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Accumulation of Adenosine Cyclic 3′,5′-Monophosphate in Rat Cerebral Cortical Slices

Stimulatory Effect of *Alpha* and *Beta* Adrenergic Agents after Treatment with 6-Hydroxydopamine, 2,3,5-Trihydroxyphenethylamine, and Dihydroxytryptamines

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SUMMARY

HUANG, M., HO, A. K. S., AND DALY, J. W.: Accumulation of adenosine cyclic 3′,5′-monophosphate in rat cerebral cortical slices. Stimulatory effect of *alpha* and *beta* adrenergic agents after treatment with 6-hydroxydopamine, 2,3,5-trihydroxyphenethylamine, and dihydroxytryptamines. *Mol. Pharmacol.* **9**, 711–717 (1973).

The effects of norepinephrine, isoproterenol, serotonin, adenosine, veratridine, *alpha* and *beta* adrenergic antagonists, and combinations of these agents on the accumulation of cyclic [^{14}C]AMP in cerebral cortical slices labeled during incubation with [^{14}C]adenine has been ascertained with tissue obtained from control rats and from rats treated with intraventricular 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine), 2,3,5-trihydroxyphenethylamine, 5,6-dihydroxytryptamine, or 5,7-dihydroxytryptamine. Treatment with 6-hydroxydopamine increased the accumulation of cyclic [^{14}C]AMP elicited by norepinephrine, isoproterenol, and a combination of adenosine and norepinephrine. The responses to adenosine and veratridine were virtually unaffected. Both *alpha* and *beta* adrenergic receptor-mediated increases in cyclic 3′,5′-AMP accumulation appeared to be potentiated by treatment with 6-hydroxydopamine. 2,3,5-Trihydroxyphenethylamine, although much more toxic than 6-hydroxydopamine, also enhanced the accumulation of cyclic [^{14}C]AMP elicited by norepinephrine and isoproterenol. The dihydroxytryptamines caused no significant changes in cyclic [^{14}C]AMP accumulation. The extent of accumulation of cyclic AMP elicited by a catecholamine in the presence of phosphodiesterase inhibitors, such as papaverine or isobutylmethylxanthine, was also significantly higher in slices from 6-hydroxydopamine-treated animals. Selective destruction of noradrenergic terminals in the cerebral cortex by trihydroxyphenethylamines thus appears to enhance the maximal accumulation of cyclic AMP elicited in slices by norepinephrine, primarily through mechanisms involving an increased rate of cyclic AMP formation from intracellular ATP rather than through a decrease in the rate of degradation of the cyclic nucleotide.

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INTRODUCTION

The accumulation of adenosine cyclic 3′,5′-monophosphate (cyclic AMP) elicited

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in rat brain slices upon incubation with norepinephrine has recently been shown to be enhanced in tissues obtained from various brain regions from animals that had been treated with 6-hydroxydopamine (1, 2). Norepinephrine has also recently been shown to elicit the accumulation of cyclic AMP in rat cerebral cortical slices by apparent interactions with *both* classical *alpha* and *beta* adrenergic receptors (3, 4). The present paper is concerned with *alpha* and *beta* receptor-mediated activation of cyclic AMP formation in animals treated with 6-hydroxydopamine, with 2,3,5-trihydroxyphenethylamine, an isomeric amine that also apparently causes destruction of adrenergic terminals (5), and with dihydroxytryptamines, compounds which apparently have cytotoxic effects primarily on serotonin-containing cells (6–9). Results similar to those presented here have recently been reported by Perkins *et al.* (10).

MATERIALS AND METHODS

Male Sprague-Dawley rats (150–200 g) obtained from Zivic Miller Laboratories were used. Compounds were purchased from commercial sources or drug companies, except for 5,6- and 5,7-dihydroxytryptamine, which were products of Regis Chemical Company (under Research Contract SA 43-ph-3021, generously provided by Dr. A. A. Manian, Psychopharmacology Service Center, National Institute of Mental Health), and 2,3,5-trihydroxyphenethylamine (5). Compounds were administered intraventricularly or intracisternally to rats as described (11, 12) in 20 μl of NaCl solution containing ascorbic acid (0.1%). Controls were treated with the same volume of NaCl-ascorbic acid solution. Animals were killed at appropriate intervals by decapitation. The brains were rapidly removed, and 260-μ slices of cerebral cortical gray matter were prepared essentially as described by Daly (13). Initial incubation and the incubation with 16 μM [^{14}C]adenine (2 μCi) for 40 min, followed by 10 min of incubation before addition of test agents and subsequent assay of cyclic [^{14}C]AMP were also conducted essentially as described previously (13). The period of incubation with test agents was 8–15 min. During this period the accumulation of cyclic [^{14}C]AMP elicited by various agents is maintained near maximal values (4). Parallel comparisons of the effects of agents in control slices and in slices from drug-pretreated animals were always made at nearly the same intervals. Results for accumulation of cyclic [^{14}C]AMP are reported as percentage conversion, i.e., the percentage of total intracellular radioactive adenine nucleotides present as radioactive cyclic AMP.

RESULTS AND DISCUSSION

Rats receiving an intraventricular injection of 6-hydroxydopamine (170–250 μg of free base) recovered completely within 1–2 days. However, such rats appeared much more sensitive to external stimuli, such as sound or handling, than controls receiving only NaCl. 2,3,5-Trihydroxyphenethylamine was much more toxic than 6-hydroxydopamine; all rats receiving an intraventricular injection of 250 μg of free base died within 1 day. At one-half this dose the rats survived, but were extremely quiescent and exhibited piloerection, gnawing activity, and lack of appetite for the remainder (5–7 days) of the experiments. Rats receiving 5,6-dihydroxytryptamine intraventricularly (100 μg of free base) also exhibited signs of marked toxicity. The animals were completely inactive for a number of days and could not seek either food or water. 5,7-Dihydroxytryptamine (100 μg of free base) was not as toxic, and the animals recovered both appetite and activity within 1–2 days. The animals did appear somewhat more reactive to stimuli than controls.

Accumulations of cyclic AMP in these experiments were ascertained using the labeling technique with radioactive adenine (13). This method, in both rat (3, 10) and guinea pig (14) cerebral cortical slices, has given results consonant with those obtained by measurement of endogenous levels of cyclic AMP. The results with Sprague-Dawley rats that had received an intraventricular injection of 6-hydroxydopamine (250 μg of free base) clearly demonstrated that during a period of 5–12 days after administration of 6-hydroxydopamine the responses of cere-

bral cortical cyclic AMP-generating system(s) both to norepinephrine, a compound with both *alpha* and *beta* adrenergic agonist activity, and to isoproterenol, a classical *beta* adrenergic agonist (15), were potentiated (Table 1). Indeed, the increase above control values of cyclic [^{14}C]AMP elicited by either of these catecholamines was approximately twice that in rats which had not received 6-hydroxydopamine. No significant response of the cyclic AMP-generating system to dopamine or serotonin was observed in slices from either control or 6-hydroxydopamine-treated animals. The responses to adenosine and to a depolarizing agent, veratridine (see ref. 16), appeared somewhat increased by prior treatment with 6-hydroxydopamine, but this apparent increase was not statistically significant ($p < 0.1$). The synergistic response to a combination of adenosine and norepinephrine was significantly increased in slices from animals previously treated with 6-hydroxydopamine, while the responses to adenosine and serotonin or adenosine and histamine combinations did not appear significantly changed. Indeed, the response to the adenosine and serotonin combination in rat cortical slices was not significantly greater than that to adenosine alone. In guinea pig cortical slices, adenosine and serotonin combinations do have synergistic effects on accumulations of cyclic AMP (17). The results indicate that in rats 6-hydroxydopamine caused a significant and selective increase in the response of cortical cyclic AMP-generating systems to catecholamines and to catecholamine-adenosine combinations but not to adenosine, veratridine, histamine, or serotonin. After intracisternal injection of 6-hydroxydopamine (200 μg of free base) in Sprague-Dawley rats or after intraventricular injection (170 μg of free base) in Wistar rats, the accumulation of cyclic AMP elicited by norepinephrine in cortical slices was also markedly enhanced (data not shown). The present findings and those of Perkins *et al.* (10) thus confirm and supplement the previous reports that 6-hydroxydopamine treatment enhances norepinephrine-mediated responses of the cyclic AMP-generating system of brain slices (1, 2).

TABLE 1

Effect of intraventricular administration of 6-hydroxydopamine on subsequent drug-elicited accumulation of cyclic [^{14}C]AMP in cerebral cortical slices from Sprague-Dawley rats

Sprague-Dawley rats received an intraventricular injection of 6-hydroxydopamine (200 μg of free base) in 20 μl of NaCl. Controls received only NaCl. The rats were killed 5–14 days later, and cortical slices were prepared and incubated as described under MATERIALS AND METHODS. No significant differences in results were observed during this time period. Each value represents the average ± standard error of the number of experiments shown in parentheses, or is the result of a single experiment. All agents were present at a concentration of 0.1 mM except for veratridine, which was 0.08 mM.

Stimulatory agent	Cyclic [^{14}C]AMP conversion	
	Control	6-Hydroxydopamine
	%	%
None	0.35 ± 0.04 (10)	0.35 ± 0.07 (11)
Norepinephrine	2.4 ± 0.2 (10)	5.2 ± 0.4 (11)[a]
Isoproterenol	1.2 ± 0.2 (8)	2.8 ± 0.3 (9)[a]
Dopamine	0.25	0.32
Serotonin	0.21	0.26
Adenosine	2.8 ± 0.3 (10)	3.4 ± 0.4 (11)
Adenosine + norepinephrine	9.1 ± 1.0 (6)	12.5 ± 1.2 (6)[b]
Adenosine + serotonin	2.8 ± 0.3 (4)	3.3 ± 0.7 (4)
Adenosine + histamine	4.6 ± 0.8 (4)	5.1 ± 0.7 (4)
Veratridine	5.8 ± 0.4 (6)	7.5 ± 1.1 (6)

[a] $p < 0.01$.
[b] $p < 0.05$.

Intraventricular administration of 6-hydroxydopamine causes neuronal degeneration (18), thereby impairing the uptake of norepinephrine into brain slices *in vitro* (19). However, lack of a mechanism for the uptake of catecholamines does not seem likely to account for the enhanced formation of cerebral cyclic AMP in response to supramaximal concentrations (0.1 mM) of isoproterenol and norepinephrine. Certainly, with isoproterenol, an amine that is not concentrated at presynaptic catecholamine uptake sites (20), the enhanced response must be due to postsynaptic mechanisms. Furthermore, cocaine, an inhibitor of neuronal uptake of norepinephrine, does not potentiate the effect of 0.1 mM norepinephrine on cyclic AMP formation (4). Cocaine did potentiate the effects of low concentrations (1 μM) of norepinephrine in control rats but not in 6-hydroxydopamine-treated rats (10).

Previous studies (2, 3) had demonstrated that accumulation of cyclic AMP elicited by norepinephrine or by a combination of norepinephrine and adenosine in rat cerebral cortical slices is apparently mediated by *alpha* and *beta* adrenergic receptors. Following intraventricular injection of 6-hydroxydopamine, the enhanced formation of cerebral cyclic AMP in respose to isoproterenol, a pure *beta* agonist (15), clearly shows that the *beta* responses were potentiated. The maximal responses to norepinephrine, a compound with both *alpha* and *beta* adrenergic agonist activity, were approximately twice the response due to isoproterenol in both control and 6-hydroxydopamine-treated animals (Table 1). Thus 6-hydroxydopamine treatment might have induced a concomitant activation of processes involved in both *alpha* and *beta* receptor-mediated formation of cerebral cyclic AMP. In order to test this hypothesis, another series of experiments was carried out, comparing norepinephrine- and isoproterenol-elicited formation of cyclic AMP in the presence of an *alpha* or a *beta* antagonist. It was found that the *percentages of inhibition* of the catecholamine-mediated increases in accumulation of cyclic [^{14}C]AMP by *alpha* and *beta* adrenergic antagonists in slices from control and 6-hydroxydopamine-treated animals were remarkably similar (Table 2).

TABLE 2

Effect of intraventricular administration of 6-hydroxydopamine on alpha and beta receptor-mediated accumulation of cyclic [^{14}C]AMP in cerebral cortical slices from Sprague-Dawley rats

Sprague-Dawley rats (Zivic Miller Laboratories) received an intraventricular injection of 6-hydroxydopamine (250 μg of free base) in 20 μl of NaCl. Controls received only NaCl. The rats were killed 5–20 days later, and cortical slices were prepared and incubated. No significant differences in results were observed during this time period. Each value represents the average ± standard error of four experiments or is the result of a single experiment. In two similar experiments with Wistar rats the effect of norepinephrine on cyclic [^{14}C]AMP was inhibited 50–60% by phentolamine and 70–80% by propranolol in slices from both control and 6-hydroxydopamine-treated animals.

Agent(s) (0.1 mM)	Cyclic AMP conversion		Inhibition by blocking agent	
	Control	6-Hydroxydopamine	Control	6-Hydroxydopamine
	%	%	%	%
None	0.33 ± 0.02	0.36 ± 0.10		
Norepinephrine	2.8 ± 0.3	4.3 ± 0.5		
Norepinephrine + phentolamine	1.8 ± 0.1	2.5 ± 0.4	40	47
Norepinephrine + dichloroisoproterenol	0.9 ± 0.1	1.4 ± 0.4	76	75
Norepinephrine + propranolol	0.8 ± 0.1	1.0 ± 0.2	80	85
Isoproterenol	1.5 ± 0.1	2.0 ± 0.2		
Isoproterenol + phentolamine	1.5	2.2	0	0
Isoproterenol + propranolol	0.4	0.6	95	92

The response elicited by isoproterenol was blocked by a *beta* antagonist, propranolol, as expected, while the response to norepinephrine was partially blocked by either *alpha* or *beta* antagonists, with the latter somewhat more effective. The data thus suggest that both *alpha* and *beta* adrenergic receptor-mediated mechanisms are associated with postsynaptic structures but not with the noradrenergic nerve terminal, since the latter is virtually destroyed by 6-hydroxydopamine (18).

The present data on enhanced responses after "denervation" are not typical of the classical adrenergic "supersensitivity," which is due to lack of presynaptic uptake processes (21). It is perhaps more like the classical "supersensitivity" of the denervated cholinergic neuromuscular junction, which is related to a greatly increased number of postsynaptic receptors (22, 23). However, whether the permanent changes in the response of the cyclic AMP-generating system to norepinephrine after 6-hydroxydopamine treatment are due to enhanced synthesis of the receptor–adenyl cyclase system or to other mechanisms is at present unknown. The enhanced response could be the result of a lower rate of degradation of cyclic AMP in the norepinephrine-sensitive compartment of the slice, i.e., lower phosphodiesterase activity in this compartment. However, in the presence of high concentrations of effective phosphodiesterase inhibitors, such as isobutylmethylxanthine and papaverine (see ref. 24), the accumulation of cyclic AMP elicited by norepinephrine or isoproterenol was still significantly higher in slices from 6-hydroxydopamine-treated rats (Table 3). Thus it appears unlikely that a decrease in phosphodiesterase activity associated with a norepinephrine-sensitive cyclic AMP-generating system is primarily responsible for the enhanced responses of this system in slices from 6-hydroxydopamine-treated animals.

In an attempt to determine whether other compounds that have been reported to have cytotoxic effects on neuronal structures would also affect the responses of the cortical cyclic AMP-generating system, an isomer of 6-hydroxydopamine, 2,3,5-trihydroxyphenethylamine, and two isomeric dihydroxytryptamines were investigated. In spite of the fact that the 2,3,5-trihydroxyphenethylamine had to be used at relatively low concentrations (125 μg of free base) because of its marked toxicity, it did potentiate the response to norepinephrine and isoproterenol (Table 4). This compound has been reported to have effects similar to 6-hydroxydopamine in noradrenergic structures (5).

5,6-Dihydroxytryptamine, which has been reported (6, 7, 9) to have selective effects on serotonin-containing neurons, and 5,7-dihydroxytryptamine, a compound with similar activity (8), are toxic substances, particularly the former. However, after administration of nearly maximal amounts by intraventricular injection, neither of these compounds appeared to have significant effects on responses of cortical slices to catecholamines, serotonin, adenosine, or adenosine–biogenic amine combinations (Table 4).

TABLE 3

Catecholamine-elicited accumulation of cyclic [^{14}C]AMP in cerebral cortical slices from Sprague-Dawley rats in the presence of phosphodiesterase inhibitors

Sprague-Dawley rats were treated with 6-hydroxydopamine or with NaCl and killed 10–17 days later, and cortical slices were prepared and incubated. For further details, see the legend to Table 2. Each value represents the average ± standard error of four experiments or the results of a single experiment.

Catecholamine (0.1 mM) and phosphodiesterase inhibitor	Cyclic AMP conversion	
	Control	6-Hydroxydopamine
No catecholamine		
No inhibitor	0.45±0.08	0.47±0.16
Isobutylmethylxanthine (1.0 mM)	0.6	0.5
Papaverine (0.5 mM)	1.1	1.2
Norepinephrine		
No inhibitor	3.0 ±0.5	5.1 ±0.6
Isobutylmethylxanthine	5.5 ±0.5	7.8 ±0.7
Papaverine	4.4 ±0.4	5.9 ±0.6
Isoproterenol		
No inhibitor	1.7 ±0.4	3.1 ±0.4
Isobutylmethylxanthine	3.8 ±0.3	6.0 ±0.7
Papaverine	3.4 ±0.4	4.8 ±0.3

TABLE 4

Effects of intraventricularly administered 2,3,5-trihydroxyphenethylamine and 5,6- and 5,7-dihydroxytryptamine on drug-elicited accumulation of cyclic [^{14}C]AMP in cerebral cortical slices from Sprague-Dawley rats

Sprague-Dawley rats (Zivic Miller Laboratories) received an intraventricular injection of either 2,3,5-trihydroxyphenethylamine (125 μg of free base), 5,6- or 5,7-dihydroxytryptamine (100 μg of free base), or NaCl. They were killed 7–10 days later, and slices were prepared and incubated as described under MATERIALS AND METHODS (see the legend to Table 1). Concentrations of all agents were 0.1 mM, except for veratridine (0.08 mM).

Agent	Cyclic [^{14}C]AMP conversion			
	Control	2,3,5-Trihydroxyphenethylamine	5,6-Dihydroxytryptamine	5,7-Dihydroxytryptamine
	%	%	%	%
None	0.27 ± 0.02 (5)	0.3, 0.2	0.3	0.4, 0.2
Norepinephrine	2.3 ± 0.2 (5)	3.6 ± 0.3 (3)[a]	2.0	3.0, 3.0
Isoproterenol	1.2 ± 0.2 (5)	2.0 ± 0.2 (3)[a]	1.4	1.4, 1.6
Serotonin	0.2, 0.2		0.2	0.2
Adenosine	2.6 ± 0.3 (5)	2.8 ± 0.3 (3)	3.0	3.6, 3.1
Adenosine + norepinephrine	9.8 ± 1.3 (4)		9.6	11.1, 11.5
Adenosine + serotonin	2.5, 2.5		2.9	3.5
Veratridine	6.1 ± 0.6 (5)	6.1	6.9	8.4, 6.2

[a] $p < 0.01$.

Responses of the cyclic AMP-generating system to serotonin are, however, minimal in rat cerebral cortical slices (see ref. 4), although they have been demonstrated in guinea pig cerebral cortical slices (14, 17, 25), in rabbit cerebellar slices (26, 27), and in slices from functional regions of squirrel monkey cerebral cortex (28).

The present results indicate that the activity of the norepinephrine-sensitive cyclic AMP-generating system of brain slices can be enhanced by inhibition of adrenergic neuronal input. Such an effect might reflect changes in postsynaptic mechanisms, as a result of a lack of neurotransmitter or of other neurotrophic factors. In order to investigate this phenomenon, the effects of other drugs which influence adrenergic mechanisms are being investigated. The results of such studies should provide a deeper insight into the mechanisms involved in the control of cyclic AMP levels in cellular entities of the central nervous system.

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