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PRELIMINARY PHARMACOLOGICAL CHARACTERIZATION OF THE BOVINE ISOLATED BRONCHIAL ARTERY STRIP: A NEW PREPARATION

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1 This paper describes a preliminary pharmacological study of the isolated helical strip of bovine bronchial artery under isotonic conditions in Krebs-Henseleit solution.

2 The tissue contracted to histamine and 5-hydroxytryptamine (5-HT), actions which were selectively antagonized by mepyramine and methysergide respectively. Histamine was not inhibited by metiamide and 5-HT was not affected by morphine.

3 Slow reacting substance of anaphylaxis (SRS-A), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 were spasmogenic, whereas PGE_1 caused relaxation. No potential antagonists of these agents were tested.

4 Carbachol, at all concentrations, caused contractions of the bronchial artery which were completely, irreversibly blocked by atropine.

5 The spasmogenic action of phenylephrine was selectively blocked by dibenamine. Vessel strips which were partially contracted to histamine, relaxed to isoprenaline and salbutamol. The action of isoprenaline was inhibited similarly by either propranolol or practolol. The evidence therefore suggests the presence of functional α, β_1 and β_2-adrenoceptors.

Introduction

The bronchial artery is a systemic artery which arises from the lesser curvature of the aortic arch and enters the lung at the bifurcation of the major bronchi. It delivers nutrients and oxygen to the lung parenchyma, nervous structures and the conducting airways (Aviado, 1965). An efficient bronchial circulation is essential for proper functioning of the lung tissue. McLaughlin, Tyler & Canada (1961) reported that if the bronchial artery is subjected to an occlusive lesion, widespread degeneration of the parenchyma occurs which closely resembles emphysema.

In allergic pulmonary disease, numerous chemical agents (pharmacological mediators) are released from the lung tissue by specific antigen (Austen & Orange, 1976; Piper, 1977; Holroyde, Burka & Eyre, 1977); these mediators are responsible, in part, for the clinical symptoms in obstructive lung disease. Some actions of many of these mediators on various bovine blood vessels, including the pulmonary vasculature, have been described previously (Eyre, 1971; Burka & Eyre, 1974a; 1977; Holroyde & Eyre, 1975). However, as far as can be ascertained there is little such information about the reactivity of bronchial blood vessels. Two earlier studies reported that in the canine bronchial artery *in vivo*, histamine caused vasodilatation, whereas the action of 5-hydroxytryptamine was biphasic (Martinez, de Letona, Castro de la Mata & Aviado, 1961; Aramendia, Martinez, de Letona & Aviado, 1962). It appears from the literature, that a bronchial vessel has never previously been studied *in vitro*, probably because the vessel, being very small, is difficult or impossible to obtain from the usual laboratory animal species. This may be the first report of a pharmacological study of the isolated bronchial artery.

Methods

Healthy lungs with the heart and thoracic aorta intact were obtained from freshly-killed mature beef cattle at a local abattoir. The entire extrapulmonary bronchial artery was located and removed between its origin close to the heart on the ventral (lesser) curvature of

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the aortic arch, and its insertion into the lung at the bifurcation of the trachea. The vessel was transported to the laboratory in ice-cold Krebs-Henseleit solution (Krebs & Henseleit, 1932) with a delay of approximately 20 to 30 min. After being trimmed of excess fat and other tissue, each vessel was cut spirally with scissors with the aid of a fine dissecting probe passed through the lumen, after the manner of Furchgott & Bhadrakom (1953) and Furchgott (1955) as modified by Eyre (1971). Each vessel strip was suspended in a 10 or 20 ml organ bath containing Krebs-Henseleit solution maintained at 37°C and gassed with a 95% O_2 and 5% CO_2 (Canox, Kitchener, Ontario). Each vessel strip was attached to a simple isotonic lever connected to a myograph detecting head/transducer unit. Contractions or relaxations of the tissues to a variety of agonists were recorded from a resting tension of 3.5 g on an E & M 4 channel desk model physiograph (Model DMPHA; Narco Biosystems Inc., Houston, Texas) after an equilibration time of 30 to 40 min, during which time the bath fluid was changed 3 to 5 times.

A further study using selective agonists and antagonists was carried out. Drugs used were phenylephrine, isoprenaline, salbutamol, carbachol, histamine and 5-hydroxytryptamine (5-HT). In the case of isoprenaline and salbutamol, a 60 to 80% maximum contraction was first induced with histamine. Dose-dependent relaxations were then produced. All other agonists were applied to the tissue at resting tone. Four point cumulative concentration-response curves were established in each muscle strip as previously described (Van Rossum, 1963) in the absence and presence of selective antagonists. At least five animals were used for each agonist-antagonist drug combination. Log concentration-response curves of the agonists were plotted before and after antagonist treatment and the effectiveness and relative specificity of each antagonist for various antagonists were estimated by measuring dose-ratios (Gaddum, Hameed, Hathway & Stephens, 1955), calculated at the EC_{50} level. The difference between the mean EC_{50} dose-ratios of each agonist in the presence and absence of antagonist were determined by Student's paired t test and considered to be significant at $P < 0.05$.

Drugs

General qualitative observations were made on the responses to prostaglandins (PGE_1) PGE_2 and $PGF_{2\alpha}$ (purchased from Sigma Chemical Co., St. Louis, U.S.A.) and to slow-reacting substance of anaphylaxis (SRS-A^{bov}), produced in this laboratory from calf lung by the method described by Burka & Eyre (1974b). SRS-A was assayed on guinea-pig ileum in the presence of atropine and mepyramine (1 μg/ml each). One unit of SRS-A was arbitrarily defined as equivalent to 5 ng histamine base contracting the ileum (Burka & Eyre, 1974b; 1977).

Semi-quantitative observations were made with the following agonists: histamine diphosphate and 5-hydroxytryptamine (serotonin) creatinine sulphate from Sigma Chemical Co., St. Louis, U.S.A.; carbamyl choline (carbachol) hydrochloride from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.; isoprenaline (isoproterenol) bitartrate from Winthrop Co., New York, New York, U.S.A.; salbutamol sulphate from Allen & Hanbury Ltd., Ware, Herts; and phenylephrine hydrochloride from Nutritional Biochemical Corp.

The following selective antagonists were employed: atropine sulphate from Nutritional Biochemical Corp.; propranolol hydrochloride Ayerst, McKenna & Harrison, Montreal, Quebec, Canada; practolol hydrochloride from I.C.I. Ltd. Macclesfield, Cheshire; dibenamine hydrochloride from K & K Laboratories, Plain View, New York, U.S.A.; mepyramine maleate from Poulenc, Montreal, Quebec, Canada; metiamide from Smith, Kline & French Laboratories, Welwyn Garden City, Herts; methysergide bimaleate from Sandoz, Basel, Switzerland and morphine hydrochloride from BDH, Toronto, Ontario, Canada.

Stock solutions of all drugs except prostaglandins were prepared at a concentration of 10^{-2} M in distilled water and final dilutions were made in Krebs-Henseleit solution before use. Prostaglandins were prepared as 1 mg/ml in 95% ethanol, stored at −5°C or less and were diluted in Krebs-Henseleit before use. Isoprenaline stock solution contained ascorbic acid (1 μg/ml).

Results

The bronchial artery strip contracted in a concentration-dependent manner to histamine (10^{-7} to 6×10^{-5} M; $n = 26$), 5-HT (5×10^{-9} to 5×10^{-5} M; $n = 25$), PGE_2 and $PGF_{2\alpha}$ (100 to 1000 ng/ml; $n = 6$) and SRS-A (100 to 200 u/ml; $n = 5$). PGE_1 (100 to 500 ng/ml; $n = 5$) produced dose-dependent relaxations of bronchial artery strips which had been partially contracted (to approximately 60% maximum) with $PGF_{2\alpha}$, PGE_2 or histamine.

The bronchial artery contracted to carbachol at a threshold concentration of 10^{-4} M. The maximal contraction, which was approximately 20 to 25% of the histamine maximum, was achieved at approximately 2×10^{-2} M carbachol ($n = 6$). Phenylephrine likewise caused the bronchial artery to contract (10^{-6} to 6×10^{-5} M; $n = 6$) the maximal response being approximately equal to 50% of the histamine maximum. Isoprenaline and salbutamol (2×10^{-8} to 5×10^{-5} M; $n = 6$ each drug) relaxed the vessel strips which were pre-contracted with histamine. At bath concentrations

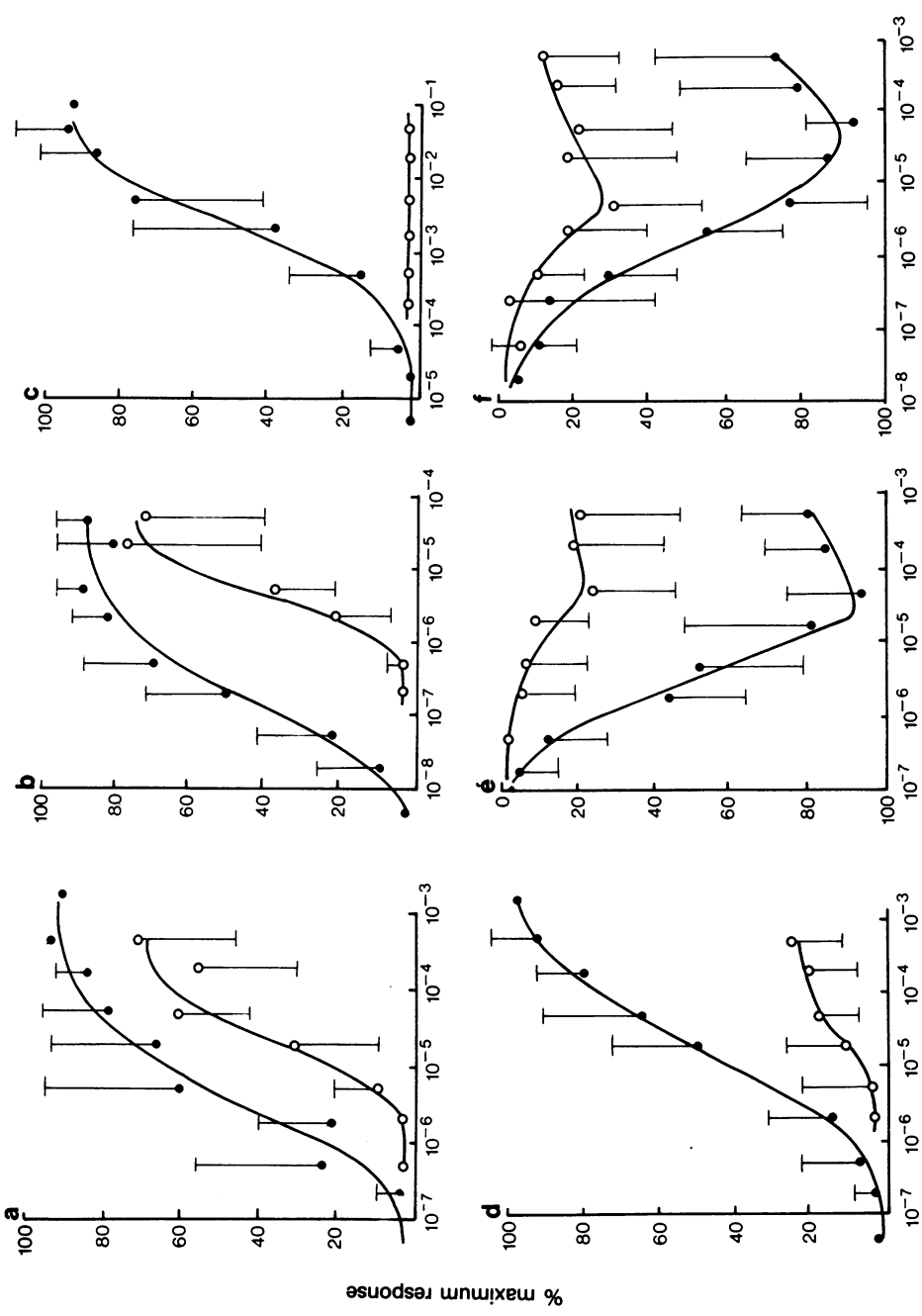

Figure 1 Effects of selective agonists and antagonists on the isolated bronchial artery strip preparation of the cow. Responses are expressed as percentages of maximum; each point is the mean of 5 observations and the vertical lines show one standard deviation. Drug doses are molar (M) final bath concentrations. (a) Log concentration-response curves for histamine (●——●) and histamine in the presence of mepyramine 2×10^{-8} M (○——○). (b) Log concentration-response curves for 5-hydroxytryptamine (5-HT) (●——●) and 5-HT in the presence of methysergide 2×10^{-8} M (○——○). (c) Log concentration response curves for carbachol (●——●) and carbachol in the presence of atropine 10^{-9} M (○——○). (d) Log concentration-response curves for phenylephrine (●——●) and phenylephrine in the presence of dibenamine 2×10^{-8} M (○——○). (e) Log concentration-response curves for isoprenaline (●——●) and isoprenaline in the presence of propranolol 5×10^{-7} M (○——○). (f) Log concentration-response curves for isoprenaline (●——●) and isoprenaline in the presence of practolol 5×10^{-8} M (○——○). In (e) and (f) responses recorded from pre-contracted state.

greater than 10^{-5} M, both isoprenaline and salbutamol became spasmogenic.

Studies with selective antagonists

Mepyramine (2×10^{-8} M) selectively blocked histamine in the bronchial artery ($n = 5$, $P < 0.05$), (Figure 1a). The mean dose-ratio was 9.55 and the mean maximum response was depressed by approximately 20 to 30%. Metiamide (2×10^{-8} M) did not antagonize the effect of histamine (mean dose-ratio = 1.5; $n = 5$).

Methysergide (2×10^{-8} M) selectively antagonized 5-HT ($n = 5$, $P < 0.05$) (Figure 1b). The mean dose-ratio was 25.1, the curves were parallel and there was no depression of the maximum. Morphine (2×10^{-7} M) failed to block the 5-HT responses but significantly potentiated histamine ($n = 5$, $P < 0.05$). The mean dose-ratio for histamine in the presence of morphine was 0.22.

Atropine at a concentration of 10^{-9} M completely abolished the carbachol contractile responses irreversibly ($n = 5$) (Figure 1c). Dibenamine (2×10^{-8} M) selectively antagonized phenylephrine ($n = 5$, $P < 0.05$) (Figure 1d) and depressed the maximum response by 70 to 90%. The same dose of dibenamine did not significantly alter the EC_{50} of histamine, although the mean maximal response to histamine was depressed by approximately 50%.

At a concentration of 2×10^{-7} M, propranolol antagonized the relaxant actions of both isoprenaline and salbutamol ($n = 5$, $P < 0.05$ for each drug); the isoprenaline curve is shown in Figure 1e. Practolol (2×10^{-8} M) significantly inhibited isoprenaline ($n = 5$, $P < 0.05$) (Figure 1f), but did not affect the salbutamol response.

Discussion

The spasmogenic action of histamine and 5-HT on the bronchial artery was in general agreement with the reported action of these amines on other bovine vessel strips, including the pulmonary (Eyre, 1971; Burka & Eyre, 1974a; 1977), mesenteric and hepatic (Holroyde & Eyre, 1975), digital (Elmes & Eyre, 1977) and coronary vasculature (Chand & Eyre, 1979). However, the present data do not agree with the results obtained by Martinez *et al.* (1961) and Aramendia *et al.* (1962), who reported that histamine caused dilatation and 5-HT caused a biphasic effect in the bronchial artery of the dog *in vivo*.

There are several possible explanations. It has been known for many years that the effects of histamine in peripheral vasculature may vary between species (Dale, 1929). Important differences may also occur between the *in vitro* actions of a drug compared with those *in vivo*. In the latter case the drug action may be affected by nervous reflexes, endogenous hormones and other chemical substances which regulate the tone of the smooth muscle of the vessel. Histamine might be expected to cause dilatation of a blood vessel under high intrinsic tone *in situ* whereas in a fully-relaxed isolated vessel strip contraction might occur.

The presence of H_1-receptors (Ash & Schild, 1966) in the bronchial artery was revealed in this study by the shift to the right of the histamine dose-response curve by 2×10^{-8} M mepyramine, but the depression of the maximum response was unexpected. The presence of H_2-receptors cannot be entirely ruled out since, although metiamide (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973) had little effect on histamine, it was used in a single, relatively low concentration of 2×10^{-8} M. Furthermore, the responses to histamine were potentiated by morphine (2×10^{-7} M), a curious finding hitherto unreported; it is possible that morphine affected some neural mechanism with a histamine-modulating role. In view of these findings, further studies of the actions of histamine are necessary.

The actions of 5-HT may vary in the same tissue, between tissues and between species (Garattini & Valzelli, 1968). It is known that 5-HT may exert both indirect and direct actions (Gaddum & Picarelli, 1957), the former being antagonised by morphine or atropine and the latter by dibenamine or lysergic acid diethylamide (LSD) derivatives. Our results suggest that 5-HT has principally direct actions on this preparation since a parallel displacement of the dose-response curve occurred with methysergide (2×10^{-8} M) whilst morphine (2×10^{-7} M) was without effect.

The spasmogenic action of carbachol in the bronchial artery is in general agreement with earlier observations in a variety of blood vessels (Su & Bevan, 1965; Eyre, 1971; Houghton & Phillips, 1973). Aviado (1965) described a dual effect of acetylcholine: vasodilatation at low concentrations and vasoconstriction with high doses. In our study, only contractile actions were recorded irrespective of the basal tone of the vessel strip, although high concentrations of carbachol were required ($> 1 \times 10^{-4}$ M). In the present study atropine (1×10^{-9} M) completely and irreversibly abolished the action of carbachol. This action was unexpected and cannot be explained by the limited data. The findings nevertheless confirm the presence of muscarinic cholinoceptors and are consistent with possible vagal innervation of the bronchial artery. This will have to be confirmed.

The bronchial artery strip is sensitive to both α- and β-adrenoceptor agonists and antagonists. Phenylephrine was spasmogenic and its effects were blocked by dibenamine (although the latter also weakly antagonized histamine). Isoprenaline and salbutamol both relaxed the partially contracted strip, the former being

about three times more potent than the latter, which is described as a selective β_2-adrenoceptor agonist. However, 5×10^{-7} M propranolol and 5×10^{-8} M practolol (a selective β_1-adrenoceptor blocking drug) suppressed the isoprenaline responses to a similar extent, despite the lower concentration of the latter drug. These preliminary data, whilst providing good evidence for the presence of β-receptors in the bronchial artery, do not accord with a simple subdivision of the receptors into β_1 and β_2 types. Further investigations are clearly warranted on the nature of the β-adrenoceptors in this tissue.

Two particularly interesting points arise from this study. The spasmogenic effects of the putative mediators of allergic hypersensitivity (histamine, 5-HT, SRS-A, prostaglandins and an acetylcholine-like drug) are consistent with the earlier hypothesis (McLaughlin *et al.*, 1961) that occlusion of the bronchial artery might contribute to airway disease. Impaired blood supply to the airways and alveoli would cause hypoxia (Guyton, 1966) which may be followed by bronchoconstriction, further (progressive) vasoconstriction and subsequent degeneration of alveolar architecture which has been associated with respiratory distress syndromes (McLaughlin *et al.*, 1961).

Secondly, it is also possible that the employment of β-sympathomimetic bronchodilator drugs (e.g. isoprenaline and salbutamol) in obstructive respiratory diseases (e.g. asthma/emphysema) may have the dual benefit of improving blood supply to the airways as well as dilating the constricted bronchi *per se*.

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