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# Effect of inosine on guinea-pig bronchial muscle

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Inosine, like other purine derivatives, shows antibronchospastic effects both *in vivo* and *in vitro*. Its activity remains in propranolol- or α-methyl-*p*-tyrosine-pretreated guinea-pigs, but is prevented by theophylline. Moreover, the interaction between 3′5AMP and inosine suggests potentiation. In view of recent findings showing that inosine inhibits phosphodiesterases, the conclusion is drawn that the antibronchospastic effect of this nucleoside depends on the increase of 3′5′AMP intracellular levels in the bronchial muscle.

The role of 3′5′AMP in smooth muscle relaxation processes is suggested by the findings of Somlyo, Haeusler & Somlyo (1970) who showed that this cyclic nucleotide causes hyperpolarization of vascular smooth muscle. Indeed, drugs able to activate adenylcyclases or inhibit phosphodiesterases are known to antagonize uterine contraction (Triner, Nahas & others, 1971), dilate coronary arteries (Kukovetz & Pöch, 1970) and the bronchial muscles (Vulliemoz, Verosky & others, 1971).

Inosine, like other purine derivatives, has been shown to inhibit phosphodiesterases (Lucacchini, Montali & others, 1972); we have checked its possible antibronchospastic effect and have found that inosine antagonizes different bronchoconstrictor agents, both *in vivo* and *in vitro*.

## MATERIALS AND METHODS

Guinea-pigs of either sex, average weight 400–500 g, were anaesthetized with urethane 1 g $kg^{-1}$ (i.p.). The trachea was cannulated and the animals were respired with a Starling micropump at a rate of 70 strokes $min^{-1}$, with an air volume of 1·5 ml $100g^{-1}$ weight, giving a positive inflow pressure of about 7 cm $H_2O$ in normal animals. The outflow tube was supplied with a water valve, acting at +15 cm $H_2O$. The inflow tube to the trachea was connected through a side arm to a Marey tambour. A light metal sheet was mounted on the tambour membrane in such a way as to leave the window of a photo-electric cell (connected to a D.C. amplifier) almost uncovered during the inflation period in normal animals. When the positive tracheal pressure rose over 7 cm $H_2O$ because bronchoconstrictor agents were given, a large part of the window was covered.

This simple device allowed us to minimize the records of the normal respiratory fluctuations and to amplify the increase in lung inflation resistance, caused by spasmogens, to a pre-established value (10 mm on the tracing = + 1 cm $H_2O$ over the "normal" resistance). An example of the recordings obtained is given in Fig. 1. Precordial ecg, blood (carotid) pressure and endotracheal pressure were simultaneously recorded on a direct-writing polygraph. Bronchoconstrictor agents (acetylcholine, bradykinin, 5-hydroxytryptamine and histamine) were injected intravenously every 10–15 min at different doses to obtain a dose-response curve (response = height of the tracing in mm).

Three doses of each agent were chosen, able to increase the lung inflation resistance of about +1, +2·5 and +6 cm $H_2O$, respectively. Then, the antibronchospastic activity of inosine, 3'5'AMP and theophylline was evaluated by repeating the highest dose of the agonist, within 10–15 min after i.v. drug treatment. Assuming the shift

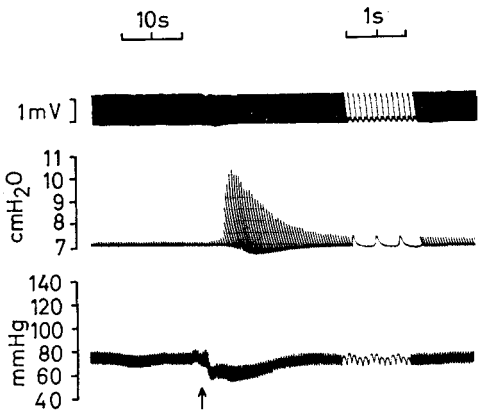


FIG. 1. Simultaneous recording of precordial ecg (top tracing), pulmonary inflation pressure (middle tracing) and carotid pressure (bottom tracing) in urethane-anaesthetized guinea-pig. The i.v. injection of histamine 2 $\mu$g kg$^{-1}$ increases the pulmonary inflation pressure from +7 to +10 cm $H_2O$.

to the right of the agonist dose-response curve, the ratio between equiactive doses before and after drug treatment was calculated. In the text this value $\times 100$ is referred to as % responsiveness. In most experiments histamine was selected as reference agonist. Some trials were made on guinea-pigs pretreated with $\alpha$-methyl-*p*-tyrosine (200 mg kg$^{-1}$, i.p.) or propranolol (1 mg kg$^{-1}$), the aim being to ascertain whether the antibronchospastic effects observed were mediated by catecholamine release. Further investigations were made on the isolated tracheal chains, set up according to Akcasu (1959) in oxygenated Tyrode solution at 37°. The contractions caused by histamine at different concentrations ($3 \times 10^{-8}$ to $1 \times 10^{-6}$ g ml$^{-1}$) were amplified 1 : 20 by an isotonic lever, load 0·5 g, and recorded on smoked paper. Inosine, 3'5'AMP and theophilline were added separately or together to establish their potency as antagonists and possible mutual interference. The % responsiveness to histamine in the presence of the test drug was calculated from the shift to the right of the agonist dose-response curve.

Freshly prepared solutions of the following drugs were used: histamine chloride; acetylcholine chloride (Roche); bradykinin (Sandoz), 5-hydroxytryptamine creatine sulphate (Vister), inosine (BDH), theophylline ethylenediamine, 3'5'AMP sodium salt (Boehringer), $\alpha$-methyl-*p*-tyrosine (K & K Lab.), propranolol HCl (ICI).

## RESULTS

In experiments to test the effect of inosine 50 mg kg$^{-1}$ on bronchospasm elicited by bradykinin (0·5–5 $\mu$g kg$^{-1}$), acetylcholine (10–50 $\mu$g kg$^{-1}$), 5-hydroxytryptamine (1–25 $\mu$g kg$^{-1}$) and histamine (1–10 $\mu$g kg$^{-1}$), inosine approximately halved sensitivity to all four spasmogens: the % responsiveness (mean of 3–4 expts) fell to 54% for 5-HT, 55% for bradykinin, 63% for acetylcholine and 66% for histamine. Thus inosine

seemed to act on the contractile mechanisms of the smooth muscle cells directly and not to behave as a selective antagonist. Inosine was then compared with 3'5'AMP and theophylline to assess the relative antibronchospastic potency. The results are summarized in Table 1. At the level of % responsiveness of 66% (corresponding to

Table 1. *Antagonism by inosine, 3'5'AMP, theophylline on the bronchial constriction by histamine in the guinea-pigs.* The results are given as ratios of histamine equiactive doses before and after drug-treatment $\times 100$, $\pm$s.e. (% responsiveness).

| Treatment | Dose mg kg$^{-1}$ | No. of expts | % Responsiveness | ED50 mg kg$^{-1}$ | b |
|---|---|---|---|---|---|
| Inosine .. .. .. | 10 | 5 | 99 ± 3 | | |
| | 20 | 6 | 74 ± 5·8 | — | −23·5 |
| | 50 | 5 | 66 ± 3·6 | | |
| 3'5'AMP .. .. .. | 5 | 5 | 100 ± 4 | 27 | −61·9 |
| | 10 | 10 | 76 ± 6·3 | | |
| | 30 | 7 | 48 ± 3 | | |
| Theophylline .. .. | 5 | 8 | 80 ± 5 | 17·4 | −54 |
| | 10 | 4 | 65 ± 7 | | |
| | 50 | 5 | 27 ± 3 | | |

the effect given by the highest dose of inosine) theophylline was 1·6 and 5 times more active than 3'5'AMP and inosine, respectively, but it appreciably reduced the arterial pressure and slightly increased cardiac rate. Its effect lasted for over 1 h. 3'5'AMP was about three times as active as inosine; however at 30 mg kg$^{-1}$ it caused considerable bradycardia (Bertelli, Bianchi & Beani, 1972), sometimes associated with A-V blockade and severe hypotension. The antagonism of 3'5'AMP to histamine bronchospasm vanished within 30 min. Inosine, although less active, did not cause appreciable changes in heart rate and blood pressure at equiactive doses; its antibronchospastic effects lasted no longer than 20–30 min.

To test possible interactions between inosine and 3'5'AMP or theophylline, the nucleoside was given together with 3'5'AMP and 20–30 min after a high dose of theophylline. As shown in Table 2, inosine, administered at 10 mg kg$^{-1}$ did not

Table 2. *Effect of 3'5'AMP plus inosine (simultaneously injected) and of inosine administered 20 min after theophylline (50 mg kg$^{-1}$) on bronchial constriction by histamine.*

| Treatment | Dose mg kg$^{-1}$ | No. of expts | % responsiveness |
|---|---|---|---|
| 3'5'AMP plus inosine .. .. | 10+ 10 | 5 | 75 ± 5 |
| 3'5'AMP plus inosine .. .. | 10+ 20 | 5 | 51 ± 4 |
| Inosine in theophylline pretreated animals .. .. | 50 | 5 | 96 ± 2 |

modify the effect of 3'5'AMP (cf. Table 1). However, when equiactive doses of the two compounds were given simultaneously (inosine 20 mg kg$^{-1}$, 3'5'AMP 10 mg kg$^{-1}$), the reduction in sensitivity of the bronchial tree to histamine was greater than the sum of the effects of the two agents: inosine or 3'5'AMP given separately at double dosage

(i.e. inosine 40 mg $kg^{-1}$ and 3'5'AMP 20 mg $kg^{-1}$) caused a 68 and 60% responsiveness respectively while the 51% reduction observed suggests potentiation. The interaction between inosine and theophylline showed an opposite pattern: inosine 50 mg $kg^{-1}$ did not further reduce the response to histamine of about 25% produced by pretreatment with theophylline 50 mg $kg^{-1}$.

To exclude that the antibronchospastic effect of inosine at least partly depended on catecholamine release (Buckley, 1970), experiments were made on α-methyl-*p*-tyrosine or propranolol pretreated animals. As previously found (Collier, James & Piper, 1965; Simke, Graerne & Sigg, 1967), propranolol 1 mg $kg^{-1}$ sensitized the bronchial tree to histamine: in ten experiments the drug increased the responsiveness to the amine to 207 ± 3%. Conversely, α-methyl-*p*-tyrosine (200 mg $kg^{-1}$, i.p., 4 h before the test) did not change the threshold doses of histamine. Neither propranolol nor α-methyl-*p*-tyrosine-pretreatment affected the antibronchospastic effect of inosine, the responsiveness showing no significant difference from that of inosine alone. Similarly, in three experiments, propranolol did not interfere with 3'5'AMP action.

An attempt to confirm the direct influence exerted by inosine on the smooth muscle was made on the tracheal chain. Both inosine and theophylline shifted to the right in parallel fashion the histamine dose-response curve. Thus, % responsiveness could be carefully calculated as ratio × 100 of the equi-effective doses of agonist before and in the presence of the antagonist. We found that preparations challenged with high or low histamine doses showed the same sensitivity to equal drug concentrations. As shown in Table 3, the ED50 value of inosine was 4·4 times higher than that of theophylline, in agreement with the lower potency of the former drug *in vivo*. In a few experiments, the tracheal chain was incubated in Tyrode solution containing theophylline 50 μg $ml^{-1}$: the histamine dose-response curves made before and in the presence of inosine 200 μg $ml^{-1}$ showed that % responsiveness to inosine of theophylline-treated preparations was less than that of control tracheal chains (Table 3).

Table 3. *Antagonism by inosine and theophylline on histamine-induced contraction of guinea-pig tracheal chain:* % responsiveness is calculated as in legends of Table 1.

| Treatment | Concentration μg $ml^{-1}$ | No of expts | % Responsiveness | ED50 μg $ml^{-1}$ | b |
|---|---|---|---|---|---|
| Inosine .. .. .. | 50 | 7 | 49 ± 7·3 | 42 | −31 |
| | 200 | 14 | 30 ± 4·1 | | |
| Theophylline .. .. | 10 | 5 | 50 ± 3 | 9·55 | −65 |
| | 50 | 5 | 4·4 ± 1 | | |
| Inosine in the presence of theophylline 50 μg $ml^{-1}$ | 200 | 5 | 71 ± 13* | — | — |

*Significantly different from % responsiveness obtained with inosine given alone, 200 μg $ml^{-1}$

Any mutual influence between 3'5'AMP and inosine could not be investigated *in vitro*, because 3'5'AMP at concentrations ranging from 20 to 250 μg $ml^{-1}$, sometimes reduced and sometimes increased the sensitivity of the preparations to histamine.

## DISCUSSION

The above findings show that inosine antagonizes various spasmogens both *in vitro* and *in vivo*. Taking into account that inosine, like theophylline, inhibits phosphodiesterases, it could be suggested that enzyme inhibition is the common action

mechanism for both drugs. There are many arguments in favour of this hypothesis.

The inhibitory activity of inosine on phosphodiesterases is more than three times weaker than that of theophylline (Lucacchini, personal communication). This observation is in agreement with the 4–5 times lower potency of inosine. Moreover, theophylline pretreatment prevents or minimizes inosine effects both *in vivo* and *in vitro*. This fact can be easily explained considering that theophylline, employed at doses sufficient to effect a high degree of enzyme inhibition, prevents the phosphodiesterases inhibiting properties of other agents (Pöch & Kukowetz, 1971). The interaction between 3'5'AMP and inosine suggests potentiation, which is known to exist between the cyclic nucleotide and the phosphodiesterase inhibitors. The mutual interference exerted by the two compounds could not be more effectively examined *in vitro*, since 3'5'AMP, as previously reported by Moore, Iorio & McManus (1968), gave erratic responses. Most probably, only when 3'5'AMP is injected *in vivo*, is it able to quickly enter the bronchial smooth muscle, thus giving relaxation.

Finally, any possible role of catecholamine release in the antibronchospastic action of inosine could be excluded because it retained its activity both in the presence of propranolol and after α-methyl-*p*-tyrosine in amounts able to cause adequate catecholamine depletion (Spector, Sjoerdsma & Udenfriend, 1965). Thus, our findings indirectly confirm the statement that the increase in 3'5'AMP intracellular levels induced by phosphodiesterase inhibition, is associated with relaxation of the bronchial smooth muscle.

Inosine, unlike theophylline and 3'5'AMP, does not present side-effects involving the cardiocirculatory system, even when administered at dosage levels capable of equally affecting the sensitivity of the bronchial tree to various spasmogens.

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