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Pharmacological studies with adenine, adenosine and some phosphorylated derivatives on guinea-pig tracheal muscle

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Adenine, adenosine and three adenine nucleotides all caused relaxation of the guinea-pig trachea. The relaxation to the nucleotides was often preceded by a contraction. The response to adenosine and the nucleotides, but not adenine, was potentiated by dipyridamole. Imidazole inhibited the response to adenine alone. Propranolol had no effect on the response to any of the compounds. It is concluded that the guinea-pig trachea does not possess a nucleotide-specific receptor as has been postulated for some other smooth muscle preparations. An alternative hypothesis postulating an adenosine-specific receptor is presented.

Studies in a variety of smooth muscle preparations have revealed an inhibitory response to nerve stimulation that is resistant to the action of cholinergic and adrenergic antagonists. Evidence has been accumulated in favour of the hypothesis that this response is mediated by 'purinergic' nerves and that the likely transmitter involved is adenosine triphosphate (Burnstock, 1972). Comparative studies with guinea-pig tracheal smooth muscle have identified the presence of an inhibitory response to field stimulation that demonstrates the features of purinergic nerve transmission (Coburn & Tomita, 1973), although studies on the nature of the transmitter involved were limited. Coleman & Levy (1974) excluded several candidate transmitters from involvement in the response. However, they were able to demonstrate an enhancement of non-adrenergic inhibitory response with a number of adenosine uptake blocking drugs. This evidence introduced the possibility that purinergic nerves were involved in the response.

These observations prompted the present study with adenine and some of its naturally occurring analogues. The purpose was to examine the pharmacological properties of the purine derivatives on guinea-pig tracheal muscle.

MATERIALS AND METHODS

Guinea-pig tracheal tubes were prepared as described by Farmer & Coleman (1970). The preparations were immersed in a physiological salt solution of the following composition (g litre^{-1}); NaCl, 8·0; $NaHCO_3$, 1·0; NaH_2PO_4, 0·32; glucose, 1·0; $MgCl_2$, 0·42; KCl, 0·2; $CaCl_2$, 0·1. The solution was maintained at 37° and gassed with room air. The intraluminal pressure of the preparation was measured with a pressure transducer. Drugs were added to the bathing fluid. The intraluminal pressure of the preparation was raised using the procedure described by Coleman & Farmer (1971). In this way the effects of drugs on the intrinsic tone of the preparation could be assessed with ease, thus excluding the need for a spasmogenic agent. Cumulative dose-response curves were constructed for the smooth muscle relaxants. The effect of various treatments on their responses was studied using different groups of tissues. Their potencies in control and treated preparations were expressed in terms of the dose required to produce 50 % of the maximal response of each preparation to isoprenaline (ED 50). ED 50 values between groups were compared for statistical significance using the Student's *t*-test.

Drugs used were adenine (Sigma), adenosine (Koch-Light), adenosine-5′-monophosphate disodium salt, (AMP, Koch-Light), adenosine-5′-diphosphate sodium salt (ADP, Koch-Light), adenosine-5′-triphosphate disodium salt (ATP, Sigma), dipyridamole (Boehringer Ingelheim), imidazole (BDH), isoprenaline hydrochloride (Pharmax), propranolol hydrochloride (ICI), quinidine sulphate (Sigma) and theophyllinel ethylenediamine (Fisons Ltd). Solutions of isoprenaline were diluted with 0·9 % w/v NaCl solution containing 0·1 % sodium metabisulphite. Other drugs were dissolved in the physiological salt solution.

RESULTS

(a) *The nature of the response.* Adenine (7·4 → 12·8 × 10^{-5} M) and adenosine (3·7 → 11·8 × 10^{-5} M) both

* Correspondence.

caused a relaxation of the preparation. Adenine was approximately twice as potent as adenosine (Table 1), but ten times less potent than theophylline. These potency differences were statistically significant ($P < 0.05$). The slopes of the dose-response curves to adenine and adenosine were similar to that of isoprenaline. However, the slope of the dose-response curve to theophylline was less than that to isoprenaline (Fig. 1).

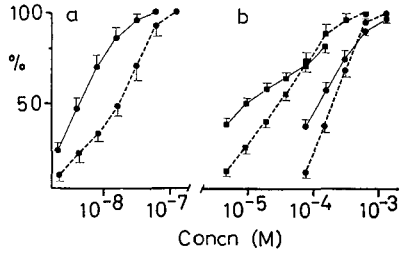

FIG. 1. The effect of imidazole on the relaxation of the guinea-pig trachea to isoprenaline, theophylline and adenine. Controls: (a) ●—● isoprenaline; (b) ■—■ theophylline; ●—● adenine. In the presence of imidazole 1.5×10^{-3} M: (a) ●- - -● isoprenaline; (b) ■- - -■ theophylline; ●- - -● adenine. Abscissa — % maximal response to isoprenaline.

The response to the phosphorylated derivatives was different to that of adenosine. In some preparations, the relaxation to threshold doses of AMP and ATP was preceded by a contraction. In general, as the dose of the nucleotide was increased, the occurrence of the contractile component became less frequent. The significance of the contractile component in relation to the total response and also the frequency of occurrence varied for each nucleotide. The effect was most evident with ADP. Five out of six preparations contracted to a threshold dose of 5.5×10^{-5} M ADP. Despite the contraction, the major response to the nucleotides was relaxation and it was this component that was subjected to further analysis. The ED 50 values for AMP and ADP were not significantly different from that for adenosine. ATP, however, was significantly less potent than both adenosine and ADP ($P < 0.05$).

(b) *Effect of propranolol.* The effect of the β-adrenoceptor antagonist, propranolol, on the relaxant response to adenine and its derivatives was studied. 1.4×10^{-7} M propranolol did not inhibit the responses to adenine or any of the derivatives studied. Similarly, propranolol did not inhibit the response to theophylline. In contrast, this dose of propranolol caused a marked inhibition of the response to isoprenaline (Table 1). The dose ratio of the effect was 29, showing that significant β-adrenoceptor blockade had occurred with this dose.

(c) *Effect of imidazole.* Imidazole has been reported to stimulate the activity of cyclic 3′,5′-nucleotide phosphodiesterase (PDE) (Butcher & Sutherland, 1962). 1.5×10^{-3} M imidazole significantly increased the ED 50 to both theophylline and adenine (Table 1). Imidazole, however, did not cause a parallel shift in the dose-response curve to these agents (Fig. 1). The inhibitory effect was most marked on threshold relaxant doses, whereas near maximally relaxant doses were not significantly affected. The result was a steepening of the dose-response curves to both theophylline and adenine. Imidazole inhibited the response to isoprenaline, causing a shift to the right of the dose-response curve and a significant increase in the ED 50 value (Table 1). Imidazole (1.5×10^{-3} M) had no effect on the responses to adenosine or its phosphorylated derivatives.

Table 1. *A comparison of the inhibitory potencies of various smooth muscle relaxants on the guinea-pig tracheal tube preparation and the effects of propranolol, dipyridamole and imidazole on their potencies.*

Compound	Control	ED50 - Molar concentration† (95% confidence limits) I	II	III
Theophylline	1.1×10^{-5} ($0.9 - 1.4$)	1.0×10^{-5} ($0.8 - 1.3$)	1.2×10^{-5} ($0.9 - 1.7$)	2.8×10^{-5}* ($2.4 - 3.3$)
Adenine	1.2×10^{-4} ($1.1 - 1.4$)	1.2×10^{-4} ($1.0 - 1.3$)	1.5×10^{-4} ($1.3 - 1.7$)	2.0×10^{-4}* ($1.9 - 2.1$)
Adenosine	2.0×10^{-4} ($1.7 - 2.3$)	1.7×10^{-4} ($1.3 - 2.1$)	4.4×10^{-4}* ($3.2 - 6.0$)	1.7×10^{-4} ($1.4 - 1.8$)
AMP	3.1×10^{-4} ($2.6 - 3.6$)	2.3×10^{-4} ($2.0 - 2.7$)	7.9×10^{-4}* ($6.2 - 10.2$)	2.3×10^{-4} ($1.7 - 3.2$)
ADP	1.5×10^{-4} ($1.2 - 1.9$)	1.4×10^{-4} ($1.2 - 1.6$)	8.1×10^{-4}* ($6.5 - 10.2$)	1.9×10^{-4} ($1.6 - 2.2$)
ATP	3.6×10^{-4} ($2.9 - 4.5$)	3.0×10^{-4} ($2.2 - 3.9$)	5.8×10^{-4}* ($5.0 - 6.6$)	2.4×10^{-4} ($2.0 - 2.9$)
Isoprenaline	5.1×10^{-9} ($3.9 - 6.8$)	1.2×10^{-7}* ($0.8 - 1.7$)	5.8×10^{-9} ($4.9 - 5.4$)	1.5×10^{-8}* ($1.3 - 1.7$)

I In the presence of propranolol 1.4×10^{-7}M
II In the presence of dipyridamole 2.0×10^{-6}M
III In the presence of imidazole 1.5×10^{-3}M
† Each value represents the mean of at least six observations.
* Significantly different from control group at $P < 0.01$.

(d) *Effect of dipyridamole.* Dipyridamole has been reported to potentiate the smooth muscle relaxant effects of adenosine (Hashimoto, Kumakura & Tanemura, 1964) possibly by the inhibition of the uptake of adenosine by the tissue (Kolassa, Pfleger & Tram, 1971). In our experiment, dipyridamole (2.0×10^{-6} M) increased the sensitivity of the trachea to the relaxant effects of adenosine, AMP, ADP and ATP. It had no effect, however, on responses to

adenine, theophylline or isoprenaline (Table 1). In the presence of dipyridamole there was no significant difference between the potency of adenosine and its nucleotides.

(e) *Effect of quinidine.* Quinidine has been reported to inhibit specifically the effects of ATP on the guinea-pig taenia coli (Burnstock, Campbell & others, 1970). No inhibitory effect on the response of the guinea-pig trachea to ATP could be demonstrated with quinidine over the dose range 1·3 to 52·0 $\times$ 10^{-5} M.

DISCUSSION

Evidence has been accumulated in favour of the hypothesis that the non-cholinergic non-adrenergic inhibitory response to nerve stimulation in various smooth muscle preparations is due to the stimulation of 'purinergic' nerves (Burnstock, 1972). It is suggested that the likely transmitter involved is an adenosine nucleotide, probably ATP. Amongst the evidence is the observation that on some smooth muscle preparations ATP and ADP are more potent relaxants than AMP and adenosine (Hashimoto & others, 1964; Satchell & Burnstock, 1975). On the guinea-pig trachea, however, ATP was significantly less potent than adenosine. This observation suggested that the trachea differed from other smooth muscle preparations in its response to ATP. Other differences were also apparent. Quinidine did not inhibit responses of the trachea to ATP in high concentrations, whereas Burnstock & others, (1970) described a specific inhibitory effect on the taenia coli. In addition, Satchell, Lynch & others, (1972) were able to distinguish between the inhibitory responses of the taenia coli to ATP and adenosine in terms of their rates of relaxation. ATP produced a rapid response that was similar in time course to the non-adrenergic inhibitory response to transmural stimulation, whilst the response to adenosine proceeded more slowly. No such differences could be detected between the rates of relaxation on the guinea-pig trachea. These differences could suggest that the guinea-pig trachea does not possess an ATP-specific receptor, as is implied for some smooth muscle preparations. Indeed, the relative lack of potency of the nucleotides on the trachea compared with the potency of known neurotransmitters such as acetylcholine and noradrenaline (Coleman & Farmer, 1971) makes it difficult to ascribe a significant neurotransmitter role for the nucleotides on the trachea. However, it is possible that the exogenous administration of nucleotides to the trachea does not reflect the action of endogeneously released transmitter due to metabolism and inactivation and that neuronal release provides a higher local concentration at the receptor.

The differential effects of imidazole and dipyridamole on the response to adenine and theophylline and also to adenosine and its nucleotides shown in these experiments suggest that the two groups of drugs mediate their smooth muscle relaxant effects in different ways. The evidence collected has led to the formulation of the hypothesis illustrated in Fig. 2, which attempts to describe a receptor system that would accommodate the drug effects seen. Essentially the hypothesis excludes the presence of a nucleotide-specific receptor, but does postulate the existence of

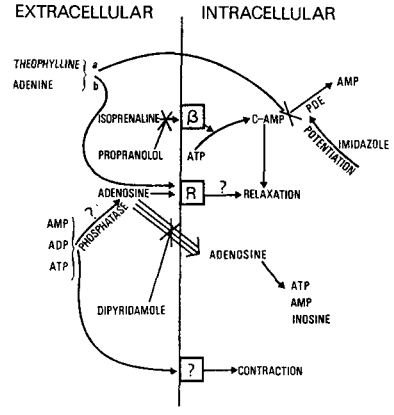

FIG. 2. Schematic representation of a hypothetical receptor system through which adenosine and its derivatives mediate their inhibitory effects on the guinea-pig trachea, compared to that of isoprenaline.

an extracellular adenosine-specific receptor (R) as previously suggested by Kolassa & others, (1971). The receptor R is distinct from the β-adrenoceptor, because propranolol did not inhibit the response to adenosine in doses that caused marked inhibition of the response to isoprenaline. It is also likely that adenosine did not cause relaxation by increasing the intracellular concentrations of cyclic adenosine -3′,5′-monophosphate (C-AMP) by other means, as imidazole did not influence the response in doses sufficient to inhibit responses to both isoprenaline and theophylline. The mechanism by which adenosine causes smooth muscle relaxation therefore remains undefined.

The evidence that the receptor R is an extracellular site comes from the observations with dipyridamole. Under normal conditions, after intravenous injection, adenosine is taken up by the lungs of the guinea-pig (Kolassa & others, 1971). Uptake of adenosine both *in vivo* and *in vitro* is inhibited by dipyridamole (Kolassa & others, 1971). Su, Bevan & Burnstock,

(1971) demonstrated that after uptake into the taenia coli, adenosine was metabolized mainly to ATP, but also to AMP and inosine. As dipyridamole sensitized the trachea to the relaxant effects of adenosine, it is likely therefore that the mechanism is the increase in the extracellular concentration of adenosine. Under these conditions the true potency of adenosine as a smooth muscle relaxant was revealed and as such was somewhat more potent than theophylline.

The observation that dipyridamole increased the sensitivity of the guinea-pig trachea to both adenosine and its nucleotides is in keeping with similar observations on the taenia coli (Satchell & Burnstock, 1975), the heart (Stafford, 1966) and arterial smooth muscle (Hashimoto & others, 1964). The fact that the effect was not confined to adenosine, suggested that the pharmacological activity of the nucleotides was closely related to that of adenosine. Two possibilities exist:

either (a) that the nucleotides interact with the receptor R itself and are also subject to tissue uptake in a manner similar to adenosine,

or (b) that they are degraded to adenosine, due to the activity of extracellular phosphatase.

It is known that the intracellular uptake of ATP is limited (Roll, Weinfeld & others, 1956). Similarly, the uptake of ADP and AMP is also less than that of adenosine. As such it is difficult to explain the effect of dipyridamole on the response to the nucleotides. It is highly likely, therefore that the second alternative is the explanation. There is corroborative evidence; in the presence of dipyridamole the molar potencies of adenosine and the nucleotides on the trachea became indistinguishable. This suggested a common metabolite. As dipyridamole did not enhance the activity of adenine, it is likely that the common metabolite is adenosine. Satchell & Burnstock (1975) also reported a similar trend towards equipotency of adenosine and its nucleotides on the taenia coli in the presence of dipyridamole. It is known that *in vivo* ATP is rapidly metabolized to adenosine and inosine (Satchell & Burnstock, 1971). Similarly it is known that AMP is rapidly broken down to adenosine when applied to the taenia coli (Satchell & Burnstock, 1975), and also Satchell & others (1972) gave evidence to suggest that in the presence of dipyridamole, the taenia coli *in vitro* will metabolize ATP to adenosine over the course of 1 min. In view of the occurrence of a contractile response before the development of the relaxation observed with the nucleotides, but not with adenosine, it is possible that the direct effect of the nucleotides on the trachea is excitatory and that this is limited by rapid metabolism to the inhibitory nucleoside, adenosine.

The fact that the response of the trachea to adenine, but not adenosine, was affected by imidazole suggested that the effect of adenine was mediated in a different manner. Imidazole had comparable effects on the responses to adenine and theophylline, suggesting that they shared a common mechanism of action. The smooth muscle relaxant effect of theophylline is usually attributed to its ability to inhibit the activity of PDE, thereby increasing the intracellular concentration of C-AMP (Butcher & Sutherland, 1962). Adenine has been shown to have a similar effect on PDE (Peers & Davies, 1971). As imidazole only inhibited responses to threshold concentrations of theophylline and adenine, it is possible that more than one effect of the drug was contributing to the response. In studies on the heart, it has long been suspected that the positive inotropic response to theophylline cannot be entirely explained by a mechanism of action involving the inhibition of PDE (McNeill, Nassar & Brody, 1969). Recent studies have suggested an extracellular effect of theophylline on the heart (Belleman & Scholtz, 1975) and it is possible that such an effect may be relevant to the smooth muscle relaxant properties of the drug. By analogy, adenine may have a similar effect. The present evidence on the trachea does not exclude the possibility that an extracellular component in the response to both theophylline and adenine does exist and that the effect is similar to that observed with adenosine. Thus it remains a possibility that both adenine and theophylline could interact with receptor R, the major difference between these drugs and adenosine being their susceptibility to the dipyridamole-sensitive adenosine uptake mechanism.

This hypothesis holds for the evidence presented in this paper. It also brings together evidence obtained in other studies concerning the mode of action of the drugs involved. Studies with potential antagonists are required to obtain further information concerning drug interactions with the postulated receptor R.

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