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Effect of nepadutant at tachykinin NK_2 receptors in human intestine and urinary bladder

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Abstract

We have characterized the action of the tachykinin NK_2 receptor antagonist nepadutant (c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β–5β)}) in the human isolated ileum, colon and urinary bladder. Nepadutant (30–1000 nM) competitively antagonized neurokinin A- or [βAla^8]neurokinin A-(4–10)-induced contractions in all tissues, with $pK_B = 8.3$ (ileum and colon) and $pK_B = 8.5$ (bladder). In contrast, the nonpeptide tachykinin NK_2 receptor antagonist SR 48968 (or (*S*)-*N*-methyl-*N* [4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl] benzamide) (30–1000 nM) produced insurmountable antagonism in all preparations. The tachykinin NK_2 receptor blockade produced by nepadutant in the colon was fully reversed by washout, whereas that produced by SR 48968 was not. Nepadutant (1 μM) greatly reduced (by 70–80%) the nonadrenergic noncholinergic (NANC) contractile off-response evoked by electrical field stimulation in the human ileum, and almost abolished it in the presence of the tachykinin NK_1 receptor antagonist GR 82334 (or: [[(*S*,*S*) Pro-Leu (spiro-γ-lactam)]^{9,10},Trp^{11}]Physalaemin (1–11)) (1 μM). The present results show that nepadutant is a potent, competitive and reversible antagonist at human tachykinin NK_2 receptors and provide further evidence that tachykinins act as excitatory NANC neurotransmitters in the human small intestine.

Keywords: Nepadutant; Tachykinin receptor; Tachykinin receptor antagonist; Intestine, human; Urinary bladder, human

1. Introduction

The tachykinins, substance P, neurokinin A and neurokinin B, are a family of neuropeptides distributed in the mammalian central and peripheral nervous system. Tachykinins produce a wide range of biological effects through the stimulation of at least three distinct receptor types, termed NK_1, NK_2 and NK_3 (Regoli et al., 1989; Guard and Watson, 1991; Maggi et al., 1993b). In the smooth muscle of the respiratory, gastrointestinal and genitourinary tract tachykinins almost invariably produce contraction, either if applied exogenously, or released from intrinsic neurons and/or from peripheral endings of capsaicin-sensitive primary afferents (Maggi, 1995a, for review). Each one of the three tachykinin receptors may be involved in mediating contractions produced by tachykinins, but the relative contribution of each receptor type varies greatly with both the tissue and the species considered (Maggi et al., 1993b, for review). In humans, the tachykinin receptors mediating smooth muscle contraction belong, for the most part, to the NK_2 type (Maggi et al., 1993b). Thus, selective antagonists of the tachykinin NK_2 receptor are regarded as possible candidates for counteracting exaggerated smooth muscle motility present in various pathological conditions in which endogenous tachykinins are thought to play a role (e.g. asthma/bronchial hyperreactivity, irritable bowel syndrome and cystitis) (Maggi et al., 1993b; Holzer and Holzer-Petsche, 1997; Holzer, 1998; Von Sprecher et al., 1998 for review). We have recently introduced a new potent tachykinin NK_2 receptor-selective antagonist bearing a glycosylated bicyclic peptide structure (nepadutant or MEN 11420; Catalioto et al., 1998). Nepadutant, which is characterized by improved bioavailability and metabolic stability (Lippi et

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al., 1998) compared to its lipophilic parent compound MEN 10627 (Maggi et al., 1994a), has been shown to block tachykinin NK_2 receptor-mediated responses in various isolated tissues from mouse, rat, guinea pig, hamster and rabbit species, and to bind with nanomolar affinity the human tachykinin NK_2 receptor stably transfected into Chinese hamster ovary (CHO) cells (Catalioto et al., 1998; Renzetti et al., 1998). However, neither potency, nor mode of interaction (competitive/noncompetitive) of nepadutant at the naïve human tachykinin NK_2 receptor has been established yet.

Therefore, the present work was undertaken to characterize the profile of action of nepadutant at the tachykinin NK_2 receptor present in the human isolated ileum and colon circular muscles, and human isolated urinary bladder detrusor muscle, as compared to the firstly introduced nonpeptide tachykinin NK_2 receptor antagonist, SR 48968 (Emonds-Alt et al., 1992). Furthermore, nepadutant was assayed for the ability to counteract tachykinin NK_2 receptor-mediated responses produced by endogenous tachykinins. For this purpose, it was used the human ileum, in which nonadrenergic noncholinergic (NANC) contractile responses elicited by electrical stimulation are putatively produced by release of endogenous tachykinins from intrinsic enteric neurons (Maggi et al., 1992; Zagorodnyuk et al., 1997). In addition to nepadutant, GR 82334 (tachykinin NK_1 receptor-selective antagonist; Hagan et al., 1991) was also used in the above experiments, to reveal a possible contribution of tachykinin NK_1 receptors in mediating NANC responses of the human ileum to electrical field stimulation.

A preliminary report of this study has been published recently (Cucchi et al., 1998).

2. Materials and methods

2.1. General

All the experiments were performed on the human isolated ileum, colon and urinary bladder. Mucosa-free circular muscle strips from human ileum were excised from 12 patients (six males and six females), age 62–80 years, undergoing radical cystectomy for invasive bladder cancer, followed by substitution of the urinary bladder with an artificial bladder obtained from a small intestine loop. The experiments with the human colon were performed on mucosa-free circular muscle strips, excised from 14 patients (five males and nine females), age 31–83 years, undergoing colectomy for carcinoma of the colon. Mucosa-free strips of detrusor muscle were excised from the urinary bladder dome of 10 patients (eight males and two females), age 60–80 years, undergoing cystectomy because of carcinoma of the bladder base. No patient received radio- or chemotherapy before intervention. In all patients, pre-anesthetic medication was intramuscular atropine (1 mg) and diazepam (10 mg). Anesthesia was induced by sodium thiopental (500 mg i.v.) and maintained with N_2O/O_2 (1/2) and halothane (0.6–1%). The patients received pancuronium bromide (6 mg i.v.) during induction of anesthesia.

All specimens appeared macroscopically normal without signs of tumor or inflammation. All muscle strips were prepared immediately after surgical removal of the organ and kept at 4°C overnight in ice-cold gassed (96% O_2 and 4% CO_2) Krebs–Henseleit solution of 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. The next day, 15–20 h after excision, the strips were placed in 5-ml organ baths filled with oxygenated Krebs–Henseleit solution at 37°C, under a resting tension of 10 mN. Mechanical activity developed by preparations was recorded isotonically (human ileum and human colon) or isometrically (human urinary bladder).

The experiments commenced after a 90–120 min equilibration period, and after having obtained three to four reproducible responses to carbachol (10 μM; human ileum and human colon) or to KCl (80 mM; human urinary bladder). After having constructed a cumulative concentration–response curve to neurokinin A (human urinary bladder) or to the tachykinin NK_2 receptor-selective agonist [βAla^8]neurokinin A-(4–10) (human ileum and human colon), a stated concentration of antagonist was added to the bath, and the curve repeated 15 min later. At least 60 min were allowed to elapse between the first and the second concentration–response curve to the agonists. All the experiments performed on human ileum and human colon were in the presence of the tachykinin NK_1 receptor-selective antagonist SR 140333 (0.1 μM; Emonds-Alt et al., 1993b), added 15 min before each concentration–response curve to the agonist. SR 140333 was used to block tachykinin receptors of the NK_1 type, whose presence has been reported in the above preparations (see Discussion). At least three concentrations of each antagonist were tested using tissues excised from at least three different patients. The reversibility of human tachykinin NK_2 receptor blockade produced by the antagonists under study was evaluated in the human colon as follows: [βAla^8]neurokinin A-(4–10) (0.1 μM) was administered to the preparations at 30-min intervals, until reproducible contractile responses were obtained (generally two to three administrations were sufficient). At this time, nepadutant or SR 48968 or vehicle were added to the bath solution, 15 min before the next challenge with the agonist. The preparations were then thoroughly washed with Krebs–Henseleit solution, which was renewed every 5 min. Administration of the agonist was repeated 30, 60, 90 and 120 min after washout of the antagonist, and the responses were compared to those obtained in control time-matched preparations, which had received the vehicle. The reversibility experiments were performed in the

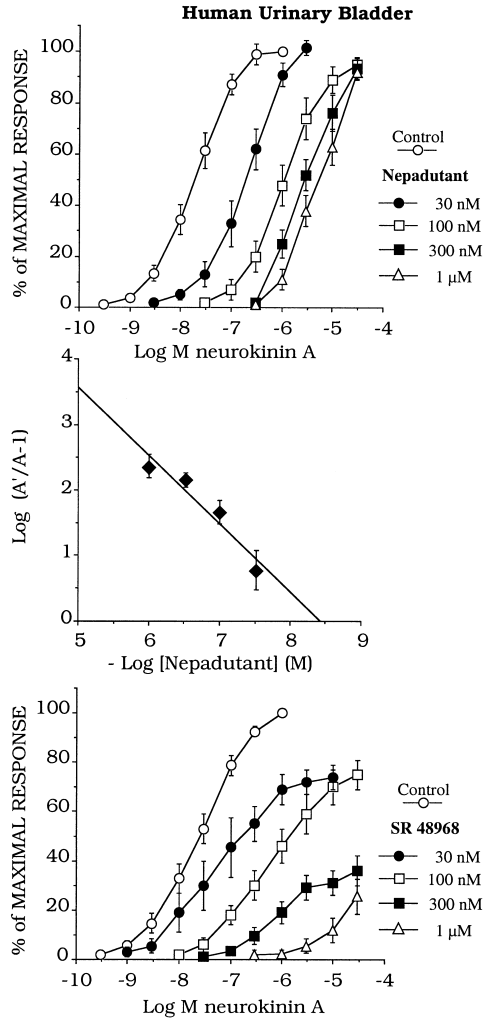

Fig. 1. Blockade by nepadutant and SR 48968 of tachykinin NK_2 receptor-mediated contractions in the human isolated urinary bladder. Upper panel: log concentration–response curves for neurokinin A in the absence and in the presence of nepadutant. Middle panel: corresponding Schild plot of agonist dose-ratios vs. nepadutant concentrations (slope = −1.02; 95% c.l. = −1.5; −0.5). Lower panel: log concentration–response curves for neurokinin A in the absence and in the presence of SR 48968. Each point is mean of three to four experiments. Vertical lines show S.E.M.

absence of an NK_1 antagonist, because we obtained evidence that the response to [βAla^8]NKA(4–10) at the concentration employed (0.1 μM) is unaffected by blockade of NK_1 receptors.

In other experiments, NANC responses to electrical field stimulation were evoked in the human ileum, in the presence of atropine (1 μM) and guanethidine (3 μM) from the beginning. The preparations were exposed to electrical field stimulation (trains of stimuli of 50 Hz, 0.25 ms pulse width, supramaximal voltage, for 20 s given every 30 min) by means of two platinum wire electrodes placed at the top and the bottom of the organ bath, and connected to a Grass S88 stimulator. The antagonists under study were assayed for the ability to reduce the neurogenic contraction developing immediately after completion of the electrical stimuli (OFF response). To this purpose, either the peak contraction or the area underlying the first 60 s of the OFF response was calculated.

2.2. Evaluation of data

Agonist activity was expressed as pD_2 ($-\log EC_{50}$). Antagonist affinity was expressed as pK_B, when ''Schild plot'' analysis (Arunlakshana and Schild, 1959) showed no significant departure from unity slope. In this case, pK_B values were estimated as the mean of the individual values

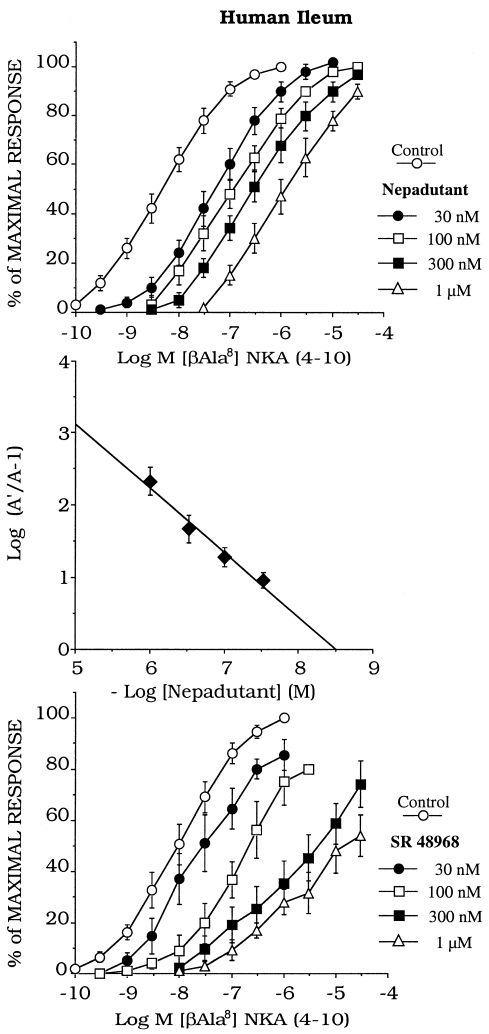

Fig. 2. Blockade by nepadutant and SR 48968 of tachykinin NK_2 receptor-mediated contractions in the human isolated ileum. Upper panel: log concentration–response curves for [βAla^8]neurokinin A-(4–10) in the absence and in the presence of nepadutant. Middle panel: corresponding Schild plot of agonist dose-ratios vs. nepadutant concentrations (slope = −0.89; 95% c.l. = −1.2; −0.5). Lower panel: log concentration–response curves for [βAla^8]neurokinin A-(4–10) in the absence and in the presence of SR 48968. All the experiments were performed in the presence of the tachykinin NK_1 receptor-selective antagonist SR 140333 (0.1 μM). Each point is mean of three to four experiments. Vertical lines show S.E.M.

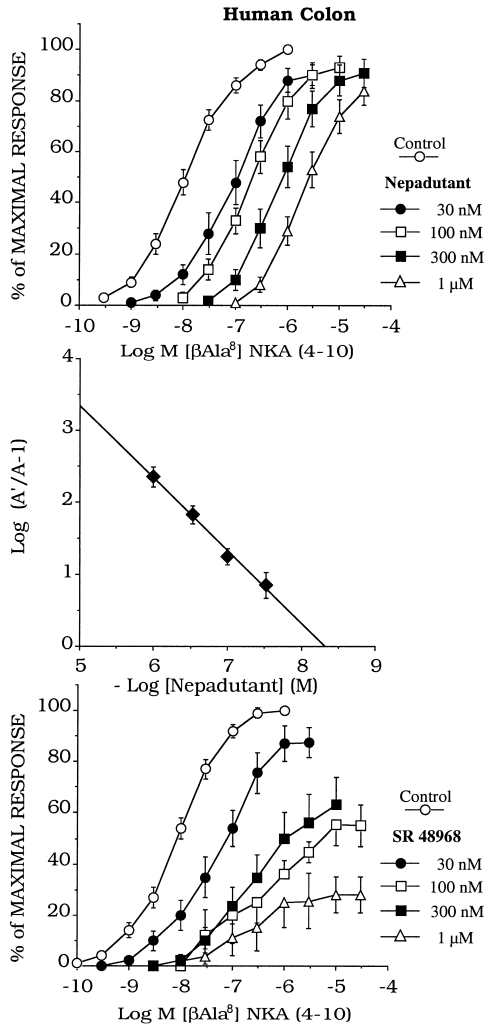

Fig. 3. Blockade by nepadutant and SR 48968 of tachykinin NK_2 receptor-mediated contractions in the human isolated colon. Upper panel: log concentration–response curves for [βAla^8]neurokinin A-(4–10) in the absence and in the presence of nepadutant. Middle panel: corresponding Schild plot of agonist dose-ratios vs. nepadutant concentrations (slope = −1.00; 95% c.l. = −1.3; −0.7). Lower panel: log concentration–response curves for [βAla^8]neurokinin A-(4–10) in the absence and in the presence of SR 48968. All the experiments were performed in the presence of the tachykinin NK_1 receptor-selective antagonist SR 140333 (0.1 μM). Each point is mean of three to four experiments. Vertical lines show S.E.M.

obtained with the equation (Kenakin, 1997; Jenkinson, 1991):

$$pK_B = \log[\text{dose ratio} - 1] - \log[\text{antagonist concentration}]$$

The antagonist potency of SR 48968 under the present experimental conditions was tentatively estimated as the ratio between two agonist concentrations (A′/A) producing 50% of the control maximal response, in the presence (A′) and in the absence (A) of the antagonist, respectively. Motor responses evoked in the human ileum by electrical field stimulation were digitized and stored on a Power MacIntosh PC by means of a Mac Lab/8e hardware device (AD Instruments, Castle Hill, Australia), and analysed by using Mac Lab Chart v. 3.6.1 software.

2.3. Statistical analysis

The values in the text, tables, or figures are expressed as mean ± 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 linear the segment between 20% and 80% of the maximal response.

2.4. Drugs

Nepadutant (MEN 11420) (or: c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β–5β)}) and [βAla^8]neurokinin A-(4–10) were synthesized at Menarini laboratories, Florence, Italy, by conventional solid-phase methods. Neurokinin A was purchased from Peninsula Laboratories (St. Helens, England), atropine from Serva (Heidelberg, Germany), tetrodotoxin from Sankyo (Japan), GR 82334 (or: [[(*S*,*S*) Pro-Leu (spiro-γ-lactam)]^{9,10},Trp^{11}]Physalaemin (1–11)) from Neosystem (Strasbourg, France), guanethidine from ICFI (Milan, Italy), N^{ω}-nitro-L-arginine (L-NOARG) and carbachol were from Sigma (St. Louis, USA). The nonpeptide antagonists SR 140333 [(*S*) 1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-n-3yl]ethyl}-4-phenyl-1-azoniabi-cyclo [2,2,2] octane chloride] and SR 48968, or (*S*)-*N*-methyl-*N*[4-acetyl-amino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl] benzamide, were kindly provided by Drs. X. Emonds-Alt and G. Le Fur, Sanofi (Montpellier, France).

3. Results

3.1. Effect of nepadutant and SR 48968 against tachykinin NK_2 receptor agonists in the human tissues

Both neurokinin A (in the human urinary bladder) and [βAla^8]neurokinin A-(4–10) (in the human ileum and

Table 1
Antagonist activity of nepadutant, MEN 10627 and SR 48968 at tachykinin NK_2 receptors in the human isolated urinary bladder, human isolated ileum and human isolated colon

Antagonist	Human urinary bladder	Human ileum	Human colon
Nepadutant	8.5[a] (8.3–8.8)	8.3[a] (8.1–8.5)	8.3[a] (8.1–8.5)
MEN 10627[c]	8.5[a] (8.3–8.7)	8.4[a] (8.3–8.5)	8.8[a] (8.6–9.0)
SR 48968	57 ± 10[b]	23 ± 5[b]	109 ± 15[b]

The values are (a) mean pK_B (antagonist dissociation constant) with 95% confidence limits (in brackets), or (b) mean ratio ± S.E.M. of two agonist concentrations (A′/A) producing 50% of the control maximum, in the absence and in the presence of SR 48968 (100 nM; 15 min before; $n = 4$ each), respectively. (c) Data are from Giuliani et al., 1996 and Patacchini et al., 1997.

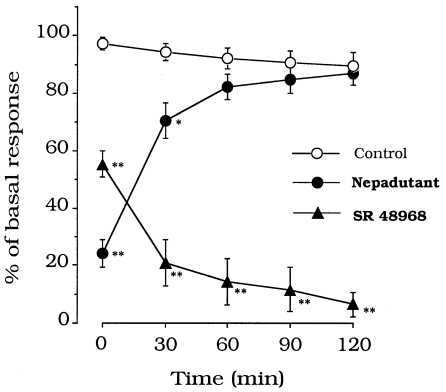

Fig. 4. Reversibility of tachykinin NK_2 receptor blockade induced by nepadutant and SR 48968 in the human isolated colon. The values represent contractile responses to single doses of [βAla^8]neurokinin A-(4–10) (0.1 μM) obtained in the absence (control) or in the presence (time = 0) of nepadutant (0.3 μM; 15 min before) or of SR 48968 (0.3 μM; 15 min before), and responses obtained after 30, 60, 90 and 120 min from washout of the antagonists. Each point is mean of five to six experiments. Vertical lines show S.E.M. * Significantly different from the corresponding control response: $P < 0.05$, and ** $P < 0.01$.

human colon) elicited reproducible contractile responses, whose estimated pD_2 values were: 7.6 ± 0.04 ($n = 20$); 8.0 ± 0.08 ($n = 22$) and 8.0 ± 0.04 ($n = 25$), respectively (Fig. 1–3). Neither nepadutant, nor SR 48968, within the concentration range they were employed, elicited any contractile response in any of the tissues examined. Nepadutant (30 nM–1 μM) potently inhibited both neurokinin A and [βAla^8]neurokinin A-(4–10)-induced responses in all tissues, producing parallel rightward shifts of the agonist curves without depressing the Emax (Figs. 1–3). The competitivity of nepadutant-induced blockade of the tachykinin NK_2 receptor in the human tissues was confirmed by the slopes of the Schild plots, which were not significantly different from −1 (Figs. 1–3). The estimated affinities (pK_B values) of nepadutant for the human tachykinin NK_2 receptor ranged from 8.3 to 8.5 (Table 1).

In contrast SR 48968, assayed in the same concentration range (30 nM–1 μM) as nepadutant, produced concentration-dependent insurmountable antagonism toward neurokinin A-mediated contractions in the human urinary bladder and toward [βAla^8]neurokinin A-(4–10) in the human ileum and human colon (Figs. 1–3). At the highest concentration (1 μM), SR 48968 depressed the maximal agonist responses by $75 \pm 7\%$, $46 \pm 8\%$ and $72 \pm 9\%$ ($n = 4$ each) in the human urinary bladder, human ileum and human colon, respectively. A quantitative estimate of the potency of SR 48968 under the present experimental conditions (15-min incubation period) is reported in Table 1. For comparison, Table 1 also shows the affinities calculated for MEN 10627, the bicyclic peptide compound from which nepadutant has been derived.

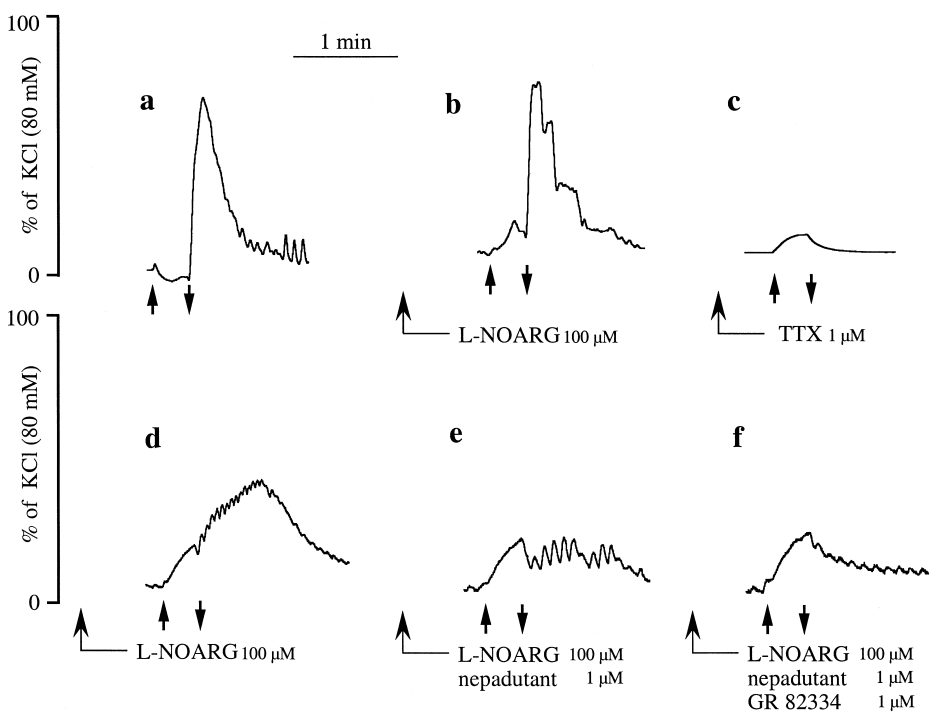

Fig. 5. Typical tracings showing the motor responses evoked by electrical field stimulation (applied at arrows) (trains of stimuli of 50 Hz, 0.25 ms pulse width, supramaximal voltage, for 20 s, given every 30 min) in the human isolated ileum in the presence of atropine (1 μM) and guanethidine (3 μM). (a) Control response to electrical field stimulation. (b and d) Effect of pretreatment with N^{ω}-nitro-L-arginine (L-NOARG; 100 μM). (c) Effect of tetrodotoxin (TTX; 1 μM). (e) Effect of nepadutant (1 μM) and (f) nepadutant in combination with GR 82334 (1 μM) on electrical field stimulation-evoked response of L-NOARG-pretreated strips.

3.2. Reversibility of tachykinin NK_2 receptor blockade produced by nepadutant and SR 48968 in the human colon

The mechanism underlying the insurmountable antagonism produced by SR 48968 was investigated by evaluating the reversibility of receptor blockade induced by the former antagonist in the human colon, as compared to the reversibility of nepadutant-induced effect. Reversibility of human tachykinin NK_2 receptor blockade was considered as the capacity of the colonic circular smooth muscle to recover the contractile response produced by a single concentration (0.1 μM) of [βAla^8]neurokinin A-(4–10), after a 15-min contact period with one of the two antagonists (see Materials and methods). Nepadutant (300 nM) inhibited [βAla^8]neurokinin A-(4–10)-induced response by 78 ± 5% ($n = 6$), whereas SR 48968 (300 nM) by 45 ± 4% only ($n = 6$; $P < 0.05$ vs. nepadutant-induced effect) (Fig. 4, time = 0). Nevertheless, while the inhibition exerted by nepadutant was fully and quickly reversed by washout (full recovery of agonist response being reached at 60 min from removal of the antagonist), the inhibition produced by SR 48968 was increased by time, despite the antagonist was no longer present in the bath solution (Fig. 4). At 120 min from removal of SR 48968, inhibition of agonist response was practically complete (94 ± 4%; $n = 6$). In control experiments, neither SR 48968, nor nepadutant (300 nM each, 15 min before) affected the contractile response to carbachol (10 μM; 99 ± 4% and 102 ± 5%, $n = 4$ each, of the control responses obtained in the absence of the antagonists, respectively).

3.3. Effect of nepadutant on electrical field stimulation-evoked NANC contractile response in the human ileum

Although in principle it cannot be ruled out that the ''cold storage'' of the tissues the night before the experiments (see Materials and methods) had produced a somewhat reduction of the neurogenic response to electrical field stimulation, it was possible to evoke cholinergic excitatory (not shown) and NANC excitatory/inhibitory response (see below) in all specimens examined. Thus, application of electrical field stimulation to human ileum preparations in the presence of atropine and guanethidine (1 and 3 μM, respectively) evoked a quite variable motor response (ON response), ranging from small relaxation to small contraction (either not exceeding ±10% of KCl-induced maximal contraction), that was followed by a phasic contractile response developing immediately after the end of the electrical field stimulation (OFF response) (Fig. 5a). Pretreatment with the inhibitor of NO synthase, L-NOARG (100 μM; 30 min before) shifted the ON response to a contraction, while leaving apparently unchanged the OFF response (Fig. 5b). Tetrodotoxin (1 μM; 15 min before) completely abolished the OFF response, whereas a small myogenic contraction (15 ± 4% of maximal response to KCl; $n = 8$) developed during application of electrical field stimulation (Fig. 5c). In L-NOARG-pretreated preparations, the peak of the OFF response averaged 53 ± 7% of KCl (80 mM). Nepadutant (1 μM; 15 min before) and the tachykinin NK_1-receptor selective antagonist GR 82334 (1 μM; 15 min before) were assayed for the ability to reduce the electrical field stimulation-evoked OFF response. To this regard, either the peak contraction or the area underlying the first 60 s of the OFF response was considered. Nepadutant greatly reduced both peak and area of electrical field stimulation-evoked OFF contraction (Fig. 5d–f), whereas GR 82334 failed to affect either of the two components (Fig. 6). However, the contemporary administration of both nepadutant and GR 82334 produced a higher inhibition of the OFF peak contraction than nepadu-

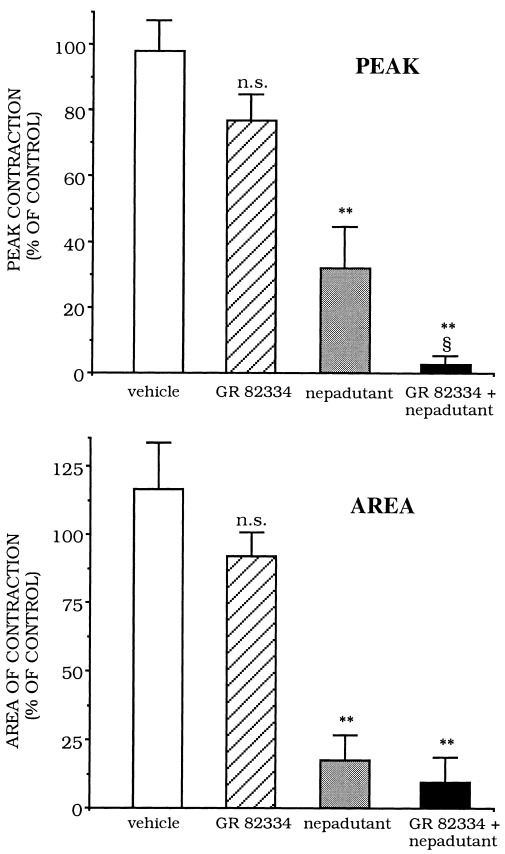

Fig. 6. Blockade by nepadutant and GR 82334 of electrical field stimulation-induced rebound contractile response in the human isolated ileum. The panels show the effects of vehicle, GR 82334, nepadutant (1 μM; 15 min before electrical field stimulation each) and GR 82334 plus nepadutant on the rebound contraction developing at the end of electrical field stimulation (trains of 50 Hz, 0.5 ms pulse width, supramaximal voltage for 20 s, given every 30 min). Both peak contraction and area under the curve were evaluated for 1 min after completion of the electrical field stimulation. All the experiments were performed in the presence of atropine (1 μM), guanethidine (3 μM) and L-NOARg (100 μM). Control contractile responses were obtained before administering drugs or vehicle. Data are mean ± S.E.M. of four to six experiments. Pretreatments are shown below the columns. ** Significantly different from the corresponding mean value obtained in vehicle-pretreated preparations; $P < 0.01$. § Significantly different from the mean value obtained in preparations pretreated with nepadutant alone; $P < 0.05$.

tant alone (Fig. 6). Thus, in the presence of both antagonists the electrical field stimulation-elicited OFF contraction of the human ileum was almost completely prevented (Fig. 6).

4. Discussion

The three human smooth muscle preparations used in the present study respond to tachykinins with a contraction that is mediated by activation of specific receptors of the NK_2 type (Maggi et al., 1988, 1990; Giuliani et al., 1991, 1993; Zeng et al., 1995). Previous studies have shown that also receptors of the NK_1 type are involved in the response to tachykinins in the human ileum (Maggi et al., 1990), whereas no evidence has been so far collected that tachykinin receptors different from the NK_2 are functionally involved in tachykinin-induced contractions of the human colon and human urinary bladder (Maggi et al., 1988, Giuliani et al., 1991, 1993; Zeng et al., 1995; Croci et al., 1998). Nevertheless, a recent radioligand binding study performed on cell membranes obtained from the circular muscle of human colon (Warner et al., 1999) has revealed the presence of a small population of tachykinin NK_1 receptors, besides tachykinin NK_2 receptors, whose functional role remains to be elucidated. Thus, to rule out any involvement of tachykinin NK_1 receptors in the human ileum and human colon we have used the tachykinin NK_1 receptor-selective antagonist SR 140333 (Emonds-Alt et al., 1993b), and the tachykinin NK_2 receptor-selective agonist [βAla^8]neurokinin A-(4–10), over the natural but less selective agonist neurokinin A.

Our results clearly show that nepadutant behaves as a competitive and reversible antagonist at the naive human tachykinin NK_2 receptor, with a potency ($pK_B = 8.3–8.5$) superimposable to the affinity measured at human tachykinin NK_2 receptors stably transfected into CHO cells ($pK_D = 8.6$; Catalioto et al., 1998; Renzetti et al., 1998). The present results obtained with nepadutant extend our previous data obtained with MEN 10627 and other bicyclic peptide antagonists in human tissues (Giuliani et al., 1996; Patacchini et al., 1997). Altogether, our data show that the bicyclic moiety present in the structure of nepadutant, MEN 10627 and in the other derivatives so far examined (Giuliani et al., 1996; Patacchini et al., 1997) provides them with nanomolar affinity for the tachykinin NK_2 receptor. To this regard, it is noteworthy that nepadutant maintains the same high affinity for the human tachykinin NK_2 receptor than the lipophilic compound from which it has been derived: MEN 10627. Compared to this latter compound, nepadutant bears a glycosylated residue [(2-acetylamino-2-desoxy-β-D-glucopyranosyl)-L-asparagine] in the place of Met, which increases its hydrophilicity (about 80 fold as compared to MEN 10627) and in vivo bioavailability (Catalioto et al., 1998). Thus, nepadutant shows higher potency and longer duration of action in vivo, than MEN 10627 (Catalioto et al., 1998; Tramontana et al., 1998), possibly due to a greater metabolic stability (Lippi et al., 1998).

Our present results also show that nepadutant is able to counteract the excitatory motor response produced by endogenous tachykinins in the human ileum. Altogether, the data collected in the experiments of electrical stimulation of the human ileum provide functional evidence that: (1) tachykinins are (most) important NANC excitatory transmitters in the human small intestine and (2) tachykinin NK_2 receptors are the main mediators of their effects. As stated in an our previous study with the human ileum (Zagorodnyuk et al., 1997), the present investigation has revealed a lower though significant contribution of tachykinin NK_1 receptors in mediating the overall contractile OFF response to electrical field stimulation. To this regard, the observation that tachykinin NK_1 receptors participate in determining the peak contraction that develops quickly after completion of the electrical field stimulation, but apparently are irrelevant for the late component of contraction (measured as a 1-min area) suggests that tachykinin NK_1 and NK_2 receptors of the human intestine could be ''specialized'' in mediating ''fast'' and ''slow'' neurotransmission, respectively, as proven to occur in the circular muscle of the guinea pig colon (Maggi et al., 1994c). Furthermore, the data arising from the experiments of electrical field stimulation of the human ileum also show that: (3) besides NO, other inhibitory neurotransmitter(s) are probably generated during the electrical field stimulation, as suggested by the occurrence of a rebound contraction after completion of the electrical stimulus. This hypothesis is supported by the observation that apamin- and L-NOARG-resistant inhibitory junction potentials could be evoked in the human ileum in sucrose-gap experiments (Zagorodnyuk et al., 1997). (4) Besides tachykinins other excitatory neurotransmitter(s) might be generated by electrical field stimulation in the human ileum, to produce the (small) contractile response obtained in the presence of nepadutant plus GR 82334 (present results). It is worth mentioning, however that we did not aim at investigating the nature of other putative NANC neurotransmitters in the human ileum.

In contrast with the results obtained with nepadutant, the nonpeptide tachykinin NK_2 receptor antagonist SR 48968 (Emonds-Alt et al., 1992) behaves as an essentially irreversible antagonist in all human tissues examined. It is noteworthy that contradictory results have been so far obtained with SR 48968 in functional experiments performed in different species, including humans. Thus, SR 48968 has been reported to produce either competitive (Emonds-Alt et al., 1992; Advenier et al., 1992; Maggi et al., 1993a; Zeng et al., 1995) or noncompetitive/insurmountable (Huber et al., 1993; Patacchini et al., 1994; Croci et al., 1998) antagonism at tachykinin NK_2 receptors, even in different tissues from the same species (Maggi et al.,1994b; Croci et al., 1995). However, there is a

general agreement that the effects produced by SR 48968 are slowly reversible/irreversible in vitro (e.g. Advenier et al., 1992; Maggi et al., 1993a; Patacchini et al., 1994) and long-lasting in vivo (e.g. Emonds-Alt et al., 1993a; Maggi et al., 1993a). Thus, we think that the observed insurmountable antagonism at tachykinin NK_2 receptors by SR 48968 can be explained by its slowly reversible binding to the receptor. On the other hand, we think that the reported competitive behaviour of SR 48968 in other tissues can be explained by one (or more) of the followings: (1) presence of a high tachykinin NK_2 receptor reserve supplying a number of unblocked receptors sufficient to achieve a maximal agonist response, (2) additional presence of unblocked tachykinin NK_1 receptors that are as well stimulated by neurokinin A, or [βAla^8]neurokinin A-(4–10) at high concentrations, and may contribute to attainment of the maximal response (see: Patacchini et al., 1994, for discussion of this point). Moreover, the possibility that the reported species-dependent heterogeneity of tachykinin NK_2 receptors (Maggi, 1995b, for review) may influence the mechanism(s) of interaction with SR 48968, cannot be ruled out. Thus, if this latter were a determining factor, the apparent competitivity of SR 48968 observed in the rabbit and hamster (Maggi et al., 1993a) might be explainable by, e.g. a faster reversibility from tachykinin NK_2 receptors belonging to these species than to other species.

In conclusion, the present data along with our previous results obtained in binding, in vivo and metabolic studies further show that nepadutant is a very promising tool to explore the role of the tachykinin NK_2 receptor in human pathophysiology.

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