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INCORPORATION AND METABOLISM OF [^{14}C]-ARACHIDONIC ACID IN GUINEA-PIG LUNGS

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1 Following infusion of [^{14}C]-arachidonic acid into guinea-pig isolated lungs more than half the administered radioactivity was retained by the lung.

2 The majority of the retained radioactivity was present in the phospholipid fraction with lesser amounts in the neutral lipid and free fatty acid fractions. When fatty acid methyl esters of the phospholipid fraction were prepared, 80% of the radioactivity co-chromatographed with methyl arachidonate.

3 Transformation to cyclo-oxygenase products and subsequent emergence in lung effluent accounted for approximately 20% of infused radioactivity.

4 After pretreatment of lungs with [^{14}C]-arachidonic acid, stimulation of arachidonic acid metabolism with injections of partially purified slow-reacting substance of anaphylaxis (SRS-A), bradykinin or antigen challenge released rabbit aorta contracting substance (RCS) and prostaglandin-like substances (PGLS) but little radioactivity. Furthermore, repeated injections of SRS-A or bradykinin released similar amounts of RCS and PGLS but diminishing amounts of radioactivity.

5 These data indicated that exogenous arachidonic acid was taken up by the lung and incorporated into phospholipids. However, this newly incorporated arachidonic acid had not equilibrated with the pool activated by SRS-A, bradykinin and antigen challenge for conversion to cyclo-oxygenase products.

Introduction

Guinea-pig isolated lungs metabolize endogenous arachidonic acid (AA) to rabbit aorta contracting substance (RCS) (a mixture of thromboxane A_2 and prostaglandin endoperoxides) and biologically active prostaglandin-like substances (PGLS) after numerous stimuli, including antigen challenge of sensitized lungs and infusions of bradykinin or injections of slow-reacting substance of anaphylaxis (SRS-A) into unsensitized lungs (Piper & Vane, 1969; Engineer, Piper & Sirois, 1977). Exogenous AA, infused or injected into the pulmonary artery is also rapidly transformed during passage through the lung to RCS and PGLS (Vargaftig & Dao Hai, 1972; Palmer, Piper & Vane, 1973) but less than 10% of injected AA appears as metabolites (Hamberg, Svensson, Hedqvist, Strandberg & Samuelsson, 1976) and the majority of infused AA remains unaccounted for by analysis of lung effluent. In the light of recent experiments with rabbit hearts and kidneys, demonstrating incorporation of [1-^{14}C]-AA into phospholipids (Isakson, Raz & Needleman, 1976), we have investigated the possibility that guinea-pig isolated lungs may similarly incorporate AA into phospholipids. The availability of incorporated AA for subsequent release with injections of SRS-A or bradykinin in unsensitized lungs and with antigen challenge of sensitized lungs has also been studied. Part of this work has been presented in a preliminary report to the Physiological Society (Al-Ubaidi, Bakhle, Jose & Seale, 1978).

Methods

Isolated perfused lungs

Unsensitized and sensitized (treated 3 weeks previously with ovalbumin grade II, 100 mg s.c. and 100 mg i.p.) male guinea-pigs (Dunkin-Hartley strain) were killed by cervical dislocation, the lungs were removed and perfused via the pulmonary artery (5 ml/min) with Krebs bicarbonate solution equilibrated with 5% CO_2 in O_2 at 37°C. The effluent superfused a rat stomach strip (RSS) and rabbit aortic strip (RbA) which were made more specific for PGLS by the continuous infusion of a mixture of antagonists to histamine, acetylcholine, catecholamines and 5-hydroxytryptamine (Piper & Vane, 1969). Indomethacin (2 μg/ml) was infused over the assay tissues to prevent endogenous prostaglandin synthesis (Eckenfels & Vane, 1972).

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Arachidonic acid (AA) was stored in *n*-hexane under nitrogen at −20°C. After evaporating *n*-hexane with a stream of nitrogen the residue was taken up in 0.9% w/v NaCl solution (saline) and converted to the sodium salt of AA with NaOH. This aqueous solution (0.5 ml) of sodium arachidonate (0.25 to 0.3 μmol; 0.25 to 1.0 μCi) was infused into the pulmonary artery over 3.5 min. From the start of the infusion, 1 min fractions (5 ml) were collected and the radioactivity was determined after adding 10 ml scintillant A per fraction. Thirty minutes after the infusion of AA, partially purified SRS-A (200 to 1500 mu; for units see Engineer, Niederhauser, Piper & Sirois, 1978) or bradykinin (2 to 6 μg) was injected into the pulmonary artery (unsensitized lungs) and repeated at 30 min intervals once or twice. Ovalbumin grade III (10 mg) was similarly injected into sensitized lungs 40 min after [^{14}C]-AA infusion. SRS-A in fractions (1 min) collected during antigen challenge was assayed on stripped longitudinal smooth muscle of guinea-pig ileum (Rang, 1964) superfused at 5 ml/min with oxygenated Tyrode solution at 37°C and blocked with mepyramine maleate and hyoscine hydrobromide (0.7 μM final concentrations). In some experiments, bovine serum albumin (BSA) (1% final concentration) was added to Krebs bicarbonate perfusate 20 min after the infusion of AA and intra-arterial injections of SRS-A or bradykinin were given 20 min later while BSA-enriched Krebs solution perfused the lungs. In 3 experiments, the lungs were pretreated with indomethacin 2 μg/ml for 15 min before the infusion of arachidonic acid.

Analysis of lung effluent

Lung effluent was acidified to pH 3 to 3.5 with HCl and extracted twice with 1.5 vol ethyl acetate (recovery of radioactivity 92.2%, $n = 2$) or with 2 vol diethyl ether (recovery of radioactivity 85.2%, $n = 2$). The dried extract was applied to t.l.c. plates and developed to 13 cm above the origin in chloroform:methanol: acetic acid (90:10:1 v/v). After drying, the plates were developed to 18 cm in hexane:diethyl ether:acetic acid (50:50:1). Standards were visualized with anisaldehyde (Keifer, Johnson & Arora, 1975). The prostaglandin-like substances were separated in the first solvent system, but did not migrate in the second system which separated arachidonic acid from hydroxyfatty acids. The dried plates were cut into 0.5 cm strips which were eluted with 1 ml methanol for 1 h before addition of 8 ml scintillant B.

Analysis of lung lipids

The lungs were weighed, homogenized in 17 vol chloroform:methanol (2:1) (containing indomethacin 2 μg/ml to limit prostaglandin synthesis during extraction) and filtered through glass wool. The homogenizer and filter were rinsed with a further 2 vol chloroform:methanol. After mixing 0.2 vol 0.12 M KCl with the filtrate, the organic phase was removed. The aqueous phase was acidified to pH 3.5 with HCl and extracted with chloroform to recover any prostaglandins which may have been present. This organic phase was added to the original and evaporated to dryness. To remove any remaining traces of water (which would interfere with subsequent chromatography), the lipid was redissolved in chloroform:methanol (1:1) and again evaporated to dryness.

The dried lipid extract was redissolved in chloroform and applied to 2 columns in series as follows: column 1 comprising 2 g silicic acid in chloroform retained phospholipids (Sweeley, 1969); column 2 comprising 2 g Florisil (partially deactivated with 8% water w/w) in diethyl ether retained free fatty acids (Radin, 1969). The columns were washed with excess chloroform (60 ml total) and the effluent containing neutral lipids was collected in glass vials. The columns were then treated separately. Column 1 was washed with 60 ml methanol to elute phospholipids; column 2 was washed with 20 ml diethyl ether:methanol (98:2) to remove any neutral lipid remaining on the Florisil and then the free fatty acids were eluted with 60 ml diethyl ether:acetic acid (96:4). Good separations were achieved since high flow rates permitted the use of relatively large volumes of solvents. Fractions (20 ml) were evaporated in glass vials and 2 ml methanol added 1 h before addition of 8 ml scintillant B. The recovery of radioactivity from the columns was 93.2 ± 0.3% (mean ± s.e. mean, $n = 3$) of that present in the chloroform:methanol extract. Trace amounts of radiolabelled AA and PGB_1 added to lipid extracts of non-radioactive lungs were eluted predominantly in the free fatty acid fraction (recovery 98.3 ± 0.3%, $n = 3$ and 85.1 ± 2.7%, $n = 4$ respectively), whereas thromboxane B_2, 6-oxo-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ were eluted in the phospholipid fraction.

Neutral lipid fractions were further analysed by t.l.c. in hexane:diethyl ether:acetic acid (60:40:1 or 85:15:1) and free fatty acid fractions in hexane: diethyl ether:acetic acid (50:50:1). Phospholipid fractions were separated into phosphatidyl ethanolamine, phosphatidyl choline and a mixture of phosphatidyl inositol, phosphatidyl serine and sphingomyelin by t.l.c. in chloroform:methanol:water (80:30:5) and chloroform:methanol:13 M ammonia:water (70:30:4:1). The dried plates were visualized by exposure to iodine vapour, cut into 1 cm strips (0.5 cm for phospholipids) and counted in 2 ml methanol (5 ml for phospholipids) and 8 ml scintillant B.

For analysis of the labelled fatty acids, the dried phospholipids were transesterified with 14% boron trifluoride in methanol (30 min at 70°C, in the presence of the anti-oxidant, butylated hydroxy toluene).

The methyl esters were extracted into light petroleum ether and washed twice with water. The dried extract was applied to a silver nitrate t.l.c. plate and developed in hexane:diethyl ether (80:20). Standards were visualized by charring with H_2SO_4. Methyl arachidonate was well separated from the methyl esters of stearic, oleic, linoleic, linolenic and hydroxy acids and from glycerides and unesterified fatty acids. Zones (1 cm) were scraped into vials and counted in 1.4 ml methanol and 10 ml scintillant B.

Liquid scintillation counting

Radioactivity was estimated in Packard Tricarb liquid scintillation counters. Counting efficiency was calculated from a sample channels ratio and all results were converted to d/min. The two scintillants used were:—A: 5 g PPO, 0.25 g dimethyl POPOP, 1 litre toluene and 0.5 litre Triton X-100; B: 5 g PPO, 0.25 g dimethyl POPOP and 1 litre toluene.

Drugs and chemicals

The following were used: [1-^{14}C]-arachidonic acid (60.2 mCi/mmol), [^{3}H]-prostaglandin E_2 (160 Ci/mmol), [^{3}H]prostaglandin E_1 (59 Ci/mmol), [^{3}H]-prostaglandin $F_{2\alpha}$ (15 Ci/mmol) and [^{3}H]-6-oxo-prostaglandin $F_{1\alpha}$ (5.9 Ci/mmol) (Radiochemical Centre, Amersham), [^{3}H]-thromboxane B_2 (125 Ci/mmol) (New England Nuclear), arachidonic acid, lipid standards, silicic acid (SIL-A-200), ovalbumin grades II and III and bovine serum albumin grade V (Sigma), Florisil (60 to 100 U.S. mesh), boron trifluoride (BDH), thin layer chromatography (t.l.c.) plates of silica gel 60 (0.2 mm on aluminium, Merck) and silica gel G + 10% silver nitrate (0.25 mm on glass, Anachem). [^{3}H]-prostaglandin B_1 (radiochemical purity 97.3%) was formed from [^{3}H]-prostaglandin E_1 by dehydration in methanolic KOH (0.5 M). Partially purified SRS-A was prepared as previously described (Engineer *et al.*, 1977). Indomethacin, bradykinin and prostaglandin standards were generous gifts from Merck, Sharp & Dohme, Sandoz and the Upjohn Company respectively.

Results

Radioactivity in lung effluent

A typical time course for the emergence of radioactivity from the lungs following a 3.5 min infusion of AA is shown in Figure 1. After 5 min 9.3 ± 0.7% (means ± s.e. mean, $n = 10$) of infused radioactivity had appeared in lung effluent and this had increased to 22.4 ± 2.8% ($n = 4$) after 50 min. The emergence

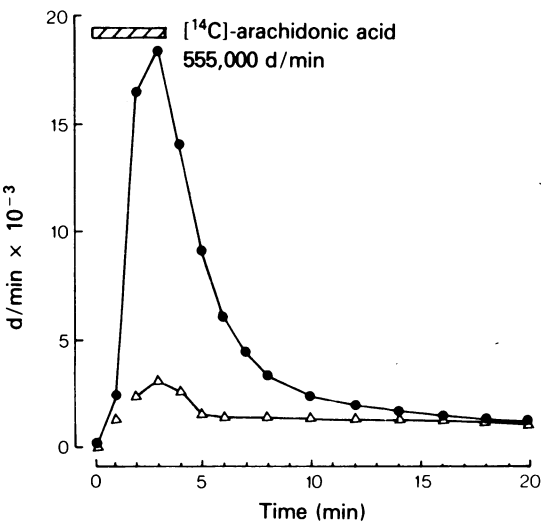

Figure 1 Efflux of radioactivity (●) from guinea-pig isolated perfused lungs with infusion of [^{14}C]-arachidonic acid (AA). The peak of radioactivity coincided with the termination of the AA infusion. Pretreatment of the lungs for 15 min with indomethacin (2 μg/ml) greatly reduced the peak of radioactivity (△). d/min values denote radioactivity in 5 ml fractions collected each minute.

of radioactivity from sensitized lungs was not significantly different from unsensitized lungs (0 to 20 min; sensitized 15.9 ± 0.3%, $n = 3$; unsensitized 16.4 ± 1.3%, $n = 8$). Simultaneous continuous bioassay during AA infusion detected RCS and PGLS in the lung effluent. In preliminary experiments it was established that the bioassay tissues did not retain significant amounts of radioactivity (less than 0.04%). Unaltered AA, estimated by t.l.c., comprised 6% (2 experiments) of lung effluent (0 to 5 min). The majority of radioactivity appeared to be cyclo-oxygenase products, their metabolites and hydroxy acids. After 15 min pretreatment of lungs with indomethacin 2 μg/ml, the peak efflux of radioactivity was greatly reduced (Figure 1) and no RCS or PGLS were detected. In one experiment indomethacin reduced radioactivity in the hydroxy acid zone to 6.7% (compared with 17.3% in untreated lungs) suggesting that a cyclo-oxygenase product (probably 12-hydroxy-heptadecatrienoic acid, HHT) was the major hydroxy acid.

When Krebs perfusate was supplemented with 1% BSA 20 min after infusion of AA, there was a two fold increase in effluent radioactivity. Analysis by t.l.c. showed that 54% of the radioactivity co-chromatographed with AA.

Radioactivity in lung tissue

More than 50% of infused AA was retained in the lung (Table 1) and that which could not be recovered from lung or effluent was lost in the perfusion apparatus since similar losses were encountered without lungs in circuit. The majority of the retained radioactivity (74 to 89%) was recovered from the phospholipid fraction. Significant amounts (9 to 24%) were also found in the neutral lipid fraction, and less than 3% was found in the free fatty acid fraction. The distribution between lipid pools was no different after transpulmonary injections of SRS-A, bradykinin (unsensitized lungs) or ovalbumin (sensitized lungs) with or without BSA enrichment of Krebs perfusate.

When fatty acid methyl esters were prepared from the phospholipid fraction (50 min perfusion) and analysed on t.l.c. plates impregnated with silver nitrate, 80% of the radioactivity co-chromatographed with methyl arachidonate and 15% with methyl esters of less unsaturated fatty acids. The distribution of radioactivity within the phospholipid, neutral lipid and free fatty acid fractions was determined by further t.l.c. analysis (30 min perfusion, average of 2 experiments). Radioactivity in phospholipids was associated with phosphatidyl choline (PC, 47%), phosphatidyl ethanolamine (PE, 21%) and other phosphatides (31%) which were mainly phosphatidyl inositol (PI) and phosphatidyl serine (PS) (chloroform:methanol: acetic acid:water; 50:30:3:2). Less than 1% of the radioactivity was associated with cyclo-oxygenase products (chloroform:methanol:acetic acid; 90:10:1). An example of the separation of phospholipids in chloroform:methanol:water is shown in Figure 2. Whereas PC was well separated from PE but incompletely from PI + PS in this solvent system, it was clearly separated from PI + PS but incompletely from PE in the second solvent system (chloroform: methanol:ammonia:water). Radioactivity in neutral lipids consisted mainly of triglycerides (62%) and diglycerides (28%); very little radioactivity was found in methyl or cholesteryl esters (less than 2%) or in monoglycerides (less than 1%). The majority of radioactivity in the free fatty acid fraction co-chromatographed with arachidonic acid (72%) and hydroxy fatty acids (9%); some of the 7% which remained near the origin may have been cyclo-oxygenase products. When [^{14}C]-AA was added to non-radioactive lungs before extraction, 98.3% of the radioactivity was recovered in the free fatty acid fraction, thus excluding possible conversion to methyl esters during the extraction procedure (Gordon, Philippon, Borgen & Kern, 1970).

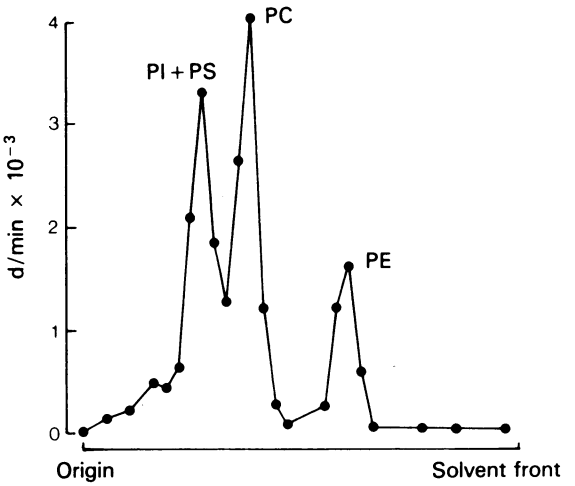

Figure 2 Thin layer chromatography analysis of phospholipid fraction of lungs 30 min after [^{14}C]-arachidonic acid infusion. The plate was developed in chloroform:methanol:water (80:30:5) to separate phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) from a mixture of phosphatides including phosphatidyl inositol (PI) and phosphatidyl serine (PS).

Table 1 Retention of radioactivity and distribution in lungs after a 3.5 min infusion of arachidonic acid (AA)

Transpulmonary injections	*Perfusion time†* (*min*)	*Retention* (*% infused*)	*Distribution (% retained)* *Phospholipid*	*Neutral lipid*	*Free fatty acid*
Nil	30	62 ± 1*	77 ± 1	20 ± 1	2.1 ± 0.4
Nil	50	56	85	13	1.9
Ovalbumin	50		81	17	1.6
Bradykinin × 2‡	86		88	10	1.3
SRS-A × 2‡	100		88	11	0.6
SRS-A × 3	120	55	87	12	0.5
Nil	120	53	89	9	0.9

* Mean ± s.e. mean, $n = 4$. † From start of [^{14}C]-AA infusion; ‡ During infusion of BSA 1%.

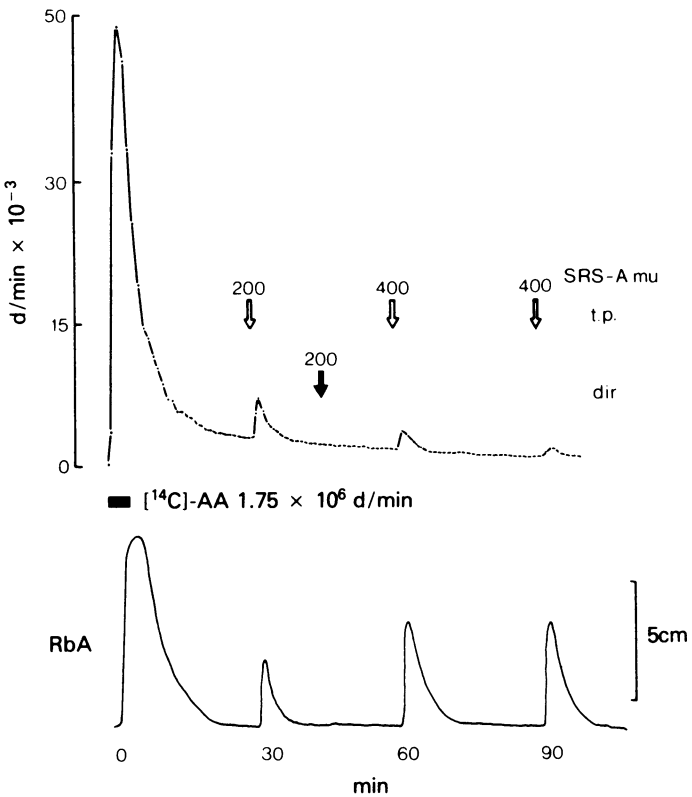

Figure 3 Release of radioactivity and rabbit aorta contracting substance (RCS) from guinea-pig isolated perfused lungs. During infusion of [^{14}C]-arachidonic acid ([^{14}C]-AA) there was a large efflux of radioactivity from the lungs (upper panel) coinciding with the formation of RCS as detected by the rabbit aorta strip (RbA). After 30 min, transpulmonary (t.p.) injection of slow-reacting substance of anaphylaxis (SRS-A) released RCS (lower panel) but only a small amount of radioactivity (upper panel). Direct (dir) administration of SRS-A (black arrow) did not contract RbA. Two larger t.p. doses of SRS-A (400 mu) at 60 and 90 min released correspondingly greater and constant amounts of biological activity but diminishing amounts of radioactivity.

Release of incorporated radioactivity

Injections of SRS-A into unsensitized lungs (6 experiments) released RCS and PGLS (matched on RSS by 1.5 min infusions of prostaglandin E_2 in range 5 to 20 ng/ml) into the lung effluent. However, there was only a small release of radioactivity (increase above basal release represents less than 1% total lung radioactivity) coinciding with this release of biological activity. The results of a typical experiment with transpulmonary (t.p.) injections of SRS-A are shown in Figure 3. At 30 min intervals after the first t.p. injection of SRS-A (200 mu), two larger t.p. doses of SRS-A (400 mu) released correspondingly greater amounts of RCS and PGLS (response of RSS not shown) than after SRS-A (200 mu), but progressively decreasing amounts of radioactivity. Similar results were obtained in a further experiment with bradykinin in which repeated t.p. injections (2 μg) released similar amounts of RCS and PGLS with each injection but diminishing amounts of radioactivity. When repeated t.p. injections of SRS-A (1.5 u) were followed by t.p. injections of bradykinin (3 to 6 μg), the pattern of diminishing release of radioactivity despite similar release of biological activity was still evident. Although 1% BSA increased the basal efflux of radioactivity from the lungs, it did not enhance the small release of radioactivity following injections of SRS-A or bradykinin.

Lung effluent collected for 6 min after an injection of SRS-A was analysed by t.l.c. (Figure 4). The two major peaks of radioactivity co-chromatographed with thromboxane B_2 and with 13, 14-dihydro-15-oxo-PGE_2. Other prostaglandin metabolites with similar R_F values to 13, 14-dihydro-15-oxo-PGE_2 in this solvent system include 15-oxo-PGE_2, 15-oxo-

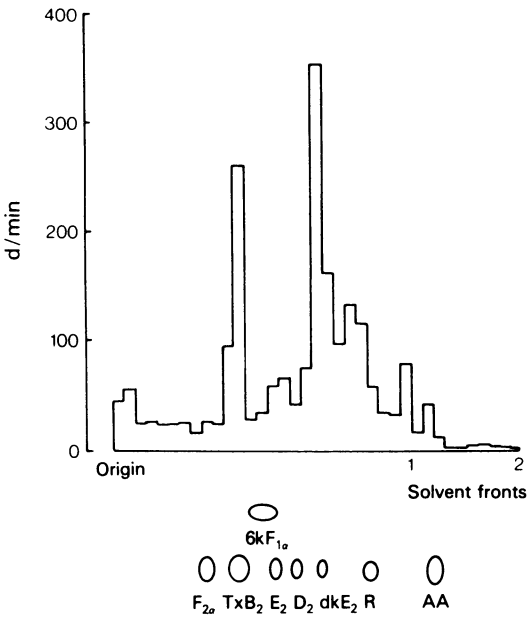

Figure 4 Thin layer chromatography analysis of lung effluent collected for 6 min after injection of SRS-A into lungs labelled with [^{14}C]-arachidonic acid. The plate was developed in chloroform:methanol:acetic acid (90:10:1) to 13 cm, then in hexane:diethyl ether:acetic acid (50:50:1) to 18 cm. The following standards were used: arachidonic acid (AA), ricinoleic acid (R), 13, 14-dihydro-15-oxo-PGE_2 (dkE_2), PGD_2, E_2 and $F_2\alpha$, thromboxane B_2 (TxB_2) and 6-oxo-$PGF_{1\alpha}$ ($6kF_{1\alpha}$).

PGE_1 and 13, 14-dihydro-15-oxo-$PGF_{2\alpha}$ (results not shown). The sum of radioactivity in zones corresponding to 6-oxo-$PGF_{1\alpha}$, prostaglandins $F_{2\alpha}$, E_2 and D_2 and AA amounted to less than 10%.

In sensitized lungs (2 experiments), injection of ovalbumin produced substantial release of RCS and PGLS (matched on RSS by infusions of PGE_2 20 to 50 ng/ml) and small amounts of radioactivity (less than 0.5% total lung radioactivity). This small release was greater than the release of radioactivity from unsensitized lungs following injection of ovalbumin (less than 0.2% total lung radioactivity). After pretreatment of the lungs with indomethacin (1 μg/ml), antigen challenge produced a three fold increase in SRS-A generation but a smaller release of radioactivity and no RCS or PGLS were detected.

Discussion

These results show that following infusion of [^{14}C]-AA into guinea-pig isolated lungs, the majority of radioactivity was retained in lung tissue. More than 70% of this retained radioactivity was incorporated in phospholipids and smaller amounts were found in neutral lipid and free fatty acid fractions. Radioactivity recovered in the free fatty acid fraction was 7% after 10 min (Al-Ubaidi *et al.*, 1978), 2% after 30 min and less than 1% after 100 min. This time-dependent depletion of radioactivity from the free fatty acid fraction may have been due to continuing incorporation into phospholipids or metabolism of AA by cyclo-oxygenase and subsequent loss in the lung effluent. Further analysis of the phospholipid fraction indicated that 80% of radioactivity was still arachidonate and approximately half of this was in phosphatidyl choline.

The present results in guinea-pig lungs are similar to findings in other experimental models. Isakson *et al.* (1976) demonstrated that 84% of radioactivity removed from the vascular bed of rabbit hearts and kidneys during infusion of [^{14}C]-AA was incorporated into phospholipids. Although the uptake of radioactivity by human platelets from platelet-rich plasma containing trace amounts of [^{14}C]-AA varied between 12 to 40%, approximately 90% of incorporated radioactivity was in the phospholipids (Bills, Smith & Silver, 1976). When guinea-pig spleen slices were incubated with [^{14}C]-AA for 30 min, the radioactivity was mainly incorporated into phospholipids (Flower & Blackwell, 1976). Hence, despite the use of different techniques and different tissues in each of these studies, there was a consistent finding that the majority of incorporated [^{14}C]-AA was found in the phospholipid fraction.

In the present experiments approximately 20% of infused radioactivity had emerged from the lung after 50 min. Rapid metabolism of some of the infused AA to cyclo-oxygenase products was demonstrated by the following: bioassay detected RCS and PGLS in lung effluent; t.l.c. analysis showed that only 6% of effluent radioactivity was unaltered AA; indomethacin (2 μg/ml) inhibited RCS and PGLS production and greatly reduced effluent radioactivity. However, only 9% of infused radioactivity had emerged from the lung after 5 min, indicating that conversion to cyclo-oxygenase products accounted for only a small proportion of infused AA. This finding is consistent with a previous study of guinea-pig isolated lungs (Hamberg *et al.*, 1976) in which less than 10% of injected AA appeared as metabolites in the effluent.

Having demonstrated uptake of [^{14}C]-AA by the lung and predominant incorporation into phospholipid, we studied its availability for subsequent metabolism by cyclo-oxygenase. In order to stimulate AA metabolism, we used partially purified SRS-A, repeated injections of which release similar amounts of RCS and PGLS on each occasion (Engineer *et al.*, 1977). Although the total turnover of AA induced by

SRS-A (as measured by bioassay) was substantial (of similar magnitude to turnover occurring during AA infusion in some experiments), simultaneous efflux of radioactivity from the lung was always small, representing less than 1% of the total lung radioactivity. Our attempts to augment effluent radioactivity during stimulation by 'trapping' released AA with BSA (Isakson, Raz, Denny, Wyche & Needleman, 1977) were unsuccessful.

Small amounts of radioactivity were also released from [^{14}C]-AA-labelled rabbit hearts with hormonal stimulation (Isakson *et al.*, 1976). During bradykinin infusion, the increase of radioactivity above basal release was only 2000 ct/min although the hearts had retained 88% of 5 μCi AA (equivalent to $\sim 8 \times 10^6$ ct/min). In contrast to the small release observed in guinea-pig lungs and rabbit hearts, when labelled platelets were aggregated with thrombin, there was a major decrease in phospholipid radioactivity coinciding with the appearance of labelled oxygenated products of AA (Bills *et al.*, 1976). A significant reduction (10 to 20%) in phospholipid AA was also evident after mechanical vibration of [^{14}C]-AA-labelled guinea-pig spleen slices (Flower & Blackwell, 1976). These marked differences in release of radioactivity may have reflected the varying stimuli used. They may also be related to differences between the tissue types studied.

The small release of radioactivity from guinea-pig lungs with SRS-A stimulation may have been due to dilution of [^{14}C]-AA with unlabelled endogenous AA. Alternatively, incorporation of [^{14}C]-AA may have occurred predominantly in one cell population and SRS-A-induced AA metabolism may have occurred predominantly in another. A more interesting effect was observed with repeated t.p. injections of SRS-A. The total amount of cyclo-oxygenase products released with each injection of SRS-A was similar but the efflux of radioactivity diminished. This progressive diminution could not be attributed to depletion of total lung radioactivity since large amounts were subsequently recovered from the lungs but may have been related to the time-dependent depletion of radioactivity in free fatty acids. Thus, we concluded that within 2 h of infusion the newly incorporated AA had not equilibrated with the pool stimulated by SRS-A. It is not known whether this non-equilibration reflected preferential distribution of infused AA between different cell populations or between different subcellular pools. It may be possible to achieve a more favourable distribution for subsequent release by injections of SRS-A after time intervals greater than 2 h but gradual accumulation of interstitial fluid during prolonged perfusion may render the lungs unsuitable for further experimentation. Our results with two other stimulators of AA metabolism (i.e. bradykinin in unsensitized and antigen challenge in sensitized lungs) were no different from the above findings with SRS-A and experiments in which two stimuli (i.e. SRS-A and bradykinin) were given to the same lungs did not indicate whether SRS-A and bradykinin were activating different pools of arachidonic acid.

Although there was only a small release of radioactivity following t.p. injections of SRS-A, t.l.c. analysis of the lung effluent showed two major peaks, one of which co-chromatographed with thromboxane B_2 and the other with 13, 14-dihydro-15-oxo-PGE_2. However, this solvent system does not resolve the numerous pulmonary metabolites of prostaglandins produced by 15-hydroxy prostaglandin dehydrogenase activity. We suggest that a large proportion of this unidentified peak of radioactivity may be a metabolite(s) of thromboxane B_2. Dawson, Boot, Cockerill, Mallen & Osborne (1976) reported that the major metabolites of endogenous AA in guinea-pig lungs during anaphylaxis were thromboxane B_2 and its 13, 14-dihydro-15-oxo-metabolite. Bioassay techniques have also revealed qualitative similarities between activation of AA metabolism following t.p. injections of partially purified SRS-A and anaphylaxis (Engineer *et al.*, 1977). It is possible that the nature of the stimulus does not influence the pattern of endogenous AA metabolism in the lung.

Recently it has been reported that rat basophilic leukaemia cells released slow-reacting substance (SRS) during incubation with calcium ionophore A23187 (Jakschik, Falkenhein & Parker, 1977). When radio-labelled AA was included in the incubation medium, a small percentage of the radioactivity was recovered in the SRS-containing fractions, suggesting that SRS may have been synthesized from arachidonic acid. Both calcium ionophore-induced SRS and SRS-A can be produced by guinea-pig lung (Piper & Seale, 1978) and, to date, it has not been possible to differentiate between these two slow-reacting substances. Therefore, we investigated the possibility that incorporated [^{14}C]-AA may be a precursor for SRS-A production in guinea-pig lungs. However, indomethacin, which has been shown to potentiate SRS-A generation (Engineer *et al.*, 1978), increased three fold the formation of SRS-A with a decrease in effluent radioactivity, suggesting that recently incorporated AA is not involved in SRS-A synthesis. Similar inferences were drawn from experiments in which cromoglycate reduced SRS-A production with no effect on radioactivity recovered from lung effluent (Dawson & Tomlinson, 1974).

Studies of the transformation of exogenous AA during passage through the pulmonary circulation permit some observations concerning the metabolism of AA but it may not be correct to assume that endogenous AA is metabolized in a similar fashion. Since many of the pathophysiological situations which we endeavour to investigate involve metabolism of endogenous AA,

a technique for radioactive labelling of endogenous pools would be desirable. It was possible to incorporate [^{14}C]-AA into the phospholipids of guinea-pig isolated lungs but its release by various stimuli up to 2 h after incorporation did not closely reflect total AA turnover.

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