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# 5-HYDROXYTRYPTAMINE INDUCES RELAXATION OF GOAT PULMONARY VEINS: EVIDENCE FOR THE NONINVOLVEMENT OF M AND D-TRYPTAMINE RECEPTORS

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**1** Reactivity of goat isolated pulmonary arteries and veins to a variety of vasoactive agents was investigated.

**2** Bradykinin (Bk), acetylcholine (ACh), noradrenaline (NA), prostaglandin $A_1$ ($PGA_1$), $PGE_2$, $PGF_{2\alpha}$ and histamine induced dose-dependent contractile responses on veins; 5-hydroxytryptamine (5-HT) induced veno-relaxation. Angiotensin was virtually inactive.

**3** Angiotensin, NA, Bk, histamine and 5-HT produced concentration-dependent contractile responses on the arterial strips; these arteries failed to respond to ACh, $PGA_1$, $PGE_2$ or $PGF_{2\alpha}$.

**4** The pulmonary veno-relaxant response to 5-HT was found to be resistant to propranolol, indomethacin, metiamide, cimetidine, methysergide, atropine and morphine. These findings appear to exclude the involvement of adrenergic mechanisms and prostaglandin generation as well as activation of classical M and D-tryptamine receptors by 5-HT in the goat pulmonary veins. This response may be mediated via a presynaptic inhibitory 5-HT receptor.

**5** 5-HT-induced arterial contractile responses were antagonized by methysergide, showing the occurrence of classical D receptors in the goat pulmonary artery.

**6** It is concluded that differential reactivity of pulmonary arteries and veins to vasoactive agents may play differential role(s) in the pathophysiology of pulmonary disease. 5-HT may exert an anti-inflammatory activity in goat pulmonary veins.

## Introduction

Recently, there has been some interest in the autonomic and autacoid pulmonary pharmacology of farm animals (Eyre, 1971; 1975,a,b; Hanna & Eyre, 1978; Chand, DeRoth & Eyre, 1979; Chand & Altura, 1980). During the course of one of these investigations, 5-hydroxytryptamine (5-HT) was found to relax goat trachea (Chand *et al.*, 1979). A preliminary study, in our laboratory, indicated that goat pulmonary veins were also relaxed by 5-HT. The pharmacological characterization of this unusual relaxant response to 5-HT on goat pulmonary veins was therefore undertaken.

## Methods

Seven adult goats of either sex, weighing 50 to 150 lbs, were killed by shooting and exsanguination. The lungs and heart were immediately dissected out and placed in ice-cold oxygenated Krebs-Henseleit solution. The remainder of the experimental procedures were identical to those described earlier (Eyre, 1975, a, b; Chand *et al.*, 1979). In brief, pulmonary veins and arteries were isolated, dissected out and cut into spiral strips. Each strip was longitudinally dissected into twin 'identical strips'. From each animal, 3 to 6 pairs of pulmonary vein strips were used within 12 to 24 h. Tissues were mounted in pairs in 15 ml isolated organ baths, containing Krebs-Henseleit solution, mixed with 95% $O_2$ and 5% $CO_2$ at 37°C. The composition of the Krebs-Henseleit solution was (mmol/l): NaCl 118, KCl 4.70, $CaCl_2.2H_2O$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25.0 and glucose 10.0. The strips were allowed to equilibrate for 2 h under a resting load of 2 g. Single or cumulative dose-response curves to one or two agonists were recorded with a Narco Isotonic Myograph Transducer connected to a Fisher Recordall Series 5000 pen recorder via a Narco Physiograph. 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. Thirty min later

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the concentration-response curves were re-established in the presence of antagonists. One to three concentrations of an antagonist were tested on each strip. The second strip of each pair served as a control to monitor any time-related change in the responsiveness of the tissues to the agonist. The effectiveness and specificity of the antagonist was 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).

*Drugs*

Drugs used in this study were: histamine diphosphate, bradykinin triacetate (Bk), noradrenaline bitartrate (NA), 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma Chemical Co., St. Louis, Mo); $PGA_1$, $PGE_2$, $PGF_{2\alpha}$ (Upjohn Co., Kalamazoo, Mich.); angiotensin II (angiotensin-amide, Hypertensin, Ciba-Geigy Co.); morphine sulphate (E. Lily Co.); metiamide, cimetidine (S.K.F.); mepyramine maleate (Poulenc Ltd, Montreal, Quebec); propranolol hydrochloride (Ayerst Laboratories, N.Y.); methysergide hydrogen maleinate (Sandoz Canada Ltd. Montreal) and indomethacin (Merck Frost Laboratories).

## Results

The reactivity of goat pulmonary arteries and veins to vasoactive agents is summarized in Table 1. Arterial strips were insensitive to acetylcholine (ACh), $PGA_1$, $PGE_2$, and $PGF_{2\alpha}$ ($10^{-7}$ to $10^{-4}$ M), but reacted with concentration-dependent contractions to Bk, angiotensin, 5-HT, NA and histamine. Veins failed to respond to angiotensin; they reacted with concentration-dependent contractions to Bk, NA, ACh, $PGF_{2\alpha}$, $PGE_2$, $PGA_1$ and histamine; 5-HT produced relaxations (Figure 1a, b). In most of the vein strips (30/40), the resting tone usually recovered in 15 to 45 min following frequent washings after 5-HT-induced relaxations. In the remainder of the veins, tone was restored to resting level by the addition of histamine. 5-HT also relaxed vein strips which were pre-contracted with histamine (Figure 1b).

Mepramine ($10^{-8}$ M) antagonized contractions to histamine (dose-ratio = 27 ± 7, $n$ = 5). Metiamide and cimetidine ($10^{-6}$ to $10^{-5}$ M) did not alter responses to either histamine or 5-HT (dose-ratio = 1 ± 0, $n$ = 5). The concentration-response curves to 5-HT were not affected by methysergide ($10^{-9}$ to $10^{-6}$ M), indomethacin ($10^{-6}$ to $5 \times 10^{-5}$ M), propranolol ($10^{-6}$ to $5 \times 10^{-5}$ M) (Figure 1a), atropine ($10^{-7}$ to $10^{-6}$ M) or morphine ($10^{-6}$ to $10^{-4}$ M) (dose-ratio = 1 ± 0; $n$ = 5 with each dose of each antagonist). However the 5-HT-induced contractions on the artery were selectively blocked by methysergide ($10^{-8}$ to $10^{-6}$ M) without influencing contractile responses to Bk and histamine (Figure 2). The histamine-induced arterial contractions were reversed by the addition of mepyramine in a concentration-related manner. (Figure 2).

## Discussion

In this study, goat pulmonary arteries and veins exhibited a remarkable differential reactivity to a variety of vasoactive agents. For example, arterial strips were unresponsive to ACh, $PGA_1$, $E_2$ and $F_{2\alpha}$, whereas veins were insensitive to angiotensin. Both arteries and veins reacted with contractions to Bk and histamine. 5-HT induced contractions of arteries and relaxations of veins.

The qualitative and quantitative differences in the reactivity of pulmonary veins and arteries to vasoactive agents in goats (this study) and other mammals (Somlyo & Somlyo, 1970; Eyre, 1971; 1975a, b; Joiner, Kadowitz, Davis & Hyman, 1975; Su & Bevan, 1976; Hanna & Eyre, 1978; Chand & Altura,

**Table 1** Comparison of the reactivity of goat isolated pulmonary arteries and veins to vasoactive agents

| *Vasoactive agent* | n | *Pulmonary artery* | | *Pulmonary vein* | |
|---|---|---|---|---|---|
| | | *Threshold conc.* (M) | $EC_{50}$(M) | *Threshold conc.* (M) | $EC_{50}$(M) |
| Bradykinin | 5 | $1.2 \pm 0.5 \times 10^{-12}$ | $5.4 \pm 1.2 \times 10^{-8}$ | $2.3 \pm 0.3 \times 10^{-12}$ | $4.7 \pm 2.2 \times 10^{-8}$ |
| Angiotensin | 4 | $9.8 \pm 0.9 \times 10^{-9}$ | $4.9 \pm 2.1 \times 10^{-9}$ | unresponsive | unresponsive |
| 5-HT | 7 | $1.4 \pm 0.7 \times 10^{-8}$ | $3.1 \pm 0.6 \times 10^{-7}$ | $^{R}8.5 \pm 2.9 \times 10^{-10}$ | $^{R}3.3 \pm 1.5 \times 10^{-8}$ |
| Noradrenaline | 4 | $2.8 \pm 0.9 \times 10^{-8}$ | $9.1 \pm 1.5 \times 10^{-7}$ | $2.3 \pm 1.2 \times 10^{-9}$ | $8.7 \pm 2.1 \times 10^{-7}$ |
| Acetylcholine | 7 | unresponsive | unresponsive | $1.5 \pm 0.6 \times 10^{-7}$ | $2.3 \pm 0.6 \times 10^{-6}$ |
| $PGE_2$ | 4 | unresponsive | unresponsive | $1.5 \pm 0.7 \times 10^{-7}$ | $5.6 \pm 1.5 \times 10^{-6}$ |
| $PGA_1$ | 5 | unresponsive | unresponsive | $2.9 \pm 1.2 \times 10^{-7}$ | $6.5 \pm 0.9 \times 10^{-6}$ |
| $PGF_{2\alpha}$ | 5 | unresponsive | unresponsive | $2.4 \pm 0.8 \times 10^{-7}$ | $2.3 \pm 0.7 \times 10^{-6}$ |
| Histamine | 7 | $2.3 \pm 0.9 \times 10^{-7}$ | $2.5 \pm 0.8 \times 10^{-6}$ | $9.8 \pm 1.2 \times 10^{-8}$ | $3.9 \pm 0.4 \times 10^{-6}$ |

Values are mean ± s.e. mean. R = Relaxation

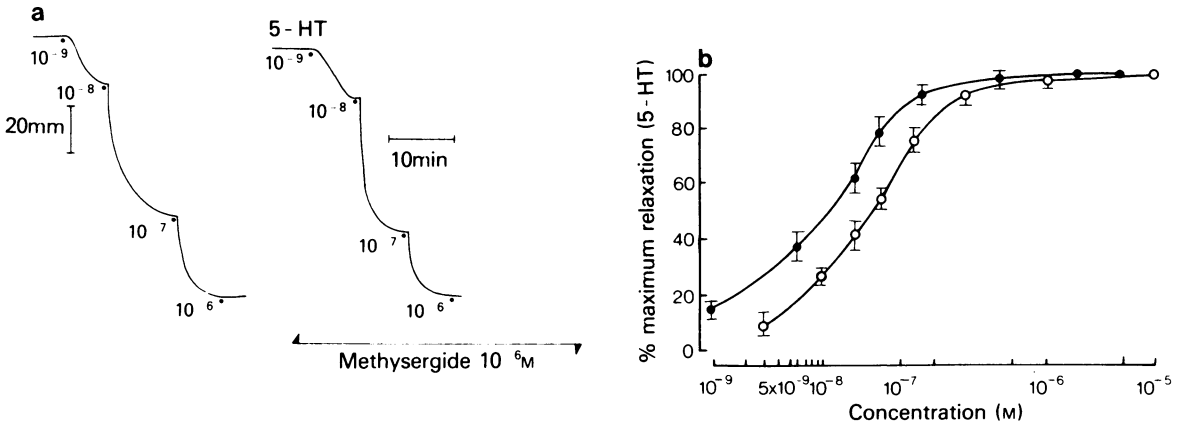


**Figure 1** (a) Failure of methysergide ($10^{-6}$ M) to antagonize 5-hydroxytryptamine (5-HT) induced relaxation of goat isolated pulmonary vein under a resting of 2 g. Tissue bathing fluid contained indomethacin ($5 \times 10^{-6}$ M). Drug doses are expressed in molar concentration (M). (b) Concentration-response (relaxation) curves to 5-HT of goat isolated pulmonary vein under resting tension (2 g) (●) and of histamine ($5 \times 10^{-6}$ M to $10^{-5}$ M)-contracted (○) veins. Each point represents the mean value of 4 to 7 observations. Vertical bars indicate s.e. mean.

1980) may be very important in the patho-physiological responses of this region of the circulation; for example, in the development of congestion and oedema in the lungs, and in hypoxic pulmonary vasoconstriction.

5-HT is a well known potent constrictor of the pulmonary blood vessels in several species (Somlyo & Somlyo, 1970; Eyre, 1971; 1975a, b; Joiner *et al.*, 1975; Su & Bevan, 1976; Chand & Altura, 1980). However, there is a paucity of data describing its inhibitory actions in blood vessels (Somlyo & Somlyo, 1970; Vargaftig & Lefort, 1974; McGrath, 1977; Fenuik, Humphrey & Watts, 1979). In low concentrations, 5-HT relaxes isolated pulmonary veins of sheep and goats (Eyre, 1975a, b; Chand & Altura, 1980; this study).

Traditionally, 5-HT is considered to act on at least two distinct types of receptor namely, M-neuronal and D-muscular tryptamine receptors. M-neuronal receptor-mediated responses are antagonized by morphine, cocaine and atropine, whereas D-tryptamine receptor-mediated responses are blocked by methysergide, dibenamine and dibenzyline, lysergic acid diethylamide and 2-bromolysergic acid diethylamide (Gaddum & Picarelli, 1957). The antagonism of methysergide to 5-HT-induced contractile responses of goat pulmonary arteries demonstrates the existence of classical D receptors in this tissue. This observation is not inconsistent with similar findings in the pulmonary blood vessels of several other mammalian species (Somlyo & Somlyo, 1970; Eyre, 1971, 1975a; Joiner *et al.*, 1975; Su & Bevan, 1976; Hanna & Eyre, 1978; Chand & Altura, 1980). 5-HT-induced relaxations of the goat pulmonary veins were not blocked by indomethacin (a potent blocker of prostaglandin synthesis), propranolol ($\beta$-blocker), metiamide, cimetidine ($H_2$-histamine receptor antagonists), atropine, morphine or methysergide, excluding the possibility of prostaglandin generation by 5-HT (Alabaster & Bakhle, 1976) or activation of $\beta$-adrenoceptors, $H_2$-histamine and muscarinic receptors as well as classical M and D-receptors. Similar observations have also been made in the dog saphenous vein and sheep pulmonary vein (Eyre, 1975a; Feniuk *et al.*, 1979; Chand & Altura, 1980). In view of these findings, the possibility of the existence of presynaptic neuronal inhibitory 5-HT receptors in the goat pulmonary veins should be considered.

The physiopathological significance of the inhibitory actions of 5-HT on vascular smooth muscle is not yet clearly understood. Interestingly, goat lung contains large quantities of 5-HT (Sadavongvivad, 1970). Tryptamine analogues have been considered as aetiological factors in the induction of pulmonary oedema and emphysema in goats and cattle (Carlson, Dyer & Johnson, 1968; Carlson, Yokoyama & Dickinson, 1972). However, the results of *in vitro* studies appear to suggest that in the caprine and ovine species, tryptamine analogues may be acting as anti-inflammatory agents at least in the pulmonary veins (Eyre, 1975a, b; Chand & Altura, 1980; this study). These investigations seem to suggest that goats and sheep have acquired a possible physiological, evolutionary beneficial response to 5-HT in comparison with closely related ruminants; i.e., bovine species (Eyre, 1971; 1975b).

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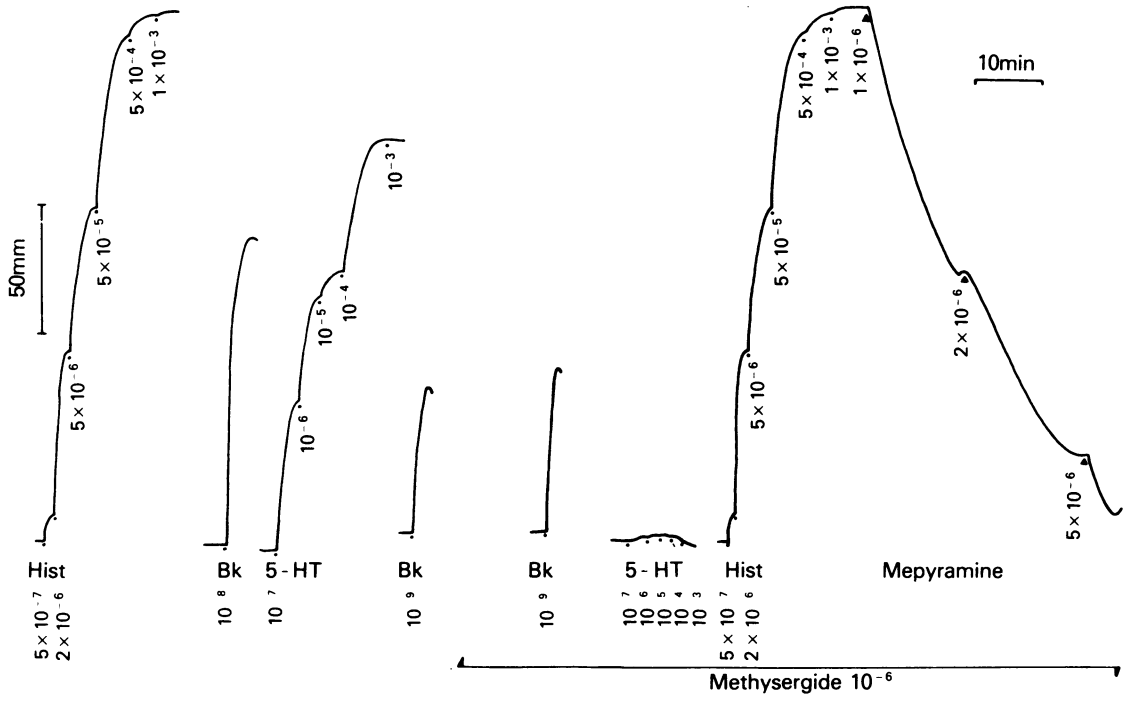


**Figure 2** Contractile responses of goat isolated pulmonary arterial strip to histamine (Hist), bradykinin (Bk) and 5-hydroxytryptamine (5-HT). Methysergide completely blocked 5-HT-induced contractions without altering responses to Bk and histamine. Mepyramine induced dose-related reversal of the pre-existing contraction to histamine. Drug doses are expressed in molar concentrations (M). Resting tension = 3 g.

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