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Norepinephrine-Sensitive Systems Generating Adenosine 3′,5′-Monophosphate: Increased Responses in Cerebral Cortical Slices from Reserpine-Treated Rats

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SUMMARY

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The magnitude of accumulations of cyclic AMP elicited by various concentrations of norepinephrine was increased by 40–60% in cerebral cortical slices obtained from Sprague-Dawley rats 2 days after the first of two daily treatments with reserpine. The enhanced response of cortical cyclic AMP-generating systems to norepinephrine developed between 5 and 48 hr and disappeared between day 9 and day 16 after reserpine. The apparent EC_{50} of norepinephrine was not altered to a measurable extent by prior treatment with reserpine. The enhancement in the response was similar throughout a 4–90-min stimulation of slices. The response to isoproterenol or a norepinephrine-phentolamine combination was increased by 50–70%, while the response to a norepinephrine-propranolol combination was not significantly increased. This suggests that reserpine treatment may have caused a partly selective increase in the *beta* adrenergic component of the response to catecholamines. The response of cyclic AMP-generating systems to veratridine was not increased by reserpine, but there was about a 30% increase in the response to adenosine. The response to a combination of norepinephrine and a phosphodiesterase inhibitor, Ro 20-1724, was enhanced by about 40% in cortical slices from reserpine-treated rats, while the responses to combinations of either serotonin, dopamine, or histamine with the phosphodiesterase inhibitor were not enhanced above responses in control slices. It is proposed that the decreased availability of the neurotransmitter, norepinephrine, in reserpine-treated rats results in an enhanced concentration or responsiveness of norepinephrine-sensitive adenylate cyclase. Another possibility is a selective decrease in phosphodiesterase activity associated with this cyclase. The data do not permit an unambiguous choice between these alternatives.

INTRODUCTION

The responses of norepinephrine-sensitive cyclic AMP-generating systems in brain tissue appear to be closely interelated with availability of norepinephrine as a neurotransmitter in vivo. Thus destruction of central noradrenergic terminals by treatment of rats with 6-hydroxydopamine results in a compensatory 2-fold increase in the magnitude of accumulation of cyclic AMP elicited in brain slices by norepinephrine (1–4). Depletion of central stores of norepinephrine

by acute or chronic treatment of rats with reserpine also elicits an increase in the accumulation of cyclic AMP produced by norepinephrine in slices from various brain regions (5, 6). The present paper examines in some detail the character of the altered response of cyclic AMP-generating systems in cerebral cortical slices from reserpine-treated rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150–200 g) were obtained from Taconic Farms, Germantown, N. Y. Reserpine (Serpasil) was obtained as a 2.5 mg/ml solution from Ciba and was administered by intraperitoneal injection at a dose of 5 mg/kg. Sham injections contained 0.9% NaCl. The phosphodiesterase inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) was kindly provided by Hoffmann–La Roche. All other compounds were obtained from commercial sources.

Rats were killed by decapitation. Their brains were rapidly removed and placed in ice-cold Krebs-Ringer solution. Cerebral cortical tissue was removed, and 260-μm slices were prepared with a McIlwain chopper. The slices from one or two rats were incubated at 37° in Krebs-Ringer solution for 15 min, collected by filtration through nylon mesh, and placed in 10 ml of solution containing 13 μM [^{14}C]adenine (3 μCi) for an incubation of 45 min. In experiments in which both radioactive and total cyclic AMP were assayed, a solution containing 4 μM [^{3}H]adenine (20 μCi) was used for labeling. Tissue was washed twice after labeling, incubated for another 20–30 min, collected on nylon mesh, and divided into equal portions. These portions were then incubated with solutions containing test agents, usually for 30 min, followed by collection on nylon mesh and homogenization in cold 8% trichloracetic acid. All incubations were performed at 37° in Krebs-Ringer bicarbonate buffer aerated with 95% O_2–5% CO_2. The percentage conversion of radioactive adenine nucleotides to cyclic AMP was determined as previously described (7). Simultaneous determination of radioactive and total cyclic AMP, the latter by the Gilman assay (8), was carried out essentially as previously described (9).

RESULTS

The accumulation of cyclic AMP elicited by norepinephrine was 40–60% greater in cerebral cortical slices from reserpine-treated rats than in slices from control rats (Figs. 1 and 2). Accumulations of radioactive and total cyclic AMP were enhanced to similar extents (Table 1). The apparent EC_{50} of norepinephrine appeared to be shifted to a lower value (Fig. 1), but in view of the variability of the results this apparent shift is not significant. The accumulation of cyclic AMP elicited by norepinephrine was not significantly greater in slices obtained 5 hr after reserpine (Table 2). The enhanced response seen 2 days after reserpine was still manifest at 9 days, but had disappeared at 16 days. The present dosage schedule for reserpine resulted in a large reduction of brain norepinephrine at 2 days (results not shown; see ref. 10).

Responses of cyclic AMP-generating systems to the depolarizing agent veratridine or to serotonin were not enhanced in slices from reserpinized rats (Table 3). The response to adenosine, however, showed a small but significant enhancement in slices from reserpine-treated rats. In the presence of a phosphodiesterase inhibitor (Ro 20-1724), other biogenic amines (serotonin, dopamine, and histamine) as well as norepinephrine significantly raised the accumulation of cyclic AMP over the concentrations obtained with the inhibitor alone in cortical slices from control rats (Table 3). However, only the accumulations elicited by the combination of norepinephrine and Ro 20-1724 were higher in slices from the reserpine-treated animals as compared with control animals. The accumulation of cyclic AMP produced by norepinephrine was enhanced by about 40% in slices from reserpine-treated rats in both the presence and absence of the phosphodiesterase inhibitor (Table 4). The accumulation of cyclic AMP elicited by norepinephrine was increased 2-fold by the presence of the phosphodiesterase inhibitor in slices from either control or reserpine-treated animals (Table 4).

The response to isoproterenol, a pure *beta* adrenergic agonist, was enhanced by 70% in slices from reserpinized rats (Table 5). The accumulation elicited by isoproterenol was

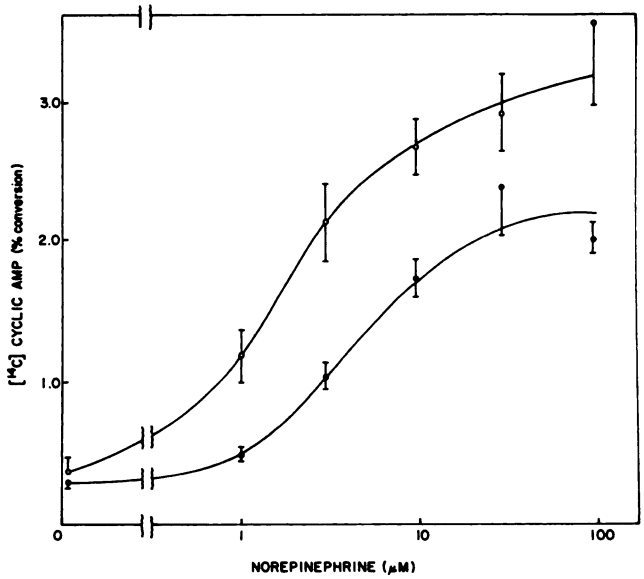

FIG. 1. *Accumulation of cyclic AMP elicited by norepinephrine in rat cerebral cortical slices*

Rats either received sham injections or were treated with reserpine 2 days and 1 day before death. In six separate experiments cortical slices from two reserpine-treated rats (○——○) were pooled, as were slices from two control rats (●——●). After labeling with [^{14}C]adenine the slices were divided into six equal portions, which were then incubated with concentrations of norepinephrine between 0 and 100 μM for 30 min. The data are expressed as means ± standard errors of the percentage conversion of total incorporated radioactivity to cyclic AMP.

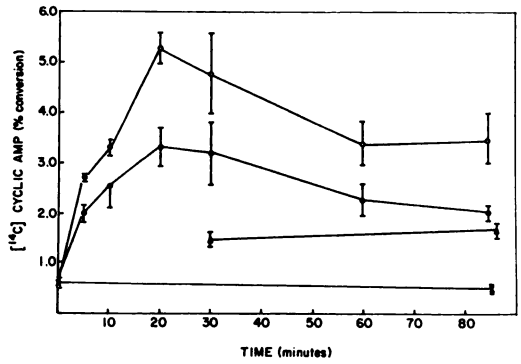

FIG. 2. *Time course for accumulation of cyclic AMP elicited by norepinephrine in rat cortical slices*

Rats either received sham injections or were treated with reserpine 3 days and 1 day before death. In three separate experiments cortical slices were pooled from two reserpinized rats (○——○), as were slices from two control rats (●——●). After labeling with [^{14}C]adenine as described in MATERIALS AND METHODS, the slices were incubated with 100 μM norepinephrine for times ranging from 0 to 90 min. In another three experiments control slices were incubated with 3 μM norepinephrine (▲——▲). Values for slices incubated without norepinephrine are also shown (×——×). Data are presented as means ± standard errors.

not significantly different from that produced by a combination of norepinephrine and the *alpha* adrenergic antagonist phentolamine either in control slices or in slices from reserpine-treated animals. The response to a combination of norepinephrine and the *beta* adrenergic antagonist propranolol was not significantly enhanced in slices from reserpine-treated animals.

DISCUSSION

A variety of treatments alter the responsiveness of cyclic AMP-generating systems in noradrenergically innervated cells. Such alterations would appear to represent compensatory increases in the responses of receptor-modulated systems caused by the absence or reduction of normal functional noradrenergic activation of the system. In pineal gland the "supersensitivity" of cyclic AMP-generating systems after surgical denervation, immunosympathectomy, destruction of noradrenergic terminals with 6-hydroxydopamine, or reduction of norepinephrine concentrations with reserpine appears due primarily to an enhanced amount or activity of norepinephrine-sensitive cyclases

rather than to reductions in the associated phosphodiesterases (11–13). In brain the mechanism of "supersensitivity" of cyclic AMP-generating systems to norepinephrine seen after treatment of animals with 6-hydroxydopamine (1–4) or reserpine (5, 6) is not yet defined.

The character of the enhanced responses of norepinephrine-sensitive cyclic AMP-generating systems in brain slices from reserpine-treated rats has now been examined in detail for the first time. The enhanced response to norepinephrine was present in slices 48 hr after the first of two injections of reserpine and was maintained for at least 9 days, finally returning to control concentrations by day 16 after reserpine (Tables 1 and 2). In 6-hydroxydopamine-treated animals, enhanced maximal responses to norepinephrine were not observed until 96 hr after the drug treatment (1) and were then maintained for at least 20 days (3, 4). Thus the enhanced responsiveness appears to develop more quickly after reserpine, and to persist for a shorter period of time. The maximal response to norepinephrine was increased by about 50% in slices from reserpine-treated rats (Fig. 1). In view of the variability of the results, no definitive statements as to possible slight alterations in the EC_{50} of norepinephrine can be made. Even in the presence of cocaine the EC_{50} values were still not significantly different in slices from control and reserpine-treated rats (results not shown).

TABLE 1

Accumulation of radioactive and total cyclic AMP elicited in rat cortical slices by norepinephrine

Four pairs of rats either received sham injections or were treated with reserpine 2 days and 1 day before death. After labeling with [^{3}H]adenine the cortical slices from each rat were divided into two portions and incubated for 30 min with or without 100 μM norepinephrine. [^{3}H]Cyclic AMP and total cyclic AMP were assayed as previously described (9). Values are means ± standard errors.

Stimulant	[^{3}H]Cyclic AMP			Total cyclic AMP		
	Control	Reserpine	Change	Control	Reserpine	Change
	cpm/mg protein		%	*pmoles/mg protein*		%
None	1500 ± 217	1680 ± 280		69 ± 15	56 ± 12	
Norepinephrine	6500 ± 155	9300 ± 775	+43[a]	330 ± 12	497 ± 21	+50[b]

[a] $p < 0.02$ (two-tailed t-test) with respect to controls.
[b] $p < 0.001$ (two-tailed t-test) with respect to controls.

TABLE 2

Accumulation of cyclic AMP elicited by norepinephrine in rat cortical slices at various intervals after treatment with reserpine

Rats either received sham injections or were treated with reserpine. In separate experiments individual rats (numbers in parentheses) were killed 5 hr after a single injection or 9 or 16 days after the second of two daily injections. After labeling with [^{14}C]adenine the cortical slices were incubated with or without 100 μM norepinephrine for 30 min. Results are expressed as means ± standard errors.

Time after injection	Stimulant	[^{14}C]Cyclic AMP	
		Control	Reserpine
		% conversion	
5 hr	None	0.30 ± 0.01 (5)	0.33 ± 0.04 (5)
	Norepinephrine	3.46 ± 0.36 (5)	3.47 ± 0.45 (5)
9 days	None	0.51 ± 0.03 (6)	0.51 ± 0.03 (6)
	Norepinephrine	3.06 ± 0.16 (6)	4.72 ± 0.59 (6)[a]
16 days	None	0.44 ± 0.06 (3)	0.49 ± 0.06 (3)
	Norepinephrine	2.53 ± 0.78 (3)	2.37 ± 0.23 (3)

[a] $p < 0.02$ (two-tailed t-test) with respect to control.

TABLE 3

Accumulation of cyclic AMP elicited by various stimulants in rat cortical slices

Rats either received sham injections or were treated with reserpine 2 days and 1 day before death. After labeling with [^{14}C]adenine the cortical slices from two rats were divided into five equal portions, followed by incubation with drugs (100 μM) for 30 min. In another set of experiments a phosphodiesterase inhibitor (Ro 20-1724, 250 μM) was present during the 30-min incubation. Data are expressed as means ± standard errors for four or six sets of experiments.

Stimulant	[^{14}C]Cyclic AMP			
	No phosphodiesterase inhibitor		With phosphodiesterase inhibitor	
	Control	Reserpine	Control	Reserpine
	% conversion		*% conversion*	
None	0.36 ± 0.06	0.48 ± 0.02	1.72 ± 0.29	1.61 ± 0.12
Norepinephrine	2.25 ± 0.20	3.60 ± 0.23[a]	6.44 ± 0.74	8.91 ± 0.73[b]
Serotonin	0.39 ± 0.09	0.53 ± 0.07	2.16 ± 0.26	1.86 ± 0.31
Dopamine			3.79 ± 0.73	3.06 ± 0.41
Histamine			2.37 ± 0.22	1.92 ± 0.25
Adenosine	2.26 ± 0.13	2.88 ± 0.22[b]		
Veratridine	5.95 ± 0.28	6.05 ± 0.31		

[a] $p < 0.001$ (two-tailed t-test) with respect to controls.
[b] $p < 0.05$ (two-tailed t-test) with respect to controls.

The maximal response to norepinephrine was enhanced by about 40% in slices from reserpine-treated rats in *both* the presence and absence of cocaine. Thus, although the potentiation of responses was nearly twice as great in slices from rats treated with 6-hydroxydopamine (1, 3) as in slices from animals given reserpine, in both cases the affinity of the receptors for norepinephrine does not appear to be greatly altered.

Experiments with α-methyl-p-tyrosine did not reveal an enhanced responsiveness of norepinephrine-sensitive cyclic AMP-generating systems 48 hr after inhibition of the synthesis of norepinephrine.[1]

Prior treatment with reserpine did not significantly affect the total incorporation of radioactive adenine into slices (results not shown). In addition, the specific activity of cyclic AMP in norepinephrine-stimulated slices labeled during a prior incubation with radioactive adenine was not significantly different in experiments with slices from control and reserpine-treated rats (Table 1). Thus determination of percentage conversions of radioactive nucleotides to cyclic AMP in slices from control and reserpine-treated rats provides a valid method for comparison of responses of the cyclic AMP-generating systems (see ref. 14).

Maximal concentrations of cyclic AMP were attained after 20 min of stimulation by norepinephrine in slices from both control (see ref. 15) and reserpine-treated rats (Fig. 2). The rates of both rise and decline of cyclic AMP were similar in control and reserpine-treated preparations. The reason for the decline in cyclic AMP in the continued presence of a stimulant in brain slices is not yet clear. It could be the result of enhanced phosphodiesterase activity (16–19), the gradual depletion of precursor ATP, or refractoriness of receptor mechanisms. It is surprising that at low concentrations of the stimulatory agent, norepinephrine, no such decline in maximal levels was observed over a period of 90 min (Fig. 2).

The responses of cyclic AMP-generating systems to other stimulatory agents were examined in slices from control and reserpine-treated animals (Table 3). Responses to serotonin, dopamine, and histamine were not significantly increased after reserpine administration. These amines had significant effects on concentrations of cyclic AMP in rat cortical slices only in the presence of the phosphodieseterase inhibitor Ro 20-1724.

[1] Unpublished experiments.

The response to adenosine was significantly increased in slices from reserpine-treated rats, although to a lesser extent than the response to norepinephrine. Since adenosine has been proposed to mediate the accumulation of cyclic AMP elicited by depolarizing agents, such as veratridine (20, 21), it is interesting that the response to veratridine was not altered in slices from reserpine-treated rats (Table 3). Adenosine and norepinephrine have greater than additive effects on the accumulation of cyclic AMP in rat cerebral cortical slices (14, 22), indicating that these agents interact to some extent with a common cyclic AMP-generating compartment. Thus an increase in the response to adenosine in a norepinephrine-sensitive compartment might not be totally unexpected. The response of cyclic AMP-generating systems to adenosine in slices from 6-hydroxydopamine-treated rats, however, was not significantly increased (1, 3), although a trend toward higher responses to adenosine was evident (3). Responses to electrical stimulation were significantly enhanced in cerebellar slices from guinea pigs treated with 6-hydroxydopamine and in cerebral cortical and cerebellar slices from animals treated with reserpine (23, 24). Responses to veratridine were not altered in cerebral cortical slices from 6-hydroxydopamine-treated rats (3).

The concept of *alpha* and *beta* adrenergic receptors developed for the peripheral autonomic nervous system has been extended to

TABLE 4

Effect of phosphodiesterase inhibitor (Ro 20-1724) on accumulation of cyclic AMP elicited by norepinephrine in rat cortical slices

Rats either received sham injections or were treated with reserpine 2 days and 1 day before death. After labeling with [^{14}C]adenine the cortical slices from each rat were divided into four equal portions and incubated for 30 min with or without norepinephrine and/or Ro 20-1724. The numbers of separate experiments are shown in parentheses. Data are expressed as means ± standard errors.

Norepinephrine (3 μM)	Phosphodiesterase Inhibitor (250 μM)	[^{14}C]Cyclic AMP	
		Control	Reserpine
		% conversion	
−	−	0.51 ± 0.06 (4)	0.48 ± 0.13 (4)
−	+	1.25 ± 0.11 (4)	1.46 ± 0.17 (4)
+	−	1.76 ± 0.13 (8)	2.51 ± 0.21 (8)[a]
+	+	4.28 ± 0.30 (8)	5.75 ± 0.23 (8)[b]

[a] $p < 0.01$ (two-tailed t-test) with respect to controls.

[b] $p < 0.002$ (two-tailed t-test) with respect to controls.

TABLE 5

Accumulation of cyclic AMP elicited by norepinephrine and isoproterenol in rat cortical slices: effects of alpha and beta adrenergic antagonists

Rats either received sham injections or were treated with reserpine 2 days and 1 day before death. In six separate experiments cortical slices from two reserpine-treated rats were pooled, as were slices from two control rats. After labeling with [^{14}C]adenine slices were divided into five equal portions, which were then incubated with the various stimulants and antagonists (all at 100 μM). Data are presented as means ± standard errors.

Drugs	[^{14}C]Cyclic AMP		Change
	Controls	Reserpine	
	% conversion		%
None	0.27 ± 0.02	0.44 ± 0.04	
Isoproterenol	1.30 ± 0.11	2.21 ± 0.21	+70[a]
Norepinephrine	2.59 ± 0.12	3.87 ± 0.39	+49[a]
Norepinephrine + phentolamine	1.41 ± 0.10	2.16 ± 0.32	+53[b]
Norepinephrine + propranolol	0.91 ± 0.18	0.79 ± 0.16	−17

[a] $p < 0.01$ (two-tailed t-test) with respect to controls.

[b] $p < 0.05$ (two-tailed t-test) with respect to controls.

the central nervous system. In contrast to the peripheral sympathetic system, both *alpha* and *beta* adrenergic stimulation leads to an accumulation of cyclic AMP in rat cerebral cortical slices (14, 15, 22). The present data suggest that the increase in *beta* adrenergic response to norepinephrine in slices from reserpine-treated rats is significantly greater than the increase in the *alpha* adrenergic response (Table 5). This is in marked contrast to the results with slices from 6-hydroxydopamine-treated rats, where there were similar increases in both *alpha* and *beta* adrenergic responses (1, 3).

It is clear that an increase in the maximal accumulation of cyclic AMP elicited by norepinephrine could result from higher norepinephrine-sensitive adenylate cyclase activity, a reduction in phosphodiesterase activity, an increase in the concentration of ATP, or a combination of such alterations in the compartment associated with the norepinephrine-sensitive cyclase. Unfortunately there appears to be no unambiguous means of distinguishing between increased adenylate cyclase activity and decreased phosphodiesterase activity in the brain slice system. With brain, in contrast to pineal and other tissue, homogenization results in significant, perhaps variable, loss of the norepinephrine sensitivity of adenylate cyclases (25–27). Thus a study of norepinephrine-sensitive cyclase activity in homogenates would not be expected to yield meaningful results. Dopamine-sensitive adenylate cyclase activity in mouse striatal homogenates was not increased after 6-hydroxydopamine or after inhibition of norepinephrine synthesis by α-methyltyrosine (28). Phosphodiesterase activity measured in homogenates represents the total from all cell types, of which the phosphodiesterase associated with the norepinephrine-sensitive cyclase may be only a small fraction. A significant alteration in phosphodiesterase activity associated with a minor norepinephrine-sensitive system might thus be masked by the lack of change in other phosphodiesterases. A priori it would be expected that kinetic analysis of formation and degradation of cyclic AMP in the intact brain slice would be informative. In an ideal system the rate of accumulation of cyclic AMP during stimulation by norepinephrine would be a function of the activity of both cyclases and phosphodiesterase. The half-times of rise and decline of cyclic AMP concentrations, the latter after removal of norepinephrine, would be a function only of the phosphodiesterase activity. Thus, if ideal kinetics were followed, the half-time to maximal accumulation of cyclic AMP should be greater in slices from reserpine-treated rats if the phosphodiesterase activity were lower in these preparations. It is clear from Fig. 2 that the half-time to maximal concentrations of cyclic AMP is at best only marginally greater in slices from reserpine-treated rats The brain slice, however, is not an ideal system, and the kinetics of cyclic AMP accumulation and disappearance is dependent in large measure on the diffusion rates of the stimulatory agents into and out of the slices (9). Indeed, the half-time to maximal accumulation of cyclic AMP was only slightly increased when norepinephrine was used in combination with the phosphodiesterase inhibitor Ro 20-1724 (results not shown).

The accumulation of cyclic AMP elicited by norepinephrine is potentiated 2–3-fold by the presence of a phosphodiesterase inhibitor, Ro 20-1724, in slices from both control and reserpine-treated rats (Table 4). The response to norepinephrine is enhanced by 30–40% in slices from reserpine-treated rats compared to control slices in both the presence and absence of the phosphodiesterase inhibitor. Similar results were seen with slices from 6-hydroxydopamine-treated rats, using papaverine or isobutylmethylxanthine as the phosphodiesterase inhibitor (3). Such data could be interpreted to indicate that the enhanced response in slices from treated rats is due primarily to increased activity of the adenylate cyclase. However, since it is uncertain to what extent the phosphodiesterases were inhibited in the intact cell, the results remain inconclusive. Thus, in brain, the nature of regulation of the responsiveness of norepinephrine-sensitive cyclic AMP-generating systems to chronic variations in functional input has not been defined.

The persistence of the enhanced response to norepinephrine for at least 9 days after

reserpine makes it possible to correlate changes in behavior with the increased responsiveness of cyclic AMP-dependent systems. With the present dosage schedule the overt effects of reserpine (sedation, ptosis, diarrhea, etc.) completely disappeared by the third day although amine levels remain depressed for several more days (10). This discrepancy has been attributed to replenishment of a small "functional" pool of norepinephrine before the total levels recover (29). The present results suggest that, in addition to the proposed replenishment of a functional pool of norepinephrine by increased levels of tyrosine hydroxylase (30), enhanced responsiveness of a norepinephrine-sensitive cyclic AMP-generating system is manifest during the period after reserpine treatment, when rats regain normal activity or become hyperactive. Indeed, the responsiveness of norepinephrine-sensitive cyclic AMP-generating systems in slices from whole rat brains exhibits an excellent positive correlation with the extent of hyperactivity after 8 days of chronic reserpine treatment (6).

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