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Effect of niflumic acid on noradrenaline-induced contractions of the rat aorta

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1 The effects of niflumic acid, an inhibitor of calcium-activated chloride channels, were compared with the actions of the calcium channel antagonist nifedipine on noradrenaline-evoked contractions in isolated preparations of the rat aorta.

2 The cumulative concentration-effect curve to noradrenaline (NA) was depressed by both nifedipine and niflumic acid in a reversible and concentration-dependent manner. The degree of inhibition of the maximal contractile response to NA (1 μM) produced by 10 μM niflumic acid (38%) was similar to the effect of 1 μM nifedipine (39%).

3 Contractions to brief applications (30 s) of 1 μM NA were inhibited by 55% and 62% respectively by 10 μM niflumic acid and 1 μM nifedipine.

4 In the presence of 0.1 μM nifedipine, niflumic acid (10 μM) produced no further inhibition of the NA-evoked contractions. Thus, the actions of niflumic acid and nifedipine were not additive.

5 In Ca-free conditions the transient contraction induced by 1 μM NA was not inhibited by niflumic acid (10 μM) and therefore this agent does not reduce the amount of calcium released from the intracellular store or reduce the sensitivity of the contractile apparatus to calcium.

6 Niflumic acid 10 μM did not inhibit the contractions produced by KCl (up to 120 mM) which were totally blocked by nifedipine. Contractions induced by 25 mM KCl were completely inhibited by 1 μM levcromakalim but were unaffected by niflumic acid.

7 It was concluded that niflumic acid produces selective inhibition of a component of NA-evoked contraction which is probably mediated by voltage-gated calcium channels. These data are consistent with a model in which NA stimulates a calcium-activated chloride conductance which leads to the opening of voltage-gated calcium channels to produce contraction.

Keywords: Niflumic acid: noradrenaline-evoked contraction; vascular smooth muscle; calcium-activated chloride current

Introduction

It has been proposed that several cellular mechanisms may mediate the contractile response to noradrenaline in vascular smooth muscle (e.g. Bolton & Large, 1986; van Breeman & Saida, 1989). One general scheme is that noradrenaline depolarizes smooth muscle to produce opening of voltage-dependent calcium channels. In rabbit portal vein and ear artery smooth muscle cells noradrenaline (NA) acts on α_1-adrenoceptors to activate a non-selective cation current and to release calcium from caffeine-sensitive intracellular stores (presumably the sarcoplasmic reticulum, SR) to evoke a calcium-activated chloride current ($I_{Cl(Ca)}$) (Byrne & Large, 1988; Amédée *et al.*, 1990; Wang & Large, 1991). Since in smooth muscle the chloride equilibrium potential (between -20 and -30 mV, Aickin, 1990) is considerably more positive than the resting membrane potential (approximately -50 to -70 mV) activation of $I_{Cl(Ca)}$ will lead to membrane depolarization. The subsequent opening of voltage-dependent calcium channels (VDCCs) and calcium influx will produce vasoconstriction (Byrne & Large, 1988; Amédée & Large, 1989). Consequently the putative physiological role of $I_{Cl(Ca)}$ is to link α-adrenoceptor stimulation to activation of VDCCs with subsequent influx of calcium ions. However, stimulation of the non-selective cation current would also lead to depolarization and contraction and the present experiments were designed to assess the contribution of $I_{Cl(Ca)}$ to the contractile response to NA in the rat aorta.

Despite the abundant evidence for $I_{Cl(Ca)}$ in isolated cells there is little known about the function of $I_{Cl(Ca)}$ in contractile mechanisms in smooth muscles. A suitable method to evaluate the role of $I_{Cl(Ca)}$ in smooth muscle would be to use a selective inhibitor of calcium-activated chloride channels against agonist-induced contractions. Whereas most blockers of $I_{Cl(Ca)}$ are neither potent nor selective (Hogg *et al.*, 1994) niflumic acid inhibits $I_{Cl(Ca)}$ in micromolar concentrations in smooth muscle cells dissociated from rat and rabbit portal vein, canine and guinea-pig trachea, rabbit oesophagus and coronary artery (Pacaud *et al.*, 1989; Janssen & Sims, 1992; Akbarali & Giles, 1993; Hogg *et al.*, 1994; Lamb *et al.*, 1994). It appears that niflumic acid inhibits $I_{Cl(Ca)}$ by blocking the chloride channel once it has opened (Hogg *et al.*, 1994). Moreover, electrophysiological experiments on single cells indicate that in concentrations up to 50 μM, niflumic acid did not (1) interact with α-adrenoceptors, (2) inhibit VDCCs, (3) deplete the intracellular calcium store or inhibit the noradrenaline-induced release of calcium from the SR, (4) reduce the NA-evoked non-selective cation current, or (5) evoke a potassium current (Hogg *et al.*, 1994). Therefore it appears that niflumic acid is a good candidate as a selective inhibitor of $I_{Cl(Ca)}$ and in the present study we have investigated the effect of this agent on NA-induced contractions in strips of rat aorta. This is a well described preparation in which about half of the NA-induced contraction is blocked by calcium-channel antagonists (Orallo *et al.*, 1991). Moreover, NA produces depolarization of rat aorta (W.A. Large, unpublished data). If $I_{Cl(Ca)}$ was a pivotal link between receptor activation and the opening of VDCCs then it would be expected that the fraction of the NA-induced contraction which was sensitive to calcium channel blockers would also be inhibited by niflumic acid. The results show that niflumic acid reduced NA-induced contractions in a con-

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centration-dependent manner consistent with a role of $I_{Cl(Ca)}$ in producing the opening of VDCCs. A brief account of this work has been presented to the British Pharmacological Society (Criddle *et al.*, 1995).

Methods

Experiments were carried out on segments of thoracic aorta isolated from male Wistar rats (250–350 g) which had been killed by stunning and exsanguination. Each segment of aorta was cut into rings approximately 0.5 cm long and these were suspended in organ chambers containing 30 ml of Krebs-Henseleit solution (composition in mM; NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 2.5, EDTA 0.026 and glucose 11) bubbled with 95% O_2 and 5% CO_2 at 37°C. Each ring was suspended by two stainless steel stirrups passed through the lumen. One stirrup was anchored inside the organ chamber and the other was connected to a force transducer (FTA 10; Hewlett-Packard Co., Palo Alto, U.S.A.) for the measurement of isometric force on a Hewlett-Packard 7754A recorder. All rings were mounted under a resting tension of 1 g and left to equilibrate for 1 h during which time the tissues were washed regularly.

The effects of niflumic acid and nifedipine on NA-induced contractions were investigated using two protocols. Initially, NA (0.001 to 1 μM) was applied cumulatively. Once the concentration-effect curve had been constructed the tissue was washed with PSS every 10 min for 1 h. Tissues were then exposed to niflumic acid (3 and 10 μM), nifedipine (0.1 and 1 μM) or the equivalent solvents for 30 min and then re-exposed to cumulative applications of NA in the continued presence of the respective agents.

Experiments were also performed in which the aortic segments were exposed to a single, maximally effective concentration of NA (1 μM) to elicit a transient contraction. Tissues were exposed to NA until the rate of tension development had slowed (approximately 30 s) and were then washed with PSS. After consistent contractions to NA had been obtained the tissues were exposed to niflumic acid (3 and 10 μM), nifedipine (1 μM) or the equivalent solvents for 15 min and then re-exposed to NA in the continued presence of the above agents. Further experiments were performed investigating the effect of niflumic acid and nifedipine on KCl-induced contractions. Experiments were performed in which a stable contraction in response to 25 mM or 60 mM KCl was obtained and the tissue was then exposed to niflumic acid (3 and 10 μM) for 10 min per concentration in the continued presence of KCl. After the last application of niflumic acid the tissue was exposed to either 0.1 μM nifedipine (to tissues bathed in 60 mM KCl) or 1 μM levcromakalim (to tissues bathed in 25 mM KCl). Additional experiments were performed on the effect of niflumic acid on contractions produced by the cumulative addition of KCl (5.9–120 mM) to the tissue bath. Pairs of aorta were exposed to increasing concentrations of KCl for 5 min per concentration. After the addition of 120 mM KCl the tissues were washed with normal PSS for 30 min and then exposed to either niflumic acid (10 μM) or the equivalent concentration of vehicle. Cumulative responses were then constructed in the presence of these agents.

Ca-free experiments

Since NA is known to mobilize Ca from intracellular Ca-stores, the effects of niflumic acid on NA-induced contraction in Ca-free PSS were investigated. The protocol used was based on that described by Greenwood & Weston, (1993). Briefly. tissues were bathed in normal PSS and exposed to NA (1 μM) for 10 min. Following washout of NA and relaxation to basal levels, tissues were exposed to Ca-free PSS (3 washes) for 15 min, after which time NA was reapplied for 10 min and the ensuing contraction recorded. Aortae were then re-exposed to normal PSS for 20 min to refill the intracellular calcium stores, then washed in Ca-free PSS once more for 15 min before re-application of 1 μM NA. Paired tissues were exposed to niflumic acid (10 μM) or the appropriate concentration of solvent for 15 min prior to the final application of NA.

Drugs and solutions

The following solutions were used: noradrenaline, niflumic acid, nifedipine (all Sigma) and levcromakalim (SmithKline Beecham). Noradrenaline was prepared as a stock solution in 0.1 N HCl containing a small amount of ascorbic acid and was diluted to the desired concentration. Nifedipine stock solution was prepared in 70% ethanol under conditions of reduced illumination and all experiments with nifedipine were performed under similar conditions. Niflumic acid was prepared as a stock solution (10 mM) in dimethyl sulphoxide (DMSO).

Ca-free PSS was produced by omitting $CaCl_2$ and $MgSO_4$ and adding 20 mM $MgCl_2$ while the NaCl concentration was reduced to 82 mM to maintain the osmolarity. EDTA was increased to 0.1 mM in the solution to chelate free Ca. In experiments on K-induced contractions, NaCl was replaced by an isomolar concentration of KCl.

Analysis of data

Data are expressed as the mean of n observations ± s.e.mean. Cumulative concentration-effect curves were constructed and the contraction produced by each concentration of NA was expressed as a % of the maximum NA-evoked contraction. Contractions produced in the presence of niflumic acid and nifedipine were also expressed as a % of the maximum obtained contraction.

Results

Effects of niflumic acid and nifedipine on cumulatively-applied NA-evoked contractions

Cumulative application of noradrenaline (NA) from 1 nM to 1 μM to aortic segments bathed in Ca-containing PSS produced sustained contractions which reached a maximum value at 1 μM NA of 1.4 ± 0.1 g (Figure 1, $n=11$). Exposure of rat aortae to niflumic acid (3 and 10 μM) did not affect basal tension (Figure 1a). However, 3 μM niflumic acid reduced the NA-evoked contraction at all concentrations of NA and produced a depression of the maximum contraction of 25 ± 6% (Figure 1a and b; $n=7$). Exposure of the tissue to 10 μM niflumic acid produced further inhibition of the NA-induced response and reduced the maximal contraction by 38 ± 5% (Figure 1a and b). Time-matched exposure of the tissues to DMSO at the relevant concentrations did not affect the NA-evoked contraction ($n=6$). The inhibitory effect of niflumic acid was reversible and the amplitude of the NA-induced contractions approached control values after approximately 30 min washout of 10 μM niflumic acid.

If the inhibition of NA-induced contractions by niflumic acid was due to the blockade of chloride channels which produce sufficient membrane depolarization to open VDCCs then calcium channel antagonists should inhibit NA-evoked contractions by a similar extent. From Figure 1c it can be seen that the calcium channel antagonist nifedipine also inhibited the responses to NA. Exposure of the strips to 0.1 μM nifedipine inhibited the contractions produced by cumulative application of NA in a manner qualitatively similar to that of niflumic acid. Nifedipine 1 μM did not have a significantly ($P<0.05$) greater effect on the NA-evoked contractions than 0.1 μM nifedipine (Figure 1c). The effects of niflumic acid and nifedipine were also quantitatively similar and the maximal reduction of NA-evoked contractions produced by nifedipine was 34 ± 4% ($n=5$) and 39 ± 4% with respectively 0.1 μM and 1.0 μM nifedipine ($n=5$).

If the niflumic acid-sensitive mechanism is responsible for

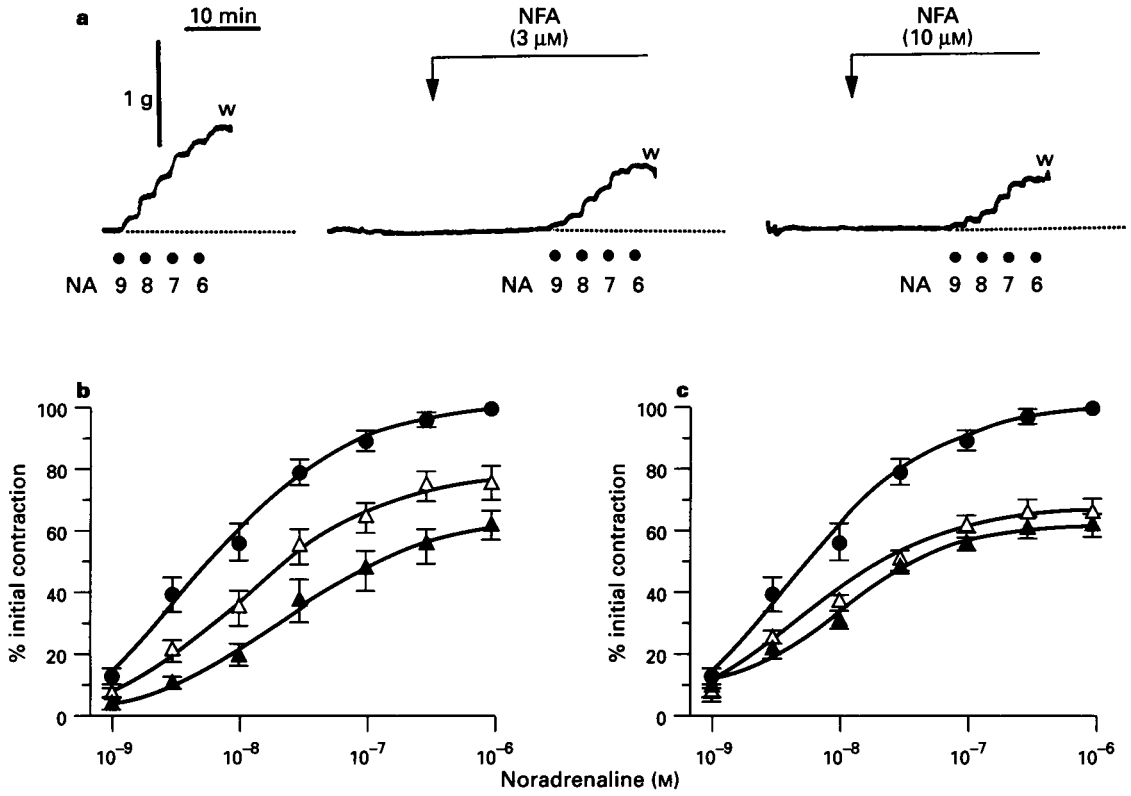

Figure 1 Effect of niflumic acid and nifedipine on noradrenaline (NA)-evoked contractions. (a) Representative trace showing the inhibitory effect of niflumic acid (NFA, 3 μM and 10 μM) on the contraction of rat aorta induced by the cumulative application of NA (numbers represent the addition of each log increment in NA concentration). Niflumic acid was applied from the point denoted by the downward arrow; w = wash out of NA. (b) Concentration-effect curve for NA in the absence (control, ●) and presence of niflumic acid (3 μM, △; 10 μM, ▲). (c) Concentration-effect curve for NA in the absence (control, ●) and presence of nifedipine (0.1 μM, △; 1 μM, ▲). In both (b) and (c) the abscissa scale shows the NA concentration (M) on a log scale and the ordinate scale shows the induced contraction as a % of the initial contraction. Each point is the mean of 5–11 tissues ± s.e.mean.

the indirect opening of VDCCs then the actions of maximally effective concentrations of niflumic acid and nifedipine should not be additive. Figure 2 illustrates the results from a series of experiments in which the effects of 10 μM niflumic acid and 0.1 μM nifedipine on NA-evoked contractions were tested independently and then combined. It can be seen that the inhibitory effect of both niflumic acid and nifedipine together was not significantly greater than the effect of nifedipine alone (Figure 2) and therefore the effects of these two agents were not additive.

Effects of niflumic acid and nifedipine on NA-induced phasic contractions

In physiological circumstances blood vessels are exposed to transiently high concentrations of neurotransmitter released from sympathetic nerve terminals. In an attempt to construct a representative situation, segments of aorta were briefly exposed to a relatively high concentration of NA (1 μM). This produced an immediate and rapid development of tension (mean value 0.8 ± 0.1 g; $n = 11$) which slowed after approximately 30 s exposure after which time the NA was washed out. After a washout period re-application of NA produced a similar response to the initial exposure and use of this protocol produced reproducible responses for over 90 min. Figure 3a shows a typical experiment in which the effect of niflumic acid was investigated on the contraction produced by a brief application of NA. Niflumic acid produced a concentration-dependent and reversible inhibition of the NA-elicited contraction (Figure 3a and b). Niflumic acid 3 and 10 μM inhibited NA-evoked phasic contractions by 14 ± 8% and 55 ± 5%, respectively (Figure 3b, $n = 7$ for each concentration). Pretreatment of the tissues with DMSO alone had no effect on

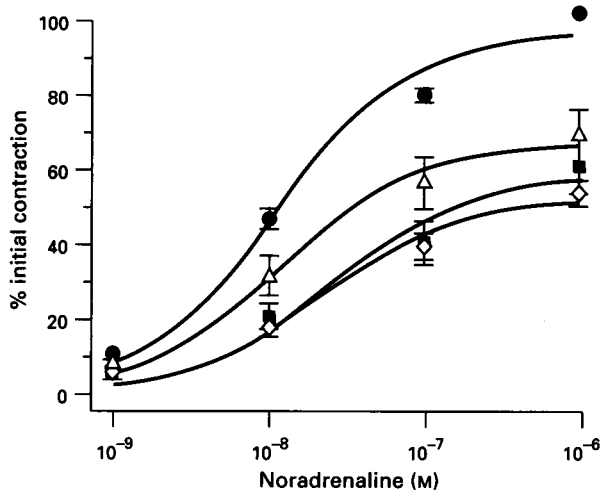

Figure 2 Lack of an additive effect of niflumic acid and nifedipine on noradrenaline (NA)-induced contractions. Concentration-effect curves for NA were constructed by the cumulative application of NA in the absence of any drug (●) and repeated in the presence of 10 μM niflumic acid (△), 0.1 μM nifedipine (■) and 0.1 μM nifedipine plus 10 μM niflumic acid (◇). Each point is the mean of 6 tissues ± s.e. mean. Abscissa scale, NA concentration on a log scale. Ordinate scale, induced contraction as a % of the maximum contraction.

the NA-evoked phasic contraction. Similar to the effects of 10 μM niflumic acid, nifedipine (1 μM) depressed NA-induced phasic contractions by a comparable amount with a mean

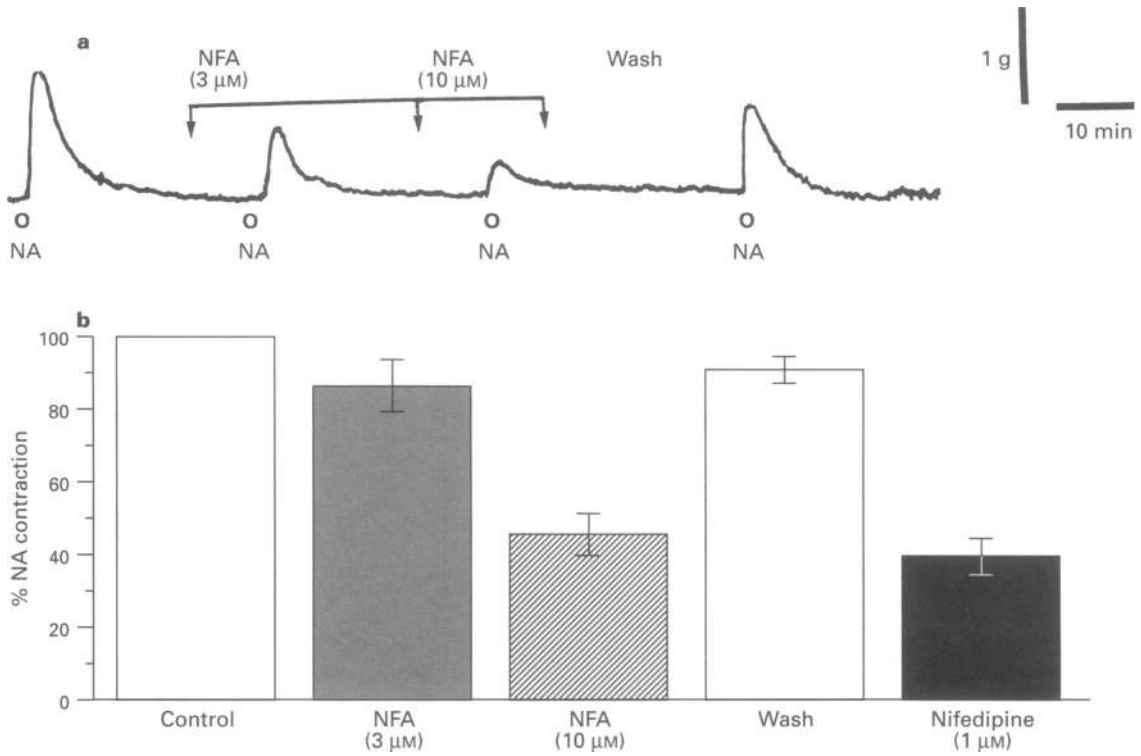

Figure 3 Effect of niflumic acid and nifedipine on the contraction produced by the brief application of 1 μM noradrenaline (NA). (a) A representative trace showing the contraction produced by the brief application of 1 μM NA at the point denoted by the open circle for 30 s in the absence and presence of niflumic acid (NFA, 3 and 10 μM). (b) Histogram showing the % reduction of the phasic contraction by niflumic acid (NFA) 3 μM and 10 μM and nifedipine 1 μM.

inhibition of 62±5% (Figure 3b, $n=4$). The inhibitory effects of 10 μM niflumic acid and 1 μM nifedipine were significantly greater ($P<0.05$) against contractions produced by brief application of 1 μM NA compared with the cumulative application of NA (see previous section).

Effects of niflumic acid on NA-evoked contractions in Ca-free conditions

In normal PSS solution 1 μM NA elicited a sustained contraction of 1.19±0.15 g ($n=12$). Under Ca-free conditions the contraction induced by a 10 min exposure to NA was transient and was 45.5±6.7% of the contraction in normal Ca-containing PSS ($n=12$). Pre-exposure to 10 μM niflumic acid did not modify the ensuing contraction induced by a further application of 1 μM NA in Ca-free PSS; the mean contraction in niflumic acid was 96±14% ($n=6$) of the control contraction compared to 97±11% of the control value in the presence of solvent equivalent ($n=6$).

Effects of niflumic acid and nifedipine on KCl-induced contraction

The similarity of the effects of niflumic acid and nifedipine is consistent with the hypothesis that the former agent blocks chloride channels opened by NA. However, it is possible that niflumic acid might act in whole tissues as a calcium-channel blocker or potassium-channel opener although single cell data would argue against this proposal. Thus, experiments were performed investigating the effects of niflumic acid and nifedipine against contractions produced by various concentrations of KCl to test the selectivity of niflumic acid. Exposure of rat aortic segments to 60 mM KCl produced a mean contraction of 1.77±0.23 g ($n=11$) which was stable over a 30 min period. Application of niflumic acid (1, 3 and 10 μM) for 10 min per concentration to the tissue bath in the continued presence of KCl did not relax the established tension (Figure 4a, $n=5$); however, subsequent addition of nifedipine (0.1 μM) to the bath produced a rapid and full relaxation of the developed contraction (Figure 4a, $n=5$).

It has been established (Hamilton *et al.*, 1986) that an agent which activates a potassium channel does not relax a contraction produced by a high KCl concentration (>40 mM) but does relax spasms produced by low KCl concentrations (<40 mM). Application of 25 mM KCl elicited contractions of 1.12±0.9 g ($n=12$) which were completely relaxed by 1 μM levcromakalim (Figure 4, $n=6$) in agreement with the data of Greenwood & Weston, (1993). However, niflumic acid (1 and 10 μM) did not relax the induced tone (Figure 4b, $n=7$).

To establish fully the lack of effect of niflumic acid on KCl-induced contractions in comparison to its observed effect on NA-evoked tone full concentration-effect curves for KCl (5.9 mM to 120 mM) were constructed in the presence and absence of niflumic acid (10 μM). Niflumic acid did not affect the contraction produced by any concentration of KCl ($n=4$). Thus, the concentration-effect curve for KCl in the presence of niflumic acid was not shifted from that constructed in the absence of niflumic acid (Figure 5). These data indicate that the inhibitory effect of niflumic acid on NA-evoked contractions is not due to the direct block of VDCCs or the opening of potassium channels.

Concurrent insensitivity of NA-induced contraction to niflumic acid and nifedipine in some preparations

During certain experimental periods the inhibitory effect of niflumic acid on NA-induced contraction was greatly reduced or absent although the amplitude of the NA-evoked contraction was not significantly different ($P<0.05$) from the contractions recorded in niflumic acid-sensitive tissues. Interestingly, the effect of nifedipine was also changed in the aortae of these animals. For example, in one group of experiments the mean phasic contraction induced by 1 μM NA in the presence of 10 μM niflumic acid ($n=11$) and 0.1 μM nife-

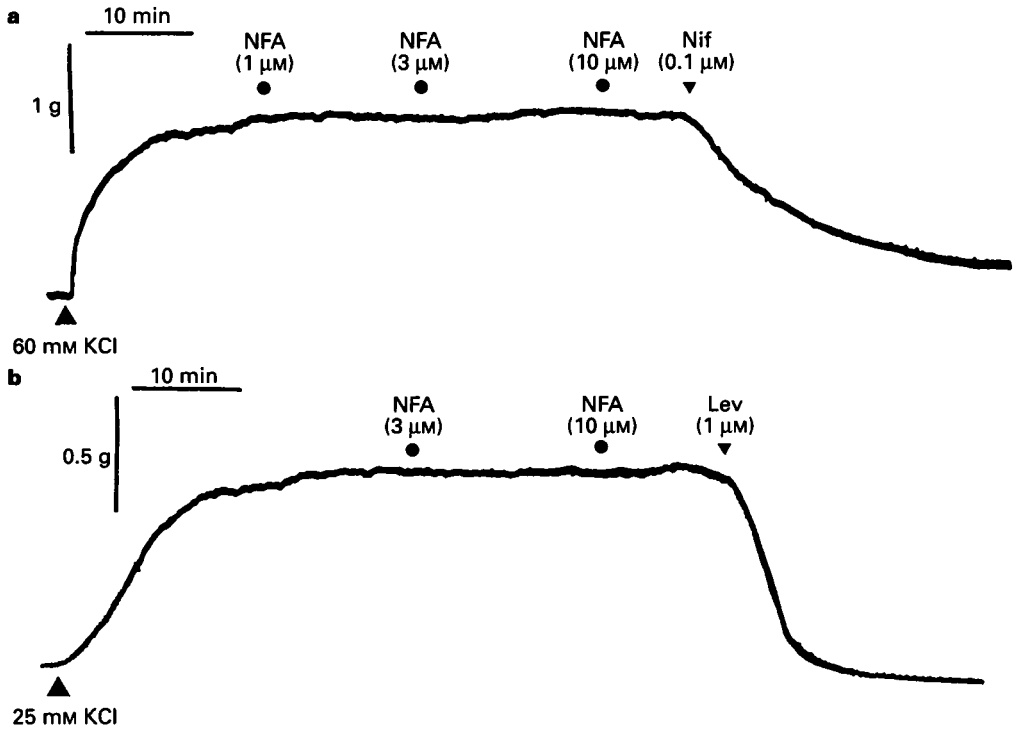

Figure 4 Lack of effect of niflumic acid on contractions produced by 25 mM and 60 mM KCl. (a) A typical trace of the contraction produced by 60 mM KCl and the lack of effect of 1, 3 and 10 μM niflumic acid (NFA) on the developed tone. Application of 0.1 μM nifedipine (Nif) at the point denoted by ▼ produced a total reduction of the contraction induced by 60 mM KCl. (b) Addition of 25 mM KCl to the organ bath produced a contraction which was not relaxed by 3 and 10 μM niflumic acid (NFA). Levcromakalim (LEV, 1 μM) applied at the point denoted by ▼ produced full relaxation of the established tone.

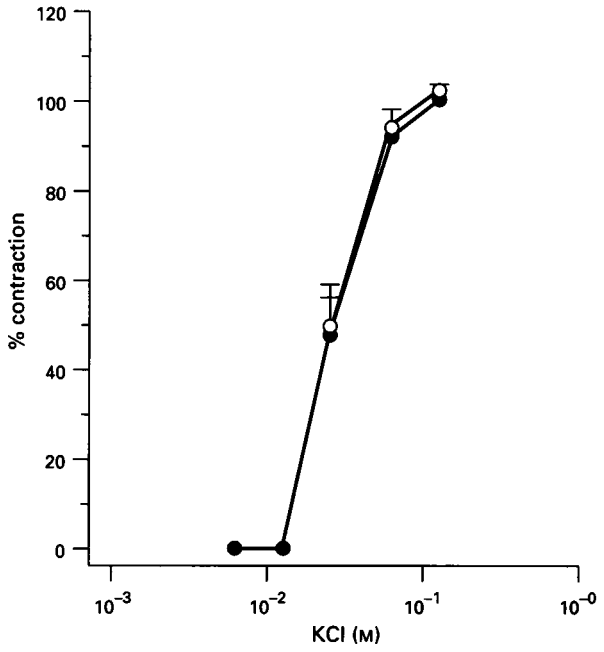

Figure 5 Effect of niflumic acid on the contractions produced by KCl. Concentration-effect curves for KCl were constructed by the cumulative addition of KCl (5.9 to 120 mM) in the absence (○) and presence of 10 μM niflumic acid (●). The abscissa scale shows the KCl concentration (M) and the ordinate scale shows the induced contraction as a % of the maximum contraction obtained.

dipine ($n=8$) was respectively $100\pm5\%$ and $92\pm7.0\%$ of the control response. In these tissues where the NA-evoked contractions were insensitive to both niflumic acid and nifedipine, KCl-induced contractions were totally blocked by 0.1 μM nifedipine. Thus, in these tissues no component of the NA-induced contraction appeared to involve a niflumic acid-sensitive mechanism or VDCCs.

Discussion

The results of the present work show that niflumic acid produced a concentration-dependent and reversible inhibition of NA-evoked contractions in rat aorta. Since niflumic acid did not inhibit KCl-induced contractions, which were relaxed totally by 0.1 μM nifedipine, it can be concluded that the relaxant effect of niflumic acid was not due to the direct blockade of VDCCs. These results agree with the data on calcium currents in isolated cells (Pacaud *et al.*, 1989; Akbarali & Giles, 1993; Hogg *et al.*, 1994; Janssen & Sims, 1995). Moreover, niflumic acid ($\leqslant 10$ μM) did not relax contractions induced by 25 mM KCl which were fully relaxed by levcromakalim. Therefore the inhibitory effect of niflumic acid cannot be attributed to the opening of potassium channels (see the discussion of potassium channel openers in Hamilton *et al.*, 1986). These data also agree with the findings in single smooth muscle cells that concentrations of niflumic acid up to 50 μM did not activate a potassium current (Hogg *et al.*, 1994). The observation that niflumic acid did not decrease the transient contraction in calcium-free PSS indicates that neither the release of calcium from the SR nor the sensitivity of the contractile apparatus to calcium ions were reduced by niflumic acid. Also, it should be noted that nifedipine did not inhibit the transient contraction produced by NA in calcium-free conditions in rabbit aorta (Bray *et al.*, 1991) or rat mesenteric artery (Quast & Baumlin, 1991). Overall, the results suggest that the relaxant effect of niflumic acid is not due to a non-specific effect on smooth muscle (e.g. block of VDCCs or opening of potassium channels) but rather that niflumic acid inhibits selectively a membrane mechanism which is activated by NA.

The experimental evidence indicates that niflumic acid in-

hibits a membrane mechanism associated with the opening of VDCCs. The maximum relaxant effects of niflumic acid and nifedipine were similar whether NA was added cumulatively or in a single high concentration. Furthermore, the effects of niflumic acid and nifedipine were not additive. Thus, the addition of 0.1 μM nifedipine and 10 μM niflumic acid together did not produce a greater relaxation than 0.1 μM nifedipine alone. The parallelism between the effects of the two agents also extended to 'insensitive' tissues where neither niflumic acid nor nifedipine produced significant relaxation of NA-induced responses. Presumably in these 'insensitive' preparations the contractions to NA were not mediated by the niflumic acid-sensitive mechanism or VDCCs and these results illustrate that NA utilizes several mechanisms to produce smooth muscle contraction (see *e.g.* Bolton & Large, 1986; van Breeman & Saida, 1989). Since niflumic acid reduced a component of the NA-induced contraction which utilized VDCCs but did not block VDCCs directly another mechanism must be inhibited by niflumic acid to reduce the NA-evoked contraction. In single smooth muscle cells niflumic acid did not prevent the interaction of NA with the α-adrenoceptor (Hogg *et al.*, 1994) which was confirmed by the data on NA-evoked responses in calcium-free conditions in the present study. Therefore it would seem that niflumic acid blocks the membrane mechanism which links the α-adrenoceptor to the opening of VDCCs. The concentrations of niflumic acid (3–10 μM) that relaxed NA-evoked contractions in rat aorta are similar to those that inhibited $I_{Cl(Ca)}$ in single isolated cells. The estimated concentration of niflumic acid to inhibit by 50% the amplitude of spontaneous calcium-activated chloride currents was about 2–3 μM (Hogg *et al.*, 1994) which also significantly reduced contractions elicited by NA in the present work. Moreover, 10 μM niflumic acid reduced $I_{Cl(Ca)}$ by about 80% in rabbit portal vein cells (Hogg *et al.*, 1994) and coronary artery cells (Lamb *et al.*, 1994), and produced a near maximal inhibition of contractions evoked by NA. Consequently both the qualitative and quantitative data suggest that the reduction of the NA-induced contraction by niflumic acid is due to inhibition of $I_{Cl(Ca)}$. Interestingly, the degree of inhibition by both niflumic acid and nifedipine was significantly greater against a brief application of NA compared to when NA was applied cumulatively. This suggests that the niflumic acid-sensitive mechanism ($I_{Cl(Ca)}$) plays a greater role in the generation of a phasic contraction in response to a rapid application of a high concentration of NA for a brief period of time compared to the tension developed by the cumulative addition of NA when NA is in contact with the tissue for much longer. It is possible that the contribution of the various cellular mechanisms to the overall contraction produced by NA depends on the concentration and the contact time of NA with the tissue. Also the contraction of rat aorta, evoked by endothelin, which has been shown to elicit $I_{Cl(Ca}$, in vascular smooth muscle cells (Klöckner & Isenberg, 1991), is decreased by indanyloxyacetic acid (Iijima *et al.*, 1991) which blocks $I_{Cl(Ca)}$ in single smooth muscle cells (Greenwood *et al.*, 1995). Overall the results from whole tissue experiments implicates a role for $I_{Cl(Ca)}$ in producing contraction in vascular smooth muscle.

In summary, niflumic acid inhibited the component of the NA-induced contraction of the rat aorta which was sensitive to the calcium channel antagonist nifedipine. These results are consistent with a scheme in which α-adrenoceptor stimulation leads to activation of $I_{Cl(Ca)}$, which is inhibited by niflumic acid, and subsequent opening of VDCCs. In addition to this mechanism, NA contracts rat aorta by a process which does not involve either $I_{Cl(Ca)}$ or VDCCs. For example, that part of the contractile response resistant to niflumic acid and nifedipine may be mediated by influx of calcium through the non-selective cation channel (see Byrne & Large, 1988; Wang & Large, 1991). It will be interesting to investigate the effects of niflumic acid on agonist-induced contractions in other smooth muscle tissues.

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