Nepadutant Pharmacokinetics and Dose-Effect Relationships as Tachykinin NK₂ Receptor Antagonist Are Altered by Intestinal Inflammation in Rodent Models

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ABSTRACT

Tachykinin NK₂ receptor antagonists could reduce motility and symptoms during gastrointestinal diseases characterized by local inflammation such as diarrhea or colitis; however, if these conditions change pharmacodynamic and pharmacokinetic characteristics of NK₂ receptor antagonists is unknown. We investigated the effect of the peptide NK₂ receptor antagonist nepadutant on spontaneous intestinal motility or [βAla₈]NKA(4-10)-induced colonic and bladder contractions in rodent models of intestinal inflammation (enteritis induced by castor oil and rectocolitis induced by bacterial toxins in mice). In the castor oil model, the oral/intraduodenal bioavailability of nepadutant was also determined. The intrarectal (i.r.) administration of nepadutant (30 nmol/kg) did not reduce [βAla₈]NKA(4-10) (10 nmol/kg i.v.)-induced colonic and bladder contractions in normal animals, but the same dose of nepadutant produced an inhibitory effect in the two organs following rectocolitis; in contrast, nepadutant is equieffective by the intravenous route in normal and colitic animals. In this model, nepadutant (100 nmol/kg i.r. or i.v.) decreased spontaneous colonic hypermotility, without affecting motility in controls. The intraduodenal administration of nepadutant (30 nmol/kg), which was ineffective on [βAla₈]NKA(4-10) (10 nmol/kg i.v.)-induced colonic and bladder contractions in control animals, abolished bladder contractions in castor oil-pretreated animals. In this latter group, the oral and intraduodenal bioavailability of nepadutant showed a 7- to 9-fold increase with respect to controls. Oral administration of nepadutant, in nanomolar or subnanomolar dosage, reduced diarrhea induced by bacterial toxins in mice. It is concluded that intestinal inflammation increases nepadutant absorption in the intestine, enhancing its activity. These results suggest that a drug with a limited oral bioavailability could be used for treating gastrointestinal diseases associated with a local inflammation.

Tachykinins (TKs) are a family of neuropeptides that include Substance P, neurokinin A (NKA), neurokinin B, and two elongated forms of NKA: neuropeptide-Y, and neuropeptide-α. TKs share a common C-terminal sequence Phe-Xaa-Gly-Leu-MetNH₂ that confers affinity and activity at their receptors termed NK₁, NK₂, and NK₃. Substance P, NKA, and neurokinin B possess the highest affinity for NK₁, NK₂, and NK₃ receptors, respectively; however, the selectivity of natural TKs for these receptors is quite limited, and an extensive cross talk can occur between different TKs and their receptors (Maggi, 2000). In the enteric nervous system TKs are contained in both capsaicin-sensitive extrinsic sensory fibers and in capsaicin-resistant intrinsic neurons. Several nociceptive and/or inflammatory stimuli induce the release of TKs from capsaicin-sensitive neurons, whereas the release from intrinsic neurons seem to participate in the modulation of physiological processes (Holzer and Holzer-Petsche, 1997a,b).

Tachykinin NK₂ receptors participate in a variety of physiological and pathophysiological events in the gastrointestinal tract. It has been shown that NK₂ receptors are located on both excitatory and inhibitory neural pathways regulating intestinal motility (Portbury et al., 1996; Vannucchi et al., 2000) and the resulting motor effect of selective NK₂ receptor antagonists can vary depending on the physiological status of the viscus. NK₂ receptor antagonists can induce both prokinetic and inhibitory motor effects (Lecce et al., 1998; Onori et al., 2000), the latter being more easily evidenced following intestinal inflammation/irritation (Croci et al., 1994, 1997; Tramontana et al., 1994) or pharmacological manipulations aimed to remove neural inhibitory mechanisms (Giuliani et al., 1993, 1996; Holzer et al., 1998; Lecce et al., 1998). Beyond

ABBREVIATIONS: TK, tachykinin; NKA, neurokinin A; HPLC, high-performance liquid chromatography; MS, mass spectrometry; AUC, area under the curve; i.d., intraduodenal; i.r., intrarectal; PE, polyethylene; L-NAME, L-nitro-L-arginine methyl ester.
their modulatory effect on intestinal motility, NK2 receptor antagonists can also affect the processes of water absorption/secrection across the intestinal wall. During diarrhea or other intraluminal stimuli, water secretion prevails over absorption and this process can produce a life-threatening body dehydration; NK2 receptor antagonists abolished diarrhea-induced hypersecretion without modifying water fluxes in physiological conditions (Eutamene et al., 1995, 1997). Other beneficial effects of NK2 receptor antagonists following intestinal inflammation include anti-inflammatory effects associated with a reduction of tissue injury (Mazelin et al., 1998; Cutrufo et al., 2000), and the inhibition of visceral hyperalgesia (Kiss et al., 1999; Olivar et al., 1999; Toulouse et al., 2000). All these effects contribute to define a number of intestinal diseases in which NK2 receptor antagonists could prove beneficial effects in humans, such as irritable bowel syndrome or other diseases characterized by local inflammatory processes (Holzer, 1998).

In most cases, the preclinical characterization of a given compound is carried out on normal animals or in excised tissues from these animals, despite the fact that the clinical testing of the efficacy of such a compound will occur in defined pathological conditions. On the other hand, it is known that in certain pathological conditions both the pharmacodynamic and the pharmacokinetic properties of xenobiotics can be substantially modified (Gardiner et al., 1995; Hathaway et al., 1999). In the present study we have investigated the pharmacodynamic and pharmacokinetic properties of nepadutant, a selective NK2 receptor antagonist (Catalioto et al., 1998), in rodent models of intestinal inflammation, namely, castor oil- or bacterial toxin-induced diarrhea, and acetic acid-induced rectocolitis. The pharmacological activity of nepadutant was assessed in vivo through the simultaneous recording of the intraluminal pressure rise induced by a selective NK2 receptor agonist on the inflamed organ (colon) versus a reference organ (urinary bladder). Results from both pharmacodynamic and pharmacokinetic studies indicate that intestinal inflammation increases systemic bioavailability of nepadutant following its enteric administration. This picture justifies the antidiarrheic effect of nepadutant orally administered at dosages in the nanomolar or subnanomolar range.

Materials and Methods

Effect of Castor Oil on Nepadutant Pharmacokinetics Following Its Intraduodenal or Oral Administration. Male Sprague-Dawley CD rats (Harlan, Correzzana, Italy) weighing 350 to 400 g were used throughout the study. The day before the experiments animals underwent surgical procedures under ether anesthesia. An incision was made in the neck and the right jugular vein was cannulated with a silastic catheter to permit blood sampling. For intraduodenal treatment, another incision was made in the abdomen and a polyethylene catheter was inserted into the duodenum. The catheter was then exposed on the back of the animal and the abdomen was sutured. After recovering from anesthesia, the animals were housed singly. The day of the experiment, castor oil (10 ml/kg) was administered orally 2 h before the administration of nepadutant (42 μmol/kg in a volume of 2.5 ml/kg, either orally or intraduodenally). Blood samples (about 0.5 ml) were collected in heparinized tubes just before and 0.083, 0.167, 0.3, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h after nepadutant administration, from each animal. Samples were then centrifuged at 5000 rpm for 10 min at 4°C and the obtained plasma was stored at −20°C until analysis. Plasma (0.25 ml) was added to a known amount of internal standard (10 ng in 40 μl), mixed with 0.25 ml of HPLC-grade water, and then loaded onto a Water Oasis cartridge, which had been preconditioned with 3 ml of methanol and 3 ml of water. The cartridge was washed with 1.3 ml of water, 1.3 ml of water/methanol (50% v/v), and nepadutant was finally eluted with 3 ml of methanol. The methanol was evaporated to dryness under a helium stream and the residue redissolved in 100 μl of a mixture of water/methanol/acetonitrile (66:5:29, v/v/v), containing 0.2% formic acid. Twenty microliters of the reconstituted sample were injected into the chromatographic system. For the calculation of plasma concentration a calibration curve (nepadutant concentration between 0.42 and 454 nM) was prepared in rat plasma and processed as described above. The HPLC/MS/MS system consisted of a solvent delivery pump equipped with an automatic sample injector (model 200; PerkinElmer, Milano, Italy), a Luna C18 HPLC column (3 μM, 4.6 × 50 mm) (Phenomenex, Macclesfield, Cheshire, UK), and an MS/MS detector API 2000 (PE Sciex, Thornhill, ON, Canada). The mobile phase consisted of a mixture methanol/water (75:25) 10 mM ammonium acetate, pH 4, with formic acid. The HPLC system operated at room temperature at a flow rate of 700 μl/min. After splitting, 100 μl/min was introduced into the MS apparatus. Concentration values were calculated from the weighted linear regression of the calibration curve data. The mean recovery of nepadutant standard was 108 ± 7% and the lower limit of quantitation was 0.42 pmol/ml. The pharmacokinetic parameters were estimated from the individual plasma concentrations. Maximum plasma concentration (Cmax) and time to the maximum concentration (Tmax) were as observed experimentally. The terminal half-life (t1/2) was calculated by linear regression of the experimental plasma concentration values at the last three or four collection times. The area under the plasma concentration-time curve from zero to infinity (AUC) was estimated by trapezoidal rule and extrapolated to infinity. Absolute bioavailability after oral and intraduodenal administration was estimated by the ratio of mean AUC obtained after each enteral administration route (as corrected for the dose) to the mean AUC obtained after intravenous administration of 2.1 μmol/kg of nepadutant (8851 nmol · h/ml).

Effect of Nepadutant on [βAla8]NKA(4-10)-Induced Colonic and Bladder Contractions in Animals with Acetic Acid-Induced Diarrhea. Male Wistar rats (Charles River, Calco, Italy) weighing 350 to 400 g were used throughout the study; they had free access to water until the day of experiment and to food until the day before. Animals were treated by gavage with castor oil (10 ml/kg) or an analog amount of special formula (0.5% v/v carboxymethylcellulose, 0.4% v/v Tween 80, in a solution of 0.9% NaCl) immediately before the induction of the urethane anesthesia. Vehicle was administered to exclude that changes in the activity of nepadutant in castor oil-treated animals could be attributed to a “volume” effect rather than a specific effect of castor oil. Special formula was chosen among the possible vehicles because its viscosity was similar to that of castor oil. The method for recording intracolonic and intravesical pressures was the same as described above. Three hours after castor oil administration, a basal response to [βAla8]NKA(4-10) (10 nmol/kg i.v.) was determined in atropine-pretreated animals (1.4 μmol/kg/ml i.v. as a bolus, administered 15 min before saline or acetic acid, followed by infusion of 1.4 μmol/ml in a volume of 300 μl/h), and thereafter the NK2 receptor agonist challenge was repeated at 30-min intervals 10, 40, 70, 100, 130, 160, 190, 220, and 250 min after nepadutant administration. Nepadutant (30 nmol/kg/ml) was administered intraduodenally (i.d.) through a direct injection into the duodenum at about 3 cm below the pilorus, 3 h and 20 min from vehicle or castor oil administration. A schematic drawing of the experimental schedule is shown in Fig. 1A.

Effect of Nepadutant on [βAla8]NKA(4-10)-Induced Colonic and Bladder Contractions in Animals with Acetic Acid-Induced Rectocolitis. Male Wistar rats (Charles River) weighing 350 to 400 g were used throughout the study; they had free access to water until the day of experiment and to food until the day before.
until the dose of 10 nmol/kg; thereafter the interval was increased to 30 min. In these experiments, animals received the NK₁ receptor antagonist SR 140333 (1 μmol/kg i.v., 5 min before the first agonist challenge), to avoid the direct stimulation of NK₁ receptors by the highest doses of [αAla₈]NKA(4-10). A schematic drawing of the experimental schedule is shown in Fig. 1B.

To explore the effect of nepadutant in animals intrarectally pre-treated with saline or acetic acid (15 min before the first agonist challenge), on [αAla₈]NKA(4-10)-induced colon and bladder contractions, a dose of the agonist attaining about 50% of the maximal contractile effects in both organs, selected on the basis of previous dose-response experiments (10 nmol/kg i.v.), was administered at 30-min intervals, the first agonist challenge was given 25 min before and then repeated again at 5, 30, 60, 90, 120, 150, and 180 min after nepadutant administration. These experiments were carried out in atropine-pretreated animals (1.4 μmol/kg/ml i.v. as a bolus, administered 15 min before saline or acetic acid, followed by infusion of 1.4 μmol/ml in a volume of 300 μl/h) to reduce spontaneous colon hypermotility induced by acetic acid. Nepadutant (100 nmol/kg/ml, dissolved in saline) was administered i.r. 5 min before the second agonist challenge (40 min after i.r. saline or acetic acid) through the same catheter used for acetic acid or saline administration. A schematic drawing of the experimental schedule is shown in Fig. 1D.

In a separate series of experiments the effect of the systemic administration of nepadutant (1 and 3 nmol/kg i.v.) was assessed in rats that had received the i.r. administration of saline or acetic acid as described above. Each animal received two doses of nepadutant: the first one (1 nmol/kg) was administered 5 min before the second challenge of [αAla₈]NKA(4-10) (10 nmol/kg i.v.), whereas the second one (3 nmol/kg) was given 90 min after the first dose. A schematic drawing of the experimental schedule is shown in Fig. 1C.

**Effect of Nepadutant on Colonic Hypermotility Induced by Acetic Acid.** The procedures for intracolonic pressure recordings are described in the previous section. Soon after the setup, rats were administered with ω-nitro-l-arginine methyl ester (l-NAME, 3.9 μmol/kg i.v. as bolus, followed by the infusion of 3.9 μmol/h/330 μl). Sixty minutes following l-NAME treatment, vehicle (saline solution, 0.9% NaCl, 0.5 ml/rat) or a saline solution of acid acetic (7.5% v/v, 0.5 ml/rat) was intracolonically administered. Pretreatment with l-NAME was performed to increase spontaneous colonic motility even in rats intrarectally treated with vehicle; this was done to assess the effect of nepadutant on intestinal motility in the absence of inflammation. Colonic motility was recorded during a 30-min period before the i.r. or intravenous administration of vehicle or nepadutant (basal predrug). Afterward, vehicle (saline, 1 ml/kg i.v. or i.r.) or nepadutant (0.1 μmol/kg/ml i.v. or i.r.) was administered and colonic motility was recorded up to 120 min after administration of drugs. At the end of this period, hexamethonium (13.5 μmol/kg/ml i.v.) was administered as a bolus, and 10 min later three increasing doses of [αAla₈]NKA(4-10) (1, 3, and 100 nmol/kg i.v.) were sequentially administered to each animal at 30-min intervals. A schematic drawing of the experimental schedule is shown in Fig. 1E.

**Evaluation of Data from Functional Experiments.** The effect of nepadutant on [αAla₈]NKA(4-10)-induced colonic and bladder contractions in animals with acetic acid-induced rectocolitis or castor oil-induced diarrhea was evaluated at the various time points from the antagonist (or vehicle) administration as percentage of the basal response to the agonist calculated as maximal amplitude of contractions occurring in periods of 30 min before and after nepadutant administration in saline- or acetic acid-treated animals and by

Fig. 1. Schematic drawings representing the experimental schedules used in this study. A, experiments summarized in Fig. 3. B, experiments summarized in Fig. 4. C, experiments summarized in Fig. 5. D, experiments summarized in Fig. 6. E, experiments summarized in Fig. 7. F, experiments summarized in Fig. 8. β indicates the i.v. challenge of [αAla₈]NKA(4-10) (10 nmol/kg).

Animals were anesthetized with urethane (1.2 g/kg s.c.) and the left jugular vein was cannulated with a polyethylene catheter (PE50) for drug administrations. Fecal pellets were hand-pushed out from the distal colon by gentle pressure exerted through the skin in the cephalexoral direction. Afterwards, a polyethylene catheter (PE50) for acid acetic or saline inactivation and a latex balloon (approximate length 2 cm when empty, capacity >1.5 ml) tied to another polyethylene catheter (PE90) were inserted through the anus into the rectum. The balloon was filled with 0.5 ml of water and secured to the tail to avoid the propulsion of the balloon and catheters. Following laparotomy, the urinary bladder was exposed and cannulated through a polyethylene catheter (PE90) inserted into the proximal urethra, the ureters were tied to avoid bladder urine accumulation, and the bladder was filled with a constant volume (0.5 ml) of physiological saline solution (0.9% NaCl w/v). The colonic and bladder catheters were connected to pressure transducers and the intraluminal pressures were recorded through a polygraph integrated with a MacLab apparatus.

A dose-response study for [αAla₈]NKA(4-10) (0.03–300 nmol/kg i.v.) was performed in animals intrarectally pretreated with saline (0.9% NaCl, 0.5 ml/rat) or a saline solution of acid acetic (7.5% v/v, 0.5 ml/rat). Fifteen minutes following acid or saline administration, the agonist was administered in the same preparation at increasing doses (0.5 log units). The time interval between each dose was 20 min.
comparing the effect of the treatments with time-matched vehicle-treated animals.

Antidiarrhoeal Effects of Nepadutant on Bacterial Toxin-Induced Diarrhea in Mice. Male NMRI mice weighing 25 to 30 g (Elevage Janvier, Le Genest-Saint-Ise, France) were used in these experiments. Animals were placed in individual cages (20 × 18 × 15 cm), and the floor of the cages was covered with a preweighed white filter paper allowing the direct observation of fecal material expelled and complete collection of feces each 60 min for 120 min. Nepadutant was administered by gavage (10 ml/kg) 30 min before Escherichia coli STa toxin (70 ng/mouse, by gavage) or Clostridium difficile toxins A and B (6 ng/mouse). Each pool of fecal excretion was weighed and then was heated at 120°C for 24 h to evaluate its water content. A schematic drawing of the experimental schedule is shown in Fig. 1F.

Drugs. Drugs used were atropine sulfate salt, α-nitro-l-arginine methyl ester, and hexamethonium from Sigma (St. Louis, MO). βAla8[NKA(4-10) and nepadutant ([(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dap-Leu](c(2 β-5β)) were synthesized by conventional solid phase methods at the Chemistry Department of Menarini Ricerche (Florence, Italy). SR 140333, (S)-1-[2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxypyphenylacetyl)-piperidin-3-yl]-ethyl]-4-phenyl-1azoniabicyclo[2.2.2]octane chloride, was a kind gift from Dr. X. Emonds-Alt (Sanofi, Montpellier France).

Statistics. All data are expressed as mean ± S.E.M. of the given number (n) of experiments. Results were compared by means of one-way or factorial (two-way, treatment × time, or treatment × dose) analysis of variance: post hoc test (Fisher’s least-significant difference) was carried out when the F for drug treatment of the analysis of variance was significant (P < 0.05). In the post hoc test, a P value < 0.05 was considered statistically significant.

Results

Effect of Castor Oil on Nepadutant Pharmacokinetics Following Its Intraduodenal or Oral Administration. Following administration of nepadutant (42 µmol/kg i.d. or per os) in controls or castor oil-treated rats, its maximal plasma concentration was reached at 3 to 4 h from treatment (Tmax); however, both the maximal concentration (Cmax) and the AUC were significantly increased in castor oil-treated rats compared with the control group (Table 1; Fig. 2). Therefore, the plasma half-life of nepadutant was also larger in castor oil-treated animals, although this effect was statistically significant only following the i.d. administration (Table 1). Pharmacokinetic parameters of nepadutant (2.1 µmol/kg) following the i.v. administration are also displayed in Table 1 for comparison.

Effect of Nepadutant on βAla8[NKA(4-10)-Induced Colonic and Bladder Contractions in Animals with Castor Oil-Induced Diarrhea. Nepadutant (30 nmol/kg i.d., in atropine-pretreated animals) did not significantly modify βAla8[NKA(4-10) (10 nmol/kg i.v.)-induced colonic and bladder contractions in control rats (Fig. 3, A and B), whereas in animals with castor oil-induced diarrhea, nepadutant consistently inhibited the agonist-induced bladder contractions without reducing the colonic response (Fig. 3, C and D). Therefore, nepadutant produced a larger inhibitory effect toward βAla8[NKA(4-10) in the urinary bladder but not in the colon of castor-oil-treated animals compared with controls (Fig. 3, E and F).

Effect of Nepadutant on βAla8[NKA(4-10)-Induced Colonic and Bladder Contractions in Animals with Acetic Acid-Induced Rectocolitis. In SR 140333 (1 µmol/kg i.v.)-pretreated rats, [βAla8[NKA(4-10) (0.03–300 nmol/kg i.v.) induced a dose-dependent increase of intracolonic and intravesical pressures (Fig. 4, A and B). In animals with acetic acid-induced rectocolitis, the maximal contractile response to the NK2 receptor agonist in the colon was reduced compared with controls (Fig. 4A), without any significant change in the ED50: 24 nmol/kg (11–52 nmol/kg, 95% confidence limit) for controls and 8 nmol/kg (2–38 nmol/kg, 95% confidence limit) for rats with rectocolitis. In contrast, the dose-response curve to βAla8[NKA(4-10) for inducing urinary bladder contractions did not change in the two experimental groups both in terms of maximal effect and ED50 (9, 3–32 nmol/kg, 95% confidence limits in controls; and 11, 6–19 nmol/kg 95% confidence limits in animals with rectocolitis) (Fig. 4B). On the basis of these experiments an agonist dose

TABLE 1

<table>
<thead>
<tr>
<th>Route</th>
<th>Pretreat.</th>
<th>Nep Dose</th>
<th>Tmax</th>
<th>Cmax</th>
<th>t1/2</th>
<th>AUC</th>
<th>F%</th>
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<td></td>
<td></td>
<td>µmol/kg</td>
<td>h</td>
<td>nM</td>
<td>h</td>
<td>nmol·h/ml</td>
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<tr>
<td>i.v.</td>
<td>Vehicle</td>
<td>2.1</td>
<td></td>
<td></td>
<td>6.7</td>
<td>8831 ± 414</td>
<td>100</td>
</tr>
<tr>
<td>i.d.</td>
<td>Vehicle</td>
<td>42</td>
<td>4.0</td>
<td>± 2.2</td>
<td>73</td>
<td>6.3 ± 1.9</td>
<td>913 ± 33</td>
</tr>
<tr>
<td>i.d.</td>
<td>Castor oil</td>
<td>42</td>
<td>3.3</td>
<td>± 0.7</td>
<td>1131 ± 381**</td>
<td>19.3 ± 4.1**</td>
<td>6452 ± 933*</td>
</tr>
<tr>
<td>per os</td>
<td>Castor oil</td>
<td>42</td>
<td>2.7</td>
<td>± 0.7</td>
<td>1401 ± 220**</td>
<td>11.7 ± 0.9</td>
<td>8463 ± 2635*</td>
</tr>
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* P < 0.05 and ** P < 0.01 versus vehicle.

Fig. 2. Time course of plasma concentrations (nmol/ml) of nepadutant following the i.v. (2.1 µmol/kg) or i.d. (42 µmol/kg) routes in control animals (CTR-i.v. and CTR-i.d., respectively) or the i.d. or oral routes (42 µmol/kg) in castor oil-pretreated rats (CO-i.d. and CO-p.o., respectively). The y-axis is in a log scale. Each point and bar represents the mean ± S.E.M. of three experiments.
of 10 nmol/kg was selected to study the effect of nepadutant in both experimental groups.

Systemic administration of nepadutant (1 nmol/kg i.v.) in atropine-pretreated animals significantly reduced [βAla⁸]NKA(4-10) (10 nmol/kg i.v.)-induced urinary bladder contractions in both rats with rectocolitis and controls (Fig. 5, B and D), without affecting colonic contractions (Fig. 5, A and C). At the 3-nmol/kg dose nepadutant consistently antagonized [βAla⁸]NKA(4-10)-induced response of both organs in the two experimental groups (Fig. 5, A and C). No quantitative differences were detected in the inhibitory effect of systemic administration of nepadutant between animals with rectocolitis and controls (Fig. 5, E and F).

The i.r. administration of nepadutant (100 nmol/kg in atropine-pretreated animals) did not significantly reduce [βAla⁸]NKA(4-10) (10 nmol/kg i.v.)-induced colonic and bladder contractions in control rats (Fig. 6, A and B), whereas in animals with rectocolitis, nepadutant consistently inhibited the contractile effect of the NK₂ receptor agonists in both organs (Fig. 6, C and D). The inhibitory effect of nepadutant on [βAla⁸]NKA(4-10)-induced colonic and bladder contractions was significantly larger in animals with rectocolitis compared with the control group (Fig. 6, E and F).

Effect of Nepadutant on Colonic Hypermotility Induced by Acetic Acid. In L-NAME- (3.9 μmol/kg i.v. as bolus, followed by the infusion of 3.9 μmol/h/330 μl) pre-treated rats, the i.r. administration of acetic acid (7.5% v/v, 0.5 ml/rat) increased the number of high-amplitude (>15 mm Hg) colonic contractions induced by balloon distension (0.5 ml) (7.7 ± 1.6–22.6 ± 1.7 in 30 min, P < 0.01, n = 40) compared with the control group (intrarectal saline, 0.5 ml) (4.4 ± 0.6–6.3 ± 0.8 contractions in 30 min, N.S., n = 40). The i.r. administration of nepadutant (100 nmol/kg) reduced (at 60 min from administration) acetic acid-induced motility without modifying the basal motility in the control group (Fig. 7A). Likewise, the i.v. administration of nepadutant significantly reduced (at 60 and 120 min from administration) acetic acid-induced motility without modifying the basal motility in the control group (Fig. 7B).

In the same preparations used for assessing the effect of nepadutant on acetic acid-induced colonic motility, the colonic contractions induced by the i.v. administration of 10, 30, and 100 nmol/kg [βAla⁸]NKA(4-10) were consistently reduced in the rectocolitis group by the NK₂ receptor antagonist when administered i.r. (Fig. 7C). In contrast, the i.v. administration of nepadutant antagonized [βAla⁸]NKA(4-10)-induced colonic contractions both in acetic acid-treated animals and in controls (Fig. 7D).

Antidiarrhoeal Effects of Nepadutant on Bacterial Toxin-Induced Diarrhea in Mice. The administration of E. coli heat-stable toxin STa (70 ng/mouse per os) or C. difficile toxins A and B (6 ng/mouse per os) increased fecal water content: in both models the effect peaked at about 60 min from treatment. Pretreatment with nepadutant (0.03–3 μmol/kg per os, 30 min before) reduced E. coli toxin-induced diarrhea at both 60 and 120 min by about 50%; however, this effect was not dose-related (Fig. 8). Likewise diarrhea induced by C. difficile toxins was reduced by nepadutant (0.03–3 nmol/kg per os, 30 min before) by about 50 to 70% at both 60 and 120 min; however, even in this case the effect...
was not dose-related despite of 1000-fold lowering of the antagonist dose (Fig. 8).

**Discussion**

Nepadutant is a potent and selective peptide NK₂ receptor antagonist that displays in vivo activity following i.v., intranasal, i.r., i.d., and oral administration in rodents. In particular, in anesthetized rats, urinary bladder contractions induced by 1 nmol/kg i.v. of the selective NK₂ receptor agonist [βAla⁸]NKA(4-10) were dose dependently reduced by the i.v. (0.3–10 nmol/kg), i.r. (30 and 100 nmol/kg), or i.d. (100 and 300 nmol/kg) administration of nepadutant (Catalioto et al., 1998). Likewise, nepadutant (administered i.v.) was a potent antagonist of [βAla⁸]NKA(4-10)-induced colonic contractions in rats (Lecci et al., 1997). Since nepadutant behaves as a competitive and surmountable antagonist in rats (Catalioto et al., 1998), a loss of its activity due to the increase of the agonist dose could be predicted. Indeed, in normal rats increasing the dose of [βAla⁸]NKA(4-10) up to 10 nmol/kg reduced the in vivo antagonist activity of nepadutant either following the i.v. (at 60 min from administration, 1 nmol/kg nepadutant reduced by 50 and 20% the contractions elicited by 1 or 10 nmol/kg of the agonist, respectively) or i.r. routes (the maximal inhibition induced by 100 nmol/kg nepadutant was 75 and 30% of the responses induced by the administration of 1 or 10 nmol/kg of the agonist, respectively) (Catalioto et al., 1998; this study). However, in rats with acetic acid-induced rectocolitis, the i.r. administration of 100 nmol/kg nepadutant almost completely abolished (96% inhibition) bladder contractions induced by 10-nmol/kg dose of the NK₂ receptor agonist. A similar enhancement of the antagonist activity of i.r. nepadutant (100 nmol/kg) by rectocolitis on the agonist (10 nmol/kg)-evoked colonic contractions was also recorded: the 95% inhibition detected in acetic acid-treated animals resulted significantly larger than that (50%) observed in controls.

It may be speculated that the increased antagonist activity of i.r. nepadutant in the rectocolitis group may involve
changes in the properties of NK2 receptors linked to inflammation. Decreased smooth muscle contractility in response to various spasmines, including Substance P, is a common feature of colitis (Grossi et al., 1993; Myers et al., 1997; Tsukamoto et al., 1997), and present results on NK2 receptor-mediated colonic contractions are in line with this previous evidence. However, there are several arguments excluding that inflammation induces changes in the properties of NK2 receptors. In fact, although the maximal contractile effect induced by Ala8-NKA(4-10) in the colon was lower in animals with rectocolitis compared with controls, the same did not occur for the ED50 values, which were similar in both experimental groups. Moreover, the inhibitory effect of i.v. administration of nepadutant on NK2 receptor-induced colonic contractions almost perfectly overlapped with that recorded in control animals, indicating that following the systemic administration the antagonists reduces at a similar extent [Ala8-NKA(4-10)]-induced contractions in animals with rectocolitis and controls. Therefore, the larger inhibitory effect of i.r. nepadutant in rats with rectocolitis seems largely ascribable to an increased bioavailability of the drug: the observation that a comparable enhancement in the inhibitory action of nepadutant was also observed for NK2 receptor-induced bladder contractions indicates that rectocolitis increases the systemic bioavailability of nepadutant following its i.r. administration.

Inflammatory stimuli reverse net colonic water absorptive properties into secretory ones (Eutamene et al., 1995, 1997): this effect could theoretically decrease the colonic absorption of xenobiotics. However, the net secretory function of the colon during inflammation is the consequence of increased water fluxes through the intestinal wall, a process that could be responsible for the increased absorption of nepadutant during rectocolitis. Interestingly, colonic water hypersecretion induced by interleukin-1β or overdistension is reduced by NK2 receptor antagonist, cholinergic antagonists, and by inhibitors of nitric-oxide synthase (Eutamene et al., 1995, 1997), suggesting that the blockade of NK2 receptors could be a self-limiting factor in the colonic absorption of NK2 receptor antagonists. Indeed, the present results would exclude that such effect occurs at a biologically significant extent since, following the i.r. administration of nepadutant, the inhibition of [Ala8-NKA(4-10)]-induced colonic contraction was enhanced in acetic acid-treated animals either after pretreatment with atropine or L-NAME. These findings fit with the concept that intestinal secretion and absorption are differently regulated (Chang and Rao, 1994) and that during inflammatory processes, tachykinin and cholinergic antagonists, or nitric-oxide synthase inhibitors can reduce water secretion but not absorption of drugs.

The concept that, following enteric routes of administration, the increased antagonist activity of nepadutant in animals with intestinal inflammation is indeed due to an enhanced absorption is supported by the results obtained in the model of castor oil-induced diarrhea. Nepadutant intraduodenally administered at a dose (30 nmol/kg) having no effect on [Ala8-NKA(4-10)] (10 nmol/kg)-induced bladder contractions in control animals consistently inhibited this response in animals pretreated with castor oil. Pharmacokinetic data indicated that both the peak of plasma concentrations of nepadutant and the area under the curve were increased by a severalfold factor in castor oil-treated rats compared with controls. It has been reported that inflammation can decrease oxidative metabolism of xenobiotics by altering the expression and/or the activity of several families of cytochromes (Blobner et al., 1999; Poloyac et al., 1999); however, since, even in the absence of inflammation, the oxidative metabolism does not affect the stability of nepadutant (Catalioto et al., 1998), the increased plasma levels of nepadutant following castor oil administration are likely to represent an increased intestinal absorption of the drug.

The present results indicate that the increased systemic bioavailability of nepadutant following enteric administration during intestinal inflammation could have therapeutic relevance. In fact, the i.r. administration of nepadutant, at doses (100 nmol/kg) having no effect on colonic contractions induced by stimulation of NK2 receptors in control rats reduced both the amplitude of these contractions and the fre-
quency of high-amplitude colonic contractions in animals with acetic acid-induced rectocolitis, suggesting that in this experimental group, nepudant reduced the effects of both the exogenous and endogenous tachykinins acting via NK2 receptors. Likewise, an antidiarrheic effect of nepudant (orally administered) could be demonstrated in bacterial toxin-induced diarrhea in mice. The effect of nepudant was observed at extremely low doses (0.03 nmol/kg was already effective): this could be due in part to the very high affinity of this compound for the mouse NK2 receptor (pKᵢ = 9.8 in the mouse urinary bladder), higher than that measured at the rat NK2 receptor (pKᵢ = 9 in the rat urinary bladder), and also to the noncompetitive behavior of the antagonist at the mouse NK2 receptor (Catalioto et al., 1998). However, the possibility that the antidiarrheic effect of NK2 receptor antagonist is also mediated by a local effect on intestinal mucosa cannot be excluded, since oral administration of very low doses (about 0.3 nmol/kg) of saredutant (a nonpeptide NK2 receptor antagonist) reduced castor oil-induced diarrhea, whereas a 10-fold higher dose was necessary to reproduce this effect following subcutaneous administration (Croci et al., 1997).

In conclusion, the present results indicate that intestinal inflammation/diarrhea increases intestinal absorption and, consequently, the systemic bioavailability of nepudant following its enteric administration. Since nepudant (administered by enteric routes) reduce exaggerated motility and diarrhea in rodents with intestinal inflammation at doses having no effect in normal animals, NK2 receptor antagonists with a limited oral bioavailability could be used for treating gastrointestinal diseases associated with a local inflammation/alteration of intestinal permeability.

References

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