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Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor responses in the isolated mesenteric vascular bed of the rat

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1 The effects of niflumic acid, an inhibitor of calcium-activated chloride currents, were compared with the actions of the calcium channel blocker nifedipine on noradrenaline- and 5-hydroxytryptamine (5-HT)-induced pressor responses of the rat perfused isolated mesenteric vascular bed.

2 Bolus injections of noradrenaline (1 and 10 nmol) increased the perfusion pressure in a dose-dependent manner. Nifedipine (1 μM) inhibited the increase in pressure produced by 1 nmol noradrenaline by $31 \pm 5\%$. Niflumic acid (10 and 30 μM) also inhibited the noradrenaline-induced increase in perfusion pressure and 30 μM niflumic acid reduced the pressor response to 1 nmol noradrenaline by $34 \pm 6\%$.

3 The increases in perfusion elicited by 5-HT (0.3 and 3 nmol) were reduced by niflumic acid (10 and 30 μM) in a concentration-dependent manner and 30 μM niflumic acid inhibited responses to 0.3 and 3 nmol 5-HT by, respectively, $49 \pm 8\%$ and $50 \pm 7\%$. Nifedipine (1 μM) decreased the pressor response to 3 nmol 5-HT by $44 \pm 9\%$.

4 In the presence of a combination of 30 μM niflumic acid and 1 μM nifedipine the inhibition of the pressor effects of noradrenaline (10 nmol) and 5-HT (3 nmol) was not significantly greater than with niflumic acid (30 μM) alone. Thus the effects of niflumic acid and nifedipine were not additive.

5 In Ca-free conditions the transient contractions induced by 5-HT (3 nmol) were not reduced by 30 μM niflumic acid, suggesting that this agent does not inhibit calcium release from the intracellular store or the binding of 5-HT to its receptor.

6 Niflumic acid 30 μM did not inhibit the pressor responses induced by KCl (20 and 60 μmol) which were markedly reduced by 1 μM nifedipine. In addition, 1 μM levcromakalim decreased pressor responses produced by 20 μmol KCl. These data suggest that niflumic acid does not block directly calcium channels or activate potassium channels.

7 It is concluded that niflumic acid selectively reduces a component of noradrenaline- and 5-HT-induced pressor responses by inhibiting a mechanism which leads to the opening of voltage-gated calcium channels. Our data suggest that the Ca^{2+}-activated chloride conductance may play a pivotal role in the activation of voltage-gated calcium channels in agonist-induced constriction of resistance blood vessels.

Keywords: Niflumic acid; calcium-activated chloride current; vascular smooth muscle; contraction mechanism; voltage-dependent calcium channel; noradrenaline; 5-hydroxytryptamine

Introduction

In recent years there has been increasing interest in the properties of calcium-activated chloride currents ($I_{Cl(Ca)}$) in single smooth muscle but the lack of selective blockers of $I_{Cl(Ca)}$ has hindered the systematic analysis of the functional role of this mechanism in smooth muscle (see Large & Wang, 1996). However, recently niflumic acid has been shown to inhibit selectively $I_{Cl(Ca)}$ in single isolated cells of various smooth muscle cell types (Pacaud *et al*., 1989; Janssen & Sims, 1992; Akbarali & Giles, 1993; Hogg *et al*., 1994; Lamb *et al*., 1994). The concentration of niflumic acid needed to inhibit $I_{Cl(Ca)}$ by 50% is 2–7 μM (Hogg *et al*., 1994) and in concentrations up to 50 μM niflumic acid does not inhibit voltage-dependent calcium channels (VDCCs) or activate K^+ channels (see Criddle *et al*., 1996 for further discussion of the selectivity of niflumic acid). Furthermore, there is evidence to suggest that niflumic acid blocks $I_{Cl(Ca)}$ directly by interacting with a specific site in the open chloride channel (Hogg *et al*., 1994) and this feature in addition to its relative selectivity suggests that niflumic acid may be a useful probe to investigate the functional role of $I_{Cl(Ca)}$.

Recently, we have shown that niflumic acid reduces the amplitude of noradrenaline-induced contractions of rat aorta (Criddle *et al*., 1996). These inhibitory effects occurred with micromolar concentrations of niflumic acid, were reversible, and of a similar order of magnitude to nifedipine-induced inhibition of noradrenaline-evoked contractions (Criddle *et al*., 1996). The data from this work suggest that the reduction by niflumic acid of the noradrenaline-evoked contractions does not involve established inhibitory mechanisms (e.g. α-adrenoceptor blockade, activation of a K-current, block of VDCCs or inhibition of calcium-release). Moreover, since the degree of inhibition of noradrenaline-induced contractions by niflumic acid and nifedipine were similar and the actions of niflumic acid and nifedipine were not additive it was proposed that niflumic acid inhibited a mechanism which linked the α-adrenoceptor to VDCCs. Since the concentration of niflumic acid needed to inhibit $I_{Cl(Ca)}$ in single cells and noradrenaline-evoked contractions in rat aorta was similar it seemed that the reduction by niflumic acid of the mechanical response could be attributed to block of $I_{Cl(Ca)}$. Thus, the proposed model was that noradrenaline activiated $I_{Cl(Ca)}$ which caused membrane depolarization to produce the opening of VDCCs and subsequent Ca^{2+} influx which contributed to the overall mechanical response.

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In the present study, we have investigated the effects of niflumic acid in the rat isolated mesenteric vascular bed, a preparation containing resistance vessels which are important in the physiological control of arterial blood pressure. We have compared the effect of niflumic acid with that of nifedipine on the pressor responses to noradrenaline and to another vasoconstrictor agent, 5-hydroxytryptamine (5-HT).

Methods

The rat superior mesenteric vascular bed was isolated according to McGregor (1965). Male Wistar rats (250–350 g) were killed by stunning and cervical dislocation, and the mesenteric vascular bed was cannulated and perfused at a flow rate of 4 ml min^{-1} with physiological salt solution (PSS) by a peristaltic pump (Lifecare Model 4, Abbott/Shaw). The PSS had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.026 and glucose 11 and was bubbled with 95% O_2/5% CO_2 at 37°C. Perfusion pressure was measured with a pressure transducer connected to a preamplifier and chart recorder. Drugs were either dissolved in the PSS and perfused at the desired concentration or were administered as bolus injections directly into the perfusion stream (volume $\leqslant 30$ μl).

Preparations were left to equilibrate for 30 min at which time injections of 60 μmol KCl were applied every 5 min until consistent responses were obtained. The effects of niflumic acid or nifedipine were then investigated by use of the following protocol. Injections of KCl (20 and 60 μmol), noradrenaline (1 and 10 nmol) and 5-HT (0.3 and 3 nmol) were applied at 5 min intervals until reproducible responses were recorded. Then the mesenteric vascular bed was perfused with increasing concentrations of niflumic acid or nifedipine (pre-exposure time of 15 min) and the responses to the pressor agents repeated. In separate experiments, the effect of the solvent in which niflumic acid and nifedipine were dissolved was tested by injecting the equivalent concentration of vehicle into the perfusate. The effects of the K-channel opener, levcromakalim, on pressor responses to raised KCl were also tested.

In order to ascertain whether the effects of niflumic acid and nifedipine were additive, separate experiments were performed in which the effects of 30 μM niflumic acid and then a combination of 30 μM niflumic acid and 1 μM nifedipine were assessed on pressor responses to 3 nmol 5-HT and 10 nmol noradrenaline (dose interval of 5 min).

Ca-free experiments

Experiments were also performed to investigate the effects of niflumic acid on the pressor responses to 3 nmol 5-HT in Ca^{2+}-free PSS. The protocol involved obtaining stable responses to KCl (60 μmol) and 5-HT (0.3 nmol) in normal PSS, then switching to Ca-free (15 min exposure period) and repeating the doses of KCl and 5-HT. The mesenteric vascular beds were then re-exposed to normal Ca-containing PSS ('refilling' period for 30 min) and KCl and 5-HT were then reapplied. Subsequently the preparations were perfused with Ca-free PSS containing 30 μM niflumic acid for a further 15 min before the injections of KCl and 5-HT were repeated.

Drugs and solutions

The following solutions were used: 5-hydroxytryptamine (5-HT), noradrenaline, niflumic acid and nifedipine (Sigma). Levcromakalim was a gift from 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 in PSS. 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 prepared in accordance with the study of Quast & Baumlin (1991) and had a similar composition to normal PSS but $CaCl_2$ and $MgSO_4$ were omitted and 20 mM $MgCl_2$ was added to maintain the stability of the tissue in zero calcium conditions. The NaCl concentration was reduced to 82 mM to maintain osmolarity and EDTA was increased to 0.1 mM in the solution to chelate free Ca.

Analysis of data

Data are expressed as the mean of n observations ± s.e.mean. Inhibitory effects of niflumic acid and nifedipine are expressed as % of control responses in the absence of drug. Statistical analysis was performed by use of paired Student's t test and values were taken to be significantly different when $P<0.05$.

Results

The basal perfusion pressure in the rat mesenteric vascular bed was 37 ± 2 mmHg ($n=38$). Bolus injections of KCl produced a transient elevation of perfusion pressure in a dose-dependent manner (Figures 1a and b) with mean values of 16 ± 2 mmHg and 100 ± 9 mmHg produced by 20 and 60 μmol KCl, respectively ($n=38$). Bolus injection of noradrenaline and 5-HT also increased the perfusion pressure transiently (Figure 1a and b) with mean pressor responses of 66 ± 7 mmHg and 170 ± 13 mmHg evoked by respectively 1 and 10 nmol noradrenaline ($n=20$), and 49 ± 7 mmHg and 126 ± 16 mmHg induced by, respectively 0.3 and 3 nmol 5-HT ($n=18$). In some tissues the β-adrenoceptor antagonist, propranolol (1 μM), and the cyclo-oxygenase inhibitor, indomethacin (1 μM), were included in the perfusate and neither agent had an effect on the contractile responses to any of the agonists used.

Effects of niflumic acid and nifedipine on the pressor responses to noradrenaline

Niflumic acid produced a concentration-dependent inhibition of the pressor response to noradrenaline. Thus, 10 and 30 μM niflumic acid reduced the increase in perfusion pressure elicited by 1 nmol noradrenaline by 9 ± 6% and 34 ± 6%, respectively (Figures 1a and 2a; $n=6$). Interestingly, niflumic acid was less effective at decreasing pressor responses produced by 10 nmol noradrenaline (Figure 2) with 30 μM niflumic acid reducing the response to 10 nmol noradrenaline by 20 ± 4%. If the hypothesis that activation of α-adrenoceptors by noradrenaline causes membrane depolarization (due to activation of $I_{Cl(Ca)}$ and consequent Cl^- efflux) to produce the opening of VDCCs is correct, then the degree of inhibition of noradrenaline-evoked constriction produced by a direct blocker of VDCCs should be similar to that produced by niflumic acid. The response to noradrenaline was relatively insensitive to lower concentrations of the VDCC antagonist nifedipine (0.01–0.1 μM). However, 1 μM nifedipine (which completely blocked responses to 20 μmol KCl; Figure 5b) decreased the pressor responses to 1 and 10 nmol noradrenaline by 31 ± 4% and 20 ± 8%, respectively (Figure 2b, $n=5$). Therefore, 1 μM nifedipine and 30 μM niflumic acid reduced the noradrenaline-evoked increase in perfusion pressure by a similar degree for each dose of noradrenaline. Injection of either DMSO or ethanol at concentrations equivalent to the concentration used with niflumic acid or nifedipine had no effect on the resting perfusion pressure or on pressor responses induced by agonists.

Effects of niflumic acid and nifedipine on the pressor responses to 5-HT

Niflumic acid produced a greater degree of inhibition of the increases in perfusion pressure produced by 5-HT compared to those evoked by noradrenaline. Thus, 10 and 30 μM niflumic acid inhibited the increase in perfusion pressure induced by 0.3 nmol 5-HT by 26 ± 9% and 49 ± 8% (Figures 1b and 3a,

$n=8$) which was significantly greater that its effect on noradrenaline-induced pressor responses (see Figure 2a). Furthermore, 10 and 30 μM niflumic acid inhibited increases in perfusion pressure evoked by 3 nmol 5-HT by a similar amount, $25\pm9\%$ and $51\pm7\%$, respectively ($n=8$, Figure 3a). Nifedipine also had a significantly greater effect on 5-HT- induced compared to noradrenaline-induced increases in perfusion pressure. Thus, 1 μM nifedipine reduced the pressor responses to 0.3 and 3 nmol 5-HT by $47\pm7\%$ and $44\pm9\%$, respectively (Figure 3b, $n=5$). Consequently the inhibitory effects of 1 μM nifedipine and 30 μM niflumic acid against 5-HT-induced responses were quantitatively similar, as was found for responses evoked by noradrenaline.

Effect of a combination of niflumic acid and nifedipine on the pressor responses to noradrenaline and 5-HT

If niflumic acid decreases the pressor responses to agonists by inhibiting $I_{Cl(Ca)}$ which leads to opening of VDCCs and subsequent contraction, then not only should the inhibitory effects of niflumic acid and nifedipine be equivalent but also the actions should not be markedly additive. It is worth pointing out that from single cell work 30 μM niflumic acid inhibited $I_{Cl(Ca)}$ by about 80–90% (Hogg *et al*., 1994) and therefore some chloride channels will be available for activation by agonists with this concentration of niflumic acid. We compared the ability of 30 μM niflumic acid to inhibit agonist-induced pressor responses alone and in combination with maximal concentrations of nifedipine. Niflumic acid (30 μM) inhibited the pressor effects of noradrenaline (10 nmol) and 5-HT (3 nmol) by $25\pm3\%$ and $53\pm7\%$, respectively (Figure 4; $n=8$). When 1 μM nifedipine was applied in conjunction with 30 μM niflumic acid, the reductions of the responses to noradrenaline and 5-HT were respectively $41\pm12\%$ and $68\pm6\%$ ($n=5$). These inhibitory effects, although slightly greater than with niflumic acid alone, were not statistically significant ($P>0.05$, Figure 4).

Effects of niflumic acid and nifedipine on the pressor responses to KCl

Nifedipine ($\leqslant1$ μM) and niflumic acid (10 and 30 μM) did not affect basal perfusion pressure and niflumic acid did not inhibit KCl-induced pressor responses (Figures 1a, b and 5a). In some preparations niflumic acid (10 and 30 μM) appeared to potentiate the vasoconstrictor effect to 20 μmol KCl (Figures 1a, b and 5a), although this effect was variable and was not statistically significant ($P>0.05$). The responses to 60 μmol KCl were not inhibited by niflumic acid (Figures 1 and 5a) but nifedipine produced a concentration-dependent inhibition of pressor responses evoked by 20 and 60 μmol KCl (Figure 5b, $n=13$). This indicates that the pressor responses to KCl are mediated by the direct opening of VDCCs which are not blocked by niflumic acid. Furthermore, the pressor responses to 20 μmol KCl but not 60 μmol KCl were inhibited by the K^+ channel opener levcromakalim. Thus, application of 20 μmol KCl increased the perfusion pressure by 29 ± 4 mmHg under control conditions and by 15 ± 4 mmHg in the presence of 1 μM levcromakalim ($n=5$). This degree of inhibition was similar to that obtained for the effect of 1 μM levcromakalim on isolated mesenteric vessels precontracted with 30 mM KCl (Criddle *et al*., 1994).

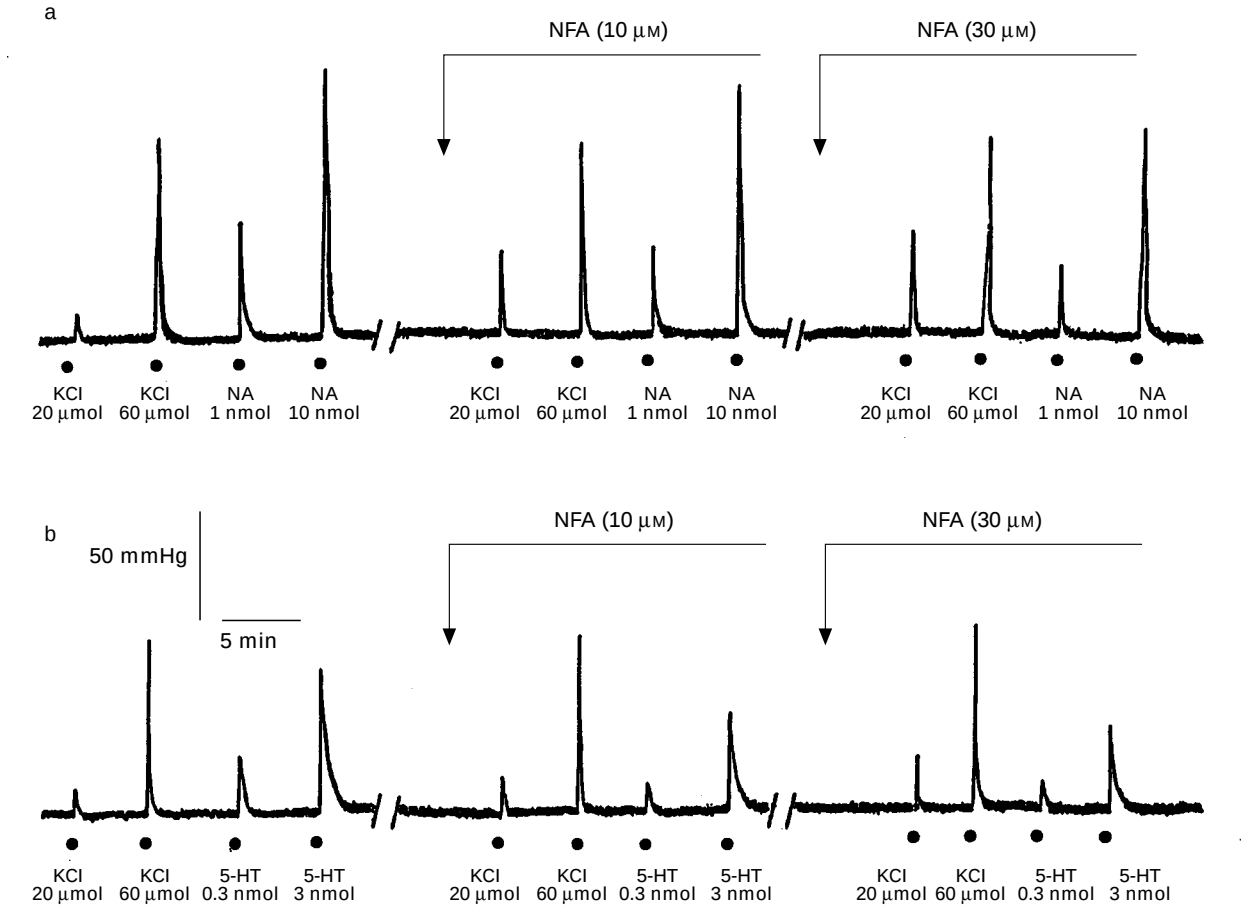

Figure 1 The effect of niflumic acid (NFA, 10 and 30 μM) on the pressor responses induced by KCl (20 and 60 μmol) and (**a**) noradrenaline (NA, 1 and 10 nmol) and (**b**) 5-HT (0.3 and 3 nmol). Increase in perfusion pressure is shown as an upward deflection and niflumic acid was applied for the period denoted by the horizontal bar and downward arrow.

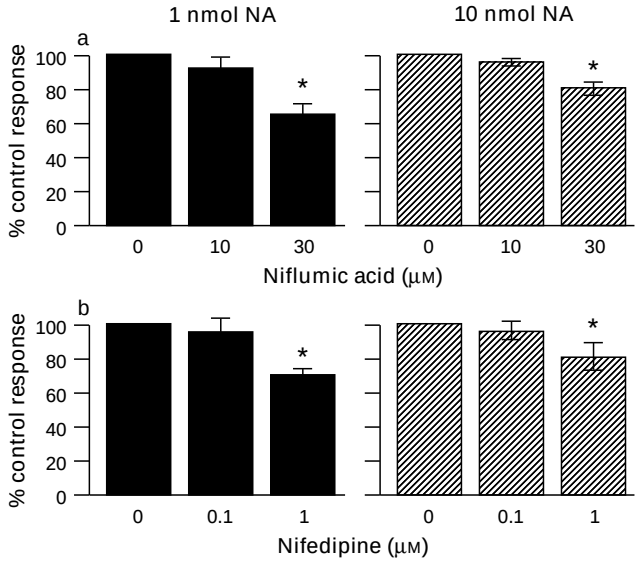

Figure 2 Effects of niflumic acid (**a**) and nifedipine (**b**) on pressor responses induced by noradrenaline (NA, 1 and 10 nmol) in the mesenteric vascular bed of the rat (data are expressed as mean ± s.e.mean; *represents significant difference from control at $P<0.05$).

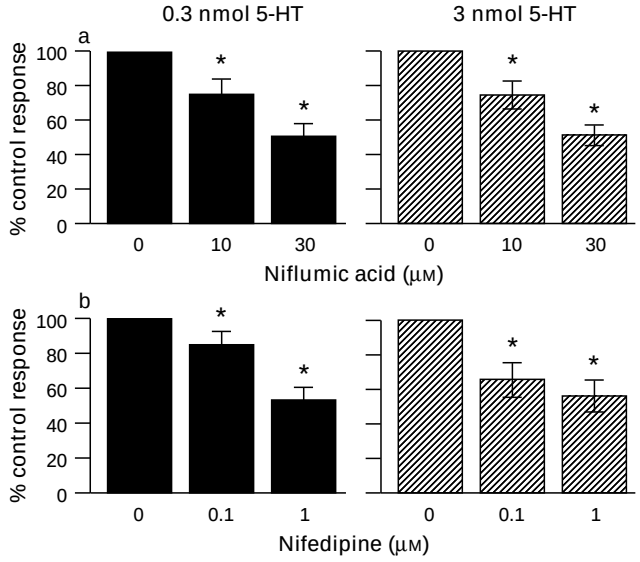

Figure 3 Effects of niflumic acid (**a**) and nifedipine (**b**) on pressor responses induced by 5-HT (0.3 and 3 nmol) in the mesenteric vascular bed of the rat (data are expressed as mean ± s.e.mean; *represents significant difference from control at $P<0.05$).

Effects of niflumic acid on the pressor responses to 5-HT in Ca^{2+}-free PSS

It is possible that niflumic acid may inhibit the interaction of agonists with their receptors or alter the sensitivity of contractile filaments to Ca^{2+} ions. This hypothesis can be tested by investigating the effect of niflumic acid on contractions produced by agonists in external solutions which contain zero calcium (see Methods). Previously we have shown that niflumic acid does not reduce the noradrenaline-evoked contractions of rat aorta in Ca^{2+}-free external solutions (Criddle *et al*., 1996). In the present study we investigated whether niflumic acid compromised the responses to 5-HT in Ca^{2+}-free bathing solutions. In Ca^{2+}-free bathing solution injection of 60 μmol KCl did not produce any increase in perfusion pressure ($n=10$), suggesting that under these conditions the influx of calcium through VDCCs is abolished. In comparison, 5-HT evoked reproducible pressor responses in the absence of external calcium, illustrating that 5-HT mobilizes Ca^{2+} ions from internal calcium stores. The mean increase in pressure evoked by 3 nmol 5-HT in Ca^{2+}-free conditions was 11 ± 4 mmHg and these responses represented $18\pm4\%$ ($n=6$) of the control effect produce in Ca^{2+}-containing conditions. Niflumic acid (30 μM) did not affect the pressor responses to 5-HT in Ca^{2+}-free PSS ($113\pm20\%$ of control responses, $n=6$) in contrast to the effect of niflumic acid on 5-HT-induced pressor responses in Ca^{2+}-containing conditions (see Figures 1b and 3a). Thus, it appears that niflumic acid does not inhibit the interaction of 5-HT with its receptor or its ability to release Ca^{2+} from internal stores.

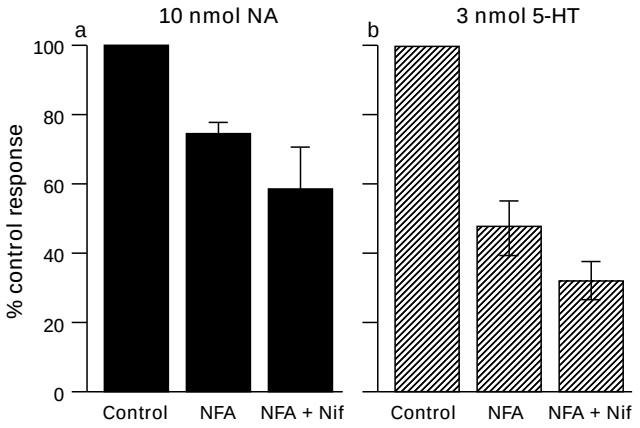

Figure 4 Effects of niflumic acid (NFA, 30 μM) alone and in combination with nifedipine (Nif; 1 μM) on pressor responses induced by (**a**) noradrenaline (NA, 10 nmol) and (**b**) 5-HT (3 nmol) in the mesenteric vascular bed of the rat (data are expressed as mean ± s.e.mean; response in presence of niflumic acid and niflumic acid + nifedipine were not significantly different, $P>0.05$).

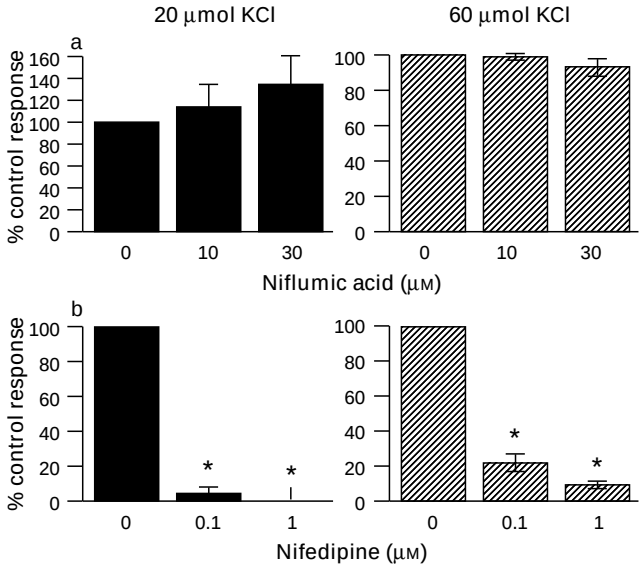

Figure 5 Effects of niflumic acid (**a**) and nifedipine (**b**) on pressor responses induced by KCl (20 (solid columns) and 60 (hatched columns) μmol) in the mesenteric vascular bed of the rat (data are expressed as mean ± s.e.mean; * represents significant difference from control at $P<0.05$).

Discussion

The present work demonstrates that niflumic acid inhibits the pressor responses to both noradrenaline and 5-HT in the perfused isolated mesenteric vascular bed of the rat. Niflumic acid (30 μM) and maximal concentrations of nifedipine inhibited the pressor responses to noradrenaline and 5-HT by a similar amount. Moreover, the actions of niflumic acid and nifedipine were not additive when applied in combination and, therefore, it is possible that niflumic acid inhibited responses to the agonists by blocking VDCCs. However, whereas the contractile responses to both 20 μmol and 60 μmol KCl in the rat mesenteric vascular bed were completely blocked by nifedipine, niflumic acid had no significant effect on responses to these raised K concentrations. These data support observations in single smooth muscle cells that niflumic acid (up to concentrations of 50 μM) does not inhibit VDCCs directly (Hogg *et al*., 1994). Furthermore, comparison of the actions of niflumic acid with those of levcromakalim, which inhibited the response to 20 μmol KCl (but not to 60 μmol KCl which is consistent with this agent acting as a K^+-channel opener, Hamilton *et al*., 1986) supports the observation that niflumic acid does not activate a K^+-current at concentrations used in this study (see Greenwood & Large, 1995). The present work has also shown that niflumic acid did not inhibit the pressor responses of the mesenteric vascular bed to 5-HT in Ca^{2+}-free solutions, which suggests that niflumic acid does not inhibit Ca-release or block the 5-HT receptor. Thus, the selective effects of niflumic acid on agonist-induced responses compared to K-induced contractions in the mesenteric vascular bed agree with the data obtained in the rat aorta (Criddle *et al*., 1996).

We have previously shown in the rat aorta that the contraction induced by noradrenaline is reduced by niflumic acid by approximately 55% (Criddle *et al*., 1996), whereas, in the rat mesenteric vascular bed the inhibitory effect of niflumic acid against noradrenaline-induced responses was less (20–35%). However, in both preparations the degree of inhibition by niflumic acid was similar to that of nifedipine, supporting the hypothesis that niflumic acid blocks a mechanism that results in the opening of VDCCs (possibly $I_{Cl(Ca)}$). The relatively weak effect of nifedipine against noradrenaline-induced contractions in the present study agrees with previous observations in rat isolated mesenteric resistance vessels (Cauvin *et al*., 1988) and in the isolated mesenteric vascular bed (Quast & Baumlin, 1991), indicating that only a minor part of the contraction to noradrenaline involves the entry of Ca^{2+} through VDCCs. In comparison, Nilsson *et al*. (1994) showed that in rat small mesenteric arteries felodipine reduced the noradrenaline-induced increase in force and intracellular calcium by approximately 50%. While the above discrepancies may be due to differences in experimentation, we have shown in the rat aorta (Criddle *et al*., 1996) that the nifedipine-sensitive component of the noradrenaline-induced contraction also varies between tissues. This observation probably reflects the fact that noradrenaline utilises different mechanisms to contract vascular smooth muscle and the contribution of these mechanisms to the overall contractile response is likely to vary not only between different tissues but also possibly between different preparations. In comparison to noradrenaline, 5-HT-induced pressor responses in the mesenteric vascular bed were inhibited markedly by nifedipine, suggesting that Ca^{2+}-influx through VDCCs contributes to a major part of the 5-HT-evoked vasoconstriction. Although the degree of inhibition produced by niflumic acid varies between tissues for the same agonist and also between agonists in the same tissue, it is significant that the reduction of agonist-induced responses by niflumic acid is always the same as that produced by nifedipine. Consequently the mechanism that is inhibited by niflumic acid utilizes VDCCs to produce contraction.

Worley *et al*. (1991) have shown that in single smooth muscle cells of the rabbit basilar artery 5-HT increases the open probability of single calcium channels. However, the present study shows that niflumic acid blocks 5-HT-induced pressor responses without affecting KCl-evoked responses, suggesting that the reduction of the 5-HT-evoked contraction by niflumic acid cannot be explained by the direct block of VDCCs. Therefore, the present data suggest that niflumic acid blocks a mechanism that links the 5-HT receptor to the opening of VDCCs. This hypothesis is supported by the observation in the rabbit basilar artery that the contraction, but not the depolarization, evoked by 5-HT is markedly inhibited by prior incubation with nifedipine (Clark & Garland, 1993).

Since electrophysiological studies in single cells have shown that niflumic acid is a selective blocker of $I_{Cl(Ca)}$, the data from the present work suggest that inhibition of this mechanism accounts for the reduction of the 5-HT-induced pressor responses by niflumic acid. However, although 5-HT activates $I_{Cl(Ca)}$ in epithelial cells of the rat choroid plexus (Garner *et al*., 1993), it has not yet been shown that this mechanism is linked to 5-HT receptors in smooth muscle cells. Hughes & Bolton (1995) have shown that 5-HT evokes a non-selective cation current in single smooth muscle cells of the rabbit ear artery which might represent a depolarizing mechanism that would lead to the opening of VDCCs. It is not known whether niflumic acid blocks the 5-HT-activated non-selective cation current, but it has been shown that niflumic acid at concentrations $\leqslant 50$ μM does not inhibit either noradrenaline-activated non-selective cation current in rabbit portal vein smooth muscle cells (Hogg *et al*., 1994) or the acetylcholine-activated cation current in guinea-pig ileum (Chen *et al*., 1993). Therefore the evidence suggests that niflumic acid inhibits 5-HT-evoked contractions by blocking a chloride mechanism, but further experiments are required to elucidate the membrane mechanisms activated by 5-HT in mesenteric arteries to support this proposal. Whatever the mechanism of action of niflumic acid, this compound is a potent inhibitor of agonist-induced contractions in vascular smooth muscle and appears to have a novel mechanism of action which confers an inhibitory profile that is distinct from established vasorelaxants.

In conclusion, niflumic acid selectively inhibited agonist-induced vasoconstrictor effects in the isolated mesenteric vascular bed of the rat, in a similar manner to our previous findings in rat aorta (Criddle *et al*., 1996). If our interpretation of the mechanism of action of niflumic acid is correct, then the results suggest that activation of $Cl_{(Ca)}$ currents may constitute an important common mechanism whereby vasoconstrictor agents produce contraction of vascular smooth muscle.

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