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Release of slow reacting substance from various tissues by A23187†

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Release of slow reacting substances (SRS, leukotrienes) from lung in response to antigen (Brocklehurst 1960) or divalent cationic ionophores (Bach & Brashler 1974; Piper & Seale 1979) has been well documented. In addition to lung, SRS can be released from various sensitized guinea-pig tissues by antigen challenge (Brocklehurst 1960). However, limited information exists regarding comparative amounts of SRS capable of being released from tissues other than lung by ionophores. In the present investigation, we used A23187† (Wong et al 1973) to survey a variety of tissues from guinea-pig for their ability to synthesize and release SRS.

Methods and materials

Male, Hartley guinea-pigs (Murphy Breeding Laboratories, Plainfield, IN) 250–300 g were decapitated. Lungs, pulmonary artery, thoracic aorta, kidney, ileum, spleen, heart, brain, and liver were excised and placed in separate petri dishes containing Krebs-bicarbonate solution of the following composition in mmol litre^{-1}: KCl, 4·6; $CaCl_2.2H_2O$, 1·8; KH_2PO_4, 1·2; Mg $SO_4.7H_2O$, 1·2; NaCl, 118·2; $NaHCO_3$, 24·8; and dextrose 10·0. The tissues were cleaned and freed of excess fat, connective tissue, and blood. Poorly perfused areas were discarded. All tissues except pulmonary arteries and aortas were cut into 1 mm cubes with a McIlwain tissue chopper. The arteries were minced with scissors. Due to the paucity of tissue, 100 mg aorta and 65 mg pulmonary artery were chosen as optimal sample size. All other tissues were divided into 500 mg aliquots. The tissue samples were placed in vials containing 2·25 ml of Krebs solution with 1×10^{-6} M indomethacin to optimize release of SRS (Walker 1973). The vials were then incubated at 37 °C for 5 to 10 min after which A23187 (250 µl, 1×10^{-4} M) was added to make a final concentration of 1×10^{-5} M. The incubation was continued for an additional 45 min. The incubation medium was then decanted and centrifuged at 3000 *g* at 4 °C for 5 min. The supernatant solutions were collected and assayed for SRS by a computerized bioassay using the guinea-pig ileum (Fleisch et al 1979). SRS was quantitated by comparison with an inhouse standard and the results expressed in terms of arbitrary units. For the bioassay, male, Hartley guinea pigs, 4–6 weeks old, were decapitated. A section of terminal ileum was removed, the lumen cleaned, and the tissue divided into 2·5 cm segments. The ilea were mounted in 10 ml tissue baths containing Krebs-bicarbonate solution maintained at 37 °C and aerated with 95% O_2 and 5% CO_2. The composition of Krebs-bicarbonate solution was as above except for $CaCl_2.2H_2O$ which was 1·2 mmol litre^{-1}. All studies with guinea-pig ileum were in the presence of 1×10^{-6} M atropine, to minimize spontaneous activity, and 1×10^{-6} M pyrilamine, an antihistamine. Isometric measurements were made with a Grass FT03C force displacement transducer and recorded on a Grass polygraph as changes in grams of force. A passive force of 0·5 g was applied to the tissues.

Drugs used were: indomethacin, atropine sulphate, bradykinin triacetate, (Sigma Chemical Co., St Louis, MO), pyrilamine maleate (ICN Pharmaceuticals, Inc., Plainview, N.Y.), A23187 (Eli Lilly and Company, Indianapolis, IN) and FPL 55712‡ (Gift of Fisons Ltd, Leicestershire, U.K.).

Results

Release of slow reacting substance by A23187. As previously demonstrated (Piper & Seale 1979; Fleisch & Haisch 1980), A23187 releases large amounts of SRS from fragmented guinea-pig lung (Table 1). Compared with lung, smaller amounts of SRS were released from aorta (12·7%), kidney (10·4%), ileum (6·1%), spleen (4·6%) and heart (3·0%). The ionophore did not release amounts of SRS from brain or liver that could be detected by the bioassay. In contrast, a substantial amount of SRS was elaborated from pulmonary artery in response to A23187. When calculated on a tissue weight to weight basis, pulmonary artery released 20% more SRS than lung.

To prove that the substance released from the various organs was indeed SRS, we demonstrated that its ability to contract guinea-pig isolated ileum was antagonized by FPL 55712, a selective SRS antagnoist (Augstein et al 1973). A sample polygraph tracing is shown in Fig. 1. The substance released from the pulmonary artery by ionophore contracted the ileum as did bradykinin. However, in the presence of 1×10^{-6} M FPL 55712, the former response was antagonized whereas the contraction elicited by bradykinin was unaffected.

† 2-[3β,9α,11β-trimethyl)-8-(2-pyrrolecarboxymethyl)-1,7-dioxaspiro[6.6]undecyl-2β-methyl]-5-methylaminobenzoxazole-4-carboxylic acid.

‡ 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-benzopyran-2-carboxylate.

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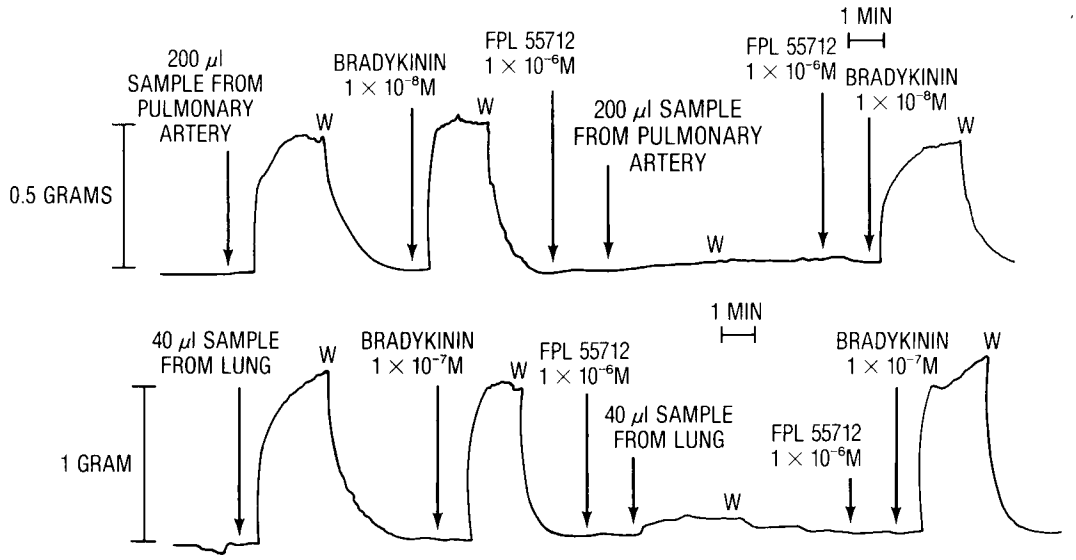

FIG. 1. FPL 55712 selectively blocked ileal contractions to SRS released from pulmonary artery (top) and lung (bottom) by A23187. Responses of ilea to bradykinin were not antagonized by this agent.

Table 1. Release of SRS from guinea-pig tissues by A23187*

Tissue	Lung	Pulmonary artery	Thoracic aorta	Kidney	Ileum	Spleen	Heart	Brain	Liver
Tissue weight (mg)	500	65	100	500	500	500	500	500	500
SRS/500 mg tissue	70·5±6·3†	84·5±6·1	8·9±2·0	7·3±0·8	4·3±0·9	3·2±0·7	2·1±0·3	0·9±0·3	0·5±0·2

* A23187, 1×10^{-5} M, for 45 min.
† Mean value, in arbitrary units, ± standard error for 6 determinations.

Discussion

Slow reacting substances, or leukotrienes, released as a consequence of immediate hypersensitivity reactions are thought responsible, in part, for the acute bronchoconstriction characteristic of asthma (Lewis et al 1981). Our study represents an initial attempt to determine the universality of SRS release, that is, to quantitate the relative amounts of SRS capable of being produced by various tissues. This would provide two important pieces of information: (1) whether any tissue, other than lung, could release relatively large amounts of SRS and (2) the relative amounts of SRS each tissue could conceivably contribute to a generalized anaphylactic reaction.

We chose A23187 as the releasing agent since under optimal conditions it appears to indicate the total potential release of SRS (Conroy et al 1976; Fleisch & Haisch 1980). Our results clearly show that lung and pulmonary artery were able to release the largest quantities of SRS among the nine tissues examined. Results with the pulmonary artery and aorta provide an interesting contrast between two systemic arteries. Whether or not this has physiological implications or merely represents the closer proximity of the pulmonary artery to the lung cannot be deduced from these experiments. As indicated above, the thoracic aorta, kidney, intestine, spleen, and heart released smaller amounts of SRS than either the lung or pulmonary arteries. However, the significance of this observation may be solely that these tissues are able to synthesize and release SRS. For if so, then there exists the possibility to eventually ascribe a physiological function to the leukotrienes in this group of tissues. Our inability to detect levels of SRS in brain and liver suggests a minor role, if any, for SRS in these organs. However, the guinea-pig ileum may not be sufficiently sensitive to assay the amounts of SRS released from brain and liver.

In conclusion, the present study demonstrated release of SRS in vitro by a variety of tissues in response to A23187. With this as a foundation, future studies can concern the physiological, pharmacological, and even pathological consequences of this find. If leukotrienes are indeed widely distributed throughout the body, then perhaps they function in a more general way than currently appreciated.

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Identification of arachidonate metabolites in normal and malignant human lung

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Many tumours (see Bennett 1979) and normal tissues produce substantial amounts of prostaglandins. The prostaglandins in human normal lung and pulmonary carcinomas have not been formally identified previously; this paper describes our results with gas chromatography-mass spectrometry.

Materials and methods

Preparation of tissues. Tissues from five histologically confirmed carcinomas (two well differentiated and one poorly differentiated squamous carcinomas, and two undifferentiated large cell carcinomas), and five specimens of normal lung tissue removed with the tumours, were provided by a pathologist within 15 min of surgery. Each sample was cut into small pieces, washed in Krebs solution, and any carbon deposits > 3 mm were removed. Weighed amounts (1–3·5 g) were homogenized for 30 s at room temperature (20 °C) in Krebs solution (0·1 g ml^{-1}, Silverson homogenizer) to obtain new synthesis of cyclo-oxygenase and lipoxygenase products (collectively called eicosanoids) from released precursors (Bennett et al 1973).

Following chloroform extraction and evaporation (Unger et al 1971) part of each sample was bioassayed on rat gastric fundus against PGE_2. The remainder was dissolved in dichloromethane for purification on an LH20 column. Non-polar impurities were first removed by dichloromethane, and the eicosanoids eluted with methanol. These were evaporated to dryness, dissolved in 10 ml double-distilled water, acidified to pH 3·0 with 1 M hydrochloric acid, and percolated through Amberlite XAD-2 columns.

Unwanted substances were eluted with 15 ml distilled water followed by 5 ml n-heptane. The eicosanoids were eluted with 10 ml methanol, and the solvent removed by evaporation first at 40 °C under nitrogen, and then desiccated under vacuum. The residue was dissolved in 200 μl methanol—chloroform (1:1 v/v) and applied as a narrow band on a silica gel G thin-layer chromatography plate (200 × 100 × 0·2 mm Merck; ethyl acetate–acetone–glacial acetic acid, 95:5:1 by volume, Andersen 1969). Authentic prostanoid standards were applied to the zone next to each biological sample. The plates were developed to 15 cm from the origin and 1 cm zones were eluted twice with 5 ml methanol which was then evaporated. Zones corresponding to authentic arachidonic acid (AA) and 12-hydroxy-eicosatetraenoic acid (12-HETE) were pooled and the residues re-chromatographed as described above, using diethyl ether–light petroleum–acetic acid (50:50:1 by volume). This gave better separation of the zones corresponding to AA and 12-HETE which were eluted as described above. No search was made for leukotrienes (Samuelsson et al 1979).

The chloroform extraction method gives recoveries >70% with PGE, F and A compounds (Unger et al 1971). Recoveries on subsequent purification were about 60–80%.

Chemical derivatization. *O*-Methyloximes were formed by dissolving residues in 100 μl pyridine containing methyloxime hydrochloride (Aldrich) 5 mg ml^{-1}, and heating at 60–80 °C for 1 h. The pyridine was removed under vacuum for 30 min, and the *O*-methyloximes converted into the corresponding methylesters by dissolving them in 100 μl methanol and treating with 200 μl freshly redistilled diazomethane. After vortexing, the samples were evaporated under nitrogen at room

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