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Effect of inhibition of synthesis and receptor antagonism of SRS-A in cardiac anaphylaxis

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1 The effects of infusions of the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 1.1×10^{-7} mol min^{-1}) and the antagonist of slow-reacting substance of anaphylaxis (SRS-A) FPL 55712 (1.2×10^{-7} mol min^{-1}) on the coronary constriction and the release of SRS-A, leukotriene C_4-like immunoreactivity, thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ from perfused anaphylactic guinea-pig hearts were investigated.

2 Both NDGA and FPL 55712 in the concentrations used induced an increase in basal coronary flow, but did not prevent the coronary flow reduction in the early phase (0–4 min) after antigen injection. On the other hand, NDGA and FPL 55712 inhibited the less pronounced long-lasting coronary flow reduction in the later phase of cardiac anaphylaxis.

3 NDGA decreased the release of SRS-A from the anaphylactic guinea-pig hearts below or close to the detection limit of the bioassay and simultaneously diminished the release of leukotriene C_4-like immunoreactivity. On the other hand, FPL 55712 did not influence the amounts of leukotriene C_4-like immunoreactivity released in cardiac anaphylaxis.

4 Neither NDGA nor FPL 55712 affected the release of immunoreactive thromboxane B_2 (TXB_2) from anaphylactic guinea-pig hearts. Release of 6-keto-prostaglandin $F_{1\alpha}$ after challenge, however, was decreased by NDGA, while FPL 55712 had no significant effect.

5 These results suggest, that SRS-A may be a relatively more important mediator in the late phase of coronary constriction occurring during cardiac anaphylaxis, while the effects of other mediators, particularly vasoconstrictor cyclo-oxygenase products, seem to prevail in the early phase.

Introduction

Release of various mediators like histamine (Schild, 1937; Schild, 1939; Mongar & Schild, 1952; Brocklehurst, 1960; Chakravarty, 1960a; Feigen, Vaughan Williams, Petersen & Nielsen, 1960; Giotti, Guidotti, Mannaioni & Zilletti, 1966; Levi, 1972), slow-reacting substance of anaphylaxis (SRS-A) (Brocklehurst, 1960; Chakravarty, 1960b; Liebig, Bernauer & Peskar, 1975), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Liebig *et al.*, 1975; Levi, Allan & Zavecz, 1976), PGD_2 (Anhut, Bernauer & Peskar, 1978), thromboxane B_2 (TXB_2) (Anhut, Bernauer & Peskar, 1977; Allan & Levi, 1981), the stable degradation product of the vasoconstrictor TXA_2 (Moncada & Vane, 1977), and 6-keto-$PGF_{1\alpha}$ (Peskar, Steffens & Peskar, 1979; Allan & Levi, 1981), the stable degradation product of the vasodilator PGI_2 (Moncada & Vane, 1977), in cardiac anaphylaxis has been repeatedly shown. We have previously demonstrated (Anhut *et al.*, 1977) that inhibition of fatty acid cyclo-oxygenase by indomethacin results in inhibition of the initial phase of anaphylactic coronary constriction, but does not prevent the coronary constriction occurring later in the time course of cardiac anaphylaxis. These results suggested that vasoconstrictor cyclo-oxygenase products are largely responsible for the reduction in coronary flow observed immediately after antigen injection, but other mediators, particularly SRS-A, may be more important for the long-lasting coronary constriction in the later phase of cardiac anaphylaxis. Recently, leukotrienes (LTs), which are formed from arachidonic acid via the lipoxygenase pathway (Samuelsson, Borgeat, Hammarström & Murphy, 1980), have been identified as constituents of SRS-A (Murphy, Hammarström & Samuelsson, 1979; Morris, Taylor, Piper & Tippins, 1980b). Exogenous LTC_4 and LTD_4 are, in fact, potent coronary constrictors (Terashita, Fukui, Hirata, Terao, Ohkawa, Nishikawa & Kikuchi, 1981; Letts & Piper, 1982; Burke, Levi, Guo & Corey, 1982). In order to investigate further the contribution of endogenous SRS-A

to anaphylactic coronary flow reduction we have now used the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) (Tappel, Lundberg & Boyer, 1953; Hamberg, 1976) and the SRS-A antagonist, FPL 55712 (Augstein, Farmer, Lee, Sheard & Tattersall, 1973). We have studied the effects of these compounds on coronary flow as well as on anaphylactic release of biologically active SRS-A and LTC_4-like immunoreactivity. The use of a radioimmunoassay for LTC_4, developed recently (Aehringhaus, Wölbling, König, Patrono, Peskar & Peskar, 1982), permitted quantification of LTC_4-like immunoreactivity in the presence of the antagonist FPL 55712, when bioassay of SRS-A is not possible. Finally, since exogenous leukotrienes release 6-keto-$PGF_{1\alpha}$, but not TXB_2 (Terashita *et al.*, 1981), from guinea-pig isolated perfused hearts, we have additionally examined the effects of NDGA and FPL 55712 on the release of these cyclo-oxygenase products during cardiac anaphylaxis. Some of these results have been reported at the Annual Symposium on Leukotrienes and Other Lipoxygenase Products, London, 1982 (Wittenberg, Wölbling, Aehringhaus, Patrono, Peskar & Peskar, 1983).

Methods

Isolated hearts of ovalbumin-sensitized male guinea-pigs (350–550 g body weight) were perfused and challenged as described previously (Liebig *et al.*, 1975). The hearts were perfused at a constant pressure of 55 cmH_2O. Heart rate and contraction force were recorded continuously on a Watanabe multichannel recorder. Coronary flow was measured by direct determination of the perfusate volume per collection period. After an equilibration period of 25–30 min, heart perfusates were collected in one 4 min and three 2 min collection periods before antigenic challenge and three 1 min, two 2 min and four 4 min collection periods after challenge. Perfusates were collected for a total of 23 min after antigen injection. In experiments with NDGA the compound was infused into the aortic inflow cannula at a constant rate of 1.1×10^{-7} mol min^{-1} resulting in final concentrations in the perfusates between 1.2×10^{-5} M and 5.4×10^{-6} M. FPL 55712 was infused at a rate of 1.2×10^{-7} mol min^{-1} resulting in final perfusate concentrations between 1.2×10^{-5} M and 7.7×10^{-6} M. Preliminary experiments had shown that the concentration of NDGA used is sufficient to decrease the release of SRS-A into the heart perfusates below or close to the detection limit of the bioassay. The infusion of FPL 55712 was selected to result in a final perfusate concentration, which is about two to four times higher than necessary to diminish significantly the effects of 2×10^{-8} M of exogenous LTC_4 and LTD_4 on the function of guinea-pig isolated perfused hearts (Letts & Piper, 1982). In addition, several experiments had demonstrated, that a lower concentration of FPL 55712 (2.4×10^{-8} mol min^{-1}) did not affect anaphylactic coronary constriction. The drug infusions were started 15 min before antigenic challenge and lasted throughout the experiment. NDGA was dissolved in ethanol (33×10^{-3} M) and FPL 55712 was dissolved in distilled water (1.9×10^{-3} M). Control experiments demonstrated that the solvents in the concentrations used did not significantly affect the symptoms of cardiac anaphylaxis and mediator release.

Bioassay of SRS-A on the guinea-pig isolated ileum treated with mepyramine (3.5×10^{-6} M) and atropine (6.9×10^{-7} M) was performed as described previously (Liebig *et al.*, 1975). Results were expressed in LTD_4-equivalents, since the major biological activity of guinea-pig SRS-A is LTD_4 (Morris *et al.*, 1980b). NDGA, in the concentration used, did not interfere with the assay. Since bioassay of SRS-A was not possible, when the SRS-A antagonist FPL 55712 was present in the perfusates, samples of perfusates of control and drug-treated hearts were additionally analysed by a radioimmunoassay for LTC_4 (Aehringhaus *et al.*, 1982). Results were expressed in terms of LTC_4-like immunoreactivity. However, the anti-LTC_4 antibodies do not only bind LTC_4, but also LTD_4 and possibly other LTs, which might occur in the perfusates. Thus, the radioimmunoassay, like bioassay, does not permit absolute quantification of leukotrienes present in the perfusates without formal identification of leukotrienes released by the guinea-pig hearts. The general validity of the radioimmunological determination of LTC_4 in the heart perfusates was, however, demonstrated by a significant correlation of the results with data obtained by bioassay of SRS-A (Wittenberg *et al.*, 1983). TXB_2 and 6-keto-$PGF_{1\alpha}$ in the perfusates were determined radioimmunologically, as described previously (Anhut *et al.*, 1977; Peskar *et al.*, 1979). NDGA and FPL 55712 in the concentrations used did not interfere with the various radioimmunoassays.

Means ± s.e.mean were calculated. Statistical analysis was performed by means of Student's *t* test. Changes in coronary flow after challenge were compared with basal flow immediately before challenge using the *t* test for paired values.

Materials

FPL 55712 (sodium 7-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy)-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) was kindly provided by Fisons Pharmaceutical Laboratories, Loughborough, England. LTD_4 was a generous gift from Dr J. Rokach, Merck-Frosst Laboratories,

Pointe Claire-Dorval, Canada. Ovalbumin, nordihydroguaiaretic acid, atropine sulphate and mepyramine maleate were from Sigma, St Louis, Mo., USA.

Results

Cardiac function

Antigenic challenge of sensitized isolated perfused hearts of guinea-pigs resulted in a characteristic biphasic coronary constriction with an initial more severe phase (0–4 min) and a less pronounced long-lasting reduction in coronary flow in the later phase (Figure 1). Infusion of NDGA (1.1×10^{-7} mol min^{-1}) induced a significant increase in basal coronary flow of $164 \pm 14\%$ ($P < 0.001$ as compared to controls) (Figure 1). Challenge of NDGA-perfused sensitized hearts was followed by a significant coronary constriction in the early phase of anaphylaxis, while the flow reduction in the later phase was abolished (Figure 1).

Infusion of FPL 55712 (1.2×10^{-7} mol min^{-1}) into guinea-pig hearts induced an increase in basal flow of $74 \pm 22\%$ ($P < 0.001$ as compared to controls) (Figure 1). After antigenic challenge changes in coronary flow were observed, which were comparable to those in NDGA-treated hearts (Figure 1).

The effects of NDGA and FPL 55712 on heart rate and contraction force before and after antigenic challenge are shown in Table 1. NDGA in the concentration used decreased basal contraction force slightly, but not significantly. Neither NDGA nor FPL 55712

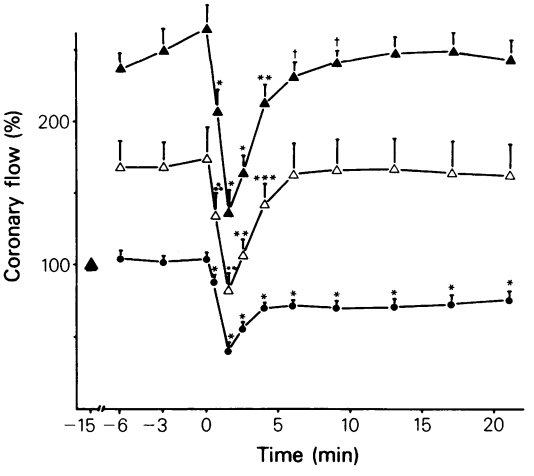

Figure 1 Coronary flow before and after antigenic challenge of sensitized guinea-pig hearts under control conditions (●, $n = 27$), during infusion of nordihydroguaiaretic acid (NDGA) (▲, 1.1×10^{-7} mol min^{-1}, $n = 11$) or FPL 55712 (△, 1.2×10^{-7} mol min^{-1}, $n = 9$). Drug infusions were started 15 min before challenge. Coronary flow before drug infusion was 9.5 ± 0.4 ml and was set 100%. Antigen was injected at time 0. $^{*}P < 0.001$; $^{**}P < 0.005$; $^{***}P < 0.025$; †$P < 0.05$ as compared to coronary flow immediately before challenge.

Table 1 The effects of nordihydroguaiaretic acid (NDGA) and FPL 55712 on heart rate and contraction force of sensitized guinea-pig hearts before and after antigenic challenge

	Control		*NDGA*		*FPL 55712*	
Time	*HR (%)*	*CF (%)*	*HR (%)*	*CF (%)*	*HR (%)*	*CF (%)*
1 min before challenge	101 ± 2.6	113 ± 11.3	99 ± 8.4	94 ± 11.3	101 ± 3.0	101 ± 21.5
1 min after challenge	146 ± 2.3	205 ± 17.2	164 ± 5.6*	187 ± 20.4	147 ± 1.2	242 ± 55.0
3 min after challenge	133 ± 12.0	129 ± 13.0	154 ± 15.0	148 ± 26.1	148 ± 1.1	124 ± 15.6
21 min after challenge	107 ± 4.0	71 ± 10.5	116 ± 4.9	66 ± 11.3	118 ± 8.7	107 ± 24.8

Heart rate (HR) and contraction force (CF) before and after antigenic challenge of sensitized guinea-pig hearts under control conditions ($n = 25$), during infusion of NDGA (1.1×10^{-7} mol min^{-1}, $n = 8$) or FPL 55712 (1.2×10^{-7} mol min^{-1}, $n = 5$). Infusions of drugs or solvents were started 15 min before challenge. Heart rate and contraction force before drug infusions were 182 ± 5 beats min^{-1} and 22.7 ± 1.8 mN, respectively, and were taken as 100%. $^{*}P < 0.005$

induced significant changes in the contraction force during the time course of cardiac anaphylaxis as compared to controls (Table 1). FPL 55712 (1.2×10^{-7} mol min^{-1}) did not significantly affect resting heart rate and anaphylactic changes of heart rate. NDGA (1.1×10^{-7} mol min^{-1}) increased the heart rate immediately after challenge, but had no significant effects on heart rate later in the time course of cardiac anaphylaxis and on resting heart rate (Table 1).

Mediator release

SRS-A was not detected in heart perfusates obtained before antigenic challenge. After challenge large amounts of SRS-A determined by bioassay were found (Figure 2). Infusion of NDGA (1.1×10^{-7} mol min^{-1}) resulted in a highly significant decrease of SRS-A release (Figure 2).

When selected samples of heart perfusates were analysed for LTC_4-like immunoreactivity, the results of the bioassay of SRS-A in perfusates collected before and after antigenic challenge as well as the inhibitory effect of NDGA were confirmed (Figure 3). In addition, it could be shown that FPL 55712 had no significant effect on the release of LTC_4-like immunoreactivity (Figure 3).

Levels of immunoreactive TXB_2 and 6-keto-$PGF_{1\alpha}$ in basal heart perfusates were either below or close to the detection limits of the radioimmunoassays (1.1×10^{-10} M and 9×10^{-11} M, respectively). However, large amounts of these cyclo-oxygenase products were found in the heart perfusates after antigen injection. As shown in Figure 4 neither

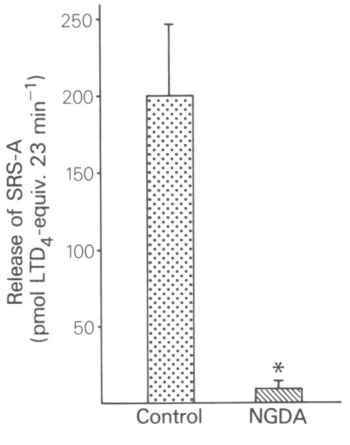

Figure 2 Effect of nordihydroguaiaretic acid (NDGA) (1.1×10^{-7} mol min^{-1}, $n = 11$) on release of biologically active SRS-A, expressed in leukotriene (LT) D_4-equivalents, from anaphylactic guinea-pig hearts. Controls, $n = 10$. $^*P < 0.001$.

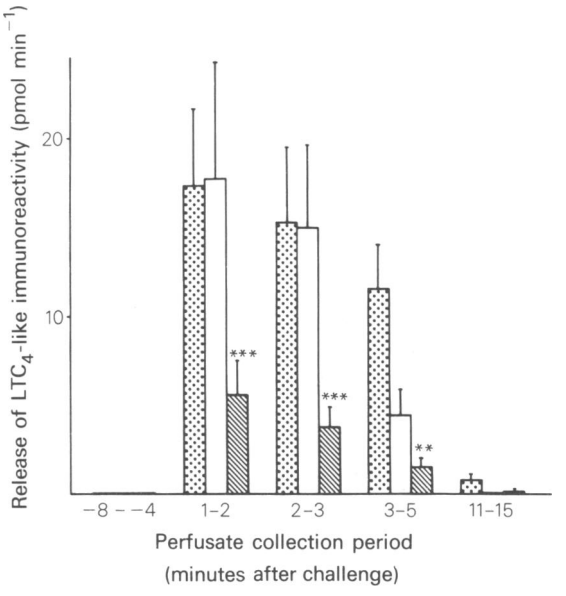

Figure 3 Effects of nordihydroguaiaretic acid (NDGA) (hatched columns, 1.1×10^{-7} mol min^{-1}, $n = 10$) and FPL 55712 (open columns, 1.2×10^{-7} mol min^{-1}, $n = 9$) as compared to controls (stippled columns, $n = 16$) on the release of leukotriene (LT)C_4-like immunoreactivity from anaphylactic guinea-pig hearts, $^{**}P < 0.01$, $^{***}P < 0.05$.

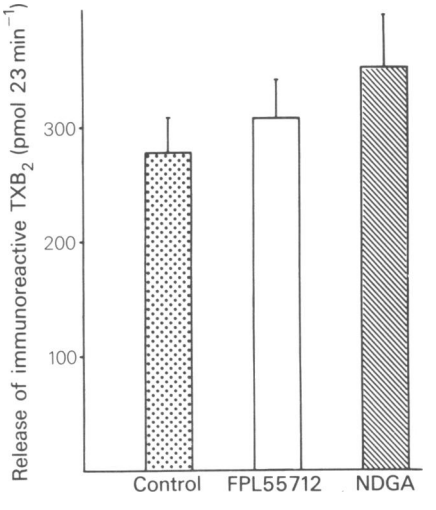

Figure 4 Effects of nordihydroguaiaretic acid (NDGA) (hatched column, 1.1×10^{-7} mol min^{-1}, $n = 11$) and FPL 55712 (open column, 1.2×10^{-7} mol min^{-1}, $n = 9$) as compared to controls (stippled column, $n = 27$) on the release of immunoreactive thromboxane (TX)B_2 from anaphylactic guinea-pig hearts.

NDGA nor FPL 55712 had a significant effect on anaphylactic release of immunoreactive TXB_2. On the other hand, NDGA significantly diminished release of immunoreactive 6-keto-$PGF_{1\alpha}$, while FPL 55712 had no significant effect on the release of the degradation product of PGI_2 (Figure 5).

In control experiments ($n = 7$) it was shown that injection of ovalbumin into non-sensitized guinea-pig hearts did not result in changes of coronary flow and release of SRS-A, LTC_4-like immunoreactivity, TXB_2 and 6-keto-$PGF_{1\alpha}$.

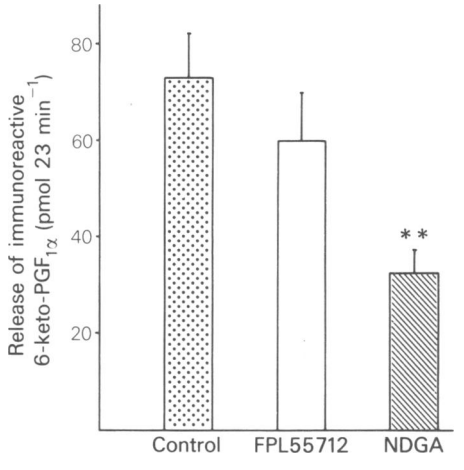

Figure 5 Effects of nordihydroguaiaretic acid (NDGA) (hatched column, 1.1×10^{-7} mol min^{-1}, $n = 11$) and FPL 55712 (open column, 1.2×10^{-7} mol min^{-1}, $n = 8$) as compared to controls (stippled column, $n = 27$) on the release of immunoreactive 6-keto-$PGF_{1\alpha}$ from anaphylactic guinea-pig hearts, $^{**}P < 0.01$.

Discussion

Both the lipoxygenase inhibitor NDGA and the SRS-A antagonist, FPL 55712, in the concentrations used abolished the long-lasting coronary flow reduction in the later phase of cardiac anaphylaxis but did not prevent the coronary constriction immediately after antigen injection. The qualitatively comparable effects of both drugs on anaphylactic changes of coronary flow suggest a role for endogenous SRS-A as a mediator of the late phase of anaphylactic coronary constriction. This assumption is supported by the parallel inhibitory effect of NDGA on cardiac release of SRS-A and LTC_4-like immunoreactivity. Similarly, NDGA has been shown to inhibit SRS-A release from anaphylactic guinea-pig lung (Morris, Piper, Taylor & Tippins, 1980a). While FPL 55712 does not significantly affect release of immunoreactive LTC_4, the concentration of FPL 55712 used can be expected from the results of Letts & Piper (1982) to antagonize significantly the coronary vasoconstrictor effects of rather high LT concentrations. Interestingly, although cardiac release of SRS-A reaches a maximum only slightly later than release of cyclo-oxygenase products (Liebig *et al.*, 1975), the vasoconstrictor effect of SRS-A as compared to TXA_2 and prostaglandins seems to be more important in the later phase of anaphylactic coronary flow reduction. Thus, concentrations of SRS-A released into the heart perfusates during the time course of cardiac anaphylaxis do not seem to correlate necessarily with the contribution of SRS-A to anaphylactic coronary constriction. In this context the observation may be relevant to the findings by Letts & Piper (1982), that infusion of high concentrations of exogenous leukotrienes into guinea-pig isolated hearts results in a reduction of coronary flow rates, which do not recover to pre-infusion values even one hour after the end of the leukotriene infusion.

Furthermore, experiments with the dual inhibitor of lipoxygenase and cyclo-oxygenase BW755C (3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride) (Higgs, Flower & Vane, 1979) have shown, that infusions of 7.4×10^{-7} mol min^{-1} result in complete inhibition of release of immunoreactive 6-keto-$PGF_{1\alpha}$ and TXB_2, but do only slightly, but not significantly, decrease release of biologically active SRS-A and LTC_4-like immunoreactivity (Wittenberg, Wölbling & Peskar, 1982). Simultaneously, the infusion of BW755C inhibited the early phase of anaphylactic coronary flow reduction, but had much less effect on the later phase. Similarly, it has been demonstrated previously (Anhut *et al.*, 1977), that addition of indomethacin to the perfusion medium (2.8×10^{-6} M) abolishes the anaphylactic release of immunoreactive TXB_2 and $PGF_{2\alpha}$ and simultaneously inhibits the early phase of anaphylactic coronary constriction, but does not affect the later phase. These results support the view that SRS-A may be a major mediator of the indomethacin-resistant phase of anaphylactic coronary constriction. Levi *et al.* (1976) described almost complete inhibition of anaphylactic coronary flow reduction by a higher concentration (1.4×10^{-5} M) of indomethacin. However, these authors measured total coronary flow for a period of 10 min after challenge only, and might, therefore, have not been able to discriminate between the indomethacin-sensitive and indomethacin-resistant phase of anaphylactic coronary flow reduction.

Both NDGA and FPL 55712 in the concentrations necessary for the observed effects on anaphylactic coronary flow changes induce a significant increase in basal coronary flow. If this effect is due to inhibition

of endogenous vasoconstrictor agents, only very small amounts of SRS-A could be involved, since biologically active SRS-A and immunoreactive LTC_4 could not be detected in heart perfusates collected before antigenic challenge. Nevertheless, a possible contribution of vasoconstrictor lipoxygenase products to basal coronary tone in isolated perfused guinea-pig hearts cannot be excluded from our results. On the other hand, the increase in basal coronary flow may be non-specific, as both compounds in higher concentrations have effects not related to inhibition of lipoxygenase and antagonism of SRS-A, respectively (Krell, Osborn, Falcone & Vickery, 1981; Welton, Hope, Tobias & Hamilton, 1981; Scott-Miller & McMillan, 1982). It remains to be investigated, to which extent a possible non-specific increase in basal coronary flow could contribute to the drug effects observed in cardiac anaphylaxis.

While the release of TXB_2 from anaphylactic guinea-pig hearts is not influenced by either NDGA or FPL 55712 in the concentrations used, release of 6-keto-$PGF_{1\alpha}$, the stable degradation product of the vasodilator PGI_2, is significantly reduced by NDGA. From our results we cannot exclude a direct effect of NDGA on the enzymes of PGI_2 synthesis in the heart. Alternatively, the decreased release of 6-keto-$PGF_{1\alpha}$ into the heart perfusates may be a consequence of the inhibition of SRS-A release by NDGA. In fact, release of 6-keto-$PGF_{1\alpha}$ by exogenous leukotrienes from isolated perfused guinea pig hearts has been demonstrated (Terashita *et al.*, 1981). On the other hand, the lack of effect of NDGA on TXB_2 release suggests that endogenous SRS-A does not liberate TXB_2 from isolated anaphylactic guinea-pig hearts. This assumption is supported by results (Terashita *et al.*, 1981) showing that exogenous leukotrienes do not release TXB_2 from guinea-pig isolated perfused hearts. In the guinea-pig lung, however, exogenous LTC_4 (Folco, Hansson & Granström, 1981) as well as SRS-A (Engineer, Morris, Piper & Sirois, 1978) have been demonstrated to release TXA_2, indicating an organ specificity as to the cyclo-oxygenase products released by leukotrienes. The possible release of other vasoconstrictor cyclo-oxygenase products like PGD_2 and $PGF_{2\alpha}$ by SRS-A from guinea-pig isolated perfused hearts, as suspected by Letts & Piper (1982), remains to be investigated.

Contrary to NDGA the SRS-A antagonist FPL 55712 does not affect anaphylactic release of SRS-A determined as LTC_4-like immunoreactivity. However, from data by Letts & Piper (1982) on the antagonism of exogenous leukotrienes by FPL 55712, significant inhibition of the coronary constrictor effects of endogenous leukotrienes by the concentration of FPL 55712 used can be expected. Contrary to the results with NDGA, anaphylactic release of 6-keto-$PGF_{1\alpha}$ is not significantly decreased in the presence of FPL 55712. Several explanations are possible for this result. The concentration of FPL 55712, which significantly inhibits the effect of endogenous SRS-A on anaphylactic coronary constriction, may not be sufficient to antagonize the SRS-A effects on PGI_2 release. Furthermore, a major fraction of PGI_2 could be released by antigenic challenge independent of SRS-A release. Thus, although NDGA in the concentration used induced marked inhibition of SRS-A release (95%), it inhibited release of 6-keto-$PGF_{1\alpha}$ by only 55%. Therefore, if the SRS-A-dependent fraction of PGI_2 release is only partially antagonized by FPL 55712, total anaphylactic PGI_2 release can be expected to decrease only slightly.

The decrease in cardiac contraction force induced by exogenous leukotrienes is significantly antagonized by FPL 55712 (Letts & Piper, 1982; Burke, Levi, Guo & Corey, 1982). In the present experiments FPL 55712 tended to antagonize the decrease in contraction force observed in the late phase of cardiac anaphylaxis, although the effect was not significant as compared to the controls. It might be more difficult to antagonize the effect of endogenous SRS-A on cardiac contraction force than the effect of exogenous leukotrienes. Furthermore, the leukotriene-induced coronary constriction may be more susceptible to antagonism by FPL 55712 than the decrease in contraction force. Thus, in the sheep *in vivo* the decrease in regional systolic ventricular wall shortening after injection of LTD_4 was only partially antagonized by FPL 55712, while the LTD_4-induced coronary constriction was completely abolished (Michelassi, Landa, Hill, Lowenstein, Watkins, Petrau & Zapol, 1982). In the experiments with NDGA the slight negative inotropic effect of the drug in the concentration used may mask effects resulting from inhibition of SRS-A release. In addition, effects on the release of other mediators by NDGA and FPL 55712 cannot be excluded. Thus, for example, the release of platelet activating factor, another mediator with negative inotropic and coronary constrictor effects, should be considered; as its release from isolated anaphylactic guinea-pig hearts has been described recently (Burke, Levi, Hanahan & Pinckard, 1982).

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