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Potentiation of dopaminergic transmission by phosphodiesterase inhibitors and cyclic nucleotides

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Experiments were made to determine whether cyclic AMP plays a role in transmission at identified dopaminergic synapses in the water snail *Planorbis corneus*. Intracellular stimulation of a specific dopamine neuron produces direct inhibitory postsynaptic potentials (ipsps) in a number of other neurons. These ipsps, which are mediated by dopamine, were potentiated by as much as 120% by caffeine, theophylline or dibutyryl cyclic AMP, although they were unaffected by cyclic AMP and prostaglandin E_1. Caffeine and theophylline also potentiated the inhibitory response to dopamine, applied to the postsynaptic neurons by perfusion or iontophoresis, but the effects were generally much smaller (maximum potentiation 30%). The results provide evidence that postsynaptic cyclic AMP is involved in transmission at these synapses, but that the phosphodiesterase inhibitors may also have a presynaptic effect.

The postsynaptic actions of catecholamines are possibly mediated through cyclic nucleotides (McAfee & Greengard, 1972; Iversen, 1975; Greengard, 1976). The evidence is particularly good for the superior cervical ganglion, where the dopamine-mediated slow ipsps, elicited by preganglionic nerve stimulation, are associated with an increase in the amount of cyclic AMP in the postganglionic neurons (Kebabian, Bloom & others, 1975; Greengard, 1976). There is evidence that dopamine and noradrenaline act by the same mechanism in the mammalian cns, where many of the criteria listed by Greengard (1976) for mediation of a postsynaptic potential by a cyclic nucleotide have been tested (e.g. Gähwiler, 1976).

In the central ganglia of the gastropod mollusc *Planorbis corneus* there is a specific dopamine-containing neuron which makes excitatory and inhibitory synaptic connexions with a number of other neurons. The postsynaptic potentials appear to be mediated monosynaptically by release of dopamine (Berry & Cottrell, 1973, 1975). The results of this investigation suggest that the inhibitory postsynaptic responses to stimulation of the dopamine neuron may be mediated by a dopamine-sensitive adenylate cyclase.

MATERIALS AND METHODS

Specimens of *Planorbis corneus* were obtained from Gerrard & Haig Ltd., East Preston, Sussex, and maintained in aquaria at room temperature (16–20°).

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The central ganglia were removed and pinned to the base of a 5 ml Perspex chamber which was perfused with physiological saline (Berry, 1972) at room temperature. Double barrelled microelectrodes were used to record intracellularly from the dopamine neuron, in the left pedal ganglion, and from its postsynaptic neurons in the visceral ganglion (Pentreath, Berry & Cottrell, 1974). Both barrels contained 0·6 M K_2SO_4 and had resistances of about 5 megohm; one barrel was used for recording potential, the other for passing current. Single barrelled microelectrodes containing 200 mg ml^{-1} dopamine hydrochloride were used for iontophoresis of dopamine onto the postsynaptic neurons. Injection current (50 nA, 1 s) was monitored on the oscilloscope. In some experiments visceral nerve trunks were stimulated using a plastic suction electrode. Records were made on a Brush 220 series two-channel ink recorder. Conventional amplifying and stimulating equipment was used.

Drugs were dissolved in the saline immediately before use. The following drugs were used: dopamine hydrochloride (Koch–Light), caffeine, theophylline (BDH), cyclic AMP, dibutyryl cyclic AMP (Sigma), prostaglandin E_1 (Upjohn Company).

RESULTS

Each action potential elicited by intracellular stimulation of the dopamine neuron produces a unitary ipsp in postsynaptic neurons in the visceral ganglion (Fig. 1a). At high frequency of firing the ipsps summate to produce a smooth hyperpolarization (Fig. 1a, b). The dopamine neuron was hyper-

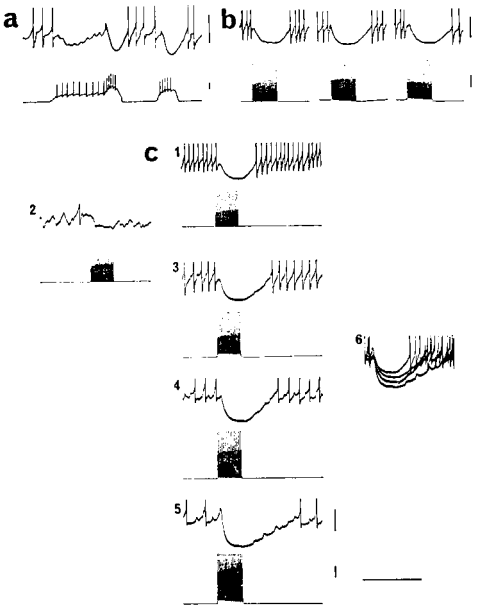

FIG. 1. Potentiation by caffeine of the inhibitory postsynaptic potentials (upper traces in each record) produced by stimulation of the dopamine neuron (lower traces). a–c are each from a different preparation. a—The dopamine neuron produces unitary ipsps which summate smoothly at high frequency. b—If the stimulus parameters for the dopamine neuron are kept constant, then a relatively constant postsynaptic response is obtained for several hours. Each response was separated by 1 h. In control experiments the amplitude of the postsynaptic potential was never seen to increase with time, and if the neuron was in any way damaged the responses steadily declined. c_1—Control response. c_2—Partial blockade of response 5 min after addition of 2×10^{-3} M caffeine (note the spontaneous ipsps). c_3–c_5, Potentiation after washing for 10, 20 and 40 min respectively. c_6—Composite record showing $c_{1,\ 3-5}$ superimposed for clarity. Voltage scales: 25 mV. Time scale represents 10 s for each record.

polarized by 20 mV to prevent spontaneous firing and was stimulated at about 5 s^{-1} by short depolarizing pulses, each of which produced one action potential. The stimulus parameters were kept constant, and summated postsynaptic responses of constant amplitude were obtained for several hours (Fig. 1b). The summated potentials were used rather than unitary ipsps in these experiments because their amplitude could be more accurately measured.

When the phosphodiesterase inhibitors theophylline or caffeine were added to the bath there was a gradual increase in both the amplitude and duration of the inhibitory responses. Caffeine was more effective than theophylline; 2×10^{-4} M caffeine produced approximately a 20% increase in the amplitude of response after 30 min perfusion, whereas theophylline at 2×10^{-4} M produced a 10% increase.

Large increases (up to 120%) could be obtained with higher concentrations of caffeine (5×10^{-3}M). At these concentrations caffeine first produced apparently non-specific effects on the postsynaptic neurons; action potential firing was reduced or abolished, there was increased spontaneous inhibitory input, and the postsynaptic response to stimulation of the dopamine neuron was reduced (Fig. 1c_2). However, if the preparation was exposed to the caffeine for no more than about 10 min, and was then washed with fresh saline, there was a progressive increase in the postsynaptic response beyond the control level (Fig. 1c). The increase became maximal after washing for 10–50 min, and the response returned to control level after 2–4 h when it could again be potentiated by further treatment with caffeine.

If the caffeine was left in the bath, or if it was washed off after more than about 15 min, there was no recovery of the ipsps even after 8 h, although other synaptic input was not affected, and the spike amplitude and membrane resistance were normal.

The results were similar in each of nine experiments.

Measurements of membrane resistance were made throughout the experiments by observing the potentials produced by constant-current hyperpolarizing pulses of 1 s duration applied through one barrel of the microelectrode. The resistance of the postsynaptic neurons before treatment with caffeine did not differ from their resistance at the time when the postsynaptic potentials were maximally potentiated. The potentiation did not therefore appear to be attributed to an increase in membrane resistance. The amplitude and duration of action potentials in the dopamine neuron were also unaffected by caffeine.

The effects of caffeine and theophylline were tested on non-dopaminergic inhibitory input to the postsynaptic neurons of the dopamine cell. Stimulation of a visceral or parietal nerve trunk produces ipsps which may be mediated by glutamate (Berry & Cottrell, 1975). Neither caffeine nor theophylline had any effect on this input.

Cyclic AMP, added to the bath at a concentration of 5×10^{-3} M, did not produce a hyperpolarization of the postsynaptic neurons or have any effect on the postsynaptic response to stimulation of the dopamine neuron. This could be due to failure of the substance to penetrate the membrane of the postsynaptic neurons, or to its rapid destruction by

intracellular phosphodiesterase before it reached its site of action. Dibutyryl cyclic AMP, which penetrates membranes more readily, also failed to affect the membrane potential of the postsynaptic neurons, but at 5×10^{-4} M it caused a marked increase in the amplitude of the ipsps elicited by stimulation of the dopamine neuron (Fig. 2a). Again this potentiation could not be attributed to an increase either in membrane resistance or in the amplitude and duration of presynaptic action potentials (Fig. 2b).

Prostaglandin E_1 (PGE_1), which affects the adenylate cyclase activity of many tissues, and has been shown to abolish the dopamine-induced hyperpolarization in superior cervical ganglia of the rabbit (McAfee & Greengard, 1972), had no effect on the ipsps mediated by the dopamine neuron.

The postsynaptic neurons were hyperpolarized by dopamine which was applied by perfusion (10^{-5} M) or iontophoresis. In the presence of caffeine or theophylline (5×10^{-3} M) the responses were first reduced in amplitude but were potentiated after washing for 10–50 min. However, the increases were generally small and never more than 30%.

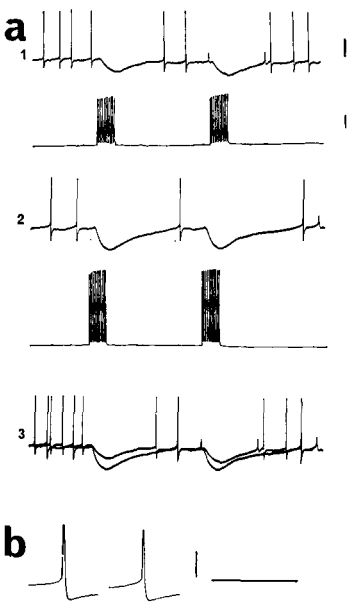

FIG. 2. a. Potentiation by dibutyryl cyclic AMP of the inhibitory response (upper traces) to stimulation of the dopamine neuron (lower traces). a_1—Control responses. a_2—Increased response 15 min after the addition of 5×10^{-4} M dibutyryl cyclic AMP. a_3—Records $a_{1,2}$ superimposed for clarity. b—Action potentials recorded from the dopamine neuron before (left) and 30 min after (right) the addition of 5×10^{-4} M dibutyryl cyclic AMP. This substance (and caffeine and theophylline) produced no measurable increase in amplitude or duration of the presynaptic spike. Voltage scales: 25 mV. Time scale represents 10 s for a, and 0·4 s for b.

DISCUSSION

Some importance has been attached to enhancement of the slow inhibitory potentials in superior cervical ganglia by caffeine and theophylline, and to the mimicking of the potentials by cyclic AMP; this has been regarded as evidence that a cyclic nucleotide mediates the postsynaptic potential (see Greengard, 1976).

The results of the present study are at least suggestive of a similar situation at identified dopaminergic synapses in the water snail *Planorbis corneus*. There was an increase in response to applied dopamine, and a striking increase in inhibitory postsynaptic response to stimulation of the dopamine neuron following treatment with caffeine. The relatively long time periods and high concentrations of the drug necessary to produce this increase may be explained by its low rate of penetration of cell membranes and also by the fact that its site of action may be confined to fine dendritic processes of the postsynaptic neurons which lie in the neuropile (Triestman & Levitan, 1976). The amplification of the postsynaptic potentials by high concentrations of dibutyryl cyclic AMP, which may be due to the elevated concentrations of this substance in the postsynaptic neurons, also argues for a role of cyclic AMP in the response.

However, there are certain inconsistencies. For example, caffeine and theophylline produced relatively small increases in response to applied dopamine. Cyclic AMP or dibutyryl cyclic AMP did not mimic the postsynaptic potentials produced by stimulating the dopamine neuron, and PGE_1 did not antagonize the responses. This may be due to failure of these substances to reach the site of action of dopamine on the postsynaptic membrane, and could probably be overcome by intracellular injection (Triestman & Levitan, 1976).

The results indicate that the phosphodiesterase inhibitors may have a presynaptic effect. For example they may act by prolonging action potentials in presynaptic nerve terminals (S.-Rózsa & Kiss, 1976), increasing the amount of dopamine available for synaptic release via induction of tyrosine hydroxylase (Harris, Baldessarini & others, 1974), increasing the quantal content of dopamine (Mambrini & Benoit, 1963), or blocking re-uptake of dopamine. There are also possible postsynaptic effects which do not involve cyclic AMP; for example, increase in the

resistance of the postsynaptic membrane. It was not possible to detect a change in postsynaptic resistance or an increase in the amplitude or duration of the presynaptic spike, but the measurements were made in the cell body, and may not reflect changes in the region of the synapse.

Although the results suggest that transmission at these specified dopaminergic synapses is mediated by a cyclic nucleotide, more data are necessary to determine the relative importance of pre- and post-synaptic effects of the phosphodiesterase inhibitors.

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