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Adenosine 3′,5′-Monophosphate: Electrophysiological Evidence for a Role in Synaptic Transmission

Abstract. *Synaptic potentials and changes in resting membrane potentials of superior cervical ganglia of the rabbit were measured in the presence of adenosine 3′5′-monophosphate and agents that affect its metabolism. Adenosine 3′5′-monophosphate and its mono- and dibutyryl derivatives caused a hyperpolarization of the postganglionic neurons. Theophylline potentiated the slow inhibitory postsynaptic potential that follows synaptic transmission, as well as the hyperpolarization of postganglionic neurons caused by exogenous dopamine. Conversely, prostaglandin E_1 inhibited both the slow inhibitory postsynaptic potential and the dopamine-induced hyperpolarization. We hypothesize that the slow inhibitory postsynaptic potential as well as the dopamine-induced hyperpolarization result from increased amounts of adenosine 3′5′-monophosphate in the postganglionic neurons. The dibutyryl derivative of guanosine 3′5′-monophosphate caused a depolarization of the postganglionic neurons, which is consistent with the possibility that guanosine 3′5′-monophosphate mediates synaptic transmission at muscarinic cholinergic synapses.*

Studies in our laboratory have implicated adenosine 3′,5′-monophosphate (cyclic AMP) in the physiology of synaptic transmission in the mammalian superior cervical sympathetic ganglion (*1–4*). Stimulation of preganglionic fibers causes an increase in the amount of cyclic AMP in this ganglion (*1, 2*). In addition, dopamine, a putative neurotransmitter in the ganglion (*2–6*), increases the amount of ganglionic cyclic AMP (*3*) and causes a hyperpolarization of the postganglionic neurons (*5, 6*) (see Fig. 1). The effects of dopamine, both on cyclic AMP (*3*) and on postganglionic membrane potential (*5, 6*), are antagonized by α-adrenergic blocking agents. To account for these and other results, we have suggested (*2–4*) that dopamine, released from interneurons during activity, causes an increase in the amount of cyclic AMP in the postganglionic neurons and that it is this increased cyclic AMP which is responsible for the slow inhibitory postsynaptic potential (slow-IPSP) that follows preganglionic stimulation. We now report the results of electrophysiological studies designed to test certain predictions made by this hypothesis, namely, (i) that cyclic AMP would hyperpolarize the postganglionic neurons; (ii) that theophylline, a phosphodiesterase inhibitor which potentiates the accumulation of cyclic AMP in the ganglion (*7*), would also potentiate the slow-IPSP as well as the hyperpolarization due to dopamine; and (iii) that prostaglandin E_1 (PGE_1), which has been shown to affect adenylate cyclase activity of almost all tissues studied, might alter the slow-IPSP as well as the hyperpolarization due to dopamine.

Changes in membrane potential of postganglionic neurons in superior cervical sympathetic ganglia of the rabbit were measured by the sucrose gap technique (*8*). Preganglionic stimulation of this ganglion results in the generation of an initial brief excitatory postsynaptic potential (initial EPSP), followed successively by a slow-IPSP that reaches a maximum within 600 msec, and a slow excitatory postsynaptic potential (slow-EPSP) lasting

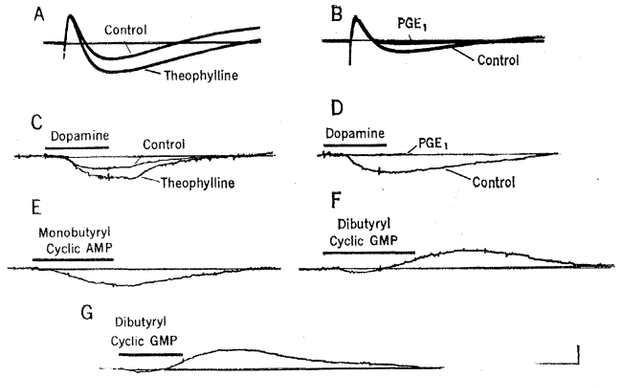

Fig. 1. (A to F) Effect of cyclic nucleotides, and of agents which affect cyclic AMP metabolism, on synaptic and resting membrane potentials recorded by means of the sucrose gap technique from postganglionic neurons of the superior cervical sympathetic ganglion of the rabbit. (A and B) Oscillographic traces of electrotonically conducted synaptic potentials elicited in response to a single supramaximum stimulus to the preganglionic nerve. Hexamethonium chloride (600 μ*M*) was present to abolish propagated responses. (A) Responses obtained in Locke solution and after 30 minutes of superfusion with Locke solution containing 1.5 m*M* theophylline are superimposed. (B) Responses obtained in normal Locke solution and after 15 minutes of superfusion with Locke solution containing 3×10^{-7} *M* PGE_1 are superimposed. Results similar to those illustrated here were obtained when *d*-tubocurarine (125 μ*M*) was used instead of hexamethonium chloride to abolish propagated responses. This dose of *d*-tubocurarine also abolished the initial EPSP. (C and D) Resting membrane potential changes in response to a brief period of superfusion with dopamine. (C) Responses to 50 μ*M* dopamine before (control) and 30 minutes after the start of superfusion with 2 m*M* theophylline are superimposed. (D) Responses to 200 μ*M* dopamine before (control) and 20 minutes after the start of superfusion with 6×10^{-7} *M* PGE_1 are superimposed. (E and F) Changes in membrane potential in response to a brief period of superfusion with 2.5 m*M* monobutyryl cyclic AMP (E) or 25 μ*M* dibutyryl cyclic GMP (F). (G) Change in membrane potential of the cervical vagus nerve in response to a brief period of superfusion with 200 μ*M* dibutyryl cyclic GMP. The duration of superfusion with Locke solutions containing dopamine or cyclic nucleotides is indicated by the solid bars. All records are d-c recording, hyperpolarization downward. Calibration marks: (A and B) 1 second, 800 μv; (C to G) 2 minutes, 400 μv.

approximately 30 seconds. The slow-IPSP, but not the initial EPSP, was potentiated in amplitude and duration in the presence of theophylline (Fig. 1A). Theophylline did not inhibit either the amplitude or the duration of the slow-EPSP (not shown). The hyperpolarization of the postganglionic neurons induced by exogenous dopamine was also potentiated by theophylline (Fig. 1C). The response to dopamine was tested by switching for 3 minutes from a superfusate of normal Locke solution to one containing 50 μM dopamine (prepared 2 to 5 minutes previously) and then by returning to the normal Locke solution. Theophylline (1 to 5 mM) in nine experiments caused an increase of 44 ± 7 percent (mean $\pm$ S.E.M.) in the amplitude of the slow-IPSP, and an increase of 54 ± 9 percent in the amplitude of the hyperpolarization induced by 50 to 200 μM dopamine. The effect of the theophylline could be reversed by superfusion of the ganglion with normal Locke solution for 90 minutes.

In each of 15 ganglion preparations studied, $10^{-7}M$ to $10^{-6}M$ PGE_1 virtually abolished the slow-IPSP within 10 to 20 minutes and substantially reduced the slow-EPSP, but had no effect on the initial EPSP. (A transient hyperpolarization of the postganglionic neurons of 5 to 10 minutes duration, was usually observed on starting PGE_1 superfusion.) The effect of $3 \times 10^{-7}M$ PGE_1 on the slow-IPSP is illustrated in Fig. 1B. A concentration of PGE_1 of $1 \times 10^{-8}M$ caused a 50 percent decrease in the amplitude of the slow-IPSP. In nine ganglion preparations, the effect of PGE_1 was tested on the hyperpolarization induced by 50 to 200 μM dopamine. It was found that PGE_1 abolished, or largely reduced, the dopamine-induced hyperpolarization at the same concentrations as were effective in abolishing the slow-IPSP (Fig. 1D). The effects of PGE_1 could be largely reversed on prolonged superfusion with normal Locke solution.

Monobutyryl cyclic AMP, applied in a concentration of 1 to 2.5 mM, hyperpolarized 8 of 11 ganglia tested (Fig. 1E). Qualitatively similar results were obtained with cyclic AMP and dibutyryl cyclic AMP, but not with adenosine 5′-monophosphate, adenosine, or butyric acid. Cyclic AMP and its derivatives were never observed to cause depolarization. The lack of any response, by some of the preparations, to the direct application of cyclic AMP or its derivatives may be due to a low permeability of neuronal membranes to cyclic AMP.

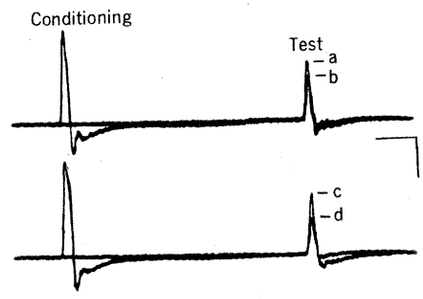

Fig. 2. Compound action potentials, elicited by submaximum stimulation of the preganglionic (cervical sympathetic) nerve, and recorded from platinum bipolar electrodes on the postganglionic (internal carotid) nerve 2 to 3 mm distant from the pole of the ganglion. Upper row, superimposed traces a and b recorded during superfusion of the ganglion with normal Locke solution. Lower row, superimposed traces c and d recorded 24 minutes after start of superfusion with Locke solution containing 5 mM theophylline. Traces a and c, response to a single test stimulus only. Traces b and d, response to a conditioning stimulus followed 600 msec later by response to a test stimulus. Conditioning stimuli were just maximum for the S_a elevation [see (*10*)]; voltage of the test stimuli was one-half that of the conditioning stimuli; pulse width for both kinds of stimuli was 0.5 msec. Calibration mark: 200 μv, 100 msec. Bandwidth, 2 hz to 10 khz.

Studies have shown that acetylcholine can cause an increase in the amount of guanosine 3′,5′-monophosphate (cyclic GMP) in heart and brain tissue (*9*). Moreover, available evidence indicates the existence, on postganglionic neurons of the superior cervical ganglion, of muscarinic-type receptors that respond to acetylcholine by causing a prolonged depolarization of the neurons (*10*). These same receptors are probably involved in the generation of the slow-EPSP (*10*). In view of the possibility that cyclic GMP might mediate this muscarinic depolarizing action of acetylcholine, we have studied the effect of cyclic GMP and dibutyryl cyclic GMP on the resting membrane potential of postganglionic neurons. Cyclic GMP itself did not cause a change in membrane potential. However, exposure of the ganglia to dibutyryl cyclic GMP, in low concentrations (2.5 to $5.0 \times 10^{-5}M$) for 4 minutes, caused a small, transient hyperpolarization followed by a depolarization of the postganglionic nerve cells in each of seven preparations tested (Fig. 1F). A depolarization of several millivolts could be achieved by maintaining the ganglion in solutions of dibutyryl cyclic GMP for longer periods of time (5 to 10 minutes). Higher doses (100 to 250 μM) of dibutyryl cyclic GMP greatly enhanced the rate of depolarization of the postganglionic neurons but either had no effect on, or decreased the size of, the transient hyperpolarization. These observations are in contrast to those made on liver slices where both cyclic AMP and cyclic GMP caused a hyperpolarization (*11*). Conceivably, cyclic GMP may mediate the slow-EPSP and, thereby, increase the responsiveness of the postganglionic neurons to subsequent excitatory input. If so, this would indicate that cyclic AMP and cyclic GMP function in opposite directions, that is, in a push-pull fashion to exert long-term control over neuronal excitability in the sympathetic ganglion.

Dopamine, theophylline, PGE_1, cyclic GMP, and cyclic AMP and its butyryl derivatives, when tested on axons of the cervical vagus nerve in the same concentrations that had been used on the ganglion, were found to cause little or no effect on the membrane potential. High concentrations of dibutyryl cyclic GMP (1 to $4 \times 10^{-4}M$) had an effect on the vagus nerve (Fig. 1G) similar to that which had been observed on the ganglion with lower concentrations.

We have been able to demonstrate consistently and reproducibly, with each of 13 preparations, that the excitability of postganglionic neurons in the superior cervical ganglion was diminished during the period of the slow-IPSP and that this inhibition was markedly potentiated by theophylline. These effects are illustrated in Fig. 2 where the unconditioned response to a submaximum test stimulus is compared with the response to the same strength of stimulus applied 600 msec after a stronger conditioning stimulus, that is, at the time of maximum development of the slow-IPSP. The upper portion of Fig. 2 contains two superimposed oscillographic traces of compound action potentials derived from the postganglionic nerve with platinum bipolar recording electrodes. The test response in trace a was elicited without a prior conditioning stimulus and therefore did not occur during a slow-IPSP. The test response elicited 600 msec after a conditioning response (trace b) and, therefore, during the slow-IPSP, was reduced 24 percent in amplitude and 20 percent in area compared to the unconditioned test response. The lower portion of Fig. 2 contains two superimposed oscil-

lographic traces of compound action potentials obtained under experimental conditions identical to those of the upper portion of Fig. 2, except that the ganglion had been superfused for 24 minutes with 5 m*M* theophylline. The unconditioned test response (trace c) and the conditioning response (early part of trace d) were unaffected by theophylline. In contrast, the conditioned test response (later part of trace d) was considerably more reduced in amplitude (46 percent) and area (44 percent) than prior to theophylline. This increased inhibition of synaptic transmission after a conditioning stimulus, observed in the presence of theophylline, can be attributed to the potentiation of the slow-IPSP achieved by this phosphodiesterase inhibitor (Fig. 1A).

Our results indicate that cyclic AMP can mimic the electrophysiologic effects of dopamine, a putative ganglionic neurotransmitter. Cyclic AMP has been found to mimic the hyperpolarizing action of β-adrenergic agonists on the Purkinje cells of the rat cerebellum (*12*) and on the smooth muscle cells of the rabbit pulmonary artery (*13*). In the case of the Purkinje cells, theophylline potentiated, and PGE_1 blocked the inhibition of spontaneous discharge caused by application of exogenous norepinephrine (*12*).

Our electrophysiological data support the hypothesis that cyclic AMP plays a role in synaptic transmission in sympathetic ganglia. There appear to be both direct excitatory and interneuron-mediated inhibitory input from the preganglionic fibers to the postganglionic neurons of the superior cervical ganglion (*10, 14*). Our evidence supports the idea (*2–4*) that the slow-IPSP is generated by an increase in the amount of cyclic AMP in the postganglionic neurons in response to dopamine released from the interneurons. The hyperpolarization of the postganglionic neurons makes them less responsive to subsequent excitatory input. According to this scheme, cyclic AMP mediates dopaminergic transmission and, thereby, modulates cholinergic transmission in the ganglion, the modulation being of an inhibitory type that produces a negative feedback and limits the effectiveness of subsequent excitation.

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Polychlorinated Biphenyls: Metabolic Behavior of Pure Isomers in Pigeons, Rats, and Brook Trout

Abstract. *The metabolic behavior of pure mono-, di-, tetra-, and hexachlorobiphenyl isomers in pigeons, rats, and brook trout was investigated. Excreta from these animals were extracted and examined by chromatographic and mass spectrometric techniques. The results showed conversion of the 4-chloro-, 4,4′-dichloro-, and 2,2′,5,5′-tetrachlorobiphenyl isomers into monohydroxylated derivatives by the rat and pigeon whereas no hydroxymetabolites were detected in the excreta of the brook trout. No hydroxylated products of 2,2′,4,4′,5,5′-hexachlorobiphenyl were detected in the excreta of pigeons, rats, or brook trout.*

Polychlorinated biphenyls (PCB) are now recognized as almost universally distributed pollutants which are generally considered to be quite resistant to chemical and enzymatic degradation. Recent evidence suggests that chloro-

Table 1. Data on hydroxylated metabolites from rat urine and pigeon excreta. Thin-layer chromatography on silica; solvent A, hexane-acetone, 2.5 : 1; solvent B, benzene–ethyl acetate, 12 : 1.

Compound administered*	Hydroxychlorobiphenyl R_F in solvent†		Mass spectrum	
	A	B	Molecular ion	Number of chlorine atoms in the metabolite
4-Chlorobiphenyl	0.5	0.5	204	1
4-Chlorobiphenyl	< 0.3		220	1‡
4,4′-Dichlorobiphenyl	0.5§	0.6	238‖	2
2,2′,5,5′-Tetrachlorobiphenyl†	0.55§	0.55	306	4

* No hydroxymetabolites could be detected in the excreta of rats or pigeons treated with 2,2′,4,4′,5,5′-hexachlorobiphenyl. † The PCB isomers move with the solvent front in these systems. ‡ This compound was found in rat urine only (that is, not in pigeon excreta). § Several minor bands in this region were extracted together; peaks due to impurities were present in the mass spectrum at different temperatures. Compounds were made visible by viewing with ultraviolet light or by spraying a portion of the plate with a 1 percent solution of 2,4,7-trinitro-9-fluorenone in acetone. ‖ The accurate mass was determined for this compound. Mass calculated for $C_{12}H_8Cl_2O$: 237.9952; mass found, 237.9959.