REFERENCES

Bailey, D. N., Shaw, R. F., Guba, J. J. (1978) J Anal. Toxicol. 2: 233–237

Bailey, D. N., Guba, J. J. (1980) Clin. Chem. 26: 437–440

Burns, R. S., Lerner, S. E. (1978) Clin. Toxicol. 12: 463–481

Cone, E. J., Vaupel, D. B., Yousefnejad, D. (1980) Fed. Proc. Fed. Am. Soc. Exp. Biol. 39: 302.

Cone, E. J., Buchwald, W., Yousefnejad, D. (1981) J. Chromatogr. 223: 331–339

Domino, E. F. (1978) in: Petersen, R. C., Stillman, R. C. (eds) Phencyclidine (PCP) Abuse: An Appraisal, NIDA Research Monograph No. 21. U.S. Government Printing Office, Washington, D.C., pp 18–43.

James, S. H., Scholl, S. H. (1976) Clin. Toxicol. 9: 573–582

Lin, D. C. K., Fentiman, Jr., A. F., Foltz, R. L. (1975) Biomed. Mass Spectrom. 2: 206–214

Litchfield, J. T., Wilcoxon, F. (1949) J. Pharmacol. exp. Ther. 96: 99–113

Martin, B. R., Vincek, W. C., Balster, R. L. (1980) Drug Met. Dispos. 8: 49–54

McCarthy, D., Potter, D. (1963) Pharmacologist 5: 265

Misra, A. L., Pontani, R. B., Bartolomeo, J. (1979) Res. Commun. Chem. Pathol. Pharmacol. 24: 431–445

Sioris, L. J., Krenzelok, E. P. (1978) Am. J. Hosp. Pharm. 35: 1362–1367

Su, T.-P., Cone, E. J., Shannon, H., Vaupel, D. B. (1980) Res. Commun. Sub. Abuse 1: 85–98

Vaupel, D. B., Cone, E. J. (1981) in: Problems of Drug Dependence 1980, NIDA Research Monograph No. 34. U.S. Government Printing Office, Washington, D.C., pp 180–186

Wong, L. K., Bieman, K. (1976) Clin. Toxicol. 9: 583–591

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Histamine tachyphylaxis in canine isolated airways: role of endogenous prostaglandins

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The basal tone of respiratory smooth muscle in vitro is related to the endogenous production of small amounts of prostaglandins (Coburn et al 1974; Orehek et al 1975; Duncan et al 1980). Agonist contraction or mechanical stimulation enhanced release of these mediators (Orehek et al 1973; Gryglewski et al 1976). On the basis of these data it has been proposed that the locally released prostaglandins are important in the maintenance of resting tone and antagonize the response of respiratory smooth muscle during contraction.

Recently Anderson et al (1979) proposed another hypothesis for the role of prostaglandins in airway muscle namely, receptor desensitization. These authors reported that canine isolated tracheal muscle when contracted with histamine became refractory and this tachyphylaxis could be reversed by exposure to the anti-inflammatory agent indomethacin. While many reports have related increased prostaglandin production to the desensitization of β-adrenoceptors (Gryglewski & Ocetkiewicz 1974; Douglas et al 1977) few have suggested their role as mediators in histamine receptor desensitization (Krzanowski et al 1980). We have determined whether histamine was tachyphylactic in canine bronchi and if the tachyphylaxis was associated with altered prostaglandin synthesis.

Methods

Animals and tissues. Male mongrel dogs 13–23 kg, were anaesthetized with intravenous sodium pentobarbitone (30 mg kg^{-1} i.v.). The chest cavity was opened and a lung lobe was removed and immersed in cold Tyrode solution of composition mM: NaCl, 139·2; KCl, 2·7; $CaCl_2$, 1·8; $MgCl_2$, 0·49; $NaHCO_3$, 11·9; NaH_2PO_4, 0·4 and glucose, 5·5; pH 7·4.

Bronchi from the 5th generation were dissected out, spirally cut and equilibrated in Tyrode solution in 10 ml organ baths under an initial load of 5 g. In four experiments, lung parenchymal strips were also cut from the lung lobes and placed in similar tissue baths under a 1 g load. All preparations were allowed to equilibrate for 90 min in Tyrode solution at 37 °C gassed with 5% CO_2 in O_2. Force was measured isometrically with Statham strain gauges (Model UC3) and was displayed on Honeywell two-channel pen recorders (Electronik 19). After each experiment the tissues were dried (65 °C) for 12 h and weighed. The change in basal tone of each preparation was determined from the records by calculating the resting tone (g mg^{-1} tissue dry weight) at the beginning and end of the equilibration period (equilibration ratio) as well as before and after an incubation with indomethacin (incubation ratio).

Concentration-effect curves to acetylcholine. Concentration-effect curves were produced by adding acetylcholine (1 to 200 μM) in a volume <0·5 ml, in random order, to the tissue bath. When the response to the agonist reached a plateau, the bath fluid was exchanged for fresh Tyrode solution and the preparation was allowed to return passively to its resting tone. Contractions produced by

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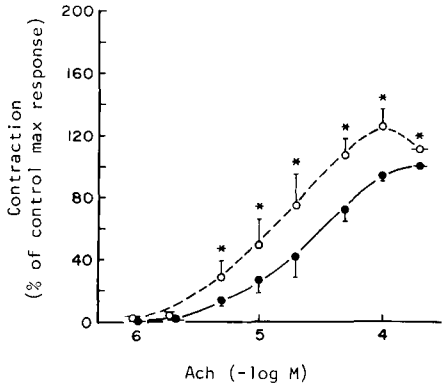

FIG. 1. Concentration-effect curves to acetylcholine in canine bronchial preparations from adult male dogs before (●) and after (○) a 30 min incubation with indomethacin (17 μM). Values are expressed as a percent of maximal response to acetylcholine (50 μM) before indomethacin treatment and are the mean ± s.e.m. of 8 experiments. * denotes values significantly different from initial response ($P < 0.01$).

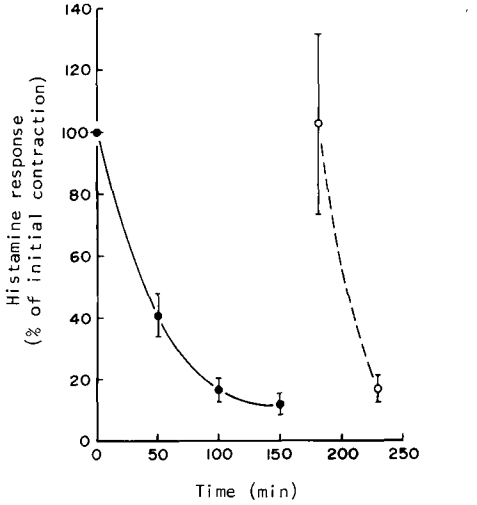

FIG. 2. Effects of histamine in canine bronchial preparations from adult male dogs before (●) and after (○) a 30 min incubation with indomethacin (17 μM). Preparations were contracted at 50 min intervals with histamine (50 μM). Values are expressed as a percent of initial response to histamine. The data are the mean ± s.e.m. of 8 experiments.

acetylcholine were expressed as a percentage of the maximal force per mg tissue dry weight (g mg^{-1} dry wt). Once concentration-effect curves were established, preparations were incubated with indomethacin (17 μM; final bath concentration) for 30 min and, following washout, concentration-effect curves were repeated.

Tachyphylaxis to histamine. The reproducibility of responses to histamine in bronchial and parenchymal preparations was examined by repeatedly adding the same concentration of histamine (10 to 50 μM) to the bath at 50 min intervals. Once the response reached a plateau, the bath fluid was exchanged for fresh Tyrode. Preparations were then washed at 15 min intervals until the next concentration of histamine was addded to the bath.

Tachyphylaxis and prostaglandins. Preparations were contracted on four occasions 50 min apart with histamine (50 μM). These tissues were then incubated with indomethacin (17 μM) for 30 min and following washout, histamine (50 μM) was again added on two occasions 50 min apart. In an alternative protocol, bronchial spirals were bathed continuously with indomethacin (1·7 μM) and histamine was added to the bath with a 50 min interval between challenges.

Results

During the equilibration period bronchial spirals significantly relaxed but the resting tone of parenchymal preparations remained essentially unchanged. The equilibration ratios for bronchial and parenchymal preparations were 0·57 ± 0·04 (n = 8) and 0·93 ± 0·02 (n = 5), respectively. Indomethacin (17 μM for 30 min) did not alter the basal tone in either preparation either before or after exposure to histamine or acetylcholine. The incubation ratio was 0·96 ± 0·06 (n = 8).

Concentration effect-curves to acetylcholine. Bronchial preparations contracted to acetylcholine in a concentration-dependent manner (Fig. 1). The force induced with a maximally-effective concentration of agonist was 5·25 ± 0·85 g. When normalized for tissue dry weight, the efficacy of acetylcholine was 0·09 ± 0·02.

Tachyphylaxis to histamine. Tissues contracted to histamine but less vigorously than to acetylcholine. In eight preparations the mean force developed to the initial histamine concentration (50 μM) was 1·28 ± 0·28. The normalized efficacy of histamine was 0·03 ± 0·01 g mg^{-1} tissue dry weight. When the same concentration of histamine (50 μM) was added to the organ bath repeatedly, the response decreased (Fig. 2). Frequent washing of the tissues did not reverse the tachyphylaxis. The tachyphylaxis was specific for the histamine receptor since preparations refractory to histamine were also refractory to the H_1 agonist, 2-(2-pyridyl) ethylamine (50 μM; n = 2) and vice versa. The force developed to an initial exposure of 2-(2-pyridyl) ethylamine (50 μM) was 0·69 g. Preparations in which acetylcholine concentration-effect curves were produced responded to histamine but the latter response was always tachyphylactic.

Tachyphylaxis and prostaglandins. Neither an incubation for 30 min with indomethacin (17 μM; n = 2) nor continuous exposure of the tissue to indomethacin (1·7 μM; n = 2) prevented the loss of response to histamine (Fig. 2). Responses to acetylcholine were enhanced after indomethacin treatment (Fig. 1).

Discussion

Respiratory tissues from dogs produce measurable amounts of prostaglandins and there are quantitative and qualitative changes in production when these tissues are contracted

(Yamaguchi et al 1976). The products of arachidonic acid metabolism have been shown to affect basal tone and response to contractile agonists in airway muscle preparations from female guinea-pigs (Orehek et al 1975; Duncan et al 1980). The inhibition of endogenous prostaglandins does not alter the basal tone of dog respiratory tissue (Coburn et al 1974; Yamaguchi et al 1976; this report). However, inhibition of prostaglandin synthetase with indomethacin significantly affects responses of these tissues to acetycholine and these results are consistent with results of experiments where airway preparations from guinea-pigs were used (Farmer et al 1974; Brink et al 1980). These data suggest that endogenous prostaglandins do not contribute to inherent tone in canine bronchial smooth muscle. Enhanced contractility after indomethacin treatment suggests that either cyclooxygenase products are released which modulate contractile responses (Orehek et al 1973, 1975) or lipoxygenase products, formed in greater quantity by the diversion of arachidonic acid after cyclooxygenase inhibition augment tissue contractility (Adcock & Garland 1980). However, results from guinea-pig airways contracted with histamine do not support the latter hypothesis (Brink et al 1981). Significantly, responses to acetylcholine were always reproducible although prostaglandins have been shown to be released (Yamaguchi et al 1976). In contrast, responses to histamine in dog bronchi were tachyphylactic. This tachyphylaxis was specifically associated with H_1 receptors since responses to 2-(2-pyridyl) ethylamine were also tachyphylactic; these tissues were also tachyphylactic to histamine. Anderson et al (1979) showed a similar phenomenon in dog tracheal preparations. These authors proposed that the tachyphylaxis to histamine was due to the production of a cyclooxygenase product. If prostaglandins are responsible for histamine desensitization then histamine concentration-effect curves after an incubation with indomethacin or in the presence of the anti-inflammatory agent should be reproducible. In addition, any stimulus which releases the cyclooxygenase products should desensitize the histamine receptor. Our data show that (a) histamine tachyphylaxis was present after an incubation or continuous exposure to indomethacin and that (b) prior exposure to other agonists does not reduce histamine responses (Krzanowski et al 1980). Since other contractile agonists release prostaglandins, it is difficult to understand why only those prostaglandins released after a histamine-induced contraction would lead to a desensitization of the H_1 receptor. While Anderson et al (1979) and Krzanowski et al (1980) demonstrated a similar pattern of desensitization to a high concentration of histamine, they failed to show the reproducibility of the histamine contractions after the anti-inflammatory drug treatment. Our results in dog bronchial muscle show that after inhibition of cyclooxygenase, tissue responses to histamine were on the average restored (note large s.e.m.) but once again became tachyphylactic. The presence of histamine tachyphylaxis in dog tracheal and bronchial preparations implies that histamine concentration-effect curves yield inaccurate estimates of drug potency and efficacy (Antonissen et al 1980; Chand & Eyre 1980). Interestingly, tachyphylaxis to histamine was not demonstrable in lung parenchymal strips. However, the complex composition of these preparations makes this phenomenon difficult to interpret.

Our results with dog bronchial tissues show that histamine tachyphylaxis is not reversed by inhibition of endogenous prostaglandin production, thus desensitization is due to another mechanism.

REFERENCES

Adcock, J. J., Garland, L. G. (1980) Br. J. Pharmacol. 69: 167–169

Anderson, W. H., Krzanowski, J. J., Polson, J. B., Szentivanyi, A. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 117–125

Antonissen, L. A., Mitchell, R. W., Kroeger, E. A., Kepron, W., Stephens, N. L., Bergen, J. (1980) J. Pharmacol. Exp. Ther. 213: 150–155

Brink, C., Duncan, P. G., Midzenski, M., Douglas, J. S. (1980) J. Pharmacol. Exp. Ther. 215, 426–433

Brink, C., Duncan, P. G., Douglas, J. S. (1981) Ibid. 217, 592–601

Chand, N., Eyre, P. (1980) Res. Commun. Chem. Pathol. Pharmacol. 28: 245–254

Coburn, R. F., Hitzig, B., Yamaguchi, T. (1974) Fed. Proc. Fed. Am. Soc. Exp. Biol. 33: 451P

Douglas, J. S., Lewis, A. J., Ridgway, P., Brink, C., Bouhuys, A. (1977) Eur. J. Pharmacol. 42: 195–205

Duncan, P. G., Brink, C., Adolphson, R. L., Douglas, J. S. (1980) J. Pharmacol. Exp. Ther. 215: 434–442

Farmer, J. B., Farrar, D. G., Wilson, J. (1974) Br. J. Pharmacol. 44: 328–330

Gryglewski, R. J., Ocetkiewicz, A. (1974) Prostaglandins 8: 31–42

Gryglewski, R. J., Dembinska-Kiec, A., Grodzinska, L., Panczenko, B. (1976) in: Lung Cells and Disease, edited by A. Bouhuys. Amsterdam: Elsevier/North Holland, 289–308

Krzanowski, J. J., Anderson, W. H., Polson, J. B., Szentivanyi, A. (1980) Arch. Int. Pharmacodyn. 247: 155–162

Orehek, J., Douglas, J. S., Lewis, A. J., Bouhuys, A. (1973) Nature New Biol. 245: 84–85

Orehek, J., Douglas, J. S., Bouhuys, A. (1975) J. Pharmacol. Exp. Ther. 194: 554–564

Yamaguchi, T., Hitzig, B., Coburn, R. F. (1976) Am. J. Physiol. 230: 1737–1743