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METABOLISM OF ARACHIDONIC ACID AND ITS ENDOPEROXIDE (PGH_2) TO MYOTROPIC PRODUCTS IN GUINEA-PIG AND RABBIT ISOLATED LUNGS

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1 Conversion of arachidonic acid (AA) and its endoperoxide (PGH_2) to myotropic metabolites has been studied in isolated Krebs-perfused lungs of guinea-pig and rabbit. A continuous differential bioassay technique was used to measure myotropic metabolites in the lung perfusate.

2 AA was metabolized in guinea-pig lungs to thromboxane A_2 (TxA_2), prostacyclin (PGI_2) and small amounts of a prostaglandin E_2 (PGE_2)-like substance. The amounts of products were dose-related over the AA range used (1 to 10 μg). PGH_2 was detected only after AA (10 μg).

3 Rabbit lungs converted AA (2.5 to 10 μg) to the same products in similar relative proportions but the amounts were 15 to 25% of those produced by guinea-pig lungs.

4 Indomethacin (10 nM) preferentially inhibited metabolism of AA to prostaglandins in guinea-pig lungs but preferentially inhibited metabolism to TxA_2 in rabbit lungs. Higher concentrations (50 nM) greatly reduced conversion to all the myotropic metabolites in lungs from both species.

5 Imidazole (50 μM) selectively inhibited conversion of AA to TxA_2 and increased conversion to PGI_2 in rabbit lungs. A similar effect was produced in guinea-pig lungs but with much higher concentrations of imidazole (2.5 to 5 mM).

6 PGH_2 (800 ng) was converted in guinea-pig lung to TxA_2 (100 ng) and very small amounts of PGI_2 (10 to 16 ng) but only unchanged PGH_2 and some PGE_2 were present in the lung perfusate after injection of PGH_2 in rabbit lung.

7 It is concluded that guinea-pig and rabbit lung differ in their ability to metabolize AA to myotropic substances and also in their response and sensitivity to drugs which interfere with prostaglandin and TxA_2 synthesis. The lungs do not appear to have an important role in converting PGH_2 to PGI_2.

Introduction

Arachidonic acid (AA) is the major unsaturated fatty acid component of membrane phospholipids and is released by the enzyme phospholipase A_2. AA is metabolized by either a lipoxygenase to 12-hydroperoxy-arachidonic acid (HPETE) and its stable end-product 12-hydroxyarachidonic acid (HETE), or via cyclo-oxygenase to prostaglandin endoperoxide (PGG_2). This endoperoxide is converted to PGH_2 which can be metabolized by three main pathways to thromboxane A_2 (TxA_2), prostacyclin (PGI_2) and the stable prostaglandins PGE_2, $PGF_{2\alpha}$ and PGD_2 (Samuelsson, Goldyne, Granström, Hamberg, Hammarström & Malmsten, 1978). The major metabolites produced vary between tissues. Thus platelets for example, do not contain PGI_2 synthetase and transform AA predominantly to TxA_2 and HETE (Samuelsson *et al.*, 1978; Tansik, Namm & White, 1978), while many vascular beds, including the coronary circulation, metabolize AA to PGI_2 but not TxA_2 (Schrör, Moncada, Ubatuba & Vane, 1978).

AA is metabolized in the pulmonary circulation of guinea-pig isolated lungs to a mixture of myotropic substances consisting principally of TxA_2 and PGI_2 with some PGE_2 (Boot, Cockerill, Dawson, Mallen & Osborne, 1977; Alabaster & Hawkeswood, 1978a, b). The lung therefore provides a good test system to study the effect of drugs, which interfere with synthesis of prostaglandins and TxA_2 at different sites, on conversion of AA to active substances.

The purpose of the present work was to compare the conversion of AA to myotropic substances in isolated lungs of rabbit and guinea-pig, and to investigate the effect of indomethacin which inhibits cyclo-oxygenase (Vane, 1971) and imidazole which inhibits thromboxane synthetase (Moncada, Bunting, Mullane, Thorogood, Vane, Raz & Needleman, 1977).

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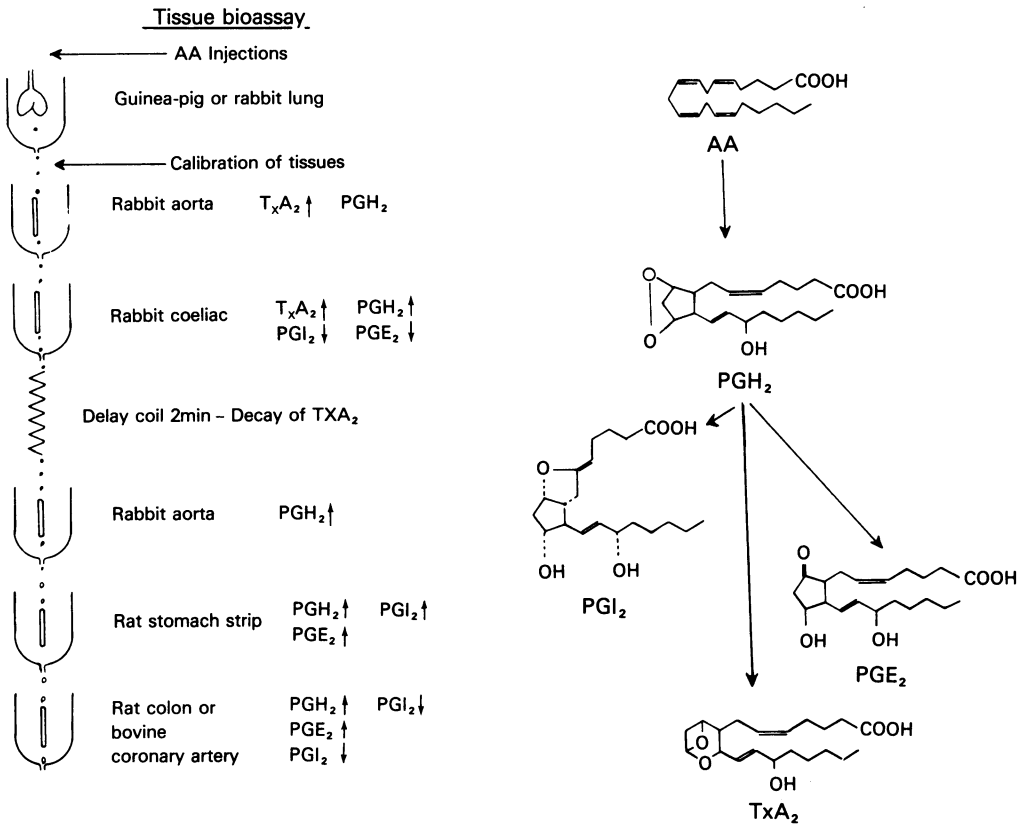

Figure 1 Bioassay of arachidonic acid (AA) metabolites from lung. The perfusate from isolated lungs superfused the cascade of tissues shown. The tissues were calibrated by injecting the following myotropic metabolites of AA directly over the tissues at the top of the cascade, thromboxane A_2 (TxA_2), prostaglandin H_2 (PGH_2), prostaglandin I_2 (PGI_2) and prostaglandin E_2 (PGE_2). The type of response obtained on the tissues is indicated (↑ contraction; ↓ relaxation). AA was injected into the pulmonary artery. The delay coil incorporated in the cascade removed any labile TxA_2 from the perfusate after quantitation, to allow more accurate assay of the more stable prostaglandins present. The pathways of AA metabolism to the principal myotropic products are also shown.

Since the immediate precursor of both TxA_2, PGI_2 and the stable prostaglandin is the endoperoxide PGH_2, the conversion of this substance in the pulmonary circulation has also been studied. Some of the work described in this paper has previously been communicated to the Physiological Society (Alabaster, 1979).

Methods

Perfused lungs

Male albino guinea-pigs weighing 450 to 650 g were anaesthetized with pentobarbitone (60 mg/kg i.p.). The thorax was opened and heparin 500 i.u. injected into the heart. The pulmonary artery was cannulated via the right ventricle and the majority of the heart including the left atria was cut away to allow free outflow from the pulmonary veins. The trachea was cannulated and the lungs dissected free. The lungs were inflated with air, suspended by the tracheal cannula in a heated chamber, and perfused through the pulmonary artery with gassed (95% O_2, 5% CO_2) Krebs bicarbonate solution (37°C) at 7 ml/min. Composition of Krebs bicarbonate solution was (mM); $NaHCO_3$ 25, NaCl 120, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5.6. Perfusion pressure was measured by a Statham pressure transducer attached to a side arm on the pulmonary artery cannula. Male rabbits (New Zealand Whites) weighing 1.7 to 2.4 kg were anaesthetized with pentobarbitone (60 mg/kg i.v.) and urethane (1 ml of 25% solution/kg i.p.) and the lungs prepared as described for the

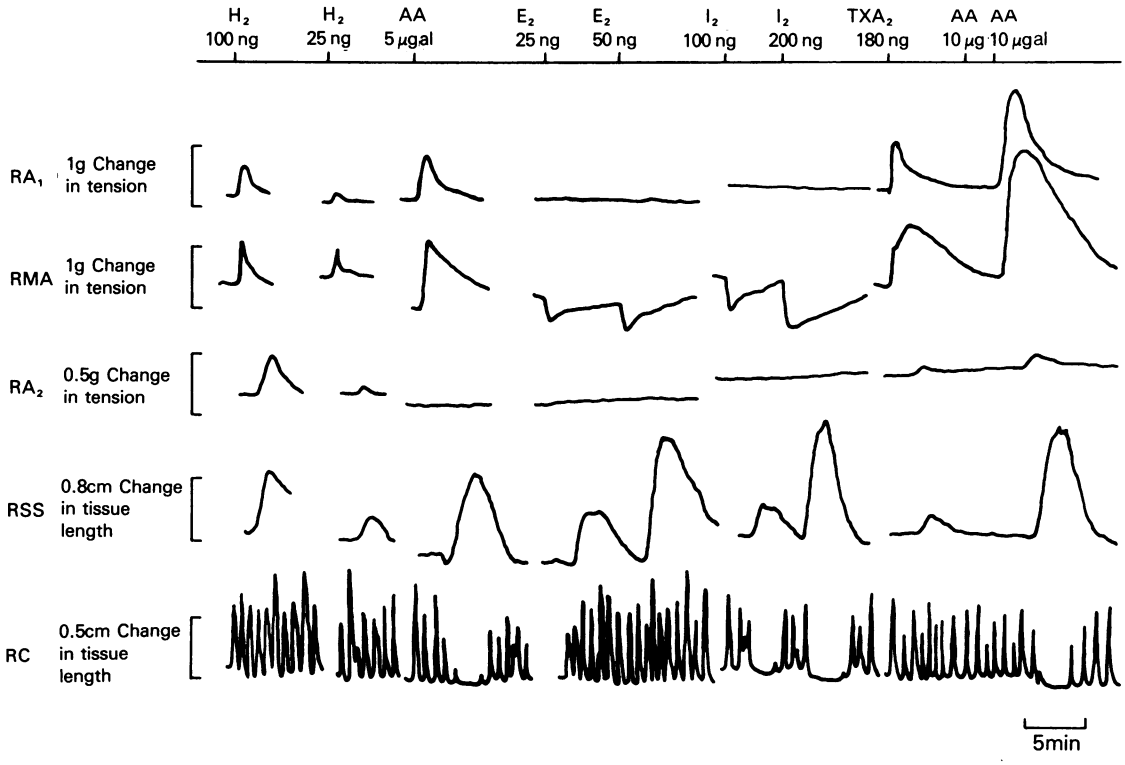

Figure 2 Conversion of arachidonic acid (AA) to myotropic products in guinea-pig isolated lung. Perfusate from guinea-pig lung superfused a rabbit aorta (RA_1) and a rabbit mesenteric artery (RMA). The perfusate then passed through a delay coil to allow the labile thromboxane A_2 (TxA_2) to decay before superfusing a second rabbit aorta (RA_2), a rat stomach strip (RSS) and a rat colon (RC). Calibration of the tissues and lettering are as described in Figure 1. AA (5 μg) injected into the lung (al) produced a contraction of the RA_1, RMA and RSS and inhibited the spontaneous activity of the RC. Since there was no PGH_2 present in the perfusate as indicated by the lack of contraction of the RA_2, the response in the RA_1 was due solely to TxA_2. AA(10 μg) injected directly over the tissues had no effect but injected into the lung (al) was converted to larger amounts of TxA_2 and a small amount of PGH_2 was detected in the perfusate as indicated by the contraction of RA_2. (The small contraction produced on the RA_2 by large concentrations of generated TxA_2 reflects the presence of a small amount of unconverted PGH_2 rather than TxA_2 surviving passage through the delay coil.)

guinea-pig. In most experiments the lungs could be perfused for about 3 h without signs of oedema, as indicated by a stable perfusion pressure and visual observation. Lungs which became oedematous during the course of an experiment did not give consistent conversion of injected AA and were discarded.

Bioassay

Pharmacologically active substances present in the lung perfusate after an injection of AA into the pulmonary artery were assayed by a continuous superfusion bioassay technique described by Vane (1964; 1969). The technique was modified to include a delay circuit such that the labile TxA_2 was removed from the perfusate, after quantitation, to allow more accurate measurement of the more stable prostaglandins present (Alabaster & Hawkeswood, 1978b). The following tissues were used: rabbit aortic spiral strip, rabbit coeliac or mesenteric artery, rat fundic strip, rat colon and bovine coronary artery. Details of the preparation, responses recorded and relative sensitivities of these tissues to AA metabolites have been described previously (Bunting, Moncada & Vane, 1976; Omini, Moncada & Vane, 1977; Alabaster & Hawkeswood, 1978b). The sequence of bioassay tissues used and the nature of response produced by AA metabolites on each tissue is summarised in Figure 1. To increase the specificity of the tissues for TxA_2 and prostaglandins released from the lung, a

mixture of antagonists was superfused over the bioassay tissues such that the resulting concentrations in the Krebs solution were: mepyramine maleate 0.1 μg/ml, hyoscine hydrobromide 0.1 μg/ml, propranolol hydrochloride 1 μg/ml, methysergide bimaleate 0.2 μg/ml, phentolamine 0.1 μg/ml and indomethacin, 0.5 μg/ml.

Calibration of the assay tissues

In all experiments the assay tissues were calibrated to PGE_2, $PGF_{2\alpha}$, PGH_2, TxA_2 and PGI_2. The endoperoxide (PGH_2) was prepared from ram seminal vesicle microsomes and AA according to the method of Ubatuba & Moncada (1977) and stored in dry acetone at −70°C. TxA_2 was generated from human platelet microsomes and PGH_2 (Needleman, Moncada, Bunting, Vane, Hamberg and Samuelsson, 1976) and aliquots of the incubation mixture immediately injected over the assay tissues. The following mixture incubated for 2 min at 0°C was found to give 90–95% conversion to TxA_2: PGH_2 200 ng, human platelet microsomes 50–60 μg protein and phosphate buffer (pH 7.8) 70 μl.

Drugs

The following drugs were used: arachidonic acid (Sigma Grade I), mepyramine maleate (May and Baker), hyoscine hydrobromide (BDH), methysergide bimaleate (Sandoz), phentolamine mesylate (Ciba), propranolol hydrochloride (I.C.I.), imidazole and indomethacin (Merck, Sharp and Dohme).

Arachidonic acid was made up in ethanol (50 mg/ml), stored at −5°C and diluted daily with 0.2% sodium carbonate before use. Imidazole solutions were adjusted to pH 7.4 with 0.1 N HCl.

Results

Metabolism of arachidonic acid to myotropic substances in guinea-pig and rabbit lungs

AA (1 to 10 μg) injected into the pulmonary circulation of guinea-pig isolated lungs was transformed into substances which contracted the rabbit aorta (RA_1), rabbit mesenteric (or coeliac) artery and rat stomach strip but relaxed the bovine coronary artery and inhibited the spontaneous contractions of the rat colon. The bioassay tissues were calibrated with standard myotropic metabolites of AA and the design of the bioassay cascade allowed the amounts of TxA_2 PGI_2 and PGH_2 produced from AA to be measured (see Figure 2). The amount of PGE_2 produced from AA could not be measured directly but was assessed by subtracting the contribution of the PGI_2 from the response on the rat stomach strip. (The contraction of the rat stomach strip is due to the presence of PGI_2, PGE_2 and possibly small amounts of $PGF_{2\alpha}$ and PGD_2.) The measurement of PGE_2 by this method was therefore an estimate rather than an accurate determination. An estimate of the amount of PGE_2 was considered possible since contractions to standard injections of PGE_2 and PGI_2 were found to be additive on the rat stomach strip and the slope of the dose-response curve to PGE_2 was unchanged in the presence of a fixed amount of PGI_2.

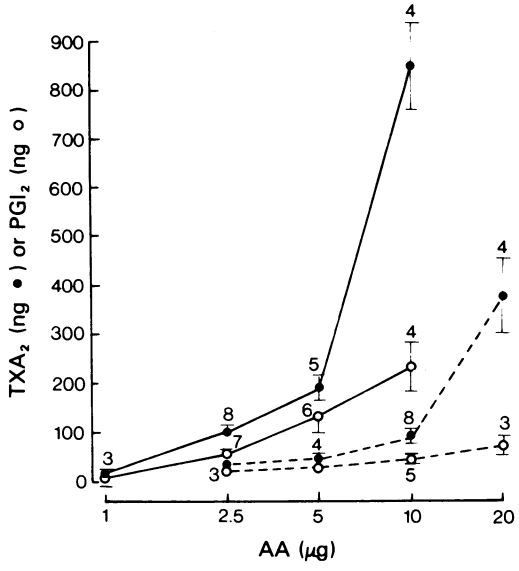

Figure 3 Effect of increasing the concentration of arachidonic acid (AA) on conversion to thromboxane A_2 (TxA_2) (●) and prostaglandin I_2 (PGI_2) (○) in guinea-pig lungs (solid lines) and rabbit lungs (broken lines). The points represent mean values (ng detected in lung perfusate) and numbers beside each point refer to the number of experiments; vertical lines show s.e. mean.

The amounts of myotropic products formed from AA in the lung were dose-related. Figure 3 shows the increasing amounts of TxA_2 and PGI_2 produced by increasing concentrations of AA injected into the pulmonary artery. AA (1 to 10 μg) in guinea-pig lungs was transformed to TxA_2 (21 to 845 ng), PGI_2 (25 to 240 ng) and PGE_2 (5 to 50 ng). No PGH_2 was detected (lower limit of sensitivity of assay was 16 to 20 ng) in lung perfusate after AA 1 to 5 μg but PGH_2 (30 to 70 ng) was detected in 3 out of 5 lungs after AA 10 μg.

AA (2.5 to 20 μg) was also converted in rabbit lung to TxA_2, PGI_2 and a PGE_2-like substance in similar

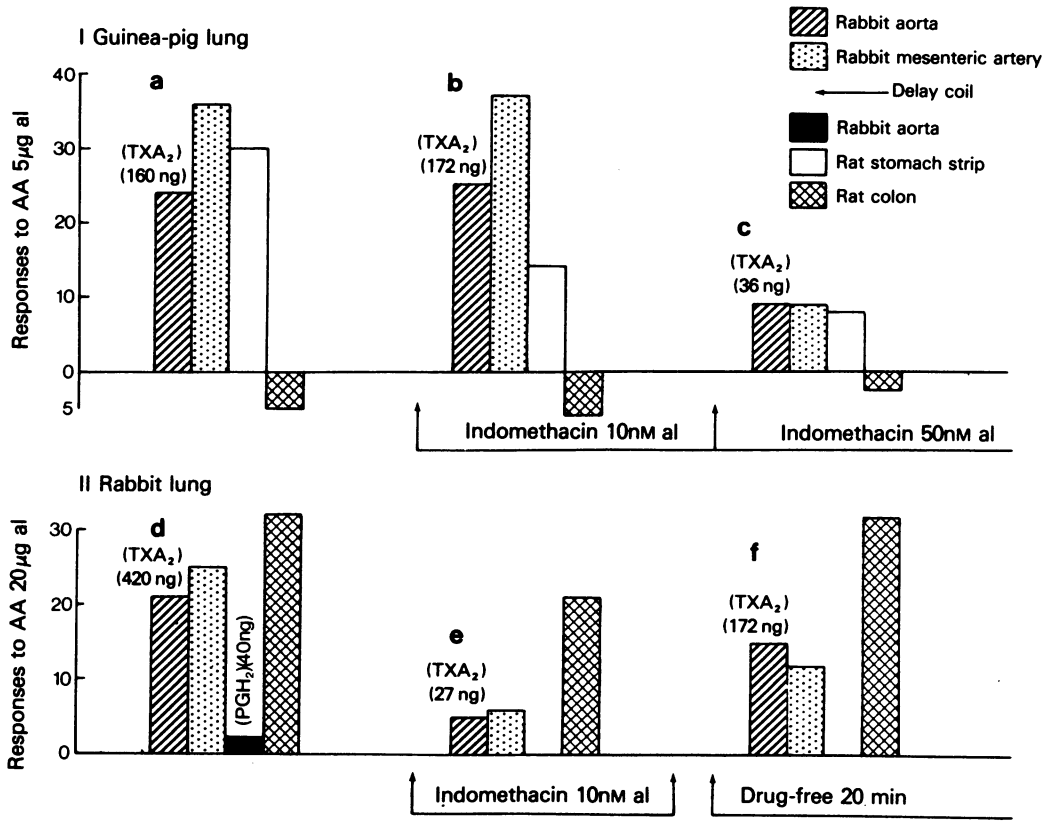

Figure 4 Effect of indomethacin on conversion of arachidonic acid (AA) to myotropic substances in guinea-pig (I) and rabbit isolated lungs (II). Perfusate from lungs superfused a cascade of bioassay tissues as shown in Figure 1. The columns represent in arbitrary units, contractile responses of a rabbit aorta (hatched column), rabbit mesenteric artery (stippled column), and after a delay coil, a second rabbit aorta (solid column), and a rat stomach strip (open column) and duration of inhibition of spontaneous activity of a rat colon (cross-hatched column) to AA injected into the lungs. Control responses (a) were obtained to AA 5 μg injected into guinea-pig lungs (al) and this injection was repeated after a 20 min pulmonary infusion of indomethacin 10 nM (b) and indomethacin 50 nM (c). Similarly control responses to AA (20 μg) were obtained in rabbit lungs (d) and repeated in the presence of indomethacin 10 nM (e) and after 20 min drug-free lung perfusion (f). The tissues were calibrated as described in Figure 1 and the number on top of the columns represent the ng equivalents of TxA_2 or PGH_2 as indicated.

relative proportions, but the amounts of myotropic substances produced were some 15 to 25% of those in guinea-pig lung (see Figure 3).

Perfusion pressure

AA (1 to 10 μg) injected into the pulmonary circulation of guinea-pig lungs produced small increases in lung perfusion pressure of 5 to 20 mmHg for 2 to 4 min. However, in rabbit lungs injections of AA (2.5 to 20 μg) produced larger increases (20 to 40 mmHg) in perfusion pressure lasting 3 to 6 min. The increase in perfusion pressure produced by AA was greater than 40 mmHg in about 10% of rabbit lungs, and flow rate through these lungs was reduced for 1 to 2 min. The results from these lungs were not used since quantitation could not be accurate.

Effects of indomethacin and imidazole on arachidonic acid conversion in guinea-pig and rabbit lungs

Control responses of the assay tissues were obtained following the injection of various concentrations of AA injected into the pulmonary artery. The drug was then infused continuously through the pulmonary circulation (0.07 ml/min) and the AA injections repeated. The tissues were calibrated to PGE_2, PGH_2, PGI_2 and TxA_2 before and during the drug infusion to establish that tissue sensitivity was not affected by the drug.

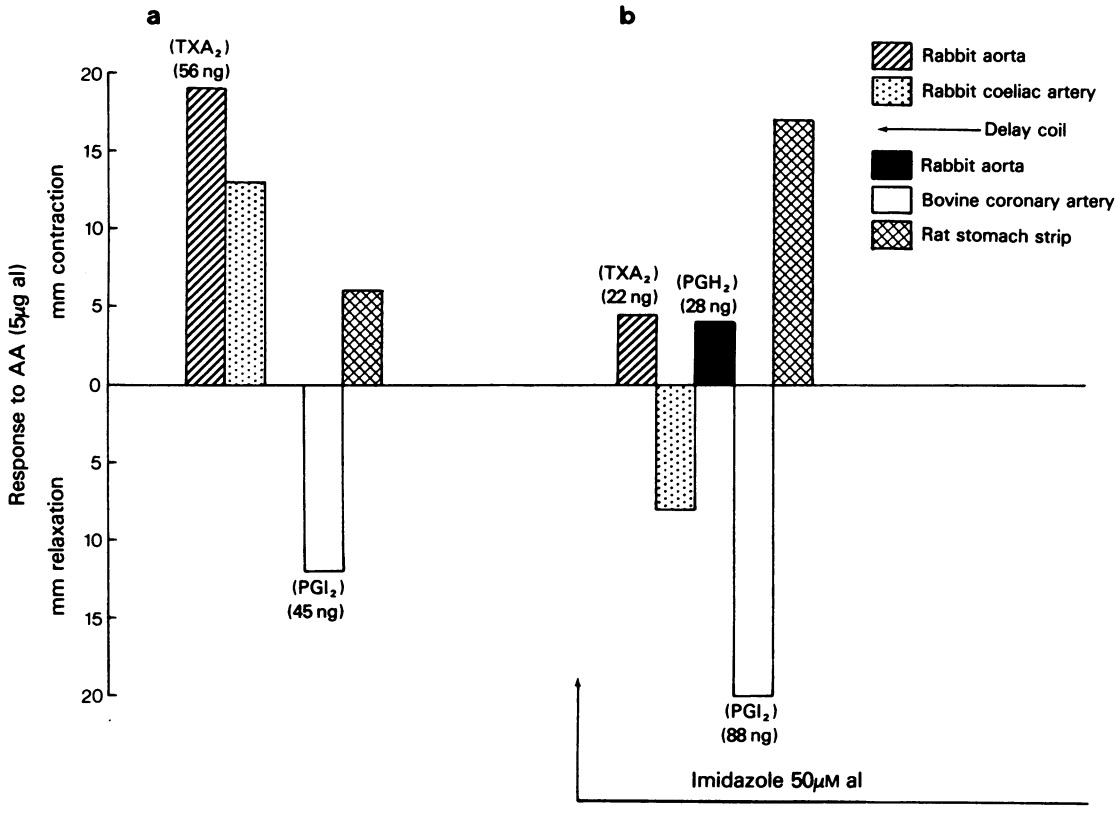

Figure 5 The effect of imidazole on conversion of arachidoic acid (AA) to myotropic substances in rabbit isolated lungs. Perfusate from the lungs superfused a cascade of bioassay tissues as shown in Figure 1. The columns represent responses of a rabbit aorta (RA_1) (hatched column), a rabbit coeliac artery (RCA) (stippled column), and after a delay coil, a second rabbit aorta (RA_2) (solid column), a bovine coronary artery (BCA) (open column) and a rat stomach strip (RSS) (cross-hatched column) to injections of AA (5 μg) into the lung (al) in the absence of drug (a) and during a pulmonary infusion of imidazole 50 μM (b). Conversion of AA to thromboxane A_2 (TxA_2) measured on the RA_1 was reduced by imidazole but conversion to prostaglandin I_2 (PGI_2) measured on the BCA was increased. Prostaglandin H_2 (PGH_2), measured on the RA_2, was also detected in the perfusate. The lack of TxA_2 and the presence of PGH_2 (and PGE_2) and increased amounts of PGI_2 in the perfusate after AA in the presence of imidazole is also confirmed by the reversal of the response of the RCA. (Figure 1 shows the direction of response of metabolites of AA on this tissue.)

Indomethacin Indomethacin (5 to 10 nM) infused into guinea-pig lungs ($n = 5$) reduced the conversion of AA to prostaglandin-like substances (PGE_2 and PGH_2) but had little effect on conversion to TxA_2. This preferential inhibition of conversion of AA to PGE_2 and PGH_2 occurred at all concentrations of AA used (1 to 10 μg). Higher concentrations (50 nM) of drug greatly reduced the conversion of AA to all the myotropic metabolites measured. In contrast, low concentrations of indomethacin (10 nM) preferentially inhibited the conversion of AA to TxA_2 in rabbit lungs ($n = 4$). Higher concentrations (50 nM) similarly reduced the conversion of AA to all myotropic metabolites. The results of two experiments comparing the effect of indomethacin in guinea-pig and rabbit lungs are shown in Figure 4.

Imidazole Imidazole (50 μM) infused over the assay tissues had no effect on tissue responses to AA injected into rabbit lungs. However, the same concentration infused through the pulmonary circulation markedly reduced the amount of TxA_2 measured in the perfusate and increased the amount of PGH_2 and PGI_2 ($n = 4$). The results obtained from one experiment are shown in Figure 5.

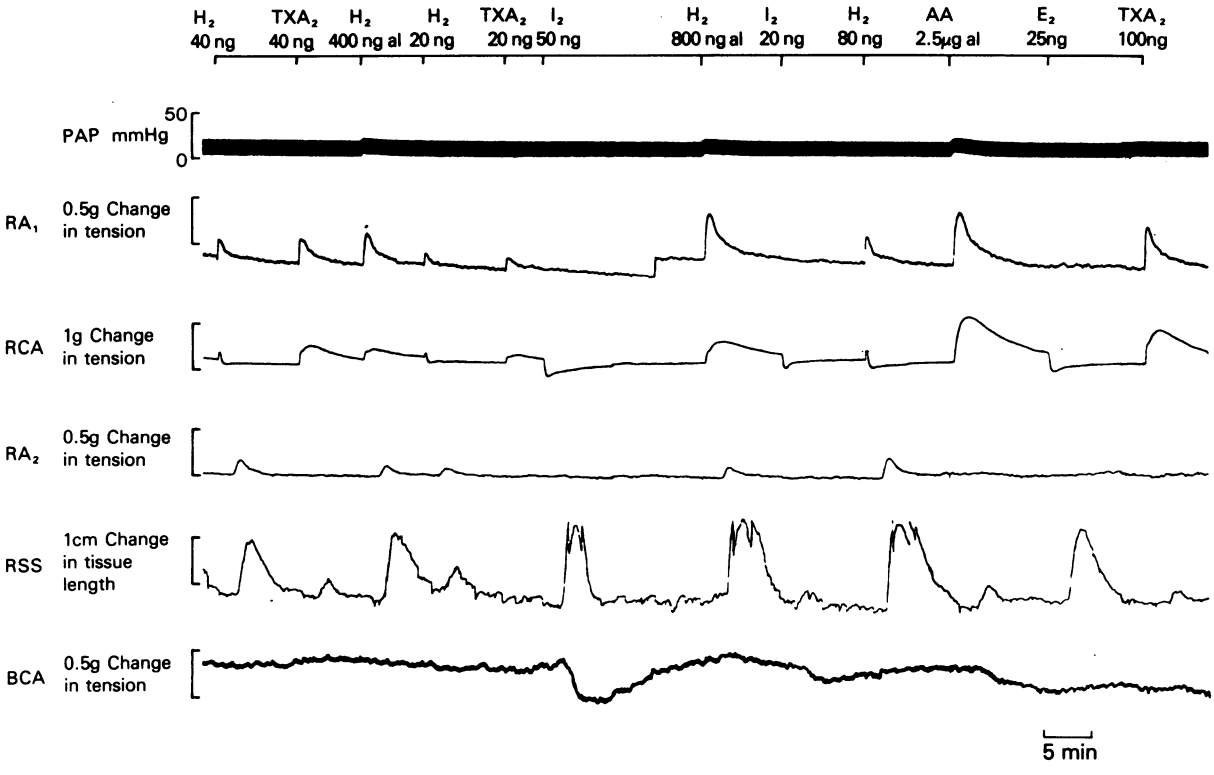

Figure 6 Conversion of prostaglandin H_2 (PGH_2) to myotropic substances in guinea-pig isolated lung. The upper tracing shows pulmonary perfusion pressure (PAP) and the lower tracings show responses of a rabbit aorta (RA_1), rabbit coeliac artery (RCA), and, after the delay coil, a second rabbit aorta (RA_2), a rat stomach strip (RSS) and bovine coronary artery (BCA) continuously superfused with the perfusate from guinea-pig lung. Calibration of the tissues and lettering are as described in Figure 1. PGH_2 (400 ng and 800 ng) injected into the lung (al) produced a contraction of the RA_1, RCA, RA_2 and RSS consistent with the presence of TxA_2 and PGH_2 (and PGE_2) but did not produce a relaxation of the BCA indicating the absence of PGI_2. In contrast AA 2.5 μg al produced contractions of the RA_1 and RCA without affecting the RA_2 showing the presence of TxA_2 (160 ng), and a relaxation of the BCA equivalent to 32 ng PGI_2.

A similar effect on AA conversion was produced by imidazole in guinea-pig lungs ($n = 7$) but the concentration of imidazole required was 50 to 100 fold higher than that used in rabbit lungs. The high concentrations of imidazole (2.5 to 5 mM) tended to affect the base-line and sensitivity of the vascular tissues used for bioassay and quantitation was unreliable

Metabolism of prostaglandin H_2 to myotropic substances in guinea-pig and rabbit lungs

PGH_2 (100 ng) injected into the pulmonary circulation of guinea-pig lungs ($n = 3$) had no effect on any of the assay tissues used. The lower limit of sensitivity of the bioassay was TxA_2 25 ng, PGI_2 20 ng and PGE_2 10 ng. PGH_2 (200 to 400 ng) injected into guinea-pig lungs ($n = 4$) was converted to TxA_2 (20 to 40 ng) but was not converted to any detectable PGI_2. In 5 lungs, PGH_2 (800 ng) was converted to TxA_2 101 ± 13 ng, PGI_2 10 to 16 ng (in only 3 of the 5 lungs since this amount tended to be below the limit of sensitivity of the bovine coronary arteries used) and small amounts of PGE_2 (20 to 30 ng) and unconverted PGH_2 (10 to 25 ng). An experiment showing the pulmonary conversion of PGH_2 (800 ng) in guinea-pig lung is described in Figure 6. In this lung no PGI_2 was detected although the bovine coronary artery used was sensitive to 20 ng PGI_2 injected directly over the tissues.

In contrast, PGH_2 (200 to 800 ng) was not converted to any detectable TxA_2 or PGI_2 in rabbit lungs and only unchanged PGH_2 and some PGE_2 was present in the perfusate, which together accounted for only 6 to 8% of the PGH_2 injected. Thus after injec-

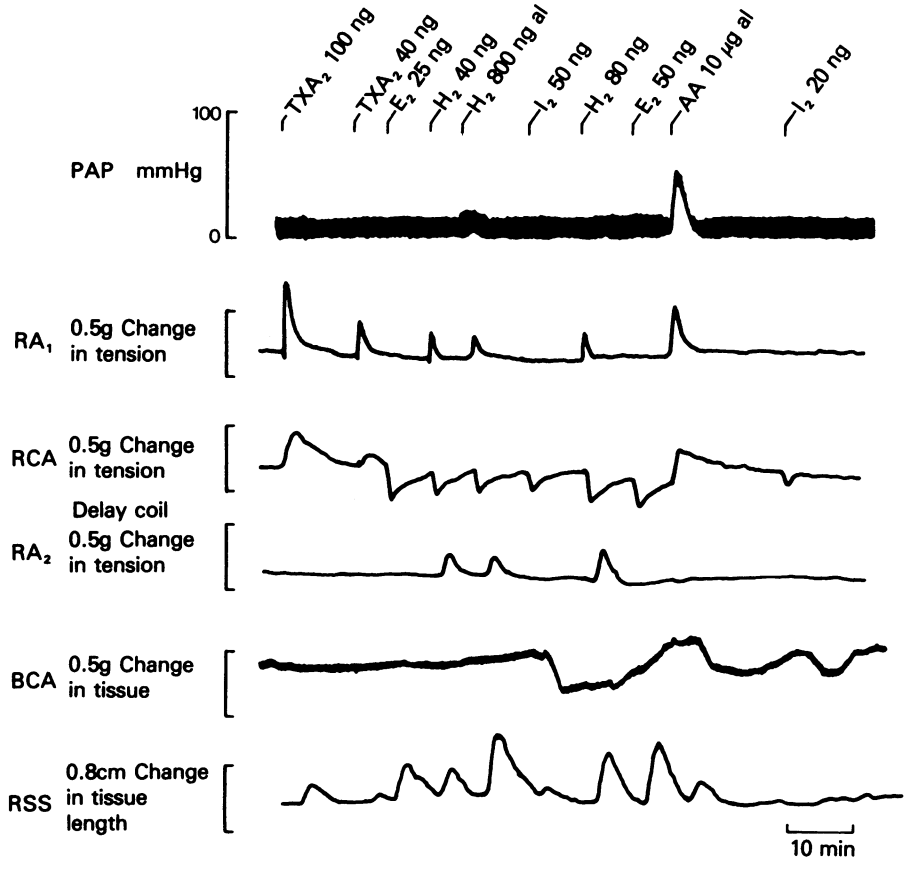

Figure 7 Lack of conversion of prostaglandin H_2 (PGH_2) to thromboxane A_2 (TxA_2) and prostaglandin I_2 (PGI_2) in rabbit isolated lungs. The upper tracing shows pulmonary perfusion pressure (PAP) and the lower tracings shows responses of a rabbit aorta (RA_1), a rabbit coeliac artery (RCA), a second rabbit aorta after the delay coil (RA_2), a bovine coronary artery (BCA) and a rat stomach strip (RSS) continuously superfused with the perfusate from rabbit lung. Calibration of the tissues and lettering are as described in Figure 1. PGH_2 (800 ng) injected into the lung (al) produced a contraction of the RA_1 and RA_2 and relaxation of the RCA consistent with the presence of 36 ng PGH_2 in the perfusate. The BCA did not relax, indicating the absence of PGI_2 and the contraction of the RSS was attributed to PGH_2 and a PGE_2-like substance. In contrast AA 10 μg al produced contractions of the RA_1 and RCA but not the RA_2 showing the presence of TxA_2 (50 ng) and a relaxation of the BCA indicating the presence of PGI_2 (about 30 ng).

tion of PGH_2 (800 ng) into rabbit lungs ($n = 5$) only PGH_2 46 ± 9 ng and PGE_2 26 ± 10 ng was detected in the perfusate. An experiment showing the lack of conversion of PGH_2 to TxA_2 and PGI_2 in rabbit lungs is shown in Figure 7.

Discussion

Metabolism of AA to myotropic substances in perfused lungs of guinea-pig and rabbit was found to be qualitatively similar in that TxA_2 and PGI_2 were the predominant metabolites formed, PGH_2 appearing in the perfusate only after high concentrations of AA (10 to 20 μg). However, at all the concentrations studied, the degree of conversion of AA to TxA_2, PGI_2 and PGE_2 was much smaller in rabbit lungs than in guinea-pig lungs. This probably reflects a species difference in the activity of the metabolizing enzymes rather than a difference in AA uptake or binding, since rabbit lung microsome preparations were intrinsically less active than guinea-pig lung microsomes in converting PGH_2 to TxA_2 (Sun, Chapman & McGuire, 1977). The concentration of imidazole

required to inhibit AA conversion to TxA_2 in guinea-pig lungs was 50 to 100 fold that required in rabbit lungs which may indicate that guinea-pig lung thromboxane synthetase is the more active enzyme. Metabolism of AA via the cyclo-oxygenase system has also been shown to be much less in human and rat perfused lungs compared to guinea-pig lungs (Al-Ubaidi & Bakhle, 1979). The guinea-pig lung is therefore apparently atypical in its degree of metabolism of AA and as such may give misleading results in predicting the potential clinical efficacy and potency of drugs which interfere with prostaglandin and TxA_2 synthesis.

Imidazole selectively inhibits TxA_2 synthetase in platelets (Moncada *et al.*, 1977a) and was found to redirect AA metabolism in guinea-pig lungs from TxA_2 to prostaglandin-like substances (Nijkamp, Moncada, White & Vane, 1977). In rabbit lungs, imidazole markedly reduced AA conversion to TxA_2 at concentrations which produced an increase in conversion to PGI_2. These results emphasise the clinical potential of a selective thromboxane synthesis inhibitor. Such a compound would not only reduce the production of the pro-aggregatory TxA_2 but would reinforce this anti-thrombotic effect by increasing the levels of the potent vasodilator and anti-aggregatory substance PGI_2.

Indomethacin, a non-steroidal anti-inflammatory drug which inhibits cyclo-oxygenase (Vane, 1971) produced qualitatively different effects on AA metabolism in rabbit and guinea-pig lungs. In rabbit lung, conversion of AA to TxA_2 was preferentially inhibited by low concentrations while in guinea-pig lung, low concentrations of indomethacin inhibited AA conversion to prostaglandin-like substances without affecting conversion to TxA_2. A similar differential inhibition was reported in guinea-pig lung where metabolites in lung perfusate, in response to an antigen challenge before and after indomethacin. were extracted and measured by GLC-MS (Dawson, 1977). After indomethacin, little 6-keto $F_{1\alpha}$ was detected and the major products were TxB_2 and its metabolite. The fact that indomethacin at low concentrations produced the reverse effect in rabbit lung, preferentially inhibiting TxA_2 synthesis, implies that the enzymes involved in the metabolism of AA must either have different distributions or locations in rabbit compared to the guinea-pig lung, or that the enzyme kinetics of the complex multiple enzyme system are different in the two species and the results obtained may be reflecting differences in affinity of the enzymes for the common substrate PGH_2. However, at higher concentrations of indomethacin, when cyclo-oxygenase is inhibited to a greater degree, conversion of AA to all myotropic metabolites is markedly reduced consequent to a marked reduction in PGH_2 supply.

PGH_2 is rapidly and specifically converted into PGI_2 by vascular microsome preparations, arterial rings and isolated blood vessel segments (Gryglewski, Bunting, Moncada, Flower & Vane, 1976; Bunting, Gryglewski, Moncada & Vane, 1976; Moncada & Vane, 1978). However, the conversion of PGH_2 to PGI_2 in isolated perfused lungs of guinea-pig was very small, only 10 to 16 ng PGI_2 being detected from an injection of PGH_2 800 ng (1 to 2% conversion). Conversion of PGH_2 to TxA_2 was greater (12.5%) and was evident at much lower concentrations of PGH_2. These surprising results may reflect a difference in penetration of PGH_2 to the different sites of location of PGI_2 synthetase and TxA_2 synthetase. PGI_2 synthetase is located principally in the intima and decreases in concentration progressively from the intima to the adventitia of vascular tissue (Moncada, Herman, Higgs & Vane, 1977b). The cell types responsible for synthesis of TxA_2 and the location of synthesis in lung is not clear. However cultured human lung fibroblasts have been shown to synthesize TxA_2, but not PGI_2, from AA (Bryant, Feinmark, Makheja & Bailey, 1978) and PGH_2 (Hopkins, Sun & Gorman, 1978). Vascular tissue does not synthesize TxA_2 (Tansik *et al.*, 1978).

Isolated rabbit lungs did not transform PGH_2 (800 ng) to any detectable TxA_2 or PGI_2 although both these metabolites are produced from PGH_2 by microsomal preparations of rabbit lung (Sun *et al.*, 1977). The PGH_2 and PGE_2-like substance detected in the perfusate together accounted for only 6 to 8% of the PGH_2 injected into the lung. The PGE_2 may have been formed by chemical breakdown of PGH_2 rather than by enzymatic conversion (Nugteren & Hazelhof, 1973). If a pulmonary transport system for PGH_2, as opposed to the more lipid soluble precursor AA, was absent in rabbit lung, much more PGH_2 (and spontaneous breakdown products) would be expected in the lung perfusate. Experiments with radiolabelled PGH_2 will determine if the PGH_2 becomes non-specifically bound within the pulmonary circulation or rapidly metabolized to products which would not be detected by the assay tissues used in these experiments. Although PGI_2 passes through the pulmonary circulation unchanged (Dusting, Moncada & Vane, 1978), PGI_2 produced intracellularly from PGH_2 may be rapidly converted to the biologically inactive metabolite 6 keto-$PGF_{1\alpha}$. However, this seems unlikely in view of the measurable conversion of AA to PGI_2 in the lung.

A species difference is also apparent in the coronary circulation of rabbits and guinea-pigs. In perfused rabbit hearts, PGH_2 in concentrations up to 2 μg was not converted to any detectable PGI_2, where the limit of sensitivity was 0.2 ng PGI_2 using a platelet aggregation bioassay (Needleman, Bronson, Wyche, Sivakoff & Nicolaou, 1978). In contrast, in the guinea-pig perfused heart, 50% of the injected PGH_2 (500 ng)

appeared in the perfusate as PGI_2 (Schrör *et al.*, 1978).

It appears therefore that the ability of the peripheral vascular circulation to transform PGH_2 to PGI_2 varies according to the species and the particular vascular bed under study. It has been suggested that endoperoxides released from platelets during aggregation or adhesion are utilised by the vascular endothelial cells to generate PGI_2, thus preventing the accumulation of platelets on the vascular wall under normal circumstances and maintaining a haemostatic balance between platelet-derived TxA_2 and PGI_2 derived from the blood vessels (Bunting *et al.*, 1976; Moncada & Vane, 1978). The results of experiments described in this paper indicate that the lungs, of rabbit and guinea-pig at least, do not have an important role in the conversion of PGH_2 produced in blood into PGI_2. However, the lungs may well produce PGI_2 from endogenous or exogenous AA for release into the systemic circulation. There is evidence that the lungs generate PGI_2 spontaneously in concentrations expected to have anti-platelet activity (Gryglewski, Korbut & Ocetkiewicz, 1978; Moncada, Korbut, Bunting & Vane, 1978). It seems likely therefore that this PGI_2 is generated from endogenous or exogenous AA.

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