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Selective inhibitory effects of niflumic acid on 5-HT-induced contraction of the rat isolated stomach fundus

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1 The effects of niflumic acid (NFA), an inhibitor of calcium-activated chloride currents $I_{Cl(Ca)}$, were compared with the actions of the voltage-dependent calcium channel (VDCC) blocker nifedipine on 5-hydroxtryptamine (5-HT)- and acetylcholine (ACh)-induced contractions of the rat isolated fundus.

2 NFA (1–30 μM) elicited a concentration-dependent inhibition of contractions induced by 5-HT (10 μM) with a reduction to 15.5±6.0% of the control value at 30 μM. 1 μM nifedipine reduced 5-HT-induced contraction to 15.2±4.9% of the control, an effect not greater in the additional presence of 30 μM NFA.

3 In contrast, the contractile response to ACh (10 μM) was not inhibited by NFA in concentrations $\leqslant$100 μM, although this response was partly inhibited by nifedipine (1 μM) to 67.6±11.8% of the control value.

4 NFA (1–30 μM) did not affect contraction induced by either 20 mM or 60 mM KCl, suggesting that this drug was not acting *via* blockade of VDCCs or activation of potassium channels. In contrast, 3,5-dichlorophenylamine-2-carboxylic acid and 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid were less selective in their inhibitory effects, inducing reductions of 60 mM KCl-induced contraction at concentrations $\geqslant$10 μM.

5 Our results show that NFA can exert selective inhibitory effects on the chloride-dependent 5-HT-induced contractions of the rat fundus. The data support the hypothesis that activation of $Cl_{(Ca)}$ channels leading to calcium entry *via* VDCCs is a mechanism utilized by 5-HT, but not by ACh, to elicit contraction of the rat fundus.

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Abbreviations: ACh, acetylcholine; DCDPC, 3,5-dichlorophenylamine-2-carboxylic acid; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid; DMSO, dimethyl sulphoxide; 5-HT, 5-hydroxytryptamine; $I_{Cl(Ca)}$, calcium-activated chloride currents; VDCC, voltage-dependent calcium channels

Introduction

In recent years there has been growing interest in the possible physiological role of calcium-activated chloride channels ($Cl_{(Ca)}$) in the contractile responses induced by neurotransmitters in smooth muscle (see Large & Wang, 1996). Fenamates such as niflumic acid (NFA) have been shown to selectively inhibit $I_{Cl(Ca)}$ in isolated smooth muscle cells (Pacaud *et al*., 1989; Janssen & Sims, 1992; Akbarali & Giles, 1993; Hogg *et al*., 1994; Lamb *et al*., 1994) aiding a functional evaluation of these channels in smooth muscle.

We have previously shown that NFA reduces the amplitude of noradrenaline-induced contractions in aorta (Criddle *et al*., 1996). Since the magnitude of inhibition was comparable to that produced by nifedipine, and not further increased by a combination of NFA and nifedipine, we proposed from these functional data that activation of $Cl_{(Ca)}$ by neurotransmitters may lead to a depolarization-induced entry of calcium into the cell *via* voltage-dependent calcium channels (VDCCs) causing contraction of vascular smooth muscle. Subsequent studies with noradrenaline in rat aorta and endothelin in pulmonary artery have provided data to support this hypothesis (Hyvelin *et al*., 1998; Lamb & Barna, 1998), although important recent evidence in pulmonary artery has also suggested that niflumic acid may exert relaxant effects independently of chloride channel blockade (Kato *et al*., 1999). Whether this constitutes a common excitatory mechanism utilized by neurotransmitters to contract smooth muscle in general remains to be determined.

Despite electrophysiological studies showing the existence of $I_{Cl(Ca)}$ in a variety of non-vascular smooth muscle, including trachea (Janssen & Sims, 1992), myometrium (Arnaudeau *et al*., 1994), ileum (Ito *et al*., 1993) and oesophagus (Akbarali & Giles, 1993; Wang *et al*., 1994), little information is available at present regarding the possible functional role of $Cl_{(Ca)}$ channels in visceral smooth muscle. Previously we found that the pressor effects of 5-hydroxytryptamine (5-HT) in the rat mesenteric vascular bed were sensitive to NFA, with approximately 50% of the response being inhibited by a concentration of 30 μM (Criddle *et al*., 1997). In the present study we decided to evaluate the effects this $I_{Cl(Ca)}$ blocker on 5-HT-induced contraction of the rat fundus, a non-vascular smooth muscle preparation classically used to assay 5-HT (Vane, 1957). We have compared the effects of NFA and the voltage-dependent calcium channel (VDCC) blocker nifedipine on contractions elicited by 5-HT and acetylcholine (ACh). In addition, we have also evaluated the effects of other putative chloride channel blockers 3,5-dichlorophenylamine-2-carboxylic acid (DCDPC), a structural analogue of NFA, and

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the stilbene derivative, 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS). Some of these results have previously been presented to the British Pharmacological Society (Criddle *et al*., 1999).

Methods

Male Wistar rats (250–350 g) were killed by stunning and cervical dislocation. The abdomen was opened and the stomach removed. The fundus was dissected and washed in fresh Tyrode's solution. Following this longitudinal strips (2 cm long) were carefully prepared and mounted vertically in an organ bath (10 ml capacity) containing Tyrode's solution bubbled with air (37°C, pH 7.4). Tissues were mounted under an initial resting tension of 2 g and left to equilibrate for a period of 1 h, following which a further 2 g was applied before starting the experimental protocol. Tension changes were recorded using isometric force transducers (Grass Model FT0.3, Quincy, MA, U.S.A.) connected to a Grass chart recorder (Model 5D).

The effects of NFA were assessed on contractions induced by KCl (60 mM), 5-HT (10 μM) and ACh (10 μM) with each agonist being evaluated in a separate experimental group. Following stable contractions to the contractile agent, NFA was applied in increasing concentrations and further responses obtained (contact time of 15 mins). Since DMSO was used as a solvent for stock solutions of NFA, the effects of this solvent at equivalent concentrations were assessed as time-matched controls, on 5-HT-induced contraction of the fundus. In addition, the effects of 1 μM nifedipine and a combination of 1 μM nifedipine and 30 μM NFA were also assessed on 5-HT-induced contraction. Similar experiments were also performed to assess the effects of NFA on 20 mM KCl induced contractions, to examine any potential action to induce potassium conductances. In this protocol 3 μM levcromakalim was employed as a positive control.

In separate experiments, the effect of extracellular chloride withdrawal from the bathing solution on the responses of the contractile agonists was assessed. Initially control contractions to 5-HT (10 μM) and ACh (10 μM) were obtained. The tissues were then bathed in a modified Tyrode's solution ($0Cl^-$), in which all chloride salts had been substituted by their gluconate equivalents. Since application of $0Cl^-$ solution to the preparation caused a transient contraction, reapplication of 5-HT or ACh was carried out only when basal levels of tone were restored. In addition, the reversibility of any effect on agonist-induced contraction by $0Cl^-$ solution was assessed, by reintroducing normal (Cl^--containing) Tyrode's solution to the bath and repeating the application of 5-HT or ACh.

Solutions and drugs

The following drugs were used: 5-hydroxytryptamine (5-HT), acetylcholine (ACh), niflumic acid, DIDS and nifedipine were purchased from Sigma Chemical Company. Levcromakalim and DCDPC were generous gifts from SmithKline Beecham Ltd and Dr I. Greenwood, respectively. Nifedipine stock solution was prepared in 70% ethanol under conditions of reduced illumination and all experiments with nifedipine were performed under similar conditions. NFA was prepared as a stock solution (10 mM) in dimethyl sulphoxide (DMSO) and diluted on the day of the experiment in Tyrode's solution.

The bathing solution was a standard Tyrode's solution of the following composition (mM); NaCl 136, KCl 5, $MgCl_2$ 0.98, $CaCl_2$ 2, NaH_2PO_4 0.36, $NaHCO_3$ 11.9, glucose 5.5. In solutions in which the potassium concentration was raised (60 mM), the NaCl concentration was concomitantly reduced to maintain osmolarity. The chloride-free solution was of the same composition as standard Tyrode's solution, except that all the chloride salts were replaced by their gluconate equivalents. The pH was always maintained constant throughout the experimental period at 7.4.

Analysis of data

Data are expressed as the mean of n observations ± s.e.mean. Inhibitory effects are expressed as per cent of control responses in the absence of the drug. Statistical analysis was performed using a Student's t-test and values were taken to be significantly different when $P<0.05$.

Results

Effects of NFA and nifedipine on contractions induced by 60 mM KCl

Since NFA has been shown to possess actions apart from blockade of $I_{Cl(Ca)}$ when in sufficient concentration, such as the induction of K^+-currents and blockade of Ca^{2+}-channels (Hogg *et al*., 1994; Greenwood & Large, 1995), we initially assessed the selectivity of this drug by evaluating its effects on 60 mM KCl-induced contractions. In concentrations up to 30 μM, NFA did not inhibit the contractions induced by KCl, in contrast to nifedipine (1 μM) which almost completely blocked this response (Figure 1, $n=6$). 100 μM NFA, however, started to produce an inhibitory effect on KCl-induced contraction, possibly indicating non-specific effects of the drug (data not shown). Thus only concentrations <100 μM were used in subsequent experiments to assess the action of NFA on 5-HT-induced contraction.

Effects of NFA and nifedipine on contractions induced by 5-HT

Application of 5-HT (10 μM) to the rat fundus induced a tonic contraction (1.52 ± 0.40 g, $n=8$) with an oscillatory component superimposed (Figure 2a). In the presence of NFA (1–

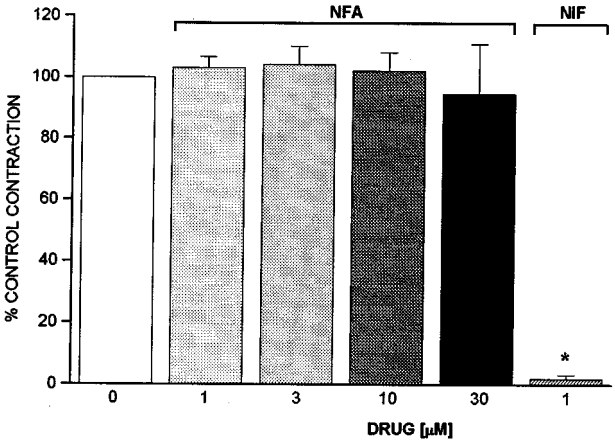

Figure 1 Comparative effects of niflumic acid (NFA 1–30 μM) and nifedipine (NIF 1 μM, $n=6$) on the contraction of the rat isolated fundus induced by 60 mM KCl. Values are shown as the mean ± s.e.mean of six experiments, and results are shown to differ significantly from the control when $P<0.05$ (*).

30 μM) the tonic contractions were concentration-dependently inhibited with a maximal reduction to 15.5±6.0% of the control value at the highest concentration of NFA used (Figure 2b). The inhibitory effect of NFA was fully reversible on washout of the drug from the tissue. In control paired tissues exposed only to the solvent DMSO at equivalent concentrations, the response to 5-HT did not diminish over the experimental period ($n=8$). Interestingly, whilst the tonic contraction of the response was inhibited by NFA, the oscillatory component of the 5-HT response remained largely unaffected (Figure 2a).

In separate experiments 1 μM nifedipine, which completely inhibited the contraction induced by 60 mM KCl (Figure 1, $n=6$), reduced the 5-HT-induced contraction to 15.2±4.9% of the control (Figure 3a,b, $n=6$). In contrast to the effects of NFA, nifedipine completely blocked the oscillatory component of the 5-HT response (Figure 3a). The effects of NFA and nifedipine were not additive such that in the combined presence of 1 μM nifedipine and 30 μM NFA, the 5-HT contraction was decreased to 10.4±4.7% of the control, a reduction not significantly ($P>0.05$) greater than that elicited by nifedipine alone (Figure 3a,b, $n=8$).

Effects of NFA and nifedipine on acetylcholine-induced contractions

ACh (10 μM) induced stable, non-oscillatory contractions of the rat fundus of mean amplitude 3.11±0.44 g ($n=7$) (Figure 4a). In contrast to the inhibitory effects on 5-HT-induced contraction, NFA (10–100 μM) did not inhibit contractions elicited by 10 μM ACh (Figure 4a,b, $n=7$). In separate experiments 1 μM nifedipine inhibited the contractions induced by ACh (10 μM) to 67.6±11.8% of the control value ($n=4$).

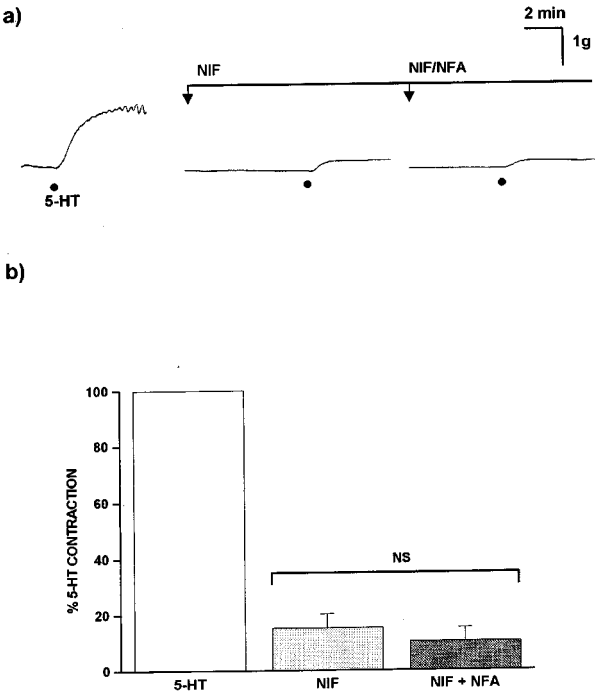

Figure 3 (a) Representative trace and (b) mean data showing the lack of additive inhibitory effect of a combination of nifedipine and niflumic acid (NIF 1 μM/NFA 30 μM), compared with that of nifedipine (NIF, 1 μM) alone, on the contraction of the rat isolated fundus induced by 5-HT (●, 10 μM). Values are shown as the mean±s.e.mean of eight experiments. N.B. The difference between the responses to 5-HT in the presence of NIF alone and NIF/NFA together was not significant (NS).

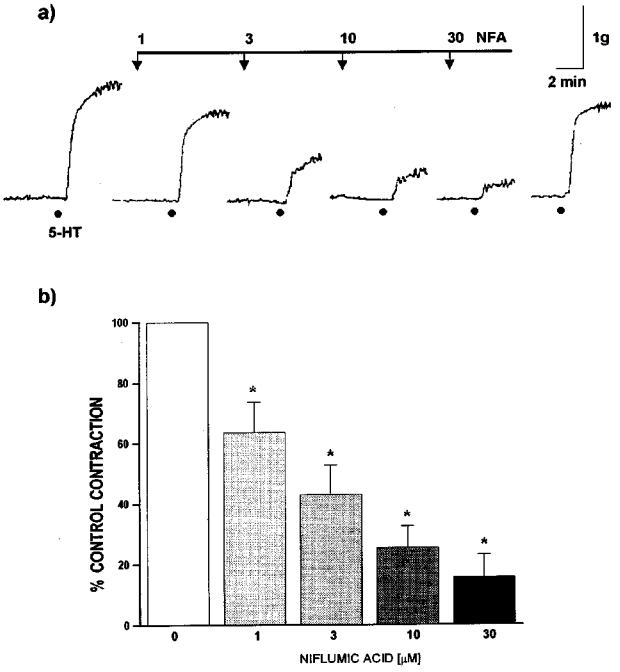

Figure 2 (a) Representative trace and (b) mean data showing the inhibitory action of niflumic acid (NFA, 1–30 μM) on the contraction of the rat isolated fundus induced by 5-HT (●, 10 μM). Values are shown as the mean±s.e.mean of eight experiments, and results are shown to differ significantly from the control when $P<0.05$ (*). N.B. The recovery of the response to 5-HT, after a washout period of 1 h, following exposure to NFA has been included in the representative trace.

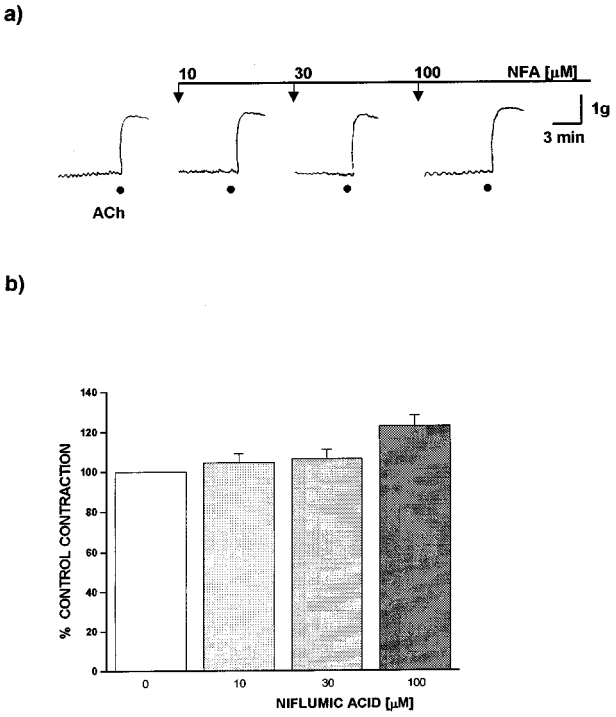

Figure 4 (a) Representative trace and (b) mean data showing the lack of inhibitory effect of niflumic acid (NFA, 10–100 μM) on contractions of the rat isolated fundus induced by ACh (●, 10 μM). Values are shown as the mean±s.e.mean of seven experiments, and results are shown to differ significantly from the control when $P<0.05$ (*).

Effects of 0Cl⁻ solution on the contractile response to 5-HT and acetylcholine

Addition of 0Cl⁻ (gluconate-substituted) physiological solution to the rat fundus elicited a small transient contraction which returned to basal levels within a few minutes. In the continued presence of 0Cl⁻ the contractile responses to 5-HT (10 μM) and ACh (10 μM) were reduced to 32.2±6.0% ($n=10$) and 68.2±11.0% ($n=6$) of their respective control values (Figure 5). These reductions were fully reversible on washout with normal (Cl^--containing) Tyrode's solution.

Effects of NFA and levcromakalim on 20 mM KCl-induced contractions

Twenty mM KCl produced reproducible contractions of mean amplitude 3.11±0.37 g (Figure 6a, $n=6$). NFA (30 μM) did not inhibit this response, with the contraction being 111.6±11.2% of the control value (Figure 6a,b, $n=6$). In contrast the ATP-dependent K^+-channel opener levcromakalim (3 μM) completely abolished this response (Figure 6a,b, $n=6$).

Effects of DCDPC and DIDS on 5-HT-, ACh- and KCl-induced contractions

In separate experiments the effects of DCDPC, a structural analogue of NFA, and DIDS, a stilbene derivative, were evaluated on the contractions induced by 5-HT (10 μM), ACh (10 μM) and KCl (60 mM). DCDPC (10–100 μM) concentration dependently inhibited the contractions induced by all three spasmogens, with reductions to 13.9±9.3%, 19.6±7.9% and 28.1±12.2% of the control responses to 5-HT, ACh and KCl, respectively, at the highest concentration tested (Figure 7a–c).

In contrast, DIDS (1–300 μM) was without inhibitory effect on ACh-induced contraction (Figure 8b), whilst contractions elicited by 5-HT were inhibited only at the highest concentration used (300 μM) to 68.3±6.4% of the control value (Figure 8a, $n=6$). The contraction induced by 60 mM KCl, however, was more sensitive to the action of DIDS, with inhibition occurring at concentrations $\geqslant$30 μM (Figure 8c, $n=6$).

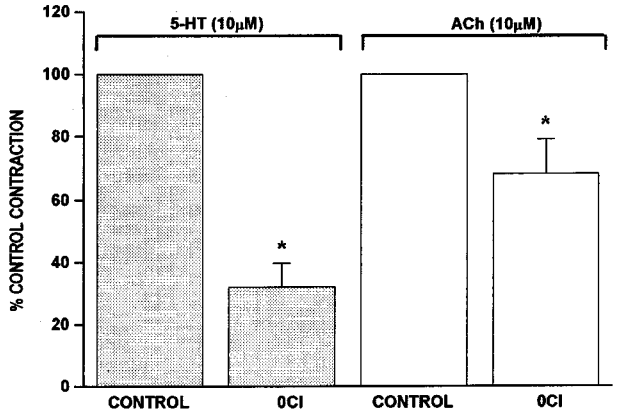

Figure 5 Inhibitory effects of 0Cl⁻ solution (gluconate-substituted) on the contractions of the rat isolated fundus induced by 5-HT (10 μM, $n=10$) and ACh (10 μM, $n=6$). Values are shown as the mean±s.e.mean of n experiments, and results are shown to differ significantly from the control when $P<0.05$ (*).

Discussion

The present study has shown that NFA exerts selective, reversible and concentration-dependent inhibitory effects on 5-HT-induced contraction of the rat stomach fundus. These effects occurred at concentrations previously shown to inhibit $I_{Cl(Ca)}$ in a variety of smooth muscle cell types (Pacaud *et al*., 1989; Janssen & Sims, 1992; Akbarali & Giles, 1993; Hogg *et al*., 1994). Recently, a detailed study in the rat pulmonary artery has demonstrated that NFA inhibits $I_{Cl(Ca)}$ in isolated myocytes, and both depolarizations and contractions elicited by 5-HT in the intact vessel (Yuan, 1997). Certain evidence exists to support the possibility that 5-HT-induced contraction in the rat fundus, probably *via* the 5-HT_{2B} subtype (Kursar *et al*., 1994; Foguet *et al*., 1992; Baxter *et al*., 1994; Hoyer *et al*., 1994), involves the activation of $I_{Cl(Ca)}$. For example, stimulation of rat fundus 5-HT_{2B} receptors expressed in *Xenopus* oocytes has been shown to directly activate $Cl_{(Ca)}$ channels (Foguet *et al*., 1992), whilst 5-HT-induced activation of $I_{Cl(Ca)}$ occurs in epithelial cells of the rat choroid plexus (Garner *et al*., 1993). However, a NFA-sensitive activation of $I_{Cl(Ca)}$ has yet to be shown in isolated myocytes of the rat fundus.

Higher concentrations of NFA than those employed in the current study (>50 μM) have been shown to inhibit VDCCs and activate K^+-channels in rabbit portal vein (Hogg *et al*., 1994) and activate K_{ATP} in rat portal vein (Kirkup *et al*., 1996). In the rat fundus we found that NFA in concentrations $\leqslant$30 μM did not inhibit contractions to either 20 mM or 60 mM KCl, in agreement with previous studies in rat aorta (Criddle *et al*., 1996), mesenteric vascular bed (Criddle *et al*., 1997), rabbit colon (Patacchini *et al*., 1996) and rat pulmonary artery (Yuan, 1997). This would appear to indicate that at these concentrations NFA is not interacting with either Ca^{2+}

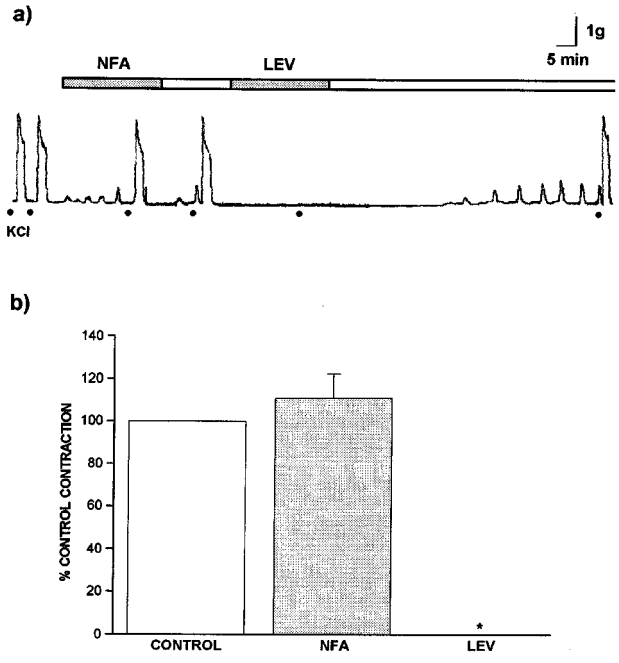

Figure 6 (a) Representative trace and (b) mean data showing the effects of niflumic acid (NFA, 30 μM) and levcromakalim (LEV, 3 μM) on the contraction of the rat isolated fundus induced by 20 mM KCl (●). Values are shown as the mean±s.e.mean of five experiments, and results are shown to differ significantly from the control when $P<0.05$ (*). N.B. A washout period has been included after the inhibitory effect of levcromakalim to show recovery of the control response to 20 mM KCl (●).

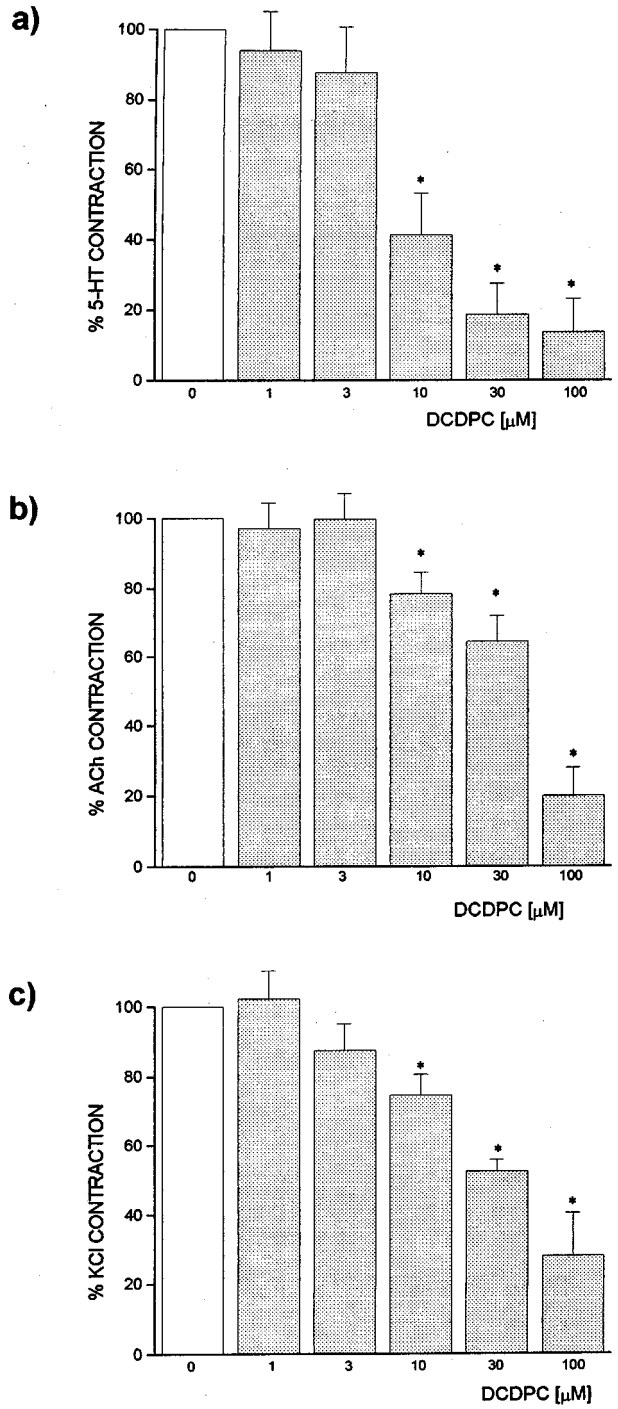

Figure 7 Effects of 3,5-dichlorophenylamine-2-carboxylic acid (DCDPC; 1–100 μM) on the contraction of the rat isolated fundus induced by (a) 5-HT (10 μM, $n=9$), (b) ACh (10 μM, $n=6$) and (c) KCl (60 mM, $n=6$). Values are shown as the mean$\pm$s.e.mean of n experiments, and results are shown to differ significantly from the control when $P<0.05$ (*).

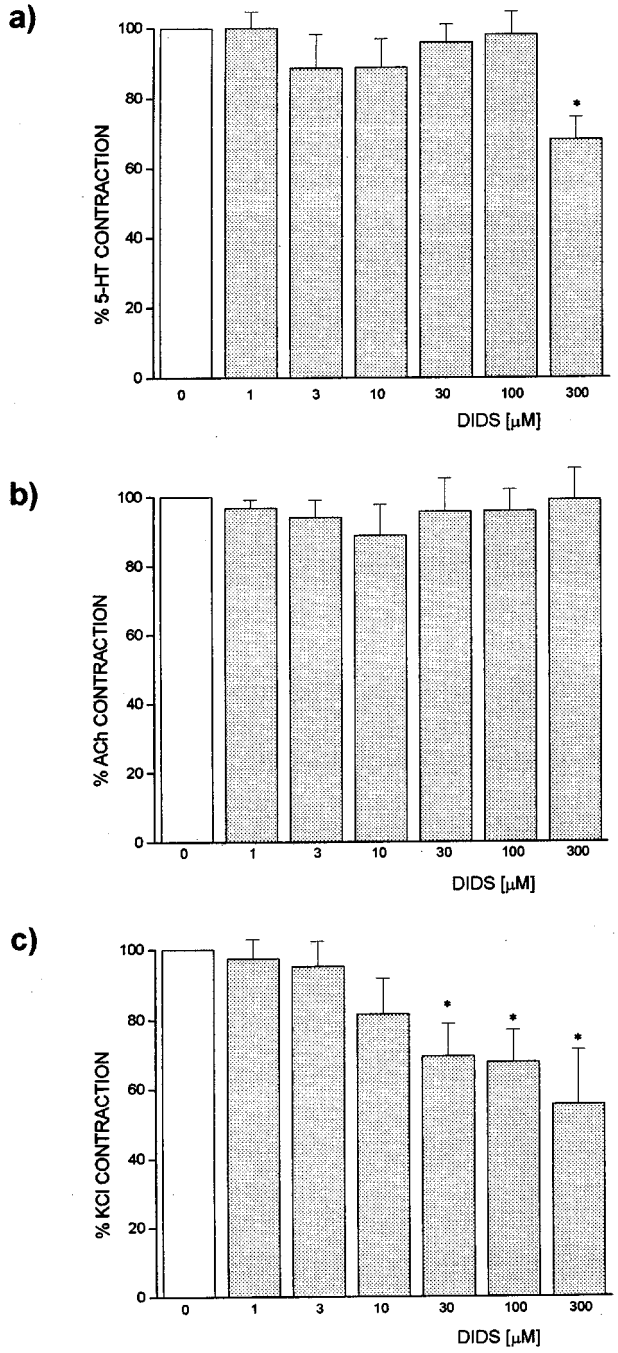

Figure 8 Effects of 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS; 1–300 μM) on the contraction of the rat isolated fundus induced by (a) 5-HT (10 μM, $n=6$), (b) ACh (10 μM, $n=8$) and (c) KCl (60 mM, $n=6$). Values are shown as the mean$\pm$s.e.mean of n experiments, and results are shown to differ significantly from the control when $P<0.05$ (*).

or K^+ channels in the functional protocols of this study. The lack of inhibitory effects of NFA contrasted with the profiles of action of the VDCC blocker nifedipine and the K^+-channel opener levcromakalim, which completely blocked 60 and 20 mM KCl-induced contractions of the fundus, respectively.

Our current data indicate that the magnitude of the inhibitory effect of NFA on 5-HT responses in the fundus ($\sim$85% with 30 μM) was greater than that previously found in the mesenteric vascular bed ($\sim$50% with 30 μM; Criddle *et al.*, 1997) and pulmonary artery ($\sim$55% with 50 μM; Yuan, 1997). This may reflect a slightly greater importance of the activation of $I_{Cl(Ca)}$ in the contractile process elicited by 5-HT in the rat fundus compared with these blood vessels, possibly as a consequence of the different subtypes of receptors activated in these tissues (Humphrey *et al.*, 1993; Hoyer *et al.*, 1994). In accord with our previous findings in mesenteric arteries (Criddle *et al.*, 1997), the contractile response to 5-HT in the rat fundus was inhibited to a similar extent by the VDCC blocker nifedipine, the magnitude of which was in close agreement with earlier studies in this tissue using nitrendipine and isradipine (Secrest *et al.*, 1989; Smaili *et al.*, 1991). That the effects of nifedipine and NFA were not additive in the rat

fundus is in accord with the hypothesis that activation of Cl_{Ca} channels, leading to depolarization, opens VDCCs to contract smooth muscle (Criddle *et al*., 1996). This is supported by the findings of an earlier study in rabbit basilar artery showing that the contraction, but not the depolarization, induced by 5-HT in rabbit basilar artery is greatly inhibited by nifedipine (Clark & Garland, 1993). In addition, several studies in pulmonary artery have reported a similar proportional inhibition of agonist-induced contractions occuring with NFA and nifedipine, using angiotensin II and endothelin-1 as contractile agents (Guibert *et al*., 1997; Hyvelin *et al*., 1998), although it should be noted that NFA-induced inhibition of endothelin-1-mediated contraction in this tissue may occur independently of blockade of chloride channels (Kato *et al*., 1999). However, in the present study, although nifedipine and NFA blocked a similar proportion of the 5-HT response in the rat fundus their actions were clearly fundamentally different. For example, the oscillatory component of the 5-HT-induced contraction persisted when the tonic contraction had been substantially reduced by NFA, whereas it was completely blocked by nifedipine.

In contrast to the sensitivity of 5-HT-induced contraction, the ACh-induced response was not inhibited by NFA, even at a high concentration of 100 μM. This lack of effect was shared by nifedipine, as previously reported in this preparation (Secrest *et al*., 1989; Smaili *et al*., 1991). Accordingly, there was little chloride-dependency exhibited by the ACh contraction, in contrast to the 5-HT-induced response which was greatly reduced when extracellular chloride was replaced by gluconate ions. These observations support the argument that that there is little or no involvement of $I_{Cl(Ca)}$ in ACh-induced contraction in the rat fundus. Whilst the authors are currently unaware of any published study examining this possibility, certain evidence from other gastrointestinal smooth muscle would appear to support this hypothesis. For example, depolarization induced by carbachol is much less affected by reductions in extracellular chloride (substituted by benzenesulphonate) than by reductions in extracellular sodium ions in isolated ileum (Bolton, 1972), whilst the contraction induced by ACh in canine gastric smooth muscle (Sims, 1992) and the depolarization to carbachol in rabbit jejunum (Benham *et al*., 1985) appear to be mediated *via* activation of a non-selective cation conductance.

The other putative $Cl_{(Ca)}$ channel blockers tested in this study, DCDPC and DIDS, proved to be less selective in their inhibitory effects than NFA. For example, although DCDPC inhibited the contractions produced by 5-HT to approximately 14% of the control in common with NFA, it also inhibited contractions to both KCl and ACh within a similar concentration range i.e. 10–100 μM. DCDPC has previously been shown to inhibit $ICl_{(Ca)}$ in single cells from rabbit portal vein with a similar potency to NFA (Greenwood & Large, 1997), however, it also activated a K^+ conductance at much lower concentrations. In addition, the indiscriminate inhibitory effects observed in our study may also be related to the ability of this compound to inhibit smooth muscle non-selective cation channels at similar concentrations (Chen *et al*., 1993). The structurally-distinct chloride channel blocker DIDS exhibited a different profile of action, preferentially depressing the contractile response to KCl, with only a weak inhibition of the 5-HT-induced response apparent at a concentration of 300 μM. A recent study has suggested that DIDS is more active at suppressing volume-activated chloride currents than $I_{Cl(Ca)}$ in smooth muscle (Greenwood & Large, 1998), possibly explaining the comparative lack of effect of this compound on 5-HT-induced contraction compared with NFA observed in the present study. Further detailed electrophysiological experiments in single cells of the rat fundus would be required to resolve the apparent differences between the chloride channel blockers evaluated in the present functional study.

In conclusion, our results show that NFA exerts selective inhibitory effects on 5-HT-induced contraction of the rat fundus. The comparative inhibitory actions of nifedipine and NFA in this tissue are in accord with similar observations on agonist-induced contraction of vascular smooth muscle (Criddle *et al*., 1996; 1997; Guibert *et al*., 1997; Hyvelin *et al*., 1998), and the current data support the hypothesis that activation of $Cl_{(Ca)}$ channels leading to the entry of extracellular calcium ions through VDCCs, is an excitatory mechanism utilized by 5-HT, but not by ACh, to contract the rat fundus. However, conclusions derived purely from contractile studies must remain cautious, especially in the light of recent evidence casting doubt on the selectivity of currently available chloride channel blockers (Kato *et al*., 1999).

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References

AKBARALI, H.I. & GILES, W.R. (1993). Ca^{2+} and Ca^{2+}-activated Cl^- currents in rabbit oesophageal smooth muscle. *J. Physiol.,* **460,** 117–133.

ARNAUDEAU, S., LEPETRE, N. & MIRONNEAU, J. (1994). Chloride and monovalent ion-selective cation currents activated by oxytocin in pregnant rat myometrial cells. *Am. J. Obstet. Gynaecol.,* **171,** 491–501.

BAXTER, G.S., MURPHY, O.E. & BLACKBURN, T.P. (1994). Further characterization of 5-hydroxytryptamine receptors (putative 5-HT_{2B}) in rat stomach fundus longitudinal muscle. *Br. J. Pharmacol.,* **112,** 323–331.

BENHAM, C.D., BOLTON, T.B. & LANG, R.J. (1985). Acetylcholine activates an inward current in single mammalian smooth muscle cells. *Nature,* **316,** 345–347.

BOLTON, T.B. (1972). The depolarising action of acetylcholine or carbachol in intestinal smooth muscle. *J. Physiol.,* **220,** 647–671.

CHEN, S., INOUE, R. & ITO, Y. (1993). Pharmacological characterization of muscarinic receptor-activated cation channels in guinea-pig ileum. *Br. J. Pharmacol.,* **109,** 793–801.

CLARK, A.H. & GARLAND, C.J. (1993). Ca2+ channel antagonists and inhibition of protein kinase C each block contraction but not depolarization to 5-hydroxytryptamine in rabbit basilar artery. *Eur. J. Pharmacol.,* **235,** 113–116.

CRIDDLE, D.N., SCARPARO, H.C., SANTOS, G.C.M. & LEAL-CARDOSO, J.H. (1999). Selective inhibitory effects of niflumic acid on 5-HT-induced contraction of the rat isolated fundus. *Br. J. Pharmacol.,* **126,** 103P.

CRIDDLE, D.N., SOARES DE MOURA, R., GREENWOOD, I.A. & LARGE, W.A. (1996). Inhibitory actions of niflumic acid on noradrenaline-induced contraction of the rat aorta. *Br. J. Pharmacol.,* **118,** 1065–1071.

CRIDDLE, D.N., SOARES DE MOURA, R., GREENWOOD, I.A. & LARGE, W.A. (1997). Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor responses in the isolated mesenteric vascular bed of the rat. *Br. J. Pharmacol.,* **120,** 813–818.

FOGUET, M., HOYER, D., PARDO, L.A., PAREKH, A., FLUXEN, F.W., KALKMAN, H.O., STÜHMER, W. & LÜBBERT, H. (1992). Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J.,* **11,** 3481 – 3487.

GARNER, C., FENIUK, W. & BROWN, P. (1993). Serotonin activates Cl^- channels in the apical membrane of the rat choroid plexus epithelial cells. *Eur. J. Pharmacol.,* **239,** 31 – 37.

GREENWOOD, I.A. & LARGE, W.A. (1995). Comparison of the effects of fenamates on Ca-activated chloride and potassium currents in rabbit portal vein smooth muscle cells. *Br. J. Pharmacol.,* **116,** 2939 – 2948.

GREENWOOD, I.A. & LARGE, W.A. (1997). Inhibition Ca^{2+}-activated Cl^--currents in smooth muscle cells by compounds structurally similar to niflumic acid. *Br. J. Pharmacol.,* **123,** 324P.

GREENWOOD, I.A. & LARGE, W.A. (1998). Properties of a Cl^- current activated by cell swelling in rabbit portal vein vascular smooth muscle cells. *Am. J. Physiol.,* **275,** H1524 – H1532.

GUIBERT, C., MARTHAN, R. & SAVINEAU, J.P. (1997). Oscillatory Cl- current induced by angiotensin II in rat pulmonary arterial myocytes: Ca^{2+} dependence and physiological implication. *Cell Calcium,* **21,** 421 – 429.

HOGG, R., WANG, Q. & LARGE, W.A. (1994). Action of niflumic acid on evoked and spontaneous calcium-activated chloride and potassium currents in smooth muscle cells from rabbit portal vein. *Br. J. Pharmacol.,* **112,** 977 – 984.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.,* **46,** 157 – 203.

HUMPHREY, P.P.A., HARTIG, P. & HOYER, D. (1993). A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol. Sci.,* **14,** 233 – 236.

HYVELIN, J.M., GUIBERT, C., MARTHAN, R. & SAVINEAU, J.P. (1998). Acellular mechanisms and role of endothelin-1-induced calcium oscillations in pulmonary arterial myocytes. *Am. J. Physiol.,* **275,** L269 – L282.

ITO, S., OHTA, T. & NAKAZATO, Y. (1993). Inward current activated by carbachol in rat intestinal smooth muscle cells. *J. Physiol. Lond.,* **470,** 395 – 409.

JANSSEN, L.J. & SIMS, S.M. (1992). Acetylcholine activates non-selective cation and chloride conductances in canine and guinea-pig tracheal myocytes. *J. Physiol.,* **453,** 197 – 218.

KATO, K., EVANS, A.M. & KOZLOWSKI, R.Z. (1999). Relaxation of endothelin-1-induced pulmonary artery constriction by niflumic acid and NPPB: mechanism(s) independent of chloride channel block. *J. Pharmacol. Exp. Ther.,* **288,** 1242 – 1250.

KIRKUP, A.J., EDWARDS, G., GREEN, M.E., MILLER, M., WALKER, S.D. & WESTON, A.H. (1996). Modulation of membrane currents and mechanical activity by niflumic acid in rat vascular smooth muscle. *Eur. J. Pharmacol.,* **317,** 165 – 174.

KURSAR, J.D., NELSON, D.L., WAINSCOTT, D.B. & BAEZ, M. (1994). Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine2B receptor. *Mol. Pharmacol.,* **46,** 227 – 234.

LAMB, F.S. & BARNA, T.J. (1998). Chloride ion currents contribute functionally to norepinephrine-induced vascular contraction. *Am. J. Physiol.,* **275,** H151 – H160.

LAMB, F.S., VOLK, J.A. & SHIBATA, E.F. (1994). Calcium-activated chloride current in rabbit coronary artery myocytes. *Circ. Res.,* **75,** 742 – 750.

LARGE, W.A. & WANG, Q. (1996). Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. *Am. J. Physiol.,* **271,** C435 – C454.

PACAUD, P., LOIRAND, G., MIRONNEAU, C. & MIRONNEAU, J. (1989). Calcium-activated chloride current in rat vascular smooth muscle cells in short-term primary culture. *Pflügers Arch.,* **413,** 629 – 636.

PATACCHINI, R., SANTICIOLI, P. & MAGGI, C.A. (1996). Effect of NFA on electromechanical coupling by tachykinin NK_1 receptor activation in rabbit colon. *Eur. J. Pharmacol.,* **303,** 197 – 204.

SECREST, R.J., SCHOEPP, D.D. & COHEN, M.L. (1989). Comparison of contractions to serotonin, carbamylcholine and prostaglandin $F_{2\alpha}$ in rat stomach fundus. *J. Pharmacol. Exp. Ther.,* **250,** 971 – 978.

SIMS, S.M. (1992). Cholinergic activation of a non-selective cation current in canine gastric smooth muscle is associted with contraction. *J. Physiol.,* **449,** 377 – 398.

SMAILI, S.S., JURKIEWICZ, N.H., GARCIA, A.G. & JURKIWICZ, A. (1991). Comparison of the effect of calcium withdrawal from the medium and of blockade of extracellular calcium entry by isradipine on the contractile responses of the isolated rat stomach. *Brazilian. J. Med. Biol. Res.,* **24,** 953 – 956.

VANE, J.R. (1957). A sensitive method for the assay of 5-HT. *Br. J. Pharmacol.,* **12,** 344 – 349.

WANG, Q., AKBARALI, H.I. & GOYAL, R.K. (1994). Calcium-activated chloride currents in opossum esophageal circular muscle. *Gastroenterology,* **106,** A586.

YUAN, X.-J. (1997). Role of calcium-activated chloride current in regulating pulmonary vasomotor tone. *Am. J. Physiol.,* **272,** L959 – L968.

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