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European Journal of Pharmacology 415 (2001) 61–71

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Effect of a tachykinin NK_2 receptor antagonist, nepadutant, on cardiovascular and gastrointestinal function in rats and dogs

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Received in revised form 2 January 2001; accepted 26 January 2001

Abstract

The effect of the tachykinin NK_2 receptor antagonist, nepadutant (MEN 11420 or (c{[(β-D-GlcNAc)Asn–Asp–Trp–Phe–Dpr–Leu]c(2β–5β)})) was assessed on cardiovascular function (unanaesthetized rats and anaesthetized dogs) and gastrointestinal motor activity (fasted unanaesthetized dogs). The selective tachykinin NK_2 receptor agonist, [βAla^8]neurokinin A (4–10), up to 100 nmol/kg, i.v., did not produce changes on mean blood pressure or heart rate in unanaesthetized rats. Nepadutant did not affect blood pressure and heart rate up to 10 μmol/kg, whereas saredutant (SR 48968 or ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl] benzamide), a nonpeptide antagonist, produced a transient reduction of mean blood pressure and heart rate. Nepadutant up to 20 μmol/kg, i.v. neither caused changes of cardiovascular and respiratory parameters in anaesthetized dogs nor induced any changes in left ventricular systolic pressure, left ventricular dP/dt or of electrocardiogram (lead II) waveforms. Intravenous administration of neurokinin A (9 nmol/kg) in unanaesthetized dogs stimulated gastrointestinal motility for 20–25 min. Nepadutant at 0.1 μmol/kg suppressed the stimulant effects of neurokinin A but, up to a dose of 10 μmol/kg, did not produce significant changes in the basal migrating motor complexes. We conclude that tachykinin NK_2 receptors do not participate in the physiologic regulation of resting cardiovascular and respiratory functions and that they do not regulate the fasted pattern of gastrointestinal motility. The cardiovascular changes induced by the nonpeptide tachykinin NK_2 receptor antagonist, saredutant, likely arise from nonspecific effects unrelated to tachykinin NK_2 receptor blockade.

Keywords: Tachykinin NK_2 receptor antagonist; Nepadutant; Saredutant; Cardiovascular parameter; Respiratory effect; Gastrointestinal motility; Migrating motor complex; (Rat); (Dog)

1. Introduction

Tachykinin NK_2 receptors are abundantly expressed in the peripheral nervous system (Tsuchida et al., 1990) and participate in nonadrenergic noncholinergic (NANC) regulation of several visceral function in the airways, genitourinary and gastrointestinal tract (Maggi et al., 1993b, for review): from this, a possible role for tachykinin NK_2 receptor antagonists in the treatment of various human diseases (asthma, irritable bowel syndrome and cystitis) has been hypothesized.

Several data indicate that tachykinin NK_2 receptors could be expressed in the heart and blood vessels and mediate effects, which, in principle, could affect cardiovascular function. Tachykinin NK_2 receptors mediate powerful contraction of certain isolated blood vessels (D'Orleans-Juste et al., 1986), can modify cardiac rhythm and performance in isolated guinea-pig heart (Lundberg et al., 1985; Hoover et al., 1998) and in the perfused dog heart in vivo (Thompson et al., 1998). It has been reported that administration of a selective NK_2 receptor agonist induces tachycardia in anaesthetized rats, likely ascribable to catecholamine release from sympathetic nerves/adrenals (Couture et al., 1989). Roccon et al. (1996) reported that i.v. administration of a selective NK_2 receptor agonist had no effect on blood pressure in conscious freely moving guinea pigs. On the other hand, Floch et al. (1996) showed that the same agonist exerts a blood pressure lowering effect in pithed and vagotomized guinea pigs in which

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00794-4

arterial blood pressure was pharmacologically elevated by infusion of norepinephrine. Finally, Lam and Wong (1996) reported that intraarticular infusion of a selective tachykinin NK_2 receptor agonist caused vasodilation in the rat knee joint microvasculature and Ferrell et al. (1997) presented evidence that tonic activation of tachykinin NK_2 receptors by endogenous tachykinins could regulate blood flow in the microcirculation. With the exception of the latter study, there is little evidence to date that tachykinin NK_2 receptors, activated by endogenous tachykinins play a physiological role in regulating cardiovascular function.

With regard to the gastrointestinal tract there are a number of data, which indicate that tachykinin NK_2 receptors mediate the transmitter function of this family of peptides acting as NANC excitatory neuromuscular transmitters in various species (Holzer, 1998, for review). Tachykinin NK_2 receptor antagonists inhibit the atropine-resistant reflex contractions induced by balloon distension in guinea-pig colon (Giuliani et al., 1993a) and rat duodenum in vivo (Giuliani et al., 1996). Moreover, tachykinin NK_2 receptor antagonists, such as nepadutant (Catalioto et al., 1998), exert a modulatory role on colonic visceral hyperalgesia induced by experimental inflammation or stress in animals (Buéno et al., 1997, for review; Kiss et al., 1999; Olivar et al., 1999; Toulouse et al., 2000). From these preclinical data, it is anticipated that these drugs could represent a novel type of treatment in patients suffering from irritable bowel syndrome. The available data (Holzer and Holzer-Petsche, 1997, for review) suggest that endogenous tachykinins, via NK_2 receptors, should not play a major role in normal intestinal motor activity and that this transmitter system could rather be involved in generating patterns of exaggerated intestinal motor activity of pathophysiological rather than physiological relevance. However, the effect of potent and selective tachykinin NK_2 receptor antagonists on integrated patterns of gastrointestinal motor activity, such as the migrating motor complex, have not been reported yet.

In this study, we have used the potent and highly selective tachykinin NK_2 receptor antagonist, nepadutant, as a tool to assess whether there is any evidence for a role of a tachykininergic tone on resting cardiovascular function (in unanaesthetized rats and anaesthetized instrumented dogs) and on the propulsive motor activity in unanaesthetized fasted dogs. In a few experiments, the nonpeptide NK_2 receptor antagonist saredutant (SR 48968, Emonds-Alt et al., 1992, 1993) was studied as well.

2. Materials and methods

2.1. Binding experiments

The binding profile of nepadutant was checked on 27 unrelated receptors and ion channels at 1 μM concentration in duplicate (see Table 1 for the binding assays) and compared to the binding of the nonpeptide tachykinin NK_2 receptor antagonist, saredutant.

A conventional binding assay procedure was used and the method has been extensively detailed in Catalioto et al. (1998).

Results of binding experiments were expressed as a percentage of control specific binding and as a percentage of inhibition of specific binding obtained in the presence of the compound.

This series of experiments was performed by Cerep (Celle L'Evescault, France) according to the protocols used by this Laboratory (see Cerep catalog for methodological details).

2.2. In vivo experiments in unanaesthetized rats

All experiment involving chronically instrumented animals were carried out in accordance with the principles and guidelines of the local government and the European Union regulations.

Male albino Wistar rats (Charles-River, Calco, Italy) weighing 330–380 g were pretreated intraperitoneally (i.p.) with fentanyl (0.024 mg/kg) plus fluanisone (1.2 mg/kg) (Hypnorm, Janssen) and then anaesthetized with sodium pentobarbital (35–40 mg/kg, i.p.). Two small midline skin incisions were made in the ventral and in the dorsal neck to allow the catheterization of the vessels and exteriorization of the catheters, respectively. Silastic catheters (No 155, Dow Corning) were inserted into the left carotid artery for blood pressure measurement and into the right jugular vein for intravenous (i.v.) administration of drugs and exteriorized behind the head through a two-channel swivel device (Danuso, Milan, Italy), which was fixed on the muscle of the neck by strain sutures. Then the incisions were sutured and the animals were allowed to recover under a continuous intraarterial infusion (0.6 ml/h with a model 22 microdialysis syringe pump, Harvard) of heparinized saline (10 UI/ml) separately in a cage with free access to water.

The experiments started 18 h after surgical preparation in order to allow the recovery from the anaesthesia and surgical procedure. The carotid catheter was connected to a Transpac II pressure transducer (Abbott, Latina, Italy), the signal delivered to a 8805 D preamplifier (Hewlett-Packard, Milan, Italy) and blood pressure and heart rate were recorded by a 7758 D polygraph (Hewlett-Packard). The signal from the preamplifier was digitized in a 16-bit analog–digital converter (IDAS BM 9000, Biomedica Mangoni, Pisa, Italy) and forwarded to a Compaq 386/20e computer for acquisition and evaluation of the data.

Two protocols were used as follows: after the recording of a 30-min basal period, the animals were either treated intravenously with increasing doses of [βAla^8]neurokinin A (4–10) (0.03–100 nmol/kg in 2 ml/kg) at 15–30-min

Table 1
Percentage of inhibition for nepadutant and saredutant of specific radioligands at various receptors binding assays and IC_{50} values for the reference compounds

Receptors	Nepadutant 1 (μM)	Saredutant 1 (μM)	Reference compounds	IC_{50} (nM)
$GABA_A$	–	–	muscimol	7.3
$GABA_B$	19	–	baclofen	152
AMPA	–	–	L-glutamate	782
Glycine	–	–	strychnine	13.8
H_1	17	–	pyrilamine	10.1
Kainate	15	–	kainic acid	26.1
Muscarinic	–	–	atropine	0.87
Nicotinic	14	12	nicotine	15.4
NMDA	–	–	CGS 19755	174
NPY	–	–	NPY	1.5
Neurotensin	–	–	neurotensin	1.8
Opiate (n.s.)	–	–	naloxone	2.6
μ	–	90	DAMGO	1.0
δ	–	–	DPDPE	2.1
κ	–	20	U 50488	0.9
PCP	–	–	MK-801	5.8
5-HT (n.s.)	–	18	Serotonin	1.9
σ (n.s.)	11	25	haloperidol	4.0
Ca^{2+} (L)	–	38	nitrendipine	1.7
Ca^{2+} (L)	–	83	diltiazem	59.9
Ca^{2+} (L)	–	44	verapamil	21.5
Ca^{2+} (N)	–	–	ω-conotoxin GVIA	0.002
K^+ (ATP-sens.)	–	–	glibenclamide	1.2
K^+ (Volt.-dep.)	–	–	charybdotoxin	0.037
K^+ (Ca-dep.)	–	–	apamin	0.013
Na^+ channels (1)	–	–	tetrodotoxin	13.6
Na^+ channels (2)	–	53	veratridine	7400

In each experiment, the reference compound for the receptor studied was simultaneously tested at eight concentrations in duplicate to obtain a competition curve and to validate this experiment.
IC_{50}: Concentration required to inhibit 50% of the specific binding.
The symbol – indicates an inhibition of less than 10%.
n.s.: Nonselective.

intervals to construct a dose–response curve to the agonist (protocol A) or nepadutant, saredutant or the vehicle were intravenously administered at high doses (1–10 μmol/kg) to verify the effects of the two tachykinin NK_2 receptor antagonists at cardiovascular level (protocol B). Systolic blood pressure, diastolic blood pressure, mean blood pressure and heart rate were continuously recorded for 8 h after administration of the tachykinin antagonists or the vehicle.

At the end of the experiments, the animals were sacrificed by an excess of the anaesthetic urethane (2.5–3 g/kg, i.v.).

2.3. In vivo experiments in anaesthetized dogs

Four female Beagle dogs (Interfauna, UK), approximately 12–17 months old weighing 10–11 kg, were used. All dogs were allowed an acclimatisation period of at least 4 days prior to experimentation. They did not receive any prophylactic or therapeutic treatment during this period. The dogs were deprived of food for a minimum of 16 h prior to starting the experiment. Anaesthesia was induced by an intravenous injection of thiopental sodium (30 mg/kg) and maintained by a mixture of α − chloralose (20 ml 1%) and pentobarbital sodium (1 ml Sagatal, 60 mg/ml) given intravenously as required. Body temperature was maintained constant at 36°C by means of a homeothermic blanket and rectal probe.

The trachea was cannulated and the cannula connected to a pneumotachometer screen to allow measurement of respiratory tidal volume (ml) and the derived parameters of respiration rate (breaths/min).

The right femoral artery and a cephalic vein were cannulated for measurement of blood pressure and for drugs administration, respectively. The femoral artery was connected to a heparinized (250 U/ml) pressure transducer (L221, Bell and Howell) and the signal was delivered to a Grass 7E polygraph. Heart rate was derived electronically from the blood pressure signal.

An ultrasonic flow probe was positioned around the left femoral artery and connected to a Transonic Systems (type

T206) blood flow meter coupled to a Grass polygraph to record femoral blood flow (ml/min).

A catheter was introduced into the left carotid artery and advanced into the left ventricle in order to measure the left ventricular sistolic pressure (mmHg). The catheter was connected to a pressure transducer (Isotec) and the signal was displayed on the Grass recorder together with the first derivative, left ventricular dP/dt, which is an index for the contractile status of the myocardium. Subcutaneous needle electrodes were inserted in the appropriate limbs in order to monitor the electrocardiogram (lead II), which was displayed on the Grass polygraph.

All signals were acquired on a personal computer by using the Po–Ne–Mah data acquisition system software.

Following a 30-min stabilization period, the vehicle (0.9% saline) was administered intravenously at 8 ml/kg and, 40 min later at 40-min intervals thereafter, increasing doses of nepadutant (0.2, 2 and 20 μmol/kg, i.v.) were administered in a volume of 8 ml/kg. All doses were infused over a 10 min period using an infusion pump (22 microdialysis syringe pump, Harvard). All physiological parameters were monitored continuously.

At the end of the observation period, the animals were sacrificed with an intravenous overdose of pentobarbital sodium.

2.4. In vivo experiments in unanaesthetized dogs

Four adult Beagle dogs weighing 13–18 kg were used for these investigations. Under halothane (Flothane N.D.) anaesthesia, four strain-gauge transducers, constructed in our laboratory according to Pascaud et al. (1975), were sewn on the serosa of the antrum at 5 cm from the pylorus, on the proximal jejunum at 15 cm from the Treitz ligament and on the proximal colon at 10 cm from the ileo-colonic junction. Each transducer was sewn with its recording axis parallel to the transverse axis of the gut in order to measure selectively the contractile force of the circular muscle layer.The free ends of the strain-gauge wires were drawn subcutaneously to emerge dorsally between the scapulas. The dogs were allowed to recover during 8 days before the start of experiments.

Calibration of each strain-gauge was performed before implantation. Mechanical activity detected by the transducers was recorded by connecting the strain-gauge to a Wheatstone bridge amplifier (VT 2100, Vishay, France) linked to a potentiometric recorder (BS 273, Gould, France). The motility indexes of the antrum, jejunum and colon were determined by a microcomputer (Epson HX20) according to the technique of Hachet et al. (1986). The calculated index of motility corresponds to the area between the baseline and the contractile curve over a h of post-injection recordings and is expressed in mN min/60 min.

The animals were fed once daily at 17.00 h with a standard laboratory diet. Experiments were performed in the fasted state, i.e. from 9.00 to 17.00 h. On the gastric antrum, phases of high amplitude contractions, lasting about 20 min, occurred at intervals of 90–120 min. Gastric

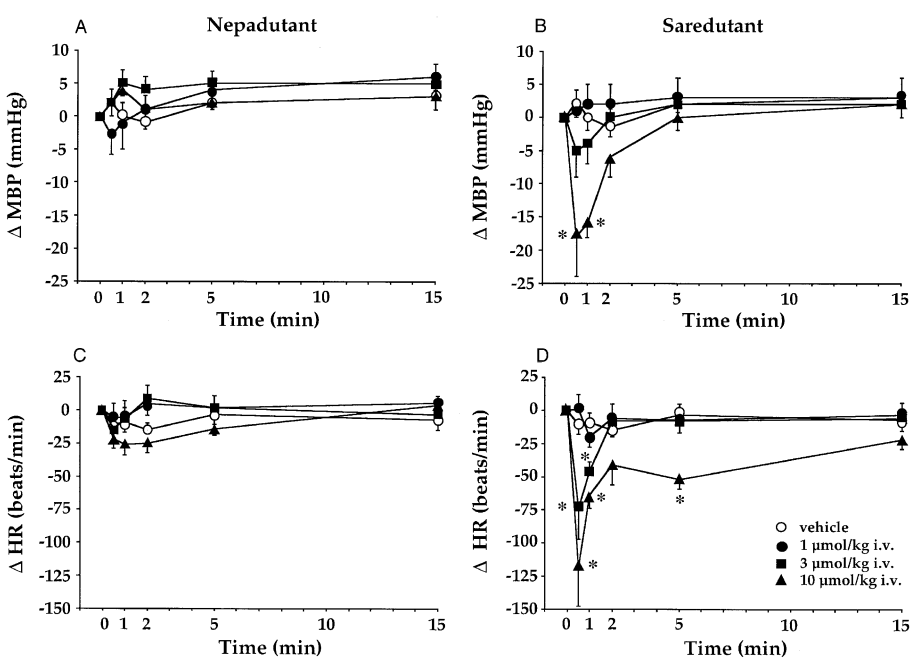

Fig. 1. Effect of intravenous administration (1–10 μmol/kg) of nepadutant (panel A and C) and saredutant (panel B and D) on mean blood pressure (MBP, upper panels) and heart rate (HR, lower panels) in unanaesthetized rats. Values are expressed as delta (δ), which is the difference between the basal values and the values at various times from administration. The figure shows the effect during the first 15 min from administration, out of the 8 h recording time, where the more pronounced changes were observed. Each value is mean ± S.E.M. of six experiments. $^{*}P < 0.05$ significantly different from the vehicle values.

migrating motor complexes were propagated on the small intestine where they consisted of two phases of contractile activity: a period of irregular activity (Phase II) lasting 30–50 min, followed by a period of regular activity (Phase III) lasting 5–6 min. These two consecutive phases of contractions were followed by a period of quiescence (Phase I). The phasic colonic contractions were grouped in phases lasting 8–10 min occurring at a rate of 3–4 per hour (Fioramonti and Buéno, 1983). In a first series of experiments performed in three dogs, nepadutant (0.1 μmol/kg) or its vehicle was administered 10 min before a bolus i.v. injection of neurokinin A at a dose of 9 nmol/kg.

In a second series of experiments, performed in four dogs, each animal received a bolus intravenous (i.v.) injection of the vehicle (5 ml of NaCl 0.9%) and three different doses (0.1, 1 and 10 μmol/kg) of nepadutant. The complete experiment cover 2 days: the first for the vehicle and the lower dose of nepadutant, the second day, at 2 days interval, for the other two doses of nepadutant. The effect of each administration was followed for 4 h. The same protocol was repeated in each dog after 1 week except for the highest dosage.

2.5. Data analysis

All values in the text, tables or figures are mean ± S.E.M., where appropriate. Statistical analysis was performed by means of Student's *t*-test for paired data or by means of one-way analysis of variance (ANOVA) followed by the Dunnett test for multiple comparison. A P level < 0.05 was considered statistically significant.

Values of the motility index as well as the interval between two consecutive gastrointestinal migrating motor complex and jejunal phase II were measured and compared using Student's *t*-test for paired values during 4 h after administration of nepadutant or its vehicle.

2.6. Drugs

[βAla^8]neurokinin A (4–10) and nepadutant or (c{[(β-D-GlcNAc)Asn–Asp–Trp–Phe–Dpr–Leu]c(2β–5β)}) were synthesized by conventional solid phase methods in the Chemistry Department of Menarini Ricerche. Neurokinin A was from Sigma-Aldrich Chimie (Saint Quentin, France).

The nonpeptide tachykinin NK_2 receptor antagonist saredutant or ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl] benzamide) was kindly provided by Drs. X. Emonds-Alt and G. LeFur (Sanofi, Montpellier, France).

For binding experiments, radiolabelled ligands were from DuPont NEN (Zaventem, Belgium) and Amersham (Buckinghamshire, UK) while cold ligands were from Sigma (St. Louis, MO, USA), RBI (Natick, MA, USA), Bachem (Bubendorf, Switzerland), ICI (Macclesfield, UK) and Bayer (Leverkusen, Germany).

3. Results

3.1. Binding experiments

Both nepadutant and saredutant have been reported to possess high affinity for tachykinin NK_2 receptors and selectivity (about three orders of magnitude) vs. tachykinin NK_1 or NK_3 receptors (Emonds-Alt et al., 1992; Catalioto et al., 1998; Renzetti et al., 1998). Experiments performed in rats (see next section) indicated a substantial difference between these two compounds in affecting resting cardiovascular parameters. Since saredutant has been reported to possess nonspecific effects on molecular targets, which may be relevant for affecting cardiovascular function (Boyle et al., 1993; Martin et al., 1993; Wang et al., 1994), we performed an extensive comparative analysis of the affinities of these two compounds in 27 different molecular targets unrelated to tachykinin receptors. The results, summarized in Table 1, indicate that at a concentration of 1 μM, saredutant possesses a sizeable (> 50% inhibition of specific binding) for μ-opioid receptors, L-type Ca^{2+} channels (diltiazem site) and Na^+ channels (veratridine site), whereas nepadutant, at the same concentration, is ineffective at these as well as at the other molecular targets tested.

Table 2
Basal values and effect of vehicle and nepadutant (20 μmol/kg, i.v.) on the various parameters recorded in anaesthetized dogs

Parameters	Basal	Effect (Δ)	
	Values	Vehicle	Nepadutant
Systolic blood pressure (mmHg)	168 ± 10	−2 ± 1	−6 ± 3
Diastolic blood pressure (mmHg)	110 ± 4	−6 ± 2	−9 ± 3
Mean blood pressure (mmHg)	129 ± 7	−5 ± 3	−8 ± 3
Heart rate (beats/min)	221 ± 11	−7 ± 3	−6 ± 1
Respiratory tidal volume (ml)	162 ± 27	−4 ± 5	−5 ± 3
Respiratory rate (breaths/min)	22 ± 1	2 ± 3	4 ± 1
LVSP (mmHg)	157 ± 13	−9 ± 3	−5 ± 3
LV$dp/dt$ (mmHg/s)	6103 ± 981	−388 ± 125	87 ± 256
Mean femoral flow (ml/min)	57 ± 20	10 ± 2	9 ± 3
Femoral resistance (mmHg/ml/min)	2.9 ± 1.0	−0.8 ± 0.4	−0.9 ± 0.5

Δ is the difference between the basal values and the values reached at the maximal effect.
Each value is the mean ± S.E.M. of four experiments.

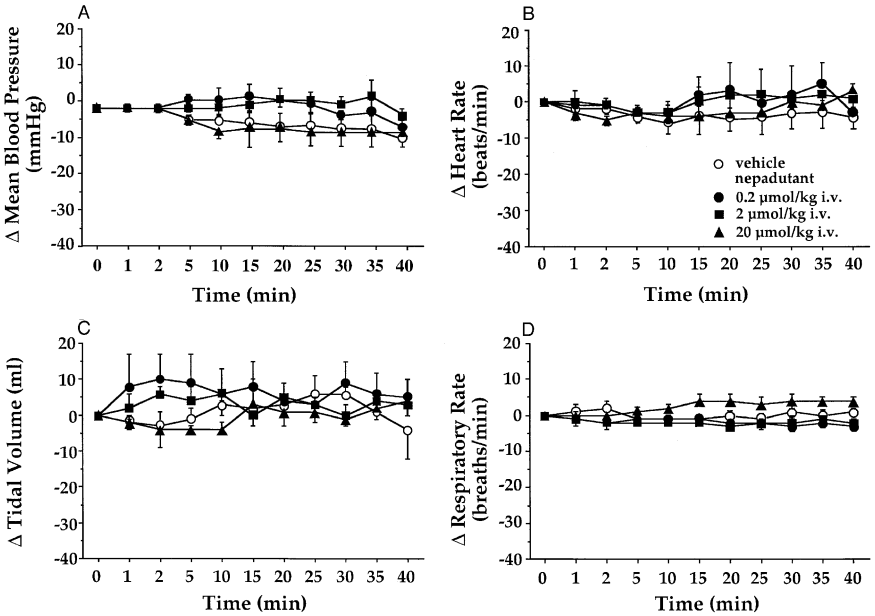

Fig. 2. Effect of intravenous administration of nepadutant (0.2, 2 and 20 μmol/kg) on mean blood pressure (A) heart rate (B), respiratory tidal volume (C) and respiratory frequency (D) in anaesthetized dogs. Values are expressed as delta (δ), which is the difference between the basal and the values at various times from administration. Each value is mean ± S.E.M. of four experiments.

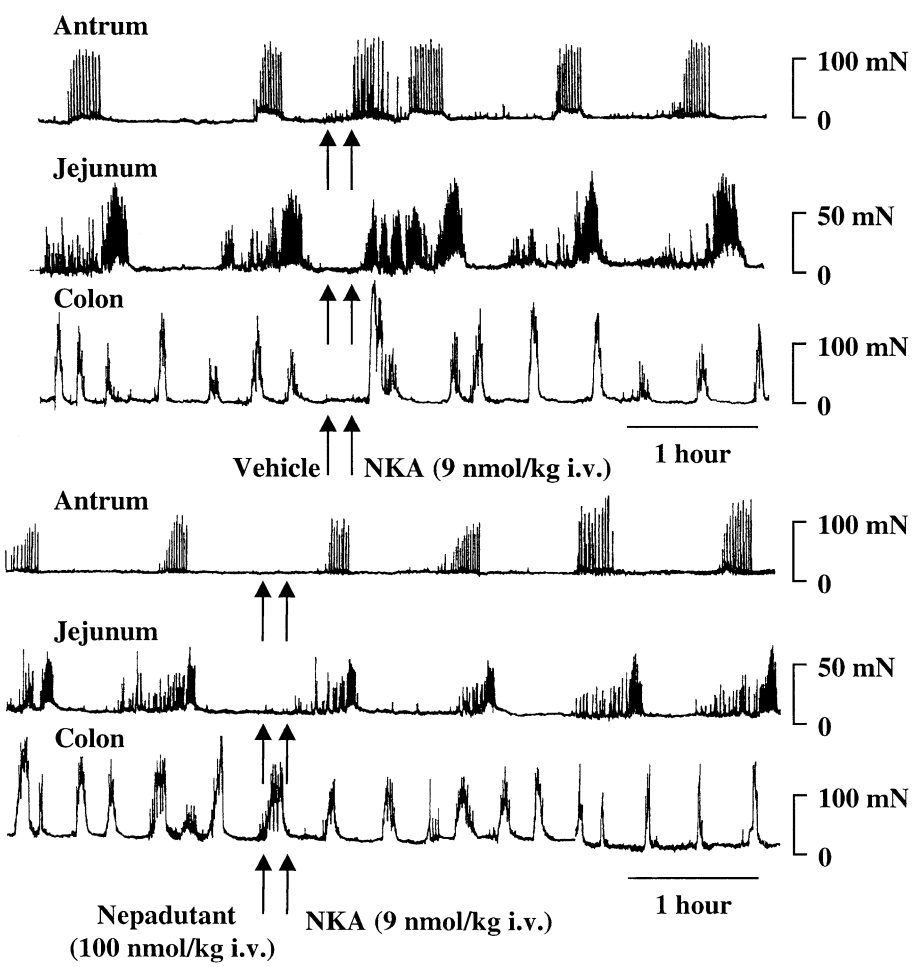

Fig. 3. Typical tracings showing the effect of nepadutant (100 nmol/kg, i.v.) on the neurokinin A (NKA)-induced gastrointestinal and colonic motor stimulation in a fasted dog. At this dose, nepadutant blocked the stimulatory effects of neurokinin A on motility.

Table 3
Effect of nepadutant on neurokinin A-induced colonic motor response in dogs

	Frequency (contractions/min)	Colonic phasic contractions	
		Amplitude (mN)	Motility index (mN min/60 min)
Control	1.2 ± 0.6	108 ± 45	56 ± 16
Neurokinin A (9 nmol/kg, i.v.)	3.9 ± 1.4	188 ± 31	107 ± 18
Neurokinin A + nepadutant (100 nmol/kg, i.v.)	1.6 ± 0.7	95 ± 38	62 ± 9

The colonic phasic contractions were measured during 1 h post-injection period.
Each value is the mean ± S.E.M. of three experiments.

3.2. Cardiovascular effects of nepadutant and saredutant in unanaesthetized rats

The selective tachykinin NK_2 receptor agonist [βAla^8]neurokinin A (4–10) (0.03–100 nmol/kg) administered at progressively increasing doses (15–30-min intervals, protocol A) in unanaesthetized rats (basal mean blood pressure 113 ± 4 mmHg and heart rate 390 ± 7 beats/min, $n = 6$), did not produce significant effects on mean blood pressure and heart rate at the highest dose (data not shown).

For experiments with the tachykinin NK_2 receptor antagonists (protocol B), the basal values for mean blood pressure and heart rate in the various groups ranged from 110 ± 7 to 117 ± 5 mmHg and from 350 ± 12 to 377 ± 14 beats/min ($n = 6$), respectively. Nepadutant, in the range 1–10 μmol/kg, did not produce significant variations of basal heart rate and mean blood pressure values up to 8 h from the i.v. administration (Fig. 1). On the contrary, saredutant, at 10 μmol/kg, i.v., induced a transient significant reduction of mean blood pressure by about 16% (−18 ± 5 mmHg) that recovered within 5 min, while heart rate was significantly reduced at 3 and 10 μmol/kg by about 21% (−74 ± 23 beats/min) and 32% (−118 ± 32 beats/min), respectively. At the highest dose, heart rate values recovered to the baseline within 15 min.

No overt behavioural changes were observed during i.v. administration of either compound.

3.3. Cardiovascular and respiratory effects of nepadutant in anaesthetized dogs

The basal values and the effect on the various parameters recorded in anaesthetized dogs are showed in Table 2. Nepadutant (0.2–20 μmol/kg, $n = 4$) induced no overt effects on arterial blood pressure and heart rate (Fig. 2). Likewise, no statistically significant effects were observed on respiratory parameters at the three doses tested.

Nepadutant did not induce any change in left ventricular systolic pressure or left ventricular dP/dt (Table 2) nor it produced changes of electrocardiogram (lead II) waveforms throughout the experiment (data not shown).

Nepadutant at the higher dose (20 μmol/kg, i.v.) induced a small increase in femoral flow and a concurrent inhibition (31 ± 17%) in femoral resistance but approximately the same variation was produced by the vehicle administration (27 ± 14%) and, therefore, they are though not to be treatment-related but to be the result of volume increase during the infusion.

3.4. Effect of nepadutant on gastrointestinal and colonic motility in fasted unanaesthetized dogs

At gastrointestinal level, the pattern of contractile activity during fasted state was characterized by the cyclic occurrence of migrating motor complexes (Figs. 3 and 4).

Table 4
Influence of increasing doses of nepadutant on the duration of the first migrating motor complex (M.M.C.) cycle, the first phase II duration and on the gastrointestinal and colonic motility indexes after its administration in unanaesthetized fasted dogs

	Dose (μmol/kg, i.v.)	M.M.C. duration (min)		Phase II duration (min)		Motility index (mN min/60 min)		
		Antrum	Jejunum	Antrum	Jejunum	Antrum	Jejunum	Colon
Vehicle	5 ml	113 ± 7	104 ± 6	46 ± 4	48 ± 4	42 ± 5	68 ± 5	61 ± 6
Nepadutant	0.1	100 ± 6	98 ± 6	50 ± 4	49 ± 5	44 ± 7	66 ± 6	59 ± 5
	1	116 ± 5	108 ± 5	53 ± 4	51 ± 5	39 ± 5	71 ± 4	68 ± 3
	10	107 ± 7	110 ± 7	45 ± 4	62 ± 5	42 ± 3	74 ± 3	62 ± 2

Values are the mean ± S.E.M of 4—8 determinations; each dose of nepadutant as well as the vehicle was repeated two times in each four dogs except for the highest dose which was performed only one time.
Vehicle and nepadutant were administered during the phase I; motility index values are the mean values measured over 4 h after vehicle or nepadutant administration.

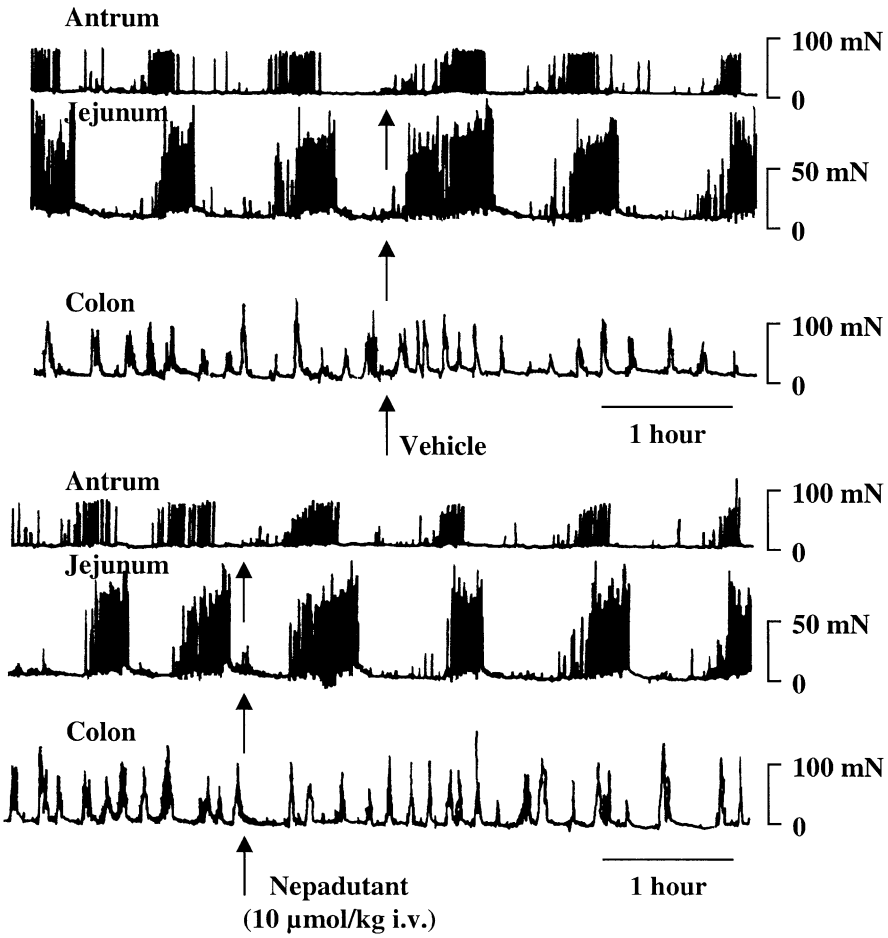

Fig. 4. Typical tracings showing the influence of high dose (10 μmol/kg, i.v.) of nepadutant on gastrointestinal and colonic motility in a fasted dog. Note that this dose did not affect the gastrointestinal migrating motor complex as well as the cyclic propulsive groups of colonic contractions.

Intravenous (i.v.) administration of vehicle (saline) did not modify any parameter of gastrointestinal or colonic motility (Fig. 3). Neurokinin A at 9 nmol/kg, i.v. powerfully stimulated gastric and intestinal motility for 20–25 min with a pattern of phasic, high amplitude (130 ± 39 and 50 ± 25 mN, respectively, $n = 3$) contractions, which resemble the cyclic pattern characterizing the migrating motor complexes. Neurokinin A (9 nmol/kg, i.v.) produced at colonic level phasic high amplitude contractile responses (188 ± 31 mN) more pronounced as compared to the normal contractions of the fasted state (108 ± 45 mN). Nepadutant, at 0.1 μmol/kg, i.v., suppressed the stimulatory effects of neurokinin A at all levels investigated and in particular at colonic level it blocked the increase of either frequency, amplitude or the motility index during 1-h period after neurokinin A administration (Table 3, Fig. 3).

Intravenous injection of nepadutant at 0.1–10 μmol/kg, 10–15 min after the last gastric migrating motor complex, did not affect neither the rhythm nor the amplitude of spontaneous gastrointestinal and colonic motor activity (Table 4). In addition, there was no significant difference, even at the higher dose, on either gastric, jejunal and colonic motility indexes over a 4-h post-injection period of measurement (Fig. 4).

4. Discussion

Both nepadutant and saredutant possess high affinity for rat and dog tachykinin NK_2 receptors. In rats, the estimated affinities of these two compounds in terms of pA_2 or pKB were of 9.0 in the urinary bladder for nepadutant (Catalioto et al., 1998) and among 8.2 and 9.4 in the vas deferens, duodenum and urinary bladder for saredutant (Advenier et al., 1992; Boyle et al., 1993; Emonds-Alt et al., 1993; Maggi et al., 1994). In dogs, the estimated affinities (pKB values) of saredutant were 8.4 in the proximal colon (Parlani et al., 1996) and 8.9 in the urinary bladder (Mussap et al., 1996). MEN 10627, a less water-soluble analog of nepadutant, which possesses a superimposable spectrum of potency/selectivity for tachykinin NK_2 receptors in different species (Catalioto et al., 1998; Patacchini et al., 2000) showed a pA_2 value of 8.5 in the dog-isolated proximal colon (Parlani et al., 1996).

In both rats and dogs, doses of nepadutant and saredutant ranging between 10 and 1000 nmol/kg are sufficient

to produce a long lasting blockade of peripheral tachykinin NK_2 receptors (Maggi et al., 1993a, 1994; Giuliani et al., 1993b; Basilisco and Phillips, 1994; Lecci et al., 1997; Catalioto et al., 1998).

In a first series of experiments, we tested the cardiovascular effects of nepadutant and saredutant in unanaesthetized rats. Nepadutant did not affect resting cardiovascular parameters at a dose (10 μmol/kg) exceeding by about 1000-fold those required to produce a full blockade of peripheral tachykinin NK_2 receptors, for, e.g. that necessary to block contraction of the urinary bladder induced by a tachykinin NK_2 receptor agonist (10 nmol/kg, i.v., Catalioto et al., 1998).

The failure of nepadutant to affect resting cardiovascular parameters in unanaesthetized rats at i.v. doses largely exceeding those required to block peripheral tachykinin NK_2 receptors in this species, provides clearcut evidence that tachykinin NK_2 receptors are not involved in physiological regulation of cardiovascular function in this species. Noteworthy, at variance with the results of Couture et al. (1989), we failed to detect any significant cardiovascular effect of [βAla^8]neurokinin A (4–10), further implying that tachykinin NK_2 receptor have little or no role in regulating resting cardiovascular function in rats.

Contrary to nepadutant, saredutant induced a transient bradycardia and hypotension in conscious rats at relatively high doses (3–10 μmol/kg, i.v.). These doses are higher than those required to block peripheral tachykinin NK_2 receptor in this species by about 30–100-fold (cf. Maggi et al., 1993a). Saredutant has been reported to possess, at high concentrations, nonspecific effects on opioid receptors and ion channels (Boyle et al., 1993; Martin et al., 1993; Wang et al., 1994), a finding confirmed by the present receptor/ion channel screen data. It appears conceivable that the transient changes in cardiovascular function produced by saredutant in this study are linked to actions on molecular targets unrelated to tachykinin NK_2 receptor since relatively high plasma concentrations of the drugs were likely attained at short times after their i.v. injection.

An alternative explanation, which cannot be fully discarded presently, is an involvement of central tachykinin NK_2 receptors involved in regulating blood pressure/heart rate. In fact, peripherally administered saredutant has been reported to block certain responses in the rodent central nervous system (turning behaviour induced by intrastriatal administration of a tachykinin NK_2 receptor agonist, Poncelet et al., 1993; activation of thermonociceptive thalamic neurons, Santucci et al., 1993) and the doses reported to be active in these studies (0.12–0.5 mg/kg, i.v. or i.p.) are lower than the i.v. doses, which we found effective in producing transient changes in resting blood pressure and heart rate in rats.

Experiments with nepadutant do not provide evidence for an involvement of peripheral tachykinin NK_2 receptors in regulating resting cardiovascular function also in anaesthetized dogs. In this species, a dose of nepadutant of 100 nmol/kg was sufficient to fully block the stimulation of gastrointestinal and colonic motility induced by i.v. administered neurokinin A. At a dose 200-fold higher (20 μmol/kg), nepadutant had no effect on resting blood pressure, heart rate, electrocardiogram, cardiac contractility, vascular resistance and respiratory rate. Notably, up to a dose of 10 μmol/kg, which is 100-fold higher than that sufficient to block the stimulant effect of neurokinin A, nepadutant also had no effect on the migrating motor complex in conscious fasted dogs. This result is in keeping with the concept that endogenous tachykinins—while acting as excitatory neuromuscular transmitters in the mammalian gut (Holzer and Holzer-Petsche, 1997) do not play a major role in physiological or resting gastrointestinal motor activity. The present results in dogs mirror those recently obtained in a Phase I clinical study in healthy volunteers (Loerdal et al., 1999): nepadutant at a dose of 8 mg, i.v. (8.5 μmol) markedly inhibited the stimulation of intestinal motor activity induced by infusion of neurokinin A without affecting fasted migrating motor complex of its own. In the same study, no significant changes in blood pressure and heart rate or vital signs were observed upon administration of nepadutant.

Beyond conclusions of physiological relevance, the present results provide further ground for speculating that tachykinin NK_2 receptor antagonists, such as nepadutant, could represent a novel approach for treatment of irritable bowel sindrome. Preclinical data indicate that these compounds are effective in a number of different models of colonic visceral hyperalgesia induced by stress/inflammation of the colon (Julia et al., 1994; Toulouse et al., 2000). With regard to nepadutant, a peripheral site of action has been established by showing that this compound prevents the activation of spinal cord second-order sensory neurons induced by mechanical stimulation of the inflamed colon without affecting the stimulation of the same neurons following electrical stimulation of the pelvic nerve (Olivar et al., 1999). The present findings, in keeping with the clinical results mentioned above (Loerdal et al., 1999), also indicate that nepadutant or drugs of this class may modulate visceral hyperalgesia at doses devoid of any effect on cardiovascular and respiratory function as well as on normal intestinal transit.

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