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# Lymphocytes sensitize rat isolated atria to the inotropic and chronotropic effects of sodium arachidonate

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**1** Normal human lymphocytes ($4 \times 10^5$ ml$^{-1}$) incubated with sodium arachidonate ($8 \times 10^{-7}$ M) (NaA-L) induced a strong enhancement of the tension and frequency of spontaneously beating rat atria. Normal human lymphocytes (L) or NaA alone at $8 \times 10^{-7}$ M did not modify this contractile activity.

**2** Between $2 \times 10^{-6}$ M to $1 \times 10^{-5}$ M NaA alone increased the tension of the atria without effect on the rate. In the presence of L ($4 \times 10^5$ ml$^{-1}$) the dose-response curve to NaA shifted to the left, the potency and the efficiency of NaA were enhanced and the chronotropic action was triggered.

**3** Inhibitors of cyclo-oxygenase (indomethacin $1 \times 10^{-6}$ M or acetylsalicylic acid (ASA) $1.8 \times 10^{-4}$ M) completely blocked the positive inotropic effect induced by NaA alone. Inhibitors of lipoxygenase/s (nordihidroguaiaretic acid (NDGA) $1 \times 10^{-5}$ M or 5,8,11,14-eicosatetraynoic acid (ETYA) $1 \times 10^{-7}$ M did not modify this effect.

**4** Indomethacin and ASA did not block the positive inotropic and chronotropic effects of the lower concentration of NaA-L and significantly reduced the inotropic effect of the higher ones.

**5** NDGA and ETYA shifted to the right the inotropic and chronotropic dose-response curve to NaA-L. FPL-55712 ($1 \times 10^{-7}$ M), the slow reacting substance of anaphylaxis (SRS-A) antagonist, significantly reduced the overall inotropic and chronotropic effect of NaA-L.

**6** Direct contact of NaA-L with the atria was not necessary. Cell-free supernatants of L exposed to NaA increased the tension and the frequency of beating rat atria.

**7** The stimulatory effect of NaA-L supernatants did not occur if rat atria had been previously incubated with NDGA $1 \times 10^{-5}$ M. On the other hand, the generation of stimulatory products from NaA-L was not prevented by preincubating L with $1 \times 10^{-5}$ M NDGA. Hence SRS-A and/or other oxidative metabolites of arachidonic acid were produced by the atria.

**8** These results suggest that NaA-L react *in vitro* with spontaneous beating rat atria, inducing inotropic and chronotropic effects. Moreover, the stimulatory action of NaA itself was potentiated by L. These reactions involved a balance between cyclo-oxygenase and lipoxygenase oxidative products with a central role for SRS-A.

## Introduction

It is well established that the oxidative metabolism of arachidonic acid (AA) in the tissues occurs by at least two independent enzymatic pathways. In one, AA is transformed by a cyclo-oxygenase catalyzed reaction into endoperoxides and then prostaglandins and thromboxanes (Nugteren & Hazelhof, 1973; Hamberg & Samuelsson, 1974; Hamberg, Svensson & Samuelsson, 1975). In the second pathway, catalyzed by lipoxygenase(s), AA is converted to an unstable hydroperoxy acid and ultimately to a hydroxy acid, HETE and leukotrienes (Hamberg & Samuelsson, 1974; Nugteren, 1975). There is now evidence that slow reacting substance of anaphylaxis (SRS-A) is composed of leukotrienes $C_4$, $D_4$ (Lewis, Austen, Drazen, Clark, Marfat & Corey, 1980) and $E_4$ (Welton, Growley, Miller & Yaremko, 1981) and that these substances have a positive inotropic effect on guinea-pig isolated atria (Terashita, Fukui, Hirata, Terao, Ohkawa, Nishikawa & Kiruchi, 1981).

In addition, during the arrythmia observed in cardiac anaphylaxis, SRS-A, thromboxane $A_2$ and other vasoactive substances have been detected in the

coronary perfusate (Liebig, Beurnaur & Pesker, 1975). A positive chronotropic effect of AA on guinea-pig atria has been reported by Borbola, Susskand, Siess & Szekeres (1977) and it was attributed to peroxides produced by its metabolism. The administration of AA to the isolated perfused heart of the rat usually produced biphasic responses due to its biotransformation into active metabolic products (Belo & Talesnik, 1982).

Studies concerning the biosynthesis of prostaglandins and related lipids by lymphocytes provide equivocal data (Goldyne & Stobo, 1981). There are numerous reports that cyclo-oxygenase metabolites of AA are involved in the regulation of lymphocyte differentiation (Parker, Kelly, Falkenheim & Huber, 1979a; Parker, Stenson, Huber & Kelly, 1979b). Much of the information on these systems has come from the use of inhibitors of cyclo-oxygenase. Kennedy, Stobo & Goldyne (1980) failed to demonstrate the presence of active cyclo-oxygenase in highly purified lymphocytes. However, Parker *et al.* (1979a,b) reported that both AA and its cyclo-oxygenase and lipoxygenase products, were released from human peripheral lymphocytes as a consequence of stimulation with mitogenic lectins. The modulatory effect of AA (Kelly & Parker, 1979a) or its products (Kelly, Johnson & Parker, 1979b; Parker *et al.*, 1979b) on lymphocyte replication was exerted at the early stages of lymphocyte activation. It has also been shown that inhibitors of cyclo-oxygenase and lipoxygenase may control the production of the lymphokine interleukin-2, which is involved in the process of T cell replication. Furthermore, AA bound hydrophobically to interleukin-2 had reduced biological activity (Henriksen & Frey, 1982). Thus it appears that AA metabolism is intimately related to lymphocyte function.

Recently we have demonstrated that normal human lymphocytes activated by a mitogenic lectin or by a $Ca^{2+}$ ionophore can react *in vitro* with rat atria increasing both the tension and the frequency of contractions by a mechanism involving the release of an active substance derived from AA via the lipoxygenase catalyzed pathway (Sterin-Borda, Borda, Fink & de Bracco, 1983).

In this study we investigated whether sodium arachidonate (NaA) could trigger the reaction between normal human lymphocytes and rat isolated atria replacing the initial triggering step provided by lectin stimulation of the lymphocyte's membrane.

It will be shown that addition of NaA plus lymphocytes (NaA-L) or of cell-free supernatants of NaA-L to the atria resulted in a marked stimulation of the contractile tension and frequency. Lipoxygenase metabolites were the effectors of this reaction. The same doses of NaA in the absence of lymphocytes were ineffective. We propose that lymphocytes, by means of soluble factors, sensitize the rat isolated atria to the effect of NaA and suggest that their presence favours the production of active lipoxygenase metabolites.

## Methods

### *Effector cells*

Peripheral mononuclear leukocytes (ML) were obtained by Ficoll Hypaque centrifugation of defibrinated normal human blood (Boyum, 1968). The interphase containing the ML was washed twice with PBS, once with RPMI 1640 tissue culture medium (Gibco Lab) buffered with 10 mM HEPES solution (Gibco Lab) containing 50 $\mu g\,ml^{-1}$ gentamycin (Schering Co Essex, Argentina) and 5% heat-inactivated foetal calf serum (Gibco Lab) (RPMI-FCS). The cells were counted in a Neubauer chamber. ML contained 90–98% mononuclear cells. ML ($10-20 \times 10^6\,ml^{-1}$) were incubated at 37°C for 18 h in plastic tissue culture bottles (Falcon Plastic) to eliminate adherent cells. The resultant lymphocyte suspension (L) contained less than 1% monocytes as determined by peroxidase staining. L were washed with RPMI-FCS three times and suspended at $8 \times 10^6\,ml^{-1}$ in the same medium. These cells were used in all the experiments as effector cells unless otherwise stated. Viability was tested by exclusion of Trypan blue and effector cells containing more than 80% viable cells were used.

### *Rat isolated atria preparations*

Male albino rats of the Wistar strain weighing between 220–250 g were used. The animals were killed by decapitation. The entire hearts were excised quickly and placed in Petri dishes filled with a modified Krebs-Ringer-Bicarbonate (KRB) solution, composed as follows (mM): $Na^+$ 145, $K^+$ 6.02, $Ca^{2+}$ 1.22, $Mg^{2+}$ 1.33, $Cl^-$ 126, $HCO_3^-$ 25.3, $SO_4^{2+}$ 1.33, $PO_4^{2+}$ 1.20 and glucose 5.5. The atria were separated from the ventricles, carefully dissected, attached to a glass holder and immersed in a tissue chamber filled with 15 ml KRB with or without the effector cells. The tissue bath solution was gassed with 5% $CO_2$ in $O_2$ and kept at a constant temperature of 30°C and pH 7.4 throughout the experiments. In each bath solution, antifoam (Sigma Chemical Co) was added. This agent did not modify any of the variables explored. One end of the preparation was anchored to a glass holder and the other connected to a force transducer coupled to an ink-writing oscillograph. A constant resting tension of 750 mg was applied to the atria by means of a micrometric device and the activity of the isolated spontaneously beating

atria was analysed in terms of tension and frequency of the contractions. The atria were allowed to beat for 150 min before the reaction. Records were then taken and their values considered as initial controls, were taken as 100%. The absolute values of tension (mg) and frequency (beats per min) (Table 1) agreed with previous reports (Sterin-Borda, Cossio, Gimeno, Gimeno, Diez, Laguens, Cabeza-Meckert & Arana, 1976; Sterin-Borda, Fink, Diez, Cossio & de Bracco, 1982).

Concentration-response curves for NaA (Sigma Chemical Co) were obtained with the method described by Van Rossum (1963). Single doses were contained in volumes of 0.002–0.005 ml of the appropriate stock solution. The total volume added to the bath never exceeded 0.1 ml. The time interval between doses was that for each individual dose to produce a maximal effect (15 min).

*Supernatants of lymphocytes incubated with sodium arachidonate*

Cell-free NaA-L supernatants were obtained by incubation of $8 \times 10^6$ L suspended in 1 ml of RPMI-FCS containing 4 μl of a freshly prepared $1.5 \times 10^{-2}$ M NaA solution, for 20 min at 37°C, followed by centrifugation at 800 *g* for 10 min. The sedimented cells were discarded and 0.5 ml of the NaA-L supernatants were used immediately. The final NaA concentraiton achieved in the 15 ml bath was $1.5 \times 10^{-6}$ M.

*Drugs*

Acetylsalicylic acid (ASA), sodium arachidonate (NaA) and nordihydroguaiaretic acid (NDGA) were purchased from Sigma Chemical Co; indomethacin from Merck, Sharp and Dohme; 5,8,11,14-eicosatetraynoic acid (ETYA) was donated by Hoffman La Roche and FPL-55712 (sodium 7-[3(4-acetyl-3-hydroxy-2-propylphenoxy) -2-hydroxy propoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate) by Fisons Ltd. Stock solutions of ASA, NaA and FPL-55712 were freshly prepared in distilled water, ETYA was dissolved in ethanol and NDGA in dimethyl sulphoxide (DMSO). The drugs were diluted in the bath to achieve the final concentrations shown in Table 1. At these dilutions the solvent had no effect *per se*.

## Results

*Effect of lymphocytes treated with sodium arachidonate on the activity of rat isolated atria*

Figure 1 shows that either $4 \times 10^5$ ml$^{-1}$ normal human lymphocytes (L) or NaA ($8 \times 10^{-7}$ M) added alone to the rat isolated atria preparation did not modify its contractile activity. On the contrary, when L were preincubated at 37°C for 30 min with NaA (NaA-L) at the concentrations given above, they induced a strong enhancement of the tension and the frequency of spontaneously beating rat atria. This effect developed with time and was maximal after 15 min of reaction for tension and at 5 min for frequency.

To determine the mechanism of the reaction of NaA-L with rat isolated atria, several inhibitors acting on different pathways of arachidonic acid metabolism that could result in stimulation of the frequency and tension of contraction of the rat atria were used.

The results, shown in Figure 2, demonstrate that the inhibition of cyclo-oxygenase activity by indomethacin or ASA did not block the positive inot-

**Table 1** Influence of different drugs on the spontaneous activity of rat isolated atria and on lymphocyte viability

| *Drugs* | *Tension* (mg) | *Frequency of contractions* (beats min$^{-1}$) | n | *Lymphocyte viability* (%) | n |
|---|---|---|---|---|---|
| None | 528 ± 32 | 140 ± 7.0 | 8 | 95 ± 3 | 6 |
| Indomethacin ($10^{-6}$ M) | 539 ± 47 | 135 ± 8.0 | 6 | 89 ± 3 | 4 |
| ASA ($1.8 \times 10^{-4}$ M) | 560 ± 73 | 136 ± 6.0 | 7 | ND | |
| NDGA ($10^{-5}$ M) | 525 ± 52 | 138 ± 10 | 6 | 89 ± 2 | 5 |
| ETYA ($10^{-7}$ M) | 496 ± 43 | 129 ± 9.0 | 6 | 89 ± 3 | 4 |
| FPL-55712 ($10^{-7}$ M) | 528 ± 38 | 129 ± 10 | 7 | 91 ± 2 | 7 |

Mean ± s.e.mean values of tension measured in mg. Frequency of contractions measured in number of beats per min and lymphocyte viability measured as percentage of viable cells after 1 h incubation with the different drugs. *n* = number of preparations. ASA: acetylsalicylic acid; NDGA: nordihydroguaiaretic acid; ETYA: 5,8,11,14-eicosatetraynoic acid; FPL-55712: sodium 7-[3(4 - acetyl - 3 - hydroxy - 2 - propylphenoxy) - 2 - hydroxyproboxy] - 4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate.

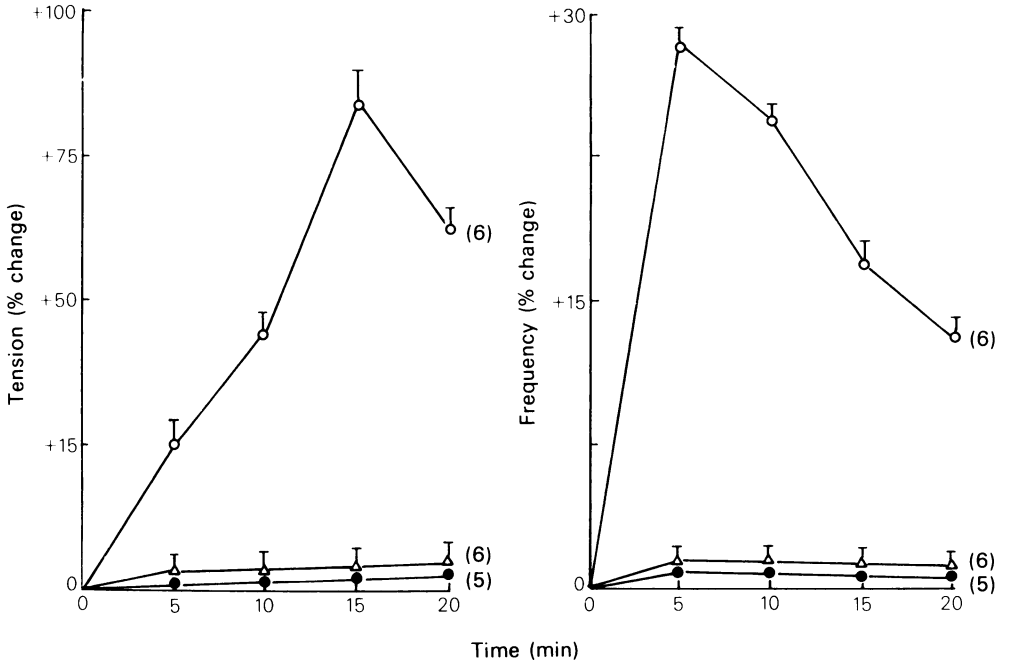


**Figure 1** Time course of the reaction of sodium arachidonate (NaA)-treated normal human lymphocytes with beating rat atria. Isolated atria were suspended in 15 ml of KRB solution to which was added: 1 ml of a $4 \times 10^6$ ml$^{-1}$ lymphocyte (L) suspension (●); 1 ml of a fresh dilution of $12 \times 10^{-6}$ M NaA in RPMI (△) or 1 ml of $4 \times 10^6$ L ml$^{-1}$ that had been preincubated for 30 min at 37°C with RPMI containing $12 \times 10^{-6}$ NaA (○). The final molarity of NaA in the organ bath was $8 \times 10^{-7}$ M. Changes in the tension and frequency are expressed as mean % of the initial KRB control values, s.e.mean shown by vertical lines. The number of experiments is indicated in parentheses.

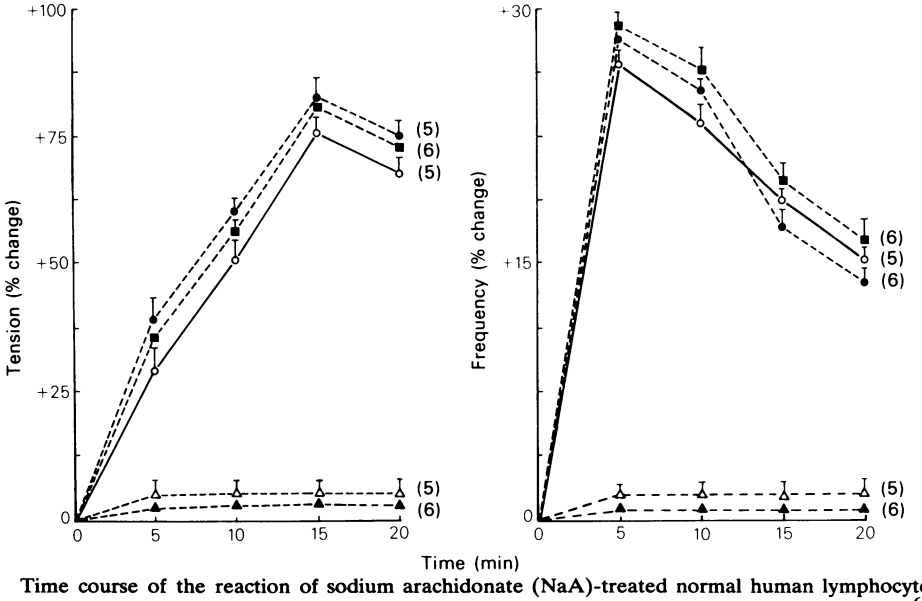


**Figure 2** Time course of the reaction of sodium arachidonate (NaA)-treated normal human lymphocytes on beating rat atria in the presence of inhibitors of arachidonic acid (AA) metabolism. Lymphocytes (L) at $4 \times 10^6$ ml$^{-1}$ that had been incubated for 30 min at 37°C in 1 ml RPMI containing $12 \times 10^{-6}$ M NaA were added to beating rat atria suspended in 15 ml KRB (○) or atria preincubated for 30 min in 15 ml KRB containing $1.8 \times 10^{-4}$ M acetylsalicylic acid (■) $1 \times 10^{-6}$ M indomethacin (●); $1 \times 10^{-5}$ M nordihydroguaiaretic acid (▲) or $1 \times 10^{-7}$ M ETYA (△). Details and conditions as in Figure 1.

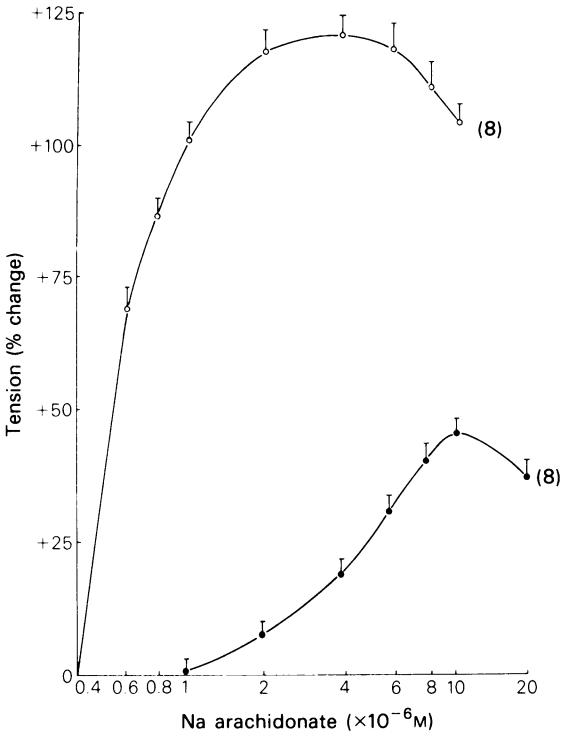


**Figure 3** Effect of sodium arachidonate (NaA) on beating rat atria in the presence or absence of lymphocytes. Rat isolated atria beating in 15 ml KRB were incubated for 30 min with 1 ml RPMI containing $4 \times 10^6$ L ml$^{-1}$ (○) or 1 ml RPMI (●). Increasing amounts of NaA were added as described in Methods.

ropic and chronotropic effects of NaA-L. On the other hand, an inhibitor of the lipoxygenase pathway of arachidonic acid metabolism (NDGA) interfered with the reaction of NaA on the atria. In addition, ETYA at concentrations that had no effect on cyclo-oxygenase activity, efficiently blocked both the inotropic and chronotropic effects of NaA-L.

*Dose-response curves to sodium arachidonate in presence and absence of lymphocytes*

Figure 3 shows dose-responses curves to NaA in the presence and in the absence of lymphocytes. It can be seen that in a range of $2 \times 10^{-6}$ M to $1 \times 10^{-5}$ M, NaA increased the tension of the atria. It had no effect on the frequency of contractions. In the presence of $4 \times 10^5$ L ml$^{-1}$, the dose-response curve to NaA shifted to the left and enhanced the potency and the efficacy of the drug. L triggered the chronotropic action of NaA (Figure 5).

As shown in Figure 4, preincubation of the atria with indomethacin or ASA, completely blocked the positive inotropic effect induced by the addition of NaA alone. In contrast, NDGA and ETYA did not modify this effect.

Figure 5 illustrates the influence of L on the inotropic and chronotropic effect of NaA. It can be seen that the inhibition of cyclo-oxygenase activity by ASA, did not modify the inotropic action of the lower concentrations of NaA and significantly reduced the inotropic effect of the higher ones. In atria that had been preincubated with FPL-55712, the inotropic dose-response curve to L plus NaA, shifted to the right and the overall effect was significantly reduced. The chronotropic effect was completely blocked. On the other hand, the chronotropic action observed with NaA in the presence of L was not altered by preincubation of the organ with ASA. Addition of both inhibitors at the same time, led to the disappearance of the positive inotropic effect observed with NaA plus L. With NDGA and ETYA the results were the same as with the antagonist of SRS-A (FPL-55712) (data not shown).

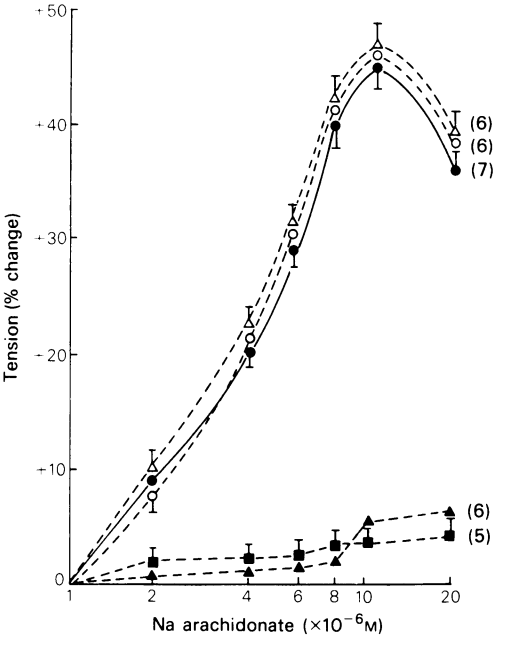


**Figure 4** Effect of inhibitors of arachidonic acid (AA) metabolism on the action of sodium arachidonate (NaA) on beating rat atria. Rat isolated atria were suspended in 15 ml KRB (●) or preincubated for 30 min with $1 \times 10^{-6}$ M indomethacin (■); $1.8 \times 10^{-4}$ M acetylsalicylic acid (▲); $1 \times 10^{-5}$ M nordihydroguaiaretic acid (○) or $1 \times 10^{-7}$ M ETYA (△). Increasing amounts of NaA were added as described in Methods.

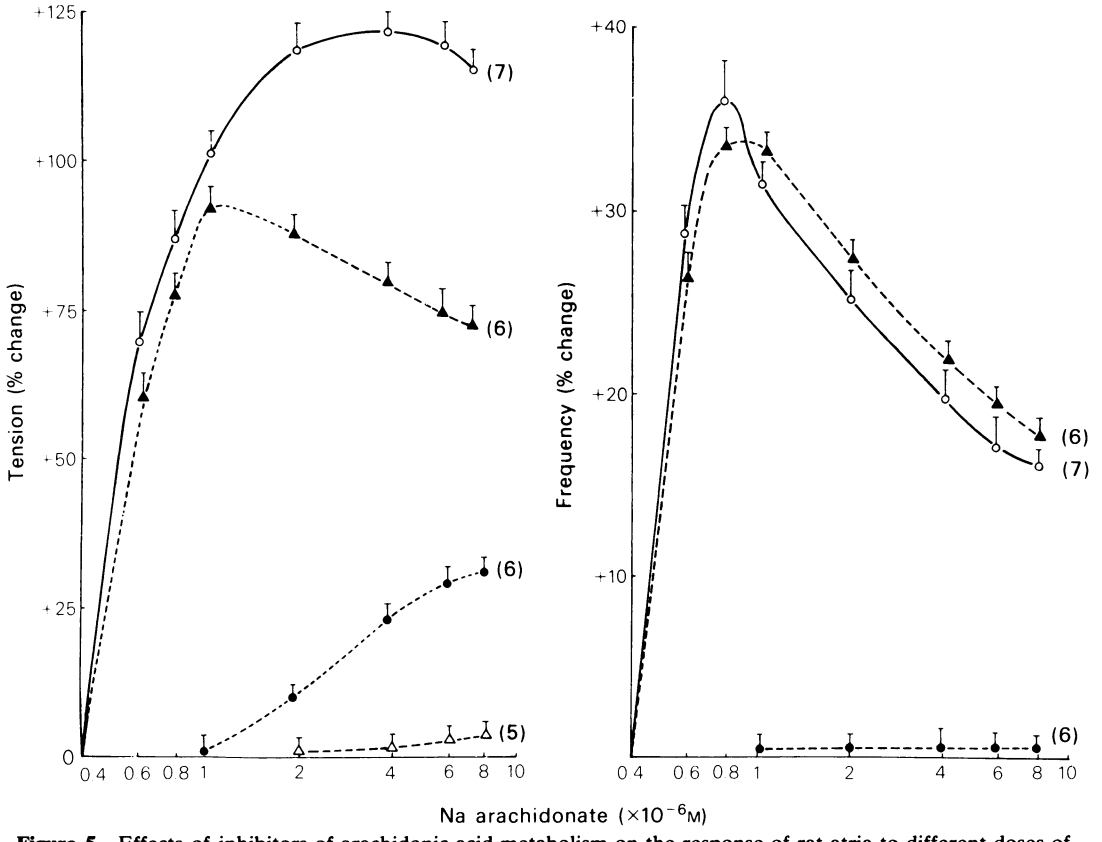


**Figure 5** Effects of inhibitors of arachidonic acid metabolism on the response of rat atria to different doses of sodium arachidonate (NaA) in the presence of lymphocytes. Rat isolated atria were suspended in 15 ml of KRB (○) or in 15 ml of KRB with $1.8 \times 10^{-4}$ M acetysalicylic acid (ASA) (▲); $1 \times 10^{-7}$ M FPL-55712 (●) or $1.8 \times 10^{-4}$ M ASA plus $1 \times 10^{-7}$ M FPL-55712 (△) and incubated for 30 min. RPMI 1 ml containing $4 \times 10^6$ L was added and the dose-response curve to NaA was constructed as described in Methods.

*Effect of cell-free supernatants from lymphocytes exposed to sodium arachidonate on the tension and frequency of beating atria*

To investigate whether contact between NaA-L and the atria was necessary for stimulation, the reaction was divided into two steps. First, L were exposed to NaA and supernatants of this reaction were collected and added to beating atria. The results shown in Table 2 demonstrate that soluble factors derived

**Table 2** Effect of supernatants from the reaction of lymphocytes (L) and sodium arachidonate (NaA) on spontaneously beating rat atria

| *Supernatants* | *Atria* | *Tension* (%) | *Frequency* (%) | n |
|---|---|---|---|---|
| L | KRB | + 5.0±2.5 | 0 | 5 |
| NaA-L | KRB | + 46.0±7.3 | + 15.9±1.3 | 5 |
| NaA-L | NDGA + KRB | + 6.0±1.9 | 0 | 4 |
| NaA-L | FPL-55712 + KRB | + 4.7±2.6 | 0 | 4 |

Supernatants (0.5 ml) of the reaction of $4 \times 10^6$ L with 2 μl of $1.5 \times 10^{-2}$ M NaA for 30 min at 37°C were added to rat atria beating in KRB or to rat atria that had been previously incubated with $10^{-5}$ M NDGA or $10^{-7}$ M FPL-55712 for 30 min. Mean ± s.e.mean. Values of tension and frequency are expressed as % change of the initial control. *n*: number of preparations. Other abbreviations see footnote to Table 1.

from the interaction of NaA with L could replace the intact L.

The effect of the NaA-L supernatants required active lipoxygenase enzymes, since it did not occur when supernatants of NaA-L were assayed on atria that had been previously treated with NDGA or FPL-55712 (Table 2). This indicates that similar compounds are the cause of stimulation both in intact NaA-L and in supernatants of NaA-L. Furthermore, if NDGA $1 \times 10^{-5}$ M was added to the L during 30 min and then washed, exposure of NDGA-pretreated L to NaA generated active stimulatory supernatants (data not shown).

## Discussion

The experiments described in this paper show that normal lymphocytes that have been incubated with NaA (NaA-L) react *in vitro* with spontaneously beating rat atria inducing positive inotropic and chronotropic effects. Moreover, the inotropic stimulatory reaction of NaA itself was potentiated by the presence of normal human peripheral lymphocytes in the incubation media.

The time allowed for the reaction between NaA and the lymphocytes was very short (30 min) and the changes in the contractile parameters of the atria were observed soon after their exposure to NaA-treated effector cells (Figure 1). Thus it appears that the early events of lymphocyte activation were involved.

Lipoxygenase products mediated the reaction of NaA-L and rat atria, since pretreatment of the atria with lipoxygenase inhibitors, but not with cyclo-oxygenase inhibitors eliminated both the chronotropic and the inotropic effects of L at concentrations of NaA that had no effect of their own (Figure 2).

Recently we have demonstrated that human lymphocytes stimulated by phytohaemaglutinin (PHA) and the ionophore A-23187 were able to induce a strong enhancement of the frequency and tension of rat atria, as a consequence of the release of derivates of lipoxygenase pathway of AA metabolism (Sterin-Borda *et al.*, 1983).

That reaction had many points in common with the one described in this paper: it occurred shortly after exposure of the effector cells to the stimulus and it involved lipoxygenase products.

It has been shown that stimulation of normal lymphocytes with PHA leads to a prompt release of AA to the surrounding medium (Parker *et al.*, 1979a). It could be postulated that while PHA-stimulation triggers a reaction that results in the release of AA, NaA treatment of lymphocytes bypasses that initial step and provides the substrate for the following steps of the stimulatory cascade.

The effect of NaA itself on the atria is different from that of NaA-L, both in quality and magnitude (Figures 3, 4, 5). Cyclo-oxygenase products were the mediators of the direct inotropic effect of NaA. This is supported by the experiments shown in Figure 4 demonstrating that incubation of the atria with indomethacin and ASA abrogated the action of NaA while NDGA at concentrations that had no effect on the cyclo-oxygenase pathway (Hamberg & Samuelsson, 1974; Hamberg, 1976) did not inhibit the reaction.

On the other hand, NaA-L affected the contractile behaviour of the atria inducing positive inotropic and chronotropic effects by different mechanisms depending on the dose of NaA supplied.

Two aspects of the reaction of NaA must be emphasized: the appearance of a chronotropic effect which occurred solely when L were added to the preparation together with NaA (Figures 1, 2, 5) and the sensitization of the reaction of the atria to NaA (Figures 3, 5). These effects were evident both when NaA-L were used (Figures 1, 2) or when L were added to the atria and then increasing amounts of NaA were added to construct dose-response curves (Figures 3, 5).

At low doses, SRS-A was the main metabolite involved, since FPL-55712 eliminated both the positive inotropic and chronotropic effects of NaA-L (Figure 5). At the concentration used, this compound has been shown to antagonize the effect of SRS-A on guinea-pig trachea (Burka & Paterson, 1981) and on human airways (Ghelani, Holroyde & Sheard, 1980). At high doses of NaA, lymphocytes enhanced the inotropic effect of NaA by mechanisms that involved both the cyclo-oxygenase and the lipoxygenase pathways (Figure 5). In these conditions, inhibition of cyclo-oxygenase activity partially reduced the positive inotropic effect of NaA-L and preincubation of the atria with both FPL-55712 plus ASA was necessary to eliminate the stimulatory effect of NaA-L (Figure 5).

Results with cell-free supernatants of NaA-L indicate that soluble factors are generated by the influence of NaA on lymphocytes and that these factors can trigger positive inotropic and chronotropic effects on beating atria. Hence, close contact between intact lymphocyte and myocardium is not necessary.

The fact that inhibition of the atrium lipoxygenase(s) or blockade of their SRS-A receptors prevented the action of the soluble stimulatory factors derived from NaA-L, suggests that the oxidative metabolites of AA are produced by the enxymatic battery of the atria. SRS-A was probably the main metabolite involved, because FPL-55712 inhibited the reaction of NaA-L supernatants as well as that of intact L.

Furthermore, inactivation of the possible lipoxy-

genase enzymes of the L, prior to treatment of L with NaA did not block the appearance of active soluble factors in the supernatants.

We propose that upon exposure of L to NaA, soluble factors are released from the lymphocytes and that these factors in turn influence the effect of NaA on the atria, triggering the lipoxygenase oxidative pathway. In other words, we suggest that presentation of NaA to the atria in association with soluble lymphocyte-derived factors, provides a signal to the atrial tissue that differs from the signal given by NaA alone and that this affects the oxidative metabolic route followed thereafter.

The results of this work provide additional data regarding non-conventional effects of cells of the immune system on unrelated tissues. While the results of the *in vitro* reaction described here are very difficult to extrapolate to *in vivo* conditions, the fact that lymphocytes may enhance the cardiostimulatory effect of NaA, influencing the route followed for its metabolism must be taken into account for the interpretation of the role of infiltrating lymphocytes in several cardiac diseases.

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