J. Pharm. Pharmac., 1976, **28**, 294–297 *Received September* 4, 1975

Mediation of prostaglandin E_2 in the biphasic response to ATP of the isolated tracheal muscle of guinea-pigs

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ATP, at a dose higher than 0·1 μg ml^{-1}, showed a biphasic action consisting of an initial increase followed by a gradual decrease of muscle tension in the isolated tracheal strip-chains of guinea-pigs. The pattern of this biphasic response to ATP varied with the level of basal tone of the preparation at the moment of application of ATP. A similar biphasic action was obtained by prostaglandin (PG) E_2 among the various active substances studied including acetylcholine, histamine, catecholamines and various types of PG. Indomethacin (0·1 μg ml^{-1}) and aspirin (30 μg ml^{-1}) completely abolished the ATP-induced inhibitory response observed in the presence of histamine (10 μM). Polyphloretin phosphate (100 μg ml^{-1}) also significantly depressed the inhibitory response to ATP or PGE_2. It is concluded that the response to ATP of the preparation is mediated by PGE_2 released via the stimulation of its biosynthesis.

Exogenously applied adenosine 5′-triphosphate (ATP) produces various pharmacodynamic effects in a variety of smooth muscle preparations but its mode of action is not yet clearly established. Burnstock (1972) reported ATP to be the transmitter released from non-adrenergic inhibitory neurons in the gastrointestinal smooth muscle. The presence of non-adrenergic inhibitory neurons was also demonstrated in the guinea-pig tracheal muscle by Coburn & Tomita (1973), and Coleman & Levy (1974) proposed ATP as a transmitter in these.

Recently, Needleman, Minkes & Douglas (1974) have reported that ATP acts as a potent releaser of a prostaglandin (PG)-like substance in a wide variety of isolated organs. We now describe the responses to ATP of the tracheal strip-chain preparation of guinea-pigs, and the influence on the ATP-induced response of PG synthetase inhibitors, indomethacin and aspirin (Vane, 1971), and a PG antagonist, polyphloretin phosphate (Eakins, Karim & Miller, 1970). A brief report has already been made (Kamikawa & Shimo, 1975).

MATERIALS AND METHODS

Male guinea-pigs, 250 to 400 g, were killed by a blow on the head and the trachea excised. Transverse strips, 2–3 mm wide, were prepared, which included tracheal smooth muscle and ends of cartilage. Two strips were tied in alignment and suspended in a 30 ml organ bath containing modified Krebs-Ringer solution (composition mM; NaCl, 120; KCl, 4·7; $CaCl_2$, 2·0; $MgCl_2$, 1·2; $NaHCO_3$, 25; KH_2PO_4, 1·2; glucose, 14). The solution at 37° was bubbled with 5% carbon dioxide in oxygen at pH 7·4.

Changes in muscle tension were recorded on a Recticorder using an isometric transducer (Nihon Kohden). Preparations were allowed to equilibrate under a tension of 0·5 or 0·2 g for at least 1 h.

Drugs used were: adenosine 5′-triphosphate disodium salt, (±)-isoprenaline hydrochloride (Sigma), histamine dihydrochloride, atropine sulphate (Wako Pure Chem.), indomethacin, propranolol hydrochloride, chlorpheniramine maleate (Sankyo), aspirin (Mitsui Toatsu), phentolamine (Ciba), theophylline (Tokyo Kasei), acetylcholine chloride (Daiichi), prostaglandin E_1, E_2, $F_{2\alpha}$ (Ono), and polyphloretin phosphate (AB Leo). Indomethacin and aspirin were dissolved in 50% ethanol. All other drugs were dissolved in the physiological saline. Concentrations of drugs were calculated as salt and are given as the final concentration in a 30 ml bath.

RESULTS

ATP, at a concentration higher than 0·1 μg ml^{-1}, generally showed a biphasic action consisting of an initial, transient increase followed by a gradual decrease of tension. As shown in Fig. 1, however, the biphasic action of ATP was variable depending on the level of basal tone which was checked by an application of isoprenaline (0·1 μM). In the muscle with lower tone which showed little decrease of tension with isoprenaline, the initial increase was more marked than the following decrease of tension (Fig. 1, A). Some preparations having such a low tone showed only a tonic increase of tension (Fig. 2, B). In the muscle with higher tone, which showed a great decrease of tension with isoprenaline, the subsequent decrease was predominant (Fig. 1,

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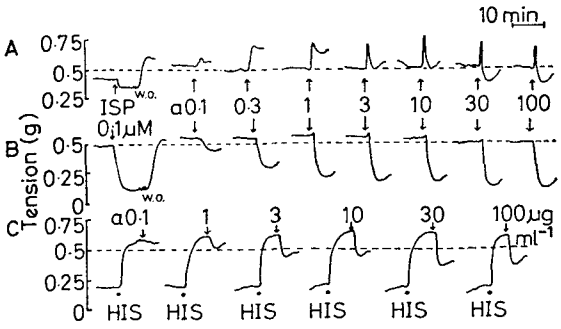

FIG. 1. Dose-response relations of ATP in the guinea-pig tracheal strip-chains. Initial tension is 0·5 g in A and B, and 0·2 g in C. A; biphasic responses to ATP (a) (0·1–100 μg ml^{-1}) in the low tone preparation in which the basal tone decreased little with isoprenaline (ISP, 0·1 μM). B; predominant inhibitory responses to ATP in the high tone preparation in which the basal tone decreased markedly with ISP. C; inhibitory responses to ATP in the presence of histamine (HIS, 10 μM applied at dots). Dotted lines indicates 0·5 g level of muscle tension. W.O.: washing out ISP.

B). In 63 preparations examined with ATP 10 μg ml^{-1}, a tonic increase of tension without any decrease was observed in 16, a biphasic action with the predominant initial increase of tension in 19, a biphasic action with the predominant decrease in 25, and a pure decrease of tension in 3 preparations. On the other hand, ATP caused only a decrease of tension without any initial increase

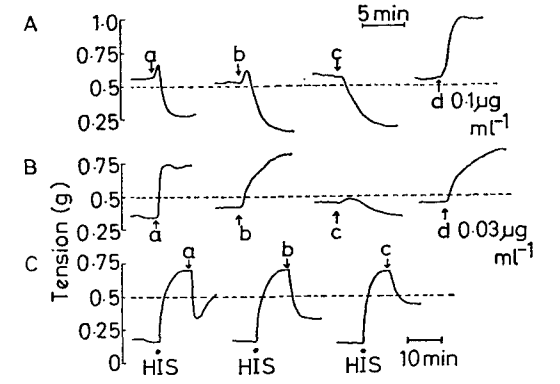

FIG. 2. Comparisons of the responses to ATP(a), PGE_2(b), PGE_1(c) and $PGF_{2\alpha}$(d) of the guinea-pig tracheal strip-chains. Initial tension is 0·5 g in A and B, and 0·2 g in C. A, responses to ATP (10 μg ml^{-1}), PGE_2 (0·1 μg ml^{-1}), and $PGF_{2\alpha}$ in the high tone preparation. B, responses to ATP, PGE_2 and PGE_1, at concentrations described in A, and to $PGF_{2\alpha}$ in the low tone preparation. C; inhibitory responses to ATP, PGE_2 and PGE_1, at concentrations described in A, in the presence of histamine (HIS, 10 μM applied at dots). In each preparation, the responses to ATP were similar to those obtained by PGE_2. Dotted lines indicate the 0·5 g level of muscle tension.

whenever the basal tone was increased by an application of histamine (10 μM, Fig. 1, C). The inhibitory response to ATP under these conditions depended on its concentration in the medium. In general, the response is greater as the ATP concentration is increased (Fig. 1, C). There were no signs of tachyphylaxis in the response to ATP 10 μg ml^{-1}.

These responses to ATP depending on the basal tone, were different from those obtained by acetylcholine, catecholamines and histamine, and were not affected by atropine (1 μM), propranolol (1 μg ml^{-1}), phentolamine (10 μg ml^{-1}), theophylline (10 μg ml^{-1}) and chlorpheniramine (1 μg ml^{-1}). Among the several types of PG studied, only PGE_2 showed a similar pattern of the biphasic response depending on the basal tone. As shown in Fig. 2, PGE_2 showed the same biphasic (A), excitatory (B), or inhibitory patterns (C) with or without histamine as those after ATP under the same conditions. But PGE_1 predominantly showed a decrease of tension and $PGF_{2\alpha}$ only an increase of tension in all preparations.

Effects of indomethacin and aspirin on the response to ATP

The effects of indomethacin and aspirin were investigated only in preparations in which the basal tone was increased by an application of histamine 10 μM, since these drugs markedly decreased the basal tone and therefore their influences on the biphasic action of ATP were not clearly estimated. Both indomethacin (0·1 or 1 μg ml^{-1}, n = 16) and aspirin (30 μg ml^{-1}, n = 6) completely abolished the inhibitory response to ATP (10 μg ml^{-1}) as shown in Fig. 3, A and B. Some antagonistic effect of indomethacin remained even 2 h after washing out the drug, while that of aspirin was fully reversible. On the other hand, these drugs, at the doses described, did not modify the inhibitory response to ISP or PGE_2, but slightly potentiated the contractile response to histamine. The solvent (0·15 ml of 50% ethanol) for these drugs did not influence the response to ATP.

Effect of polyphloretin phosphate (PPP) on the response to ATP and PG

PPP had no effect on the tracheal tone below 100 μg ml^{-1}, but at higher doses showed a gradual increase followed by decrease of tension. Fig. 4, A and B shows the inhibitory effect of PPP (100 μg ml^{-1}) on a biphasic response to ATP (10 μg ml^{-1}, A) and PGE_2 (0·1 μg ml^{-1}, B). The tension decreasing effects of ATP and PGE_2 were depressed by 67·5 $\pm$ 6·3% (n = 16) and 47·8 $\pm$ 8·7% (n = 6) respectively, by the preincubation with PPP for 15 min, and fully

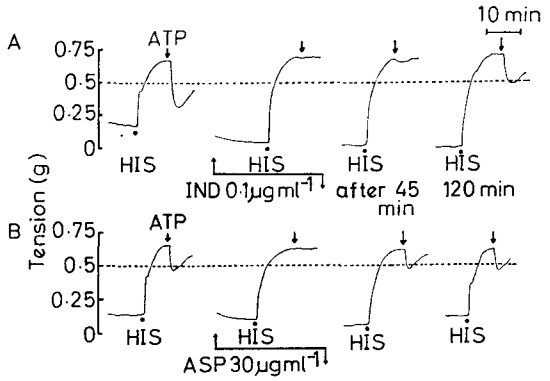

FIG. 3. Antagonistic effects of indomethacin and aspirin on the inhibitory response to ATP (10 μg ml^{-1}) in the presence of histamine (HIS, 10 μM) of the guinea-pig tracheal strip-chains. Initial tension is 0·2 g. Indomethacin (IND, 0·1 μg ml^{-1}, A) and aspirin (ASP, 30 μg ml^{-1}, B) were applied at 20 min before an application of histamine. The antagonistic effect of indomethacin was partially removed at 2 h after washing out the drug, while that of aspirin was removed immediately.

restored by washing out the drug. The excitatory responses to ATP, PGE_2 and $PGF_{2\alpha}$ were also depressed at higher concentrations of PPP (up to 300 μg ml^{-1}, n = 5). These reversible inhibitory effects were also observed in the presence of histamine (10 μM, Fig. 4, C and D), in which case the inhibitory response to ATP (10 μg ml^{-1}) and PGE_2 (0·1 μg ml^{-1}) was reversibly inhibited by 43 $\pm$ 8·2% (n = 5) and 41 $\pm$ 12·3% (n = 5) respectively, by the preincubation with PPP 100 μg ml^{-1}. However, the responses to ATP and PGE_2 were not completely abolished even at concentrations of PPP up to 300 μg ml^{-1}.

DISCUSSION

Reports on the pharmacological action of ATP on bronchial smooth muscle are few. Bianchi, De Natale & Giaquinto (1963) and Collier, James & Schneider (1966) have reported that ATP produces a dilation of the bronchial tree *in vivo* and *in vitro* after small doses and constriction after high doses. Coleman & Levy (1974) observed a small excitatory response to ATP in the guinea-pig tracheal muscle *in vitro*. Using the tracheal strip-chains of guinea-pigs, we have shown that ATP causes variable biphasic responses depending on the level of basal tone of the preparation. A smilar biphasic reponse to ATP has been reported by Furchgott (1966) in the rabbit aortic strip, and Coleman & Levy (1974) also observed that the inhibitory response to ATP is unmasked in the presence of dipyridamole which is known to block the uptake of adenosine and potentiate responses to adenyl compounds in several tissues. These responses to ATP observed in the present experiments apparently differed from those to catecholamines, acetylcholine and histamine; a similar biphasic response was obtained only with PGE_2. Previous reports have shown that E types of PG only relax isolated tracheal muscle from various species (Main, 1964; Sheard, 1968; Türker & Khairallah, 1969). But Lambley & Smith (1975) found the response to PGE_2 in guinea-pig tracheal chains depends on the pre-existing level of tone; when this was high, PGE_2 caused relaxation, but when the muscle was relaxed, it caused contraction. This is in good agreement with our results.

The inhibitory response to ATP in the presence of histamine was completely antagonized by pre-incubation with indomethacin or aspirin. Collier & others (1966) also observed that aspirin and fenamates effectively antagonized ATP-induced broncho-constriction. Since these drugs are known to inhibit the synthesis of PGs in tissues (Vane, 1971), these results suggest that the response to ATP of the guinea-pig tracheal muscle is mediated by PGs released via the stimulation of its biosynthesis. This suggestion is supported by the findings of Needleman

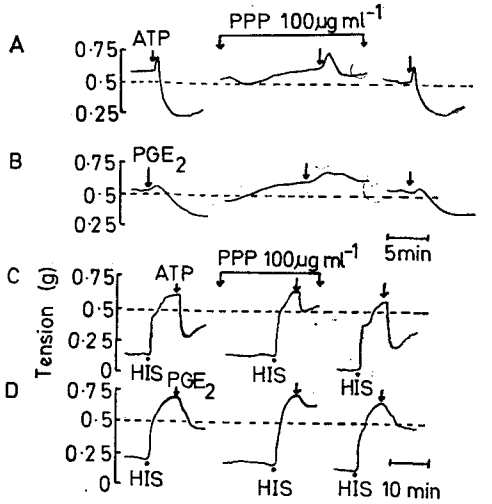

FIG. 4. Effects of polyphloretin phosphate (PPP, 100 μg ml^{-1}) on the biphasic responses (A and B), and the inhibitory responses in the presence of histamine (HIS, 10 μM, C and D) to ATP (10 μg ml^{-1}) and PGE_2 (0·1 μg ml^{-1}). Initial tension is 0·5 g in A and B, and 0·2 g in C and D. PPP applied at 15 min before the application of ATP or PGE_2, and 10 min before an application of histamine. Inhibitory responses to ATP and PGE_2 of the preparation with or without histamine were inhibited by the preincubation of PPP to the same degree. Dotted lines indicate 0·5 g level of muscle tension. ATP (A and C) and PGE_2 (B and D) applied at arrows.

& others (1974) who showed the inhibitory effect of indomethacin on the release of PG-like substance by ATP. Furthermore, this is also supported by the present results concerning the antagonistic effects of PPP upon the inhibitory response to ATP or PGs, in which PPP ($100\,\mu g\,ml^{-1}$) inhibited the ATP and PGE_2 induced responses to a similar degree. PPP did not inhibit the excitatory response to $PGF_{2\alpha}$ until PPP concentration was increased up to $300\,\mu g\,ml^{-1}$, while it inhibited the PGE_2 induced response at a lower concentration of PPP. Previous studies demonstrated that PPP specifically antagonized the smooth muscle stimulating action of both $PGF_{2\alpha}$ and PGE_2 on several isolated smooth muscle preparations, but not the inhibitory actions of PGE_2 on the circular gastrointestinal muscle of man and guinea-pigs (Eakins & others 1970; Eakins, 1971; Bennett & Posner, 1971). Also, in bronchial smooth muscle, it has been reported that PPP antagonized the $PGF_{2\alpha}$-induced bronchoconstriction in several species (Mathé, Strandberg & Åström, 1971; Mathé, Strandberg & Fredholm, 1972), but not the PGE_2-induced bronchodilation (Mathé & others, 1971) and rabbit tracheal relaxation (Eakins, 1971). This finding concerning the effect of PPP on the response to PGE_2 differs from our present results, but its causes may be the differences of animal species and experimental condition (*in vitro* and *in vivo*, or isometric and isotonic conditions), From our present findings, it is concluded that the inhibitory response to ATP of the guinea-pig tracheal muscle is fully mediated possibly by PGE_2. We think it unlikely that $PGF_{2\alpha}$ is involved in the initial excitatory response to ATP in the low tone preparation since ATP caused an inhibitory response without any excitatory response in higher tone preparations with or without histamine, while $PGF_{2\alpha}$ always caused only an excitatory response even in these preparations. Furthermore Needleman & others (1974) also did not obtain any evidence for $PGF_{2\alpha}$ release by ATP.

The hypothesis of Coleman & Levy (1974) that ATP may be a transmitter released from non-adrenergic inhibitory neurons in the guinea-pig trachea is not supported since we found the inhibitory action of ATP to be indirect and mediated by PGE_2. There are several reports supporting the hypothesis that PGs play a role in the maintenance of tone in bronchial and tracheal smooth muscle (Farmer, Farrar & Wilson, 1972, 1974; Orehek, Douglas & others, 1973; Lambley & Smith, 1975). Our results support this, since indomethacin, aspirin and high doses of PPP itself decreased the basal tone, and a biphasic action of ATP mediated by PGE_2 depended on a level of basal tone, even under isometric conditions. Also, we consider that endogenous ATP may act as a trigger for PG synthesis responsible for the maintenance of tracheal muscle tone.

Acknowledgements

We wish to thank Professor S. Katori, Kitazato University School of Medicine, and Dr. B. Fredholm AB Leo, Helsingborg, for supplying PPP, and Ono Pharmaceutical Mfg. Co. for prostaglandins.

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