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Renal vascular effects of leukotriene C_4 in the isolated perfused kidney of the rat

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1 The vascular effects of leukotriene C_4 (LTC_4) were investigated in the isolated perfused kidney of the rat.

2 LTC_4 (6.4×10^{-10} to 3.2×10^{-8} mol kg^{-1} min^{-1} given over 5 min) resulted in a prompt, dose-dependent increase in renal vascular resistance in a recirculating system, which lasted for more than 60 min.

3 LTC_4 was 10 to 20 fold and 1000 to 2000 fold, respectively, less active on a molar basis than noradrenaline and angiotensin II in eliciting renal vasoconstriction.

4 The vascular response to LTC_4 was blocked dose-dependently by FPL 55712, an antagonist of slow reacting substance of anaphylaxis. OKY 1581, a specific thromboxane synthetase inhibitor, and indomethacin, a cyclo-oxygenase inhibitor, did not influence the LTC_4 response.

5 LTC_4 given in a single-pass perfusion system resulted in a short lasting response with baseline values for renal vascular resistance reached after 4 min.

6 These results show that LTC_4 is a short acting renal vasoconstrictor with less potency than noradrenaline and angiotensin II. Its pressor effects seem to be mediated by specific leukotriene receptors and independent of cyclo-oxygenase products. The long-lasting effect in the recirculating arrangement, in contrast to the single pass system, is compatible with formation of active metabolite(s).

Introduction

Leukotriene C_4 (LTC_4) which is naturally formed from arachidonic acid through the 5-lipoxygenase pathway (Samuelsson *et al.*, 1980), has been identified recently as a major active constituent of slow reacting substance of anaphylaxis (SRS-A) (Nakane *et al.*, 1978; Morris *et al.*, 1980). LTC_4 has been shown to be converted into its metabolites, leukotriene D_4 and E_4 (LTD_4 and LTE_4) by the enzymes γ-glutamyl transpeptidase and dipeptidase, respectively (Bergström & Hammarström, 1981; Örning & Hammarström, 1982). Furthermore, the formed LTE_4 can be converted into leukotriene F_4 (LTF_4) by addition of a glutamyl residue (Bergström & Hammarström, 1982). Following this biochemical elucidation of leukotrienes their biological activities have been studied extensively in various tissues and organs. It was found that these leukotrienes are potent bronchoconstrictors in guinea-pigs and man (Holme *et al.*, 1980; Piper & Samhoun, 1981; Weiss *et al.*, 1981; 1982; Kaijser, 1982) and enhance vascular permeability when injected intradermally (Drazen *et al.*, 1980; Soter *et al.*, 1983). These compounds have also been shown to cause cardiac dysfunction, which is characterized by a significant reduction in cardiac contractility and coronary flow (Burke *et al.*, 1982; Letts & Piper, 1982) and to be potent vasoconstrictors in the cutaneous microcirculation (Drazen *et al.*, 1980) and the mesenteric vascular beds (Feigen, 1983). Furthemore, systemic vasoconstrictor effects of intravenously administered LTC_4 and LTD_4 were also observed in rats. In this model the vasoconstrictor potencies were similar to those of noradrenaline and angiotensin (Pfeffer *et al.*, 1983).

Despite these well-known bioactivities in the lung, the heart and the peripheral vasculature, the effects of leukotrienes on the renal haemodynamics are still controversial. Feigen (1983) reported that bolus injections of 3 and 10 μg of LTC_4 and LTD_4 into the renal artery produced a small increase in renal blood flow in the dog, suggesting that leukotrienes are feeble vasodilators in the kidney. In contrast, marked reductions of renal blood flow, in response to bolus injections of 5×10^{-12} to 10^{-9} mol of LTC_4 into the renal artery in pigs (McLeod *et al.*, 1984) and to

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intravenous infusions of 2 $\mu g\,kg^{-1}\,min^{-1}$ of LTC_4 in the rat (Badr *et al.*, 1984; Filep *et al.*, 1985), were recently observed. These findings suggest that leukotrienes are potent vasoconstrictors in the kidney. On the other hand, in spontaneously hypertensive rats an intravenous injection of 20 $\mu g\,kg^{-1}$ of LTD_4 did not alter renal blood flow, while it elicited a significant decrease in renal vascular resistance (Zukowska-Grojac *et al.*, 1983). The diverse responses of renal vasculatures to leukotrienes may be partly due to species differences. Moreover, all of these experiments were performed under *in vivo* conditions and, in general, the kidneys *in vivo* are known to be exposed to multiple nervous and humoral factors that can obscure primary actions of agents.

In the present study, we have therefore investigated the effects of LTC_4 on the renal vasculature and its pharmacological properties in the isolated perfused kidneys of the rat and compared them with those of the well-characterized renal vasoactive agents, noradrenaline and angiotensin II so as to assess the relative potency of LTC_4. In addition, inhibitors of cyclo-oxygenase and of thromboxane synthetase as well as an antagonist of SRS-A have been examined to evaluate the mechanism of LTC_4-induced renal responses.

Methods

Kidney perfusion in vivo

The study was carried out in male Wistar rats weighing between 350 and 400 g, fed standard laboratory rat diet and allowed free access to tap water. After anaesthesia with pentobarbitone (40 to 50 $mg\,kg^{-1}$, intraperitoneally), heparin (100 u) was injected into right jugular vein to prevent blood coagulation during the subsequent operative procedure. The kidneys were then isolated and perfused according to the method of Bowman & Maack (1972) as modified by Nakane *et al.* (1978). Briefly, the abdomen was opened and the right urether catheterised with polyethylene-10 tubing. The right renal artery was cannulated with a 19 G needle via the superior mesenteric artery and the aorta (Nishiitsutsuji-Uwo *et al.*, 1967), so as to avoid interruption of blood flow to the kidney, and the right kidney was then excised and placed in the perfusion apparatus in which 75 ml of recirculating medium was continuously gassed with 95% O_2 and 5% CO_2. The left kidney was taken out and weighed. The temperature of the perfusate flowing to the arterial catheter was maintained at 37°C. The renal vein was not cannulated in the present experiments. A blood-free Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5.5 mM glucose, 6% bovine serum albumin (Cohn's fraction V, Sigma, St. Louis, USA) and creatine (15 $mg\,l^{-1}$) was used for the perfusate. The kidney in the chamber was perfused with a peristaltic pump (Cole-Parmer, Chicago, USA). Perfusion pressure was maintained within a range of 90 to 100 mmHg; perfusate flow was 20 to 30 $ml\,min^{-1}$. After allowing 20 min from the beginning of the perfusion for the stabilization of the kidney, the one-hour-observation period began. Renal perfusion pressure (RPP) and renal perfusate flow (RPF) were determined by reading directly on the mercury manometer and the flow meter, respectively, set in line with the arterial cannula (Nakane *et al.*, 1978). Renal resistances were calculated in terms of $RPP \times RPF^{-1}$ and expressed as mmHg $min\,ml^{-1}$. To estimate the viability of the perfused kidney, urine and perfusate were sampled for the measurements of creatinine clearance. Changes in perfusate volume and in sodium and potassium concentrations due to sampling and urinary losses were replaced as previously described (Nakane *et al.*, 1978). For the study in the single pass system, the perfused kidney was moved outside the perfusion chamber 1 min before the administration of each agent and the volume loss from the renal vein was supplemented by perfusate which had been kept at 37°C and oxygenated beforehand.

Drug administration

The experimental groups were designated as follows:
Experiment 1: Following 20 min of observation, 6.4×10^{-10} to 3.2×10^{-8} $mol\,kg^{-1}\,min^{-1}$ of LTC_4 were administered into the arterial tubing over a 5 min period by an infusion pump (B. Braun, Melsungen, F.R.G.), with each kidney receiving only one concentration of the compound.
Experiment 2: Noradrenaline (1.2×10^{-10} to 4.7×10^{-9} $mol\,kg^{-1}\,min^{-1}$) or angiotensin II (3.8×10^{-13} to 1.9×10^{-11} $mol\,kg^{-1}\,min^{-1}$) were administered as described for LTC_4 above, to compare the intensities of the renal responses among these three agents.
Experiment 3: 3.8×10^{-8} to 3.8×10^{-7} $mol\,kg^{-1}$ of FPL 55712 were infused into the perfusate reservoir for 10 min before and 10 min after the start of LTC_4 infusion (5.3×10^{-9} $mol\,kg^{-1}$, 5 min). OKY 1581 (7.3×10^{-4} $mol\,kg^{-1}\,min^{-1}$) or indomethacin (2.1×10^{-7} $mol\,kg^{-1}\,min^{-1}$) was added to the perfusate in the same manner as FPL 55712 infusion.
Experiments 1 to 3 were performed in the recirculating system and all drugs used in these series were dissolved in 0.5 ml of Krebs-Ringer bicarbonate buffer solution, which had been proved to have no influence on RPP and RPF by itself.
Experiment 4: LTC_4 (3.6×10^{-8} $mol\,kg^{-1}$), noradrenaline (5.9×10^{-9} $mol\,kg^{-1}$) or angiotensin II (2.4×10^{-11} $mol\,kg^{-1}$) were injected as a bolus into the arterial arm of the single pass system with a 10 μl Hamilton syringe. In this series of experiments, each

agent was dissolved in 10 μl of Krebs-Ringer bicarbonate buffer solution, which did not affect RPP and RPF when given during observation periods.

The duration of the effect was described by calculating its half-life. For this 3–6 observation points from the maximum of the effect to base line were fitted on a straight line after log transformation and the time elapsed between the maximum and half of the maximum of the effect was determined.

Materials

LTC_4 and OKY 1581 (sodium (E)-3-(4-(3-pyridylmethyl)phenyl)-2-methyl-2-propenoate) were generous gifts from Ono Pharm. Co Ltd., Osaka, Japan. LTC_4 was purified before use, as previously described (Metz *et al.*, 1982). Briefly, following the purification by high-performance liquid chromatography (h.p.l.c.), the LTC_4 fraction was collected and immediately lyophylized under nitrogen (N_2) and shielded from light. H.p.l.c was performed with a Waters 6000 A pump (Waters Assoc., Milford, U.S.A.), Beckman 160 absorbance detector (Beckman Instr. Inc., Berkeley, U.S.A.) set at 280 nm, 5 μm Nucleosil column (25 cm length); (Nagel, Düren, F.R.G.) and Waters WISP 710 B injector. The solvent of the mobile phase was composed of 67% methanol, 33% water and 0.08% acetic acid and adjusted to a final pH of 6.2 with ammonium hydroxide. Purified LTC_4 was dissolved in Krebs-Ringer bicarbonate buffer solution (pH 7.4) at appropriate concentrations. This buffer had been equilibrated with N_2 gas and stored in a freezer at −80°C up to the experimental date. The purity of LTC_4 after these procedures was checked every week and only LTC_4 with a purity greater than 98% was used for the present study. FPL 55712 (sodium 7-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxyl-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) was kindly supplied by Fisons Pharm. Lab., Loughborough, England. Angiotensin II was purchased from Sigma, St. Louis, U.S.A. and noradrenline from Hoechst, Frankfurt, F.R.G. Indomethacin was provided by Sharp & Dohme, Munich, F.R.G.

Statistical analysis

All results are expressed as means ± s.e.mean. For statistical evaluation, analysis of variance was performed followed by Student's *t* test for unpaired observations (Campbell, 1974). *P* values less than 0.05 were accepted as significant.

Results

Renal vascular effect of leukotriene C_4 on recirculating system

A representative response of the renal vasculature of the perfused kidney to intra-arterial LTC_4 (9.6×10^{-9} mol kg^{-1} min^{-1}, 5 min) in the recirculating system is illustrated in Figure 1, which shows that the effect of LTC_4 was characterized by moderate eleva-

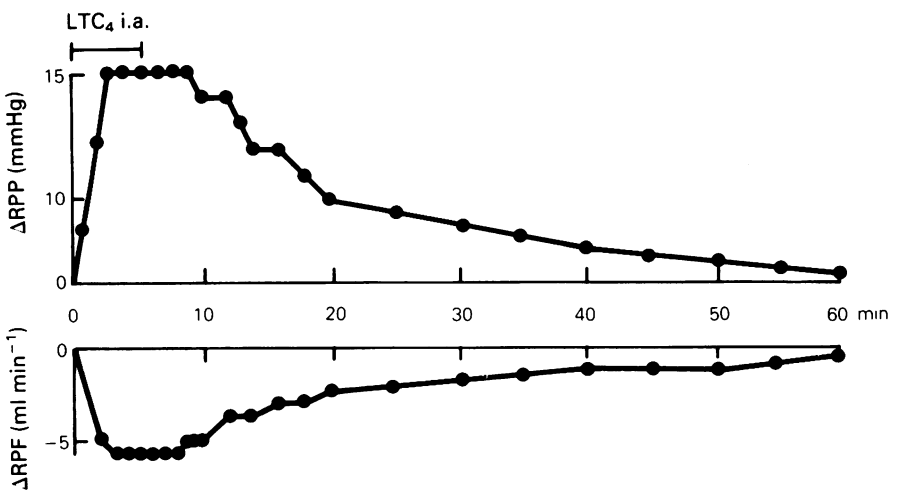

Figure 1 Changes in renal perfusion pressure (Δ RPP) and renal perfusate flow (Δ RPF) of an isolated perfused kidney following a 5 min infusion of leukotriene C_4 (LTC_4, 9.6×10^{-9} mol kg^{-1} min^{-1}) in a closed circuit.

tion of renal perfusion pressure (RPP) produced by an increase in total renal vascular resistance (RVR), since renal perfusate flow (RPF) actually declined. This response was qualitatively similar to those observed within the range of doses tested (LTC_4: 6.4×10^{-10} to 3.2×10^{-8} mol kg^{-1} min^{-1}, 5 min), i.e., the first portion of the pressor response was relatively steep in shape and short in duration (2 to 4 min), the second part was the peak phase and persisted for 3 to 10 min, followed by a long-lasting gradual decline (the third portion). There was no significant correlation between the peak durations and the doses of LTC_4 infused. The increased

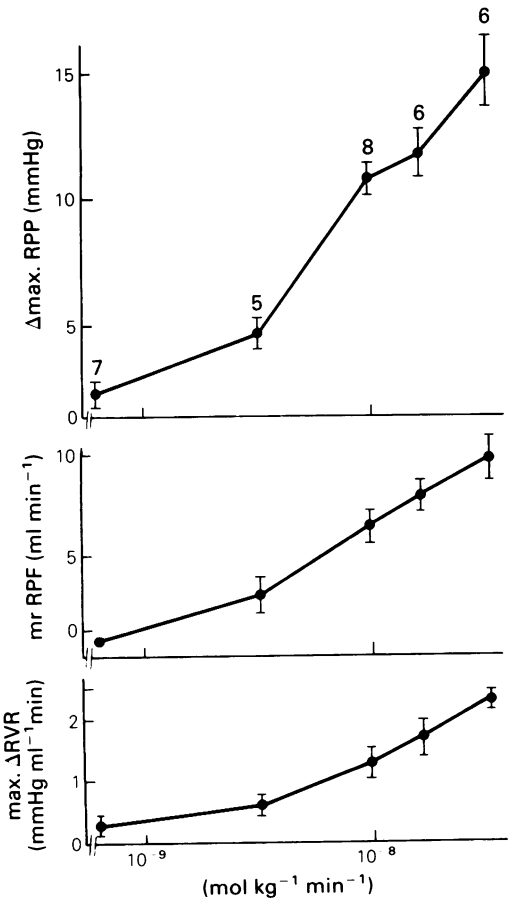

Figure 2 Dose-dependency of the maximal responses to various doses of leukotriene C_4 (LTC_4) (6.4×10^{-10} to 3.2×10^{-8} mol kg^{-1} min^{-1}) given over 5 min into the renal artery of the isolated perfused kidney in a closed circuit. Abbreviations: max Δ RPP, maximal change of renal perfusion pressure; mr RPF, maximal reduction in renal perfusate flow; max Δ RVR, maximal change in renal vascular resistance. Values are mean with s.e.mean shown by vertical lines; number of kidneys used is shown beside the points.

RPP as well as the reduced RPF did not recover completely within the observation period (over 1 h) in the recirculating system.

Figure 2 shows maximal increments of RPP (max. Δ RPP) and RVR (max. Δ RVR) and maximal reduction of RPF, resulting from intra-arterial infusions of LTC_4, in the range of 6.4×10^{-10} to 3.2×10^{-8} mol kg^{-1} min^{-1} (5 min) into the recirculating system. At the highest dose, LTC_4 administration increased RPP by 15.1×3.0 mmHg accompanied by a decrease in RPF of 8.8 ± 2.0 ml min^{-1} and accordingly an increase in RVR of 2.3 ± 0.2 mmHg ml^{-1} min, while at the lowest dose (6.4×10^{-10} mol kg^{-1} min^{-1}) these values of RPP, RPF and RVR were 2.2 ± 1.4 mmHg, 1.58 ± 0.59 ml min^{-1} and 0.27 ± 0.1 mmHg ml^{-1} min, respectively. These pressor responses were apparently dose-dependent with ED_{50} values of approximately 5.6×10^{-9} mol kg^{-1} min^{-1}.

Responses to noradrenaline and angiotensin II

As shown in Figure 3, the maximal renal pressor response to intra-arterial LTC_4 was compared with those produced by well-known renal vasoconstrictors, noradrenaline (1.2×10^{-10} to 4.7×10^{-9} mol kg^{-1} min^{-1}, 5 min, i.a.) and angiotensin II (3.8×10^{-13} to 1.9×10^{-11} mol kg^{-1} min^{-1}, 5 min, i.a.). The renal pressor responses to noradrenaline and angiotensin II were clearly dose-dependent and quantitatively quite different. The rank order of potencies for these agonists were consistently angiotensin II > noradrenaline > LTC_4. On a molar basis, LTC_4 was approximately 10 to 20 fold and 1000 to 2000 fold less potent than noradrenaline and angiotensin II respectively in evoking renal vasoconstriction of the perfused kidney.

Effects of inhibitors

Figure 4 demonstrates that treating the perfused kidney with FPL 55712 resulted in a dose-dependent inhibition of the renal vasoconstriction induced by LTC_4 (9.6×10^{-9} mol kg^{-1} min^{-1}, 5 min, i.a.) and the degrees of inhibition were 19.4%, 47.6% and 78.6% at doses of 3.8×10^{-8}, 9.5×10^{-8} and 3.8×10^{-7} mol kg^{-1} min^{-1} of FPL 55712, respectively.

Neither indomethacin (2.1×10^{-7} mol kg^{-1} min^{-1}) nor OKY 1581 (7.3×10^{-4} mol kg^{-1} min^{-1}) had an effect on changes of RPP or RPF induced by LTC_4 (5.3×10^{-9} mol kg^{-1} min^{-1}) (Figure 4). None of the inhibitors alone had an effect on RPP during a 60 min observation period (data not shown).

Renal vascular effects of leukotriene C_4 in the single pass system

Single pass experiments were carried out in order to ascertain whether the renal pressor effects observed in

the recirculating system (shown in Figure 1) were of a direct nature or produced by potent further metabolites in this experimental model.

Figure 5 shows typical pressor effects produced by 3.6×10^{-8} mol kg^{-1} of LTC_4, 5.9×10^{-9} mol kg^{-1} of noradrenaline and 2.4×10^{-11} mol kg^{-1} of angiotensin II injected into the arterial arm of the kidney as a bolus in the single pass system. RPP increased rapidly after bolus injections of LTC_4, noradrenaline and angiotensin II, reached its maximum rapidly within 20 to 50 s and completely returned to pre-injection level by 3 to 8 min. This is in contrast to the long-lasting effect (>60 min) of LTC_4 in the recirculating system (Figure 1). These data, obtained from single pass system, showed that the half-life of the LTC_4 effect was much shorter (1.5 min) than that expected from the

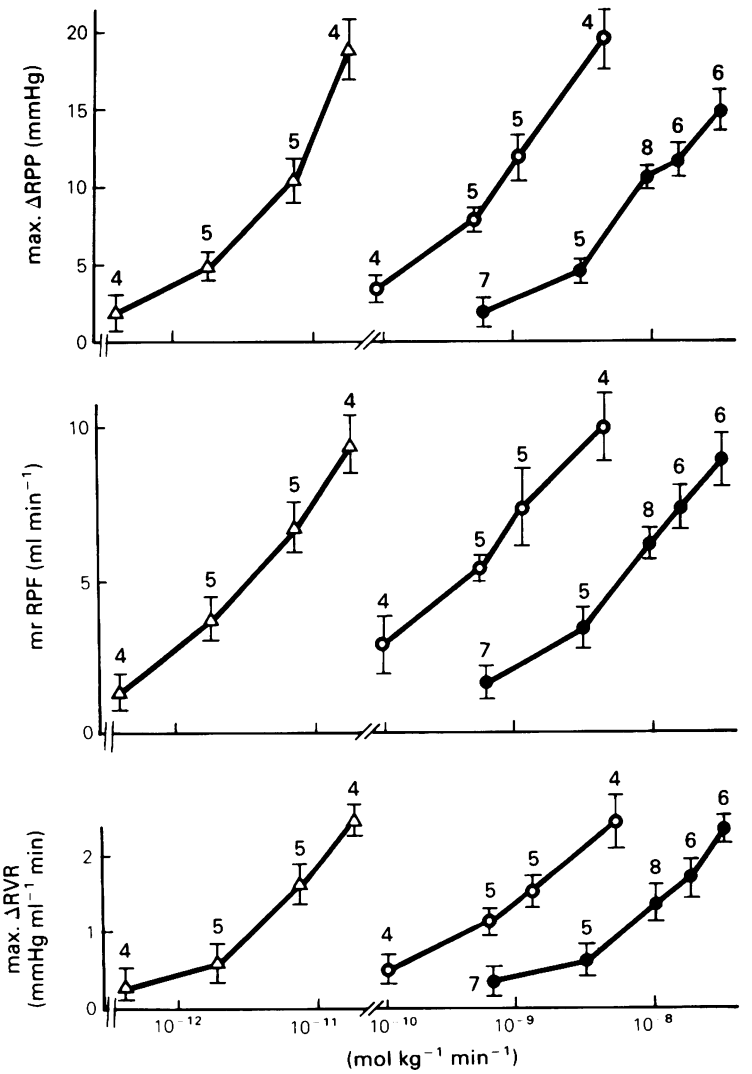

Figure 3 Comparison of renal vascular responses to leukotriene C_4 (LTC_4, 6.4×10^{-10} to 3.2×10^{-8} mol kg^{-1} min^{-1}, ●) with those to noradrenaline (1.2×10^{-10} to 4.7×10^{-9} mol kg^{-1} min^{-1}, ○) and angiotensin II (3.8×10^{-13} to 1.9×10^{-11} mol kg^{-1} min^{-1}, Δ) given over 5 min into the renal artery in the closed circuit perfused kidney. Abbreviations: max Δ RPP; maximal change in renal perfusion pressure; mr RPF, maximal reduction in renal perfusate flow; max Δ RVR, maximal change in renal vascular resistance. Values are mean with s.e.mean shown by vertical lines; number of kidneys used is indicated by each point.

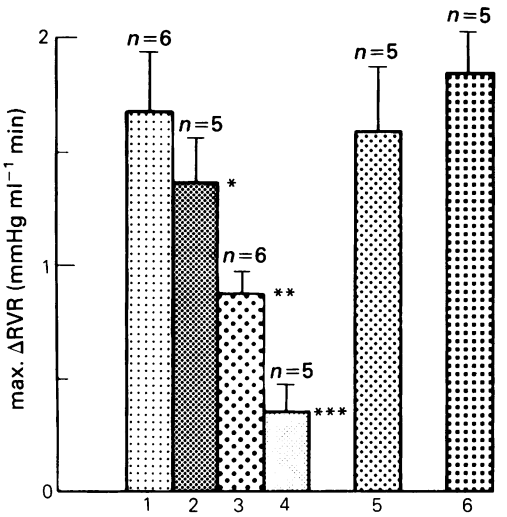

Figure 4 Effects of FPL 55712, indomethacin and OKY 1581 on the leukotriene C_4 (LTC_4)-induced maximal change in renal vascular resistance (max Δ RVR) in closed circuit perfusion: LTC_4 was given into the renal artery over 5 min in a dose of 5.3×10^{-9} mol kg^{-1} min^{-1}, the other compounds for 20 min i.v. Values are mean with s.e.mean shown by vertical lines, *n* refers to the number of kidneys. Statistical analysis by ANOVA and unpaired *t* test: $*P<0.05$; $**P<0.01$; $***P<0.005$. Column (1) LTC_4 (5.3×10^{-9} mol kg^{-1} min^{-1}); (2) LTC_4 + FPL 55712 (3.8×10^{-8} mol kg^{-1} min^{-1}); (3) LTC_4 + FPL 55712 (9.5×10^{-8} mol kg^{-1} min^{-1}); (4) LTC_4 + FPL 55712 (3.8×10^{-7} mol kg^{-1} min^{-1}); (5) LTC_4 + indomethacin (2.1×10^{-7} mol kg^{-1} min^{-1}); (6) LTC_4 + OKY 1581 (7.3×10^{-4} mol kg^{-1} min^{-1}).

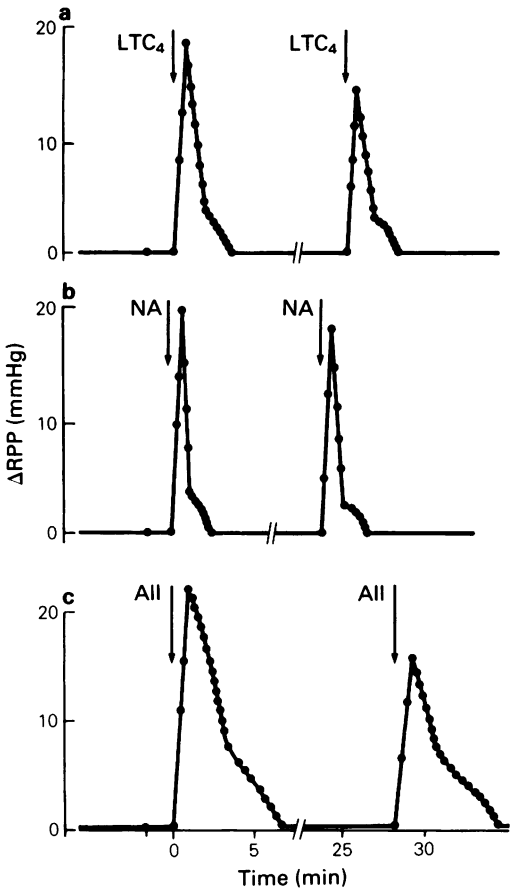

Figure 5 Time course of renal vascular response to intra-arterial leukotriene C_4 (LTC_4, a, 3.6×10^{-8} mol kg^{-1}), noradrenaline (NA, b, 5.9×10^{-9} mol kg^{-1}) and angiotensin II (AII, c, 2.4×10^{-11} mol kg^{-1}) expressed as change in renal perfusion pressure (Δ RPP) in an open circuit. Each compound was given as a bolus injection into the renal artery and measurements were taken every 10 s until RPP had returned to base line. After waiting for about 25 min the experiment was repeated.

studies carried out in the recirculating system. The half-life of the effect of noradrenaline and angiotensin II was about 1–3 min.

Discussion

The response of the kidney to LTC_4 was characterized by a dose-related increase in RVR that produced an elevation in RPP. This vasoconstrictor effect of LTC_4 occured with a potency that was one to three orders of magnitude less than that of noradrenaline and of angiotensin II, respectively. In other experimental models the reported effects of leukotrienes on the renal vascular beds and their intensities have been controversial (Feigen, 1983; Zukowska-Grojac *et al.*, 1983; Badr *et al.*, 1984; McLeod *et al.*, 1984). In the present study, we confirmed the renal vasoconstrictor effect of LTC_4 in rat isolated kidneys perfused with blood-free Krebs-Ringer solution, which are not affected by systemic hormonal and neural factors.

SRS-A (leukotrienes) can be produced in asthma, immediate hypersensitivity reactions (Brocklehurst, 1960; Kellaway & Trethewie, 1940) and possibly during non-immunological forms of tissue injury (Lewis & Austen, 1981), although there are no reports on actual concentrations of leukotrienes under these circumstances. In the present study, a larger quantity of LTC_4 was required to evoke renal vasoconstriction than that expected under physiological conditions as estimated by Örning & Hammarström (1982). These authors speculated that the physiological concentra-

tions of LTC_4 are lower than 1–5 μM. On this basis, we surmise that LTC_4 might not play an important role under physiological conditions; however, in pathological states, it may contribute to the regulation of vascular resistance.

In the present study, neither indomethacin, a cyclo-oxygenase inhibitor nor OKY 1581, a specific thromboxane synthetase inhibitor (Smith & Jubitz, 1981) modified the LTC_4-induced effects, whereas these effects were antagonized dose-dependently by FPL 55712, an SRS-A antagonist (Augstein *et al.*, 1973). Therefore, the LTC_4-induced response is not mediated by cyclo-oxygenase products in the perfused kidney. In this respect, our results are consistent with those described by Pong *et al.* (1983), who inferred the presence of specific LTC_4 receptors, although isolated perfused kidneys are capable of producing thromboxane A_2 (Morrison *et al.*, 1978) which has been reported to modify biological activities of leukotrienes (Hamel *et al.*, 1982).

The effect of LTC_4 was short-acting and disappeared with a half-life of 1.5 min in the single pass system, while a long-lasting response was observed in the recirculating system. It is possible that LTC_4 recirculates in the latter system, and thereby produces a long-range effect. It is, however, more likely that LTC_4 is converted to further potent metabolites. In fact, we have recently observed the conversion of LTC_4 to LTD_4 and further to LTE_4 in the latter system (Yoshizawa & Frölich, unpublished observations). It has also been demonstrated that LTC_3 is degraded to LTD_3 and further to LTE_3 in the isolated perfused rat kidney, catalysed by γ-glutamyl transferase and dipeptidase, respectively (Ormstad *et al.*, 1982).

In conclusion, LTC_4 is a short-acting vasoconstrictor that acts directly on specific leukotriene receptors. The compound may be converted to further potent metabolites within the kidney. Thus, LTC_4 and presumably its metabolites may participate in the regulation of renal vascular tone under pathological conditions where leukotriene synthesis is enhanced.

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