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Increased Synthesis of Prostaglandin-Like Material During Histamine Tachyphylaxis in Canine Tracheal Smooth Muscle

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We have previously demonstrated a rapidly developing tachyphylaxis to histamine in isolated canine tracheal smooth muscle [1,2]. This tachyphylaxis can be reversed in a time- and dose-dependent fashion with a variety of prostaglandin synthesis inhibitors [2]. Furthermore, when the development of tachyphylaxis is prevented by pretreatment with indomethacin, addition of PGE_2 (but not PGB_2, PGD_2 or $PGF_{2\alpha}$) in the subnanomolar range can selectively reduce the response to histamine, thus duplicating the tachyphylactic response [2]. These findings suggested indirectly that the development of tachyphylaxis to histamine may be a result of increased prostaglandin production.

In this study we present evidence that decreasing responses to histamine are associated with increasing production of prostaglandin-like material. These results lend further support to the hypothesis that prostaglandins, presumably of the E series, are produced by tracheal smooth muscle in response to histamine and serve as mediators of this tachyphylaxis.

Strips of canine tracheal smooth muscle were isolated from adult mongrel dogs anesthetized with intravenous pentobarbital (30 mg/kg). Two tracheal strips (5-7 mm x 20-25 mm each) were tied together in series, to increase tissue mass (283.7 ± 33 mg) and suspended in a 1.0 ml constantly perfused microbath at a resting tension of 1-2 grams. Krebs-Ringer solution (NaCl, 117.0 mM; KCl, 4.0 mM; $NaHCO_3$, 25 mM; $MgSO_4 \cdot 7H_2O$, 2.4 mM; NaH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; and dextrose, 11.0 mM) was bubbled with 95% O_2, 5% CO_2 and perfused through the bath at a rate of 1.5 ml/min by a Buchler polystaltic pump. The entire system was maintained at 37° in a Precision Scientific full-view incubator. Following a 30-min equilibration period, four consecutive bolus doses of histamine (180 μg) were administered to the tracheal preparation and the perfusate was collected for 10 min following the initiation of each contraction. A 15-min interval was allowed, following the return to baseline of each contraction, before the next histamine application. The contractile responses were measured with a Grass FT03C force displacement transducer and recorded with a Grass polygraph.

The Krebs superfusates were adjusted to pH 4.5 with 2 M citric acid and extracted with 10 ml of ice cold ethyl acetate [3]. The ethyl acetate layer was removed and evaporated to dryness under vacuum in a Buchler evaporator. Dried extracts were reconstituted in 200 µl of Krebs buffer and assayed on a rat stomach strip previously incubated in a perfused micro-bath with Krebs buffer containing: atropine, 0.1 µg/ml; phenoxybenzamine, 0.1 µg/ml (Smith Kline and French Labs, Philadelphia, PA); methysergide, 0.3 µg/ml (Sandoz Pharmaceuticals, E. Hanover, NJ); mepyramine, 0.1 µg/ml; propranolol, 0.2 µg/ml [4]; indomethacin, 1.0 µg/ml; and procaine, 4.0 µg/ml [5] (Sigma Chemical Co., St. Louis, MO).

A standard curve was prepared for each experiment by the addition of 0.3-3.0 ng of PGE_2 (The Upjohn Co., Kalamazoo, MI) to volumes of Krebs buffer equal to the collected perfusates (15 ml). The standards were extracted and bioassayed by the same procedure as the samples. To estimate recovery, non-extracted standards of PGE_2 were compared to the extracted standards.

The results shown in Figure 1 indicate that the contractile responses to repeated bolus administrations of histamine (180 µg) decline rapidly to 15% of the initial response by the fourth administration. Prostaglandin-like activity, determined from aliquots collected

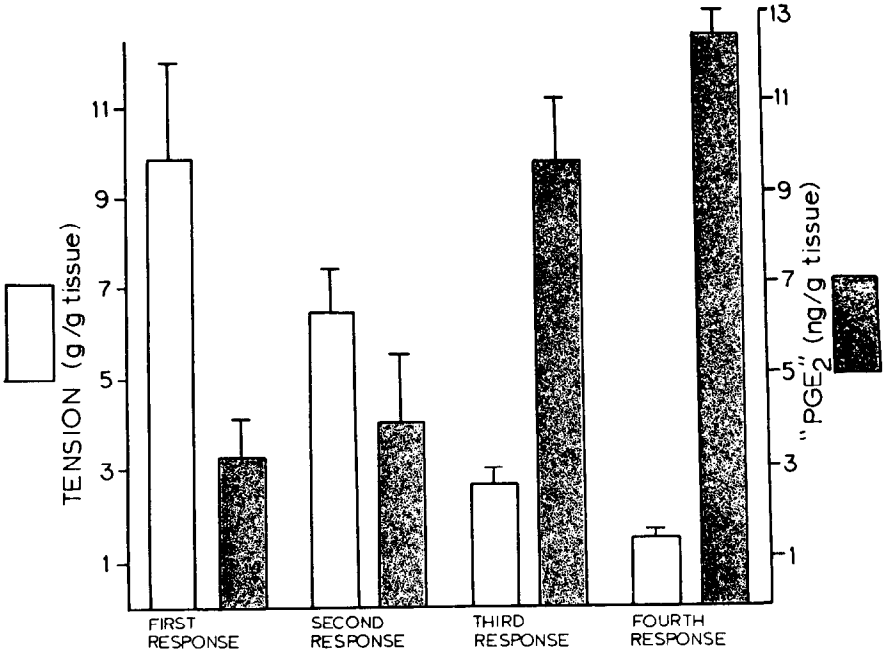

Fig. 1. The development of histamine tachyphylaxis and the production of prostaglandin-like activity ("PGE_2") in canine tracheal smooth muscle. Responses to four consecutive administrations of histamine (180 µg bolus) introduced into the perfusing solution were determined at 15-min intervals. Responses (open bars) are plotted in grams contraction/gram of tissue (wet weight) as means ± S.E.M. (n=3). Production of prostaglandin-like activity (shaded bars) was determined during each of the histamine responses and expressed as "PGE_2" ng/gram tissue (wet weight) produced during the 10-min collection period. Prostaglandin-like activity was bioassayed using PGE_2 standards which were added to equal volumes of Krebs-Ringer solutions and treated as samples. Results are plotted as means ± S.E.M. (n=3).

during each of the contractile responses and expressed in terms of equivalent amounts of PGE_2, increased from 3.29 ± 0.84 ng/g/10 min produced during the first histamine challenge to 12.45 ± 0.61 ng/g/10 min produced during the fourth histamine response. Thus, as contraction decreases to 15% of the control response, production of prostaglandin-like activity increases 280%. There was no detectable prostaglandin-like activity produced during an equivalent collection period prior to the first histamine challenge.

Perfusates from two experiments were pooled, extracted and concentrated as described above, and applied to silica gel thin layer chromatography plates previously activated at 110° for 60 min. The plates were developed to a height of 11 cm in a mixture of chloroform: methanol:acetic acid (16:1:1). Standards (10 µg) of PGA_2, PGE_2, and $PGF_{2\alpha}$ were applied to adjacent lanes. The sample lane was divided into 10 mm sections, each section scraped, dissolved in Krebs-Ringer buffer (200 µl) and assayed on the rat stomach strip. All recoverable biological activity from the sample migrated with the same Rf as the PGE_2 standard. Standards were visualized with iodine vapor.

The addition of a prostaglandin antagonist [6], SC19220 (1-acetyl-2-(8-chloro-10,11-dihydrodibenz[b,f][1,4]oxazepine-10-carbonyl)hydrazine; G.D. Searle and Co., Chicago, IL), to the Krebs-Ringer fluid superfusing the rat stomach strip blocked the response to the crude extracts providing additional evidence that the material was a prostaglandin. Furthermore, treating the tracheal strip with indomethacin (2.8 µM) for one hr following the development of tachyphylaxis eliminated the production of prostaglandin-like material and restored the response of the tracheal strip to histamine (data not shown).

It has been demonstrated [7] that no detectable difference exists between crude ethyl acetate extracts from canine tracheal smooth muscle and extracts where prostaglandins of the E series were separated by thin layer chromatography when these extracts were bioassayed on a rat stomach strip. The major prostaglandin produced by canine tracheal smooth muscle is therefore considered to be PGE_2 and consequently it is PGE_2 which most likely mediates the tachyphylactic response.

We have demonstrated previously [1,2] that the development of histamine tachyphylaxis can be prevented and reversed by a number of prostaglandin synthesis inhibitors and that exogenous addition of PGE_2 (but not $PGF_{2\alpha}$) can mimic the tachyphylactic response suggesting indirectly that the development of tachyphylaxis to histamine was a result of increased prostaglandin production. Here we have demonstrated for the first time that there is a progressive increase in the production of prostaglandin-like material associated with decreasing tracheal smooth muscle contractile responses to repetitive administrations of histamine.

In conclusion, these data support our hypothesis that the development of tachyphylaxis to histamine in canine tracheal smooth muscle is the result of increasing histamine-

stimulated PGE_2 production. This conclusion is consistent with the proposed role of prostaglandins as modulators of airway reactivity [8,9].

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