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Generation of prostaglandin E-like material by the guinea-pig trachea contracted by histamine

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Challenges of the superfused (1 ml min^{-1}) trachea with histamine (100–200 μg) result in the release of prostaglandin E-like material (3–25 ng in terms of prostaglandin E_2) but no prostaglandin F-like activity has been detected in the superfusate. This release is blocked by indomethacin (1μg ml^{-1}) and then the contractile action of histamine is enhanced. It is concluded that the release of a prostaglandin E-like material by histamine from tracheal smooth muscles is a self-defensive mechanism protecting against the strong constriction of airways. The maximal relaxation of trachea by isoprenaline (50–500 μg) is not accompanied by the release of a prostaglandin-like material.

Prostaglandins (PG) of E and F series are known to evoke the opposite effects in the airways smooth muscles. Horton (1969) has proposed that asthmatic bronchospasm may be due to overproduction of bronchoconstrictor PGFs at the expense of bronchodilatator PGEs. An alternative explanation is the hypersensitivity towards a bronchoconstrictor action of $PGF_{2\alpha}$ in asthmatic patients (Mathé, Hedqvist & others, 1973). The fact that inhibitors of PG biosynthesis (Vane, 1971; Flower, Gryglewski & others, 1972) precipitate asthmatic attacks in aspirin-sensitive patients (Smith, 1973, Szczeklik, Gryglewski & Czerniawska-Mysik, 1974) is consistent with the possibility that PGEs play a more important role than PGFs in maintaining bronchial tone in those patients.

PGEs, PGFs, their precursors (Piper & Vane, 1969; Piper, 1974) and their metabolites (Mathé & Levine, 1973) are released from guinea-pig perfused lungs during anaphylactic shock. PG-like material is also released from unshocked guinea-pig lungs by massage, hyperventilation and infusion of particles (Piper, 1974), as well as by an infusion of 5-hydroxytryptamine, acetylcholine and histamine into the pulmonary circulation (Bakhle & Smith, 1972).

Orehek, Douglas & others (1973) have reported that histamine liberates both PGEs and PGFs from the contracting guinea-pig trachea. These authors suggest that the airways tone may be modulated by a continuous intramural synthesis of both types of prostaglandins by the smooth muscle. We report here that histamine releases mainly PGE-like material during contraction of the trachea smooth muscle of the guinea-pig.

MATERIAL AND METHODS

Guinea-pig trachea spirally cut (GPT) (Constantine, 1965), rat stomach strip (RSS) (Vane, 1957) and rat colon (RC) (Regoli & Vane, 1964) were superfused in cascade (Vane, 1964) with oxygenated Tyrode solution (37°) at 1 ml min^{-1}. The changes in tone of all three tissues were recorded isotonically with auxotonic levers (initial load 2–3 g) using Harvard transducers type 386 connected to a Watanabe multirecorder.

The stomach and colon preparations were used to bioassay PGE-like and PGF-like activity in the superfusate (Piper & Vane, 1969, Vane, 1971). The set of the assay tissues was used only when the colon was 5–8 times more sensitive to $PGF_{2\alpha}$ than the stomach strip. The sensitivity and selectivity of the assay tissues to PG were enhanced by the treatment with combined antagonists (Gilmore, Vane & Wyllie, 1968). The Tyrode solution contained the following concentrations of antagonists ($\mu g\ ml^{-1}$): phenoxybenzamine 0·1, propranolol 2·0, atropine 0·1, methysergide 0·1, diphenhydramine 10·0 and indomethacin 1·0. Indomethacin or diphenhydramine could be infused over the assay tissues alone or over the trachea. Trachea was challenged with histamine (1–200 μg) or isoprenaline (50–500 μg) administered as single injections. During the isoprenaline challenge the concentration of diphenhydramine in the Tyrode solution was lowered to 0·1 $\mu g\ ml^{-1}$. Isoprenaline was completely removed from the trachea superfusate by a column of activated aluminium oxide. This does not remove PG (unpublished observation of R. Korbut). The calibration doses of PGE_1, PGE_2 and $PGF_{2\alpha}$ and the control doses of histamine and isoprenaline were injected into the superfusate leaving the trachea.

Drugs: Atropine sulphate (Medexport), diphenhydramine hydrochloride (Brocades), histamine dihydrochloride (Polfa), indomethacin (Polfa), isoprenaline hydrochloride (Sigma), methysergide (Sandoz), phenoxybenzamine (Smith, Kline and French), propranolol hydrochloride (Polfa).

Prostaglandins E_1, E_2 and $F_{2\alpha}$ (salt) were kindly supplied by Dr. John E. Pike, Upjohn Company, Kalamazoo, U.S.A.

Concentrations of all drugs, except for prostaglandins and indomethacin, are calculated for salts. Superfusing fluid (Tyrode solution) of the following composition was used (g $litre^{-1}$): NaCl 8·0, KCl 0·2, $CaCl_2$ (anhydrous) 0·2, $MgCl_2$ (anhydrous) 0·01, $NaHCO_3$ 1·0, NaH_2PO_4 0·05, glucose 1·5.

RESULTS

Challenges of four tracheas with single injections of histamine 1–25 μg produced no detectable release of PG-like material. Out of three tracheas challenged with 50 μg of histamine in one there was a release of a material contracting the two rat preparations. Out of twelve tracheas which were challenged with 100–200 μg of histamine, ten released a material contracting the assay tissues. The relative potency of this material to contract the stomach and colon fit relative potencies of authentic PGE_2 or PGE_1, but not of $PGF_{2\alpha}$ (Fig. 1). The peak amounts of the released material ranged from 3 to 25 ng ($n = 10$, mean $\pm$ s.e., 7·2 $\pm$ 1·8) as matched by PGE_2. Only in one experiment could the presence of PGF-like substance in the superfusate be suspected. In all experiments the assay tissues were able to detect 0·5 — 1·0 ng of PGE_2 or $PGF_{2\alpha}$.

The prostaglandin-like character of the released material was confirmed by the fact that it was not released by tracheas pretreated with indomethacin (1 $\mu g\ ml^{-1}$), while in four control experiments three consecutive challenges with histamine each resulted in a release of PGE-like material. The assay tissues did not contract to histamine because of the blockade with diphenhydramine (10–20 $\mu g\ ml^{-1}$). The stomach strip and colon were sometimes relaxed by histamine but then the assay tissues were discarded. The treatment of trachea with indomethacin not only prevented the release of the PGE-like material by histamine, but also increased the contractile action of histamine by 11–100% ($n = 10$, mean $\pm$ s.e., 37·6 $\pm$ 8·1) (Fig. 1). The pretreatment

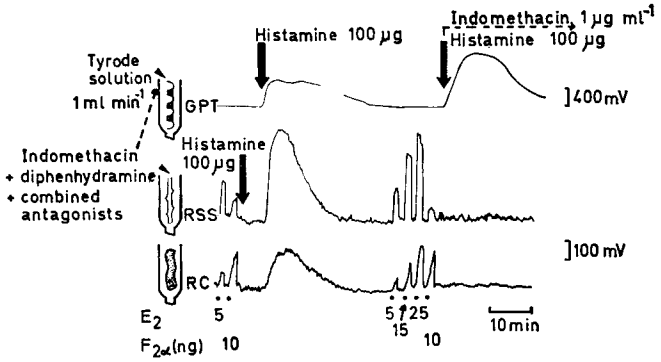

FIG. 1. The release of PGE-like substance from the guinea-pig trachea (GPT) contracted by histamine (100 μg) and its blockade by indomethacin (1 μg ml^{-1}). GPT was superfused (1 ml min^{-1}) in cascade with the rat stomach strip (RSS) and the rat colon (RC), which differentiated between PGE_2 and $PGF_{2\alpha}$, and were not contracted by histamine (see methods). The challenge of GPT with histamine resulted in the release of a material which contracted RSS and RC. The contraction of RSS and RC could be matched with 25 ng of PGE_2 (but not $PGF_{2\alpha}$) injected directly over the assay tissues (as indicated by dots). Twenty min after the superfusion of GPT with indomethacin (the recorder was stopped) the second injection of histamine was administered. No release of PGE-like substance occured but the GPT contraction was increased by 100%. Time 10 min, vertical scales in mV for RSS, RC and GPT contractions.

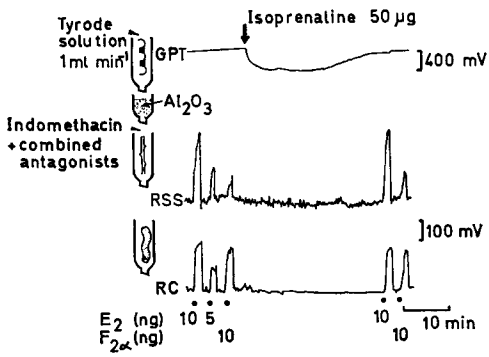

FIG. 2. The lack of the release of a PG-like material from the guinea-pig trachea (GPT) relaxed by isoprenaline (50 μg). The scheme of the experiment was the same as those described in Fig. 1, except for the aluminium oxide column incorporated into the cascade (see methods).

of trachea with diphenhydramine prevented the contractile response to histamine and the release of the PGE-like material.

Six tracheas treated with isoprenaline (50–500 μg) released no detectable amounts of either PGE-like or PGF-like substances (Fig. 2).

DISCUSSION

Indomethacin is an efficient inhibitor of PG biosynthesis (Vane, 1971, Flower & others, 1972) and at the same time it potentiates the contractile response of GPT to high dosage of histamine (Orehek & others, 1973 and this paper). We suppose that this may be explained by the release of a bronchodilator PGE-like material in an average amount of 7 ng (assayed as PGE_2) by tracheas non-treated with indomethacin when challenged with histamine (100–200 μg). These tracheas release no detectable amounts ($> 0{\cdot}5$–$1{\cdot}0$ ng in terms of $PGF_{2\alpha}$) of PGF-like material. Orehek & others (1973) were the first to have shown that histamine liberates PG from tracheal smooth

muscles. They have reported that both PGEs and PGFs are found in the superfusate of the histamine-contracted guinea-pig trachea. A difference between our experiments and those of Orehek & others (1973) is their use of thin-layer chromatography to separate PG in the pooled superfusates collected from several tracheas, while we have differentiated directly the presence of PGE-like or PGF-like materials in the course of each experiment.

Cell-free homogenates of guinea-pig lungs incubated with arachidonic acid contain more $PGF_{2\alpha}$ than PGE_2 (Vane, 1971), but this is presumably related to the greater rate at which PGE_2 is catabolized by homogenates (Änggård & Samuelsson, 1967). The anaphylactic bronchoconstriction releases from the sensitized guinea-pig lungs PGE_2 and $PGF_{2\alpha}$ along with histamine (Piper & Vane, 1969; Piper, 1974), but Mathé & Levine (1973) have demonstrated the preponderance of the PGE_2 metabolite in the perfusate; the PGE_2: $PGF_{2\alpha}$ ratio being approximately 6:1. Also, the effluent from the unshocked and unsensitized guinea-pig lungs contains small amounts of PGE_2 but not $PGF_{2\alpha}$ (Piper & Vane, 1969). Karim, Sandler & Williams (1967) have shown that bronchial tissue, unlike lung parenchyma, contains predominantly PGEs and only small amounts of PGFs. Finally the potentiation of the histamine-induced tracheal contraction by indomethacin is in agreement with our finding that the contracted trachea releases mainly a PGE-like substance. Perhaps the release of PGEs by the airways' smooth muscles is a self-defensive mechanism protecting airways against over constriction. In aspirin-sensitive asthma, severe attacks of bronchospasm may be induced by aspirin, indomethacin, mefenamate (Smith, 1973) and by other inhibitors of PG biosynthesis (Szczeklik & others, 1974). One possibility is that these drugs block the bronchodilator PG mechanism (i.e. biosynthesis of PGEs), which is normally activated by endogenous histamine.

PG are generated by the contracting smooth muscles of the trachea, but even the maximal relaxation induced by isoprenaline (500 μg) is unable to release a PG-like substance from the guinea-pig trachea.

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