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ATYPICAL TRYPTAMINE RECEPTORS IN SHEEP PULMONARY VEIN

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1 Both the pulmonary artery and vein of the sheep contracted dose-dependently to histamine, carbamoylcholine, prostaglandin $F_{2\alpha}$, noradrenaline and bradykinin and relaxed in the presence of isoprenaline or prostaglandin E_1.

2 The effect of 5-hydroxytryptamine (5-HT) on the artery was consistently to produce dose-dependent contractions without tachyphylaxis. The effect on the vein was biphasic. 5-HT 5×10^{-10} to 5×10^{-8} M relaxed the partially constricted vein. 5-HT 10^{-7} to 10^{-6} M caused brief venoconstriction followed by relaxation. 5-HT $> 10^{-6}$ M caused dose-related contraction of the vein.

3 Methysergide effectively blocked the contractile response of the artery to 5-HT, but only weakly inhibited the contractions of the vein (dose-ratio <20).

4 Each of ten antagonists tested failed to inhibit the 5-HT-induced relaxation of the vein. Sheep pulmonary vein possesses tryptamine receptors which mediate relaxation and which are not of the classical M- or D-type. These receptors appear not to be involved directly or indirectly with responses to acetylcholine, catecholamines, histamine or prostaglandins.

Introduction

Dietary tryptophan is reported to be a principal aetiological factor in the development of a form of acute inflammatory reaction of bovine lung characterized by oedema, congestion, bronchospasm and emphysema: known as acute bovine pulmonary emphysema (Dickinson, Spencer & Goreham, 1967; Blake & Thomas, 1971). The suggested pathogenesis is that tryptamine analogues are synthesized from tryptophan in the digestive tract, probably in the rumen contents (Schatzmann & Gerber, 1972). 5-Hydroxytryptamine (5-HT) (Eyre, 1972; Schatzmann & Gerber, 1972), tryptamine (Schatzmann & Gerber, 1972) and 3-methylindole (Carlson, Yokoyama & Dickinson, 1972) have been related to bovine pulmonary emphysema. Carlson, Dyer & Johnson (1968) experimentally reproduced the disease in cattle by feeding DL-tryptophan to cattle, whereas this amino acid led to no observable clinical or pathological effects in sheep.

It was important to screen a number of tryptamine antagonists in order to suggest suitable therapy for the disease in cattle (to be published elsewhere). It was also of pharmacological interest to study the actions of tryptamine analogues and antagonists on sheep pulmonary blood vessels as a possible means of explaining the peculiar species difference.

Methods

Lungs were obtained from healthy sheep of mixed breed, age and of either sex which were killed with pentobarbitone sodium. The principal pulmonary artery and vein were dissected out, cut spirally and mounted individually in similar organ-baths of 20 or 30 ml volume in Krebs-Henseleit solution, gassed with 5% CO_2 in O_2 at 35° C as previously described (Eyre, 1971). Movements of the mucle strips were recorded with isotonic myograph transducers and an E & M Model 4 Physiograph. All tissues were exposed at random to at least three concentrations of agonists for 2 min or until maximum response, every 10-15 minutes. Three-point dose-response curves to each of two or three predetermined agonists were established in each strip, 5-HT being included in every case. Before injection of 5-HT, the muscle strip was sub-maximally contracted (approximately 50%) by histamine or carbamoylcholine (Eyre, 1973).

Antagonists were applied when the agonist dose-effect relationship was stable. A specific antagonist was added to each bath and the drug left in contact with the tissue for at least 30 min before agonist dose-response curves were re-established. The activity of an antagonist was estimated by measuring the dose-ratio of each agonist, i.e. the ratio of doses of agonist which give equal responses in the presence and absence of

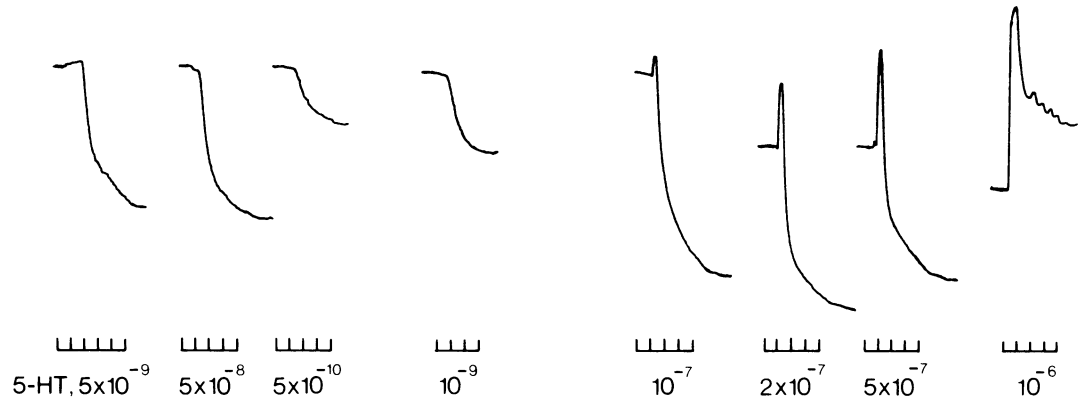

Figure 1 Isolated helical strip of sheep pulmonary vein in 20 ml Krebs-Henseleit solution gassed with 5% CO_2 in O_2 at 35° C. Preparation is partially contracted (approximately 50%) by histamine prior to each addition of 5-hydroxytryptamine (conc. range 5 x 10^{-10} – 10^{-6} M). Marker shows injection times and 0.5 minutes.

antagonist (Gaddum, Hameed, Hathway & Stephens, 1955).

Drugs

The following drugs were used: histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, carbamoylcholine chloride, noradrenaline bitartrate, isoprenaline hydrochloride, bradykinin triacetate, tryptamine hydrochloride, methysergide bimaleate, dibenamine hydrochloride, propranolol hydrochloride, atropine sulphate, morphine sulphate, mepyramine maleate, burimamide, indomethacin, sodium meclofenamate and phloretin phosphate dimer.

Results

5-HT (5×10^{-10} to 5×10^{-8} M) induced dose-dependent relaxation of the sheep pulmonary vein which had been partially contracted by histamine (10^{-8} to 10^{-7} M). 5-HT concentrations between approximately 10^{-7} and 10^{-6} M caused brief contractions rapidly followed by relaxation (net response = relaxation) and concentrations of 5-HT > 10^{-6} M caused only contractile responses in the partially contracted vein (Figures 1 and 2).

Tryptamine caused qualitatively similar effects to 5-HT (four observations each drug) but was quantitatively weaker (equipotent molar ratios: 5-HT = 1; tryptamine = 155).

Methysergide (5×10^{-7} M) inhibited all contractile effects of 5-HT irrespective of the dose of 5-HT used. However, methysergide >5×10^{-6} M itself contracted the vein strip, which precluded measurements using greater concentrations of the antagonist. Dibenamine (10^{-8} to 10^{-6} M) did not inhibit 5-HT-induced contractions of the vein.

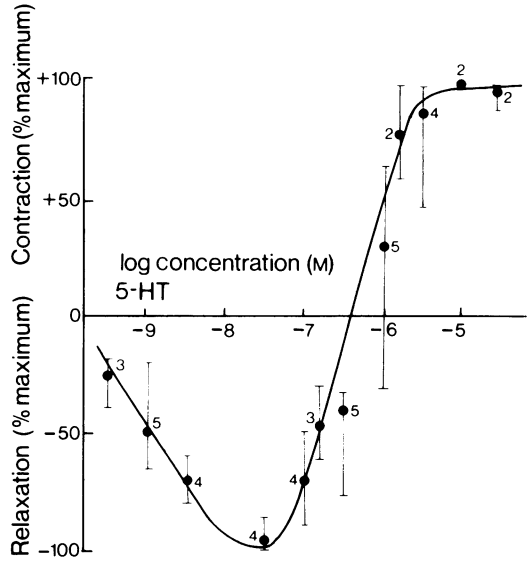

Figure 2 Log concentration (M) response curve (% maximum) to 5-hydroxytryptamine in isolated helical strips of sheep pulmonary vein in 20 ml Krebs-Henseleit solution gassed with 5% CO_2 in O_2 at 35° C. Numbers on graph refer to numbers of observations. Vertical bars represent ranges of values.

Ten antagonists were used in an attempt to block 5-HT-induced relaxation. No antagonist tested had any measurable inhibitory action on the relaxant response 5-HT. Specific antagonists used, selectively blocked their complementary agonists (Table 1). For example, propranolol inhibited the

Table 1 Antagonism of histamine, carbachol, prostaglandins E_1 (PGE_1) and $F_{2\alpha}$ ($PGF_{2\alpha}$), bradykinin, noradrenaline, isoprenaline and 5-hydroxytryptamine (5-HT) on isolated helical smooth muscle strip of sheep pulmonary vein

		*Dose ratios of agonists**							
Antagonist	*Conc. (M)*	*Histamine*	*Carbachol*	*PGE_1*	*$PGF_{2\alpha}$*	*Bradykinin*	*Noradrenaline*	*Isoprenaline*	*5-HT*
Methysergide	10^{-7}	–	–	–	–	–	–	–	1.0(2)
	2×10^{-6}	2.2(2)	–	–	–	–	1.5(2)	–	1.5(4)
Dibenamine	10^{-7}	–	–	–	–	–	20.0(2)	–	1.2(2)
	2×10^{-6}	1.8(2)	–	–	–	–	31.0(2)	–	1.4(2)
Propranolol	10^{-7}	–	–	1.5(2)	–	–	–	42.0(2)	1.1(2)
	2×10^{-6}	3.0(2)	2.5(2)	1.6(3)	–	–	–	75.0(3)	1.5(3)
Atropine	2×10^{-7}	–	55.0(3)	–	–	–	–	–	1.2(3)
	2×10^{-6}	2.5(2)	92.0(2)	–	–	–	–	–	1.0(2)
Morphine	2×10^{-7}	1.5(3)	–	–	–	–	–	––	1.1(4)
	5×10^{-6}	2.1(2)	3.0(2)	–	–	–	–	–	1.0(3)
Mepyramine	10^{-8}	151.0(3)	2.5(2)	–	–	–	–	–	1.0(3)
	10^{-7}	510.0(3)	–	–	–	5.5(2)	–	–	1.0(3)
Burimamide	10^{-6}	1.0(3)	–	–	–	–	–	–	0.9(3)
	5×10^{-5}	0.9(3)	–	–	–	–	–	–	1.0(3)
Indomethacin	5×10^{-7}	–	–	1.5(3)	–	–	–	–	1.5(3)
	5×10^{-5}	–	–	1.5(2)	1.0(2)	–	–	–	1.5(2)
Meclofenamate-Na	10^{-7}	–	6.1(2)	5.0(2)	2.0(2)	5.5(2)	–	–	1.1(2)
Phloretin phosphate	3×10^{-5}	1.5(2)	–	18.5(2)	–	–	–	–	1.2(2)
dimer	1.5×10^{-4}	1.4(2)	–	25.1(2)	6.5(2)	–	–	–	1.4(2)

* Dose-ratio is the ratio of equiactive doses (concentrations) of agonist in the presence and absence of antagonist. Each figure is a mean and numbers of observations are in parentheses.

effect of isoprenaline; atropine the effects of acetylcholine; mepyramine the actions of histamine and phloretin phosphate dimer inhibited prostaglandin E_1 in the same preparation, without blocking 5-HT. It was interesting that indomethacin itself relaxed the pulmonary vein (possibly due to inhibition of endogenous prostaglandin production) but when the vessel was partially contracted again by histamine or acetylcholine the superimposed relaxant effect of 5-HT was unimpaired. The three classical antagonists of 5-HT (methysergide, dibenamine and morphine) did not inhibit the relaxant actions of 5-HT in this tissue. Drugs which are known to block prostaglandin synthesis (indomethacin and meclofenamate), or which block prostaglandin receptors (phloretin phosphate) did not impair the relaxant effect of 5-HT.

By contrast, the sheep pulmonary artery was conistently contracted by 5-HT (threshold dose 10^{-8} to 10^{-6} M) dose-dependently and without tachyphylaxis. The arterial response was specifically antagonized by methysergide (dose-ratio = 120 at 10^{-6} M) but not by morphine (up to 10^{-5} M) nor by dibenamine (up to 5×10^{-6} M).

Discussion

Two kinds of tryptamine receptors have been classified according to antagonists. Gaddum & Piccarelli (1957) suggested there were neuronal receptors (possibly in ganglia or nerve fibres) which are blocked by morphine and atropine, the so-called M-receptors. A second type of receptor was postulated in smooth muscle, the D-receptors, which are blocked by dibenzyline and lysergic acid diethylamide (LSD) derivatives.

From the data presented here it appears that the sheep pulmonary artery carries only D-receptors (muscular), blocked by methysergide, though unexpectedly not blocked by dibenamine. Contractile actions of tryptamine analogues on sheep pulmonary veins may also be mediated by D-receptors, whereas the 5-HT-induced relaxant effect appears to be mediated via a third type of tryptamine receptor which may not be pharmacologically related to the classical M- and D-types (on the basis of antagonists at least), nor involved directly or indirectly with responses to acetylcholine, catecholamines, histamine or prostaglandins.

Wurzel (1966) and Bakhle & Smith (1974) described tryptamine receptors in rabbit aorta and rat pulmonary artery respectively which do not fit into the original Gaddum & Picarelli classification. Receptors of the rabbit aorta were inhibited non-specifically by dibenzyline and dichloriso-proterenol, but not by morphine or LSD. Receptors of the rat pulmonary artery were blocked both by morphine and methysergide. As far as can be ascertained the present paper may be the first to describe pulmonary vasodilatation mediated by tryptamine receptors which are refractory to blockade. As Wurzel (1966) pointed out, the Gaddum-Picarelli convention for tryptamine receptors leaves much to be desired. Most autacoid receptors have been classified by both specific agonists and specific antagonists; usually on the basis of a structure-action relationship. Gaddum & Picarelli's 'D and M' classification is based neither on structurally-related agonists nor specific antagonists. The so-called atypical nature of certain tryptamine receptors (Wurzel, 1966; Bakhle & Smith, 1974; and this paper) may be more imagined than real. More extensive studies of tryptamine receptors in many tissues are indicated. This may then permit a re-appraisal of the classification.

The pulmonary vein is some 10 to 100 times more sensitive to 5-HT than the artery in both cow and sheep (Eyre, 1970; and this paper). The response of bovine pulmonary blood vessels to tryptamine analogues is purely contractile. It is probable that pulmonary venospasm and increased capillary permeability contribute to the tryptamine-induced inflammatory response of bovine lung. On the other hand, venodilatation is the most sensitive pulmonary vascular tryptaminergic response of sheep and it therefore seems reasonable to suggest that in this species the naturally-occurring tryptamine analogues may be in a sense counterinflammatory. It is interesting to think of this as a possible evolutionary advantage that sheep have acquired over their bovine relatives.

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