Br. J. Pharmac. (1979), **66**, 331–336

RELAXANT RESPONSE OF GOAT TRACHEA TO 5-HYDROXYTRYPTAMINE MEDIATED BY D-TRYPTAMINE RECEPTORS

N. CHAND, L. DeROTH & P. EYRE*

Département d'anatomie et physiologie animales, Faculté de Médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec, Canada J2S 7C6, and Department of Biomedical Sciences,* University of Guelph, Guelph, Ontario N1G 2W1, Canada

1 Goat isolated trachea contracted in response to carbachol, histamine and 2-pyridylethylamine (an H_1-receptor agonist) and relaxed after application of isoprenaline, 5-hydroxytryptamine (5-HT) and phenylephrine.

2 Mepyramine, a selective H_1-receptor antagonist, blocked histamine- and 2-pyridylethylamine-induced contractions. In high doses it also exhibited some nonspecific antagonism to carbachol. After H_1-receptor blockade, 4-methylhistamine and dimaprit (specific H_2-agonists) relaxed the carbachol-contracted trachea.

3 Propranolol, a β--adrenoceptor blocker, antagonized relaxation in response to isoprenaline and phenylephrine. In high doses, it produced a reversal of the phenylephrine response.

4 Indomethacin enhanced contractions in response to carbachol and histamine.

5 Relaxation to 5-HT was not affected by propranolol, indomethacin, metiamide or cimetidine (H_2-blockers). These findings appear to exclude the involvement of adrenergic, prostaglandinergic and H_2-histaminergic mechanisms in the mediation of this response.

6 Atropine potentiated 5-HT-induced relaxations. This suggests the participation of a 'masked' excitatory cholinergic mechanism.

7 Methysergide, dibenamine and dibenzyline selectively antagonized or reversed 5-HT-induced relaxation. Dibenamine and dibenzyline enhanced relaxations to isoprenaline.

8 This investigation showed (i) a relaxant response of goat trachea to 5-HT, mediated via D-muscular tryptamine receptors; (ii) a small population of excitatory M-neuronal tryptamine and α-adrenoceptors; and (iii) predominance of H_1-histamine receptors in the goat trachea.

Introduction

5-Hydroxytryptamine (serotonin: 5-HT) has been found to contract the tracheobronchial smooth muscles of cat, dog, horse, sheep and calf (Brocklehurst, 1958; Offermeier & Ariens, 1966; Eyre, 1969; Statkov, 1969; Aitken & Sanford, 1970; Chand & Eyre, 1977; 1978). This biogenic amine is inactive on the trachea and bronchus of rat, rabbit, pig and neonatal piglets (Chand & Eyre, 1977; Chand & DeRoth, 1978a, b). Surprisingly, 5-HT has also been reported to antagonize acetylcholine-induced contractions on rhesus monkey bronchioles, and to relax human bronchioles (Brocklehurst, 1958), and bronchus (Mathe, Aström & Persson, 1971) and sheep pulmonary vein (Eyre, 1975).

5-HT is considered to act on at least two distinct types of receptors, namely M-neuronal and D-muscular tryptamine receptors. The former type of 5-HT receptors are antagonized by morphine, cocaine and atropine, whereas D-tryptamine receptors are blocked by dibenamine, dibenzyline, methysergide, lysergic acid diethylamide and 2-bromolysergic acid diethylamine (Gaddum & Picarelli, 1957).

There has been some interest in the autonomic and autacoid pharmacology of airways of herbivores (Eyre, 1969; 1973; Aitkin & Sanford, 1970; Chand & Eyre, 1977; 1978). During the course of a similar investigation, 5-HT was found to relax goat trachea. The observation was considered interesting. This report describes the pharmacology of this and other drug responses in the goat trachea.

Methods

Fifteen adult goats of either sex weighing 14 to 35 kg were killed by a rapid intravenous injection of pentobarbitone sodium (40 mg kg) followed by exsanguination. The trachea was immediately dissected out in

0007-1188/79/060331-06 $01.00

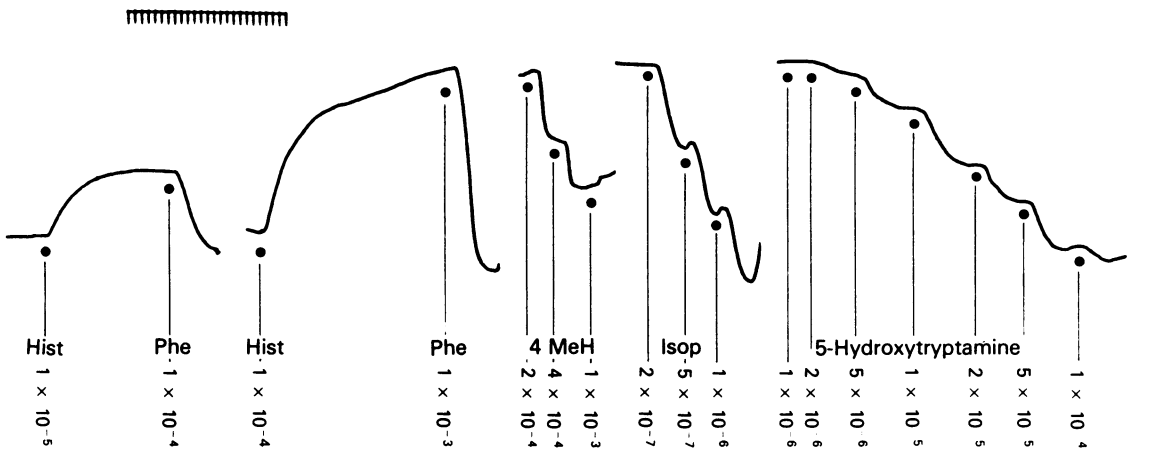

Figure 1 Isolated tracheal muscle of goat in Krebs solution mixed with 5% CO_2 in O_2, at 37°C. Resting tension = 3 g. Histamine- (Hist) contracted trachea relaxed to phenylephrine (Phe). Relaxant responses to 4-methylhistamine (4MeH), isoprenaline (Isop) and 5-hydroxytryptamine were recorded from the carbachol (5×10^{-7} M) contracted muscle (50 ± 10% max). Drug doses are expressed as final molar bath concentrations. Time marker indicates min.

ice-cold, oxygenated Krebs solution. The remainder of the experimental procedures were identical to those described earlier (Chand & Eyre, 1978; Eyre, 1969). A thoracic segment of the trachea was cut into individual cartilaginous rings from which the tracheal muscle was dissected out. From each animal, 6 to 12 pairs of tracheal muscle preparations were used within 6 to 24 h. Each pair of tracheal muscle preparations was mounted in similar 10 or 20 ml isolated tissue baths containing Krebs–Henseleit solution, bubbled with 5% CO_2 in O_2, maintained at 37°C. The strips were allowed to equilibrate for at least 1 h under a resting tension of 3 g. Single or cumulative dose-response curves to one to two agonists were recorded with a Narco Isotonic Myograph Transducer connected to a Narco Physiograph. Both strips of each pair normally produced approximately equal responses to the agonists; any pair which exhibited marked differences in the responsiveness to agonists was discarded.

After establishing dose-response curves to agonists on both strips of each pair, a predetermined concentration of an antagonist was added to one of the tissues. After 30 min, dose-response curves were re-established. The second strip of each pair served as a control to monitor any time-related change in the responsiveness of the tissues to the agonists. The effectiveness and specificity of the antagonist were determined by measuring the dose-ratio, i.e. the ratio of equiactive doses of agonist in the presence and absence of antagonist (Gaddum, Hameed, Hathway & Stephens, 1955).

The following drugs were used in this study; histamine diphosphate, isoprenaline bitartrate, 5-hydroxytryptamine creatinine sulphate, carbamylcholine chloride (carbachol), atropine sulphate (Sigma Chemical Co., St. Louis, Mo); morphine sulphate (Allen & Hanburys, Toronto, Ontario); dimaprit, 2-pyridylethylamine (2-PE), 4-methylhistamine (4-MeH), dibenzyline, metiamide and cimetidine (S.K.F.); mepyramine maleate (Poulenc Ltd, Montreal, Quebec); methysergide bimaleate (Sandoz Canada Ltd, Quebec); propranolol hydrochloride (Ayerst Laboratories Inc., New York) and indomethacin (Merck Frost Laboratories, Montreal, Quebec).

Drug doses are expressed as final molar (M) bath concentration.

Results

Goat trachea contracted to carbachol (10^{-8} to 10^{-5} M), histamine (10^{-6} to 10^{-3} M) and 2-pyridylethylamine (10^{-5} to 10^{-3} M) in a dose-related manner.

Tracheae that were partially (50 ± 10% max) contracted to carbachol (2×10^{-7} to 5×10^{-7} M) or histamine (10^{-5} to 10^{-4} M), relaxed to isoprenaline (10^{-7} to 10^{-4} M), 5-HT (10^{-6} to 5×10^{-4} M) and phenylephrine (10^{-5} to 10^{-3} M) in a dose-dependent fashion (Figures 1 and 2). The ED_{50} values of isoprenaline and 5-HT were 7×10^{-7} M and 8×10^{-6} M respectively. Thus, 5-HT was about an 11 times less potent relaxant of goat trachea than isoprenaline (Figure 2). Dimaprit and 4-methylhistamine (10^{-5} to

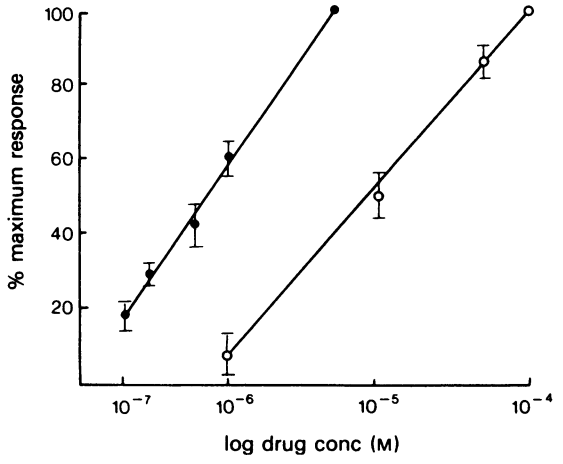

Figure 2 Dose-response curves for the relaxant effects of isoprenaline (●) and 5-hydroxytryptamine (○) on the goat trachea partially contracted to carbachol (50 ± 10% max). Each point represents the mean value from trachea of 9 to 11 animals; vertical lines show s.e. mean.

10^{-3} M) produced variable effects: weak relaxation ($n = 5$), weak contraction ($n = 4$) or relaxation followed by contraction ($n = 5$).

Mepyramine (10^{-6} M) antagonized contractions to histamine (dose-ratio = 100 ± 11, mean ± s.e. mean, $n = 7$) and 2-PE without modifying contractions to carbachol. Higher doses (10^{-5} M) strongly inhibited histamine (dose-ratio = 200 ± 35, $n = 5$) but also exhibited weak antagonism of carbachol (dose-ratio = 5 ± 2, $n = 5$). In the presence of mepyramine (10^{-5} M), 4-MeH and dimaprit (10^{-5} to 10^{-3} M) produced relaxations of varying magnitude on the carbachol-contracted trachea.

The antagonistic activity of several potential antagonists (expressed as dose-ratios) towards relaxant responses to 5-HT and isoprenaline has been summarized in Table 1 and Figures 3 and 4.

Atropine (10^{-7} M) strongly antagonized carbachol (dose-ratio = 250 ± 11) and weakly enhanced relaxations to 5-HT (Table 1 and Figure 3a).

Morphine (10^{-6} to 10^{-5} M) did not affect contractions to carbachol and relaxation to isoprenaline, but at a high dose (10^{-5} M), exhibited a small potentiation of 5-HT-induced relaxation (Table 1).

Table 1 Dose-ratios of 5-hydroxytryptamine (5-HT) and isoprenaline on goat trachea, in the presence of antagonists

Antagonist	*Bath conc.* (M)	*Dose-ratios (mean + s.e. mean)* 5-HT	*Isoprenaline*
Atropine	10^{-7}	0.65 ± 0.15* (5)	1 (5)
	10^{-6}	0.67 ± 0.2* (4)	1 (4)
Morphine	10^{-6}	1 (3)	1 (3)
	10^{-5}	0.9 ± 0.16 (4)	1 (4)
Metiamide	5×10^{-6}	1 (3)	1 (3)
	5×10^{-5}	1 (3)	1 (3)
Cimetidine	5×10^{-6}	1 (3)	1 (3)
	5×10^{-5}	1 (3)	1 (3)
Propranolol	5×10^{-7}	1 (5)	8.25 ± 1.18* (5)
	1×10^{-6}	1 (5)	16 ± 5* (5) XA
	1×10^{-5}	1 (5)	63 ± 14* (5)
Indomethacin	5×10^{-6}	1.12 ± 0.12 (4)	1 (4)
	5×10^{-5}	1.35 ± 0.25 (5)	1 (4)
Dibenamine	5×10^{-6}	8.75 ± 2.5* (5)	0.75 ± 0.12 (4)
	2×10^{-5}	R(2); 33 ± 6* (5)	0.6* ± 0.1 (5)
Dibenzyline	5×10^{-6}	5.75 ± 1.25* (5)	0.90 ± 0.05 (5)
	2×10^{-5}	R(3); 25 ± 3* (3)	*0.65 ± 0.12 (5)
Methysergide	1×10^{-6}	24 ± 5* (9)	1 (9)
	5×10^{-6}	57 ± 11* (5)	1 (5)
	1×10^{-5}	R(2); 87 ± 23* (3)	1 (5)
	2×10^{-5}	Irreversible (3); R(3); 120 ± 25 (3)	1 (9)

R = Reversal to contraction.
* $P < 0.05$.

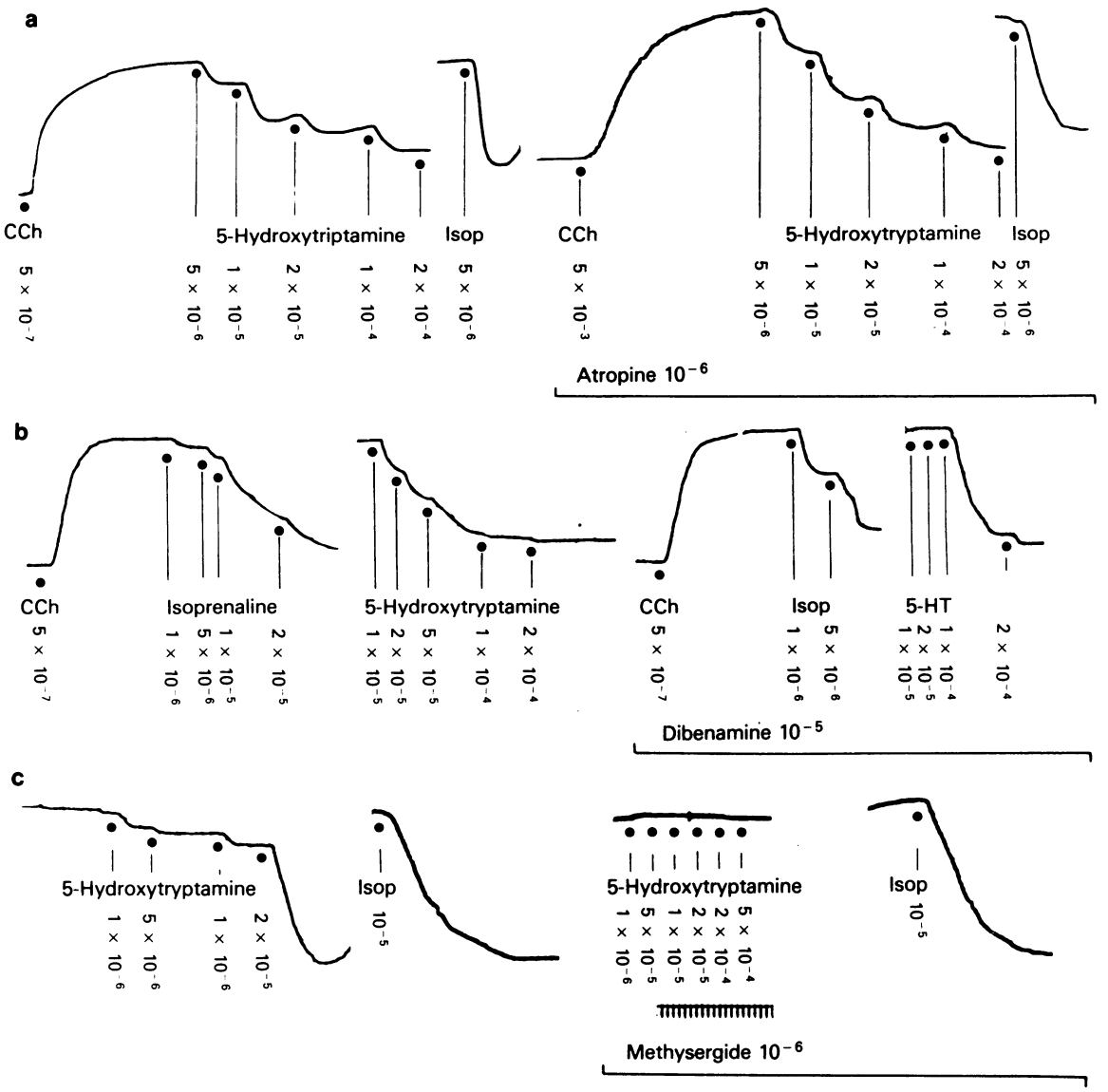

Figure 3 Isolated tracheal muscle of goat in Krebs solution mixed with 5% CO_2 in O_2, at 37°C. Resting tension = 3 g. Relaxant responses to isoprenaline (Isop) and 5-hydroxytryptamine (5-HT) recorded from partial contractions to carbachol (50 ± 10% max), in the presence of indomethacin (5×10^{-6} M) (a, b, c). (a) Atropine antagonized carbachol (CCh) and potentiated 5-HT-induced relaxation without modifying relaxation to isoprenaline. (b) Dibenamine antagonized relaxation to 5-HT and potentiated relaxations to isoprenaline. (c) Methysergide inhibited 5-HT-induced relaxation without altering relaxation to isoprenaline. Drug doses are expressed as final molar bath concentrations. Time marker indicates intervals in min.

Metiamide and cimetidine (5×10^{-6} to 5×10^{-5} M) produced a slight potentiation of histamine-induced contraction (dose-ratio = 0.95 ± 0.18, $n = 7$) without affecting contractions to carbachol and relaxations to isoprenaline or 5-HT (Table 1).

Propranolol (5×10^{-7} to 10^{-5} M) antagonized relaxations to isoprenaline and phenylephrine without altering 5-HT responses (Table 1). In the presence of propranolol (10^{-5} M), phenylephrine (5×10^{-3} to 10^{-2} M) produced threshold contractions (4/7).

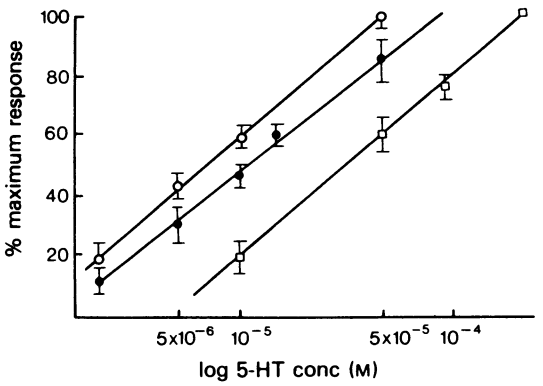

Figure 4 Dose-response curves to 5-hydroxytryptamine (5-HT) alone (●); in the presence of atropine, 10^{-6} M (○); in the presence of atropine (10^{-6} M) + methysergide (10^{-6} M) (□). Atropine produced a parallel shift of the 5-HT dose-response curve to the left, showing potentiation of relaxant response to 5-HT. Addition of methysergide (10^{-6} M) to the tissue bath again shifted the 5-HT dose-response curve to the right, showing competitive antagonism. Each point represents the mean values of preparations from seven to nine animals; vertical lines show s.e. mean.

Indomethacin (1×10^{-6} to 5×10^{-6} M) enhanced (25 to 75%) contractions to histamine and carbachol but did not modify relaxations to 5-HT or isoprenaline (Table 1, Figure 3a, b, c).

Dibenamine and dibenzyline both antagonized or reversed 5-HT-induced relaxation; and also enhanced relaxations to isoprenaline (Table 1, Figure 2b).

Methysergide (10^{-6} to 2×10^{-5} M) strongly and selectively antagonized or reversed relaxations to 5-HT without affecting isoprenaline responses (Table 1, Figure 3c).

After D-receptor blockade by dibenamine, dibenzyline or methysergide, the weak contractions to 5-HT on some strips (11/27) were subsequently antagonized by atropine (10^{-7} M).

Discussion

It is well known that 5-HT produces highly variable effects on mammalian airway smooth muscle (Brocklehurst, 1958). It contracts tracheobronchial smooth muscle of horse, sheep and cattle (Offermeier & Ariens, 1966; Eyre, 1969; Chand & Eyre, 1977, 1978) and relaxes bronchioles and bronchus of man (Brocklehurst, 1958; Mathe *et al.*, 1971) and goat trachea (this study).

Indomethacin, a potent prostaglandin synthetase inhibitor (Vane, 1971) and propranolol (β-adrenoceptor antagonist) did not alter 5-HT-induced relaxations of the goat trachea. Therefore, the possible release of catecholamines (Fozard & Mwaluko, 1976) and of prostaglandin-like substances (Alabaster & Bakhle, 1970) by 5-HT seems to play little or no role in the mediation of 5-HT-induced relaxation of goat trachea.

Methysergide, dibenamine and dibenzyline, well-known D-muscular tryptamine-receptor antagonists (Gaddum & Picarelli, 1957), strongly and selectively antagonized or reversed relaxations to 5-HT of the goat trachea. Earlier, methysergide has been reported to antagonize 5-HT-induced relaxations and contractions of the human bronchus (Mathe *et al.*, 1971).

Atropine enhanced relaxations to 5-HT of the goat trachea. This finding seemed to suggest the involvement of a 'masked' excitatory cholinergic mechanism in this tissue. The excitatory cholinergic mechanism is known to operate through 'M'-neuronal tryptamine receptors in guinea-pig ileum (Brownlee & Johnson, 1965); calf trachea (Offermeier & Ariens, 1966) and tracheobronchial tree of sheep (Eyre, 1969).

As opposed to the goat trachea (this study), several well-known D- and M-tryptamine receptor antagonists have been reported to be ineffective in antagonizing 5-HT-induced contractions of cat trachea (Statkov, 1969) and relaxations of sheep pulmonary vein (Eyre, 1975).

Dibenamine and dibenzyline (α-adrenoceptor antagonists) were found to enhance relaxation to isoprenaline of the goat trachea. This finding coupled with the phenylephrine-induced contractions after β-blockade, appears to suggest the occurrence of some α-adrenoceptors in the goat trachea. Similarly, a scanty population of excitatory α-adrenoceptors is known to exist in the airway smooth muscles of several species (Fleisch, Maling & Brodie, 1970; Persson & Johnson, 1970; Mathe *et al.*, 1971; Chand & DeRoth, 1978b, c).

Goat tracheal contractions to histamine and 2-pyridylethylamine (a selective H_1-agonist) (Durant, Ganellin & Parsons, 1975) were antagonized by mepyramine (a selective H_1-receptor antagonist). Similarly, H_1-histamine receptor mediated contractions of trachea of horse, sheep and pig have been described earlier (Eyre, 1969; Chand & Eyre, 1977, 1978; Chand & DeRoth, 1978a, b, c).

After H_1-receptor blockade, 4-methylhistamine and dimaprit (relatively selective H_2-agonists) (Chand & Eyre, 1975; Parsons, Owen, Ganellin & Durant, 1977) relaxed goat trachea. Cimetidine and metiamide (H_2-antagonists) (Chand & Eyre, 1975) weakly enhanced histamine-induced contractions. These observations seem to suggest a preponderance of H_1-excitatory and a scanty population of 'inhibitory' H_2-receptors in this tissue. The occurrence of H_2-inhibitory receptors has already been demonstrated in the airway smooth

muscles of several species (Eyre, 1973; Chand & Eyre, 1977; 1978; Chand & DeRoth, 1978a, b, c).

The authors thank the following companies for the generous gifts of drugs: Poulenc Ltd., Montreal, Canada for mepyramine maleate; Smith, Kline and French Canada Ltd., Montreal, Quebec for dibenzyline, 4-methylhistamine, 2-pyridylethylamine, dimaprit, cimetidine and metiamide; Merck Laboratories, Quebec for indomethacin; Ayerst Laboratories, N.Y., for propranolol; Sandoz (Canada) Ltd., Quebec, for methysergide bimaleate.

The study was supported in part by the Natural Sciences and Engineering Research Council of Canada, Grant A5937 and by the Ontario Ministry of Agriculture and Food.

References

AITKEN, M.M. & SANFORD, J. (1970). The effect of drugs on bovine and pulmonary vascular tissue. *Br. J. Pharmac.*, **38**, 443P.

ALABASTER, V.A. & BAKHLE, Y.S. (1970). The release of biologically active substances from isolated lungs by 5-hydroxytryptamine. *Br. J. Pharmac.*, **40**, 582P.

BROCKLEHURST, W. (1958). The action of 5-hydroxytryptamine on smooth muscle. In *5-Hydroxytryptamine*. ed. Lewis, G.P., pp. 172–176. London: Pergamon.

BROWNLEE, G. & JOHNSON, E.S. (1965). The release of acetylcholine from the isolated ileum of the guinea-pig induced by 5-hydroxytryptamine and dimethylphenylpiperazinium. *Br. J. Pharmac.*, **29**, 689–700.

CHAND, N. & DEROTH, L. (1978a). Actions of histamine and other substances on the airway smooth muscle of swine (*in vitro*). *Vet. Sci. Commun.* (in press).

CHAND, N. & DEROTH, L. (1978b). *In vitro* comparison of drug responses on swine and rabbit airways. *Physiologist*, **21**, 19.

CHAND, N. & DEROTH, L. (1978c). Occurrence of H_2-inhibitory receptors and α-excitatory adrenoceptors in guinea-pig lung. *Proc. XI Annual Conf. Indian Pharmac. Soc.* (in press).

CHAND, N. & EYRE, P. (1975). Classification and biological distribution of histamine receptor sub-types. *Agents & Actions*, **5**, 277–295.

CHAND, N. & EYRE, P. (1977). Spasmolytic actions of histamine in airway smooth muscle: An atypical H-receptor. *Fedn Proc. Fed. Am.* **36**, 1022.

CHAND, N. & EYRE, P. (1978). Spasmolytic action of histamine in airway smooth muscle of horse. *Agents & Actions*, **8**, 191–198.

DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1975). Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. med. Chem.*, **18**, 905–909.

EYRE, P. (1969). The pharmacology of sheep tracheobronchial muscle: a relaxant effect of histamine on the isolated bronchi. *Br. J. Pharmac.*, **36**, 409–417.

EYRE, P. (1973). Histamine H_2-receptors in the sheep bronchus and cat trachea. The action of burimamide. *Br. J. Pharmac.*, **48**, 321–323.

EYRE, P. (1975). Atypical tryptamine receptors in sheep pulmonary vein. *Br. J. Pharmac.*, **55**, 329–333.

FLEISCH, J.H., MALING, H.M. & BRODIE, B.B. (1970). Evidence for existence of α-adrenergic receptors in the mammalion trachea. *Am. J. Physiol.*, **218**, 596–599.

FOZARD, J.R. & MWALUKO, G.M.P. (1976). Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. *Br. J. Pharmac.*, **57**, 115–125.

GADDUM, J.H., HAMEED, K.A., HATHWAY, D.E. & STEPHENS, F.F. (1955). Quantitative studies of antagonists for 5-hydroxytryptamine. *Q. J. exp. Physiol.*, **40**, 49–74.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptors. *Br. J. Pharmac. Chemother.*, **12**, 323–328.

MATHE, A.A., ASTRÖM, A. & PERSSON, N.A. (1971). Some broncho-constricting and broncho-dilating responses of human isolated bronchi: evidence for the existence of α-adrenoceptors. *J. Pharmac. exp. Ther.*, **23**, 905–910.

OFFERMEIER, J. & ARIENS, E.J. (1966). Serotonin—I. Receptors involved in its action. *Archs int. Pharmacodyn.*, **164**, 192–215.

PARSONS, M.E., OWEN, D.A.A., GANELLIN, C.R. & DURANT, G.J. (1977). Dimaprit-[S-[3-(N,N-dimethylamino)propyl]isothiourea]. A highly specific histamine H_2-receptor agonist—Part I. Pharmacology. *Agents & Actions*, **7**, 31–37.

PERSSON, H. & JOHNSON, B. (1970). Adrenergic receptors in the guinea-pig trachea and lung. *Acta Pharmac. tox.*, **28**, 49–56.

STATKOV, P. R. (1969). Lack of evidence for M- and D-serotonin receptors in the cat's isolated tracheal smooth muscle. *Pharmacology*, **2**, 176–180.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232–235.

(Received October 9, 1978. Revised November 30, 1978.)