EVIDENCE AGAINST DOPAMINE AS THE MEDIATOR OF THE RISE OF CYCLIC AMP IN THE SUPERIOR CERVICAL GANGLION OF THE RAT

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Received May 28,1975

SUMMARY. In isolated intact superior cervical ganglia of the rat dopamine is able to increase the levels of adenosine 3', 5'-monophosphate (cAMP) only if used in concentrations about two orders of magnitude higher than norepinephrine or epinephrine. The methylxanthines papaverine and theophylline have no or liminal effects on cAMP levels in control ganglia or ganglia incubated with dopamine. However, papaverine potentiates the norepinephrine-induced accumulation of cAMP both in ganglia and incubation media. Repeated incubations with norepinephrine in the presence of papaverine show a rapid decrement of the response and a sustained fall of cAMP in ganglia and media. The catecholamine induced rise was blocked by ß-, and partially inhibited by α-adrenergic antagonists.

The role of adenosine 3', 5'-monophosphate (cAMP) in the sympathetic peripheral nervous system has been the subject of numerous investigations in the past four years (1-8). It has been shown that electrical stimulation, catecholamines and histamine induce an accumulation of cAMP in the ganglia. Moreover, evidence from experiments with slices of bovine superior cervical ganglia and isolated intact rabbit ganglia has led to the suggestion that cAMP is involved in dopamine-mediated inhibition in the ganglion (3,6). However, direct evidence that dopamine alone causes an elevation of cAMP in the intact ganglion is as yet lacking; in addition it is unclear whether the

catecholamine-induced rise in cAMP is causally related to catecholaminergic inhibition of transmission in the intact ganglion.

We present evidence that dopamine in concentrations of 10^{-5} to 10^{-3} M does not alter the cAMP-concentration in ganglia of the rat, even in the presence of theophylline, a potent phosphodiesterase inhibitor.

Moreover, we show that norepinephrine causes a large increase in the cAMP-content both in ganglia and in the medium when papaverine, another potent cAMP-phosphodiesterase inhibitor (9), is present. The effect of norepinephrine was abolished by a ß-adrenergic blocking agent, and partially blocked by an α-adrenergic blocking agent.

MATERIALS AND METHODS. Male Sprague-Dawley rats were killed by cervical dislocation and the ganglia were rapidly removed, desheathed, and kept on ice until used. Incubation procedures were essentially the same as described previously (5, 10). The cAMP content of ganglia was determined according to Gilman (11) after purification of extracts on Dowex 1X-2 columns (4). The incubation media were lyophilized, taken up in 50 mM acetate buffer pH 4.0 and assayed for cAMP by the same method.

RESULTS AND DISCUSSION. Concentrations of dopamine up to 10^{-3} M did not affect cAMP levels in ganglia, only at 10^{-2} M concentration did there appear a moderate accumulation of the nucleotide at a time when norepinephrine (10^{-4} M) and epinephrine (10^{-5} M) induced increases were maximal (figure 1).

Theophylline (0.5 mM), which alone was without significant effect on the cAMP content, was unable to potentiate the response to dopamine. The time course of the accumulation induced by high concentrations of dopamine (10^{-2} M)

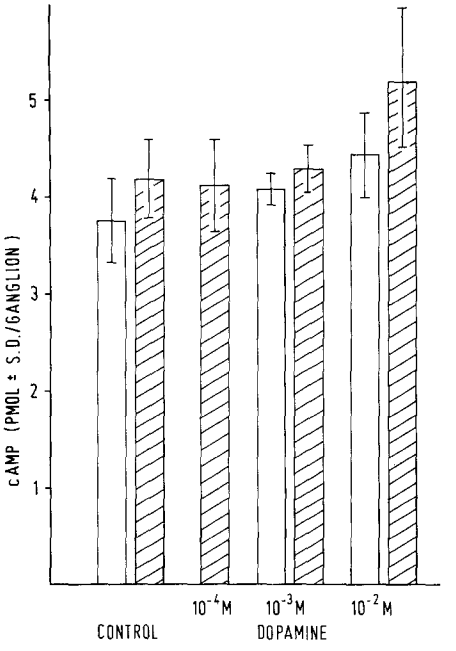

Figure 1:
Effect of dopamine on levels of cyclic AMP in superior cervical ganglia after five minutes of incubation with the amine in the medium at the concentrations indicated. Hatched bars represent ganglia incubated for 20 min in the presence of theophylline (0.5 mM) prior to the addition of dopamine (n = 4).

differed notably from that described for epinephrine and norepinephrine (4), and showed a sustained increase of cAMP concentrations for at least 30 min (figure 2).

In view of the high concentration required, the effect probably represents non-specific and indirect actions of dopamine rather than specific activation of a dopamine-sensitive adenylate cyclase.

It cannot be ruled out, however, that exogenous dopamine has little effect on the cAMP-content because barriers (physical, metabolic, uptake) prevent dopamine from reaching the receptors. But electrophysiological experiments of

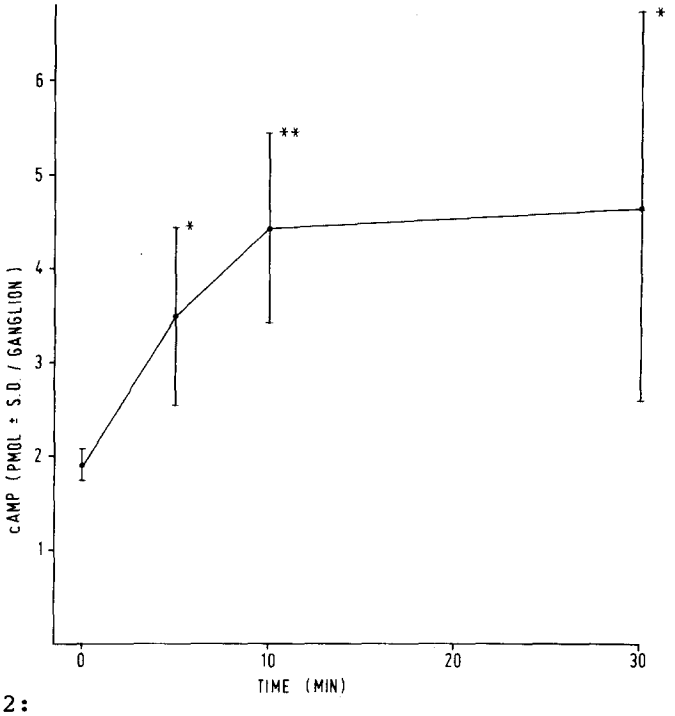

Figure 2:

Time course of accumulation of cAMP in freshly dissected ganglia on incubation with dopamine (10^{-2} M). (n = 4)
* significantly different from controls, $p \leq 0.05$
** significantly different from controls, $p \leq 0.02$

McAfee and Greengard (12) show that exogenous dopamine does cause hyperpolarization of postganglionic neurons of superior cervical ganglion cells of the rabbit and hence does penetrate into the ganglia.

Norepinephrine, which is a potent activator of adenylate cyclase in rat superior cervical ganglia (4), in combination with papaverine (10^{-4} M) caused a more than additive accumulation of cAMP in ganglia (figure 3).

Papaverine alone did not affect the cAMP-content when ganglia were incubated with it for 6 minutes. Release of cyclic AMP from the tissue into the medium was also en-

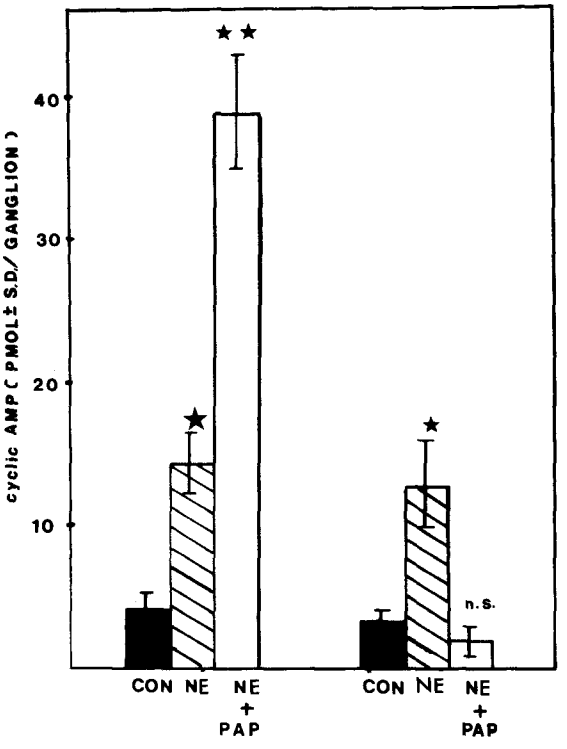

Figure 3:

Concentration of cAMP in ganglia after the first and the fourth of a series of stimulation with norepinephrine (NE, 10^{-4} M) or norepinephrine + papaverine (PAP, 10^{-4} M). Ganglia were incubated for 15 min in the presence of the drugs between incubation periods of 20 min in drug-free medium (n = 6). *$p < 0.01$; ** $p < 0.001$ compared to controls; n.s. = non significant.

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hanced when ganglia were incubated in the presence of norepinephrine or in the presence of norepinephrine and papaverine (figure 4).

On repeated incubations with alternating washes with drug-free incubation medium, the cyclic nucleotide content fell after the second incubation period and reached values near the detection limit after the fourth incubation. The fall was only detectable when ganglia were incubated with the combination norepinephrine/papaverine. No significant

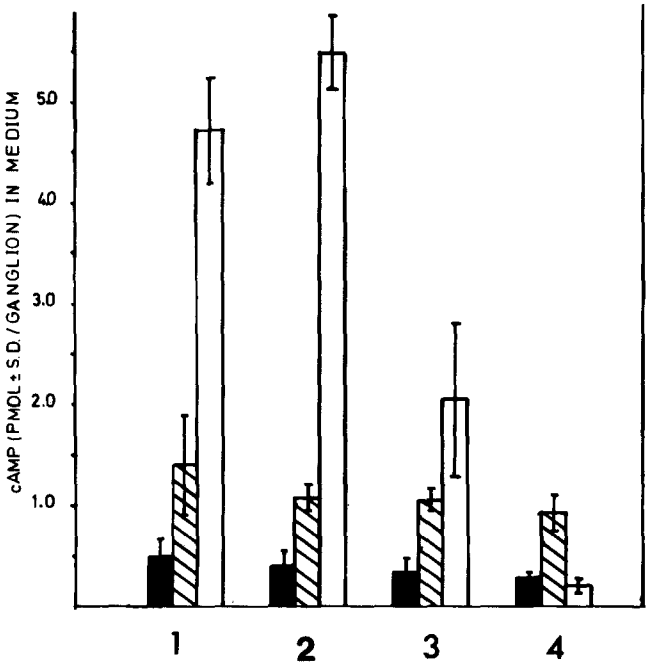

Figure 4:
Release of cyclic AMP into the incubation medium after sequential stimulation of ganglia with norepinephrine ☐ or norepinephrine + papaverine ▨. Stimulation period: 15 min with an additional washing period of 20 min. Concentration of norepinephrine: 10^{-4} M; papaverine: 10^{-4} M (n = 6 ± S.D.). ■ = control. Numbers under the bars represent the period of stimulation.

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changes were observed during control incubations and during incubations with norepinephrine alone. At the end of the entire incubation period, the cyclic nucleotide content of the ganglia exposed to norepinephrine alone was still elevated , while the cAMP content of ganglia incubated with norepinephrine/papaverine was below the control values (figure 3).

A decrease in the cAMP concentration after sequential stimulations of adenylate cyclase has also been observed in brain slices (13). The apparent refractoriness of the

adenylate cyclase to the catecholamine may be due to the exhaustion of a limited pool of ATP, the substrate of adenylate cyclase, within the membrane compartment (14-16). A limited supply of ATP can also explain the observation that the rate of conversion of radioactive adenine into cAMP in the ganglion during electrical stimulation falls rapidly with increasing frequency, or when more than 2400 pulses were given (17).

Pharmacological evidence from previous studies (4, 6) led to the conclusion that two types of catecholamine receptors, alpha and beta, can lead to elevations in cAMP in the peripheral nervous system, and that dopamine activates adenylate cyclase by a mechanism involving an α-adrenoceptor.

In the rat superior cervical ganglion however, alpha adrenergic agents such as dopamine and phenylephrine in reasonable concentrations were without effect on the concentration of cAMP in the ganglion (2,4). Preincubation with 10^{-5} M propranolol abolished the norepinephrine-induced rise of cyclic AMP, but 10^{-5} M phentolamine inhibited the rise by only 50% (figure 5).

These observations can be interpreted as indicating that both types of receptors may be present in the ganglia but that ß-receptors for adenylate cyclase prevail in this species while dopamine does not activate the enzyme. Moreover, it was described recently that propranolol has an ID 50 of 2.2×10^{-7} M suggesting nonspecific effects of 10^{-5} M phentolamine (10).

The present experiments in rat superior cervical ganglia

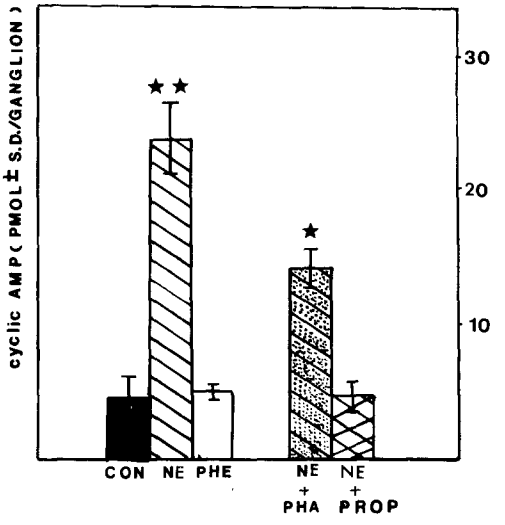

Figure 5:

Effects of norepinephrine (NE, 10^{-4} M) and phenylephrine (PHE, 10^{-2} M) on cyclic AMP levels in ganglia and effects of phentolamine (PHA, 10^{-5} M) and propramolol (PROP, 10^{-5} M) on the accumulation of cyclic AMP in ganglia induced by norepinephrine (10^{-4} M) (n = 6).
*$p \leq 0.01$; ** $p \leq 0.001$ significance of difference from controls.

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fail to support the concept of a "dopamine-sensitive adenylate cyclase" (8) which supposedly mediates the slow inhibitory postsynaptic potential (sIPSP) in the ganglion.

From this and other investigations (4,7) it can be suggested that, if cyclic AMP is actually linked with slow inhibitory postsynaptic potential (sIPSP), norepinephrine is more likely to be responsible for this action because of its marked potency in stimulating adenylate cyclase and its known capacity to influence transmission through the ganglion.

Acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 70 and SFB 138).

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