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Effect of sodium rhein on electrically-evoked and agonist-induced contractions of the guinea-pig isolated ileal circular muscle

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1 This study examined the effects of sodium rhein (0.03–30 μM) on the contractions of the isolated circular muscle of guinea-pig ileum induced by acetylcholine (100 nM), substance P (3 nM) and electrical stimulation (10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration). The effect of sodium rhein was also evaluated on the ascending excitatory reflex using a partitioned bath (oral and anal compartments). Ascending excitatory enteric nerve pathways were activated by electrical field stimulation (10 Hz for 2 s, 20 mA, 0.5 pulse duration) in the anal compartment and the resulting contraction of the guinea-pig intestinal circular muscle in the oral compartment was recorded.

2 Sodium rhein (0.3, 3 and 30 μM) significantly potentiated ($52\pm11\%$ at 30 μM) acetylcholine-induced contractions. In the presence of tetrodotoxin (0.6 μM) or ω-conotoxin GVIA (10 nM) sodium rhein (3 and 30 μM) did not enhance, but significantly reduced ($49\pm10\%$ and $44\pm8\%$, respectively, at 30 μM) acetylcholine-induced contractions.

3 Sodium rhein (0.3, 3 and 30 μM) significantly increased ($65\pm11\%$ at 30 μM) substance P-induced contractions. In the presence of tetrodotoxin (0.6 μM), ω-conotoxin GVIA (10 nM) or atropine (0.1 μM), sodium rhein (3 and 30 μM) significantly reduced ($50\pm10\%$, $55\pm8\%$ and $46\pm10\%$, respectively, at 30 μM) substance P-induced contractions.

4 N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) abolished the potentiating effect of sodium rhein on acetylcholine and substance P-induced contractions. At the highest concentration (30 μM), sodium rhein, in presence of L-NAME, reduced the acetylcholine ($30\pm6\%$)- or substance P ($36\pm6\%$)-induced contractions.

5 Sodium rhein (30 μM) significantly potentiated ($29\pm9\%$) the electrically-evoked contractions. L-NAME (100 μM), but not phentolamine, enhanced the effect of sodium rhein. Sodium rhein (30 μM) significantly increased ($32\pm9\%$) the ascending excitatory reflex when applied in the oral, but not in the anal compartment.

6 These results indicate that sodium rhein (i) activates excitatory cholinergic nerves on circular smooth muscle presumably through a facilitation of Ca^{2+} entry through the N-type Ca^{2+} channel, (ii) has a direct inhibitory effect on circular smooth muscle and (iii) does not affect enteric ascending neuroneural transmission. Nitric oxide could have a modulatory excitatory role on sodium rhein-induced changes of agonist-induced contractions and an inhibitory modulator role on sodium rhein-induced changes of electrically-induced contractions.

Keywords: Anthraquinone; laxative; senna; cholinergic nerves; enteric nervous system; intestinal motility; rhein; nitric oxide

Introduction

Senna preparations are obtained from the leaves and pods of *Cassia acutifolia* or *Cassia angustifolia* and are widely used to treat constipation. The major constituents are sennoside A and B that are catabolized by the microorganism in the colon to aglycone rhein (Capasso & Gaginella, 1997). These compounds act upon both the secretion and motility to cause laxation and these actions are largely independent of one another (Leng-Peschlow, 1992). Several studies have demonstrated, both *in vitro* and *in vivo*, the secretory effect of sennosides or their active ingredients, and various mechanisms of action have been proposed. These include inhibition of Na^+,K^+- ATPase (Wanitschke & Karbach, 1988), stimulation of prostaglandins (Beubler & Kollar, 1985), 5-hydroxytryptamine (Capasso *et al*., 1986; Beubler & Schirgi-Degen, 1993) and nitric oxide biosynthesis (Izzo *et al*., 1996; 1997b) and involvement of Ca^{2+} (Donowitz *et al*., 1984).

By contrast there is still some uncertainty as to the exact mode of action of sennosides and their active metabolites on intestinal motility. This is probably due to the difficulty to reproduce *in vitro* their stimulating effect on intestinal motility observed *in vivo* (Leng-Peschlow, 1992). Indeed previous studies *in vitro* have documented that rhein decreases intestinal contractility in the rat intestine (Odenthal & Ziegler, 1988), but recently it has been shown that increased intestinal peristalsis in the guinea-pig ileum (Nijs *et al*., 1993) and increased circular smooth muscle contractility in the rat intestine is produced by sennoside aglycones (Rumsey *et al*., 1993). However, it is still not clear whether sennosides derivatives act on smooth muscle, nerves or via activation of intrinsic reflexes.

The aim of the present study was to verify if the effect of rhein on circular smooth muscle was due to a direct action on smooth muscle or to an action at the neuromuscular junction or on neuroneural transmission. For this purpose, the effect of sodium rhein has been evaluated on (i) acetylcholine, substance P- and electrically-induced contractions on circular smooth muscle and on (ii) the ascending excitatory reflex produced by electrical stimulation, using a two compartment bath in which sodium rhein can be applied on enteric nerves without interfering with the recording of the smooth muscle contractility (Izzo *et al*., 1997a).

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Methods

Male guinea-pigs weighing between 300 and 350 g were killed by asphyxiation with CO_2. The ileum was removed, flushed of luminal contents and placed in organ baths containing warm (37°C), aerated (95%O_2 : 5% CO_2) Krebs solution (composition in mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose 11). The mechanical activity of the circular muscle was recorded isotonically (load 0.5 g) with a transducer connected to a 'Gemini' recording apparatus (Ugo Basile, Comerio, Italy). At the end of each experiment, circular muscle was stimulated with KCl 80 mM. This concentration of KCl produced a circular muscle contraction corresponding to total occlusion of the ileal lumen.

Experiments on circular smooth muscle contractility

Ileal rings (3–4 mm wide) were suspended in 10 ml organ baths by means of two stainless steel hooks and were connected to an isotonic transducer. After a 1 h equilibration period, acetylcholine (100 nM) or substance P (3 nM) was added to the bath and left in contact with the tissue for 30 and 60 s, respectively, and then washed out. In some experiments ileal rings were stimulated electrically (10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration) by a pair of electrodes placed around the ileal rings. Krebs was renewed after each electrical response. The conditions of electrical stimulation and the concentration of acetylcholine and substance P used were selected so that contractile responses were similar in amplitude. The interval between additions of the contractile agents or electrical stimulation was 10 min. Stable and reproducible contractions were obtained for a time period of two and half hours (15 contractions). After at least three stable control contractions (variation less than 5%), the responses were repeated in the presence of increasing (non cumulative) concentrations of sodium rhein (0.03–30 μM) added 10 min before the contracting stimuli (after washing the tissue). In some experiments tetrodotoxin (0.6 μM), atropine (0.1 μM), ω-conotoxin (10 nM), phentolamine (1 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) or N^G-nitro-D-arginine methyl ester (D-NAME 100 μM) were included in the Krebs solution. The effect of these drugs was also evaluated on agonist- and electrically-induced contractions (contact time: 20 min). These concentrations were selected on the basis of previous works (Rand & Li, 1993; Izzo *et al*., 1997a). In order to determine the time course of sodium rhein, in preliminary experiments electrically-induced contractions were repeated every 150 s.

Experiments on neuroneural transmission

Neuroneural transmission was studied using a partitioned bath, as previously described (Izzo *et al*., 1997a). In brief, the middle part of an ileal segment (40–60 mm) was cut open for 10–15 mm along the mesenteric border and pinned flat to strip off the mucosa and submucosa. A removable partition with a soft edge was pressed on the flat part of the preparation to divide the bath into an oral and anal compartment which contained the oral and the anal end of the intestinal segment, respectively. The enteric nerve pathways were activated by electrical field stimulation (10 Hz for 2 s, 20 mA, 0.5 ms pulse duration) via a pair of platinum electrodes placed around the intestine in the anal compartment, 30 mm from the partition. Contractions were obtained with stimulations every 10 min. Krebs was renewed after each contractile response. Stable and reproducible contractions were obtained for a time period of two and half hours. When at least three stable contractions had been obtained (variation less than 5%), sodium rhein (0.03–30 μM) was added to a single compartment in increasing (non cumulative) concentrations (contact time: 10 min for each concentration) in each preparation.

In order to test the seal between the two compartments, at the end of each experiment, atropine (10 μM) was applied to the anal bath while the circular muscle motorneurones were activated by electrical stimulation in the oral bath (10 Hz for 0.1 s, 45 mA, 0.5 ms pulse duration). The absence of inhibition by atropine applied to the anal compartment was taken as evidence of an effective seal between the two compartments.

Drugs

Drugs used were: acetylcholine chloride, atropine sulphate, substance P acetate, tetrodotoxin, phentolamine hydrochloride, N^G-nitro-L-arginine methyl ester hydrochloride, N^G-nitro-D-arginine methyl ester hydrochloride (Sigma, Milan, Italy), ω-conotoxin GVIA (RBI, Milan, Italy). Sodium rhein was prepared in our laboratory, by the action of sodium hydroxide (1 M) on rhein (Madaus, Koln). The drugs were dissolved in distilled water and added in a volume less than 0.1% of the bath volume.

Statistical analysis

Results are given as mean ± s.e.mean of n experiments (n indicates the number of tissues and coincides with the number of animals). Comparisons between two sets of data were made by Student's t test for paired data. For comparison of one control with several experimental groups, a one way analysis of variance was used followed by the modified t test according to Bonferroni. A P value less than 0.05 was regarded as significant.

Results

The contractile response of ileal circular muscle to electrical stimulation (10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration), acetylcholine (100 nM) or substance P (3 nM) was 39 ± 5% (n = 15), 38 ± 6% (n = 25) and 43 ± 5% (n = 35) of the contraction produced by 80 mM KCl respectively. In the same way electrical field stimulation (10 Hz for 2 s, 20 mA, 0.5 ms pulse duration) in the anal compartment produced a contraction of circular muscle recorded in the oral compartment which was 36 ± 5% (n = 10).

Sodium rhein (0.03–30 μM) usually did not elicit a response from the ileal circular muscle, but at the 30 μM concentration it produced small monophasic contractions only in 5 cases of 30 experiments (data not shown). Sodium rhein markedly increased (25–52%, $P < 0.05$) the amplitude of the contraction of ileal circular muscle to acetylcholine (Figures 1 and 2). The response was concentration-dependent for the concentrations ranging from 0.03 to 3 μM. However statistical significance was achieved for the 0.3–30 μM concentrations. With either tetrodotoxin (0.6 μM) or ω-conotoxin (10 nM) added to the Krebs solution, sodium rhein significantly inhibited the acetylcholine induced contractions in a concentration-dependent manner (Figure 2). Neither tetrodotoxin nor ω-conotoxin *per se* modified acetylcholine-induced contractions (less than 2% inhibition in each case).

Sodium rhein (0.03–30 μM) concentration-dependently also potentiated the substance P-induced contractions (15–65% increase, $P < 0.05$) (Figure 3). Tetrodotoxin (0.6 μM), ω-

conotoxin (10 nM), atropine (0.1 μM), or atropine plus tetrodotoxin, did not affect significantly ($P>0.2$) substance P-induced contractions ($3\pm4\%$ inhibition $n=5$, $1\pm5\%$ inhibition $P>0.2$ $n=5$, $2\pm4\%$ inhibition $n=5$ and $5\pm4\%$ inhibition $n=5$) but, added to the Krebs solution, changed the potentiating effect of sodium rhein to a significant ($P<0.05$, for 3 and 30 μM) and concentration-related inhibitory effect (Figure 3).

L-NAME (100 μM) (but not D-NAME 100 μM), added to Krebs solution, abolished the potentiating effect of sodium rhein on acetylcholine- and substance P-induced contractions (Figures 4 and 5). In the presence of L-NAME, sodium rhein, at the highest concentration tested (30 μM) significantly ($P<0.05$) inhibited the acetylcholine ($30\pm6\%$ inhibition)- and the substance P ($36\pm10\%$ inhibition)-induced contractions (Figures 4 and 5). When L-NAME was tested in the presence of atropine, it did not modify the effect of sodium rhein on substance P-induced contractions (data not shown).

Sodium rhein significantly ($P<0.05$) potentiated ($29\pm9\%$, $n=5$) the electrically-induced contractions only at the highest concentration (30 μM) tested (Figure 6). The potentiating effect of sodium rhein on electrically-induced contractions was further on augmented by L-NAME (100 μM), while D-NAME (100 μM) was ineffective (Figure 6). L-NAME *per se* increased the electrically-induced contractions ($30\pm5\%$ ($n=5$), $P<0.05$) without modifying acetylcholine (1 ± 3 inhibiton, ($n=5$), $P>0.2$)- or substance P ($2\pm5\%$ increase, ($n=5$), $P>0.2$)-induced contractions. Figure 7 shows the time course of the potentiating effect of sodium rhein (30 μM) on electrically-induced contractions and indicates that the maximal effect was reached after a contact time of 10 min.

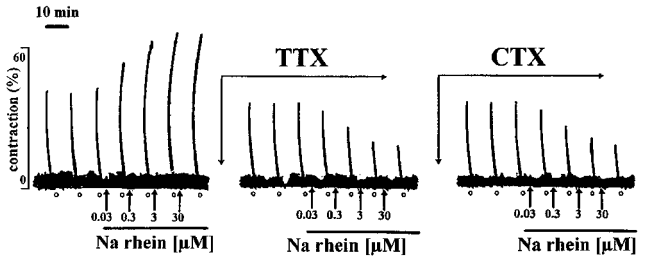

Figure 1 Traces showing the effect of sodium rhein on acetylcholine (100 nM)-induced contractions on isolated circular muscle alone or in presence of tetrodotoxin (TTX, 0.6 μM) or ω-conotoxin (CTX, 10 nM). Acetylcholine was left in contact with the tissue for 30 s and then washed out. Sodium rhein was added in single increasing concentrations after washing the tissue and left in contact for 10 min. The responses are shown expressed as a percentage of the contraction to 80 mM KCl.

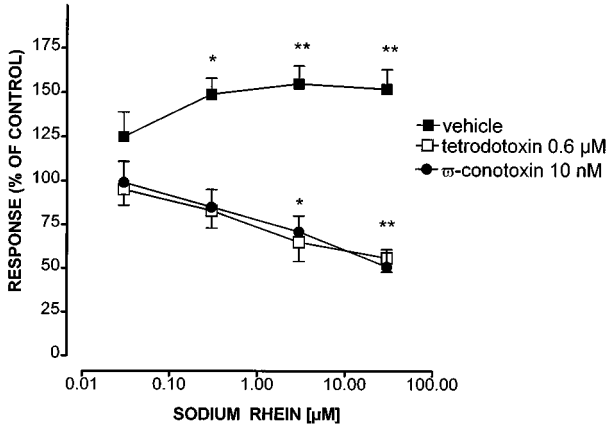

Figure 2 Effect of increasing concentration of sodium rhein (0.03–30 μM) on acetylcholine (100 nM)-induced contractions on isolated circular muscle alone (vehicle) or in presence of tetrodotoxin 0.6 μM or ω-conotoxin GVIA 10 nM. The ordinates show the percentage of control response. Each point represents the mean of 5 experiments; vertical lines show s.e.mean. $*P<0.05$ and $**P<0.01$ *vs* control.

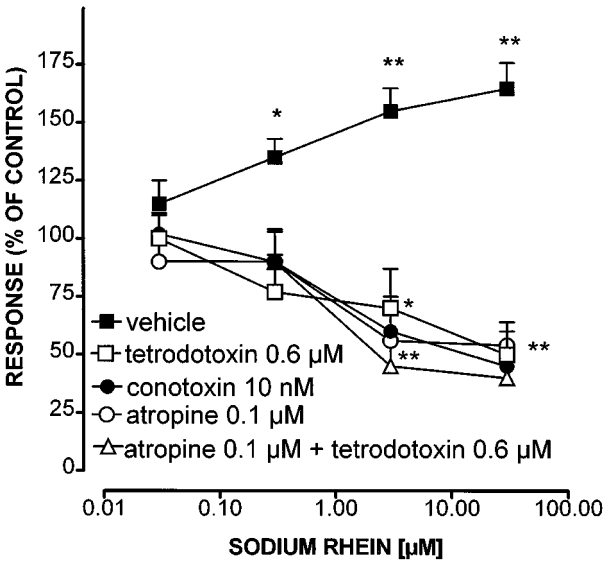

Figure 3 Effect of increasing concentrations of sodium rhein (0.03–30 μM) on substance P (3 nM)-induced contractions of isolated circular muscle alone (vehicle) or in presence of tetrodotoxin 0.6 μM, ω-conotoxin GVIA 10 nM, atropine 0.1 μM or atropine 0.1 μM plus tetrodotoxin 0.6 μM. The ordinates show the percentage of control response. Each point represents the mean of 5 experiments; vertical lines show s.e.mean. $*P<0.05$ and $**P<0.01$ *vs* control.

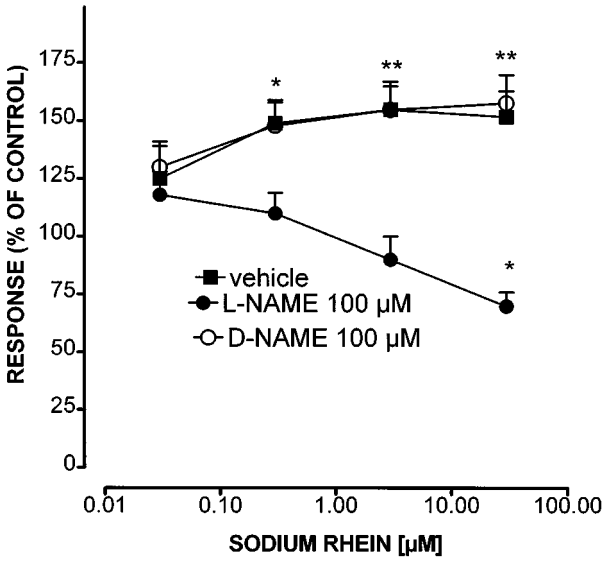

Figure 4 Effect of increasing concentrations of sodium rhein (0.03–30 μM) on acetylcholine (100 nM)-induced contractions of isolated circular muscle alone (vehicle) or in presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) or N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM). The ordinates show the percentage of control response. Each point represents the mean of 5 experiments; vertical lines show s.e.mean. $*P<0.05$ and $**P<0.01$ *vs* control.

Phentolamine (1 μM) did not modify the effect of sodium rhein (30 μM) on electrically-induced contractions (% increase of sodium rhein: 29 ± 9, $n=5$; % increse of sodium rhein in presence of phentolamine: 30 ± 5, $n=5$) and on acetylcholine-induced contractions (data not shown). Furthermore, acetylcholine and electrically-induced contractions were not affected by phentolamine (data not shown).

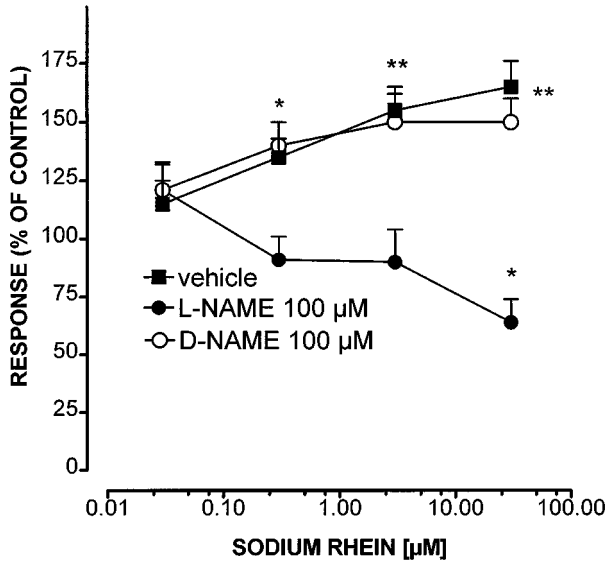

Figure 5 Effect of increasing concentrations of sodium rhein (0.03–30 μM) on substance P (3 nM)-induced contractions of isolated circular muscle alone (vehicle) or in presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) or N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM). The ordinates show the percentage of control response. Each point represents the mean of 5 experiments; vertical lines show s.e.mean. $*P<0.05$ and $**P<0.01$ *vs* control.

Results showing the effect of sodium rhein on the ascending excitatory reflex produced by electrical stimulation are shown in Figure 8. Sodium rhein significantly ($P<0.05$) potentiated (32 ± 9% increase) the nerve mediated-contraction only when applied to the oral compartment and at the highest concentration tested (30 μM).

Discussion

The importance of circular muscle for peristaltic activity of the intestine is well established (Kosterlitz & Lees, 1964); when fluid is infused continuously into the lumen, the intestine gradually distends until the circular muscle at the oral end of the intestine contracts and a wave of contraction propagates aborally along the intestine. It is also well known that rhein, an

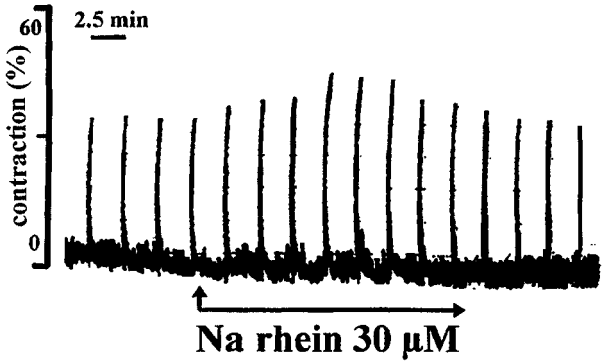

Figure 7 Trace showing the time course of the potentiating effect of sodium rhein (30 μM) on electrically-induced contractions on isolated circular muscle. Sodium rhein was left in contact with the tissue for 30 min. The responses are shown as a percentage of the contraction induced by 80 mM KCl.

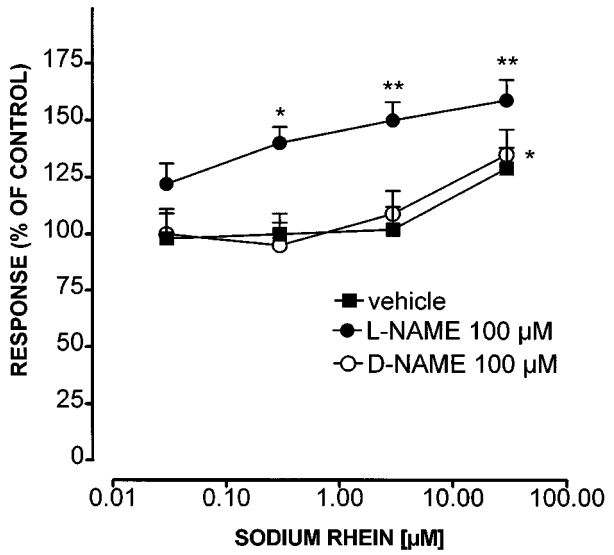

Figure 6 Effect of increasing concentration of sodium rhein (0.03–30 μM) on electrically-induced contractions of isolated circular muscle alone (vehicle) or in presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) or N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM). The ordinates show the percentage of control response. Each point represents the mean of 5 experiments; vertical lines show s.e.mean. $*P<0.05$ and $**P<0.01$ *vs* control.

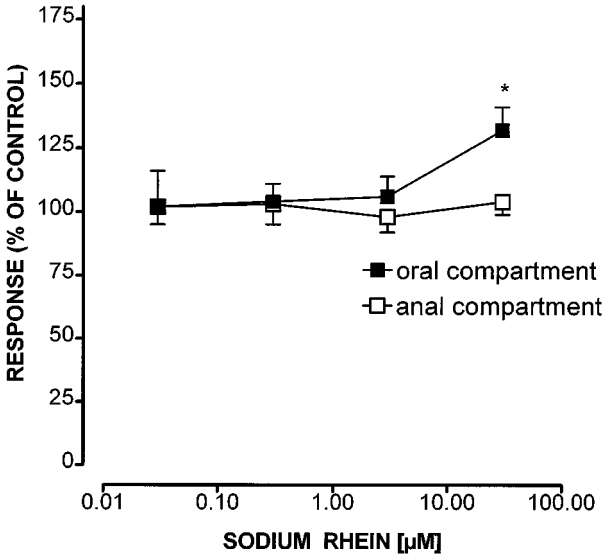

Figure 8 Effect of increasing concentration of sodium rhein (0.03–30 μM) added either to the oral or the anal compartment, on the ascending excitatory response elicited by electrical stimulation of enteric pathways. The electrodes were placed on the intestine in the anal compartment about 30 mm from the partition. Each point represents the mean and vertical lines s.e.mean of 5 experiments. $*P<0.05$ *vs* control.

active metabolic product derived from microbial fermentation of the natural occurring sennosides, induces motor changes in the colon resulting in a faster passage of faeces (Capasso & Gaginella, 1997). However, only recently it has been demonstrated *in vitro* that sennosides derivatives increase circular muscle contractility (Rumsey *et al*., 1993) and peristalsis (Nijs *et al*., 1993), but it is not clear if this effect is due to a direct action on circular muscle, to activation of circular muscle motorneurons or to a multisynaptic pathways involving nicotinic synapses.

Our data show that sodium rhein is able to potentiate the contractions produced by acetylcholine and substance P on guinea-pig isolated circular muscle. This effect seems to be mediated by enteric neurones as sodium rhein, in the presence of tetrodotoxin, inhibited acetylcholine- and substance P-induced contractions. These data indicate that, while sodium rhein activates excitatory neurotransmission, it has a direct inhibitory effect on circular smooth muscle. However sodium rhein significantly affected enteric neurotransmission at 0.3 μM, while a higher concentration (3 μM) was required to achieve a direct inhibitory significant effect on smooth muscle. In addition tetrodotoxin and ω-conotoxin *per se* did not modify acetylcholine- and substance P-induced contractions, indicating that the contractile effect of both agonists results from a direct action on the smooth muscle.

The circular muscle of the guinea-pig small intestine contains a large number of mononeurons which are immunoreactive for choline acetyltransferase, the enzyme which mediates the synthesis of acetylcholine (Steele *et al*., 1991) and there is ample evidence that acetylcholine is the primary excitatory transmitter to the circular muscle (Burks, 1994). In the circular muscle preparation used in our experiments, transmission along the enteric ganglia could be ruled out, as hexamethonium did not modify the electrically-induced contractions (Izzo A.A. unpublished results). We have shown that the main excitatory neurotransmitter responsible for the potentiating effect of sodium rhein is acetylcholine because sodium rhein, in the presence of atropine, inhibits the substance P-induced contractions. In addition, the effect of atropine and tetrodotoxin were not additive, thus indicating a neural origin of acetylcholine.

Acetylcholine is stored in cholinergic neurones within presynaptic or prejunctional vesicles (Burks, 1994). In response to invasion of the cholinergic nerve terminal by a depolarizing nerve action potential, Ca^{2+} enters the nerves, largely through the voltage-dependent N channels, to activate docking proteins which cause fusion of vesicle membranes and then exocytosis into the synaptic or junctional space (Burks, 1994). It is most likely that sodium rhein produces the release of acetylcholine by acting on these channels, as the potent N-type Ca^{2+} channel blocker ω-conotoxin (McCleskey *et al*., 1987), like atropine or tetrodotoxin, changes the potentiating effect of sodium rhein to an inhibitory action on smooth muscle. This is the first evidence indicating that N-type Ca^{2+} channels modulate the pharmacological effect of anthraquinones, although it has been shown that nifedipine, a blocker of L-type Ca^{2+} channels, potentiates the antidiarrhoeal activity of indomethacin on rhein-induced diarrhoea (Yamauchi *et al*., 1993). It is unlikely that sodium rhein affects action potentials at the axonal terminals as sodium rhein does not inhibit electrically-induced contractions.

Sodium rhein also potentiates electrically-induced contractions in isolated ileal rings. However, rhein is more effective in potentiating acetylcholine-induced contractions than the electrically-induced contractions. These results could indicate that sodium rhein releases from neural or non neural sources endogenous substances which have an inhibitory effect on electrically-induced contractions. However, it is unlikely that this effect is related to the release of noradrenaline from sympathetic nerves acting on α-adrenoceptors, as phentolamine did not modify the effect of sodium rhein on electrically-induced contractions.

NO is now recognized as perhaps the major mediator of relaxation induced by enteric inhibitory neurones (Boecxkstaens *et al*., 1991; Brookes, 1993). NO synthase-like immunoreactivity or NADPH diaphorase-like immunoreactivity (which has been identified as being identical to NO synthase) is expressed by myenteric plexus motoneurones projecting to the circular muscle of the guinea-pig ileum (Costa *et al*., 1992; Llewellyn-Smith *et al*., 1992) from which NO is released after nerve stimulation (Wiklund *et al*., 1993). Constitutive NO synthase activity is stimulated *in vivo* by senna or cascara (Izzo *et al*., 1997b). In the present study, we have shown that the NO synthase inhibitor L-NAME (Rees *et al*., 1990) potentiated electrically (but not acetylcholine or substance P)-induced contractions, indicating that endogenous NO modulates neuromuscular transmission in our experimental system. In addition L-NAME augmented the potentiating effect of sodium rhein on electrically-induced contractions, while D-NAME was without effect. These data indicate that NO could have an inhibitory modulatory role on sodium rhein-induced changes in electrically-induced circular muscle contractility. The inhibitory effect of NO is likely to be due to a direct inhibitory action of NO on intestinal muscle, but may in addition be related to the ability of NO to reduce field-stimulated acetylcholine release from enteric nerves (Knudsen & Tottrup, 1992; Hryhorenko *et al*., 1994). Previously we have shown that L-NAME reduces senna- and cascara-induced fluid secretion in the rat ligated colon *in vivo* (Izzo *et al*., 1996); thus NO mediates not only fluid secretion, but also motility changes induced by anthraquinones. However, although we have confirmed that acetylcholine is released from nerve terminals, the source of NO is unclear because previous studies have demonstrated that NO may be released from nerve terminals (Costa *et al*., 1992), epithelial cells (Tepperman *et al*., 1993), smooth muscle cells (Grider *et al*., 1992) and endothelial cells (Palmer *et al*., 1988).

In contrast to electrically-induced contractions, L-NAME inhibits the potentiating effect of sodium rhein on acetylcholine- or substance P-induced contractions. When L-NAME was tested after atropine, it did not modify the effect of sodium rhein. Thus, it is possible that sodium rhein releases NO which stimulates cholinergic transmission. Indeed NO induces contractions of the guinea-pig intestine which are abolished by tetrodotoxin or atropine (Barthò & Lefebvre, 1994a). In addition NO produces contraction in the rat isolated small intestine (Barthò & Lefebvre, 1994b) and in the opossum oesophagus (Yamato *et al*., 1992). It has been also shown that NO activates enteric neurones in basal conditions (Barthò & Lefebvre, 1994a) but inhibits them when they are activated (Kilbinger & Wolf, 1994). This dual action could explain the excitatory and inhibitory effects of NO on peristalsis, as recently described by Holzer *et al*. (1997) in the guinea-pig intestine. It is noteworthy that NO is proabsorbtive under basal conditions (Miller *et al*., 1993; Schirgi-Degen & Beubler, 1995), while it is secretagogue in pathophysiological states (Miller *et al*., 1993; Izzo *et al*., 1994a) or when secretion is stimulated by carbachol, a cholinergic agonist (Izzo *et al*., 1994b).

Rhein affects intestinal peristalsis in the guinea-pig isolated ileum (Nijs *et al*., 1993). Peristalsis involves two polarized

reflexes, the ascending contraction and descending relaxation, which both move aborally and thus propel the intraluminal content (Furness & Costa, 1987). The neural circuitry underlying these reflex responses lies entirely within the enteric nervous system (Furness & Costa, 1987). We have recently developed a method in which nerve pathways are stimulated electrically to activate synaptically excitatory motoneurones to the circular muscle, employing a two compartment bath in which drugs could be applied to the enteric nerve pathways without interfering with the recording of the smooth muscle contraction (Izzo *et al*., 1997a). This pathway involves a chain of orally directed interneurones in which nicotinic synapses are located in both compartments (Izzo *et al*., 1997a). With this experimental approach it has been possible to evaluate the effect of sodium rhein on neuroneural transmission without interfering with neuromuscular transmission or the recording of smooth muscle contractions. In the present work we have shown that sodium rhein did not inhibit the nerve-mediated contractions when applied to the anal compartment. This indicates that sodium rhein does not affect ascending neuroneural transmission and thus does not modify intestinal circular muscle contractility via activation of enteric reflexes. This is consistent with the results obtained by Frieling *et al*. (1993), who showed that the secretory effect of rhein did not involve multisynaptic pathways involving nicotinic synapses. The potentiating effect of sodium rhein when added to the oral compartment probably reflects the stimulating activities on cholinergic motoneurones previously discussed.

In conclusion, our results indicate that sodium rhein has a direct inhibitory effect on circular smooth muscle, while is able to activate excitatory cholinergic neurones on circular smooth muscle presumably through a facilitation of Ca^{2+} entry through the N-type Ca^{2+} channels. However sodium rhein does not affect ascending neuroneural transmission. Sodium rhein-induced changes in intestinal contractility are also modulated by NO, which has an excitatory action under basal conditions while it has inhibitory effect when cholinergic nerves are activated by electrical stimulation.

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References

BARTHO', L. & LEFEBVRE, R.A. (1994a). Nitric oxide induces acetylcholine-mediated contractions in the guinea-pig small intestine. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **350,** 582–584.

BARTHO', L. & LEFEBVRE, R.A. (1994b). Nitric oxide causes contraction in the rat isolated small intestine. *Eur. J. Pharmacol.,* **259,** 101–104.

BEUBLER, E. & KOLLAR, G. (1985). Stimulation of PGE_2 synthesis and water and electrolyte secretion by senna anthraquinones is inhibited by indomethacin. *J. Pharm. Pharmacol.,* **37,** 248–251.

BEUBLER, E. & SCHIRGI-DEGEN, A. (1993). Serotonin antagonists inhibit sennoside-induced fluid secretion and diarrhea. *Pharmacology,* **47** (suppl. 1), 64–68.

BOECKXSTAENS, G.E., PELCKMANS, P.A., BULT, H., DE MAN, J.G., HERMAN, A.G. & VAN MAERCKE, Y.M. (1991). Evidence for nitric oxide as mediator of non-adrenergic, non-cholinergic relaxations induced by ATP and GABA in the canine gut. *Br. J. Pharmacol.,* **102,** 434–438.

BROOKES, S.J.H. (1993). Neuronal nitric oxide in the gut. *J. Gastroenterol. Hepatol.,* **8,** 590–603.

BURKS, T.F. (1994). Neurotransmission and neurotransmitters. In *Physiology of the Gastrointestinal Tract*. ed Johnson, L.R. pp 211–242. New York: Raven Press

CAPASSO, F. & GAGINELLA, F. (1997). *Laxatives. A Practical Guide*. Milan: Springer Verlag Italia.

CAPASSO, F., MASCOLO, N., AUTORE, G., ROMANO, V. (1986). Laxatives and the production of autacoids by rat colon. *J. Pharm. Pharmacol.,* **38,** 627–629.

COSTA, M., FURNESS, J.B., POMPOLO, S., BROOKS, S.J.H., BORNSTEIN, J.C., BREDT, D.S. & SNYDER, S.H. (1992). Projections and chemical coding of neurons with immunoreactivity for nitric oxide synthase in the guinea-pig small intestine. *Neurosci. Lett.,* **148,** 121–125.

DONOWITZ, M., WICKS, J., BATTISTI, L., PIKE, G. & DE ELLIS, R. (1984). Effect of senokot on rat intestinal electrolyte transport. Evidence of Ca^{2+}-dependence. *Gastroenterology,* **87,** 503–512.

FRIELING, T., RUPPRECHT, C. & SCHEMANN, M. (1993). Rhein stimulates electrogenic chloride secretion by activation of submucosal neurons in guinea pig colon. *Pharmacology,* **47** (suppl. 1), 70–76.

FURNESS, J.B. & COSTA, M. (1987). *The Enteric Nervous System*. Edinburgh: Churchill Livingstone.

GRIDER, J.R., MURTHY, K.S., JIN, J.G. & MAKHLOUF, G.M. (1992). Stimulation of nitric oxide from muscle cells by VIP: prejunctional enhancement of VIP release. *Am. J. Physiol.,* **262,** G774–G778.

HOLZER, P., LIPPE, I.TH., LOTFI TABRIZI, L., LENARD JR, L. & BARTHO', L. (1997). Dual excitatory and inhibitory effect of nitric oxide on peristalsis in the guinea pig intestine. *J. Pharmacol. Exp. Ther.,* **280,** 154–161.

HRYHORENKO, L.M., WOSKOWSKA, Z., FOX-THRELKELD, J.-A.E.T. (1994). Nitric oxide (NO) inhibits release of acetylcholine from nerves of isolated circular muscle of the canine ileum: Relationship to motility and release of nitric oxide. *J. Pharmacol. Exp. Ther.,* **271,** 918–926.

IZZO, A.A., GAGINELLA, T.S., MASCOLO, N., BORRELLI, F. & CAPASSO, F. (1996). N^G-nitro-L-arginine methyl ester reduces senna- and cascara-induced diarrhoea and fluid secretion. *Eur. J. Pharmacol.,* **301,** 137–142.

IZZO, A.A., GAGINELLA, T.S., MASCOLO, N. & CAPASSO, F. (1994a). Nitric oxide as a mediator of the laxative action of magnesium sulphate. *Br. J. Pharmacol.,* **113,** 228–232.

IZZO, A.A., MASCOLO, N., COSTA, M. & CAPASSO, F. (1997a). Effect of papaverine on synaptic transmission in the guinea-pig ileum. *Br. J. Pharmacol.,* **121,** 768–772.

IZZO, A.A., MASCOLO, N., DI CARLO, G. & CAPASSO, F. (1994b). N^G-nitro-L-arginine methyl ester modulates intestinal secretion and motility produced by carbachol. *Eur. J. Pharmacol.,* **271,** 31–35.

IZZO, A.A., SAUTEBIN, L., ROMBOLA', L. & CAPASSO, F. (1997b). The role of constitutive and inducible nitric oxide synthase in senna- and cascara-induced diarrhoea in the rat. *Eur. J. Pharmacol.,* **353,** 93–97.

KILBINGER, H. & WOLF, D. (1994). Increase by NO synthase inhibitors of acetylcholine release from guinea-pig myenteric plexus. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **349,** 543–545.

KNUDSEN, M.A. & TOTTRUP, A. (1992). A possible role of the L-arginine-nitric oxide pathway in the modulation of cholinergic transmission in the guinea-pig taenia coli. *Br. J. Pharmacol.,* **107,** 837–841.

KOSTERLITZ, H.W. & LEES, G.M. (1964). Pharmacological analysis of intrinsic intestinal reflexes. *Pharmacol. Rev.,* **16,** 301–339.

LENG PESCHLOW, E. (1992). Senna and its rational use. *Pharmacology,* **44,** S1–S92.

LLEWELLYN-SMITH, I.J., SONG, Z.M., COSTA, M., BREDT, D.S. & SNYDER, S.H. (1992). Ultrastructural localization of nitric oxide synthase immunoreactivity in guinea-pig enteric neurons. *Brain Res.,* **577,** 337–342.

MCCLESKEY, E.W., FOX, A.P., FELDMAN, D.H., CRUZ, L.J., OLIVERA, B.M., TSIEN, R.W. & YOSHIKAMI, D. (1987). ω-Conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc. Natl. Acad. Sci. U.S.A.,* **84,** 4327–4331.

MILLER, M.J.S., SADOWSKA-KROWICKA, S., CHOTINUARUEMOL, S., KAKKIS, J.L. & CLARK, D.A. (1993). Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmacol. Exp. Ther.,* **294,** 11–16.

NIJS, G., DE WITTE, P., GEBOES, K., MEULEMANS, A., SCHUURKES, J. & LEMLI, J. (1993). Influence of rhein anthrone on peristaltic reflex of guinea-pig isolated ileum: involvement of prostaglandins. *Br. J. Pharmacol.,* **108,** 269–273.

ODENTHAL, K. & ZIEGLER, D. (1988). In vitro effects of anthraquinones in rat intestine and uterus. *Pharmacology,* **36** (suppl. 1), 57–65.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature,* **333,** 664–668.

RAND, M.J. & LI, C.G. (1993). Modulation of acetylcholine-induced contractions of the rat anococcygeus muscle by activation of nitrergic nerves. *Br. J. Pharmacol.,* **110,** 1479–1482.

REES, D., PALMER, R.M.J., SCHULTZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vivo and in vitro. *Br. J. Pharmacol.,* **101,** 746–752.

RUMSEY, R.D.E., SQUIRES, P.E. & READ, N.W. (1993). In vitro effects of sennoside on contractile activity and fluid flow in the perfused large intestine of the rat. *Pharmacology,* **47** (suppl. 1), 32–39.

SCHIRGI-DEGEN, A. & BEUBLER, E. (1995). Significance of nitric oxide in the stimulation of intestinal fluid absorption in the rat jejunum *in vivo*. *Br. J. Pharmacol.,* **114,** 13–18.

STEELE, P.A., BROOKES, S.J.H. & COSTA, M. (1991). Immunohistochemical identification of cholinergic neurons in the myenteric plexus of guinea-pig small intestine. *Neuroscience,* **45,** 227–239.

TEPPERMAN, B.L., BROWN, J.F. & WHILLE, B.J.R. (1993). Nitric oxide synthase induction and intestinal epithelial cell viability in rats. *Am. J. Physiol.,* **265,** G214–G218.

WANITSCHKE, R. & KARBACH, U. (1988). Influence of rhein on rat colonic Na + ,K + -ATPase and permeability in vitro. *Pharmacology,* **36** (suppl. 1), 98–103.

WIKLUND, C.U., LEONE, A.M., GUSTAFSSON, L.E. & MONCANDA, S. (1993). Release of nitric oxide evoked by nerve stimulation in guinea-pig intestine. *Neuroscience,* **53,** 607–611.

YAMATO, S., SPECHLER, S.J. & GOYAL, R.K. (1992). Role of nitric oxide in esophageal peristalsis in the opossum. *Gastroenterology,* **103,** 197–204.

YAMAUCHI, K., YAGI, T. & KUWANO, S. (1993). Suppression of the purgative action of rhein anthrone, the active metabolite of sennosides A and B, by calcium channel blockers, calmodulin antagonists and indomethacin. *Pharmacology,* **47** (suppl. 1), 22–31.

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