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Immunology

Precursor role of arachidonic acid in release of slow reacting substance from rat basophilic leukemia cells*

(ionophore A23187/eicosatetraynoic acid/indomethacin/fatty acids)

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ABSTRACT **The release of slow reacting substance (SRS) from rat basophilic leukemia cells (RBL-1) by the ionophore A23187 (5–10 μg/ml) was stimulated 5-fold by arachidonate and inhibited 78% by 5,8,11,14-eicosatetraynoate (an inhibitor of both fatty acid cyclooxygenase and lipoxygenase). Linoleic acid and linolenic acid both inhibited SRS formation, whereas indomethacin (a cyclooxygenase inhibitor) had no effect. Radiolabel from [^{14}C]- or [^{3}H]arachidonate was incorporated into SRS as indicated by comigration of radioactivity and bioreactivity in several chromatographic systems after purification to apparent radiochemical homogeneity. The radiolabeled SRS was clearly separated chromatographically from other known arachidonate metabolites. Thus, SRS appears to be a previously undescribed product of arachidonic acid metabolism, probably formed through the lipoxygenase pathway. The ability to prepare purified, biosynthetically labeled, SRS should be of considerable help in further studies of its structure, biologic function, and catabolism.**

Slow reacting substance (SRS) is a polar acidic lipid (or group of related lipids) of unknown structure with potent contractile activity for bronchial and ileal smooth muscle. It is readily distinguished from prostaglandins (PGs), thromboxane A_2, and other agents known to contract smooth muscle by its spectrum of muscle activity (1), inhibition by the specific SRS antagonist FPL 55712 (2), and various chromatographic and physicochemical criteria (3, 4). SRS is formed and released from lung, leukocytes, and other tissues during IgE-mediated allergic reactions as well as in response to a variety of nonimmunologic stimuli, the most potent of which is the calcium ionophore A23187 (1, 5–9). We have recently demonstrated that a line of rat basophilic leukemia cells (RBL-1) (10) with receptors for IgE (11) releases considerable quantities of SRS upon stimulation with A23187 (12, 13). The SRS from RBL-1 cells is very similar or identical in its biological and chromatographic properties to previously described SRSs from various cat, rat, or human tissues (12). With the availability of a cell line that makes large amounts of SRS, studies of SRS structure and biosynthesis should be considerably facilitated. Because SRS is an acidic lipid, and metabolites of arachidonic acid (AA) have recently been recognized as showing a wide range of biological and biochemical properties (reviewed in ref. 14), we have studied the possible role of AA in SRS formation in RBL-1 cells. Strong evidence implicating AA as a biosynthetic precursor of SRS will be described.

MATERIALS AND METHODS

Reagents and their sources were as follows: unlabeled fatty acids (>99% purity, Nu Chek Prep); [^{3}H]AA, [^{3}H]oleic acid, [^{3}H]-stearic acid, [^{3}H]palmitic acid, [^{3}H]$PGF_{1\alpha}$, and [^{14}C]AA, [^{14}C]linolenic acid, [^{14}C]lauric acid, and [^{14}C]PGE_2 (Amersham/Searle or New England Nuclear); 5,8,11,14-eicosatetraynoic acid (ETYA) (gift of Roche Laboratories); A23187 (gift of R. L. Hamill, Eli Lilly Research Laboratories); indomethacin (gift of Merck, Sharp, and Dohme); and phospholipids (Sigma). A23187 was dissolved in dimethylsulfoxide and diluted with incubation medium (see below) immediately before addition to cells. Over the concentration range present in our incubation mixtures dimethylsulfoxide itself had no effect on SRS formation. None of the reagents used in this study, including AA, had significant effects in our standard guinea pig ileal bioassay system.

RBL-1 cells were grown in Eagle's minimum essential medium as described by Kulczycki and Metzger (11). The cells were washed and incubated in the experimental medium (150 mM NaCl/3.7 mM KCl/3 mM Na_2HPO_4/3.5 mM KH_2PO_4/0.9 mM $CaCl_2$/5.6 mM dextrose, with or without 0.1% bovine serum albumin), usually at a density of 10^7 cells per ml, at 37° in duplicate or triplicate with the reagents stated. At the completion of the reaction the supernatants were extracted with ethanol, dried, and stored at −80° (12).

Human platelets were isolated by the method of Baenziger and Majerus (15) and incubated at a concentration of 2×10^9 cells per ml in the incubation medium used with RBL-1 cells.

Methods for the purification of SRS and other AA metabolites on Amberlite XAD-7 and various-sized silicic acid columns are described in detail elsewhere (3, 4, 12, 16). Samples from 5 to 10×10^7 cells were applied in aqueous solution at pH 7 to 15-ml Amberlite XAD-7 columns. The columns were washed with 30 ml of water and the SRS activity was eluted with 30 ml of 80% (vol/vol) ethanol (average recovery of SRS bioreactivity of 90 ± 12%, SEM, $n = 9$). For silicic acid column chromatography, samples from 5 to 10×10^7 cells were applied in a small volume of methanol/chloroform (10:90, vol/vol) to columns containing 0.5 g of silicic acid in chloroform at room temperature (16). The columns were developed successively with 10 ml of chloroform (fatty acids, cholesterol, triglycerides, 12-hydroxyheptadecatrienoic acid, 12-hydroxy-AA, and other hydroxy fatty acids), 10 ml of 5% (vol/vol) methanol in cholorform ($PGF_{2\alpha}$, PGE_2, PGA_2, and thromboxane B_2), and 6 ml of methanol followed by 4 ml of 1% (vol/vol) water in methanol (SRS and phospholipids) (16). The recovery of SRS bioreactivity from the silicic

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Abbreviations: SRS, slow reacting substance; RBL-1, rat basophilic leukemia cells; AA, arachidonic acid; ETYA, 5,8,11,14-eicosatetraynoic acid; PG, prostaglandin.

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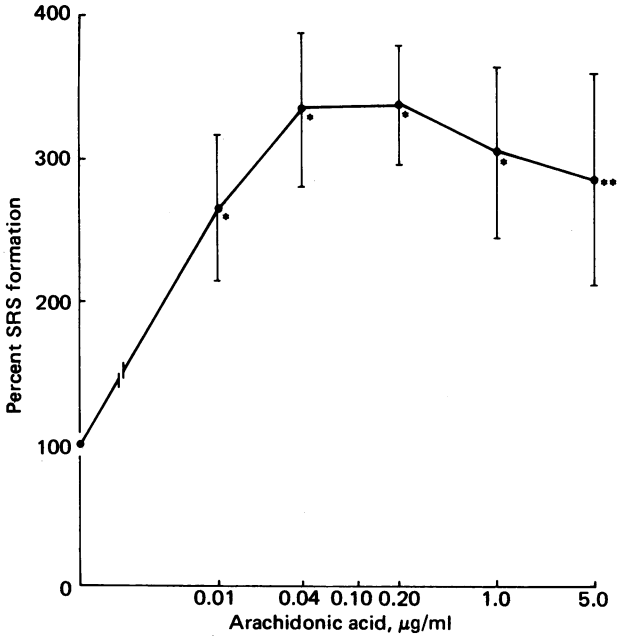

FIG. 1. Effect of arachidonic acid on SRS formation by A23187. RBL-1 cells, 10^7/ml, were incubated at 37° for 15 min in medium without albumin, with A23187 at 5 or 10 μg/ml, and without or with AA, at the concentrations indicated. The results of eight experiments (mean ± SEM) are expressed as percent of response to A23187 alone. * $P < 0.001$. ** $P < 0.02$.

acid column was 74–100%. The behavior of the various AA metabolites was verified by parallel silicic acid column and thin-layer chromatography studies with ^{14}C-labeled AA metabolites obtained biosynthetically from human platelets as well as with standard preparations of labeled AA, $PGF_{1\alpha}$, and PGE_2.

One- or two-dimensional thin-layer chromatography was performed in the dark at 24° on silica gel G 20-cm × 20-cm plates (Brinkmann) or 25-mm × 75-mm glass slides in the presence of the antioxidant 2,6-di-*t*-butyl-*p*-cresol (Fisher), keeping the drying period to a minimum. Nine different solvent systems were evaluated [solvents I, II, IV, and V of Strandberg and Uvnäs (3), solvent system C of Nugteren (17); the organic phase of ethyl acetate/trimethylpentane/water (75:150:150, vol/vol) (18) on samples esterified with diazomethane; chloroform/methanol/13.5 M NH_4OH/water (70:30:4:1, vol/vol) (16); and the two solvent systems described by Masuzawa *et al.* (19)]. After development with solvent, chromatographic plates were immediately placed together with Kodak X-omat film in lead cassettes in a nitrogen atmosphere and allowed to develop for various time periods at 24° or −70°. Areas with and without radioactivity were eluted with methanol, the radioactivities of aliquots were determined [by liquid scintillation in Scintiverse (Fisher) on a Nuclear Chicago instrument], and the remainders of the samples were evaluated for bioreactivity. DEAE-cellulose chromatography was performed as described by Takahashi *et al.* (figure 2, lower panel of ref. 9) and in the legend to our Fig. 5. Samples of SRS purified by two-dimensional thin-layer chromatography were hydrolyzed in 0.1 M NaOH containing 5% (vol/vol) methanol at 37° for 30 min (conditions that hydrolyzed phospholipids).

Smooth muscle contracting activity was measured by bioassay on sections of terminal guinea pig ileum bathed in Tyrode solution containing 0.5 μM atropine sulfate and 1 μM pyrilamine maleate (12). Throughout the paper, the terms *SRS* and *ileal* or *biological activity* refer to the contractile response in the guinea pig ileal system. Crude extracts of A23187-stimulated cells sometimes appeared to contain small amounts of prostaglandin-like reactivity as indicated by contractile responses on rat stomach muscle preparations. However, five lines of evidence indicated that the great bulk of the ileal reactivity is due to SRS (12): (*i*) the character of the ileal smooth muscle response (a slow, sustained contraction typical of SRS), (*ii*) marked inhibition by FPL 55712 (a selective SRS antagonist) with a mean inhibitory concentration of 5.7 ± 0.45 ng/ml (12), (*iii*) the relatively weak contractile activity of prostaglandins for ileal muscle as opposed to the other smooth muscle preparations, (*iv*) good recovery of ileal reactivity in polar fractions off silicic acid (prostaglandins and thromboxanes are eluted in less polar fractions), (*v*) an ability of indomethacin to reduce the generation of contractile activity for rat stomach but not guinea pig ileum (see below).

Table 1. Effect of fatty acids on SRS formation

Fatty acid	Units of SRS activity −A23187	Units of SRS activity +A23817	Percent of response to A23187 alone	*P*
None	2 ± 1	140 ± 38	100	
Arachidonic	59 ± 39	674 ± 307	543 ± 166	<0.007*
Dihomo-γ-linolenic	3 ± 1	252 ± 95	257 ± 151	0.3
Arachidic	10 ± 6	144 ± 59	157 ± 97	0.6
Linoleic	5 ± 1	42 ± 18	27 ± 11	<0.001†
Linolenic	15 ± 8	36 ± 21	26 ± 15	<0.001†

RBL-1 cells, 10^7/ml, were incubated in medium with and without fatty acid (25 μg/ml), A23187 (5 μg/ml), or both. Data are mean ± SEM of five experiments (three with 0.1% albumin and two without). Experiments with and without albumin were pooled because the data were very similar. The incubation time was 15 min.
* Statistically higher than A23187 alone.
† Statistically lower than A23187 alone.

RESULTS

As described in detail elsewhere (12), the divalent cation ionophore A23187 produces a dose- and time-related release of SRS from RBL-1 cells. In the present study, it was found that in medium containing 0.1% bovine serum albumin the addition of AA (25 μg/ml), together with A23187 (5–10 μg/ml), enhanced SRS release 466 ± 114% (SEM, $n = 9$). Because albumin binds AA, the effect of different concentrations of AA in enhancing A23187 stimulation was studied in albumin-free medium. The enhancement was concentration dependent (Fig. 1), with a maximal response at 0.04 and 0.2 μg AA/ml [an increase in ileal reactivity over A23187 alone of 336 ± 46% (mean ± SEM, $n = 8$)]. Significant stimulation was seen at AA concentrations as low as 10 ng/ml. By itself AA produced small amounts of SRS (Table 1), as might be the case if AA is a substrate for an SRS-producing enzyme, but the enzyme is largely inactive unless A23187 is present. Control studies in which AA was added to the medium immediately before harvesting the cells, to the separated cell supernatant after completion of the incubation, or directly into the bioassay chamber indicated that the potentiation caused by AA was not due to a direct effect of AA in the ileal bioassay.

The effect of the AA analog ETYA (20:4), a competitive and irreversible inhibitor of AA metabolism that acts on both the lipoxygenase and cyclooxygenase pathways (reviewed in ref. 20), also was studied. With a preincubation time of 1 min, ETYA at 10 μg/ml produced a 78 ± 6% (SEM, range 63–93%, $n = 5$) inhibition of SRS response to A23187 (10 μg/ml). Responses to AA and A23187 in combination also were markedly (>80%) inhibited. By contrast, indomethacin (0.1–10 μg/ml, preincubation 15 min), a cyclooxygenase inhibitor (20) did not

Table 2. Effect of indomethacin on SRS formation

Indomethacin, μg/ml	% of control*
0.1	172 ± 54
0.4	126 ± 21
0.7	106 ± 10
1.0	87 ± 8
10	81 ± 15

RBL-1 cells, 10^7/ml, were preincubated for 15 min with or without indomethacin, at the concentration indicated. This was followed by a 15-min incubation with A23187 at 10 μg/ml.
* Mean ± SEM (n = 4).

consistently inhibit SRS formation either in the absence (Table 2) or in the presence of AA. These observations suggest that AA may indeed be a precursor of SRS but that the cyclooxygenase pathway is probably not involved.

In contrast to AA, linoleic (18:2) and linolenic (18:3) acids at 25 μg/ml inhibited SRS formation, whereas arachidic acid (20:0) had no effect (Table 1). Dihomo-γ-linolenic acid (20:3) produced a moderate but definite increase in SRS formation in three of five experiments, but when the data were analyzed together the increase was not statistically significant (P = 0.3). The inhibition by linolenic and linoleic acid was also demonstrated by a shift of the dose response curve to A23187. No effect on the dose response to A23187 was observed with dihomo-γ-linolenic and arachidic acid (data not shown). Under appropriate conditions, linoleic and linolenic acid inhibit both the cyclooxygenase and lipoxygenase pathways of AA metabolism (20), and therefore, their ability to reduce SRS formation is consistent with a role for AA as a metabolic precursor of SRS.

In order to determine whether AA is incorporated into the SRS molecule, [^{14}C]AA was incubated with RBL-1 cells in the presence or absence of A23187. Supernatants were extracted with ethanol and partially purified on silicic acid columns or successive Amberlite XAD-7 and silicic acid columns to remove AA, PGs, and other known AA metabolites. The results from two such experiments are shown in Table 3. When the cells were incubated with A23187, 3.6% (2.9 and 4.2%) of the radioactivity originally added to the cell incubation mixture was recovered in the polar SRS-containing fractions off the silicic acid column, a 6-fold increase over the radioactivity recovered

Table 3. Silicic acid column fractionation of SRS

Solvent	% recovery of applied material: cpm	% recovery of applied material: SRS reactivity
Chloroform (10 ml)	43 ± 4	0
Chloroform/methanol (19:1) (10 ml)	42 ± 4	0
Methanol (6 ml) methanol/1% water (4 ml)	12 ± 3	88 ± 14

RBL-1 cells, 7.5 × 10^7, were incubated at 37° for 5 min with 7.4 × 10^5 cpm of [^{14}C]AA (1 μM, specific activity 60 mCi/mmol) in the presence of A23187 at 5 μg/ml (n = 2). Cell supernatants were extracted with ethanol; 50% of the recovered radioactivity (2.67 × 10^5 and 2.5 × 10^5 cpm in the two experiments or about 1/3 of the original radioactivity) was applied to 0.5-g silicic acid columns. After correction to 100% of the sample the polar fraction contained 3.6% (2.9 and 4.2%) of the radioactivity originally present in the cell suspension. In parallel experiments with control (no A23187) cells the ethanol extract and the polar fraction off the silicic acid column contained an average of 5.4% (6 and 4.7%) and 0.6% (0.72 and 0.48%) of the radioactivity originally added to the cell suspension, respectively.

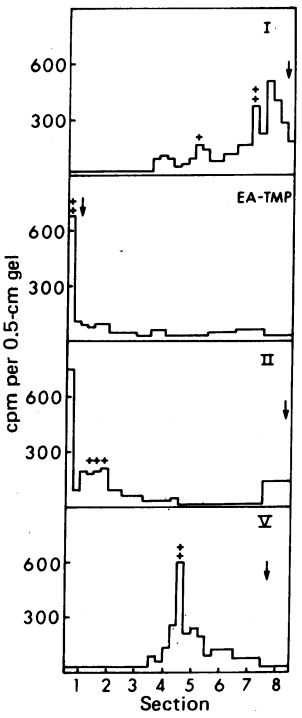

FIG. 2. SRS from cells stimulated with A23187 in the presence of [^{14}C]AA was purified on silicic acid columns and evaluated by thin-layer chromatography in solvent systems I, II, and V of Strandberg and Uvnäs (3) and the ethyl acetate/trimethylpentane/water system of Hamberg and Samuelsson (18) after esterification (designated EA-TMP in the figure). Chromatographs were divided into eight major portions and scraped, taking smaller sections in bioreactive areas. Eluted samples were divided for radioactivity counts and bioassay. Esterified samples were hydrolyzed with alkali prior to bioassay. Areas containing SRS reactivity are indicated by the + marks. Areas of $PGF_{1\alpha}$ migration are indicated by the arrows.

from control (no A23187) cells in this fraction (0.72 and 0.48%). In three experiments, ETYA reduced the radioactivity in the polar fraction from A23187-stimulated cells by 75 ± 3%.

Despite the obvious increase in release of radiolabeled polar AA metabolites from cells stimulated with A23187 there was not a linear relationship between SRS reactivity and radioactivity, suggesting considerable contamination, partial biologic inactivation, or both. Silicic acid thin-layer chromatography studies with partially purified preparations (silicic acid columns) revealed the presence of several radioactive bands. Despite considerable losses of ileal reactivity during chromatography in most of the solvent systems, correspondence between bioreactivity and a clearly defined radioactive band was demonstrable in each of the nine thin-layer chromatography systems studied. Results in four of the systems are shown in Fig. 2. SRS was resolved from $PGF_{1\alpha}$ in a number of these systems, as well as from PGE_2, AA, phospholipids, PGH_2 (a gift of Philip Needleman) and a number of products of AA metabolism obtained biosynthetically from human platelets (thromboxane B_2, 12-hydroxy-AA, 12-hydroxyheptadecatrienoic acid). Similar thin-layer chromatography results were obtained with partially purified SRS preparations from cells grown with radiolabeled AA overnight but not from cells grown with ^{14}C- or ^{3}H-labeled lauric, oleic, linoleic, palmitic, stearic, or linolenic acid or $^{32}PO_4^{2-}$.

After much effort a satisfactory separation from the point of view of both resolution and recovery was obtained in a two-dimensional system with propanol/ammonia/water (6:3:1, vol/vol) as the first solvent and propanol/water (3:1, vol/vol) as the second solvent. With appropriate precautions and sub-

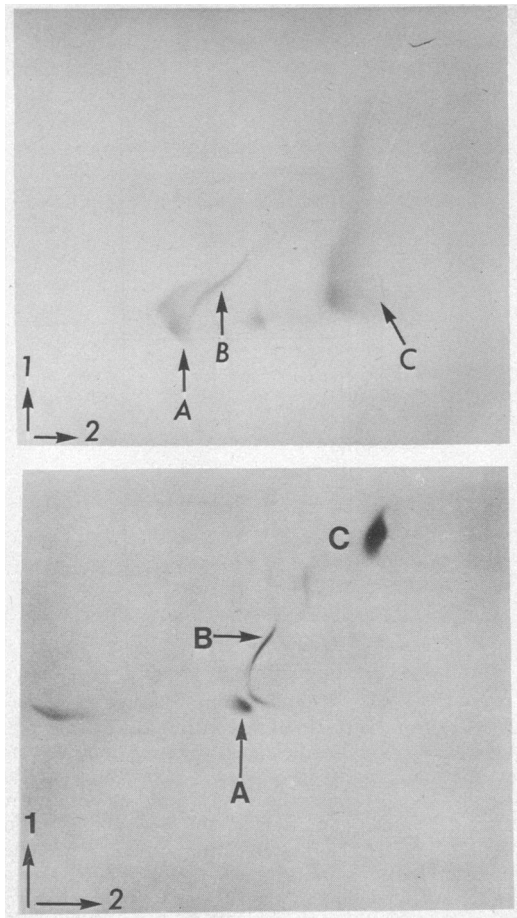

FIG. 3. Two-dimensional chromatography of SRS. (*Upper*) A ^{14}C-labeled SRS-containing sample (partially purified on a silicic acid column) containing 63,000 cpm and 40,000 units was applied in methanol to silica gel G 20-cm × 20-cm plates. Chromatography was performed in the dark with 2,6-di-*t*-butyl-*p*-cresol present. First solvent: propanol/ammonia/water (6:3:1); second solvent: propanol/water (3:1). After a brief drying period, the plate was placed together with Kodak X-omat R film in a lead cassette in a nitrogen atmosphere and allowed to develop for 3 days at 24°. Spot A contained 95% of the recovered bioreactivity, distributed over the entire area of the spot, and 14% of the recovered radioactivity; spot B, 4% and 7%, respectively; spot C, 0% and 35%, respectively. No bioreactivity was demonstrable in the other areas of the plate. (*Lower*) This is a similar experiment with 15,900 cpm and 3000 units. Spot A contained 93% of the recovered bioreactivity and 12% of the recovered radioactivity (the overall recovery of bioreactivity was 65% and of radioactivity 71%).

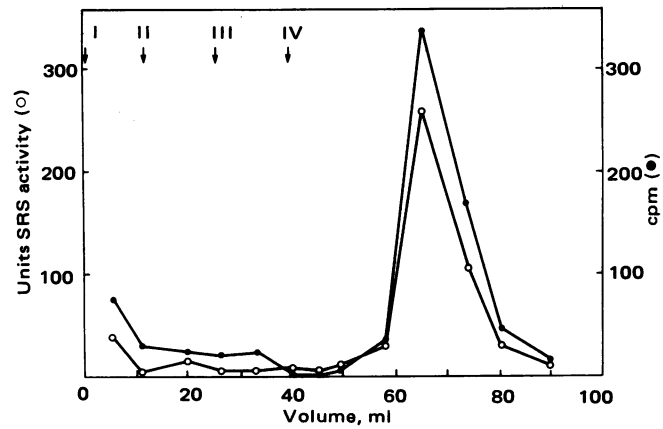

FIG. 4. DEAE-cellulose chromatography of purified SRS; ^{14}C-labeled SRS (purified by silicic acid and two-dimensional thin-layer chromatography) and unlabeled SRS (purified by Amberlite XAD-7 and silicic acid chromatography) were mixed, incubated in 5% methanol/0.1 M NaOH for 30 min at 37°, neutralized, and dried; an aliquot containing 700–750 bioassay units and 865 cpm in 1.7 ml of methanol/chloroform (1:7) was applied to a 7-ml DEAE-cellulose column packed in chloroform/methanol (7:1) and maintained at 4° in the dark. The column was eluted with four solvents: solvent I, 12 ml of chloroform/methanol (7:1); solvent II, 14 ml of chloroform/methanol (7:3); solvent III, 14 ml of methanol; solvent IV, 50 ml of methanol/2 M ammonium carbonate (10:1). A total of 15 fractions was collected. Points of addition of the different solvents are indicated by the arrows. After drying, samples were divided into equal parts for radioactivity measurement (10-min counting times) and bioassay. The values given in the figure are corrected (e.g., multiplied by 2, to correct for the use of only ½ the sample). Recovery of both applied radioactivity and bioreactivity was greater than 80%. Considering the inherent variation in the bioassay (±20%), statistical errors in counting, and possible spontaneous inactivation on the column, this represents excellent correspondence between radioactivity and bioreactivity.

stantial amounts of SRS reactivity loaded, greater than 80% recovery of radioactivity and ileal contracting activity is obtainable with this combination of solvents. In the experiment shown in Fig. 3 *upper*, spot A contained 95% and 14% of the recovered SRS reactivity and radioactivity, respectively (overall recoveries of 88% of SRS reactivity and 90% of the radioactivity applied to the two-dimensional chromatograph or 0.45% of the total AA radioactivity present in the original cell mixture). In Fig. 3 *lower*, involving a smaller number of units, 93% of the recovered ileal reactivity and 12% of the recovered radioactivity were in spot A. The overall recovery of SRS reactivity was 65% and radioactivity 71%. In three other experiments similar results were obtained with 10–14% of the radioactivity being recovered in the major SRS-reactive area. In the various experiments, areas immediately adjacent to the major SRS spot contained less than 1% of the ileal reactivity present within the spot itself, indicating that the distributions of radioactivity and bioreactivity correspond very closely. Spots corresponding to the major phospholipid classes were outside this area. Alkaline hydrolysis of material in the radioactive band resulted in some change in chromatographic behavior but no evidence for release of free AA, which is not what would be expected if the radioactivity were incorporated in the acyl portion of a phospholipid or lysophospholipid. Under comparable conditions most of the fatty acid present in phosphatidyl inositol and phosphatidyl choline was released. In addition to the major bioreactive spot a minor spot B containing 4% of the ileal reactivity but 7% of the radioactivity was found in a nearby but easily distinguishable area (Fig. 3 *upper*). Thus, there are at least two areas with SRS reactivity on the chromatograph, both of which contain radioactivity.

In control chromatographs with identically processed extracts from the supernatant or pellet of cells exposed to [^{14}C]AA in the absence of ionophore, no radioactivity was recognizable in the major bioreactive region. As an additional control, human platelets, which in our hands do not generate SRS bioreactivity, were exposed to various concentrations of A23187 (0.2, 1, 5, and 20 μg/ml) under conditions identical to those used with the RBL-1 cells and incubated over a range of time periods (0.5, 3, 5, and 15 min). Whole platelet extracts (80% ethanol) were evaluated chromatographically before and after acid/ether extraction or partial purification on silicic acid columns as described above. There was no evidence for a radioactive metabolite in the major SRS area analogous to that seen in RBL-1 cells.

The eluate from spot A, containing 14% of the radioactivity and 95% of the ileal bioreactivity, rechromatographed as a radiochemically homogeneous (>95%) substance in its original two-dimensional chromatographic system, again in the area

where biologically active SRS migrates. Apparently homogeneity and excellent correspondence of radioactivity and ileal reactivity was also seen on silica gel plates developed with propanol/water (3:1). When the eluate was mixed with unlabeled SRS and chromatographed on DEAE-cellulose, about 80% of the SRS reactivity and 80% of the radioactivity were recovered together as a single peak (Fig. 4).

DISCUSSION

From the evidence presented in this paper it is apparent that the SRS generated in RBL-1 cells by A23187 is derived directly from AA: (*i*) Unlabeled AA considerably increases the SRS response to A23187. (*ii*) When cells are exposed to both A23187 and radiolabeled AA, 0.1–0.5% of the added radioactivity copurifies with SRS. After purification to apparent radiochemical homogeneity, continued comigration of radioactivity and SRS reactivity is seen in each of the chromatographic systems examined. Purified samples from control cells or cells incubated with other radioactive fatty acids or [^{32}P]orthophosphate contain little or no radioactivity in the relevant areas of the chromatographs. The radioactive metabolite does not appear to be generated by human platelets and can be easily distinguished on chromatographic or pharmacological grounds from thromboxane B_2, thromboxane A_2, PGG_2, 12-hydroxy-AA, 12-hydroxyheptadecatrienoic acid, 5-hydroxy-AA, and common phospholipids. (*iii*) ETYA, linolenic acid, and linoleic acid decrease SRS generation.

Our results with ETYA are in contradistinction to those of Dawson and Tomlinson (21). They reported that ETYA did not cause a significant inhibition of SRS release by perfused lung preparations of antigenically stimulated guinea pigs. However, we are working with a single cell preparation in contrast to whole lung, a complex tissue. ETYA has very low water solubility and therefore the cells forming SRS in the lung could have been exposed to insufficient amounts of ETYA. On the other hand, in addition to our preliminary report that AA is a precursor of SRS in RBL-1 cells, Bach *et al.* have presented data suggesting that AA is also implicated in SRS formation in mixed rat peritoneal cells (22). Whatever the basis for the different results in our work and that of Dawson and Tomlinson (21), in RBL-1 cells, at least, SRS appears to be a novel product of AA metabolism.

With highly purified [^{14}C]SRS derived from [1-^{14}C]AA now available, tentative calculations as to the biological potency of SRS can be made, based on the specific activity of the [^{14}C]AA added to the original cell suspension, the ratio of bioreactivity and radioactivity in the purified product, and the assumptions that each molecule of radioactive SRS is derived from one molecule of radioactive AA with no loss of label and that there is no dilution of label by endogenous AA before incorporation. Assuming the validity of this calculation, one unit of SRS is derived from picogram quantities of AA, making SRS substantially more effective than histamine on a weight basis in the stimulation of guinea pig ileal smooth muscle. Utilizing a preparation of cat SRS obtained by perfusion of the paws with 48/80, Strandberg and Uvnäs estimated that 1 unit of SRS represents 1 ng or less of material (3). Their material was probably less purified than ours and had been subjected to acid/ether extraction, which may have reduced its biologic activity and may have structurally altered the molecule.

The unique chromatographic properties of SRS, particularly its relatively high polarity, suggest that it is not a close structural analog of other known AA metabolites. Whether SRS is generated through a series of enzymatic steps beginning with fatty acid cyclooxygenase or lipoxygenase or through involvement of a major new pathway remains to be seen. Over the concentration range examined, indomethacin does not significantly reduce SRS formation, making formation through the cyclooxygenase pathway unlikely. Lack of inhibition or even enhancement of SRS release by indomethacin and other antiinflammatory agents has also been noted by other investigators (23–26). Whatever the enzyme pathway involved, because SRS is synthesized by several cell types and is a potent mediator of smooth muscle reactivity, it represents still another AA metabolite of probable importance in inflammatory processes. Furthermore, the ability to prepare radiolabeled SRS of high specific activity should greatly facilitate future work on its structure, biological function, and catabolism.

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