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Some effects of leukotriene D_4 on the mechanical properties of the guinea-pig basilar artery

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1 The effects of leukotriene D_4 (LTD_4) on the mechanical properties of smooth muscle cells from the guinea-pig basilar artery were investigated in whole and chemically skinned muscle strips.

2 In strips with an intact endothelium, 5-hydroxytryptamine (5-HT; 10 μM), LTD_4 and LTC_4 (1 μM), STA_2 (1 nM–10 nM) and high K^+ (30 mM–128 mM) generated contractions. These comprised an initial phasic and subsequently generated tonic response with different amplitudes. Acetylcholine (ACh, 0.1–10 μM) inhibited and methylene blue (1–10 μM) enhanced the tonic component of these contractions in endothelium-intact muscle strips. In endothelium-denuded tissues, methylene blue had no effect on mechanical responses and ACh produced a further contraction in the presence of LTD_4.

3 When the endothelium was removed, the amplitude of contractions induced by all tested stimulants markedly increased. In intact muscle strips, the order of potency for the production of a maximum response was; 128 mM K^+ > STA_2 > LTD_4 = LTC_4 = 5-HT. Following removal of the endothelium; STA_2 > 128 mM K^+ > LTD_4 = LTC_4 ⩾ 5-HT.

4 In endothelium-denuded strips, the selective LTD_4 antagonists, ONO-RS-411 and FPL 55712 inhibited the LTD_4-induced contraction. In contrast, guanethidine, prazosin, yohimbine, atropine and mepyramine had no effect. Indomethacin and a thromboxane A_2(TXA_2) antagonist, ONO-3708 also had no effect on LTD_4-induced contractions in endothelium-denuded strips.

5 In endothelium-denuded strips, nifedipine inhibited the tonic contraction induced by LTD_4 but not the phasic component. In Ca^{2+}-free solution containing 2 mM EGTA, LTD_4 produced only the phasic contractions.

6 In saponin-treated chemically skinned muscle strips, LTD_4 had no effect on either the pCa-tension relationship or on the release of Ca^{2+} from intracellular stores. However, inositol 1,4,5-trisphosphate released Ca^{2+} from the stores and 1,2-diolein, an activator of protein kinase C, enhanced the contractions induced by 0.3 μM Ca^{2+}.

7 It was concluded that LTD_4 acts on both the endothelium and on the smooth muscle cells of the guinea-pig basilar artery. It stimulates the release of endothelium-derived relaxing factor (EDRF) which tends to inhibit the LTD_4-induced contraction. It also interacts with receptors on the smooth muscle and produces a contraction as a result of an increase in both voltage-dependent and receptor-activated Ca^2 influx and, in part, the release of Ca^{2+} from cellular storage sites.

Introduction

Leukotriene D_4 (LTD_4), a component of slow reacting substance of anaphylaxis, is a product of arachidonic acid via 5-lipoxygenase and has received much attention as the putative mediator of bronchconstriction in asthma and anaphylaxis (Hedqvist *et al.*, 1980). This substance has recently been shown to be the most potent vasoconstrictor among the 5-lipoxygenase products, particularly in the coronary and cerebral arteries of several species including man (coronary artery: Kito *et al.*, 1981; Michelassi *et al.*, 1982; Piper & Stewart, 1986; Kopia *et al.*, 1987, cerebral artery: Tagari *et al.*, 1983; Rosenblum, 1985; Busija *et al.*, 1986).

Following subarachnoid haemorrhage, the synthesis of leukotrienes in gerbil brain is increased (Kiwak *et al.*, 1985), and there is a close temporal relationship between the increase in 5-hydroxyeicosatetraenoic acid (5-HETE), a 5-lipoxygenase product, in cerebrospinal fluid and the development

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of cerebral vasospasm in patients with subarachnoid haemorrhage (Suzuki *et al.*, 1983; Nakamura *et al.*, 1984). These results may indicate that LTD_4 alone or in conjunction with other vasoactive substances is involved in the pathogenesis of cerebral vasospasm after subarachnoid haemorrhage.

In delayed cerebral vasospasm, leukocytes (Borgeat & Samuelsson, 1979), mast cells (Shimizu *et al.*, 1986) and macrophages (Rouzer *et al.*, 1980) synthesize leukotrienes which infiltrate into the wall of cerebral arteries after subarachnoid haemorrhage (Hughes & Schianchi, 1978; Liszczak *et al.*, 1983). Recently, Yokota *et al.* (1987) found that 2-(12-hydroxydodeca- 5,10-diynyl)3,5,6-trimethyl-1,4- benzoquinone (AA-861), an inhibitor of 5-lipoxygenase, was effective in maintaining cerebrovascular homeostasis after subarachnoid haemorrhage in the dog. Thus, much evidence supports the view that LTD_4 may be a putative vasoconstrictor in delayed cerebral vasospasm after subarachnoid haemorrhage. However, the mechanisms underlying the vasoconstriction induced by LTD_4 are not known.

The present study was undertaken to characterize the effects of LTD_4 on the mechanical properties of smooth muscles of the guinea-pig basilar artery. To achieve this, the role of the endothelium in LTD_4-induced contractions was investigated together with the intracellular effects of this agent by use of saponin-skinned muscle tissues.

Methods

Guinea-pigs of either sex (250–350 g) were killed by decapitation, taking care to avoid subarachnoid haemorrhage. After craniectomy the basilar artery with brain stem was removed and placed in a small chamber filled with Krebs solution. After dissecting the basilar artery, the arachnoid membrane and connective tissue were carefully removed and circular strips (0.03–0.05 mm in thickness, 0.3–0.5 mm in length and 0.1 mm in width) were prepared under a binocular microscope. To remove the endothelium, the intimal surface was gently rubbed using small knives made from pieces of razor blade (Kanmura *et al.*, 1987). Satisfactory ablation of the endothelium was histologically verified under a light microscope.

Solutions

The ionic composition of the Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.4, glucose 11.5. The solution was bubbled with 97% O_2 and 3% CO_2 and the pH was adjusted to 7.4. High K solution or Ca^{2+} free solution was prepared by isosmotic replacement of NaCl with KCl or of $CaCl_2$ with $MgCl_2$ (with 2 mM EGTA), respectively.

Recording of mechanical activity

The circularly cut preparation was set up in a small 1.0 ml chamber, through which the test solution could be changed rapidly by injecting fresh solution from one end and by sucking simultaneously from the other end with a water pump. Both ends of the preparation were fixed between pieces of Scotch double-sided tape via thin silk, and isometric tension was recorded using a strain gauge transducer (U gauge, Shinko Co., Tokyo, Japan). The temperature of the perfusate was kept at 25°C. All experiments were started after at least 90 min equilibration, under a tissue resting tension of approximately 0.5 mg.

Skinned muscle preparations

Skinned muscle preparations were obtained by exposure to saponin (40 $\mu g\,ml^{-1}$) for 20 min in relaxing solution at 25°C. The tension-pCa relationship was obtained by cumulative application of increasing Ca^{2+} concentrations in a stepwise manner. Drugs were applied during the Ca^{2+}-induced contraction after *tension* had reached a steady level.

Drugs

The chemicals used were leukotrienes C_4 and D_4 (LTC_4, LTD_4; Ono), 4-oxo-8-[p-(4-phenylbutyloxy) benzoylamino]-2-(tetrazol-5-yl)- 4H-1-benzopyran hemihydrate (ONO-RS-411; Ono), sodium 7-[3-(4-acetyl-3-hydroxy-2-propyl- phenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1- benzopyran-2-carobxylate (FPL 55712; Fisons), 9,11, epithio-11,12-methanothromboxane A_2 (STA_2; Ono), (9,11),(11,12)-dideoxa-9α,11α-dimethylmethano-11,12- methano- 13,14-dihydro-13-aza-14-oxo-15- cyclopentyl-16,17,18,19,20-pentanor- 15-epithromboxane A_2 (ONO-3708; Ono), 5-hydroxytryptamine hydrochloride (5-HT; Sigma), noradrenaline HCl (Sigma), acetylcholine-Cl (ACh, Daiichi), prazosin (Pfeizer Taito), mepyramine (Tokyo Kasei), methysergide (Sandoz), indomethacin (Sigma), nifedipine (Bayer), methylene blue (Sigma), ethyleneglycol- *bis*(β-aminoethylether)-N,N′,N′,N′-tetraacetic acid (EGTA, Dozin), phosphatidylserine (PS; beef brain) and 1,2-diolein (Serdary Research Lab.).

Leukotrienes C_4 and D_4 were stored in an atmosphere of nitrogen below −70°C protected from light, and used within a week. Inositol 1,4,5-trisphosphate (IP_3) was kindly provided by Dr M. Hirata, Dept. of Biochem., Fac. of Dentistry, Kyushu Univ.

Statistics

The measured value was expressed as the mean ± s.d. and number of observations. The statistical significance was assessed by Student's t test. P values less than 0.05 were considered significant.

Results

Effects of various stimulants before and after removal of endothelium

Figure 1a shows the effects of 128 mM K^+, 10 μM noradrenaline (NA), 10 μM 5-hydroxytryptamine (5-HT), 1 μM LTD_4 and 10 nM STA_2 on smooth muscle strips of the guinea-pig basilar artery before (intact) and after removal of the endothelium. These agonist concentrations stimulated maximum responses on both tissue conditions. In intact preparations, except for 128 mM K^+ and STA_2, the contraction was small and no contraction was evoked by application of 10 μM NA. In contrast, after removal of the endothelium, contractions evoked by all the stimulants were enhanced. Figure 1b summarizes the amplitudes of contraction evoked by various stimulants compared before and after removal of endothelium. The peak amplitude of the 128 mM K^+-induced contraction in the presence of endothelium was normalized as a relative tension of 1.0, and the maximum responses evoked by the other stimulants were compared. Contractions produced by NA, 5-HT, LTD_4 and STA_2 were all enhanced after removal of the endothelium to a greater extent than responses to 128 mM K^+. Furthermore, after endothelium removal the tonic phase of the responses was enlarged more than the phasic component.

The effects of LTC_4 were also studied and compared with changes produced by application of LTD_4 in intact and endothelium-denuded tissues. Exposure to 0.1 μM and 1.0 μM LTC_4 showed almost the same actions as those observed after application of equimolar concentrations of LTD_4 to intact and endothelium-denuded tissues (data not shown). Hereafter, we used only LTD_4.

To confirm previous findings relating to the endothelium (Furchgott & Zawadzki, 1980), the effects of ACh on tissues precontracted by LTD_4 were compared before and after removal of endothelium. As shown in Figure 2, 1 μM LTD_4 generated a small phasic and tonic contraction in the control, and application of 10 μM ACh reduced the amplitude of this tonic response. After addition of 10 μM methylene blue, a cyclic GMP synthesis inhibitor, the basal tone was gradually raised and the contraction evoked by 1 μM LTD_4 was markedly enhanced. ACh (10 μM) produced a further contraction additive with the existing LTD_4-induced response (Figure 2a). The same experimental protocols were used for the muscle strip after endothelium removal. Then the peak amplitude of the phasic component of contraction evoked by 128 mM K^+ was only slightly enlarged but the tonic response was markedly enhanced. When 10 μM ACh was applied in the presence of 1 μM LTD_4 (Figure 2b), the evoked responses were similar to those observed in the presence of methylene blue in intact muscle tissue (Figure 2a). Furthermore, application of 10 μM methylene blue did not modify the actions of LTD_4 or ACh (Figure 2b). The concentration-response relationships for LTD_4 in intact and denuded preparations are shown in Figure 3. The amplitude of phasic response of contractions evoked by 128 mM K^+ was normalized as a relative tension of 1.0.

Characteristics of the LTD_4-induced contraction

The effects of FPL 55712 and ONO-RS-411, inhibitors of the LTD_4 receptor (Obata *et al.*, 1985; Toda *et al.*, 1985), on the LTD_4-induced contraction were observed after ablation of endothelium. In the concentration range 10–100 nM, both agents inhibited contractions generated by 1 μM LTD_4 (Figure 4a,b), while the K-induced contraction evoked as a control was not affected by ONO-RS-411 or FPL 55712. Thus inhibition induced by these agents on the response to LTD_4 is not due to non-selective inhibition. Guanethidine (5 μM), prazosin (1 μM), yohimbine (1 μM), atropine (1 μM), mepyramine (1 μM), methysergide (1 μM), indomethacin (10 μM) and ONO-3708 (a thromboxane A_2 antagonist) had no effect on the LTD_4-induced contraction.

The LTD_4-induced contraction evoked in smooth muscle strips of the basilar artery following ablation of endothelium comprised small phasic and large tonic components. After application of 0.3 μM nifedipine, 128 mM K^+ produced only a contraction of small amplitude, and 1 μM LTD_4 produced a normal phasic response but the tonic component was markedly inhibited (Figure 5a). Exactly the same pattern of inhibition was observed in Ca^{2+}-free solution containing 2 mM EGTA but with greater inhibition of the tonic response (Figure 5b). This suggests that the small phasic component is produced by release of Ca^{2+} stored in the cells and that the tonic response is mainly due to Ca^{2+} influx.

Effects of LTD_4 on skinned muscle tissues

As described in the methods, the tissue was skinned by use of 40 $\mu g\,ml^{-1}$ saponin. In non-skinned endothelium-denuded controls, contractions were evoked using 128 mM K^+ and combined application of 128 mM K^+ and 10 nM STA_2 to determine the

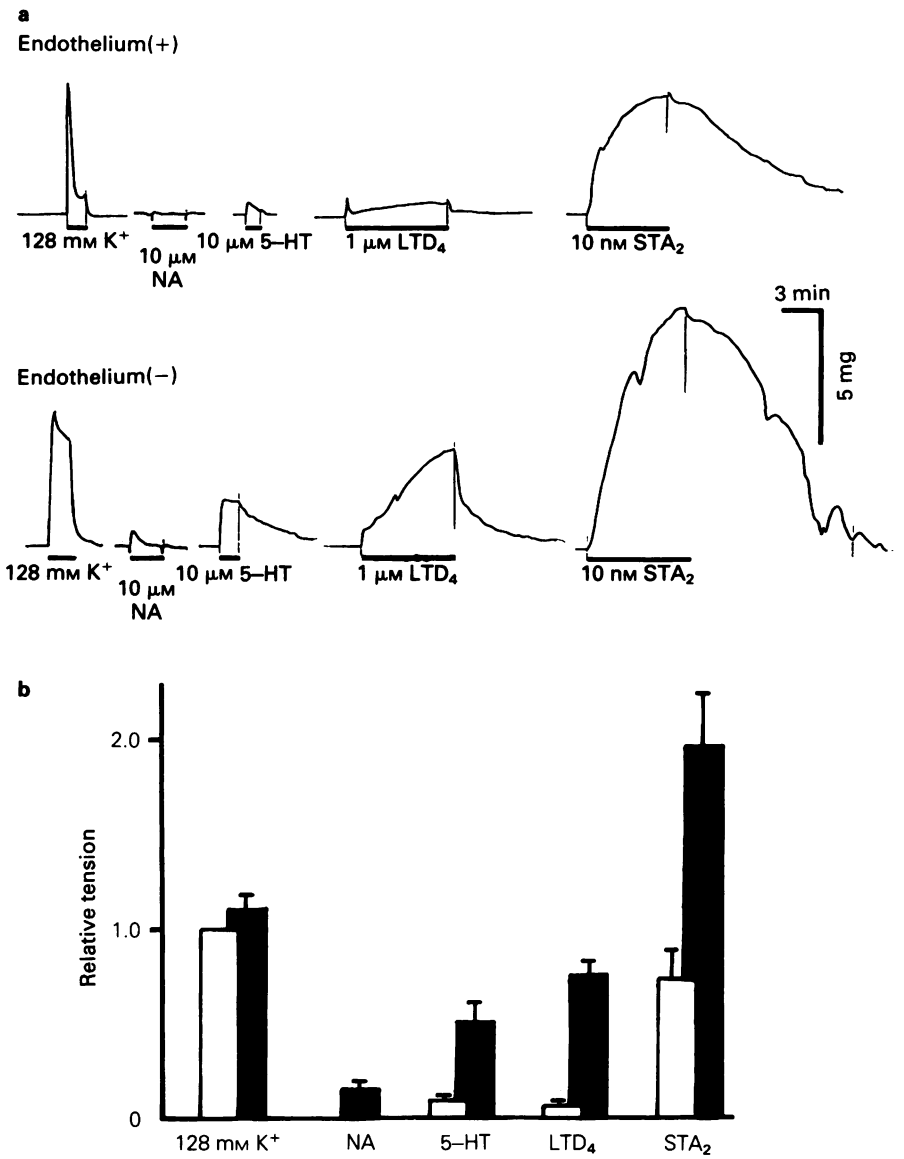

Figure 1 Effects of 128 mM K^+, noradrenaline (NA), 5-hydroxytryptamine (5-HT), leukotriene D_4 (LTD_4) and STA_2 on mechanical activity in smooth muscle strips of the guinea-pig basilar artery. (a) Typical examples of the effects of these stimulants in intact muscle strips (upper part) and endothelium-denuded strips (lower part). (b) Effects of various stimulants on contractions in the presence (open columns) and absence (solid columns) of endothelium. Vertical bars indicate s.d. (n = 5–11). The amplitude of phasic responses evoked by 128 mM K^+ in intact tissue (with endothelium) was normalized as a relative tension of 1.0.

maximum mechanical response of the tissue (Figure 6a). In skinned tissues, the minimum concentration of Ca^{2+} required to produce a contraction was 0.1 μM, and the maximum response was evoked by 10 μM. The cumulative pCa-response relationship is shown in Figure 6c. The contraction evoked by 10 μM Ca^{2+} was normalized as a relative tension of 1.0, and the maximum amplitude of mechanical changes evoked in skinned muscles was almost the same as that observed by combined application of

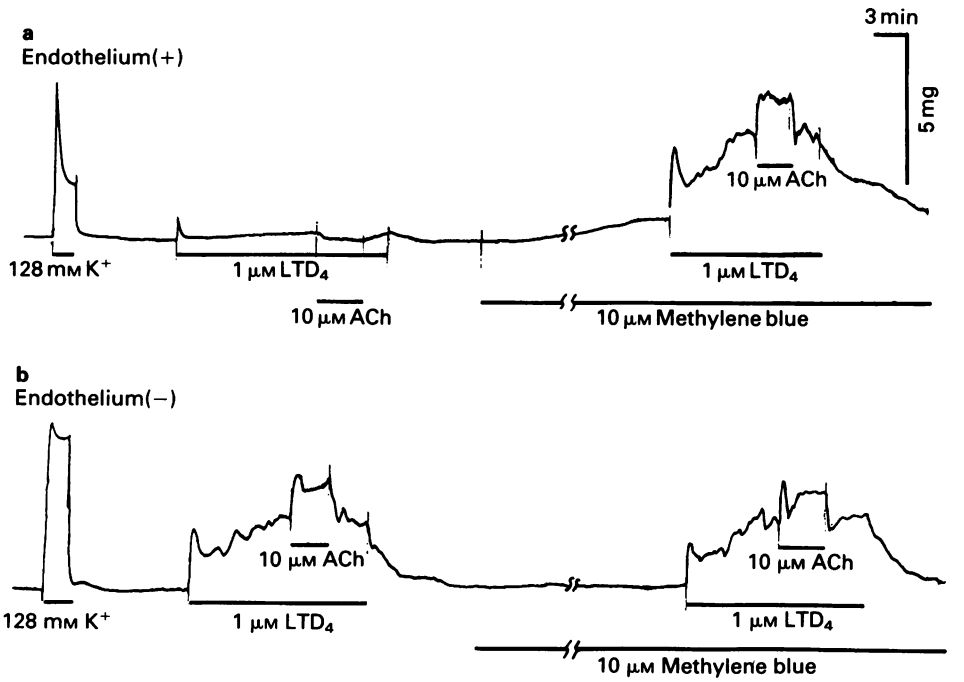

Figure 2 Effects of acetylcholine (ACh) on contractions induced by leukotriene D_4 (LTD_4) in the presence and absence of methylene blue in the guinea-pig basilar artery. (a) The endothelium-intact strip; (b) endothelium-denuded strip.

128 mM K^+ and 10 nM STA_2 in non-skinned preparations.

When 50 $\mu g\,ml^{-1}$ 1,2-diolein, an analogue of 1,2-diacylglycerol, was applied with 50 $\mu g\,ml^{-1}$ phosphatidylserine (PS) after establishing a Ca^{2+}-induced contraction (0.3 μM), the contraction gradually increased (Figure 7a). As shown in Figure 7b, 50 $\mu g\,ml^{-1}$ PS slightly enhanced the 0.3 μM Ca^{2+}-induced contraction but application of 0.3 μM LTD_4 with PS did not enhance the original contraction further. This suggests that the contractile systems in these skinned muscle tissues are intact, as estimated from the actions of 1,2-diolein, but that LTD_4 (itself) had no direct effect on the contractile proteins. PS itself slightly enhanced the Ca-induced contraction in this tissue. The mechanism underlying this phenomenon should be clarified.

The procedure used to investigate the possible effects of LTD_4 on Ca^{2+} release from intracellular stores is shown in Figure 8. Skinned tissues were first exposed to Ca^{2+}-free solution with EGTA (2 mM) to create zero Ca^{2+} conditions. A solution containing a buffered concentration of 0.3 μM Ca^{2+} was then applied. Previous work (Itoh *et al.*, 1981; 1982; 1983) had shown that such a procedure generates a small contraction and allows re-filling of the intracellular Ca^{2+} stores. Subsequent exposure to IP_3 releases Ca^{2+} from these stores and generates a contraction which can be repeated several times. In the experi-

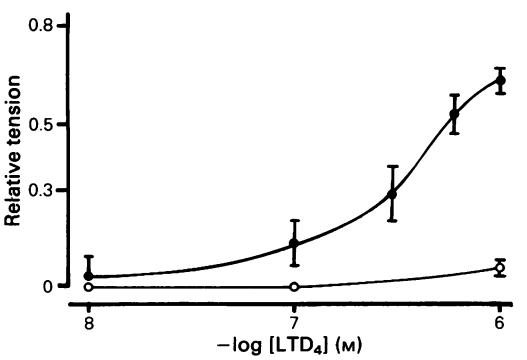

Figure 3 The concentration-response relationships to leukotriene D_4 (LTD_4) in muscle strips with and without endothelium. The maximal response to 128 mM K^+ on strips in either condition was normalized as a relative tension of 1.0. Curves were fitted by eye. (○) Control tissue (+ endothelium); (●) endothelium-denuded tissue. Each point is the mean derived from 5–7 observations with vertical lines indicating s.d.

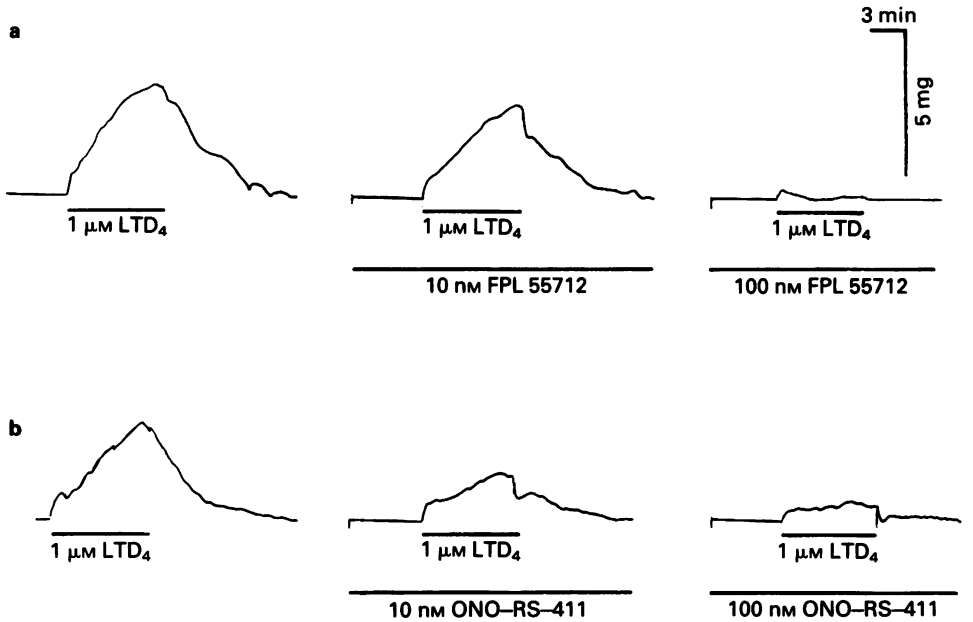

Figure 4 An example of the effects of ONO RS-411 and FPL 55712 on the contraction evoked by 1 μM leukotriene D_4 (LTD_4) in endothelium-denuded strips. The same effects were observed in 5 other preparations.

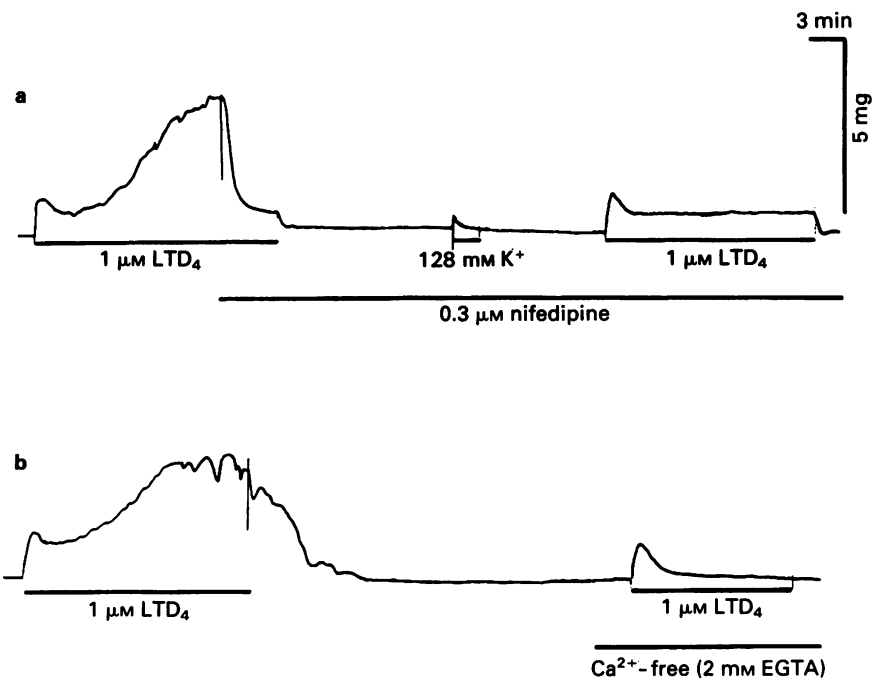

Figure 5 Effects of nifedpine and Ca^{2+}-free solution containing 2 mM EGTA on the contraction evoked by 1 μM leukotriene D_4 (LTD_4) in an endothelium-denuded strip. Bars indicate application of nifedipine (0.3 μM), Ca^{2+}-free solution and LTD_4, as appropriate. Similar results were obtained in 3 other preparations.

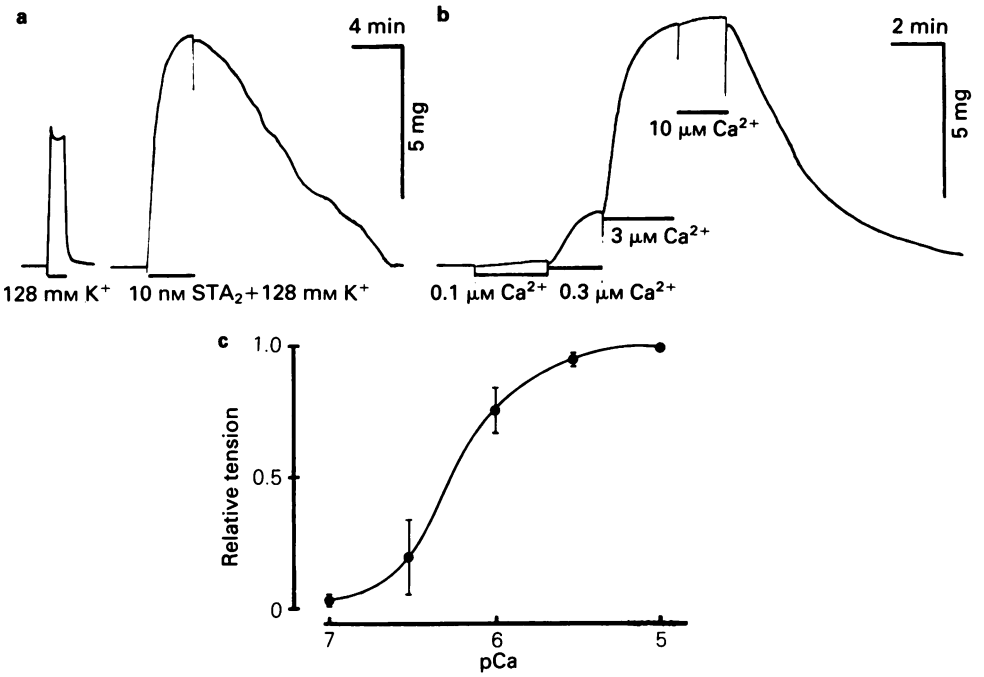

Figure 6 Ca^{2+}-induced contractions recorded from skinned muscle tissues of the guinea-pig basilar artery. The experimental procedures used to prepare the skinned muscles by use of saponin are described in the Methods. (a) The contraction evoked by 128 mM K^+ and 128 mM K^+ + 10 nM STA_2 in the intact muscle strip. (b) The contraction evoked by various concentrations of Ca^{2+} applied cumulatively. Bars indicate application of different concentrations of Ca^{2+}. (c) The relationship between concentrations of Ca^{2+} (expressed as pCa) and contraction. The contraction evoked by pCa = 5 was normalized as a relative tension of 1.0. Vertical lines indicate s.d., n = 4–5.

ment shown in Figure 8, the integrity of the store/release mechanism was tested by two successive exposures to IP_3. Subsequent exposure to LTD_4 produced no response and final challenge with IP_3 showed that the store/release mechanism was intact. This means that LTD_4 has no effect on the contractile proteins or on the intracellular Ca^{2+} stores in smooth muscle cells of the basilar artery.

Discussion

Although LTD_4 produces contractions in many smooth muscles, the findings in cerebral vessels have not been consistent with previous results ranging from no effect (Von Holst *et al.*, 1982; Kamitani *et al.*, 1985; Högestaett *et al.*, 1987) to vasoconstriction (Beckett *et al.*, 1981; Tagari *et al.*, 1983; Greenwald *et al.*, 1984; Rosenblum, 1985; Taylor *et al.*, 1986; Busija *et al.*, 1986). In the present experiments, LTD_4 showed dual actions on the guinea-pig basilar artery. In intact muscle tissues, LTD_4 produced phasic and tonic responses of low amplitude, while in endothelium denuded preparations, these phasic and tonic responses were enhanced. Treatment of intact tissues with methylene blue produced responses identical to those observed after mechanical removal of the endothelium. The endothelium derived relaxing factor (EDRF; Furchgott & Zawadzki, 1980; Furchgott, 1984) released spontaneously or after exposure to various agonists from the endothelium, may increase the amount of muscle cyclic GMP through activation of soluble guanylate cyclase (Rapoport *et al.*, 1983; Murad, 1986) and inhibit contractions due to a reduction in the free concentration of Ca^{2+} in the cytosol (Suematsu *et al.*, 1984; Kobayashi *et al.*, 1985; Itoh *et al.*, 1985). An endothelial component in the action of LTD_4 in dog renal and mesenteric arteries has been previously reported (Secrest *et al.*, 1985).

The LTD_4-induced contraction consisted of small phasic and tonic responses and was enhanced after ablation of endothelium. The phasic response evoked by LTD_4 was unchanged in Ca^{2+}-free conditions or after exposure to a Ca antagonist. Thus, this contraction was probably evoked by release of Ca^{2+}

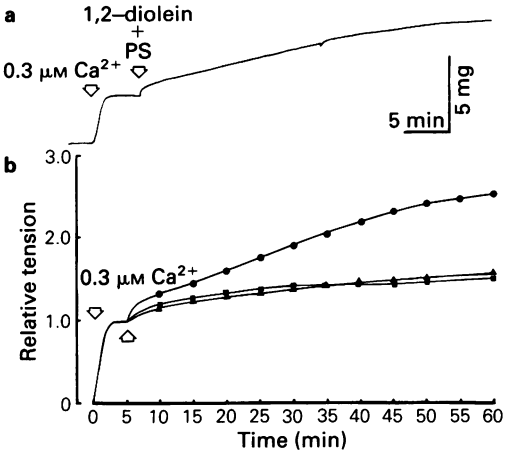

Figure 7 Effects of 1,2-diolein + phosphatidylserine (PS), leukotriene D_4 (LTD_4) + PS or PS alone on Ca^{2+}-induced contractions (0.3 μM) in skinned muscle tissues. (a) A mixture of 1,2-diolein (50 μg ml^{-1}) + PS (50 μg ml^{-1}) was applied during the generation of a sustained Ca^{2+}-induced contraction (indicated by the second arrow). (b) Effects of 1,2-diolein + PS (●), 0.3 μM LTD_4 + PS (■) and PS alone (▲) on the Ca^{2+}-induced contraction. The amplitude of contraction evoked by 0.3 μM Ca^{2+} was normalized as a relative tension of 1.0. The first arrow indicates application of 0.3 μM Ca^{2+} and the second arrow application of drugs.

from the cellular stores through the synthesis of second messengers (Berridge & Irvine, 1984; Suematsu *et al.*, 1984; Hashimoto *et al.*, 1985; Abdel-Latif, 1986; Sasaguri *et al.*, 1987). In the guinea-pig basilar artery, the phasic contraction produced by LTD_4, 5-HT, NA and STA_2 was much smaller than that induced by 128 mM K^+. This suggests that these agents induce the synthesis of relatively small amounts of IP_3. As a consequence, Ca^{2+} release from storage sites may be less than that observed in other vascular smooth muscles. Such differences in agonist-induced IP_3 may be an indication of regional differences in vascular smooth muscle tissues. However, it does not indicate that this tissue has no ability to synthesize IP_3, since histamine and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) produced a larger phasic response than that evoked by 128 mM K^+ (present authors' unpublished observations).

The tonic response evoked by LTD_4 was relatively small in intact tissues, but became larger when the endothelium was removed. The responses in endothelium-denuded tissues were markedly inhibited by nifedipine and in Ca^{2+}-free conditions. This indicates that the tonic response is caused by an increase in the free concentration of Ca^{2+} via activation of nifedipine-sensitive and -insensitive voltage-dependent Ca^{2+} channels. In the present experiments, the tonic response seen in intact tisseus and after application of nifedipine in the endothelium-denuded tissues was too small to study in detail. Thus it is not certain whether the nifedipine insensitive Ca^{2+} influx was due to receptor activated Ca^{2+} influx (Bolton, 1979), and/or a voltage-dependent Ca^{2+} influx via a channel insensitive to Ca antagonists (Bean *et al.*, 1986).

In skinned muscles, LTD_4 had no effect on Ca^{2+}-induced contractions or on the Ca^{2+} releasing mechanism from the intracellular stores. As deduced from the actions of 1,2-diolein with PS, LTD_4 does not act directly on the Ca-calmodulin activated myosin phosphorylation system (Kamm & Stull, 1985; Walsh, 1985).

In the present study, the LTD_4-induced contraction was unaffected by blockers of α-adrenoceptors, β-adrenoceptors, histamine receptors and 5-HT receptors, or by a thromboxane A_2 antagonist. However, its effects were blocked by FPL 55712 and ONO-RS-411. Therefore, LTD_4 may act on smooth muscle through the LTD_4 receptor at the surface of the membrane. The maximum contraction evoked by 10 nM STA_2 was consistently larger than that evoked by 1 μM LTD_4 in the presence and absence of endothelium. Kanmura *et al.* (1987) demonstrated that STA_2 acts on the thromboxane A_2 receptor in rabbit coronary artery and produces a contraction due to

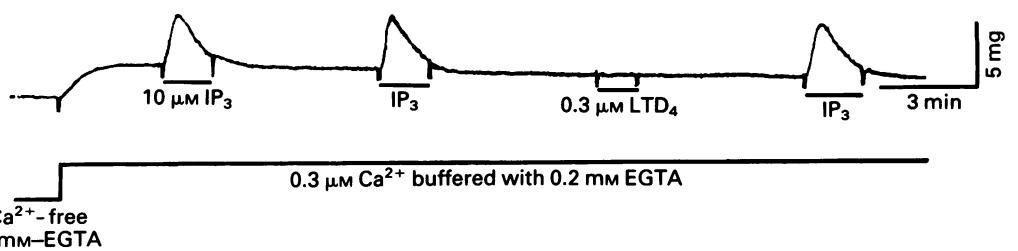

Figure 8 Effects of 10 μM inositol 1,4,5-trisphosphate (IP_3) and 0.3 μM leukotriene D_4 (LTD_4) on the Ca^{2+}-induced contraction (0.3 μM Ca^{2+} buffered with 0.2 mM EGTA) in skinned muscle tissue.

an increase in both voltage- and receptor-dependent Ca^{2+} influx. This agent also releases Ca^{2+} from ACh- and caffeine-sensitive storage sites. In the basilar artery, STA_2 produced a larger tonic response than that induced by LTD_4 and at only one hundredth of the concentration in the presence and absence of endothelium. STA_2 may release stored Ca^{2+} via an increase in the amount of IP_3, a mechanism which is activated to a lesser extent by LTD_4. After subarachinoid haemorrhage, leukocytes, mast cells and macrophages synthesize LTD_4 which is thought to induce delayed cerebral vasospasm (Borgeat & Samuelson, 1979; Rouzer *et al.*, 1980; Suzuki *et al.*, 1983; Shimizu *et al.*, 1986). In the guinea-pig basilar artery, the minimum concentration of LTD_4 required to produce a contraction was 0.1 μM in endothelium-denuded tissues and 1 μM in intact tissues. These concentrations were much higher than those of STA_2 required to produce comparable mechanical effects. Therefore, if delayed cerebral vasospasm occurs by the release of LTD_4, a damaged endothelium may be the prerequisite of such an action in marked contrast to the vasospasm induced by TXA_2 (STA_2).

In conclusion, LTD_4 has a dual action on the guinea-pig basilar artery. An indirect effect of this agent may be to stimulate the release of EDRF which inhibits the final amplitude of contraction. In addition, LTD_4 in part directly increases voltage-dependent Ca^{2+} influx and in part releases Ca^{2+} stored within the cells. In the present experiments, it was not possible to clarify whether the small contraction evoked in the presence of nifedipine was due to receptor-operated Ca^{2+} influx or to activation of voltage-dependent but Ca antagonist-insensitive Ca^{2+} channels.

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