Renal vascular effects of leukotriene C₄ in the isolated perfused kidney of the rat

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Introduction

Leukotriene C₄ (LTC₄) 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). LTC₄ has been shown to be converted into its metabolites, leukotriene D₄ and E₄ (LTD₄ and LTE₄) by the enzymes γ-glutamyl transpeptidase and dipeptidase, respectively (Bergström & Hammarström, 1981; Örning & Hammarström, 1982). Furthermore, the formed LTE₄ can be converted into leukotriene F₄ (LTF₄) 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). Furthermore, systemic vasoconstrictor effects of intravenously administered LTC₄ and LTD₄ 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 LTC₄ and LTD₄ 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 x 10⁻¹² to 10⁻⁹ mol of LTC₄ into the renal artery in pigs (McLeod et al., 1984) and to
intravenous infusions of 2 µg kg⁻¹ min⁻¹ of LTC₄ 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 µg kg⁻¹ of LTD₄ 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 LTC₄ 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 LTC₄. In addition, inhibitors of cyclooxygenase and of thromboxane synthetase as well as an antagonist of SRS-A have been examined to evaluate the mechanism of LTC₄-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 (Nishiitsuji-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% O₂ and 5% CO₂. 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; perfuse 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 perfuse 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 × 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 LTC₄ 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 × 10⁻⁸ 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 LTC₄ above, to compare the intensities of the renal responses among these three agents.

Experiment 3: 3.8 × 10⁻⁹ 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 LTC₄ infusion (5.3 × 10⁻⁹ mol kg⁻¹, 5 min). OKY 1581 (7.3 × 10⁻⁷ 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: LTC₄ (3.6 × 10⁻⁸ mol kg⁻¹), noradrenaline (5.9 × 10⁻⁷ mol kg⁻¹) or angiotensin II (2.4 × 10⁻¹⁰ 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**

LTC₄ and OKY 1581 (sodium (E)-3-(4-(3-pyridylmethyl)phenyl)-2-methyl-2-propenoate) were generous gifts from Ono Pharm. Co Ltd., Osaka, Japan. LTC₄ 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 LTC₄ fraction was collected and immediately lyophilized under nitrogen (N₂) 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, Duren, 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 LTC₄ was dissolved in Krebs-Ringer bicarbonate buffer solution (pH 7.4) at appropriate concentrations. This buffer had been equilibrated with N₂ gas and stored in a freezer at −80°C up to the experimental date. The purity of LTC₄ after these procedures was checked every week and only LTC₄ 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 noradrenaline 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 C₄ on recirculating system**

A representative response of the renal vasculature of the perfused kidney to intra-arterial LTC₄ (9.6 × 10⁻⁸ mol kg⁻¹ min⁻¹, 5 min) in the recirculating system is illustrated in Figure 1, which shows that the effect of LTC₄ was characterized by moderate eleva-

![Figure 1](image-url) Changes in renal perfusion pressure (ΔRPP) and renal perfusate flow (ΔRPF) of an isolated perfused kidney following a 5 min infusion of leukotriene C₄ (LTC₄, 9.6 × 10⁻⁸ 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 (LTC₄: 6.4 x 10⁻¹⁰ to 3.2 x 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 LTC₄ infused. The increased 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. \( \Delta \) RPP) and RVR (max. \( \Delta \) RVR) and maximal reduction of RPF, resulting from intra-arterial infusions of LTC₄, in the range of 6.4 x 10⁻¹⁰ to 3.2 x 10⁻⁸ mol kg⁻¹ min⁻¹ (5 min) into the recirculating system. At the highest dose, LTC₄ 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⁻¹, while at the lowest dose (6.4 x 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⁻¹, respectively. These pressor responses were apparently dose-dependent with ED₉₀ values of approximately 5.6 x 10⁻⁸ mol kg⁻¹ min⁻¹.

Responses to noradrenaline and angiotensin II

As shown in Figure 3, the maximal renal pressor response to intra-arterial LTC₄, was compared with those produced by well-known renal vasoconstrictors, noradrenaline (1.2 x 10⁻¹⁰ to 4.7 x 10⁻⁸ mol kg⁻¹ min⁻¹, 5 min, i.a.) and angiotensin II (3.8 x 10⁻¹⁰ to 1.9 x 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 > LTC₄. On a molar basis, LTC₄ 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 LTC₄ (9.6 x 10⁻⁸ 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 x 10⁻⁸, 9.5 x 10⁻⁸ and 3.8 x 10⁻⁷ mol kg⁻¹ min⁻¹ of FPL 55712, respectively.

Neither indomethacin (2.1 x 10⁻⁷ mol kg⁻¹ min⁻¹) nor OKY 1581 (7.3 x 10⁻⁴ mol kg⁻¹ min⁻¹) had an effect on changes of RPP or RPF induced by LTC₄ (5.3 x 10⁻⁸ 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 C₄ 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
$3.6 \times 10^{-8} \text{mol kg}^{-1}$ of LTC$_4$, $5.9 \times 10^{-8} \text{mol kg}^{-1}$ of
noradrenaline and $2.4 \times 10^{-11} \text{mol kg}^{-1}$ 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 angioten-
sin 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

![Graph](image)

**Figure 3** Comparison of renal vascular responses to leukotriene C$_4$ (LTC$_4$, $6.4 \times 10^{-10}$ to $3.2 \times 10^{-9} \text{mol kg}^{-1} \text{min}^{-1}$,
●) with those to noradrenaline ($1.2 \times 10^{-10}$ to $4.7 \times 10^{-10} \text{mol kg}^{-1} \text{min}^{-1}$, ○) and angiotensin II ($3.8 \times 10^{-11}$ to
$1.9 \times 10^{-11} \text{mol kg}^{-1} \text{min}^{-1}$, △) 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.
Less than that of elevation in RPP. This Discussion models the angiotensin II, the response effect of the effect of a with occurred (Feigen, 1983; Trethewie, 1940) and controversial beds and their intensities have been vascular 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 LTC₄ was characterized by a dose-related increase in RVR that produced an elevation in RPP. This vasoconstrictor effect of LTC₄ occurred 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 LTC₄ 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 LTC₄ 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 LTC₄ are lower than 1–5 μM. On this basis, we surmise that LTC₄ 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 cyclooxygenase inhibitor nor OKY 1581, a specific thromboxane synthetase inhibitor (Smith & Jubitz, 1981) modified the LTC₄-induced effects, whereas these effects were antagonized dose-dependently by FPL 55712, an SRS-A antagonist (Augstein et al., 1973). Therefore, the LTC₄-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 LTC₄ receptors, although isolated perfused kidneys are capable of producing thromboxane A₂ (Morrison et al., 1978) which has been reported to modify biological activities of leukotrienes (Hamel et al., 1982).

The effect of LTC₄ 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 LTC₄ recirculates in the latter system, and thereby produces a long-range effect. It is, however, more likely that LTC₄ is converted to further potent metabolites. In fact, we have recently observed the conversion of LTC₄ to LTD₄ and further to LTE₄ in the latter system (Yoshizawa & Frölich, unpublished observations). It has also been demonstrated that LTC₄ is degraded to LTD₄ and further to LTE₄ in the isolated perfused rat kidney, catalysed by γ-glutamyl transferase and dipeptidase, respectively (Ormstad et al., 1982).

In conclusion, LTC₄ 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, LTC₄ 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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