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The effect of N^6-2′-0 dibutyryl 3′, 5′ cyclic adenosine monophosphate, imidazole and aminophylline on ganglionic transmission in the superior cervical ganglion of the cat

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Summary

1. Dibutyryl cyclic AMP, injected towards the superior cervical ganglion of the cat, produced no consistent responses.

2. Imidazole, injected towards the ganglion, regularly produced facilitation, both during intermittent and continuous preganglionic stimulation. This effect was dose-dependent and lasted 2–10 minutes.

3. Aminophylline, injected towards the ganglion, regularly produced depression of ganglionic transmission, both during intermittent and continuous preganglionic stimulation. This effect was also dose-dependent and lasted 1–4 minutes. Papaverine produced the same type of response as aminophylline.

4. Imidazole potentiated the ganglionic response to 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), while aminophylline depressed it.

5. The results of the present experiments are consistent with the view that cyclic AMP may have a mediating role in the process of ganglionic transmission.

Introduction

The enzymes regulating the metabolism of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) are present in nervous tissue (Weiss & Kidman, 1969). Some evidence has already been presented that cyclic AMP may be concerned in the release of acetylcholine from the motor nerve endings (Breckenridge, Burn & Matshinsky, 1967; Goldberg & Singer, 1969). The release of acetylcholine is also influenced by dibutyryl cyclic 3′,5′-adenosine monophosphate (dibutyryl cyclic AMP) (Varagić, Žugić & Mršulja, 1972). Catecholamines which stimulate synthesis of cyclic AMP have been shown to facilitate neuromuscular transmission (Krnjević & Miledi, 1958; Jenkinson, Stamenović & Whitaker, 1968). Noradrenaline has been shown to increase both the quantal content of the endplate potential (Jenkinson *et al.*, 1968) and the level of cyclic AMP in nervous tissue (Kakiuchi & Rall, 1968a, 1968b). On the other hand, Kebabian & Greengard (1971) suggested that the cyclic AMP system might be intimately associated with the physiology of synaptic transmission. These authors have shown that an adenylcyclase is present in the mammalian superior cervical ganglion. This enzyme can be activated by low concentrations of dopamine released in response to preganglionic stimulation.

Kebabian & Greengard (1971) have therefore suggested that the physiological effects of dopamine in the ganglion, released from interneurones, may be mediated by stimulating synthesis of cyclic AMP.

It was therefore of interest to study the effect of exogenous dibutyryl cyclic AMP, as well as the effect of drug-produced changes in the level of the endogenous cyclic AMP, on transmission in the superior cervical ganglion. Changes in the endogenous cyclic AMP were supposedly produced by imidazole and aminophylline, these substances being phosphodiesterase stimulator and inhibitor, respectively.

Methods

Cats of either sex, weighing from 1·6 to 2·8 kg, were used in these experiments. They were anaesthetized with a mixture of chloralose (80 mg/kg) and urethane (500 mg/kg) injected intravenously. The superior cervical ganglion was prepared and arranged as described by Konzett & Rothlin (1955), as modified by Trendelenburg (1956). A polyethylene catheter, 0·5 mm in diameter, was introduced into the lingual artery and used for injecting drugs. A thread was put under the external carotid artery, so that it could be occluded when necessary. A drug was presumed to act on the ganglion when injected into the lingual artery during occlusion of the external carotid artery. On the other hand, a drug injected into the lingual artery while the external carotid artery was opened, predominantly acted directly on the nictitating membrane. Contractions of the nictitating membrane were recorded on a polygraph (Physiograph IV), using an E & M myograph, type A.

The cervical sympathetic was stimulated preganglionicly with a Grass S-8 stimulator. The parameters of stimulation were: square wave pulses 0·4 ms duration, 2·5 and 25 Hz frequency, with submaximal voltage output ranging from 0·25 to 5 Volts. The stimulation was applied either intermittently for 3 to 10 s at 60 s intervals, or continuously for periods of time ranging from 10 to 30 minutes. The temperature of the cat was kept constant between 35 and 36° C.

The following drugs were used: N^6-2′-*O*-dibutyryl 3′,5′-cyclic adenosine monophosphate monosodium salt (Boehringer), imidazole, aminophylline, papaverine hydrochloride and 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP).

Results

The effects of dibutyryl cyclic AMP on intermittent and continuous preganglionic stimulation

In 20 trials made in 8 experiments dibutyryl cyclic AMP in doses from 500 μg to 12·5 mg, injected towards the ganglion during intermittent preganglionic stimulation at 2·5 and 25 Hz, produced variable responses. Slight potentiation, depression or no change were observed, these responses being equally distributed. This probably means that under the conditions of our experiments dibutyryl cyclic AMP, in the doses used, did not significantly and consistently affect ganglionic transmission in the superior cervical ganglion.

In another series of 15 trials made in 8 experiments dibutyryl cyclic AMP (500 μg—12·5 mg), injected towards the ganglion during continuous submaximal pre-

ganglionic stimulation at 2·5 Hz also produced variable responses. Slight potentiation, depression or no change, were observed, these effects being equally distributed. Once again, this finding probably means that under the conditions of our experiments even large doses of dibutyryl cyclic AMP did not significantly and consistently alter ganglionic transmission in the superior cervical ganglion.

The effect of imidazole

Imidazole (50 μg−2 mg), injected towards the ganglion during intermittent submaximal preganglionic stimulation at 2·5 and 25 Hz, was regularly found to produce potentiation of the membrane response. The duration of this effect ranged from 2–10 minutes. It was also dose-dependent and it occurred immediately after injection of the imidazole. A typical experiment is shown in Figure 1. This type of response was obtained in 10 trials made in 8 experiments.

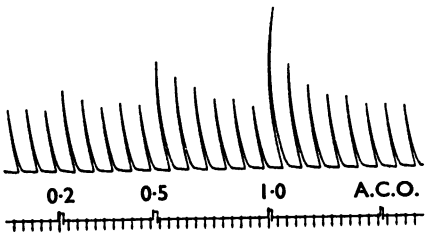

FIG. 1. The effect of imidazole on submaximal preganglionic sympathetic stimulation of the cat superior cervical ganglion. At signals, 0·2, 0·5 and 1·0 mg imidazole was injected into the lingual artery during occlusion of the external carotid artery. The parameters of intermittent stimulation were: 2·5 Hz, 0·4 ms duration, for 3 s at 60 s intervals. At A.C.O., the external carotid artery was opened. Time: 30 s intervals.

In 18 trials made in 13 experiments imidazole (50 μg−2 mg), injected towards the ganglion during continuous submaximal preganglionic stimulation at 2·5 Hz, also regularly produced a facilitatory response. This effect was dose-dependent and lasted from 2 to 4 minutes (Figure 2). There was no tachyphylaxis if the same dose of imidazole was repeatedly injected at short time intervals.

The same doses of imidazole, injected towards the ganglion without preganglionic submaximal stimulation, produced no effect on the membrane (Figure 2). Neither

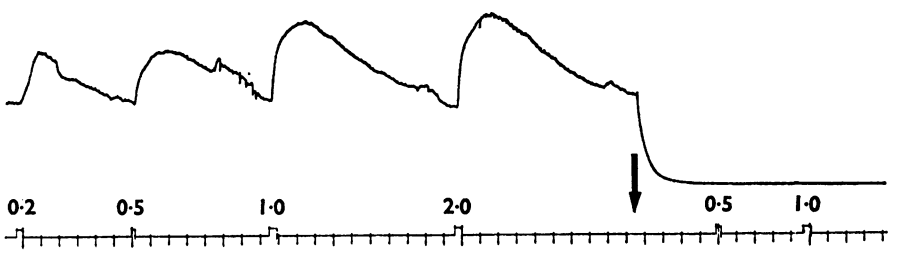

FIG. 2. The effect of imidazole on ganglionic transmission in the cat superior cervical ganglion. The preganglionic sympathetic was stimulated constantly with submaximal pulses of 2·5 Hz and 0·4 ms duration. At the arrow, stimulation stopped. At the signals, imidazole was injected in doses of 0·2, 0·5, 1·0 and 2·0 mg during stimulation, and 0·5 and 1·0 mg in the absence of stimulation. All the injections were directed towards the ganglion. Time: 30 s intervals.

did intravenous injection of imidazole during preganglionic submaximal stimulation, nor injection directly towards the membrane, produce any effect.

The effect of aminophylline

Injection of aminophylline (0·5—5 mg) towards the ganglion during intermittent preganglionic stimulation at 2·5 and 25 Hz regularly produced depression of ganglionic transmission. This effect lasted 3–4 min and was dose-dependent (Figure 3). Such a response was observed in 13 trials made in 10 experiments. No tachyphylaxis was observed if the same dose of aminophylline was repeatedly injected at short time intervals.

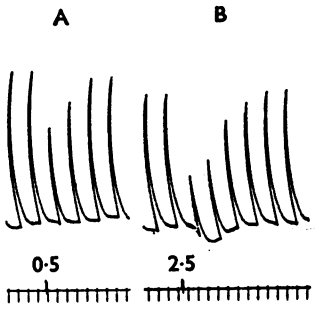

FIG. 3. The effect of aminophylline on ganglionic transmission in the cat superior cervical ganglion. The preganglionic sympathetic was stimulated intermittently with submaximal pulses of 25 Hz and 0·4 ms duration, for 5 s at 60 s intervals. At the signals in A and B, 0·5 and 2·5 mg aminophylline, respectively, were injected into the lingual artery towards the ganglion. B was taken 30 min after A. Time: 30 s intervals.

In 12 trials made in 10 experiments aminophylline (0·5—2·5 mg), injected towards the ganglion during continuous preganglionic submaximal stimulation at 2·5 Hz, also regularly produced depression of ganglionic transmission. This effect lasted only 1–2 min and was also dose-dependent (Figure 4). Exactly the same type of response was obtained after a similar injection of papaverine (0·2—0·4 mg) (Figure 4).

Comparative responses to dibutyryl cyclic AMP, imidazole and aminophylline in the same animal are shown in Figure 5.

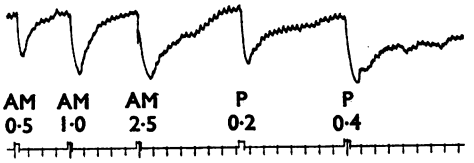

FIG. 4. The effect of aminophylline and papaverine on ganglionic transmission in the cat superior cervical ganglion. The preganglionic sympathetic was continuously stimulated with the submaximal pulses of 2·5 Hz and 0·4 ms duration. At the first, second and third signal, aminophylline (AM) was injected towards the ganglion in doses of 0·5, 1·0 and 2·5 mg, respectively. At the fourth and fifth signal, papaverine (P) was injected towards the ganglion in doses of 0·2 and 0·4 mg, respectively. Time: 30 s intervals.

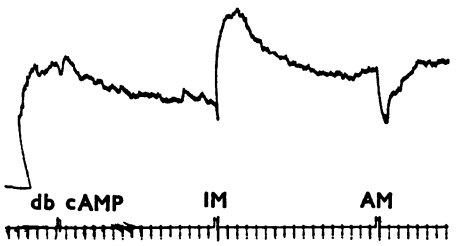

FIG. 5. The comparative effect of dibutyryl cyclic AMP (db cAMP) (5 mg), imidazole (IM) (1 mg) and aminophylline (AM) (1 mg) on ganglionic transmission during continuous submaximal preganglionic stimulation of the cervical sympathetic (2·5 Hz, 0·4 ms duration). All the drugs were injected towards the ganglion while the external carotid artery was occluded. Time: 30 s intervals.

The effect of papaverine

The effects of aminophylline were compared with those of papaverine. It was found that papaverine both during intermittent (2·5 and 25 Hz) and continuous preganglionic stimulation (2·5 Hz) regularly produced depression of ganglionic transmission. The duration of this effect ranged from 1–5 min, with doses of papaverine ranging from 100 μg to 2 mg. This type of response was obtained in 4 experiments.

In comparison with aminophylline, papaverine produced a stronger effect. Doses of papaverine of one half to one fifth those of aminophylline, produced a similar effect (Figure 4).

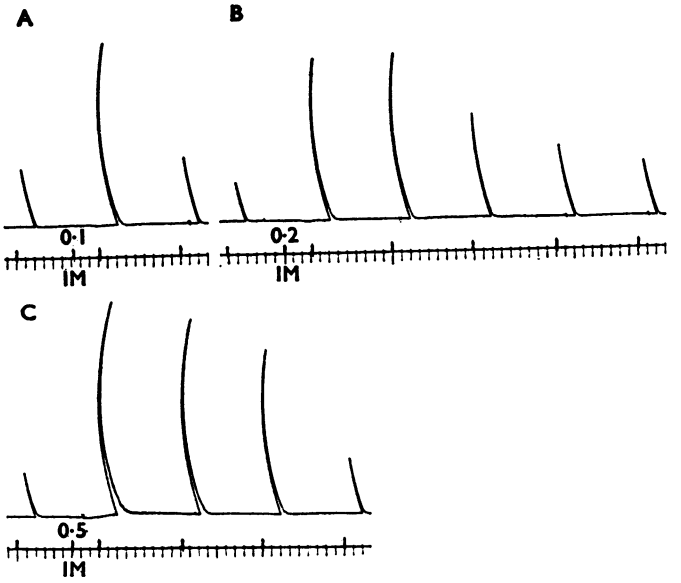

FIG. 6. The effect of imidazole on the ganglionic response to DMPP (0·5 μg injected towards the ganglion at the signals in A, B and C). At IM in A, B and C, 0·1, 0·2 and 0·5 mg imidazole was injected towards the ganglion, while the external carotid artery was occluded. Time: 30 s intervals.

Imidazole, aminophylline and DMPP responses

In 9 trials made in 7 experiments imidazole (0·1—2 mg), injected towards the ganglion, produced slight or significant potentiation of the ganglionic response to 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (0·5—4 μg). This effect was found to be dose-dependent and lasted 5–20 minutes (Figure 6).

On the other hand, aminophylline (0·5–2·5 mg) injected in the same way, depressed or blocked the ganglionic response to DMPP (0·5—1 μg) in 9 trials made in 5 experiments. This effect of aminophylline lasted 3–10 min and was dose-dependent.

Discussion

It has been shown in the present experiments that dibutyryl cyclic AMP, injected towards the ganglion even in high doses, produces no consistent response. Nucleotides are generally believed to penetrate cell membranes very poorly, if at all (Robison, Butcher & Sutherland, 1971). The permeability of the cell membrane to cyclic AMP is also very low, but it would appear that this nucleotide can penetrate some membranes at least to some extent (Robison, Butcher, Oye, Morgan & Sutherland, 1965 ; Robison *et al.*, 1971). Our previous experiments have shown that dibutyryl cyclic AMP produces pharmacological effects when added to an isolated organ bath (Varagić *et al.*, 1972 ; Varagić & Žugić, 1972).

Imidazole injected towards the ganglion, both during intermittent and continuous submaximal preganglionic stimulation, regularly produced a facilitatory response. This effect was found to be dose-dependent and to last 2–10 minutes. There was no response of the ganglion itself in the absence of preganglionic stimulation. Imidazole produced no effect on the nictitating membrane either directly or after intravenous injection, although histamine is known to stimulate the ganglion directly (Trendelenburg, 1954). Thus, the effect of imidazole is only seen during transmission. Butcher & Sutherland (1962) have shown that imidazole activates the phosphodiesterase which hydrolyses cyclic AMP. In the presence of imidazole, adrenaline does not produce its effect on the taenia coli, and it also fails to increase the ATP and creatine phosphate content of the tissue. This effect of imidazole is specific because histamine does not affect the action of adrenaline (Bueding, Bülbring, Gercken, Hawkins & Kuriyama, 1967). Preganglionic stimulation of the superior cervical sympathetic ganglion of the rabbit, under relatively physiological conditions, produced a several-fold increase in the content of cyclic AMP in the ganglion (McAfee, Schorderet & Greengard, 1971). It is possible that the increase in the concentration of cyclic AMP may be responsible for the slow hyperpolarization (slow inhibitory postsynaptic potential) of the ganglion that follows preganglionic stimulation (Kebabian & Greengard, 1971). Other experiments support this idea (Libet & Tosaka, 1970). If imidazole acts by decreasing the amount of cyclic AMP in the ganglion, and if cyclic AMP is implicated in the process of hyperpolarization in the ganglion, then a facilitatory action of imidazole is to be expected. Actually, such a result was obtained in our present experiments.

On the other hand, aminophylline and papaverine have been shown to inhibit phosphodiesterase in various tissues (Triner, Nahas, Vulliemoz, Overweg, Verosky, Habit & Ngai, 1971). Both these substances have been found in the present experiments to produce a dose-dependent and short-lasting depression in ganglionic

transmission. As an inhibitor of phosphodiesterase, papaverine is about 15 times stronger than aminophylline. Our experiments also showed that papaverine produced greater inhibition of ganglionic transmission than did aminophylline. If aminophylline and papaverine produce an increase in the content of cyclic AMP in the ganglion, and if cyclic AMP is implicated in the process of hyperpolarization in the ganglion, then the observed inhibitory action of aminophylline and papaverine is to be expected.

The present experiments therefore support the hypothesis put forward by Kebabian & Greengard (1971) that the role of increased synthesis of ganglionic cyclic AMP that follows preganglionic stimulation might be to mediate and modulate transmission in the ganglion. Because our experiments were performed with the ganglion *in situ* and under relatively physiological conditions, they do not exclude the possible physiological role of dopamine in the ganglion, as already postulated by Kebabian & Greengard (1971). Finally, changes in the metabolism of cyclic AMP are also able to modulate cholinergic transmission.

Imidazole and aminophylline affected the ganglionic response to DMPP in the same way as the responses to preganglionic stimulation, i.e. imidazole potentiated the response of the ganglion to DMPP injection, whereas aminophylline depressed it. DMPP has been shown to be a most potent nicotine-like stimulant of the ganglion, being 2–4 times as potent as nicotine (Page & McCubbin, 1953). These experiments indicate that the nicotinic receptors in the ganglion are implicated in the effects of imidazole and aminophylline on ganglionic transmission. The present experiments, therefore, indicate that cyclic AMP may have a mediating role in the process of transmission in the superior cervical ganglion of the cat.

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