Haemodynamic and abdominal motor reflexes elicited by neurotensin in anaesthetized guinea-pigs

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1 Single intraperitoneal (i.p.) injections of neurotensin (NT) ($0.14\text{--}140\text{ nmol kg}^{-1}$) in anaesthetized guinea-pigs were found to trigger transient abdominal wall contractions (TAWC) accompanied by relatively sustained increases of systemic blood pressure (BP) and heart rate (HR). The modification of the latter NT effects by various drugs and surgical manipulations was examined to obtain some insight into the nature of, and possible relationship between, these responses.

2 The abdominal motor response (i.e. TAWC) to i.p. NT (14 nmol kg^{-1}) was inhibited by prior i.v. injection of the guinea-pigs with pancuronium ($0.27\text{ }\mu\text{mol kg}^{-1}$), morphine (1.5 and $15\text{ }\mu\text{mol kg}^{-1}$), clonidine ($0.34\text{ }\mu\text{mol kg}^{-1}$), by concomitant i.p. injection of procaine 2% w/v, or by acute spinalization. It was potentiated by naloxone (2.8 and $28\text{ }\mu\text{mol kg}^{-1}$), but not affected by i.v. injection of autonomic drugs (i.e. pentolinium, prazosin, yohimbine and atropine), by capsaicin desensitization, or by acute bilateral cervical vagotomy. In spinalized animals a sustained abdominal wall contraction (SAWC) was unmasked, which was resistant to i.v. morphine, clonidine or baclofen but suppressed by i.v. pancuronium or i.p. lignocaine 2% w/v.

3 Haemodynamic responses to i.p. NT were not affected by i.v. pancuronium, morphine, naloxone, atropine, or by vagotomy. They were inhibited by i.v. pentolinium or clonidine (BP, HR), i.v. prazosin (BP), i.p. procaine 2% w/v (BP, HR), capsaicin desensitization or acute spinalization (BP, HR). Yohimbine (i.v.) potentiated BP and HR increases caused by i.p. NT.

4 These results suggest that: (1) the haemodynamic and TAWC responses to i.p. NT in this animal model, are two independent, neurally-mediated reflexes which are likely to originate from the abdominal cavity and require a functionally intact spinal cord for their full expression; (2) the neural pathways subserving both types of responses appear to be different from each other. The nature and time-response characteristics of the reflexes caused by i.p. NT, raise the possibility that i.p. NT is a noxious stimulus, at least in guinea-pigs.

Keywords: Neurotensin; abdominal muscle; motor reflexes; haemodynamic effects

Introduction

Intraperitoneal (i.p.) injection of a low volume (i.e. 0.3 ml) of saline (i.e. 0.9% w/v NaCl), added with increasing amounts of neurotensin (NT), in the anaesthetized guinea-pigs with the abdomen opened, was previously shown to cause dose-dependent increases of systemic blood pressure (BP) and heart rate (HR) (Rioux *et al.*, 1989). Similar haemodynamic effects were also elicited by topical application of minute volumes (i.e. 10 to 20 μl) of NT solution to discrete areas of the visceral peritoneum of various organs (e.g. stomach, ileum, liver). Detailed pharmacological analysis led these authors to propose that NT, directly and/or indirectly, activates abdominal visceral afferents scattered in the peritoneum, and secondarily triggers sympathetically-mediated increases of BP and HR (Rioux *et al.*, 1989). More recently, using guinea-pigs with closed-abdomen rather than opened-abdomen and commercial, isotonic peritoneal dialysis solutions instead of saline as NT vehicle for i.p. injection, the same team showed that the amplitude of the increases of BP and HR caused by single i.p. injection of NT solution is directly related to NT concentrations, whereas the duration of both effects tended to increase with the volume of NT solution used (Rioux *et al.*, 1990a). The abdominal visceral afferents believed to be the target site of action of i.p. NT in guinea-pigs were tentatively identified as capsaicin-sensitive (i.e. small diameter, tachykinins-containing afferents) (Buck & Burks, 1986), based on the blockade of i.p. NT-induced haemodynamic effects by pretreatment of the animals with

capsaicin (Rioux *et al.*, 1990a). Besides causing pressor effects and tachycardia, i.p. injection of NT in the anaesthetized guinea-pig with opened-abdomen was also claimed (i.e. based on visual observation) to induce transient, clonic-like movements of the trunk sometimes associated with partial expulsion of intestinal loops out of the abdomen (Rioux *et al.*, 1989). In anaesthetized, closed-abdomen guinea-pigs the latter motor effects of i.p. NT were also observed, although in this condition, they were clearly seen as transient abdominal wall contractions (Rioux *et al.*, 1990a).

In the present study we examined the effect of various drugs (e.g. opioids, autonomic blockers, capsaicin and others) and surgical manipulations (i.e. vagotomy, spinalization) on both the abdominal motor and cardiovascular responses to i.p. NT in anaesthetized guinea-pigs. The main objective of the study was to obtain some insight into the nature of this motor response and examine the potential relationship between the haemodynamic and abdominal motor responses to i.p. NT.

Methods

General procedures

All experiments were done on female Albino guinea-pigs (325–375 g) purchased from Charles River Canada Inc., St-Constant, Québec, Canada. The animals were housed 4–6 per cage, given guinea-pig purina chow and tap water *ad libitum*, and kept under stable environmental conditions for a few days before use.

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The animals were anaesthetized with sodium pentobarbitone ($0.22 \text{ mmol kg}^{-1}$, i.p.), tracheotomized and artificially ventilated at a constant rate of 50 strokes min^{-1} (2.8–3 ml stroke) with ambient air from rodent ventilators (New England Medical Instr., Inc.). A polyethylene catheter (PE 50) filled with saline was introduced into the left jugular vein for intravenous (i.v.) drug injection. A second catheter filled with sodium heparin (200 u ml^{-1} of saline) was inserted into the right carotid artery for direct BP recording. BP was measured with arterial pressure transducers (Spectramed P 23 XL) connected to a Grass polygraph (Model 79). HR was derived from the BP signal. Body temperature was monitored with a rectal thermoprobe coupled to a telethermometer (Yellow Spring Instr.) and kept constant at $38.0\text{--}39.0^\circ\text{C}$ with a lamp (100 W) hung over the animal. A third catheter (PE 90) was introduced into the urinary bladder (via the urethra) to prevent overdistension of the bladder and ease micturition. A fourth catheter (PE 190) was implanted in the peritoneal cavity for i.p. injection of NT or its solvent as described previously (Rioux *et al.*, 1990a). A thread was attached to the skin of the animal right lower abdominal quadrant (i.e. inguinal region) and linked almost vertically, under a resting tension of 2.5–3 g, to a force displacement transducer (Grass F 103) coupled to a Grass polygraph (Model 79), to monitor the changes of basal abdominal wall tension (AWT) (i.e. an index of abdominal muscle tension), and the number of transient (i.e. $\sim 100\text{--}300 \text{ ms}$ duration) (twitch-like) abdominal wall contraction (TAWC) induced by i.p. injection of NT. Both animal hindlimbs were secured to the bench with tape to prevent lateral displacement of the animal body during volleys of TAWC caused by i.p. NT. Additional doses of anaesthetic were given intravenously to animals during preparatory procedures only (total doses: $0.1\text{--}0.12 \text{ mmol kg}^{-1}$, i.v.) to stabilize the anaesthesia level (as judged by suppression of corneal reflexes and stability of BP recording). The time interval between the last dose of anaesthetic and testing of NT effects was set at 40–45 min throughout the study.

Dose-response curves and kinetics

Once the preparation of animals was over and all parameters stabilized, each animal was given a single i.p. injection of 0.14, 1.4, 14 or 140 nmol kg^{-1} of NT (each dose in a volume of 9 ml) or of an equal volume of NT solvent (i.e. PDS). Each solution was pre-warmed at 38°C before injection. Twelve (12) s were needed to complete an i.p. injection. The basal mean arterial pressure (MAP) (i.e. $\text{MAP} = \text{diastolic pressure} + 1/3 \text{ pulse pressure}$), heart rate (HR) and abdominal wall tension (AWT) were measured before (i.e. 30–60 s) and at different times (i.e. 10, 20, 30, 60 s, 2, 5, 10, 15 min) after i.p. injection of NT or its solvent. Changes (Δ) of MAP, HR, or AWT caused by i.p. injection of NT or its solvent were used to build dose-response curves (MAP, HR) and/or describe kinetics (MAP, HR, AWT) of NT effects. Other parameters measured were, the number of animals showing TAWC (n_1), and the number of TAWC per animal (TAWC/animal), caused by i.p. injection of NT or its solvent, over a 15 min post-i.p. injection period. These parameters were easily obtained from AWT tracings (Figure 2). The percentage of animals exhibiting TAWC and TAWC/animal values were used for describing dose- or time-response relationships. In parallel studies the animals were challenged with i.p. injection of either bradykinin (BK) or substance P (SP) (14, 140 or $1400 \text{ nmol kg}^{-1}$) (each dose given in a volume of 9 ml), to assess the response selectivity of the experimental animal model.

Animal pretreatment with selected drugs

A first series of experiments was conducted with animals pretreated with various doses of morphine, a potent analgesic drug known to suppress various nociceptive reflexes (Jaffe &

Martin, 1990) or of naloxone, an opioid receptor antagonist known to potentiate various reflexes (Duggan, 1983). A second series of experiments involved animal pretreatment with various drugs known to interfere with adrenergic and/or cholinergic neural pathways, such as pentolinium (nicotinic receptor antagonist and ganglion blocker), prazosin (α -adrenoceptor antagonist), clonidine (α_2 -adrenoceptor agonist, and centrally and peripherally acting sympatholytic), yohimbine (α_2 -adrenoceptor antagonist), atropine (muscarinic receptor blocker), and pancuronium (nicotinic receptor antagonist and neuromuscular blocker). All drugs were given by i.v. bolus injection 30 min before single i.p. injection of NT (14 nmol kg^{-1}). Only one type of drug and one drug dose were tested per animal. The volume of each injection was $0.1\text{--}0.2 \text{ ml}$, followed by 0.2 ml of saline, to rinse the catheter. Each experimental series had its own control group (i.e. animals given i.v. bolus injection of drug solvent). All these studies involved comparing, ΔMAP , ΔHR , n_1 and TAWC/animal values in control animals (i.e. drug solvent-pretreated animals) with those measured in drug-pretreated animals.

Intraperitoneal injection of a local anaesthetic

The experiments involved comparing ΔMAP , ΔHR , n_1 and TAWC/animal values measured in animals given single i.p. injection of NT (14 nmol kg^{-1}) plus procaine solvent (i.e. 0.5 ml of PDS) (control) (total volume injected: 9 ml), to those measured in animals given single i.p. injection of NT (as above) plus procaine 2% w/v. Parallel experiments involved measurement and comparison of peak pressor and tachycardic responses to i.v. bolus injection of dimethylphenylpiperazinium (DMPP) (310 nmol kg^{-1}), a ganglion stimulant, in animals first given (i.e. 2 min before) either i.p. procaine 2% w/v (as above) or i.p. procaine solvent.

Capsaicin treatment

Experimental animals were given s.c. injection of capsaicin suspension ($0.08 \text{ mmol kg}^{-1}$, once a day for two consecutive days). All treatments were done under anaesthesia with a mixture of ketamine ($0.16 \text{ mmol kg}^{-1}$) and xylazine ($0.02 \text{ mmol kg}^{-1}$) given intramuscularly (i.m.). All animals were given prophylactic doses of salbutamol (210 nmol kg^{-1} , s.c. and aminophylline ($0.06 \text{ mmol kg}^{-1}$, i.p.) 15 min before capsaicin, to prevent respiratory distress due to capsaicin-induced bronchospasms (Lundberg *et al.*, 1985). Control animals received similar amounts of capsaicin solvent (i.e. 0.4 ml of a mixture of ethanol 10% w/v, Tween 80 10% v/v and saline), and the same drug treatments (i.e. anaesthetics, bronchodilators) as experimental animals, except that no capsaicin was given. ΔMAP , ΔHR , n_1 and TAWC/animal following i.p. injection of NT were then measured in both animal groups and compared.

Vagotomy and spinalization

Acute bilateral vagotomy (at the cervical level) or complete transection of the spinal cord (between C2 and C3) (i.e. spinalization) were performed under anaesthesia according to procedures recently described (Beauchamp *et al.*, 1991). Control animals were sham-operated (i.e. exposed to the same surgical procedures except for the vagotomy or spinalization). These surgical interventions were made 45–50 min before i.p. injection of NT (14 nmol kg^{-1}) or its solvent. ΔMAP , ΔHR , n_1 and TAWC/animal values following i.p. injection of NT were then measured in both animal groups and compared to those measured in their respective controls. Additional experiments were done with spinalized guinea-pigs which, 30 min before being challenged with i.p. NT (14 nmol kg^{-1}), were given i.v. bolus injection of saline (control) or of one of the following drugs (doses in $\mu\text{mol kg}^{-1}$): pancuronium (0.27), morphine (15.0), clonidine (0.34) or baclofen (10.0). Other experiments in this series involved pre-addition

of procaine or lignocaine 2% w/v to NT solution before i.p. injection in spinalized guinea-pigs. The purpose of the latter experiments was to define better the mechanism of the relatively more sustained abdominal wall contraction observed in spinalized guinea-pigs (Figure 3) given i.p. injection of NT. Finally peak changes of MAP or HR caused by i.v. bolus injection of either noradrenaline, DMPP or pentolinium were compared in spinalized guinea-pigs and sham-operated animals to rule out any non-specific effect of spinalization on the cardiovascular or sympathetic nervous system.

Drugs, solutions and statistics

Drugs used in this study were: neurotensin triacetate (NT) (Peninsula), capsaicin, (–)-noradrenaline. HCl (NA), pentolinium tartrate, sodium heparin, procaine HCl, dimethylphenylpiperazinium iodide (DMPP), atropine methylnitrate, yohimbine HCl (all from Sigma), morphine sulphate and sodium pentobarbitone (Sabex Inc.), naloxone HCl (Endo Lab. Inc.), clonidine HCl (Boehringer), prazosin HCl (Pfizer), lignocaine HCl (Xylocard) (Astra), ketamine HCl (Rogar/STB. Inc.), xylazine (Rompun) (Bayvet), theophylline (Aminophylline) (Abbot), baclofen (lioresal) (Ciba), and salbutamol sulphate (Allen & Hanburys). Most drugs were dissolved in saline or distilled water, except for capsaicin which was dissolved in absolute ethanol. Drug solutions were made fresh daily. Drug dilutions for i.v. injections were made in saline. Drug dilutions for i.p. injections were made in commercial, sterile, isotonic peritoneal dialysis solution (PDS) (Dianeal PD 101 CAPD PDS with 0.5% dextrose) (Baxter), the composition of which was (in mM): dextrose 28.0, Na^+ 132.0, Ca^{2+} 1.62, Mg^{2+} 0.75, Cl^- 101.7, lactate 35.0. KCl (4 mM) was added to PDS solution. The pH and osmolality of PDS were 5.2 and 306 mOsm l^{-1} , respectively. Solutions of NA were acidified to pH 4.0 with HCl and added with ascorbic acid (0.5 mM) to prevent oxidation. Drug doses are given in nano-, micro-, or millimol of the salt per kg of the animal body weight, except for local anaesthetics (in %).

Statistical significances of results were determined by one-way analysis of variance (ANOVA) followed by the Dunnett's test, or by the Chi-square test or Student's *t* test for unpaired data (Tallarida & Murray, 1981). *P* values of 0.05 or less were considered significant.

Results

Dose-response curves and time courses of neurotensin effects

Single i.p. injection of 9 ml of PDS (i.e. NT solvent) in anaesthetized guinea-pigs, caused no significant changes of mean arterial pressure (MAP) or heart rate (HR), and no transient abdominal wall contraction (TAWC). However it evoked a sudden, persistent decrease of basal abdominal wall tension (AWT) (an artefactual consequence of abdominal distension caused by i.p. injection of 9 ml of fluid, in this case of PDS). On the other hand, single i.p. injection of NT (0.14, 1.4, 14 or 140 nmol kg^{-1}) (each dose given in a volume of 9 ml) elicited dose-dependent increases of MAP, HR, number of animals showing TAWC, and number of TAWC/animal. These results are shown in Figure 1. A typical tracing illustrating the changes of BP or AWT, and the occurrence of TAWC following single i.p. injection of NT (14 nmol kg^{-1}), is illustrated in Figure 2. Data describing the time sequence of cardiovascular and abdominal motor responses to i.p. injection of NT (14 nmol kg^{-1}) are presented in Table 1. Note the rapid onset (i.e. <10 s) and short times to peak effect for all the measured parameters. The basal AWT decreased to the same extent, although at a slightly lower rate in animals given i.p. injection of NT than in animals given the NT solvent. Eight (8) out of 8 animals (100%)

Figure 1 (a) Dose-response curves of pressor and tachycardic responses to single intraperitoneal (i.p.) injection of neurotensin (NT, 0.14, 1.4, 14 and 140 $\times 10^{-9}$ mol kg^{-1} , $n = 8, 7, 8$ and 6, respectively), in anaesthetized guinea-pigs. Ordinates: peak increases (+) (irrespective of time to peak) of basal mean arterial pressure (ΔMAP) (●) or heart rate (ΔHR) (○). Abscissae: $-\log \text{NT dose}$, mmHg, millimeters of mercury; b.p.m., heart beat min^{-1} . Points are mean values with s.e.mean shown by vertical bars. (b) Dose-response curves showing the percentage (%) of animals exhibiting transient abdominal wall contraction (TAWC) (% responders) (■) or number of TAWC/animal (□), following single i.p. injection of NT in anaesthetized guinea-pigs (same animals as above). Ordinates: % responders or number of TAWC/animal; abscissae: $-\log \text{NT dose}$. (■) % of responders; (□) number of TAWC per animal; mean values $\pm$ s.e.mean.

* $P < 0.05$; ** $P < 0.01$ when compared to control values (i.e. values measured in animals given single i.p. injection of NT solvent) (Dunnett's test)

† $P < 0.05$; †† $P < 0.005$ when compared to control values (Chi-square test)

challenged with i.p. injection of 14 nmol kg^{-1} of NT exhibited TAWC. $\Delta \text{TAWC/animal}$ became significant 60 s post-i.p. injection of the latter NT dose.

Single i.p. injection of bradykinin (BK) (14, 140 or 1400 nmol kg^{-1}), in anaesthetized guinea-pigs, elicited significant pressor effects (peak ΔMAP values: $+14.3 \pm 4.1$, $+13.2 \pm 5.0$, $+30.5 \pm 4.0$ mmHg, $n = 6, 6$ or 8, respectively), but only modest, variable levels of tachycardia (peak ΔHR values: $+10 \pm 3$, $+7 \pm 5$, $+21 \pm 6$ b.p.m.). TAWC were seen only in 1 out of 20 animals given i.p. injection of BK. Single i.p. injection of substance P (SP) (same dose as for BK) elicited slight decreases of MAP (peak ΔMAP values: -3.3 ± 1.1 , -8.3 ± 1.7 , -11.5 ± 1.6 mmHg, $n = 6, 6$ and 6, respectively) and HR (peak ΔHR values: -7 ± 2 , -8 ± 4 , -13 ± 3 b.p.m.), but no TAWC was seen in any of these animals.

Effects of morphine and naloxone

The pressor and tachycardic responses to single i.p. injection of 14 nmol kg^{-1} of NT were not affected by prior animal

treatment with i.v. bolus injection of 1.5 or 15.0 $\mu\text{mol kg}^{-1}$ (i.e. 1 and 10 mg kg^{-1}) of morphine. On the other hand, the number of animals exhibiting TAWC (n_1) and the number of TAWC/animal, following i.p. NT, were markedly reduced by morphine. These results are summarized in Table 2. A lower dose of morphine (i.e. 0.15 $\mu\text{mol kg}^{-1}$) was not effective in reducing the abdominal motor response to i.p. NT ($n = 8$, data not shown). Animal pretreatment with i.v. bolus injection of 2.8 or 28.0 $\mu\text{mol kg}^{-1}$ (i.e. 1 and 10 mg kg^{-1}) of naloxone, was found to markedly increase n_1 and TAWC/animal values, without altering the pressor and tachycardic responses to i.p. NT (Table 2). A lower dose of naloxone (i.e. 0.28 $\mu\text{mol kg}^{-1}$) did not modify the abdominal motor responses to i.p. NT ($n = 8$, data not shown). Combined i.v. administration of morphine (15 $\mu\text{mol kg}^{-1}$) and naloxone (28.0 $\mu\text{mol kg}^{-1}$) gave results suggesting inhibition of the morphine suppressive effect on abdominal motor responses by naloxone (Table 2).

Animal pretreatment with i.v. bolus injection of naloxone

Figure 2 Typical tracing showing the time course of changes of basal abdominal wall tension (AWT) (1st tracing from the top) and systemic blood pressure (BP) (2nd tracing from the top) in anaesthetized guinea-pigs following single i.p. injection of neurotensin (NT) (14 nmol kg^{-1}). The 3rd and 4th tracing (from the top) are continuations of 1st and 2nd tracings, respectively. The arrow indicates the end of i.p. injection of NT. Note the rapid onset of the pressor effect and occurrence of transient abdominal wall contractions (TAWC) (*) at different times after i.p. injection of NT. The slight, gradual decrease of AWT during filling of the peritoneal cavity is mainly an artefact reflecting fluid-induced distension of the abdomen. Small waves 'contaminating' AWT tracings are due to animal respiratory (i.e. diaphragm) movements. mmHg: millimeters of mercury; g, grams.

(2.8 $\mu\text{mol kg}^{-1}$) tended to increase the abdominal motor response to a single i.p. injection of a low NT dose (0.14 nmol kg^{-1}). In control (naloxone-free) animals, 1 out of 8 animals exhibited TAWC, whereas 3 out of 8 animals pretreated with naloxone, exhibited TAWC (TAWC/animal = 0.1 ± 0.1 in control animals and 17 ± 9 in naloxone-pretreated animals).

Effect of drugs interfering with cholinergic and/or adrenergic mechanisms

The results of these experiments are compiled in Table 3. Animals pretreatment with i.v. bolus injection of either pentolinium, prazosin or clonidine reduced the pressor effect elicited by i.p. injection of NT (14 nmol kg^{-1}). Pentolinium and clonidine also inhibited the tachycardic responses to i.p. NT. Of the latter 3 drugs, only clonidine was able to inhibit to some extent the abdominal motor responses to i.p. NT. The pressor and tachycardic responses to i.p. NT were increased in animals pretreated with yohimbine, but the latter drug did not affect the abdominal motor response to i.p. NT. Animal pretreatment with atropine did not affect pressor, tachycardic and abdominal motor responses to i.p. NT, whereas pancuronium completely suppressed the abdominal motor responses, without affecting the cardiovascular responses, to i.p. NT. The suppressive effect of clonidine (0.34 $\mu\text{mol kg}^{-1}$) on abdominal motor and cardiovascular responses to i.p. NT was reversed in animals given yohimbine (5.1 $\mu\text{mol kg}^{-1}$) simultaneously with clonidine.

Effects of procaine, vagotomy, capsaicin or spinalization

The results of these 4 series of experiments are shown in Table 4. The pressor, tachycardic and abdominal motor (i.e. TAWC) responses to single i.p. injection of (14 nmol kg^{-1}) were reduced (pressor) or abolished (tachycardic, TAWC), when NT solution was pre-mixed with procaine 2% w/v. On the other hand, peak pressor and tachycardic responses to i.v. bolus injection of DMPP (310 nmol kg^{-1}) in 4 animals first given (i.e. 2 min before) i.p. injection of 9 ml of procaine 2% w/v (ΔMAP and ΔHR values: $+34.7 \pm 3.3$ mmHg and $+45 \pm 12$ b.p.m., respectively), were similar to those measured in 4 animals first given i.p. injection of 9 ml of procaine solvent (i.e. PDS) (ΔMAP and ΔHR values: $+29.0 \pm 3.8$ mmHg and $+40 \pm 8$ b.p.m., respectively).

The pressor, tachycardic and TAWC responses to i.p. NT were of similar amplitude in vagotomized animals as in their controls. Animals pretreated 4 to 5 days previously with capsaicin (see Methods) exhibited reduced cardiovascular

Table 1 Time-related changes (Δ) of MAP, HR, AWT and TAWC/animal associated with i.p. injection of neurotensin (NT) in anaesthetized guinea-pigs*

Time after i.p. injection	ΔMAP (mmHg)	ΔHR (b.p.m.)	ΔAWT (g)	$\Delta\text{TAWC/animal}$
0	0	0	0	0
10 s	$+22.1 \pm 4.9^\P$	$+12 \pm 6^\dagger$	$-0.6 \pm 0.2^\dagger$	$+3 \pm 2$
20 s	$+30.4 \pm 4.2^{**}$	$+21 \pm 7^\S$	$-0.6 \pm 0.2^*$	$+2 \pm 1$
30 s	$+32.7 \pm 4.7^{**}$	$+25 \pm 8^\S$	-1.0 ± 0.1	$+2 \pm 1$
60 s	$+46.0 \pm 4.9^{**}$	$+40 \pm 10^{**}$	-1.2 ± 0.1	$+3 \pm 1^\dagger$
2 min	$+52.9 \pm 3.9^{**}$	$+49 \pm 7^{**}$	-1.0 ± 0.1	$+2 \pm 1$
5 min	$+40.2 \pm 4.1^{**}$	$+56 \pm 10^{**}$	-0.9 ± 0.1	0
10 min	$+32.4 \pm 3.3^{**}$	$+54 \pm 10^{**}$	-0.8 ± 0.1	0
15 min	$+28.6 \pm 3.3^{**}$	$+54 \pm 9^{**}$	-0.8 ± 0.2	0
ANOVA	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$

*Each animal was given a single intraperitoneal (i.p.) injection of NT (14 nmol kg^{-1}) (volume of NT solution injected = 9 ml). ΔMAP , ΔHR , ΔAWT and $\Delta\text{TAWC/animal}$ indicate increases (+) or decreases (−) of basal mean arterial pressure (MAP), heart rate (HR), abdominal wall tension (AWT) and in the number of abdominal wall contractions per animal (TAWC/animal) respectively, as measured in 8 anaesthetized guinea-pigs at different times after i.p. injection of NT. Results are expressed as mean $\pm$ s.e.mean. mmHg, millimeter of mercury; b.p.m., heart beats min^{-1} ; g, grams. Basal values of MAP, HR, AWT and TAWC/animal (i.e. before i.p. injection) were respectively 39.9 ± 1.8 mmHg, 254 ± 7 b.p.m., 2.4 ± 0.2 g and 0.

* $P < 0.05$; $^\dagger P < 0.02$; $^\S P < 0.01$; $^\P P < 0.005$; $^{**} P < 0.001$ when compared to corresponding control values (i.e. values measured in animals given single i.p. injection of 9 ml of NT solvent) (Student's *t* test)

Table 2 Effect of morphine and naloxone on pressor, tachycardic and abdominal motor responses to single i.p. injection of neurotensin (NT) in anaesthetized guinea-pigs^a

Treatment ($\mu\text{mol kg}^{-1}$)	n	MAP (mmHg)	ΔMAP (mmHg)	HR (b.p.m.)	ΔHR (b.p.m.)	n ₁	TAWC/animal
Control	8	39.9 $\pm$ 1.8	+53.2 $\pm$ 4.1	254 $\pm$ 7	+61 $\pm$ 9	8	11 $\pm$ 2
Morphine (1.5)	6	39.5 $\pm$ 1.5	+58.5 $\pm$ 4.5	268 $\pm$ 10	+42 $\pm$ 8	2†	0.3 $\pm$ 0.2*
Morphine (15.0)	10	33.3 $\pm$ 1.1*	+57.1 $\pm$ 3.5	237 $\pm$ 6	+61 $\pm$ 8	0††	0*
Naloxone (2.8)	6	38.2 $\pm$ 3.3	+51.7 $\pm$ 2.8	252 $\pm$ 5	+62 $\pm$ 10	6	43 $\pm$ 3¶
Naloxone (28.0)	8	39.5 $\pm$ 2.0	+56.4 $\pm$ 4.0	251 $\pm$ 4	+64 $\pm$ 8	8	53 $\pm$ 4¶
Morphine (15.0) + naloxone (28.0)	6	33.5 $\pm$ 1.0	+56.2 $\pm$ 2.4	237 $\pm$ 4	+60 $\pm$ 4	6§	27 $\pm$ 5¶§
ANOVA		P<0.05	NS	P<0.05	NS		P<0.01

^aAnimals were given single i.v. bolus injection of either saline (drug vehicle, control), morphine or naloxone 30 min before being given single i.p. injection of NT (14 nmol kg⁻¹). Results are expressed as mean $\pm$ s.e.mean. n, number of animals tested. MAP, basal mean arterial pressure (i.e. before i.p. injection of NT); HR, basal heart rate. ΔMAP and ΔHR indicate peak increases (+) of MAP or HR (irrespective of time to peak) over the 15 min period which follows i.p. injection of NT. mmHg, millimeters of mercury; b.p.m. heart beat per min. n₁, number of animals exhibiting transient abdominal wall contraction (TAWC) during the 15 min period of observation after i.p. injection of NT. TAWC/animal, number of TAWC per animal. NS, not statistically significant.

*P<0.05; ¶P<0.01 when compared to the control value (Dunnett's test)

†P<0.01; ††P<0.005 when compared to the control value (Chi-square test)

§P<0.01 when compared to the values measured in animals pretreated with morphine (15 $\mu\text{mol kg}^{-1}$) alone (Student's *t* test or Chi-square test)

Table 3 Effect of various drugs on pressor tachycardic and abdominal motor responses to single i.p. injection of neurotensin (NT) in anaesthetized guinea-pigs^a

Treatment ($\mu\text{mol kg}^{-1}$)	n	MAP (mmHg)	ΔMAP (mmHg)	HR (b.p.m.)	ΔHR (b.p.m.)	n ₁	TAWC/animal
Control	8	35.5 $\pm$ 1.5	+47.2 $\pm$ 1.9	250 $\pm$ 4	+49 $\pm$ 5	8	11 $\pm$ 2
Pentolinium (2.8)	8	27.4 $\pm$ 1.4*	+17.8 $\pm$ 1.7†	229 $\pm$ 6	+19 $\pm$ 4*	6	9 $\pm$ 3
Prazosin (1.9)	6	31.7 $\pm$ 1.7	+14.5 $\pm$ 2.7†	263 $\pm$ 6	+68 $\pm$ 12	5	15 $\pm$ 5
Clonidine (0.34)	8	39.0 $\pm$ 1.6	+1.6 $\pm$ 1.8†	215 $\pm$ 6†	+7 $\pm$ 5†	2§§	3 $\pm$ 3
Yohimbine (5.1)	11	38.2 $\pm$ 1.6	+66.1 $\pm$ 3.2†	259 $\pm$ 9	+93 $\pm$ 10†	9	15 $\pm$ 3
Atropine (4.1)	6	35.7 $\pm$ 2.4	+54.0 $\pm$ 4.4	245 $\pm$ 8	+68 $\pm$ 3	5	11 $\pm$ 3
Pancuronium (0.27)	5	38.6 $\pm$ 2.7	+51.2 $\pm$ 4.8	266 $\pm$ 7	+64 $\pm$ 8	0§§	0
Clonidine (0.34) + yohimbine (5.1)	8	31.0 $\pm$ 3.3	+53.8 $\pm$ 4.0§	211 $\pm$ 3†	+80 $\pm$ 11*§	6¶	10 $\pm$ 2
ANOVA		P<0.01	P<0.01	P<0.01	P<0.01		P<0.05

^aAnimals were given single i.v. bolus injection of saline (drug vehicle, control) or of one of the drugs listed above, 30 min before being given single i.p. injection of 14 nmol kg⁻¹ of NT. Results are expressed as mean $\pm$ s.e.mean. n, number of animals tested. MAP, basal mean arterial pressure (i.e. before i.p. injection of NT); HR, basal heart rate. ΔMAP and ΔHR indicate peak increases (+) of MAP or HR (irrespective of time to peak) over the 15 min period which follows i.p. injection of NT. mmHg, millimeters of mercury; b.p.m., heart beat per min. n₁, number of animals exhibiting transient abdominal wall contraction (TAWC) during the 15 min period of observation after i.p. injection of NT. TAWC/animal indicates the number of TAWC per animal.

*P<0.05; †P<0.01 when compared to respective control value (Dunnett's test)

§P<0.001 when compared to corresponding values of clonidine-pretreated animals (Student's *t* test)

§§P<0.005 when compared to the control value (i.e. saline-pretreated animals) (Chi-square test)

¶P<0.05 when compared to corresponding values of clonidine-pretreated animals (Chi-square test)

Table 4 Effect of various drug treatments or surgical interventions on pressor, tachycardic and abdominal motor responses to single i.p. injection of neurotensin (NT) in anaesthetized guinea-pigs^a

Exp. No.	Treatment	n	MAP (mmHg)	ΔMAP (mmHg)	HR (b.p.m.)	ΔHR (b.p.m.)	n ₁	TAWC/animal
1	Control	6	35.5 $\pm$ 1.8	+55.5 $\pm$ 3.0	250 $\pm$ 5	+52 $\pm$ 5	6	19 $\pm$ 4
	Procaine	6	38.3 $\pm$ 2.8	+20.0 $\pm$ 4.0§	257 $\pm$ 2	-23 $\pm$ 10§	0**	0§
2	Control	8	36.1 $\pm$ 2.0	+56.6 $\pm$ 2.6	245 $\pm$ 4	+74 $\pm$ 5	8	21 $\pm$ 6
	Vagotomy	8	32.9 $\pm$ 2.1	+52.4 $\pm$ 4.1	251 $\pm$ 6	+60 $\pm$ 7	8	10 $\pm$ 2
3	Control	8	41.4 $\pm$ 2.0	+50.6 $\pm$ 1.9	257 $\pm$ 6	+50 $\pm$ 7	8	16 $\pm$ 3
	Capsaicin	8	35.5 $\pm$ 1.9*	+16.2 $\pm$ 3.0§	250 $\pm$ 5	+20 $\pm$ 5¶	6	17 $\pm$ 4
4	Control	8	41.2 $\pm$ 1.8	+54.0 $\pm$ 5.6	258 $\pm$ 10	+67 $\pm$ 9	7	7 $\pm$ 2
	Spinalized	8	34.7 $\pm$ 1.4†	-1.5 $\pm$ 1.9§	245 $\pm$ 10	0 $\pm$ 6§	0**	0¶

^aAll animals received single i.p. injection of 14 nmol kg⁻¹ of NT. Exp. no. 1: i.p. NT solution was added with procaine 2% w/v or an equivalent volume of procaine solvent (control). Exp. no. 2: animals underwent vagotomy or sham operation (control) 45–50 min before i.p. NT. Exp. no. 3: animals were pretreated 4 days previously with capsaicin or its solvent (control) (see Methods) before i.p. NT. Exp. no. 4: animals underwent spinalization or sham operation (control) (see Methods), 45–50 min before i.p. NT. Results are expressed as mean $\pm$ s.e.mean. Other abbreviations as in Table 3.

*P<0.05; †P<0.02; ¶P<0.005 and §P<0.001 when compared to respective control value (Student's *t* test)

**P<0.005 when compared to respective control value (Chi-square test)

(MAP, HR) responses to i.p. injection of NT, but TAWC responses were not affected. Acute transection of the spinal cord (between C2–C3) 45–50 min before, completely suppressed the cardiovascular and TAWC responses to i.p. NT. Interestingly, slowly developing, relatively sustained abdominal wall contractions (SAWC) occurred in spinalized guinea-pigs given i.p. NT (Figure 3). This unexpected response was not seen in sham-operated animals. The latter animals rather exhibited the usual decrease of AWT associated with i.p. NT (see Figure 4b and Table 1). Single i.p. injection of 9 ml of NT solvent (i.e. PDS) in spinalized guinea-pigs elicited similar decreases of AWT as in sham-operated (control) animals (Figure 4a).

Effect of selected drugs on sustained abdominal wall contractions

Various drugs were tested as potential inhibitors of SAWC in anaesthetized, spinalized guinea-pigs given i.p. NT (14 nmol kg^{-1}). Results are compiled in Table 5. Animal pretreatment with i.v. bolus injection of pancuronium or pre-addition of lignocaine 2% w/v to NT solution for i.p. injection, abolished SAWC. Neither i.v. morphine, clonidine or baclofen, nor pre-addition of procaine 2% w/v to NT solution for i.p. injection, modified the amplitude of SAWC.

Effect of naloxone in spinalized animals

Intravenous bolus injection of naloxone ($5.6 \mu\text{mol kg}^{-1}$) in anaesthetized, spinalized guinea-pigs (see Methods) partially restored the pressor (ΔMAP), tachycardic (ΔHR) and TAWC responses to i.p. injection of NT (14 nmol kg^{-1}). Peak ΔMAP values in 9 naloxone-treated spinalized animals were $+15.3 \pm 3.4 \text{ mmHg}$ vs $-1.5 \pm 1.9 \text{ mmHg}$ in 8 control (i.e. saline-pretreated) spinalized animals ($P < 0.001$) (Student's *t* test). Peak ΔHR values in 9 naloxone-treated spinalized animals were $+26 \pm 12 \text{ b.p.m.}$ vs $0 \pm 6 \text{ b.p.m.}$ in 8 control animals ($P > 0.05$). Basal (i.e. pre-i.p. NT) MAP and HR values in naloxone-treated spinalized animals were $35.9 \pm 2.0 \text{ mmHg}$ and $233 \pm 4 \text{ b.p.m.}$ ($n = 9$), respectively. Corresponding values in naloxone-free spinalized animals were $34.7 \pm 1.4 \text{ mmHg}$ and $245 \pm 10 \text{ b.p.m.}$ ($n = 8$). Three (3) out of 9 naloxone-treated spinalized animals exhibited TAWC (TAWC/animal: 9 ± 4 , $P < 0.05$, Student's *t* test), whereas 0 out of 8 naloxone-free, spinalized animals exhibited TAWC. Naloxone treatment did not modify the amplitude or time course of SAWC caused by i.p. NT in spinalized guinea-pigs.

Figure 3 Typical tracing showing the time course of changes of basal abdominal wall tension (AWT) (1st tracing from the top) and systemic blood pressure (BP) (2nd tracing from the top) in anaesthetized, spinalized (i.e. acute transection of the spinal cord at the C2–C3 level) guinea-pigs, following single i.p. injection of neurotensin (NT) (14 nmol kg^{-1}). The 3rd and the 4th tracing are continuations of 1st and 2nd tracings, respectively. The arrow indicates the end of i.p. injection of NT. Note the slowly developing, relatively sustained contraction of the abdominal wall starting before the i.p. injection is over, the absence of transient abdominal wall contractions (or twitches) and absence of pressor effect after i.p. injection of NT (see Figure 1 for comparison). mmHg, millimeters of mercury; g, grams.

Effect of i.v. bolus injection of NA, DMPP or pentolinium in spinalized guinea-pigs

Peak pressor and tachycardic responses to single i.v. bolus injection of noradrenaline (NA) (7.3 nmol kg^{-1}) or DMPP (310 nmol kg^{-1}) in anaesthetized spinalized guinea-pigs were similar to those measured in control (sham-operated) guinea-pigs (ΔMAP and ΔHR values for NA in 5 control guinea-pigs: $+39.6 \pm 2.1 \text{ mmHg}$ and $+54 \pm 7 \text{ b.p.m.}$; corresponding values in 5 spinalized guinea-pigs: $+42.0 \pm 3.1 \text{ mmHg}$ and $+58 \pm 10 \text{ b.p.m.}$; ΔMAP and ΔHR values for DMPP in 4 control guinea-pigs: $+37.2 \pm 6.2 \text{ mmHg}$ and $+38 \pm 9 \text{ b.p.m.}$; corresponding values in 4 spinalized guinea-pigs: $+36.5 \pm 2.4 \text{ mmHg}$ and $+35 \pm 10 \text{ b.p.m.}$). Peak hypotensive and bradycardic responses to single i.v. bolus injection of pentolinium ($3.7 \mu\text{mol kg}^{-1}$) in 6 anaesthetized spinalized guinea-pigs (ΔMAP and ΔHR : $-13.5 \pm 1.3 \text{ mmHg}$ and $-23 \pm 2 \text{ b.p.m.}$) were similar to those measured in 6 sham-operated (control) guinea-pigs (ΔMAP and ΔHR values: $-14.8 \pm 2.7 \text{ mmHg}$ and $-32 \pm 5 \text{ b.p.m.}$).

Table 5 Effect of various drug treatments on basal abdominal wall tension (AWT) associated with single i.p. injection of neurotensin (NT) in anaesthetized, spinalized guinea-pigs*

Treatment	AWT (g)	ΔAWT (g)				
		5 s	10 s	20 s	30 s	60 s
Control (8)	2.6 ± 0.1	$+1.8 \pm 0.3$	$+1.6 \pm 0.4$	$+1.4 \pm 0.4$	$+1.0 \pm 0.4$	$+0.4 \pm 0.3$
PCR (6)	$2.9 \pm 0.1^*$	$-1.0 \pm 0.1^\dagger$	$-0.9 \pm 0.1^\dagger$	$-0.3 \pm 0.1^\dagger$	$-0.4 \pm 0.1^\dagger$	$-0.5 \pm 0.1^*$
Mor (4)	2.9 ± 0.1	$+2.5 \pm 0.7$	$+2.1 \pm 0.6$	$+1.7 \pm 0.5$	$+1.5 \pm 0.4$	$+1.1 \pm 0.2$
Clo (6)	2.8 ± 0.1	$+1.5 \pm 0.7$	$+1.8 \pm 0.7$	$+1.9 \pm 0.5$	$+1.5 \pm 0.4$	$+0.7 \pm 0.4$
Bac (4)	$2.9 \pm 0.1^*$	$+2.5 \pm 0.7$	$+1.6 \pm 0.7$	$+1.2 \pm 0.5$	$+0.8 \pm 0.4$	$+0.5 \pm 0.4$
Pro (6)	2.7 ± 0	$+0.6 \pm 0.3$	$+1.1 \pm 0.3$	$+1.1 \pm 0.2$	$+0.6 \pm 0.1$	$+0.1 \pm 0.1$
Lig (6)	2.8 ± 0.1	$-0.3 \pm 0.3^*$	$-0.1 \pm 0.2^*$	$-0.4 \pm 0.1^\dagger$	$-0.6 \pm 0.1^\dagger$	$-0.5 \pm 0.1^*$
ANOVA	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

*All animals had their spinal cord completely transected (between C2–C3) 45–50 min before receiving single i.p. injection of NT (14 nmol kg^{-1}). Thirty (30) min before i.p. NT, the animals received i.v. bolus injection of either saline (control) or of one of the following drugs (doses in $\mu\text{mol kg}^{-1}$): pancuronium (PCR) (0.27), morphine (mor) (15.0), clonidine (Clo) (0.34), or baclofen (Bac) (10.0). Procaine (Pro) (2% w/v) or lignocaine (Lig) (2% w/v) were added to NT solution before i.p. injection. AWT, basal abdominal wall tension; ΔAWT indicates increases (+) or decreases (–) of AWT at different times (over a 60 s period) after i.p. NT. g, grams. Results are expressed as mean $\pm$ s.e.mean; the number of animals per group is given in parentheses on the left.

* $P < 0.05$; $^\dagger P < 0.01$ when compared to the corresponding control value (Dunnett's test)

Figure 4 (a) Time courses of the decreases (–) of basal abdominal wall tension (AWT) in anaesthetized, sham-operated (control) (●) ($n = 4$) or spinalized (○) ($n = 4$) guinea-pigs at different times after single i.p. injection of neurotensin (NT) solvent (i.e. PDS). Ordinate scale: changes of AWT (Δ AWT); abscissa scale: time after i.p. injection of NT solvent, g, grams. Note the similar effect of i.p. injection of NT solvent on AWT in both animal groups. (b) Time courses of the decreases (–) or increases (+) of AWT in anaesthetized, sham-operated (control) (■) ($n = 8$) or spinalized (□) ($n = 8$) guinea-pigs, following single i.p. injection of NT (14 nmol kg^{-1}). Ordinate and abscissae scales, as above. Note the relatively sustained contraction of the abdominal wall in spinalized guinea-pigs compared to controls, following i.p. NT.

* $P < 0.02$; ** $P < 0.005$; † $P < 0.001$ when compared to corresponding control values (Student's t test).

Discussion

The ability of i.p. NT to induce TAWC in anaesthetized guinea-pigs, is a relatively novel observation (see Introduction). Evidence presented in this paper suggests that TAWC is a neurally-mediated reflex response that originates within the peritoneal cavity and involves the contraction of abdominal muscles. This conclusion is based on several facts. First, TAWC are suppressed by animal pretreatment with pancuronium, a neuromuscular blocker that inhibits nicotinic receptors, thus indicating that TAWC result from the activation of cholinergic motoneurons that innervate abdominal muscles. Second, the concomitant i.p. injection of procaine, a local anaesthetic, and NT, prevents TAWC caused by i.p. NT, thus suggesting that i.p. NT caused TAWC by means of activation of abdominal visceral afferents presumably making mono- and/or polysynaptic contacts with cholinergic motoneurons innervating abdominal muscles. Third, TAWC were dose-dependently suppressed by i.v. morphine, a potent opioid agonist known to suppress various nociceptive reflexes (Jaffe & Martin, 1990). Fourth, animal pretreatment with naloxone, a potent opioid receptor antagonist which potentiates various reflexes (Duggan, 1983), was shown to markedly increase the number of TAWC/animal, and to suppress the effect of morphine on TAWC. Fifth, acute spinalization was found to suppress TAWC as well as pressor and tachycardic responses caused by i.p. NT, thus indicating that the spinal cord is likely to be an important relay centre for the pressor, tachycardic and TAWC responses to i.p. NT. Whether or not supraspinal centres are involved in the latter

reflexes could not be determined in the present study, for acute transection of the spinal cord is known to cause spinal shock. However, based on the ability of naloxone to restore partially pressor, tachycardic and TAWC responses to i.p. NT in spinalized guinea-pigs, it seems reasonable to hypothesize that these reflex responses are subserved by spinal cord neural circuitry. The mechanism of the beneficial effect of naloxone in spinal shock has been discussed previously (Holaday & Faden, 1980; Faden *et al.*, 1981).

Several observations suggest that haemodynamic and TAWC responses to i.p. NT are independent reflexes that do not share the same neural pathways. This conclusion is based on the following facts: (1) animal pretreatment with pancuronium inhibits TAWC, but not haemodynamic responses to i.p. NT; (2) morphine suppresses, and naloxone potentiates, TAWC, without altering haemodynamic NT effects; (3) pentolinium, prazosin and capsaicin treatment markedly reduced haemodynamic responses to i.p. NT, without affecting TAWC. The results of capsaicin experiments also indicate that abdominal visceral afferents involved in TAWC, are unlikely to be small diameter, tachykinins-containing (i.e. capsaicin-sensitive) primary afferents (Buck & Burks, 1986), in contrast to those subserving reflex haemodynamic responses to i.p. NT (Rioux *et al.*, 1990a; this paper). That vagal afferents are not involved in TAWC (this paper) or haemodynamic responses to i.p. NT (Rioux *et al.*, 1990a; this paper), is well supported by the persistence of these responses in vagotomized animals. That acute spinalization did not inhibit the cardiovascular response to i.p. NT by causing a nonspecific depression of the cardiovascular system is indicated by the absence of amplitude differences between pressor and tachycardic responses to i.v. NA or DMPP in spinalized guinea-pigs compared to control. The similar amplitude of hypotensive effect of i.v. pentolinium in spinalized and sham-operated (i.e. control) guinea-pigs also suggests that spinalization did not completely suppress the activity of pre-ganglionic sympathetic neurones, despite the spinal shock.

Besides inhibiting haemodynamic and TAWC responses to i.p. NT, acute spinalization was also shown to reveal a different type of abdominal motor response, which we called SAWC (i.e. slowly developing, sustained abdominal wall contraction) (Figure 3). This type of abdominal motor response was absent in sham-operated or intact guinea-pigs after i.p. NT. The ability of NT to elicit SAWC is, to our knowledge, a novel biological property for NT. Various drugs were tested to unravel the mechanism by which i.p. NT causes SAWC in spinal guinea-pigs. The results showed that i.v. pancuronium, or i.p. lignocaine 2% w/v (i.e. pre-added to i.p. NT solution), completely prevent SAWC, whereas i.v. morphine, naloxone, clonidine or baclofen, or i.p. procaine 2% w/v, exert no effect. The suppression of SAWC by pancuronium clearly indicates that cholinergic motoneurons innervating abdominal muscles are involved, whereas SAWC suppression by i.p. lignocaine suggests the participation of abdominal visceral afferents. This interpretation is consistent with the fact that NT does not cross the blood-brain barrier (Nemeroff *et al.*, 1977). Consequently, the activation by i.p. NT of abdominal visceral afferents making synaptic contacts at the spinal level (i.e. presumably the ventral horn) with cholinergic motoneurons innervating abdominal muscles, is the most probable means by which i.p. NT causes SAWC in spinal guinea-pigs. However the possibility that i.p. lignocaine inhibits i.p. NT-induced SAWC by additional mechanisms (i.e. inhibition of transmission at neuromuscular junction) cannot be dismissed without further studies. The superiority of i.p. lignocaine over i.p. procaine in suppressing SAWC, is likely to reflect the difference of potency and efficacy between these 2 local anaesthetics (Ritchie & Greene, 1990). It also raises the possibility that abdominal visceral afferents subserving SAWC in spinal guinea-pigs, are less easily reached than those involved in TAWC. The inability of i.v. morphine, naloxone or clonidine to affect SAWC (in spinalized animals) even when used at doses that effectively modify TAWC (in

intact animals), suggests that the mechanism (e.g. neural circuitry) of these two types of responses may be different. Alternatively, the latter results might reflect the fact that morphine, naloxone and clonidine must act supraspinally to modify SAWC (i.e. via the activation of descending inhibitory neural pathways). Assuming that SAWC are true reflex responses, then, why do they occur in spinalized guinea-pigs, but not in sham-operated animals? In our opinion the abdominal distension associated with i.p. injection of a relatively large volume of NT solution (i.e. 9 ml), contributes to mask the detection of SAWC in sham-operated or intact guinea-pigs given i.p. NT. This hypothesis is supported by the fact that SAWC can easily be detected in intact animals given i.p. NT when lower volumes (i.e. 1 or 3 ml) of NT solution are utilized (Rioux, F., Lemieux, M., unpublished). The reason why spinalization unmasks SAWC responses to i.p. NT, despite fluid-induced abdominal distension is not clear. One possible explanation is that proprioceptive reflexes are triggered by abdominal distension, which promote abdominal muscle relaxation in intact animals, whereas such reflexes are absent in spinalized guinea-pigs (due to spinal shock), thus revealing SAWC.

The pressor and tachycardic responses to i.p. NT in anaesthetized guinea-pigs were not affected by animal pretreatment with morphine or naloxone. On the other hand TAWC responses to i.p. NT were suppressed by morphine and potentiated by naloxone. These results suggest that TAWC might be the motor component of a morphine-sensitive nociceptive reflex, whereas haemodynamic responses might be the circulatory component of the same reflex, which would involve morphine-insensitive neural pathways. The potentiation of TAWC responses (i.e. increase of the number of TAWC/animal) to i.p. NT by naloxone, also suggest that neural pathways involved in TAWC are somehow negatively regulated by endogenous opioid peptides, which could, theoretically, be released within the spinal cord following NT-induced activation of abdominal visceral afferents. This interpretation is consistent with the presence of opioid peptides in dorsal root ganglia, peripheral and central ends of primary sensory neurones, as well as in the dorsal horn of the spinal cord (Weihe *et al.*, 1988; Ribeiro da Silva *et al.*, 1990). The possibility of a tonic inhibition by endogenous opioid peptides of neural pathways involved in TAWC responses to i.p. NT cannot be ruled out completely. However, based on the inability of i.v. naloxone to elicit TAWC in animals not given NT (Rioux, F., Lemieux, M., unpublished), this last hypothesis seems less likely than the former one.

The response selectivity of our experimental model was good, for i.p. injection of BK or SP were either inactive (SP) or weakly active (BK) in mimicking the effects of i.p. NT. The weak potency of BK was somewhat surprising since BK is considered a potent analgesic substance in mammals (Garrison, 1990), and i.p. injection of BK in conscious dogs or human subjects was previously reported to be painful (Lim *et al.*, 1967; Sancilio & Cheung, 1978). Besides, BK (or SP) is known to stimulate abdominal visceral afferents in cats (Lew & Longhurst, 1986) and guinea-pigs (Beauchamp *et al.*, 1991). The precise reason for the absence of effect of i.p. SP and low potency of i.p. BK in our animal model is unknown.

The response sensitivity of our animal model to i.p. NT was probably affected by the anaesthesia and surgical stress, as barbiturates are known to depress transmission in autonomic ganglia and at the neuromuscular junction (Rall, 1990). Therefore the amplitudes of haemodynamic and abdominal motor responses to i.p. NT were probably attenuated by the anaesthesia, for both types of responses utilized barbiturate-sensitive neural pathways. On the other hand the negative influence of anaesthesia on haemodynamic responses

to i.p. NT was probably counterbalanced by concomitant reductions of baroreceptor-mediated cardiovascular reflexes. In addition, the low basal systemic BP level observed in barbiturate-anaesthetized guinea-pigs, certainly contributed to enhance pressor responses to i.p. NT, for high initial BP levels tend to attenuate the latter responses (Rioux *et al.*, 1990b). Surgical stress is the second factor which potentially modified the sensitivity of our animal model to i.p. NT, for noxious stimuli (i.e. surgery) are known to activate endogenous antinociceptive mechanisms that are known to reduce the amplitude and/or duration of nociceptive reflexes (Lumb, 1986; Besson & Chaouch, 1987). In addition the putative role of opioid peptidergic mechanisms in stress-induced analgesia (Terman *et al.*, 1984) raises the possibility that the potentiating action of naloxone on TAWC responses to i.p. NT, may depend to a large extent upon inhibition of opioid peptidergic mechanisms activated by surgical stress (Dubois *et al.*, 1981; Jaffe & Martin, 1990). The last technical point to be discussed is the use of i.p. procaine (or lignocaine) as a pharmacological tool to infer the participation of abdominal visceral afferents in haemodynamic and abdominal motor responses to i.p. NT. One could argue that i.p. procaine, in addition to causing conduction blockade in abdominal visceral afferents, also caused paralysis of cholinergic motoneurons and pre- and postganglionic sympathetic nerves, effects which could also explain the blockade of reflex haemodynamic and TAWC responses to i.p. NT. Although no direct proof is yet available to rule out this possibility, we believe that conduction blockade of abdominal visceral afferents is the most likely mechanism subserving the prevention of reflex haemodynamic and TAWC responses to i.p. NT by i.p. procaine. Our conviction is based on the following facts: (1) both the onset of NT effects and their blockade by i.p. procaine (i.e. procaine was pre-added to NT solution before i.p. injection of the latter) occurred rapidly (i.e. within seconds) after i.p. injection of NT solution, so that peritoneal visceral afferents are more likely than cholinergic motoneurons to have been reached rapidly by i.p. procaine; (2) peak pressor and tachycardic responses to i.v. bolus injection of DMPP, a ganglion stimulant, in anaesthetized guinea-pigs that first received (i.e. 2 min before) i.p. injection of 9 ml of procaine 2% w/v, were similar in amplitude to those measured in control animals (i.e. animals given i.p. injection of 9 ml of procaine solvent 2 min before i.v. DMPP), thus indicating that postganglionic noradrenergic neurones were still pharmacologically excitable 2 min after i.p. procaine; (3) reflex pressor and tachycardic responses to topical application of microlitre volumes of NT solution to discrete small area of the visceral peritoneum were previously shown, in the same animal model, to be reduced by prior (i.e. 30 s before) topical application of similar volume of lignocaine solution to the same sites as NT (Rioux *et al.*, 1989).

In conclusion, i.p. injection of NT in anaesthetized guinea-pigs caused dose-dependent pressor, tachycardic and abdominal motor responses (i.e. TAWC). Evidence were provided that haemodynamic and TAWC responses are both reflex in nature and not causally related. Both types of reflexes appear to result from the activation by i.p. NT of abdominal visceral afferents making synaptic contacts with spinal neurones. The pathophysiological significance of these results, if any, is unknown at present. However, given the nature and time-response characteristics of the reflexes elicited by i.p. NT, it seems reasonable to hypothesize that i.p. NT could be a noxious stimulus in guinea-pigs.

The authors wish to thank the Medical Research Council of Canada (Grant MT-6550) and the Canadian Heart Foundation for their financial support, and Mrs Gaétane Rioux for typing the manuscript.

References

- BEAUCHAMP, J.-F., LEMIEUX, M., DRAPEAU, G. & RIOUX, F. (1991). Mechanism of the pressor response to intraperitoneal injection of bradykinin in guinea pigs. *Peptides*, **12**, 513–521.
- BESSON, J.-M. & CHAOUCH, A. (1987). Peripheral and spinal mechanisms of nociception. *Physiol. Rev.*, **67**, 67–186.
- BUCK, S.H. & BURKS, T.F. (1986). The neuropharmacology of capsaicin. Review of some recent observations. *Pharmacol. Rev.*, **38**, 180–226.
- DUBOIS, M., PICKAR, D., COHEN, M.R., ROTH, Y.F., MACNAMARA, T. & BUNNEY, JR, W.E. (1981). Surgical stress in humans is accompanied by an increase in plasma beta-endorphin immunoreactivity. *Life Sci.*, **29**, 1249–1254.
- DUGGAN, A.W. (1983). Electrophysiology of opioid peptides and sensory systems. *Br. Med. Bull.*, **39**, 65–70.
- FADEN, A.I., JACOB, T.P. & HOLADAY, J.W. (1981). Opiate antagonist improves neurologic recovery after spinal injury. *Science*, **211**, 493–494.
- GARRISON, J.C. (1990). Histamine, bradykinin, 5-hydroxytryptamine, and their antagonists. In *The Pharmacological Basis of Therapeutics*. ed. Goodman Gilman, A., Rall, T.W., Nies, A.S. & Taylor, P., pp. 575–599. New York: Pergamon Press.
- HOLADAY, J.W. & FADEN, A.I. (1980). Naloxone acts at central opiate receptors to reverse hypotension, hypothermia and hypoventilation in spinal shock. *Brain Res.*, **189**, 295–299.
- JAFFE, J.H. & MARTIN, W.R. (1990). Opioid analgesics and antagonists. In *The Pharmacological Basis of Therapeutics*. ed. Goodman Gilman, A., Rall, T.W., Nies, A.S. & Taylor, P. pp. 485–521. New York: Pergamon Press.
- LEW, W.Y.W. & LONGHURST, J.C. (1986). Substance P, 5-hydroxytryptamine, and bradykinin stimulate abdominal visceral afferents. *Am. J. Physiol.*, **250**, R465–R473.
- LIM, R.K.S., MILLER, D.G., GUZMAN, F., RODGERS, D.W., ROGERS, R.W., WANG, S.K., CHAO, P.Y. & SHIH, T.Y. (1967). Pain and analgesia evaluated by the intraperitoneal bradykinin-evoked pain method in man. *Clin. Pharmacol. Ther.*, **8**, 521–542.
- LUMB, B.M. (1986). Brainstem control of visceral afferent pathways in the spinal cord. In *Prog. Brain Res.*, vol. 67, pp. 279–293. New York: Elsevier Science Publ., B.V.
- LUNDBERG, J.M., FRANCO-CERECEDA, A., HUA, X.-Y., HOKFELT, T. & FISCHER, J.A. (1985). Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and broncho-constrictor effects of capsaicin. *Eur. J. Pharmacol.*, **108**, 315–319.
- NEMEROFF, C.B., BISSETTE, G., PRANGE, JR, A.J., LOOSEN, P.T., STEVEN BARLOW, T. & LIPTON, M.A. (1977). Neurotensin: central nervous system effects of a hypothalamic peptide. *Brain Res.*, **128**, 485–496.
- RALL, T.W. (1990). Hypnotics and sedatives; ethanol. In *The Pharmacological Basis of Therapeutics*. ed. Goodman Gilman, A., Rall, T.W., Nies, A.S. & Taylor, P. pp. 345–382. New York: Pergamon Press.
- RIBEIRO-DA-SILVA, A., PIORO, E.P. & CLAUDIO CUELLO, A. (1991). Substance P- and enkephalin-like immunoreactivities are colocalized in certain neurons of the substantia gelatinosa of the rat spinal cord: an ultrastructural double-labeling study. *J. Neurosci.*, **11**, 1068–1080.
- RIOUX, F., LEMIEUX, M., KEROUAC, R., BERNOUSSI, A. & ROY, G. (1989). Local application of neurotensin to abdominal organs triggers cardiovascular reflexes in guinea pigs: possible mechanisms. *Peptides*, **10**, 647–655.
- RIOUX, F., LEMIEUX, M. & LEBEL, M. (1990a). Further observations on the mechanism of the cardiovascular reflexes caused by exposure of the peritoneum to neurotensin. *Peptides*, **11**, 805–816.
- RIOUX, F., LEMIEUX, M. & LEBEL, M. (1990b). Effect of initial blood pressure level on cardiovascular reflexes caused by intraperitoneal neurotensin. *Peptides*, **11**, 921–926.
- RITCHIE, J.M. & GREENE, N.M. (1990). Local anesthetics. In *The Pharmacological Basis of Therapeutics*. ed. Goodman Gilman, A., Rall, T.W., Nies, A.S. & Taylor, P. pp. 311–331. New York: Pergamon Press.
- SANCILIO, L.F. & CHEUNG, S. (1978). Analysis of the bradykinin response in dogs and its antagonism by analgesic drugs. *J. Pharmacol. Exp. Ther.*, **207**, 469–475.
- TALLARIDA, R.J. & MURRAY, R.B. (1981). *Manual of Pharmacologic Calculations with Computer Programs*. New York: Springer-Verlag.
- TERMAN, G.W., SHAVIT, Y., LEWIS, J.W., CANNON, J.T. & LIEBESKIND, J.C. (1984). Intrinsic mechanisms of pain inhibition: activation by stress. *Science*, **226**, 1270–1277.
- WEIHE, E., NOHR, D. & HARTSCHUH, W. (1988). Immunohistochemical evidence for a co-transmitter role of opioid peptides in primary sensory neurons. In *Progress in Brain Research*, vol. 74. ed. Hamann, W. & Iggo, A. pp. 189–199. New York: Elsevier Science Publishers B.V.

(Received May 30, 1991)

Revised January 8, 1992

Accepted January 10, 1992