a prostaglandin acts as the mediator between PTH stimulation of the bone cells and lysosomal enzyme release. Aspirin may therefore inhibit the PTH effect by its known ability to inhibit prostaglandin synthesis[10], but would not inhibit osteolysis induced by PGE_1, as this would not depend on intracellular prostaglandin synthesis.

Cortisone, on the other hand, does not inhibit prostaglandin synthesis[11] and its known ability to inhibit lysosomal enzyme release[12] must therefore be by another mechanism, perhaps by a direct effect on the lysosomal membrane. It could then inhibit the stimulatory effect of both PTH and PGE_1, as reported[1].

Injections of massive doses of PGE_1 do not cause an increase in the serum calcium concentration in parathyroidectomized rats, in spite of the marked osteolytic activity of PGE_1 *in vitro*[1]. We therefore suggest that prostaglandin production may be involved in the intracellular mechanism for the release of lysosomal enzymes by suitably stimulated cells, and that circulating prostaglandins may have very little, if any, *in vivo* function as stimulants of osteolysis.

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Prostaglandin Regulation of Airway Smooth Muscle Tone

PROSTAGLANDINS E and F have a strong and opposite effect on airway smooth muscle[1,2]. They are released from the isolated guinea-pig lung during anaphylaxis[3] and injection of histamine or acetylcholine into the pulmonary circulation[4], both circumstances where airway constriction probably occurs. Therefore, airway tone might be modulated by the prostaglandins released, if they reach the airway smooth muscle. We report that such modulation occurs in the isolated guinea-pig trachea.

Spirally cut tracheas[5] from female guinea-pigs (Hartley strain, 200 to 250 g) were superfused[6] at a rate of 1 ml min^{-1} with Tyrode solution at 37° C, gassed with 5% CO_2 in oxygen. The effluent superfused a rat stomach strip (Fig. 1) treated with combined antagonists to make the tissue more specific for prostaglandin detection[3]. Final antagonist concentrations (μg ml^{-1}) on the rat stomach strip were: mepyramine, 10; phenoxybenzamine, 0.1; methysergide, 0.2; propranolol, 2; atropine, 2 (80 when the agonist on the trachea was acetylcholine); and indomethacin (which increases the sensitivity of the rat stomach strip to prostaglandins[7]) 3. Histamine, acetylcholine or known quantities of prostaglandins E_2 and $F_{2\alpha}$, used for calibrating the rat stomach strip, were applied directly onto the trachea as 100 μl volumes. Contractions of both tissues were recorded isometrically with Statham strain gauges.

When the guinea-pig trachea is contracted with varying doses of histamine (twenty-two experiments) or acetylcholine (eight experiments), the rat stomach strip contracts (Fig. 1), although it is insensitive to direct application of histamine or acetylcholine. This suggests that the trachea releases a prostaglandin-like substance during its contraction. Prostaglandin release represents fresh synthesis[8] and is inhibited by anti-inflammatory drugs[9]. Indeed, indomethacin

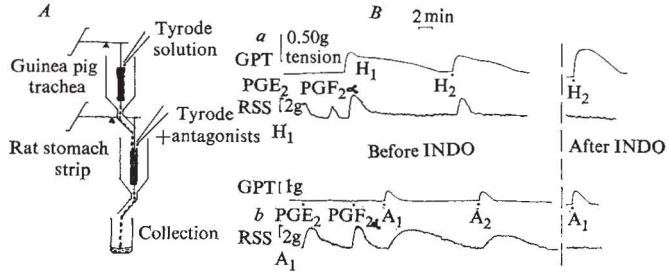

Fig. 1 *A*, Diagram of the superfused tissue technique. Tyrode solution, after superfusing the guinea-pig trachea, drips onto the rat stomach strip, treated with combined antagonists. *B*, Release of prostaglandin-like material by the guinea-pig trachea (GPT) in two different preparations, when contracted with (*a*) histamine and (*b*) acetylcholine. In both cases the rat stomach strip (RSS) is insensitive to direct application of histamine (H_1=100 μg) or acetylcholine (A_1=10 μg) but sensitive to prostaglandin E_2 (PGE_2=10 ng) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$=10 ng). When the trachea is contracted with various doses of histamine (H_1=100 μg, H_2=50 μg) or acetylcholine (A_1=10 μg, A_2=5 μg) the RSS contracts, in a dose related manner, indicating the release of a prostaglandin-like substance. After indomethacin (INDO) treatment of the trachea (3 μg ml^{-1} per 20 min) the tracheal contractions produced by histamine or acetylcholine are increased but the RSS does not contract, indicating that the release of prostaglandin-like substance is abolished.

treatment of the trachea prevents the contraction of the rat stomach strip (which was still sensitive to the application of standard prostaglandins), while the tracheal contraction induced by histamine or acetylcholine is enhanced. The released material, from several tracheas, was further characterized by chromatography and bioassay[10,11]. These data indicated that the activity on the rat stomach strip is due to the release of a prostaglandin E and prostaglandin $F_{2\alpha}$ from the trachea.

The effects of prostaglandin synthesis inhibitors on airway responses were studied in another group of experiments. Spirally cut tracheas, suspended in a 10 ml bath of Tyrode solution at (37° C and bubbled with 5% CO_2 in oxygen), were equilibrated under 3 g tension for 1 h. Changes in active tension to graded doses of histamine (seventeen experiments) or acetylcholine (twelve experiments) were recorded isometrically, with a Statham strain gauge, before and after a 20 min incubation with the prostaglandin synthesis inhibitors[9] aspirin (three experiments, 50 μg ml^{-1}) or indomethacin (thirteen experiments at both 6 μg ml^{-1} and 0.6 μg ml^{-1}, both doses being equally effective). These

Table 1 Mean Changes Produced by Aspirin or Indomethacin on Basal Tone and Histamine or Acetylcholine Induced Contractions of the Guinea-pig Trachea

Agonist	Increase in tension produced by Smallest dose of agonist. Mean ± s.e. (mg) Control	After indomethacin or aspirin	Largest dose of agonist. Mean ± s.e. (mg) Control	After indomethacin or aspirin	Decrease of tension after indomethacin or aspirin (mg)
Histamine	213.2 ± 47.3	95.0 ± 64.0 (−55.8% ± 14.8)	1,188.7 ± 148.6	2,123.3 ± 176.8 (+104.0% ± 18.5)*	440.5 ± 67
Acetylcholine	441.4 ± 84.1	188.5 ± 53.4 (−48.6% ± 15.7)*	1,322.3 ± 225.8	1,677.5 ± 252.1 (+33.9% ± 14.2)*	275.4 ± 59.3

* $P < 0.001$.

drugs reduce the basal tone, decrease the effect of the smallest doses of histamine or acetylcholine and increase the effect of the largest doses of histamine or acetylcholine (Fig. 2). The two latter effects are not due to decrease of basal tension as tension correction after indomethacin (five experiments) gave identical results. The three changes lasted several hours in spite of several washouts and were of different magnitude in the different preparations (Table 1). The discrepancies between the histamine and acetylcholine groups may be because the experiments were performed on different preparations, as experiments with acetylcholine and histamine in the same preparations, did not show significant differences.

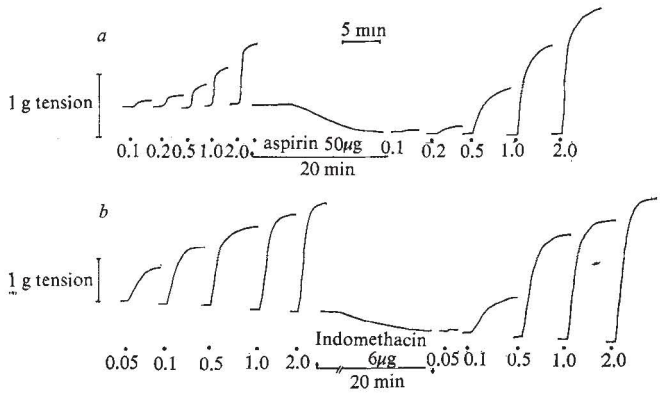

Fig. 2 Effects of aspirin or indomethacin on the histamine or acetylcholine induced contractions and the basal tone of the spirally cut guinea-pig trachea. *a*, Histamine (μg); *b*, acetylcholine (μg).

Because prostaglandin E_2 relaxes the airways[1,2], inhibition of its synthesis by indomethacin or aspirin can explain the potentiation of pronounced histamine and acetylcholine doses. Similarly, the reduction of responses to low agonist doses and the reduction of basal tone may be due to an inhibition of a continuous synthesis of prostaglandin $F_{2\alpha}$, which is an airway constrictor[1,2]. Studies in other smooth muscles have also suggested that a continuous intramural synthesis of prostaglandin maintains the resting tone[7,12].

The trachea synthesizes both types of prostaglandins but either the effect of E or the effect of F seems predominant, according to the state of tracheal muscle contraction. Two hypotheses are possible. (1) There is variation in the quantity ratio of both prostaglandins, that is, there is more F synthesized than E at the resting or low contraction state but there is more E than F at the high contraction state. (2) There is the same quantity ratio of both prostaglandins but variation in their effectiveness ratio, that is, E is more effective in relaxing tracheal muscle when highly contracted whereas F is more effective in contracting the muscle when resting or slightly contracted.

Release of prostaglandins by the agonists is specific as it can be prevented by mepyramine or atropine, but the mechanism triggering the synthesis, when the trachea contracts, is unclear. It may be related to the mechanical consequence of the contraction because mechanical stimuli release prostaglandins[8]. Prostaglandin synthesis in the guinea-pig trachea, *in vitro*, seems to be a process regulating the contraction, either enhancing or decreasing the effect of the stimuli. Such regulation could be superimposed *in vivo* on the regulation provided by the autonomic nervous system[13].

Our results suggest that tracheal strips should be treated with prostaglandin synthesis inhibitors to avoid prostaglandin interference when assessing the effects of contractile agents. Similar experiments with other tissues may provide insight into the role of prostaglandins as local regulating substances.

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Stimulation of Calcitonin Release *in vitro* by Purine and Pyrimidine Nucleotides

In view of reports concerning modulation of response of adenyl cyclase by purine nucleotides[1-4] and evidence that secretion of calcitonin is mediated by the adenyl cyclase system[5,6], we studied the possibility that purine and pyrimidine nucleotides might stimulate the release of calcitonin by parafollicular cells of the thyroid.

Guanosine triphosphate (GTP) significantly augmented release of calcitonin but guanosine' diphosphate (GDP), guanosine monophosphate (GMP) and cyclic guanosine 3′,5′-monophosphate (cyclic GMP) were ineffective (Table 1). GTP caused release in concentrations as low as 10^{-8} M,