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Effect of niflumic acid on electromechanical coupling by tachykinin NK_1 receptor activation in rabbit colon

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Abstract

We have investigated the effect of the Cl^- channel blocker, niflumic acid, on the contractile response and electromechanical coupling activated by stimulation of the tachykinin NK_1 receptor in the longitudinal muscle of rabbit proximal colon, in the presence of indomethacin (5 μM). The application of submaximal equieffective concentrations of the tachykinin NK_1 receptor-selective agonist [Sar^9]substance P sulfone (30 nM), of carbachol (300 nM) and KCl (40 mM), produced distinct phasic and tonic components of contraction. Niflumic acid (10–100 μM) preferentially and markedly inhibited the tonic component of the response to [Sar^9]substance P sulfone and to carbachol, without affecting the response to KCl. Nifedipine (1 μM) abolished the response to KCl and greatly reduced the response to [Sar^9]substance P sulfone and carbachol. The nifedipine-resistant response to [Sar^9]substance P sulfone was attenuated by niflumic acid (100 μM), while that to carbachol was unaffected. In sucrose gap experiments, superfusion with niflumic acid (100 μM), in the presence of nifedipine (3 μM), produced membrane hyperpolarization, which was totally blocked by tetraethylammonium (10 mM). Niflumic acid inhibited both depolarization and contraction induced by [Sar^9]substance P sulfone, both in the absence or in the presence of tetraethylammonium. The present findings support the idea that a niflumic acid-sensitive mechanism, probably an effect on Cl^- channels, takes part in the post-receptorial events activated by tachykinin NK_1 receptor stimulation in the longitudinal muscle of rabbit colon, and suggest that this mechanism would be more important for generating the sustained tonic than the phasic component of contraction.

Keywords: Tachykinin NK_1 receptor; Niflumic acid; Cl^- channel; Colon, rabbit

1. Introduction

Substance P is a major excitatory transmitter to the smooth muscle of the mammalian intestine (Barthò and Holzer, 1985, for review). Neuronal elements expressing substance P-like immunoreactivity are present in the myenteric plexus and project to the longitudinal and circular muscle layers of the intestine (Costa et al., 1981; Llewellyn-Smith et al., 1989). Moreover, depolarizing and physiological stimuli release substance P-like immunoreactivity from the mammalian intestine (Theodorsson et al., 1991; Schmidt et al., 1992). At the postjunctional level, the action of substance P on smooth muscle is believed to be chiefly mediated via the activation of the tachykinin NK_1 receptor, for which substance P possesses the greatest affinity amongst natural tachykinins (Maggi et al., 1993, for review). However, another tachykinin, neurokinin A, is co-released with substance P from myenteric plexus neurons (Hua et al., 1985; Schmidt et al., 1991; Shuttleworth et al., 1991) and, in most instances, smooth muscle cells co-express both tachykinin NK_1 and NK_2 receptors (Maggi et al., 1993). Since a certain degree of cross talk exists between natural tachykinins and different tachykinin receptors, substance P and neurokinin A have a limited value as tools to establish the mechanism(s) of excitation-contraction coupling activated by different tachykinin receptors in intestinal smooth muscle. One way to overcome this problem is that of using synthetic receptor-selective agonists, which possess high affinity and selectivity for tachykinin NK_1 receptors only, such as the undecapeptide substance P derivative, [Sar^9]substance P sulfone (Regoli et al., 1988).

Various studies have indicated that the recruitment of L-type voltage-dependent Ca^{2+} channels is an important mechanism for excitation/contraction coupling initiated by tachykinin NK_1 receptors in intestinal smooth muscle: the contractile response to tachykinin NK_1 receptor-selec-

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tive agonists is accompanied by a strong depolarization of the membrane, and it is largely inhibited by dihydropyridine-type Ca^{2+} channel blockers, such as nifedipine, in both the small and large intestine (e.g. Zagorodnyuk et al., 1993, 1995). On the other hand, the mechanism through which tachykinin NK_1 receptor ligands produce this final effect is uncertain: stimulation of phosphatidylinositol turnover (Holzer and Lippe, 1985; Hall and Morton, 1991), Ca^{2+} influx from the extracellular space (Mayer et al., 1990; Zagorodnyuk et al., 1994), mobilization of intracellular Ca^{2+} (Holzer and Lippe, 1984), modulation of K^+ channels (Mayer et al., 1989, 1990) and increase in cation permeability (Benham and Bolton, 1983) have all been implicated in the action of substance P and/or tachykinin NK_1 receptor-selective agonists on intestinal smooth muscle. Recently, evidence has been presented by patch clamp techniques that the selective tachykinin NK_1 receptor agonist [Sar^9]substance P sulfone activates a class of Ca^{2+}-dependent Cl^- channels in the longitudinal muscle of rabbit colon (Sun et al., 1992, 1993). This mechanism would be suited for producing depolarization of intestinal smooth muscle and, indirectly, activating the recruitment of nifedipine-sensitive voltage-dependent Ca^{2+} channels (Somlyo and Somlyo, 1994 for review).

However, it is not known at which extent such a mechanism may be relevant for producing excitation-contraction coupling of colonic smooth muscle. In this study we addressed this problem by studying the effect of niflumic acid, a blocker of Ca^{2+}-dependent Cl^- channels (White and Aylwin, 1990; Hogg et al., 1994b), on the contractile effect produced by [Sar^9]substance P sulfone on longitudinal muscle strips from rabbit colon, as compared to the responses induced by elevation of extracellular KCl and muscarinic receptor activation by carbachol. Since niflumic acid is also a cyclooxygenase inhibitor (Aussel et al., 1987; Mathieu et al., 1994), all experiments were performed in the presence of indomethacin, to exclude the contribution of endogenous prostanoids in the evoked responses.

2. Materials and methods

2.1. General

Male albino New Zealand rabbits (2.5–3.0 kg) were stunned and bled. Proximal colon segments, 3–5 cm long, were removed and placed in oxygenated Krebs solution having the following composition: NaCl, 119 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.5 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM and glucose 11 mM. From each intestinal segment four to six longitudinal muscle strips (0.7–1 cm long) were obtained, after having removed the mucosa. The preparations were placed in 5-ml organ baths, filled with oxygenated (96% O_2 and 4% CO_2) Krebs solution containing indomethacin (5 μM), at 37°C, and were connected to isotonic force transducers (load 5 mN). The experiments commenced after a 60-min equilibration period, during which the preparations received KCl (80 mM; every 20–30 min), whose maximal contractile effect was used as the internal standard.

In a first series of experiments, concentration-response curves to [Sar^9]substance P sulfone, carbachol and KCl were constructed to select submaximally equieffective concentrations of the three agents. All the responses to the peptide agonist [Sar^9]substance P sulfone were obtained in the presence of thiorphan (10 μM), to reduce its degradation by tissue endopeptidase.

The time course of the responses to [Sar^9]substance P sulfone (30 nM), carbachol (300 nM) and KCl (40 mM), each one producing a similar submaximal contractile response, was followed for 15 min. After an initial phasic contraction, which peaked within 1–2 min from administration of the agonists, a tonic component of contraction developed: in the case of the [Sar^9]substance P sulfone- and carbachol-induced response, some phasic contractions superimposed onto the tonic response, which were not considered when evaluating the height of the tonic contraction. The contractions produced by the above agonists were studied before and after incubation with niflumic acid (10, 30 and 100 μM; 30 min before). Furthermore, the effect of nifedipine (1 μM; 45 min before) and of both nifedipine and niflumic acid (100 μM; 30 min before) was studied against these single doses of [Sar^9]substance P sulfone, carbachol and KCl. Since the contractions produced by the above drugs in the presence of nifedipine were sparely reproducible, matched strips from a common colon segment were used: part of them were incubated with nifedipine alone and part with nifedipine plus niflumic acid.

2.2. Sucrose gap

Changes in membrane potential and mechanical activity of small bundles (1–1.2 mm wide and 10 mm long) of rabbit colon longitudinal smooth muscle were studied by a modified single sucrose gap, as described in previous studies (Zagorodnyuk et al., 1993, 1994, 1995). All experiments were performed in oxygenated Krebs solution containing indomethacin (5 μM), nifedipine (3 μM) and thiorphan (5 μM), which was superfused at a rate of 1 ml/min. After a 60-min equilibration period, two consecutive control responses to [Sar^9]substance P sulfone (0.3 μM for 60 s) were obtained, at a 45-min interval from each other. Niflumic acid (100 μM) was superfused for 30 min before the next challenge with [Sar^9]substance P sulfone. In a series of experiments, tetraethylammonium (10 mM), was added to the Krebs solution from the beginning of the experiments.

2.3. Statistical analysis

The values in the text, tables or figures are expressed as means ± 95% confidence limits (95% c.l.), or ± S.E.M. Statistical analysis was performed by means of Student's *t*-test for paired or unpaired data or by means of two-way analysis of variance (ANOVA), when applicable. Regression analysis of log concentration-effect curves was performed by the least squares method, considering the curves linear between 20 and 80% of the maximal response.

2.4. Drugs

Indomethacin, thiorphan, niflumic acid, tetraethylammonium, hexamethonium and nifedipine are from Sigma (St. Louis, MO, USA); [Sar^9]substance P sulfone is from Peninsula (St. Helens, UK); carbachol HCl is from Merck (Darmstadt, Germany) and atropine is from Serva (Heidelberg, Germany).

3. Results

3.1. Effect of niflumic acid on contractile responses to [Sar^9]substance P sulfone, carbachol and KCl in the rabbit isolated proximal colon

In a first series of experiments, concentration-response curves to [Sar^9]substance P sulfone, carbachol and KCl were constructed, to identify submaximally equieffective concentrations of the three agents. The tachykinin NK_1 receptor-selective agonist [Sar^9]substance P sulfone (0.3 nM to 1 μM) produced concentration-dependent contractile responses (EC_{50} = 7.6 nM, 95% c.l.: 2.1–13.1 nM; E_{max} = 79 ± 2.7% of maximal response to KCl (80 mM); n = 12). Carbachol (30 nM to 30 μM) likewise produced a concentration-dependent contraction (EC_{50} = 200 nM, 95% c.l.: 130–270 nM; E_{max} = 92 ± 2%; n = 6), as did KCl (10–80 mM) (EC_{50} = 29 mM, 95% c.l.: 20–38 mM, n = 6).

From these experiments, the concentrations of [Sar^9]substance P sulfone (30 nM), carbachol (300 nM) and KCl (40 mM) were selected, and administered to preparations as single doses. These agents produced peak phasic contractions averaging 59 ± 2 (n = 14), 70 ± 5 (n = 12) and 66 ± 4% (n = 12) of the maximal response to KCl 80 mM for [Sar^9]substance P sulfone, carbachol and KCl, respectively. The peak response to each one of the three agonists was followed by a tonic component of contraction. During a 15-min observation period, this latter component was well sustained for carbachol and KCl, while it showed a slow decay for [Sar^9]substance P sulfone (Figs. 1 and 2). At 15 min from the administration of the agonist, the tonic component of contraction averaged 25 ± 7% (n = 12), 41 ± 5% (n = 10) and 79 ± 2% (n = 12) of the maximal contraction to 80 mM KCl, for [Sar^9]substance P sulfone, carbachol and KCl, respectively (Figs. 1 and 2). Neither the phasic (62.5 ± 5% vs. 63 ± 4% of KCl 80 mM, n = 4 each) nor the tonic (41.5 ± 4 vs. 44.5 ± 4% of KCl 80 mM, n = 4 each) component of the response to carbachol (300 nM) were affected by hexamethonium (100 μM; 15 min before). On the other hand, the response to carbachol (300 nM) was completely suppressed (100% inhibition, n = 4) by atropine (1 μM; 15 min before), indicating that it is totally mediated by activation of muscarinic receptors.

Niflumic acid (10–100 μM; 30 min before) produced a concentration-dependent inhibition of both the phasic and tonic responses to [Sar^9]substance P sulfone (30 nM); for each concentration tested, the inhibitory effect was larger toward the tonic than the phasic component of contraction. The phasic contraction to [Sar^9]substance P sulfone was

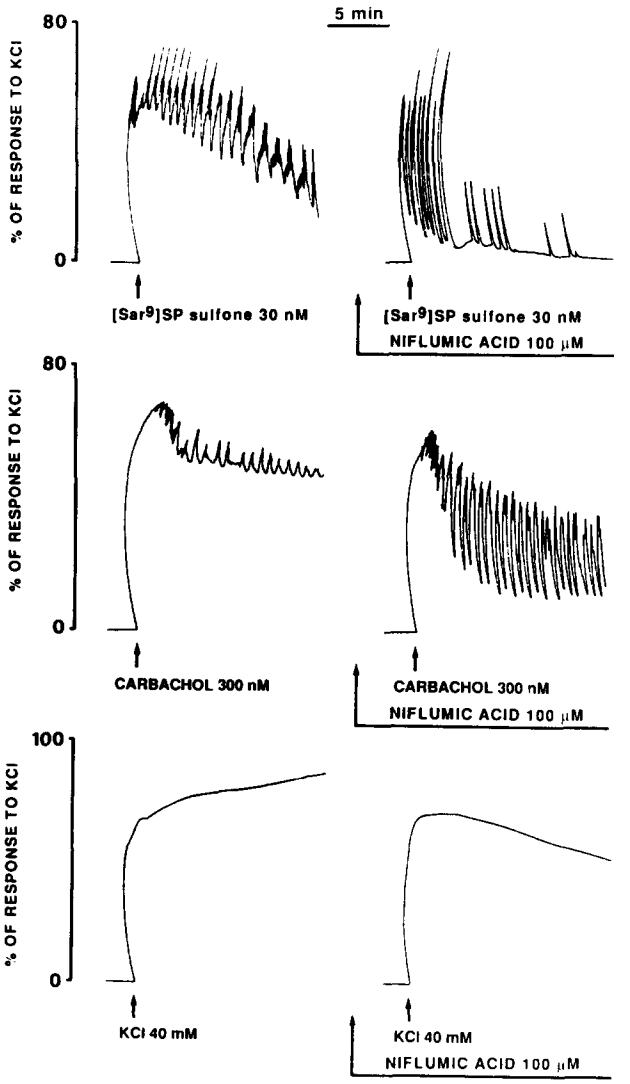

Fig. 1. Typical tracings showing the effect of niflumic acid (100 μM; 30 min before) on the contractions produced by [Sar^9]substance P sulfone (30 nM), carbachol (300 nM) and KCl (40 mM) in the rabbit isolated proximal colon.

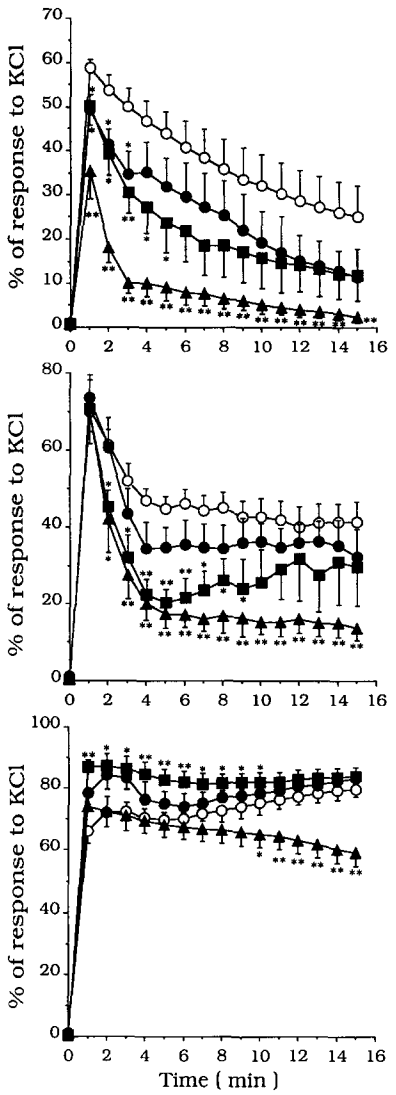

Fig. 2. Effect of niflumic acid on the time course of the contractions produced by [Sar^9]substance P sulfone, carbachol and KCl in the rabbit isolated proximal colon. Upper panel: time course of the contraction produced by [Sar^9]substance P sulfone (30 nM) in the absence (○) and after a 30-min incubation with niflumic acid 10 μM (●), 30 μM (■) and 100 μM (▲). Middle panel: time course of the contraction produced by carbachol (300 nM) in the absence (○) and after a 30-min incubation with niflumic acid 10 μM (●), 30 μM (■) and 100 μM (▲). Lower panel: time course of the contraction produced by KCl (40 mM) in the absence (○) and after a 30-min incubation with niflumic acid 10 μM (●), 30 μM (■) and 100 μM (▲). Each value in the figure is the mean ± S.E.M. of 4–12 experiments. Significantly different from the corresponding control response, * $P < 0.05$ and ** $P < 0.01$.

inhibited by 16 ± 4, 14 ± 3 and 40 ± 1% (n = 6–8) by niflumic acid at 10, 30 and 100 μM, respectively; the tonic component of contraction, measured at 15 min from bath application of the agonist, was inhibited by 55 ± 7, 53 ± 6 and 90 ± 1% (n = 6–8) by 10, 30 and 100 μM niflumic acid, respectively (Figs. 1 and 2). Niflumic acid, up to 100 μM, did not affect the peak phasic contraction to carbachol (300 nM; n = 8), but inhibited the sustained tonic contraction, producing 29 ± 9 (n = 5) and 67 ± 3% (n = 8) inhibition at 30 and 100 μM, respectively (Figs. 1 and 2).

Niflumic acid slightly increased the peak phasic response to KCl, especially at 30 μM (Fig. 2). The tonic response to KCl was slightly reduced (26 ± 4% inhibition; n = 8) by niflumic acid at 100 μM only (Figs. 1 and 2); this small effect was significantly less than the corresponding inhibitory effect observed toward the tonic contraction produced by [Sar^9]substance P sulfone or carbachol.

3.2. Effect of nifedipine and effect of niflumic acid on the nifedipine-resistant contractile responses to [Sar^9]substance P sulfone and carbachol in the rabbit isolated proximal colon

Nifedipine (1 μM; 45 min before) was tested against the contractile responses to single doses of [Sar^9]substance P sulfone (30 nM), carbachol (300 nM) and KCl (40 mM):

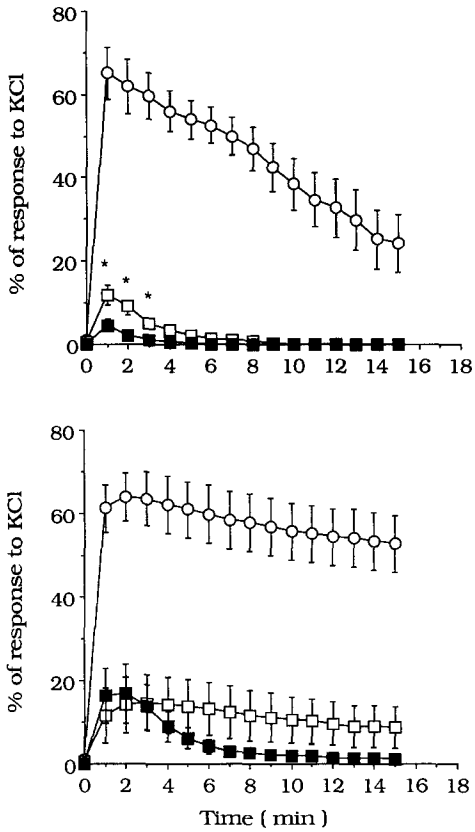

Fig. 3. Effect of niflumic acid on the nifedipine-resistant contractile response to [Sar^9]substance P sulfone and carbachol in the rabbit isolated proximal colon. Upper panel: time course of the contraction produced by [Sar^9]substance P sulfone (30 nM) in the absence (○) and after a 45-min incubation with nifedipine (1 μM) (□) and nifedipine plus niflumic acid (30 min before) 100 μM (■). Lower panel: time course of the contraction produced by carbachol (300 nM) in the absence (○) and after a 45-min incubation with nifedipine (1 μM) (□) and nifedipine plus niflumic acid (30 min before) 100 μM (■). Each value in the figure is the mean ± S.E.M. of 4–12 experiments. Significantly different from the corresponding response in the presence of nifedipine, * $P < 0.05$.

the effects of these agonists being followed over a 15-min observation period, as indicated above (Fig. 3). The contractile response to KCl was completely abolished by nifedipine ($n = 4$, not shown), indicating its total dependence upon the availability of L-type Ca^{2+} channels.

The [Sar^9]substance P sulfone-induced contraction was markedly reduced (82 ± 8% inhibition of the peak response; $n = 12$) by nifedipine and the residual response faded to baseline within 10 min from application of the agonist (Fig. 3). Likewise the carbachol-induced contraction was largely inhibited by nifedipine (76 ± 7% inhibition of peak response; $n = 12$), but in this case a small (about 15% of maximal response to the agonist in the absence of nifedipine) tonic-type sustained contraction persisted throughout the observation period (Fig. 3)

The co-incubation with niflumic acid (100 μM; 30 min before) and nifedipine (1 μM; 45 min before) produced a further reduction of the phasic response to [Sar^9]substance P sulfone, averaging 61 ± 4, 77 ± 4 and 78 ± 3% inhibition at 1, 2 and 3 min from agonist administration, respectively, as compared to the corresponding responses obtained in the presence of nifedipine alone ($P < 0.05$, $n = 8$). The peak of the nifedipine-resistant response to carbachol was unaffected by niflumic acid, although the tonic response was depressed as compared to that observed in the presence of nifedipine alone (Fig. 3); this latter inhibition, however, was not statistically significant.

3.3. Sucrose gap

In the presence of indomethacin (5 μM) and nifedipine (3 μM), superfusion with [Sar^9]substance P sulfone (0.3 μM for 1 min) produced a sustained depolarization (peak 7.13 ± 1 1 mV, $n = 5$; Fig. 4) of the membrane and an accompanying contraction (peak 3.09 ± 0.76 mN, $n = 5$; Fig. 4): both effects faded within 6–7 min from application of the agonist. Niflumic acid (100 μM for 30 min) produced a sustained hyperpolarization of the membrane (5.57 ± 1.24 mV; $n = 5$), which started after 2–3 min from application, and reached a maximum within 10–15 min. In the presence of niflumic acid, the peak depolarization produced by [Sar^9]substance P sulfone (0.3 μM) was not significantly affected (6.54 ± 1.01 mV, $n = 5$), but recovery to the resting membrane potential was significantly accelerated as compared to control (Fig. 4a). In keeping with the results of organ bath experiments performed in

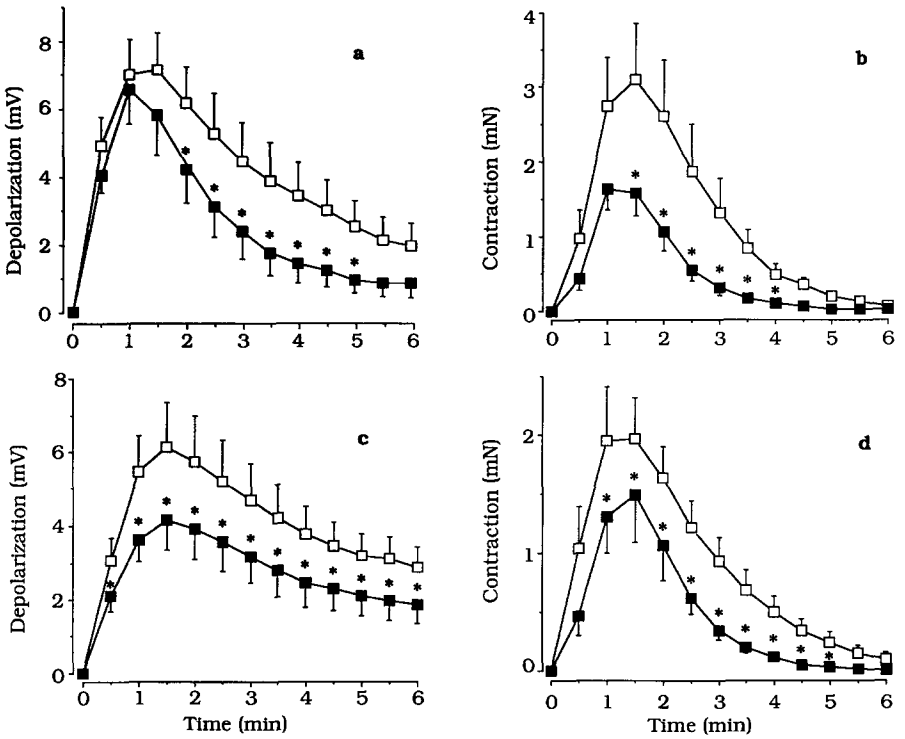

Fig. 4. Effect of niflumic acid on the nifedipine-resistant responses to [Sar^9]substance P sulfone in sucrose gap experiments with the rabbit isolated proximal colon. Upper panels: time course of the depolarization (a) and contraction (b) produced by [Sar^9]substance P sulfone (0.3 μM for 1 min) in the absence (□) and in the presence (■) of niflumic acid (100 μM for 30 min). These experiments were performed in the presence of nifedipine (3 μM). Lower panels: time course of the depolarization (c) and contraction (d) produced by [Sar^9]substance P sulfone (0.3 μM for 1 min) in the absence (□) and in the presence (■) of niflumic acid (100 μM for 30 min). These experiments were performed in the presence of nifedipine (3 μM) and tetraethylammonium (10 mM). Each value in the figure is the mean ± S.E.M. of 5–9 experiments. Significantly different from the corresponding control response, * $P < 0.05$.

the presence of nifedipine, niflumic acid significantly inhibited the contractile response to [Sar^9]substance P sulfone (Fig. 4b).

In a second series of experiments tetraethylammonium (10 mM) was added to the Krebs solution since the beginning of the experiments. In the presence of tetraethylammonium [Sar^9]substance P sulfone (0.3 μM) produced depolarization (6.13 $\pm$ 1.2 mV, $n = 6$, Fig. 4c) and contraction (1.97 $\pm$ 0.35 mN, Fig. 4d) with a time course similar to that observed in the absence of tetraethylammonium. In the presence of tetraethylammonium, niflumic acid (100 μM) did not affect the resting membrane potential, but significantly inhibited the depolarization and contraction induced by [Sar^9]substance P sulfone (Fig. 4c,d).

4. Discussion

In recent years there has been an increasing attention on the possible role played by Ca^{2+}-activated Cl^- channels in the regulation of smooth muscle cell excitability and contractility. These channels have been repeatedly demonstrated to be present in various types of smooth muscle cells (Byrne and Large, 1987a,b; Amedee et al., 1990; Janssen and Sims, 1992; Ohta et al., 1993); the Cl^- current can be activated by stimulation of membrane receptors, including α-adrenoceptors, muscarinic and tachykinin NK_1 receptors and by mobilization of Ca^{2+} from the internal store, e.g. following application of caffeine (Byrne and Large, 1987a,b; Hogg et al., 1994a; Sun et al., 1992, 1993). It has been proposed that the Ca^{2+}-activated Cl^- current would result in cell depolarization and, by recruiting voltage-sensitive Ca^{2+} channels, in Ca^{2+} influx and contraction via electromechanical coupling. The relevance of this mechanism for electromechanical coupling in smooth muscles can be evaluated quantitatively by studying the effect of blockers of the Ca^{2+}-activated Cl^- conductance on agonist-induced contractile responses. The longitudinal muscle of the rabbit proximal colon appears a suitable test object for this purpose, since the activation of a Ca^{2+}-activated Cl^- conductance in response to tachykinin NK_1 receptor agonists has been well characterized at this level in patch clamp studies (Sun et al., 1992, 1993).

Niflumic acid is one of the most potent blockers of Ca^{2+}-activated Cl^- conductance available thus far (White and Aylwin, 1990; Hogg et al., 1994b). To exclude the influence of cyclooxygenase blockade produced by niflumic acid, experiments were performed in the presence of indomethacin. The present results demonstrate that niflumic acid, in the concentration range of 10–100 μM exerts a depressant effect on the contractile response intiated by submaximally effective concentrations of [Sar^9]substance P sulfone or carbachol. For both agonists the tonic component of contraction was more sensitive to inhibition by niflumic acid, although in the case of the tachykinin NK_1 receptor agonist also the phasic response was inhibited at some extent.

In sharp contrast, the biphasic response to KCl was marginally affected by niflumic acid; this rules out that, in the range of concentrations tested, niflumic acid had exerted a nonspecific depressant effect on muscle contractility. Likewise, a direct Ca^{2+} channel inhibition by niflumic acid seems unlikely: in that case, the KCl-induced contraction should have been affected as well. These findings are reminiscent of the results presented by Criddle et al. (1995) showing that niflumic acid inhibits the noradrenaline-induced contraction of rat isolated aorta, without affecting the response to KCl. Criddle et al. (1995) found niflumic acid to be effective in rat aorta at lower concentrations (3–10 μM) than those found effective here in rabbit colon. On the other hand, niflumic acid blocks Cl^- conductance activated by tachykinin NK_3 receptor agonists in the guinea-pig myenteric neurons at concentrations similar to those used here (Bertrand and Galligan, 1994). This difference in potency would not argue in itself against an involvement of Ca^{2+}-activated Cl^- channels, because the potency of niflumic acid as Cl^- channel inhibitor is quite variable from one preparation to another, possibly reflecting heterogeneity of the channels involved (see Hogg et al., 1994b for discussion of this point).

The results of organ bath experiments are in keeping with the idea that a Cl^- channel might be involved in electromechanical coupling initiated by activation of the tachykinin NK_1 receptor in rabbit colon. To further address this point, sucrose gap experiments were designed to ascertain whether niflumic acid affects the depolarization induced by [Sar^9]substance P sulfone. As recruitment of nifedipine-sensitive Ca^{2+} channels is considered to follow the activation of Cl^- channels, these experiments were performed in the presence of nifedipine. The results indicate that niflumic acid effectively reduces, but not abolishes, the [Sar^9]substance P sulfone-induced depolarization. Niflumic acid also produced a sustained tetraethylammonium-sensitive hyperpolarization of the membrane, an effect likely ascribable to its reported action as a K^+ channel opener on large conductance Ca^{2+}-dependent K^+ channels (Ottolia and Toro, 1994; Teramoto and Brading, 1994). However, the inhibitory action of niflumic acid on depolarization induced by [Sar^9]substance P sulfone was evident also in the presence of tetraethylammonium: this dissociation indicates that niflumic acid, at the concentration tested, can affect multiple membrane conductances and calls for a word of caution in the use of this drug as a tool for studying the role of Cl^- channels in the excitation-contraction coupling in smooth muscle. An unexpected finding was the observation that the nifedipine-resistant contraction induced by carbachol or [Sar^9]substance P sulfone is inhibited by niflumic acid: at present we have no element to suggest whether this represents or not a specific effect of niflumic acid on Cl^- channels. It has to be noted that the peak of the nifedipine-resistant

contraction to carbachol was unaffected by niflumic acid, implying some degree of selectivity toward the response initiated by the tachykinin NK_1 receptor-selective agonist. This observation would also argue against a possible inhibitory effect of niflumic acid toward nonselective cation channels (Gogelein et al., 1990); neither such an effect could explain the preferential inhibitory effect of niflumic acid toward the tonic vs. phasic component of contractions induced by [Sar^9]substance P sulfone and carbachol.

In conclusion, the present findings are in keeping with the proposal that Cl^- channels may be involved in electromechanical coupling of smooth muscle of rabbit colon following stimulation of tachykinin NK_1 receptors (Sun et al., 1993), and suggest that this mechanism could be preferentially involved in maintaining more the tonic than the phasic contractile response to tachykinin NK_1 receptor agonists. However, the complex pharmacological profile of niflumic acid, and its relatively low potency in this preparation, requires the development of newer and more selective tools for a final assessment of the importance of Cl^- channels to the action of excitatory transmitters in the intestinal smooth muscle.

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