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Short communication

Effects of the CCK_A receptor antagonists SR 27897B and PD140548 on baroreflex function in conscious rats

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Abstract

Since the cardiovascular effects of cholecystokinin (CCK) seem to particularly involve the A ('peripheral') subtype of CCK (CCK_A) receptor, we examined the actions of two novel, highly selective CCK_A receptor antagonists, PD140548 (*N*-α-methyl-*N*[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-L-tryptophyl]-D-3-(phenylmethyl)-β-alanine) and SR 27897B (1-[[2-(4-(2-chlorophenyl)thiazol-2-yl)aminocarbonyl]acetic acid) on CCK-induced alterations in blood pressure and heart rate, and on the baroreceptor reflex in the conscious, instrumented rat. CCK (2 μg, i.v.) produced a pressor response and biphasic effects on heart rate involving an initial bradycardia followed by a pronounced tachycardia. Administration of PD140548 (10 mg/kg, i.v.) and SR 27897B (0.6 mg/kg, i.v.) significantly inhibited the pressor effects of CCK (35 and 47%, respectively), whilst reversing the bradycardic responses to a tachycardia. The CCK_A receptor antagonists had different effects on the baroreceptor heart rate reflex since only PD140548 caused a significant increase in the gain or sensitivity of the reflex. This effect of PD140548 on gain is likely to occur via a central mechanism and may reflect the increased lipophilicity of PD140548 relative to SR 27897B. Overall, these investigations provide new evidence for the involvement of the CCK_A receptor in cardiovascular regulation. © 1997 Elsevier Science B.V.

Keywords: CCK (cholecystokinin); CCK_A receptor antagonist; Blood pressure; Heart rate; Baroreflex; (Conscious rat)

1. Introduction

Cholecystokinin (CCK) is one of the most widely distributed of all neuropeptides and plays various physiological roles as a neurotransmitter and/or peptide hormone in the central and peripheral nervous systems (Crawley and Corwin, 1993). A considerable body of pharmacological and molecular biological evidence indicates that CCK exerts its effects via two distinct subtypes of receptors, CCK_A ('peripheral') and CCK_B ('central') (Crawley and Corwin, 1993; Wank et al., 1994). While CCK_A receptors predominate in pancreas, gall bladder and gut, the situation has changed somewhat of late with cloning, sequencing and expression studies indicating that CCK_B and gastrin receptors are essentially identical (Wank et al., 1994). The CCK_B receptor, unlike the CCK_A receptor, possesses relatively high affinity for various peptide fragments, including unsulphated CCK, CCK tetrapeptide, pentagastrin and gastrin. Within brain, where CCK_B receptors predominate, CCK appears to be involved in feeding/satiety, panic/anxiety, pain/analgesia and neurological diseases. Of course, the sulphated octapeptide has well documented physiological roles in the control of gall bladder contraction, gastric emptying and intestinal motility, and in the stimulation of biliary and pancreatic secretion (Crawley and Corwin, 1993).

In contrast to the multitude of studies pertinent to the above phenomena, the cardiovascular and respiratory effects of CCK have received surprisingly little attention. CCK is present in vagal and glossopharyngeal afferent neurones (Helke and Hill, 1988), which send their afferents centrally to the nucleus of the solitary tract, a key brainstem area for the integration of autonomic functions. Peripherally, these axons innervate the heart, lungs, carotid body, as well as the gut. Our own studies have shown that CCK_A receptors are preferentially localised to the nodose ganglion (Widdop et al., 1993; Beart et al., 1996). Moreover, the nucleus of the solitary tract contains both somata and terminals immunoreactive for CCK, and CCK_A and CCK_B receptors (Crawley and Corwin, 1993; Carlberg et al., 1993).

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CCK has actions on respiratory function (Gillis et al., 1983; Hurlé et al., 1985), while intravenous CCK causes dose-dependent bradycardia and complex changes in blood pressure, as well as having a direct action on the heart (Marker and Roberts, 1988). CCK also restores cardiovascular function and respiratory rate in severe haemorrhagic shock (Guarini et al., 1988). CCK-induced elevation of blood pressure was accompanied by dose-dependent effects on the heart, with a lower dose of CCK increasing heart rate, but a high dose causing bradycardia followed by tachycardia (Janssen et al., 1991; Bachelard et al., 1992). Additionally, generalised vasoconstriction accompanied the pressor response to CCK, all of which were markedly attenuated by the CCK_A receptor antagonist devazepide (Janssen et al., 1991; Bachelard et al., 1992). Collectively, these data indicate that the cardiovascular effects of CCK are mediated predominantly via the involvement of CCK_A receptors (Gillis et al., 1983; Hurlé et al., 1985; Marker and Roberts, 1988; Guarini et al., 1988; Janssen et al., 1991).

With developments in the medicinal chemistry of CCK receptor antagonists, we have taken the opportunity to evaluate two novel CCK_A receptor antagonists, PD140548 (Boden et al., 1993) and SR 27897B (Gully et al., 1993). Initially, we demonstrated that both agents were efficacious CCK_A receptor antagonists in the rat isolated nodose ganglion (Beart et al., 1996). In the present study, we have further investigated these compounds for their actions on CCK-induced effects on blood pressure and heart rate, and on the baroreceptor heart rate reflex in conscious rats.

2. Materials and methods

2.1. Materials

SR 27897B(1-[[2-(4-(2-chlorophenyl)thiazol-2-yl)aminocarbonyl]acetic acid) and PD140548(*N*-α-methyl-*N*[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-L-tryptophyl]-D-3-(phenylmethyl)-β-alanine) were gifts from Sanofi Recherche (Toulouse, Frnace) and Parke-Davis (Cambridge, UK). CCK was obtained from Research Plus (Bayonne, France). Stock solutions of CCK and PD140548 were made up in distilled water, while SR 27897B was dissolved in dimethyl sulphoxide; siliconised glass tubes were always used for the preparation and storage of solutions.

2.2. Surgical procedures

Male Sprague–Dawley rats were obtained from the Central Animal Services, Monash University (Melbourne, Australia). All experimental procedures were approved by the Monash University Animal Ethics Committee and performed according to the National Health and Medical Research Council of Australia guidelines for animal experimentation. Rats (aged 15–18 weeks, weighing 250–350 g) were anaesthetised (sodium methohexitone 60 mg/kg i.p., supplemented as required) and two catheters were implanted in the right jugular vein and a catheter was also inserted into the abdominal aorta via the caudal artery. Following at least 24 h recovery, experiments were performed in conscious, unrestrained rats, whereby continuous recordings were made of mean and phasic blood pressure and heart rate on a MacLab-8 data acquisition system (ADInstruments, Sydney, Australia).

2.3. Effects of the CCK_a receptor antagonists PD140548 and SR 27897B on cardiovascular parameters and baroreflex function

Initially, mean arterial pressure and heart rate responses to CCK (2 μg i.v.) were obtained in two separate groups of animals before, and 15 min and 24 h after administration of either of the CCK_A receptor antagonists, PD140548 (10 mg/kg i.v., $n = 6$) or SR 27897B (0.6 mg/kg i.v., $n = 5$). In preliminary experiments, 10-fold lower doses did not alter the cardiovascular effect of CCK. Baroreflex function was also assessed in both groups of animals, before and starting 30 min and 24 h after giving either antagonist, by constructing mean arterial pressure–heart rate curves as described previously (Head and McCarty, 1987). Phenylephrine (1–25 μg/kg i.v.) and sodium nitroprusside (1–50 μg/kg i.v.) were injected alternately through separate venous catheters in order to raise or lower blood pressure by approximately 5–50 mmHg. A sigmoidal logistic equation was then fitted to the mean arterial pressure and corresponding changes in heart rate:

$$\text{Heart rate} = P_1 + P_2/(1 + e^{P_3(\text{MAP} - P_4)})$$

where P_1 is the lowest heart rate plateau, P_2 is the heart rate range, P_3 is a curvature coefficient and P_4 is the BP_{50} value which is the mean arterial pressure value at the midpoint of the heart rate range (Head and McCarty, 1987). The average gain (i.e. slope) of the mean arterial pressure–heart rate curve between the two inflection points is given by $-P_2 \times P_3/4.56$ and the upper plateau equals P_1 + heart rate range.

2.4. Statistical analysis

Comparison of baseline mean arterial pressure and heart rate values between treatment groups was made using a Student's non-paired *t*-test. Changes in baroreflex curve parameters and responses to CCK following antagonist administration were analysed using a Student's paired *t*-test. Statistical significance was accepted at $P < 0.05$.

3. Results

Resting mean arterial pressure was slightly higher in the SR 27897B-treated group; however, there was no signifi-

Table 1
Effect of the CCK_A-receptor antagonists SR 27897B and PD140548 on baseline MAP, HR and baroreflex curve parameters in conscious Sprague–Dawley rats

	SR 27897B ($n = 5$)		PD140548 ($n = 6$)	
	baseline	post SR27897B	baseline	post PD140548
Mean arterial pressure (mmHg)	110 ± 4	108 ± 5	96 ± 4	107 ± 3
Heart rate (beats/min)	382 ± 19	382 ± 12	362 ± 9	347 ± 7
Lower plateau (beats/min)	297 ± 25	282 ± 23 [a]	285 ± 7	275 ± 9
Upper plateau (beats/min)	490 ± 18	500 ± 15	495 ± 14	469 ± 9 [a]
Range (beats/min)	193 ± 18	218 ± 18	201 ± 20	193 ± 11
BP50 (mmHg)	108 ± 2	109 ± 2	98 ± 4	102 ± 4
Gain (beats/min per mmHg)	−3.6 ± 0.3	−3.3 ± 0.1	−2.9 ± 0.2	−4.1 ± 0.5 [a]

Values are mean ± S.E.M.
[a] $P < 0.05$ versus control baseline effect.

cant difference in heart rate between the two groups (see Table 1). Prior to antagonist administration in both groups, CCK (2 μg i.v.) caused pressor responses of approximately 35 mmHg, which had returned to control levels by 5 min. In contrast, the heart rate responses were biphasic in nature with an initial bradycardia (approximately −50 to −80 beats/min) followed by modest tachycardia (approximately 20–40 beats/min) (Fig. 1a and b). Administration of PD140548 or SR 27897B significantly inhibited the pressor responses by 35 and 47%, respectively ($P <$

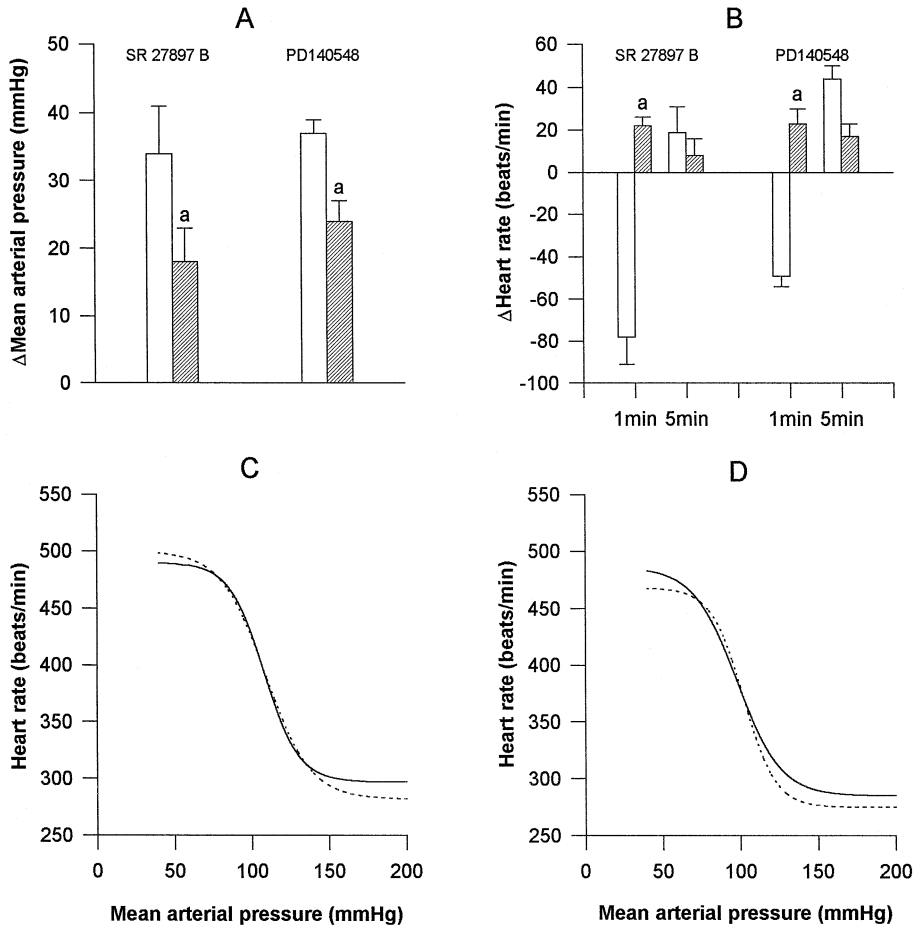

Fig. 1. (A) Mean pressor responses to CCK (2 μg iv) before (open bars) and 15 min (hatched bars) after SR 27897B (0.6 mg/kg i.v., $n = 5$) or PD140548 (10 mg/kg i.v., $n = 6$) administration; (B) changes in heart rate measured at 1 min and 5 min after CCK injection, before (open bars) and 15 min after SR 27897B and PD140548 administration (hatched bars); (C and D) baroreflex curves obtained before (solid lines) and 30 min after (dashed lines) administration of (C) SR 27897B or (D) PD140548. [a] $P < 0.01$ versus control response.

0.05), and reversed the bradycardic responses to tachycardic responses of approximately 20 beats/min ($P < 0.01$), which then waned by 5 min (Fig. 1a and b). These responses evoked by CCK had returned to control levels by 24 h (data not shown). Both antagonists did not alter resting mean arterial pressure or heart rate values (Table 1).

There were no significant differences in the basal baroreflex curve parameters between the two groups except for a slightly higher BP_{50} in the SR 27897B group, a finding which paralleled the higher basal mean arterial pressure (see Table 1). However, following antagonist administration there was a significant increase in the gain (i.e., slope) and decrease in the upper plateau of the baroreflex curve in the PD140548-treated group ($P < 0.05$) while, in the SR 27897B-treated rats, there was a significant decrease in the lower plateau ($P < 0.05$) (see Table 1 and Fig. 1c and d). These parameters had returned to control levels by 24 h (data not shown).

4. Discussion

CCK evoked an immediate pressor response which was accompanied by a biphasic heart rate response consisting of a decrease followed by an increase in heart rate, as has been reported previously (Janssen et al., 1991). The bradycardic response is in part baroreflex-mediated (Koyama et al., 1990; Janssen et al., 1991) as well as being due to a direct non-adrenergic, non-cholinergic negative chronotropic effect (Marker and Roberts, 1988). The delayed tachycardia was not temporally related to the blood pressure response, but was most likely due to baroreflex-independent inhibition of vagal tone since this was blocked by atropine (Janssen et al., 1991). However, CCK evoked a depressor response in anaesthetised dogs (Koyama et al., 1990), although this peptide had variable effects on blood pressure in anaesthetised rats (Marker and Roberts, 1988), but only pressor effects in conscious rats (present study, Janssen et al., 1991; Bachelard et al., 1992). This difference may reflect a species difference or the effect of anaesthesia.

The cardiovascular effect of CCK was likely to involve CCK_A receptors since the CCK_A receptor antagonists SR 27897B and PD 140548 significantly attenuated the pressor response to a similar extent, and similar findings were reported using the prototypical CCK_A receptor antagonist, devazepide (Janssen et al., 1991). Moreover, the initial bradycardia evoked by CCK was converted into a tachycardia in the presence of either CCK_A receptor antagonist. Thus, CCK-mediated bradycardia was more susceptible to blockade than was the pressor response evoked by this peptide, which may reflect a direct receptor-mediated vagal afferent action of CCK (Koyama et al., 1990; Widdop et al., 1993; Beart et al., 1996). Due to limited drug supplies, we were unable to increase the antagonist doses further to see if there were further reductions in the pressor responses. However, it is likely that the residual pressor response was contributed to by an increased cardiac output since there was an unopposed tachycardia at this time. Alternatively, the role of the CCK_B receptor in the cardiovascular effects of CCK is not known, so it is feasible CCK_B receptors may be involved in the refractory cardiovascular response to CCK.

The doses of SR 27897B (0.6 mg/kg i.v.), PD140548 (10 mg/kg i.v.) and devazepide ($\sim$0.075 mg/kg i.v. for 10 min infusion; Janssen et al., 1991) indicate a rank order of potency of CCK_A receptor antagonists of devazepide > SR 27897B > PD140548. However, the present study has not conducted a dose–response analysis. Nevertheless, this order is in fact identical to that described by us using the rat isolated nodose ganglion preparation (Widdop et al., 1993; Beart et al., 1996) and suggests that their in vitro potency at CCK_A receptors accurately predicts their in vivo efficacy.

The two CCK_A receptor antagonists, despite having similar effects on blood pressure and heart rate, had markedly different effects on the sensitivity of the baroreceptor heart rate reflex, since only PD140548 enhanced the gain of the baroreflex curve. Both compounds tended to cause similar small changes to the lower heart rate plateau, although only the effect of SR 27897B was significant. Thus, both compounds did not significantly alter the heart rate range. The fact that PD140548 caused a range-independent change in gain indicates there was a change in the curvature of the baroreflex curve; an effect most likely to occur via an action within the brain (Head, 1994). To our knowledge, no other similar baroreflex studies examining CCK_A receptor antagonists have been performed. In any case, our data suggest that, over the operating range of the baroreflex, CCK tonically inhibited the buffering ability of heart rate in response to blood pressure changes. In this context it appears relevant that PD140548 and SR 27897B are chemically quite different molecules possessing log ρ values of 5.46 and 4.89 (unpublished observations), respectively, indicating that PD140548 is likely to be some 3–4 times more lipophilic than SR 27897B and thus more likely to enter the brain after i.v. administration. In conclusion, our findings not only provide evidence for the involvement of CCK_A receptors in cardiovascular regulation, but they also suggest that CCK tonically modulates the sensitivity of the baroreceptor heart rate reflex, most likely by a central action (e.g. in the nucleus of the solitary tract).

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