Blood pressure reflexes following activation of capsaicin-sensitive afferent neurones in the biliopancreatic duct of rats

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1 Inflammatory diseases of the pancreas or diseases which cause obstruction within the biliary or pancreatic duct system are associated with severe pain. Although neuropeptides such as substance P are present in the biliary tree, only few capsaicin-sensitive, substance P-positive nerve fibres have been found in the ducts. In order to obtain functional evidence whether capsaicin-sensitive afferent neurones transmit nociceptive information arising from the biliopancreatic duct, blood pressure reflexes following electrical stimulation of the duct or increases in intraductal pressure were determined in barbiturate-anaesthetized rats.

2 Electrical stimulation of neurones in the biliopancreatic duct was carried out at 30 V, 3 ms, 50 Hz for 20 s. In untreated animals the electrical stimulation resulted in rises in blood pressure by up to 25 mmHg, but in about a quarter of all animals tested this response was absent. Following the administration of phentolamine ($7 \mu\text{mol kg}^{-1}$, i.p.) the blood pressure responses were changed to pronounced and reproducible depressor reflexes of -5 to -30 mmHg. Retrograde injections into the biliopancreatic duct of $300 \mu\text{l}$ of a 154 mM sodium chloride solution produced increases in intraductal pressure of approximately 10 mmHg. This elicited shortlasting falls in blood pressure of 3–15 mmHg. Phentolamine significantly augmented the fall in blood pressure to 8–30 mmHg.

3 The depressor reflexes observed in both models after the administration of phentolamine were abolished by morphine ($1 \mu\text{mol kg}^{-1}$, i.v.). The inhibition by morphine was reversed by naloxone ($3 \mu\text{mol kg}^{-1}$, i.v.). Naloxone given before morphine did not affect the depressor reflex but prevented the inhibitory action of subsequently injected morphine.

4 Acute s.c. injection of capsaicin (30 mg kg^{-1}) abolished the depressor reflexes in response to both types of nociceptive stimulation in phentolamine-treated rats. The initial pressor effects of electrical stimulation were only partly inhibited by capsaicin whereas the basal depressor reflexes in response to elevation of intraductal pressure were abolished. In rats which had received capsaicin on the day before the experiment or had been treated with capsaicin as neonates, only minor rises in blood pressure were induced by electrical stimulation at the beginning of the experiment and no changes in blood pressure occurred after the administration of phentolamine. After adult or neonatal pretreatment with capsaicin the depressor reflexes in response to increased intraductal pressure were only small and were unchanged by phentolamine.

5 The depressor reflexes following either electrical stimulation or increases in intraductal pressure were abolished by the unselective β -blocker, ($-$)-propranolol ($3 \mu\text{mol kg}^{-1}$, i.p.), and greatly reduced by the β_1 -blocker, metoprolol ($6 \mu\text{mol kg}^{-1}$, i.p.). The β_2 -preferring adrenoceptor antagonist, butoxamine ($3 \mu\text{mol kg}^{-1}$, i.p.), had no effect on the depressor responses. The reflex falls in blood pressure were also abolished by hexamethonium ($10 \mu\text{mol kg}^{-1}$, i.p.) but not by atropine ($3 \mu\text{mol kg}^{-1}$, i.p.).

6 Both models of stimulation of nociceptive afferents caused identical patterns of blood pressure responses following adrenalectomy or chemical sympathectomy. In adrenalectomized rats, the initial responses consisted of depressor reflexes which were not augmented but significantly reduced by phentolamine and further inhibited by metoprolol. In rats that had been pretreated with 6-hydroxydopamine (total dose 0.6 mmol kg^{-1}) to accomplish chemical sympathectomy, nociceptive stimulation caused rises in blood pressure. Phentolamine treatment abolished these pressor effects but revealed only minor, if any, depressor responses that were unaffected by metoprolol.

7 In summary, the hypotensive effects in both models constitute nociceptive reflexes since they are abolished by morphine and restored by naloxone. The afferent part of the reflex is mediated by nerve fibres sensitive to capsaicin. Both experimental procedures seem to elicit two, presumably separate, reflex mechanisms. Firstly, catecholamines released from the adrenal medulla elevate blood pressure or limit hypotensive responses via activation of vascular α receptors. Secondly, the reflex inhibition of the sympathetic nerve activity in the heart and the vasculature causes the nociceptive depressor reflexes.

Keywords: Pain (biliary); pain reflexes; capsaicin; afferent neurones

Introduction

Obstructive diseases of the biliary and pancreatic duct system associated with elevation of intraductal pressure are frequently accompanied by severe pain (Geenen *et al.*, 1989; Moody *et al.*, 1990; Prinz & Greenlee, 1990). Similar symptoms of pain can be observed in certain patients undergoing diagnostic or therapeutic procedures which cause an eleva-

tion of intraductal pressure such as injection of contrast media into the common bile duct during endoscopic retrograde cholangiopancreatography (Lasson *et al.*, 1988) or balloon dilatation (Jaschke *et al.*, 1992).

Experimental elevation of intraductal pressure also is painful in man (McGowan *et al.*, 1936; Chapman *et al.*, 1949)

and elicits pain-related pseudoaffective reflexes in anaesthetized animals (Woodsworth & Sherrington, 1904; Cervero, 1982a,b). Since the administration of capsaicin onto the surface of the pancreas of cats leads to cardiovascular responses similar to those commonly observed after painful stimuli (Ordway *et al.*, 1983) it might be inferred that nociceptive information arising from the biliopancreatic duct system is conveyed by capsaicin-sensitive afferent nerve fibres. Using immunological techniques, neuropeptides like substance P, calcitonin gene-related peptide or vasoactive intestinal peptide have been found in the biliary tract of several species including rat (Holzer *et al.*, 1982; Inoue *et al.*, 1992), and man (Ueno *et al.*, 1991). However, these peptides can also be contained in nerve fibres which have local effector functions and are not sensitive to capsaicin (Costa *et al.*, 1980; Holzer *et al.*, 1980; Furness *et al.*, 1982). Although the substance P content of the hepatic ducts of rats was found to be greatly reduced by capsaicin pretreatment (Holzer *et al.*, 1982) which indicates that afferent C-fibres are present in the biliary system, Sharkey *et al.* (1984) found only few or no substance P-containing capsaicin-sensitive fibres in the pancreatic duct. Thus, in the present study a functional demonstration for the role of capsaicin-sensitive afferents in the mediation of nociceptive information arising from elevations in pressure in the biliopancreatic duct system was attempted.

A preliminary account of the results has been presented to the British and Italian Pharmacological Societies (Griesbacher *et al.*, 1993).

Methods

Surgical procedures

Female Sprague-Dawley rats (200–280 g) were anaesthetized with pentobarbitone sodium (40 mg kg⁻¹, i.p.) and phenobarbitone sodium (40 mg kg⁻¹, i.p.). The trachea was cannulated to ensure unhindered respiration. One carotid artery was cannulated and connected to a Statham pressure transducer to monitor arterial blood pressure. A jugular vein was cannulated for the i.v. injection of drugs. The abdomen was opened through a midline incision.

Electrical stimulation of the biliopancreatic duct The biliopancreatic duct was carefully dissected from the surrounding pancreatic tissue and placed on a pair of bipolar platinum electrodes. Electrical stimulation was carried out at 30 V, 3 ms, 50 Hz for a period of 20 s. This stimulation was repeated three times at intervals of 5 min. The stimulation protocol was resumed 15 min after the i.p., s.c. or i.v. administration of drugs.

Elevation of intraluminal pressure in the biliopancreatic duct The biliopancreatic duct was cannulated in a retrograde direction with a piece of polyethylene tubing (0.61 mm o.d., 0.28 mm i.d. Intramedic PE-10; Becton Dickinson, Parsippany, NJ, U.S.A.). In order to increase intraluminal pressure, 300 µl of saline was injected into the duct within 15 s and the cannula was then kept closed for further 20 s. The magnitude of the ensuing increase in intraductal pressure was determined with a Statham pressure transducer connected to the cannula via a T-piece. The time intervals for the saline injections and for the administration of drugs were the same as described above for electrical stimulation of neurones in the biliopancreatic duct.

Adrenalectomy Adrenalectomy was performed 15 min before the beginning of the experiments. The adrenal stalk was ligated and the glands were removed. In sham-operated animals, only the inferior pole of the kidney was cleared of the surrounding adipose tissue.

Chemical sympathectomy The rats were pretreated with 6-hydroxydopamine (100 µmol kg⁻¹, s.c.) in the afternoons of the 6 days prior to the experiments. In order to determine the extent of the chemical sympathectomy, noradrenaline levels were determined in the right atrium, the left ventricle, and in the spleen. As control tissues, one adrenal gland and the parietal cerebral cortex were used. At the end of the experiments, the rats were killed by exsanguination. The tissues were removed, weighed, frozen immediately on dry ice, and stored at -70°C until the catecholamine content of the tissues was determined with the aluminium oxide adsorption method and h.p.l.c. analysis (Donnerer, 1988). The tissues were homogenized in 1.5 ml 0.4 M perchloric acid containing 0.5 mM sodium EDTA and 0.5 mM sodium metabisulphate. The homogenates were centrifuged for 10 min at 2 × 10⁴ m s⁻². The supernatant and 1.8 ml of 1.0 M Tris buffer (pH 8.6) were added to 100 mg of heat-activated aluminium oxide. As internal standard 10 ng dihydroxybenzylamine was added. The resulting samples were thoroughly shaken for 5 min. After centrifugation the supernatant was discarded and the aluminium oxide pellet was washed with 2 ml of 0.01 M Tris buffer. Catecholamines were eluted from the pellet with 500 µl of 0.2 M perchloric acid. The eluates from adrenal gland were diluted 10 fold with 0.2 M perchloric acid, the eluates from the other tissue samples were used undiluted. Samples of 100 µl were injected onto a reverse phase h.p.l.c. column (Merck 5 µm LiChrospher 100 RP-18, eluent: phosphate buffer pH 5.0 containing 4 mM heptanesulphonic acid and 4% v/v methanol). Noradrenaline and adrenaline were measured by electrochemical detection. The recovery, calculated from the values measured for dihydroxybenzylamine, was 50–90%.

Substances used

Pentobarbitone sodium (Nembutal) was from Sanofi (Limbourn, France), phenobarbitone sodium (Apoka, Vienna, Austria) was dissolved in a 154 mM solution of NaCl (saline). Capsaicin (Fluka, Buchs, Switzerland) was dissolved in dimethylsulphoxide at a concentration of 10 mg ml⁻¹. For the neonatal pretreatment of rats, capsaicin was dissolved in 10% (v/v) Tween 80 and 10% (v/v) ethanol in saline. 6-Hydroxydopamine hydrobromide (Sigma, St. Louis, MO, U.S.A.) was dissolved in a solution of 1% (w/v) ascorbic acid in saline at a concentration of 40 mM immediately prior to use. Phentolamine hydrochloride, metoprolol tartrate (Ciba, Basle, Switzerland), morphine hydrochloride (Diosynth, Apeldoorn, Netherlands), naloxone hydrochloride (DuPont, Geneva, Switzerland), (-)-propranolol hydrochloride (ICI, Macclesfield, U.K.), (+)-propranolol hydrochloride, butoxamine hydrochloride, hexamethonium chloride (Sigma, St. Louis, MO, U.S.A.), and atropine sulphate (Merck, Darmstadt, Germany) were dissolved in saline.

Statistical analysis

All values presented are arithmetical means ± s.e.mean. Non-parametric analysis of variance and multiple comparisons among the blood pressure responses before and after the drug administrations during the course of the experiments were made by the Quade test (Conover, 1980). The catecholamine concentrations in tissues from 6-hydroxydopamine-treated rats were compared with those determined in control animals using the Mann-Whitney U test.

Results

Blood pressure reflexes and effects of phentolamine

Electrical stimulation (30 V, 3 ms, 50 Hz, 20 s) of the biliopancreatic duct led to changes in mean arterial pressure lasting for the duration of the stimulation. Out of 43 animals

subjected to electrical stimulation of the biliopancreatic duct before and after the i.p. administration of phentolamine, 31 (72%) responded with an increase in blood pressure by 3–25 mmHg under basal conditions, whereas a smaller number of animals showed either no response (8/43, 19%) or a minute (less than 3 mmHg) fall in blood pressure (4/43, 9%). In all animals, the blood pressure response was transformed into a consistent depressor effect of 5–30 mmHg 15 min after the i.p. administration of phentolamine ($7 \mu\text{mol kg}^{-1}$, $P < 0.001$; Figures 1a–b, 2a, 3a–c, 4a–b, 5a). Phentolamine also reduced basal blood pressure itself from 131 ± 8 mmHg to 105 ± 2 mmHg ($P < 0.01$). The stimulation procedure was carried out three times at intervals of 5 min followed by a rest period of 20 min. The time course of the initial pressor responses was slightly different from that of the depressor responses following phentolamine; the pressor effects, when present, started immediately after the beginning of the electrical stimulation, whereas the depressor responses following phentolamine typically set in after a lag period of about 5 s. The depressor response remained stable for at least 90 min.

The retrograde injection of 300 μl saline into the biliopancreatic duct caused reproducible increases in intraductal pressure of about 10 mmHg. This resulted in a fall in mean arterial pressure by 3–15 mmHg which was reversed within about 1 min. Phentolamine ($7 \mu\text{mol kg}^{-1}$, i.p.) augmented the depressor response to 8–30 mmHg ($P < 0.01$; Figures 1c–d, 2e, 3d–f, 4c–d, 5e) while reducing basal blood pressure from 119 ± 4 mmHg to 91 ± 4 mmHg ($P < 0.01$). The intraductal injections of saline were carried out at the same time intervals as described above for the electrical stimulations. The ensuing blood pressure responses remained stable for at least 90 min. In order to provide experimental conditions comparable to those in the experiments using electrical stimulation of the biliopancreatic duct, phentolamine was applied in all further experiments. The depressor responses before or after phentolamine exhibited similar time courses including a certain lag period of several seconds between the beginning of the elevation in intraductal pressure and the onset of the fall in blood pressure.

Effects of morphine and naloxone

Following treatment with phentolamine, the hypotensive responses both after electrical stimulation and after increasing

Figure 1 Effects of morphine and naloxone on reflex changes in rat blood pressure (in mmHg) induced by electrical stimulation (30 V, 3 ms, 50 Hz, 20 s) of nerve fibres in the biliopancreatic duct (a and b) and by shortlasting (35 s) increases in intraductal pressure (c and d). During the course of the experiments, phentolamine (P, $7 \mu\text{mol kg}^{-1}$, i.p.), morphine (M, $1 \mu\text{mol kg}^{-1}$, i.v.) and naloxone (N, $3 \mu\text{mol kg}^{-1}$, i.v.) were administered (indicated by the arrows). Significance of difference to the effect before the previous drug administration: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS, not significant. Means $\pm$ s.e.mean; $n = 4-6$.

intraductal pressure were completely abolished by the i.v. injection of morphine ($1 \mu\text{mol kg}^{-1}$, Figure 1a and c). The inhibition caused by morphine was reversed 15–25 min after naloxone ($3 \mu\text{mol kg}^{-1}$, i.v.). In both experimental models, the hypotensive responses after the naloxone seemed to be slightly smaller than those observed before the administration of morphine; however, the difference did not achieve statistical significance. Basal blood pressure in phentolamine-treated rats (80–110 mmHg) was not significantly affected by morphine (70–100 mmHg).

In both experimental models, naloxone administered before morphine did not have any effect on the depressor reflexes in phentolamine-treated rats, but it prevented the inhibitory effect of subsequently applied morphine (Figure 1b and d).

Effects of capsaicin

The depressor reflexes in response to electrical stimulation in phentolamine-treated rats were completely abolished 15 min after acute administration of capsaicin (30 mg kg^{-1} , s.c.) (Figure 2a). The basal pressor response was only partly reduced by capsaicin, but after a subsequent i.p. administration of phentolamine the stimulation procedure failed to produce changes in blood pressure (Figure 2b). When rats were pretreated with capsaicin (30 mg kg^{-1} , s.c.) on the day before the experiment, the pressor response following electrical stimulation was absent in three out of six animals tested. In all animals, the i.p. administration of phentolamine

Figure 2 Effects of capsaicin on blood pressure reflexes (in mmHg) elicited by electrical stimulation of nerve fibres in the biliopancreatic duct (a–d) or by shortlasting increases in intraductal pressure (e–h): Phentolamine (P, $7 \mu\text{mol kg}^{-1}$) was administered i.p. Acute treatment with capsaicin (C, 30 mg kg^{-1}) during the experiment was made by s.c. injection (a, b, e, f). Pretreatment with capsaicin was made either in adult rats on the day before the experiment (30 mg kg^{-1} , s.c.; hatched columns, c and g) or in neonatal rats on the 2nd day of life (50 mg kg^{-1} , s.c.; solid columns, d and h). Drug administrations are indicated by the arrows. Significance of differences from the effect before the previous drug administration: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS, not significant. Means $\pm$ s.e.mean, $n = 4-6$.

abolished the effect of the electrical stimulation on blood pressure (Figure 2c). Rats that had been treated with capsaicin (50 mg kg^{-1} , s.c.) as neonates also responded with only small rises in blood pressure that were abolished by the subsequent administration of phentolamine (Figure 2d).

The depressor responses following elevation of intraductal pressure both before and after the i.p. administration of phentolamine were abolished by capsaicin (Figure 2e and f). When capsaicin was given before phentolamine, the α -blocker was unable to reveal any hypotensive effect (Figure 2f). The initial depressor effects induced by the elevation of intraluminal pressure in the biliopancreatic duct seemed to be smaller in rats that had been pretreated with capsaicin on the day before the experiment (Figure 2g) although the difference did not achieve statistical significance. Similarly, only small hypotensive effects were observed when the animals had been treated with capsaicin on the second day of life (Figure 2h). In both cases, these small hypotensive effects remained unchanged by phentolamine.

Acute administration of capsaicin during the course of the experiment lowered basal blood pressure by about 15 mmHg ($P < 0.05$) only when injected prior to treatment with phentolamine; in phentolamine-treated rats, it did not affect blood pressure. Blood pressure in rats treated with capsaicin on the day before the experiment (95–115 mmHg) was not significantly different from blood pressure in untreated animals (100–130 mmHg). However, rats that had received treatment with capsaicin as neonates, showed a significant ($P < 0.01$) lower blood pressure as adults (80–95 mmHg).

To ensure that the effects of the capsaicin injections were not unspecific effects of the solvent, DMSO was injected in the appropriate volume (3 ml kg^{-1} , s.c.). This had no effect

on blood pressure itself or on any of the reflex responses following either electrical stimulation of the biliopancreatic duct or elevation of intraductal pressure (data not shown, $n = 3$).

Effects of β -blockers, hexamethonium, and atropine

The reflex fall in arterial pressure following electrical stimulation of the biliopancreatic duct after the administration of phentolamine was completely blocked by the unselective β -blocker, (–)-propranolol ($3 \mu\text{mol kg}^{-1}$, i.p.), (Figure 3a). It has been reported that propranolol can also have unspecific local anaesthetic actions which, unlike the adrenoceptor blocking action, are not constrained to the active (–)-enantiomer (Barrett & Cullum, 1968). However, the depressor response ($-8 \pm 2 \text{ mmHg}$) was completely unaltered ($-8 \pm 2 \text{ mmHg}$, $n = 4$) by (+)-propranolol confirming that the inhibitory effect of (–)-propranolol was a specific action on β -adrenoceptors. The β_1 receptor antagonist, metoprolol ($6 \mu\text{mol kg}^{-1}$, i.p.), also inhibited the depressor reflex (Figure 3b) whereas the β_2 -adrenoceptor blocker, butoxamine ($3 \mu\text{mol kg}^{-1}$, i.p.), had no effect (Figure 3c).

Hexamethonium ($10 \mu\text{mol kg}^{-1}$, i.p.) abolished the response (Figure 4a). Atropine ($3 \mu\text{mol kg}^{-1}$, i.p.) seemed to reduce the response slightly (Figure 4b); however, the difference did not achieve statistical significance. The initial pressor responses before phentolamine were not affected by (–)-propranolol or atropine, but were blocked by hexamethonium (Table 1).

The depressor responses following elevation of intraductal pressure in phentolamine-treated rats were blocked by (–)-propranolol (Figure 3d), but were unchanged by (+)-propranolol ($-16 \pm 4 \text{ mmHg}$ both before and after the i.p. administration). Metoprolol greatly reduced the depressor reflexes (Figure 3e) while butoxamine had no significant effect (Figure 3f). The hypotensive responses were strongly inhibited by hexamethonium, but were not significantly affected by atropine (Figure 4c and d, respectively). The reflex falls in blood pressure observed under basal conditions, i.e. before the administration of phentolamine, were abolished by (–)-propranolol and hexamethonium, but were unaffected by atropine (Table 1).

In both experimental models, neither (–)-propranolol nor metoprolol or butoxamine, in these experiments applied following treatment with phentolamine, had any effect on basal blood pressure (80–110 mmHg) on their own. Hexamethonium and atropine both reduced blood pressure by 5–15 mmHg ($P < 0.05$).

Effects of adrenalectomy and chemical sympathectomy

Blood pressure responses to either electrical stimulation of the biliopancreatic duct or to elevation of intraductal pressure were monitored under basal conditions, following the i.p. administration of phentolamine ($7 \mu\text{mol kg}^{-1}$) and after the subsequent treatment with metoprolol ($6 \mu\text{mol kg}^{-1}$, i.p.). In sham-operated rats, both procedures elicited blood pressure responses that were comparable to those obtained in animals that had not undergone surgery in the retroperitoneal space (Figure 5a and d; compare Figure 3b and f, respectively).

In adrenalectomized rats, the initial blood pressure responses to electrical stimulation of the biliopancreatic duct consisted of depressor, rather than pressor, effects. Phentolamine significantly ($P < 0.05$) reduced these falls in blood pressure, and subsequently applied metoprolol abolished the reflex (Figure 5b). Repeated daily s.c. injections of 6-hydroxydopamine (total dose of 0.6 mmol kg^{-1}), reduced the noradrenaline concentrations in peripheral tissues by over 80–95%, whereas the catecholamine levels in the adrenal glands and in the central nervous system were not affected (Table 2). In these animals, the electrical stimulation of the biliopancreatic duct caused shortlasting rises in mean arterial

Figure 3 Effects of β -adrenoceptor antagonists on blood pressure reflexes (in mmHg) induced by electrical stimulation of the biliopancreatic duct (a–c) or by shortlasting increases in intraductal pressure (d–f) in anaesthetized rats: Phentolamine (P, $7 \mu\text{mol kg}^{-1}$) as well as (–)-propranolol (Pr, $3 \mu\text{mol kg}^{-1}$), metoprolol (Me, $6 \mu\text{mol kg}^{-1}$) or butoxamine (Bu, $3 \mu\text{mol kg}^{-1}$) were administered i.p. (indicated by the arrows). Significance of difference from the effect before the previous drug administration: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant. Means $\pm$ s.e.mean; $n = 4$ –5.

pressure similar to untreated rats (Figure 5c). These rises were abolished by phentolamine. However, the α -blocker was unable to reveal any significant depressor responses. A further administration of metoprolol was without effect.

The elevation of intraductal pressure in adrenalectomized rats was followed by consistent reflex falls in blood pressure

Figure 4 Effects of hexamethonium and atropine on reflex changes in mean arterial pressure (in mmHg) following electrical stimulation of the biliopancreatic duct (a and b) or elevation of intraductal pressure (c and d): Responses were measured before and after the i.p. administration of phentolamine (P, $7 \mu\text{mol kg}^{-1}$). A third set of painful stimuli was performed after the i.p. administration of hexamethonium (H, 10 mg kg^{-1}) or atropine (A, $3 \mu\text{mol kg}^{-1}$). Drug administrations are indicated by the arrows. Significance of difference from the effect before the previous drug administration: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$; NS, not significant. Means $\pm$ s.e. mean, $n = 5-6$.

Figure 5 Effects of adrenalectomy and chemical sympathectomy on blood pressure reflexes (in mmHg) induced by electrical stimulation of the biliopancreatic duct (a-c) or by elevation of intraductal pressure (d-f): Adrenalectomy was performed at the beginning of the experiment (hatched columns, b and e), sham-operated controls are shown in panels (a) and (d). Chemical sympathectomy was accomplished with 6-hydroxydopamine (0.6 mmol kg^{-1} divided into 6 equal doses given s.c. on the 6 days before the experiment; solid columns, c and e). During the course of the experiment, phentolamine (P, $7 \mu\text{mol kg}^{-1}$) and metoprolol (Me, $6 \mu\text{mol kg}^{-1}$) were administered i.p. as indicated by the arrows. Significance of difference from the effect before the previous drug administration: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$; NS, not significant. Means $\pm$ s.e. mean; $n = 4-5$.

Table 1 Reflex changes in systemic blood pressure (in mmHg) in anaesthetized rats induced by electrical stimulation of the biliopancreatic duct or by elevation of intraductal pressure

Electrical stimulation			Elevation of intraductal pressure		
Before	Drug	After	Before	Drug	After
21 ± 2	(-)-Propranolol	21 ± 2	-7 ± 1	(-)-Propranolol	$3 \pm 2^{**}$
16 ± 5	Hexamethonium	$-6 \pm 9^*$	-8 ± 2	Hexamethonium	$-1 \pm 2^{**}$
9 ± 1	Atropine	7 ± 3	-5 ± 1	Atropine	-7 ± 2

Electrical stimulation (30 V, 3 ms, 50 Hz) was carried out for 20 s at intervals of 5 min. Intraductal pressure was elevated by about 10 mmHg for 35 s by retrograde injections of 300 μl of saline. Blood pressure reflexes were monitored before and 15–25 min after the i.p. administration of (-)-propranolol ($3 \mu\text{mol kg}^{-1}$), hexamethonium ($10 \mu\text{mol kg}^{-1}$), or atropine ($3 \mu\text{mol kg}^{-1}$). Means $\pm$ s.e. mean; $n = 4-6$. Significance of difference to values obtained before drug: * $P < 0.05$, ** $P < 0.01$.

Table 2 Effect of 6-hydroxydopamine (6-OHDA) pretreatment on tissue concentrations of noradrenaline (NA) and adrenaline (Ad) in rats

Tissue	Catecholamine	Control	6-OHDA
Right atrium	NA (ng g^{-1} wet wt.)	$1,952 \pm 73$	$73 \pm 9^{**}$
Left ventricle	NA (ng g^{-1} wet wt.)	644 ± 34	$28 \pm 2^{**}$
Spleen	NA (ng g^{-1} wet wt.)	741 ± 47	$73 \pm 9^{**}$
Parietal cortex	NA (ng g^{-1} wet wt.)	254 ± 19	293 ± 17
Adrenal gland	NA ($\mu\text{g g}^{-1}$ wet wt.)	115 ± 20	99 ± 16
	Ad ($\mu\text{g g}^{-1}$ wet wt.)	349 ± 47	396 ± 40

Rats were pretreated with 6-OHDA (0.6 mmol kg^{-1} , s.c., divided into 6 daily doses), control rats received injections of the solvent (1% w/v ascorbic acid in saline) instead. All rats were used in experiments for the measurement of the blood pressure reflexes following either electrical stimulation of the biliopancreatic duct or elevation of intraductal pressure. During the course of these experiments, phentolamine ($7 \mu\text{mol kg}^{-1}$, i.p.) and metoprolol ($6 \mu\text{mol kg}^{-1}$, i.p.) were administered. The tissues were taken at the end of the experiments. Means $\pm$ s.e. mean; $n = 6$ for both groups. Significance of difference from control group: ** $P < 0.01$.

(Figure 5e). Unlike in sham controls (compare Figure 5d), phentolamine did not augment these hypotensive responses, but significantly ($P < 0.05$) reduced the response. The subsequent administration of metoprolol caused a further significant ($P < 0.05$) reduction of the effect. Following chemical sympathectomy, the initial blood pressure reflexes in response to elevation in intraductal pressure consisted of shortlasting rises in systemic arterial pressure (Figure 5f) which is in contrast to the effects obtained in rats with intact peripheral sympathetic neurones. Similar to the responses to electrical stimulation of the biliopancreatic duct in sympathectomized rats, phentolamine abolished these pressor effects, but could not reveal any falls in blood pressure. Metoprolol also had no further effect.

In both experimental models, the basal blood pressure was the same in adrenalectomized rats as in sham-operated controls (120–140 mmHg), but was reduced in 6-hydroxydopamine-treated rats (100–115 mmHg, $P < 0.05$). Phentolamine significantly ($P < 0.05$) reduced blood pressure in sham controls and adrenalectomized rats (to 100–120 mmHg) as well as in rats that had undergone chemical sympathectomy (to 80–100 mmHg). The subsequent treatment with metoprolol caused a further reduction in blood pressure only in adrenalectomized rats (by about 20 mmHg) but had no effect in the two other groups.

Discussion

In anaesthetized rats and rabbits, painful stimuli such as distension of the small or large intestine (Lembeck & Skofitsch, 1982; Ness & Gebhart, 1988), distension of the renal pelvis (Brasch & Zetler, 1982), electrical stimulation of the saphenous nerve (Lembeck & Donnerer, 1983), intra-arterial injection of capsaicin (Donnerer & Lembeck, 1983), or visceral or somatic administration of algescic agents (Juan & Lembeck, 1974; Holzer-Petsche, 1992) elicit reflex falls in systemic arterial pressure.

In the present experiments, electrical stimulation of the biliopancreatic duct, however, caused increases, rather than decreases, in blood pressure. Since a pressor response mediated by α -adrenoceptors was observed as part of a triphasic blood pressure reflex following the distension of the small intestine (Lembeck & Skofitsch, 1982), phentolamine was used to block effects mediated by these receptors. This treatment transformed the pressor responses into consistent and reproducible depressor reflexes. Thus, the electrical stimulation of the biliopancreatic duct is followed by the activation of more than one single effector mechanism, one of which is α -adrenergic leading to a rise in blood pressure. Since the depressor responses observed after the administration of phentolamine are similar to cardiovascular reflexes in other rat models of visceral pain, phentolamine was routinely applied in the further experiments.

An increase in intraductal pressure was produced experimentally by retrograde injection of saline into the biliopancreatic duct. The ensuing increase in intraductal pressure of about 10 mmHg may seem to be only small. However, similarly small increases in intraductal pressure have been shown to elicit pain in man especially under pathological conditions (Gaensler, 1951). Since the elevation of intraductal pressure did not induce any rises in systemic arterial pressure the procedure seems to be more selective as far as the type of blood pressure response is concerned. However, the response still contains an α -adrenergic component, since phentolamine augmented the depressor effect.

Blockade by morphine of depressor reflexes elicited by distension of the small intestine (Lembeck & Skofitsch, 1982; Clark & Smith, 1985) or the renal pelvis (Brasch & Zetler, 1982) has been used to classify these responses as nociceptive reflexes. The mode of action of morphine in preventing the reflex presumably involves both peripheral and central sites of action (Clark & Smith, 1985). It has been shown that, in

dogs and cats, narcotic analgesics can produce hypotension via a central action (Laubie *et al.*, 1974; Daskalopoulos *et al.*, 1975). This effect could interfere with blood pressure reflexes unspecifically. In the present investigation, however, morphine had only small, if any, hypotensive effects of its own which by itself do not seem to be sufficient to explain the inhibitory effects of morphine. Thus, it seems to be more likely that the morphine-induced inhibition is related to its analgesic activity and the depressor effects constitute pseudo-affective reflex responses accompanying nociceptive afferent information similar to those described in other models of visceral noxious stimulation (see Ness & Gebhart, 1990).

Indications that visceral nociceptive stimuli activate small unmyelinated fibres, i.e. C- and A δ -fibres, have already been obtained by Gernandt & Zotterman (1946). High doses of capsaicin have profound actions on these afferent neurones, leading to defunctionalization (see Fitzgerald, 1983; Holzer, 1991): acute administration of such high doses will lead to a desensitization of the afferent neurones by depletion of the neurotransmitters and also by blockade of nerve conduction. The time needed for this desensitization to come into effect varies from as little as 10 min to several days, depending on the model used (Lembeck & Donnerer, 1981). Neonatal treatment with capsaicin causes a relatively selective degeneration of the C-fibre afferents (Jancsó *et al.*, 1977). Whereas this treatment causes an almost complete degeneration of unmyelinated fibres, small myelinated fibres are affected only to a minor extent (Nagy *et al.*, 1983). The depressor responses observed in both experimental models of the present investigation were blocked by capsaicin already 15 min after its s.c. application. The nociceptive reflexes were similarly inhibited when capsaicin was administered on the day before the experiment or when rats treated with capsaicin as neonates were used. Thus, the afferent part of the reflex arc leading to the depressor responses clearly consists of activation of capsaicin-sensitive neurones.

It is interesting to note that the initial responses, i.e. the blood pressure effects before the administration of phentolamine, were also diminished by capsaicin (compare Figure 2b–d and f–h). This implies that the pressor response following electrical stimulation, at least partly involves capsaicin-sensitive nerve fibres, which is in contrast to the pressor component of the blood pressure reflex following distension of the small intestine where a contribution of such fibres was excluded (Lembeck & Skofitsch, 1982). Acute administration of capsaicin completely blocked the basal depressor responses following elevation of intraductal pressure; pretreatment with capsaicin either in adult rats on the day before the experiment or already on the 2nd day of life only partly reduced the depressor response (Figure 2g and h) while completely preventing the depressor component otherwise revealed by phentolamine. Thus, elevation of intraductal pressure may also activate some other afferent fibres not sensitive to capsaicin, but also leading to a depressor response.

The initial finding that the electrical stimulation of the biliopancreatic duct was followed, in the majority of cases, by rises in systemic arterial pressure was unexpected since nociceptive cardiovascular responses in the rat commonly are described as hypotensive effects. Such hypotensive effects were, however, readily elicited when the animals were treated with the α -adrenoceptor blocker, phentolamine. Therefore, a separate reflex mechanism comprising the stimulation of vascular α -receptors must be in operation. The same mechanism can also be demonstrated in the cardiovascular reflexes following elevation of intraluminal pressure in the biliopancreatic duct since phentolamine potentiated the depressor responses in this model. Vascular α receptors can be stimulated via two mechanisms. Firstly, α -adrenoceptor vasoconstriction can be due to the release of noradrenaline from the sympathetic postganglionic nerve fibres supplying the vasculature; secondly, the release of catecholamines from the adrenal glands into the blood can lead to the same effect. Pressor responses to painful stimuli in cats have been shown to be largely depen-

dent upon the adrenal glands (Newman, 1953). Since in adrenalectomized rats both the pressor effects of electrical stimulation and the phentolamine-induced augmentation of depressor responses following elevation of intraductal pressure are completely absent, it can be concluded that catecholamine release from the adrenal medulla also occurs in both models used in the present investigation. This mechanism may also be responsible for the fact that distension of hollow organs in rats was found to elicit typical depressor reflexes only in about two thirds of the animals tested (Brasch & Zetler, 1982; Lembeck & Skofitsch, 1982).

The transformation by an α -adrenoceptor blocker of a pressor response into a depressor effect would be in agreement with catecholamines now producing vasodilatation via the activation of vascular β -adrenoceptors. The inhibitory effects of the unselective β -blocker, propranolol, would also support this possible explanation. However, there are three facts that are in conflict with this explanation. Firstly, certain differences in the time courses of the pressor and depressor responses suggest differences in the underlying mechanisms. Secondly, elevations of intraductal pressure cause hypotensive responses even before the administration of phentolamine also suggesting an additional mechanism. Thirdly, if this mechanism were the only one leading to the depressor effects then this response would be expected to be intact in sympathectomized animals. However, in animals which were pretreated with 6-hydroxydopamine leading to a degeneration of postganglionic sympathetic nerve endings (see Kostorzewa & Jacobowitz, 1974) only very small hypotensive effects could be observed (Figure 5c and f).

Since metoprolol, which is 50–100 times more active on β_1 than on β_2 receptors (Åblad *et al.*, 1973), greatly reduces the depressor reflexes (Figure 3b and e), the response seems to be dependent, at least partly, upon β_1 -adrenoceptors. Although it was originally held that β_1 receptors were only present in the heart whereas β_2 receptors were contained only in other tissues, it is now believed that both β -adrenoceptor subtypes coexist in many tissues. Both subtypes often, but not always, mediate the same physiologic response. Thus, both β_1 and β_2 receptors in blood vessels of the rat can mediate vasodilatation (Cohen & Wiley, 1978; O'Donnell & Wanstall, 1981). On the other hand, only the β_1 receptors seem to be responsible for the positive chronotropic and inotropic effects of catecholamines in the rat heart although both subtypes are present (Juberg *et al.*, 1985). As a consequence, two possibilities exist for the explanation of depressor responses inhibited by metoprolol. Firstly, a reflex stimulation of such vascular β_1 receptors would decrease blood pressure via vasodilatation. Secondly, a fall in blood pressure could also be caused by a reflex reduction of the sympathetic nervous tone which supports blood pressure via the positive inotropic and chronotropic effects of the cardiac β_1 receptors. Both mechanisms would be inhibited by β_1 -receptor blockade. Since the vascular β_1 receptors described were found in jugular veins (Cohen & Wiley, 1978) and pulmonary artery preparations (O'Donnell & Wanstall, 1981) rather than in resistance vessels, they are unlikely to account for the observed blood pressure changes. Thus, the second explanation for β_1 -dependent depressor responses has to be favoured.

When inhibitory effects of drugs on blood pressure reflexes are to be interpreted, it might be argued that depressor reflexes would be diminished when basal blood pressure is lowered by the drug in question. However, (–)-propranolol and metoprolol abolished the depressor responses without having any effect on blood pressure by themselves whereas atropine significantly reduced blood pressure but left the depressor reflexes unaffected. This confirms that the inhibitory action of the β -blockers should be attributed to their receptor-blocking properties rather than to changes in the general level of blood pressure.

In adrenalectomized rats, the depressor responses are not augmented, but diminished by phentolamine. Thus, the nociceptive reflexes also seem to involve the reflex inhibition of the sympathetic α -adrenergic vasoconstrictor tone. Butoxamine, which exhibits some selectivity towards β_2 as compared to β_1 sites (Bieth *et al.*, 1980; Satoh *et al.*, 1993), did not have a significant effect on the depressor reflexes. Therefore, a contribution of β_2 receptors is unlikely. The crucial importance of the sympathetic nervous system for both the pressor and depressor reflexes is further confirmed by the inhibitory action of hexamethonium (Figure 4a and c, Table 2). Although hexamethonium certainly would also inhibit ganglionic transmission in the parasympathetic nervous system, a significant contribution of this part of the autonomic nervous system can be excluded since atropine was without effect (Figure 4b and d, Table 2).

The present results show that activation of capsaicin-sensitive afferent fibres in, or in the vicinity of, the biliopancreatic duct causes nociceptive cardiovascular reflex responses in anaesthetized rats and – in the absence of anaesthesia – would lead to the perception of pain. It is also demonstrated that increases in pressure in the biliopancreatic duct system cause pain involving capsaicin-sensitive afferent neurones. Increases in intraductal pressure frequently occur in obstructive diseases of the biliopancreatic duct system (Geenen *et al.*, 1989; Moody *et al.*, 1990; Prinz & Greenlee, 1990) and also occur in certain diagnostic or therapeutic procedures (Lasson *et al.*, 1988; Jaschke *et al.*, 1992). Capsaicin-sensitive afferent fibres can not only be activated by mechanical stimuli such as increases in pressure within the biliopancreatic duct system as demonstrated here, but can also be activated by bradykinin (Juan & Lembeck, 1974; Holzer-Petsche, 1992). Thus, the endogenous release of kinins in the pancreas during acute inflammation (Griesbacher & Lembeck, 1992; Griesbacher *et al.*, 1993), can also be expected to lead to activation of these fibres and consequently to symptoms of pain. A better understanding of the types of nerves leading to pain in these conditions will be important for future treatment if the transmitters of the afferent part of the reflex arcs are better defined and accessible by appropriate selective antagonists.

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References

- ÅBLAD, B., CARLSSON, E. & EK, L. (1973). Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. *Life Sci.*, **12**, 107–119.
- BARRETT, A.M. & CULLUM, V.A. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmacol.*, **34**, 43–55.
- BIETH, N., ROUOT, B., SCHWARTZ, J. & VELLY, J. (1980). Comparison of pharmacological and binding assays for ten β -adrenoceptor blocking agents and two β -adrenoceptor agonists. *Br. J. Pharmacol.*, **68**, 563–569.
- BRASCH, H. & ZETLER, G. (1982). Cerulein and morphine in a model of visceral pain: effects on the hypotensive response to renal pelvis distension in the rat. *Naunyn-Schmied. Arch. Pharmacol.*, **319**, 161–167.
- CERVERO, F. (1982a). Noxious intensities of visceral stimulation are required to activate viscerosomatic multireceptive neurons in the thoracic spinal cord of the cat. *Brain Res.*, **240**, 350–352.
- CERVERO, F. (1982b). Afferent activity evoked by natural stimulation of the biliary system in the ferret. *Pain*, **13**, 137–151.

- CHAPMAN, W.P., HERRERA, R. & JONES, C.M. (1949). A comparison of pain produced experimentally in lower esophagus, common bile duct, and upper small intestine with pain experienced by patients with disease of biliary tract and pancreas. *Surg. Gynecol. Obstet.*, **89**, 573–582.
- CLARK, S.J. & SMITH, T.W. (1985). Opiate-induced inhibition of the visceral distension reflex by peripheral and central mechanisms. *Naunyn-Schmied. Arch. Pharmacol.*, **330**, 179–183.
- COHEN, M.L. & WILEY, K.S. (1978). β_1 and β_2 receptor mechanisms in rat jugular veins: differences between norepinephrine and isoproterenol-induced relaxation. *Life Sci.*, **23**, 1997–2006.
- CONOVER, W.J. (1980). *Practical Nonparametric Statistics*. 2nd ed. pp. 295–299. New York: Wiley.
- COSTA, M., CUELLO, A.C., FURNESS, J.B. & FRANCO, R. (1980). Distribution of enteric neurons showing immunoreactivity for substance P in the guinea-pig ileum. *Neuroscience*, **5**, 323–331.
- DASKALOPOULOS, N.T., LAUBIE, M. & SCHMITT, H. (1975). Localization of the central sympatho-inhibitory effect of a narcotic analgesic agent, fentanyl, in cats. *Eur. J. Pharmacol.*, **33**, 91–97.
- DONNERER, J. (1988). Reflex activation of the adrenal medulla during hypoglycemia and circulatory dysregulations is regulated by capsaicin-sensitive afferents. *Naunyn-Schmied. Arch. Pharmacol.*, **338**, 282–286.
- DONNERER, J. & LEMBECK, F. (1983). Capsaicin-induced reflex fall in blood pressure is mediated by afferent substance P-containing neurones via a reflex centre in the brain stem. *Naunyn-Schmied. Arch. Pharmacol.*, **324**, 293–295.
- FITZGERALD, M. (1983). Capsaicin and sensory neurones – a review. *Pain*, **15**, 109–130.
- FURNESS, J.B., PAPKA, R.E., DELLA, N.G., COSTA, M. & ESKAY, R.L. (1982). Substance P-like immunoreactivity in nerves associated with the vascular system in guinea-pigs. *Neuroscience*, **7**, 447–459.
- GAENSLER, E.A. (1951). Quantitative determination of the visceral pain threshold in man: characteristics of visceral pain, effect of inflammation and analgesics on the threshold, and relationship of analgesia to visceral spasm. *J. Clin. Invest.*, **30**, 406–420.
- GEENEN, J.E., HOGAN, W.J., DODDS, W.J., TOOULI, J. & VENU, R.P. (1989). The efficacy of endoscopic sphincterotomy after cholecystectomy in patients with sphincter-of-Oddi dysfunction. *New Engl. J. Med.*, **320**, 82–87.
- GERNANDT, B. & ZOTTERMAN, Y. (1946). Intestinal pain: an electrophysiological investigation on mesenteric nerves. *Acta Physiol. Scand.*, **12**, 56–72.
- GRIESBACHER, T. & LEMBECK, F. (1992). Effects of the bradykinin antagonist, HOE 140, in experimental acute pancreatitis. *Br. J. Pharmacol.*, **107**, 356–360.
- GRIESBACHER, T. & LEMBECK, F. (1993). Increase in pressure in the biliopancreatic duct causes nociceptive blood pressure reflexes via capsaicin-sensitive afferents in the rat. *Vth Joint meeting between the British and Italian Societies of Pharmacology*, Rome, Italy. 13–16 September.
- GRIESBACHER, T., TIRAN, B. & LEMBECK, F. (1993). Pathological events in experimental acute pancreatitis prevented by the bradykinin antagonist, Hoe 140. *Br. J. Pharmacol.*, **108**, 405–411.
- HOLZER, P. (1991). Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol. Rev.*, **43**, 143–201.
- HOLZER, P., GAMSE, R. & LEMBECK, F. (1980). Distribution of substance P in the rat gastrointestinal tract – lack of effect of capsaicin pretreatment. *Eur. J. Pharmacol.*, **61**, 303–307.
- HOLZER, P., BUCSICS, A. & LEMBECK, F. (1982). Distribution of capsaicin-sensitive nerve fibres containing immunoreactive substance P in cutaneous and visceral tissues of the rat. *Neurosci. Lett.*, **31**, 253–257.
- HOLZER-PETSCH, U. (1992). Blood pressure and gastric motor responses to bradykinin and hydrochloric acid injected into somatic or visceral tissues. *Naunyn-Schmied. Arch. Pharmacol.*, **346**, 219–225.
- INOUE, N., SAKAI, H., MAGARI, S. & SANAKA, M. (1992). Distribution and possible origins of substance P-containing nerve fibres in the rat liver. *Anat. Anz.*, **174**, 557–560.
- JANCSÓ, G., KIRÁLY, E. & JANCSÓ-GÁBOR, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurons. *Nature*, **270**, 741–743.
- JASCHKE, W., KLOSE, K.J. & STRECKER, E.P. (1992). A new balloon-expandable tantalum stent (Strecker-Stent) for the biliary system: preliminary experience. *Cardiovasc. Intervent. Radiol.*, **15**, 356–359.
- JUAN, H. & LEMBECK, F. (1974). Action of peptides and other algescic agents on paravascular pain receptors of the isolated perfused rabbit ear. *Naunyn-Schmied. Arch. Pharmacol.*, **283**, 151–164.
- JUBERG, E.N., MINNEMAN, K.P. & ABEL, P.W. (1985). β_1 - and β_2 -adrenoceptor binding and functional response in right and left atria of rat heart. *Naunyn-Schmied. Arch. Pharmacol.*, **330**, 193–202.
- KOSTRZEWA, R.M. & JACOBOWITZ, D.M. (1974). Pharmacological actions of 6-hydroxydopamine. *Pharmacol. Rev.*, **26**, 199–288.
- LASSON, Å., FORK, F.-T., TRÄGÅRDH, B. & ZEDERFELDT, B. (1988). The postcholecystectomy syndrome: bile ducts as pain trigger zone. *Scand. J. Gastroenterol.*, **23**, 265–271.
- LAUBIE, M., SCHMITT, H., CANELLAS, J., ROQUEBERT, J. & DEMICHELE, P. (1974). Centrally mediated bradycardia and hypotension induced by narcotic analgesics: dextromoramide and fentanyl. *Eur. J. Pharmacol.*, **28**, 66–75.
- LEMBECK, F. & DONNERER, J. (1981). Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. *Naunyn-Schmied. Arch. Pharmacol.*, **316**, 240–243.
- LEMBECK, F. & DONNERER, J. (1983). Reflex fall in blood pressure mediated by capsaicin-sensitive afferent fibres of the rat splanchnic nerve. *Naunyn-Schmied. Arch. Pharmacol.*, **322**, 286–289.
- LEMBECK, F. & SKOFITSCH, G. (1982). Visceral pain reflex after treatment with capsaicin and morphine. *Naunyn-Schmied. Arch. Pharmacol.*, **321**, 116–122.
- MCGOWAN, J.M., BUTSCH, W.L. & WALTERS, W. (1936). Pressure in the common bile duct of man: its relation to pain following cholecystectomy. *J. Am. Med. Assoc.*, **106**, 2227–2230.
- MOODY, F.G., CALABUIG, R., VECCHIO, R. & RUNKEL, N. (1990). Stenosis of the sphincter of Oddi. *Surg. Clin. North Am.*, **70**, 1341–1354.
- NAGY, J.I., IVERSEN, L.L., GOEDERT, M., CHAPMAN, D. & HUNT, S.P. (1983). Dose-dependent effects of capsaicin on primary sensory neurons in the neonatal rat. *J. Neurosci.*, **3**, 399–406.
- NESS, T.J. & GEBHART, G.F. (1988). Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat. *Brain Res.*, **450**, 153–169.
- NESS, T.J. & GEBHART, G.F. (1990). Visceral pain: a review of experimental studies. *Pain*, **41**, 167–234.
- NEWMAN, P.P. (1953). Changes in arterial blood pressure following stimulation of the gall bladder. *J. Physiol.*, **119**, 46P–47P.
- O'DONNELL, S.R. & WANSTALL, J.C. (1981). Demonstration of both β_1 - and β_2 -adrenoceptors mediating relaxation of isolated ring preparations of rat pulmonary artery. *Br. J. Pharmacol.*, **74**, 547–552.
- ORDWAY, G.A., LONGHURST, J.C. & MITCHELL, J.H. (1983). Stimulation of pancreatic afferents reflexly activates the cardiovascular system in cats. *Am. J. Physiol.*, **245**, R820–R826.
- PRINZ, R.A., GREENLEE, H.B. (1990). Pancreatic duct drainage in chronic pancreatitis. *Hepatogastroenterology*, **37**, 295–300.
- SATO, E., NARIMATSU, A., HOSOHATA, Y., TSUCHIHASHI, H. & NAGATOMO, T. (1993). The affinity of betaxolol, a β_1 -adrenoceptor-selective blocking agent, for β -adrenoceptors in the bovine trachea and heart. *Br. J. Pharmacol.*, **108**, 484–489.
- SHARKEY, K.A., WILLIAMS, R.G. & DOCKRAY, G.J. (1984). Sensory substance P innervation of the stomach and pancreas: demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing. *Gastroenterology*, **87**, 914–921.
- UENO, T., INUZUKA, S., TORIMURA, T., SAKATA, R., SAKAMOTO, M., GONDO, K., AOKI, T., TANIKAWA, K. & TSUTSUMI, V. (1991). Distribution of substance P and vasoactive intestinal peptide in the human liver: light and electron immunoperoxidase methods of observation. *Am. J. Gastroenterol.*, **86**, 1633–1637.
- WOODSWORTH, R.S. & SHERRINGTON, C.S. (1904). A pseudo-affective reflex and its spinal path. *J. Physiol.*, **31**, 234–243.

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