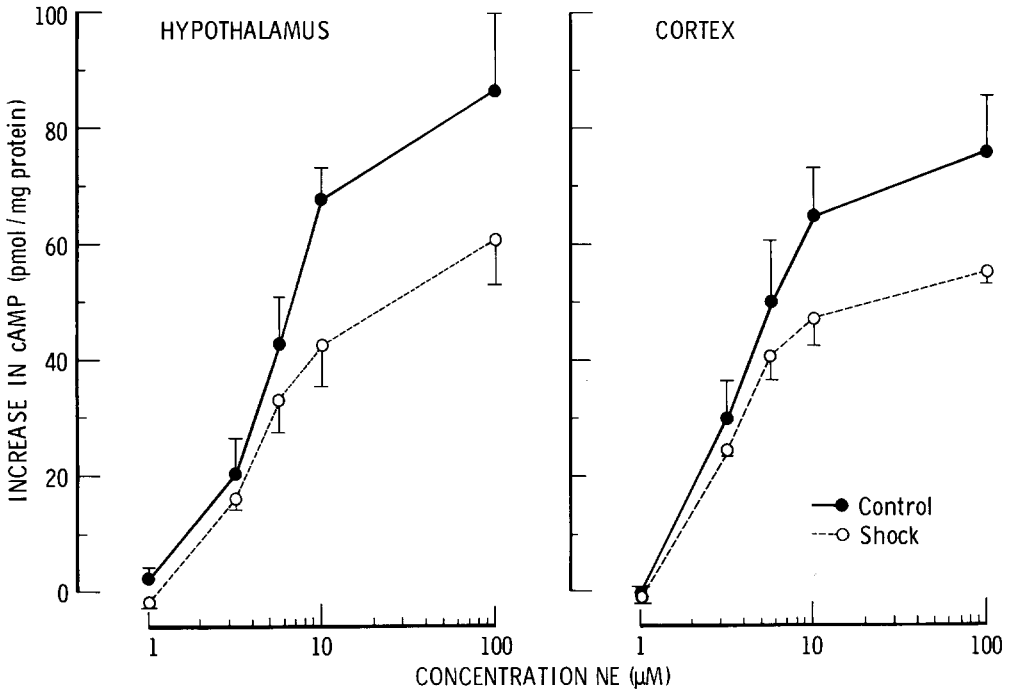

FIG. 2. Effect of chronic footshock on cAMP responses to NE in hypothalamic and cortical slices (first experiment). For details see legend of Fig. 1. EC_{50} values: Hypothalamus, control, 5.6 ± 0.5, shock, 5.6 ± 0.5; Cortex, control, 4.1 ± 0.4, shock, 3.8 ± 0.4. Basal levels: Hypothalamus, control, 23.8 ± 0.9, shock, 25.9 ± 0.8; Cortex, control, 22.2 ± 1.9, shock, 24.7 ± 1.7.

did not significantly alter the EC_{50} values of NE in either tissue (legend, Fig. 4). Reserpine treatment did not alter the responsiveness of hypothalamic tissue but did produce a marked increase in NE–stimulated cAMP formation in the cortex. The latter effect was greatest at 10^{-4} M NE and amounted to an 85.7% increase ($p<0.001$). Reserpine did not alter the EC_{50} values of NE in either tissue (legend, Fig. 4). Levels of endogenous NE in the hypothalamus and cortex of an independent group of reserpinized rats were both less than 10% of control values (values not shown).

Reserpine Plus Chronic Footshock

The control, reserpine and reserpine plus footshock

TABLE 1

REPLICATION OF EFFECT OF CHRONIC FOOTSHOCK STRESS ON CATECHOLAMINE-INDUCED cAMP ACCUMULATION IN BRAIN SLICES*

Group (N)	cAMP (pmol/mg protein)		
	Basal Level	Increase to NE (100 μM)	Increase to ISO (100 μM)
	Hypothalamus		
Control (8)	28.1 ± 4.5	82.2 ± 8.5	29.6 ± 6.5
Shock (8)	30.2 ± 3.3	74.8 ± 15.2	36.0 ± 8.4
	Cortex		
Control (8)	21.4 ± 1.7	70.7 ± 9.1	64.1 ± 6.5
Shock (8)	21.3 ± 2.0	64.2 ± 7.3	62.6 ± 4.3

*Unless designated, symbols and values in all tables are as defined in Methods.

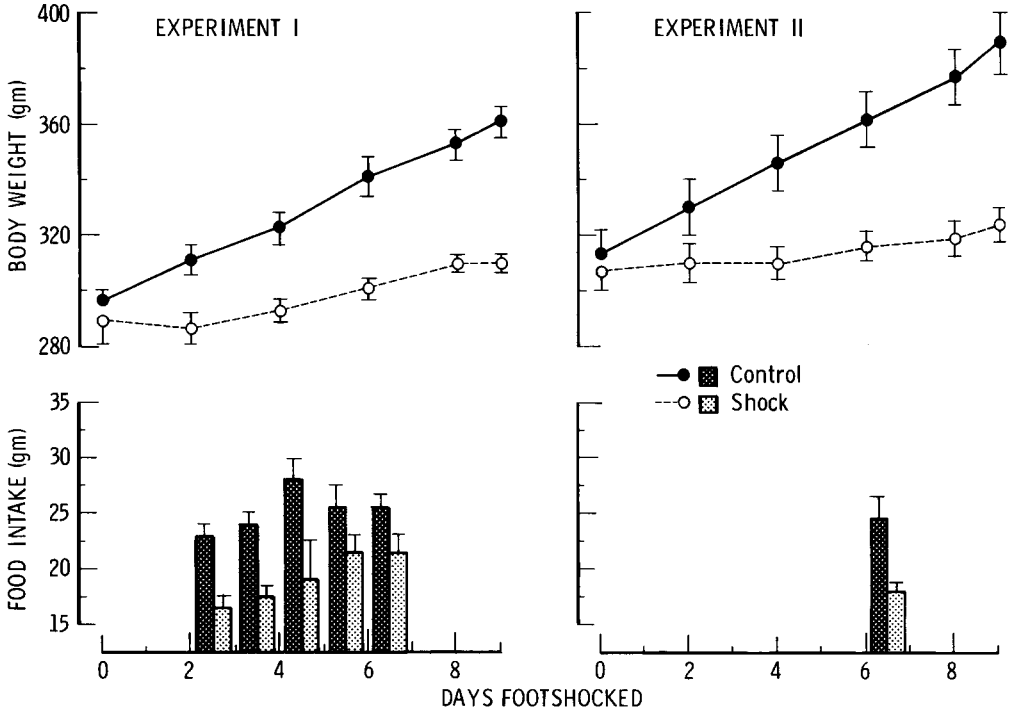

FIG. 3. Body weight gain and daily food intake in rats of the first and second chronic footshock experiments. For body weight (upper graphs) each point and vertical line is the mean and SEM of eight rats. For food intake (lower graphs) each bar and vertical line is the mean and SEM of four pairs of rats.

groups (Table 3) differed significantly in their cortical cAMP responses to maximum concentrations of NE, one-way ANOVA, $F(2,21) = 14.21$, $p<0.001$, and ISO, $F(2,21) = 11.18$, $p<0.001$. Comparison of individual means by t tests (see legend, Table 3) revealed that both reserpinized groups had significantly higher responses to both catecholamines than the control (nonreserpinized) group. No significant differences were found between the responses of rats given reserpine and those given reserpine plus footshock.

DISCUSSION

The present results do not provide convincing support for the hypothesis that emotional stress reduces central responsiveness to NE. Acute footshock had no apparent

TABLE 2

EFFECT OF CHRONIC RESTRAINT STRESS ON NE-ELICITED cAMP ACCUMULATION IN BRAIN SLICES

Group (N)	cAMP (pmol/mg protein)		
	Basal Level	Increase to NE (3.2 μM)	Increase to NE (100 μM)
	Hypothalamus		
Control (6)	25.3 ± 2.6	24.0 ± 4.9	78.0 ± 12.5
Restraint (6)	28.3 ± 3.2	19.6 ± 4.2	64.8 ± 9.4
	Cortex		
Control (6)	20.4 ± 3.2	–	78.2 ± 5.8
Restraint (6)	19.5 ± 2.7	–	71.7 ± 7.6

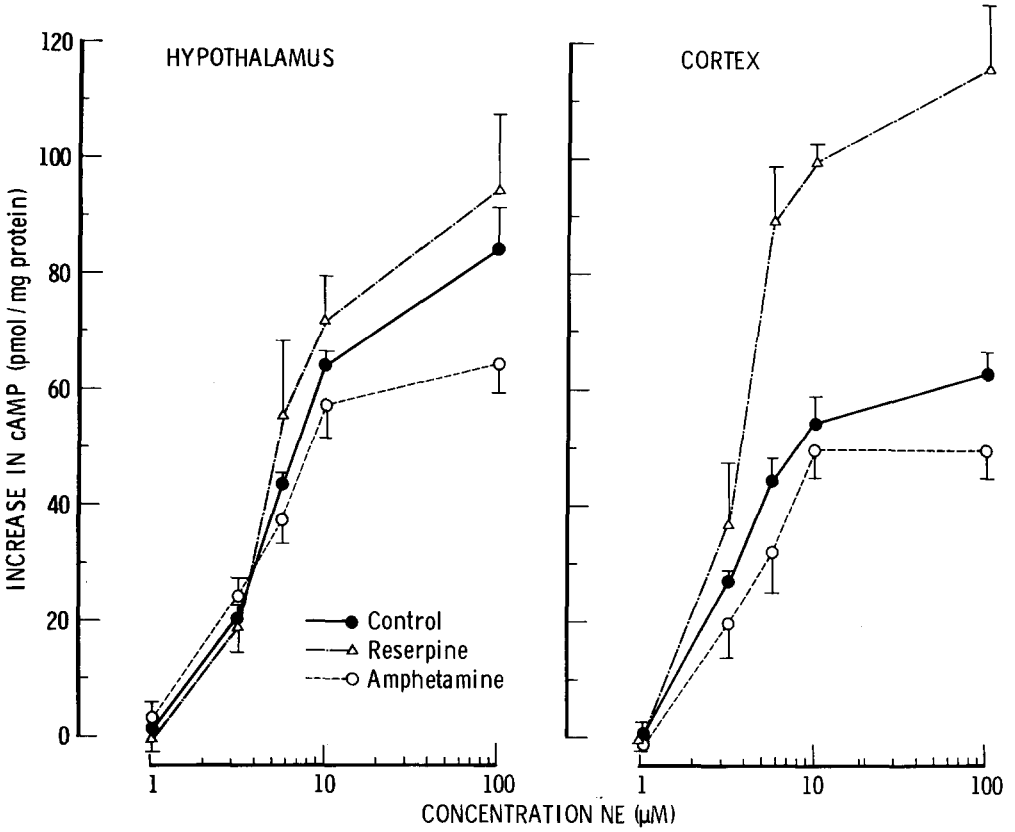

FIG. 4. Effects of chronic reserpine and amphetamine treatments on cAMP responses to NE in hypothalamic and cortical slices. Each point and vertical line is the mean and SEM of eight rats (16 rats for control group). For details see legend of Fig. 1. EC_{50} values: Hypothalamus, control, 6.2 ± 1.0, reserpine, 5.0 ± 0.3, amphetamine, 4.5 ± 0.3; Cortex, control, 3.9 ± 0.4, reserpine, 4.0 ± 0.2, amphetamine, 4.6 ± 1.0. Basal levels: Hypothalamus, control, 26.9 ± 1.7, reserpine, 30.1 ± 2.3, amphetamine, 26.1 ± 6.8; Cortex, control, 22.4 ± 1.3, reserpine, 25.4 ± 1.5, amphetamine, 24.8 ± 1.7.

TABLE 3

EFFECT OF CHRONIC FOOTSHOCK STRESS ON RESERPINE-INDUCED HYPERRESPONSIVENESS OF THE cAMP SYSTEM IN CORTICAL SLICES

Group (N)	cAMP (pmol/mg protein)		
	Basal Level	Increase to NE (100 μM)	Increase to ISO (100 μM)
Control (8)	31.8 ± 1.8	81.9 ± 3.7	65.0 ± 4.4
Reserpine (8)	40.9 ± 4.9	122.5 ± 6.0*	107.2 ± 6.8*
Reserpine + Footshock (8)	33.4 ± 3.8	113.6 ± 6.7†	99.2 ± 8.2†

*Mean differs significantly from that of control group ($p<0.01$).
†Mean does not differ significantly from that of reserpine group ($p>0.05$).

effect on the EC_{50} value or maximum response of the NE–sensitive cAMP generating system in either the hypothalamus or cerebral cortex. Chronic stress, in the form of footshock or restraint, may produce a reduction in the maximum response but this effect is small and unreliable.

A number of treatments that chronically increase brain NE availability such as imipramine [29], desmethylimipramine (DMI) [37], pargyline [38], nialamide [38], d-amphetamine [1] and electroconvulsive shock [37] have been shown to produce significant and sizable (30–75%) reductions in the cAMP response to NE of cortical and limbic forebrain regions of rats or mice. Considering the diverse nature of these agents it is improbable that the hypothesis that excessive exposure to NE leads to central subsensitivity is incorrect. Therefore it is necessary to consider various methodological problems to explain why the stress of electric footshock, which can increase brain NE utilization by as much as 200% in the rat, both acutely [34] and chronically (Stone, unpublished observations), had only weak or variable effects on responsiveness. It is unlikely that the stress was insufficiently severe since it produced significant anorexia and a marked reduction in weight gain. We have also found in a more recent study that an identical schedule of footshock produces highly significant increases in tyrosine hydroxylase activity in the adrenal medulla, hypothalamus and cerebral cortex of rats of this strain indicating that the stress is of sufficient intensity and duration to produce presynaptic neurochemical adaptation in these same brain regions (E. Stone, L. Freedman and L. Morgano, Effect of chronic footshock stress on tyrosine hydroxylase activity in the brain and adrenal medulla. In preparation.). It is also unlikely that the intermittent nature of the stress (1 hr/day) was a significant factor since 96 hr of continuous restraint stress did not produce a greater reduction than did footshock. Finally it does not appear that the cAMP system is refractory to being reduced in these animals since chronic ingestion of amphetamine reduced the maximum response to NE in both the cortex and hypothalamus in agreement with previous findings by Baudry *et al.* [1] in the mouse cortex. The factors which appear to be most likely in preventing or obscuring detection of subsensitivity in the present experiments are: (a) the use of isobutylmethylxanthine (IBMX) to inhibit the phosphodiesterase activity; (b) a low initial responsiveness to catecholamines in the rats used, i.e., a floor effect, and (c) the use of exogenous NE as a test agent to detect changes resulting from the release of endogenous NE.

The accumulation of cAMP in brain slices depends on its rate of formation via adenylate cyclase and its rate of degradation by cAMP–phosphodiesterase (PDE). IBMX, a potent PDE inhibitor, was used in the present study to simplify interpretation by minimizing the degradation of cAMP. There is reason to suspect, however, that IBMX may have obscured the detection of subsensitivity. The mechanisms underlying subsensitivity to catecholamines are not yet fully clarified and do not appear to be the same for all tissues. In some cases there is a decreased formation of cAMP which is still observable when PDE is inhibited and appears to involve a decreased density of β-adrenergic receptors. The latter has been found in the rat pineal gland made tolerant to ISO [15], the mouse cortex which is subsensitive to NE following amphetamine treatment [1] and the rat brain subsensitive to NE after chronic DMI or doxepin treatment [2]. In other cases subsensitivity appears to result from an increased PDE activity which may occur with or without apparent alterations in receptors. This has been observed for the rat pineal gland [22] and chick cortex [21], both tolerant to ISO, and for cultured astrocytoma cells subsensitive to NE [4]. Since IBMX was used in the present studies we cannot exclude the possibility therefore that stress does induce subsensitivity by increasing brain PDE activity and that IBMX obscured the effect. It is highly unlikely, however, that stress reduces the density or affinity of central noradrenergic receptors coupled to adenylate cyclase since this effect would not have been altered by IBMX unless the receptors involved were adenosine-dependent as discussed below.

A second mechanism by which IBMX may mask changes in responsiveness to NE is via the drug's antagonism of adenosine receptors [19]. Exogenous adenosine is known to potentiate the effect of NE in rat brain slices [27] possibly by augmenting the α-adrenergic response to NE [26]. Endogenous adenosine, which is released from slices into the medium during incubation [36] appears essential for the full cAMP response to NE in the rat cortex since the latter is reduced by prior addition of adenosine deaminase [30]. Since there are no data as yet on the effects of footshock or restraint stress on adenosine availability, we cannot exclude the possibility that stress affected brain levels of this nucleoside and that this change was masked by IBMX.

The ability of IBMX to block adenosine may also help to explain two anomalous findings of the present study. First, the cAMP response to NE in both brain regions was about 50% smaller than that found in previous studies not using PDE inhibitors [23, 24, 31]. A recent study, however, has shown that IBMX, by blocking adenosine, lowers the response of the rat cortex to NE [30]. In a preliminary experiment we have confirmed that this inhibition occurs in cortical slices to the extent (40%) that it can account for the smaller responses obtained in these experiments. Second, the response of the cortex to NE and ISO in the present study were similar in magnitude whereas in previous studies the responses of NE (both α and β responses) have generally been twice those of ISO (pure β response) [25]. The discrepancy may have resulted from our use of IBMX which could have caused a preferential loss of the α-response to NE by antagonism of adenosine [28]. In support of this notion, both tissues in the present study were found to be unresponsive to the α-agonist, methoxamine at 10^{-4} M (data not shown). However, it is still not clear to what extent IBMX inhibited α-activity since in the hypothalamus NE produced a far greater response than ISO (Table 1) suggesting that the former amine still retained α-stimulating properties.

A second factor that appears to have prevented development of subsensitivity after stress is a low initial responsiveness to catecholamines in the animals used. This is suggested by the responses of these rats to drugs which have been shown to induce changes in sensitivity to catecholamines in brain tissue. Reserpine, which is known to induce hyperresponsiveness in the rat cortex [6], led to a marked 90% increase, while amphetamine, which produces hyporesponsiveness in the mouse cortex [1] only produced a 20–25% decrease in the maximum cortical response to NE. The greater ease in producing supersensitivity is what one would predict if initial responsiveness were close to a lower physiological limit. Evidence that there are limits to NE-induced changes in the

responsiveness of rat brain cAMP systems has been presented by Skolnick and Daly [32]. Two findings, however, do not agree with the above interpretation. First, chronic reserpine did not enhance responsiveness to NE in the hypothalamus despite a profound depletion of NE. Thus it is difficult to argue that the weak effect of stress in this tissue is due to the initial response being close to a lower limit. The latter concept may only apply, therefore, to the NE-sensitive cAMP system of the cerebral cortex. The hypothalamic data, however, are inconclusive because they are confounded by the fact that the reserpine is far less potent in inducing supersensitivity in subcortical than cortical regions suggesting possible regional differences in receptor properties [24]. Second, in rats whose cortices were made supersensitive by reserpine, chronic footshock still did not significantly reduce either the NE– or ISO–stimulated cAMP response. The latter finding, however, is not definitive proof against a lower limit since reserpine may have reduced the amount of NE released by the shock stress. This appears to be the case since observation of reserpinized rats during footshock revealed that the animals did not show pupillary dilatation to the stress and were probably not releasing appreciable amounts of peripheral catecholamines.

A third significant factor which may have obscured detection of subsensitivity involves the use of exogenous NE as a test agent. During stress endogenous NE is released locally from nerve endings in the brain. In the test for responsiveness, brain slices are incubated in relatively high concentrations of exogenous NE. Histochemical studies have shown that under the latter conditions NE accumulates in structures that do not normally contain the endogenous amine [12]. It is possible that the exogenous amine may reach or activate a greater number of receptive sites than the endogenous amine. If the number of receptive sites activated by endogenous NE during stress constitutes a small proportion of the total number activated in vitro this would give rise to an artifactual lower limit and could obscure even total subsensitivity in the endogenous subpopulation. This interpretation raises the interesting possibility that the various procedures discussed earlier which do produce significant subsensitivity may affect a much broader population of receptive sites than does emotional stress. Further investigation of this problem requires the use of methods which activate receptors by selectively releasing endogenous NE from nerve endings in the brain slice preparation.

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