Cardiovascular Effects of Cholecystokinin-4 Are Mediated By the Cholecystokinin-B Receptor Subtype In the Conscious Guinea Pig and Dog

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Accepted for publication December 10, 1996

ABSTRACT
Panicogenic effects in humans of the selective cholecystokinin (CCKB) receptor agonist, cholecystokinin tetrapeptide (CCK4), have been reported to correlate with increases in heart rate (HR) and mean arterial pressure (MAP). Previous investigators have demonstrated that the nonselective CCKA and CCKB receptor agonist, sulfated cholecystokinin octapeptide, also produces increases in HR and mean arterial pressure. The purpose of our study is to determine if the cardiovascular changes induced by CCK4 are mediated by the CCK A or CCK B receptor subtype using selective CCK antagonists for both receptor subtypes. The rank order of potency of the CCK receptor antagonists affecting CCK4-induced HR and mean arterial pressure changes in the guinea pig corresponded to the rank order of potency for blockade of the CCKB receptor binding in rat cortex, phosphatidyl inositol turnover in AR 4-2J rat pancreatoma cells and inhibition of pentagastrin-induced acid secretion in the rat. The changes induced by CCK4 on HR, but not mean arterial pressure, appear to be species dependent as reflected by a decrease in the HR in the guinea pig and an increase in the dog. Nonetheless, the results from the antagonist studies indicate that the cardiovascular responses to CCK4 in both the guinea pig and dog are mediated by the CCKB receptor subtype.

CCK, a 33 amino acid peptide first isolated from porcine gut, is a member of a family of peptides that together with gastrin share a common carboxyl-terminal pentapeptide amino acid sequence (Mutt and Jorpes, 1968). Both N-terminal extended forms of CCK have been described as well as truncated forms such as CCKB, pentagastrin and CCK (Dockray et al., 1978; Larsson and Rehfeld, 1979; Reeve et al., 1990). These peptide fragments of CCK have been useful in pharmacologically identifying two receptor types: CCKA and CCKB (Innis and Synder, 1980; Gridler and Makhlouf, 1987; Hughes et al., 1990). These peptide fragments of CCK have been useful in pharmacologically identifying two receptor types: CCKA and CCKB (Innis and Synder, 1980; Gridler and Makhlouf, 1987; Hughes et al., 1990). CCKA receptors, found primarily in pancreatic acinar cells and in the gastrointestinal tract, have high affinity for sulfated CCK (i.e., CCKSS) and gastrin and a lower affinity for unsulfated CCKB, pentagastrin and CCK. In contrast, the CCKB receptors that predominate in brain have high affinity for pentagastrin and the tetrapeptide CCK (Hughes et al., 1990). More recently, confirmation of receptor heterogeneity has occurred with the development of selective, high affinity nonpeptide antagonists (Woodruff and Hughes, 1991) and the recent cloning of the CCKA and CCKB receptors (Wank et al., 1992a; Ulrich et al., 1993; Wank et al., 1992b; Pisegna et al., 1992; Lee et al., 1993).

The physiological functions described for the CCKA receptor in the periphery are primarily related to pancreatic secretion of amylase and insulin, although in the gastrointestinal tract pepsinogen is secreted in addition to stimulation of longitudinal smooth muscle (Woodruff and Hughes, 1991). In the central nervous system, CCKA receptors have been identified in the substantia nigra and striatum and appear to influence activity of the dopaminergic system (van Dijk et al., 1984; Hill et al., 1990). CCKA receptors have also been found in the dorsal raphe, area postrema, nucleus tractus solitarius and interpeduncular nucleus (Woodruff and Hughes, 1991).

Historically, elucidation of the physiological role of peripherally located CCKB receptors has been limited. However, with the development of such specific antagonists as CI-988 and L-365,260, the significance of this receptor is becoming more apparent. Several investigators have reported that CCKB receptors may mediate secretory and contractile responses in the guinea pig (Lucaites et al., 1991, Gridler and Makhlouf, 1990). Specific agonists that produce CCKB-mediated peripheral effects include pentagastrin and CCK (Lucaites et al., 1991).

ABBREVIATIONS: CCK, cholecystokinin; CCK, cholecystokinin tetrapeptide; CCKB, cholecystokinin octapeptide; CCKB, sulfated cholecystokinin octapeptide; CCKBS, unsulfated cholecystokinin octapeptide; PI, phosphatidylinositol; HR, heart rate; MAP, mean arterial pressure.
CCK \textsubscript{B} receptors are the predominant CCK-receptor subtype in the brain. Infusion of pentagastrin into the lateral ventricles of sheep has been shown to produce behaviors equated to fear (Della-Fera and Baile, 1979). Hughes \textit{et al.} (1990) showed that the specific receptor antagonist, CI-988, reduced anxiety in the mouse black-white test and in the marmoset-human threat test. In humans with a history of panic disorder, i.v. administered CCK\textsubscript{A} produces spontaneous panic-like symptoms that are indistinguishable from endogenous panic attacks. These symptoms have been characterized by increases in anxiety and physiological signs such as increased heart rate and blood pressure (Bradwejn \textit{et al.}, 1992). Other investigators have reported that the nonselective CCK\textsubscript{A} and CCK\textsubscript{B} receptor agonist, CCK\textsubscript{ss}, also produces increases or decreases in HR (dose dependent) and increases in mean arterial pressure of rats that are blocked by the CCK\textsubscript{A} selective antagonist, devazepide (Guarini \textit{et al.}, 1988; Janssen \textit{et al.}, 1991; Gaw \textit{et al.}, 1995). The purpose of this study is to determine which CCK receptor subtype mediates the cardiovascular responses observed by Bradwejn \textit{et al.} (1992) after administration of CCK\textsubscript{A}.

\section*{Methods}

\subsection*{Compounds}

Cholecystokinin 4 (structure H-Trp-Met-Asp-Phe-NH\textsubscript{2}) was from Bachem Feinchemikalien AG, Germany. The CCK antagonists, CP-212,454 (N-1-t-butyl) 2-[3-(3-chlorophenylethyl)-2-oxo-5-phenyl-8-methyl-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] ethanoic acid amide, CP-310,713 (N-1-t-Butyl)-2-[3-(3-carboxbenzyloxy)-2-oxo-5-cyclohexyl-8-methyl-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] ethanoic acid amide, L-365,260, devazepide and CI-988 were synthesized by Pfizer Inc. (Groton, CT).

\subsection*{Receptor Binding}

For CCK\textsubscript{B} receptor binding assays guinea pig cortex was homogenized with a Teflon homogenizer in 20 vol of 50 mM Tris HCl (pH 7.4) containing 5 mM MnCl\textsubscript{2} at 4°C and centrifuged at 100,000 \times g for 30 min. The supernatant was discarded and the pellet resuspended and spun again. The pellet was diluted to a concentration of 10 mg/ml (original wet weight) with assay buffer (10 mM HEPES, 5 mM MgCl\textsubscript{2}, 1 mM EGTA, 130 mM NaCl and 0.2 mg/ml bacitracin, pH 6.5) before use. The incubation reaction was initiated by the addition of 50 \mu M of tissue to 96-well plates containing 150 \mu M of assay buffer with 1% DMSO final concentration, 50 mM final concentration of \textsuperscript{125}I-BH-CCK\textsubscript{ss} (Du Pont NEN, Boston, MA) and the appropriate concentration of drug or vehicle. Nonspecific binding was estimated using 1 \mu M L-364,718. After a 30-min incubation the reaction was terminated by rapid filtration using a Skatron cell harvester onto GF/B filters that were soaked for 2 hr in 50 mM Tris HCl, 0.1 mg/ml bovine serum albumin. The filters were dried and counted on a Betaplate counter for 45 sec per sample.

\subsection*{Phosphatidylinositol Turnover}

AR 4-2J rat pancreatoma cells obtained from Dr. J. Putney (NIEHS, Research Triangle Park, NC) were grown in DMEM supplemented with L-glutamine and 10% fetal bovine serum (FBS). AR 4-2J cells were prelabeled with \textsuperscript{3}H-myoinositol overnight. The cells were incubated with agonists for 45 min in the presence of LiCl 10 mM and the reaction terminated by adding CHCl\textsubscript{3}:MeOH (1:2). The cells were harvested with PBS containing 3 mM EDTA, spun down and resuspended in PBS with 20 mM HEPES and 3 mg/ml d-glucose at a concentration of 1 to 5 \times 10\textsuperscript{6} cells/ml. Cells were exposed to antagonists 10 min before agonist exposure. \textsuperscript{3}H-myoinositol phosphates were isolated by a batch technique using a Dowex AG1-X8 anion exchange resin. Corrected IC\textsubscript{50}s (\textit{K}_i) were calculated by \textit{K}_i = IC\textsubscript{50}/1 + [pentagastrin]/EC\textsubscript{50} pentagastrin, where [\textit{I}] = concentration.

\subsection*{Gastric Acid Secretion}

Gastric acid secretion studies were conducted in rats using a modification of the pylorus ligation model described by Hakkinen \textit{et al.} (1991). Fasted male Sprague-Dawley rats (125–250 g) were anesthetized by inhalation with methoxyflurane (Metofane, Pitman-Moore, Inc., Chicago, IL) and the pylorus ligated. Compounds were administered in a vehicle DMSO:emulphor:saline (5:15:80) by s.c. injection (4 ml/kg). Pentagastrin was administered in a 1.99 vehicle of DMSO:saline (v:v). Rats were killed 2 hr after administration of drugs and pentagastrin and the gastric fluid was diluted with water and titrated to pH 7.0 with 0.1 N sodium hydroxide using a Radiometer TTT85 Titrator and an ABU80 Autoburette (Radiometer America, Inc., Westlake, OH). The amount of sodium hydroxide used was taken as a direct measure of the titratable acid (expressed in mEq) in the sample. The acid content was calculated per ml of gastric fluid and normalized to the time of the ligation and the body weight of the rat.

\subsection*{In Vivo Cardiovascular Studies}

\textbf{Animals.} All animal studies were conducted in accordance with protocols approved by the Pfizer Institutional Animal Care and Use Committee. Male Hartley guinea pigs were obtained from Charles River Breeding Laboratories (Wilmington, MA). Purpose-bred mongrel dogs were obtained from Hazelton Labs (Kalamazoo, MI). Animals were housed on a 12-hr light cycle (0700–1900 hr) at 27 ± 5°C. On the day before experimentation, guinea pigs (300–350 g) were anesthetized with xylazine (10 mg/kg s.c.; Mobay Corp., Shawnee, KS) and ketamine (80 mg/kg, i.m.; Parke-Davis, Morris Plains, NJ) and the right jugular vein and left carotid artery were isolated and cannulated with polyethylene tubing (0.58 mm i.d. × 0.9556 mm o.d.). Both catheters were exteriorized at the interscapular region and filled with a heparin-(500 U/ml) dextrose (50%) lock to ensure patency. Before surgery, the animals were dosed orally (1 ml) with the antibiotic combination trimethoprim: 8 mg/sulfamethoxazole: 40 mg (Roche Laboratories, Nutley, NJ). The animals were allowed to recover overnight with food and water ad libitum.

Adult mongrel dogs (8–12 kg) were anesthetized with isoflurane (1–1.5%) and nitrous oxide (0.2 liter/min) and instrumented with a...
Data Sciences, Inc. (St. Paul, MN) pressure/ECG telemetry device (model TL10M2D70-PC), with the pressure catheter tip placed in the abdominal aorta via the femoral artery and the ECG leads tunneled s.c. to the upper right thorax and the lower left inner thigh for obtaining a Lead II electrocardiogram. The telemetry transmitter body was secured s.c. on the dog’s flank. The dogs were allowed to recover from surgery for at least 2 wk and trained to lie quietly in a sling. Subjects were fasted for 12 hr before experiments.

**Cardiovascular measurements.** Animals were studied in the conscious state. Guinea pigs were placed in Plexiglas restraining tubes. Carotid catheters were connected to a Statham P231D pressure transducer (Ohmeda, Oxford, CA) positioned at the level of the heart and interfaced with a Gould (Gould Instrument Systems Inc., Valley View, OH) transducer amplifier (model 20-4615-50). The telemetered signals from dogs were transformed back to calibrated analog signals using a Data Sciences UA10 Universal Adapter D/A converter. The pulsatil waveforms were displayed on an Astromed MT95000 polygraph (Astromed, West Warwick, RI). Mean arterial pressure and heart rate were derived from a beat-to-beat analysis of the pulsatil waveform using Po-Ne-Mah model MA-1 data acquisition and analysis software (Po-Ne-Mah, Inc., Simsbury, CT). Values were averaged over 60-sec intervals during base-line periods. To increase the sensitivity of the system to acute changes in pressure and heart rate, measurements were averaged over 10-sec intervals after i.v. challenge with CCKx.

**Dose response to CCK**x. A dose of CCKx that gave a robust and reproducible increase in mean arterial pressure and decrease in heart rate after i.v. bolus administration was established. A dose response curve to CCKx was generated over a range of 1 to 160 μg/kg in guinea pigs (n = 4) and 1 to 20 μg/kg in mongrel dogs (n = 3). The peptide was dissolved in 0.9% saline vehicle at a volume of 1 ml/kg i.v. in guinea pigs or 0.1 ml/kg i.v. for dogs. The dose concentrations were delivered in a random fashion. At the end of the study, selected doses were repeated in the animals to evaluate whether there was any tolerance or tachyphylaxis to the CCKx injection.

**Experimental protocol.** After obtaining stable base-line measurements of mean arterial pressure and heart rate, the animal was challenged with an i.v. bolus injection of the 0.9% saline vehicle and changes in pressure and heart rate recorded. Fifteen min later, the maximal control cardiovascular response to an i.v. bolus injection CCKx (20 μg/kg for guinea pigs; 10 μg/kg for dogs) was measured. For guinea pig studies, the CCK receptor antagonist or vehicle (3 ml/kg) was administered by a slow i.v. push. For dog experiments, the antagonist or vehicle (2% Tween 80 in water at 4 ml/kg) was administered orally. All antagonists were prepared in a 5:5:90 (v:v:v) solution of Tween 80, propylene glycol, and water at 2% concentration. The antagonist or vehicle (2% Tween 80 in water at 4 ml/kg) was administered by a slow i.v. push. For dog experiments, the antagonist or vehicle (2% Tween 80 in water at 4 ml/kg) was administered orally. A one-way analysis of variance with an unpaired Student’s t test was used for receptor binding and gastric acid secretion studies. When examining the cardiovascular effects of CCK antagonists, a two-way analysis of variance with repeated measures using time and treatment as factors was performed. If the variances were not homogenous, a Student-Newman-Keuls method was used for pairwise multiple comparisons, otherwise a Bonferroni t test was performed. A value of P < .05 was considered significant.

**Results**

Effect of agonists and antagonists on 125I-BH-CCK8S binding to CCKB and CCKA receptors. The binding of agonists and antagonists to CCKB and CCKA receptors was examined using 125I-BH-CCK8S binding to guinea pig cortex and pancreas, respectively. Of the antagonists tested, CP-310,713 had the highest affinity and selectivity for CCKx receptors followed by CI-988, CP-212,454 and L-365,260 (table 1). Devazepide was 500-fold selective for CCKx receptors, binding with an IC50 value of 0.23 ± 0.015 nM. CCK8S did not discriminate between CCKB and CCKA receptors with IC50 values of 0.15 and 0.19 nM, respectively (table 1). Compared to CCK8S, CCK8US had an approximately 25-fold reduction in affinity for CCKx receptors, but a 1000-fold loss in binding affinity to the CCKx receptor. Pentagastrin displaced 125I-BH-CCK8S to cortex and pancreas with IC50 values of 1.3 and 1600 nM, respectively. CCK4 was selective for CCKx receptors binding with an IC50 value of 78 nM to the CCKx receptor and lacking appreciable affinity for CCKx receptors with an IC50 value of 5700 nM.

Effect of antagonists on pentagastrin-induced phosphatidylinositol turnover in rat AR 4-2J pancreatoma cells. Pentagastrin (PG) stimulated PI turnover in late passage AR 4-2J rat pancreatoma cells with an EC50 of 0.3 nM. The specific CCKx receptor antagonists, L-365,260, CI-988, CP-212,454 and CP-310,713 all showed dose-dependent inhibition of the pentagastrin-induced (0.33 nM) PI turnover with Ki values of 4.1, 2.4, 0.65 and 0.23 nM, respectively. In contrast, the selective CCKA receptor antagonist, devazepide, had little effect on pentagastrin-induced PI turnover with a Ki value > 300 nM (table 2).

Effect of antagonists on pentagastrin-induced acid secretion in rats. The specific CCKx receptor antagonists, L-365,260, CP-212,454 and CP-310,713 all showed dose-dependent inhibition of gastric acid secretion with s.c. ID50

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Guinea Pig IC50 (nM)</th>
<th>Pancreas IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Devazepide</td>
<td>120 ± 14 (7)</td>
<td>0.23 ± 0.015 (72)</td>
</tr>
<tr>
<td>L-365,260</td>
<td>8.1 ± 1.6 (22)</td>
<td>86 ± 29 (5)</td>
</tr>
<tr>
<td>CI-988</td>
<td>0.65 ± 0.03 (110)</td>
<td>490 ± 170 (7)</td>
</tr>
<tr>
<td>CP-212,454</td>
<td>0.48 ± 0.09 (4)</td>
<td>180 ± 51 (5)</td>
</tr>
<tr>
<td>CP-310,713</td>
<td>0.099 ± 0.016 (6)</td>
<td>1400 ± 280 (4)</td>
</tr>
<tr>
<td>CCK8S</td>
<td>0.15 ± 0.017 (4)</td>
<td>0.19 ± 0.013 (4)</td>
</tr>
<tr>
<td>CCK8US</td>
<td>3.7 ± 0.1 (3)</td>
<td>190 ± 38 (3)</td>
</tr>
<tr>
<td>CCK4</td>
<td>78 ± 16 (4)</td>
<td>5700 ± 850 (3)</td>
</tr>
<tr>
<td>Pentagastrin</td>
<td>1.3 ± 0.36 (4)</td>
<td>1600 ± 350 (3)</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ks (nM) ± S.E.M.*</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antagonists</td>
<td>Devazepide</td>
<td>&gt;300</td>
</tr>
<tr>
<td></td>
<td>L-365,260</td>
<td>4.14 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>CI-988</td>
<td>2.39 ± 1.27</td>
</tr>
<tr>
<td></td>
<td>CP-212,454</td>
<td>0.65 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>CP-310,713</td>
<td>0.23</td>
</tr>
<tr>
<td>Agonists</td>
<td>Pentagastrin</td>
<td>0.3 ± 0.13†</td>
</tr>
</tbody>
</table>

* Ki values were calculated as described in “Methods” and are the means of the indicated number of experiments (N).
† EC50 for pentagastrin-stimulated phosphatidylinositol turnover in rat AR 4-2J pancreatoma cells expressing CCKx-receptors.
values of 1.5, 0.80 and 0.01 mg/kg, respectively. The CCKB receptor antagonist, CI-988, blocked 60% of the pentagastrin-mediated effect with an ID50 value of 0.08 mg/kg, consistent with some reports indicating that it is a partial agonist at this receptor. The CCKA receptor antagonist, devazepide, was the least potent with an ID50 of 8.0 mg/kg when given s.c. (fig. 1).

Effect of CCK4 on heart rate and mean arterial pressure. The cardiovascular effects of CCK4 on HR and MAP were examined in the conscious guinea pig and dog. Dose-response curves in guinea pigs with i.v. bolus doses of CCK4 from 1 to 160 μg/kg were generated for changes in HR and MAP. Decreases in HR and increases in MAP were observed in a dose-dependent manner up to 20 μg/kg of CCK4. Doses of CCK4 between 20 and 160 μg/kg produced only small increases in the magnitude of both the HR and MAP responses. No tachyphylaxis was observed when CCK4 was repeatedly administered every 30 min over a 135-min period (fig. 2). Because 20 μg/kg of CCK4 produced the most consistent response, this dose was chosen for all subsequent studies to compare receptor antagonists in the guinea pig.

In the dog, the response to CCK4 was different from that of the guinea pig. Dose-response curves for both CCK4-mediated changes in HR and MAP were examined by intravenous bolus doses from 1 to 20 μg/kg. Increases in both HR and MAP were observed up to 20 μg/kg of CCK4. A dose of 10 μg/kg was chosen for subsequent studies with CCK antagonists in the dog. As in the guinea pig, no tachyphylaxis to the HR or blood pressure responses was apparent when 10 μg/kg of CCK4 was administered repeatedly over a 120-min period (see vehicle data in figs. 5 and 6).

Dose-response effect of CCKA and CCKB receptor antagonists on HR and MAP. Dose-response curves for changes in HR and MAP with specific CCKA and CCKB receptor antagonists in guinea pigs are shown in figures 3 and 4, respectively.

The specific CCKB receptor antagonists, L-365,260, CI-988, CP-212,454 and CP-310,713 produced dose-dependent blockade of HR and MAP changes induced by CCK4 in the guinea pig. The approximate ID50 in decreasing order of potency for inhibition of HR and MAP changes induced by CCK4 were 0.04 and 0.02; 0.08 and 0.03; 0.3 and 0.5 μmol/kg for CP-310,713, CI-988 and CP-212,454, respectively. The CCKA receptor antagonist, devazepide, at a single dose of 10 μmol/kg (the highest soluble dose achievable) had no effect on the HR or MAP changes induced by CCK4. The ID50 for L-365,260 and devazepide could not be determined because compound solubility precluded higher i.v. doses from being given.

Because no tachyphylaxis to CCK4 was observed in dogs, this model was used to assess the duration of activity of orally acting CCK receptor antagonists. CP-212,454 showed...
no effect on base-line HR or MAP after an oral dose of 50 mg/kg (figs. 5 and 6). Forty-five min after receiving CP-212,454, the dogs showed a 60 to 80% suppression of CCK4-induced increase in HR and MAP that lasted throughout the remainder of the 135-min observation period.

Discussion

Our study demonstrates that the cardiovascular responses to CCK4 in the guinea pig and dog are mediated by the CCKB receptor subtype. Three established models, in vitro receptor binding, inhibition of pentagastrin stimulated phosphatidylinositol hydrolysis in AR 4-2J cells and in vivo inhibition of pentagastrin-induced acid secretion, were used to rank order the affinity of antagonists for the CCKB receptor. The rank order of potencies in blocking CCK4-induced HR and MAP changes in the guinea pig corresponded closely to the rank order of their affinity and/or activity in all three models. The qualitative changes induced by CCK4 on HR, but not MAP, appear to be species dependent. CCK4 decreased the HR in the guinea pig although in the dog an increase was observed. However, in the rat, administration of CCK8S has been reported to increase HR at low doses but at higher doses there is a transient decrease followed by an increase in HR (Janssen et al., 1991). MAP is consistently increased by CCK in studies performed in our laboratory using guinea pigs and dogs and by other investigators using rats (Gaw et al., 1995).

The anxiolytic activity of CCK receptor antagonists has been shown to be mediated through the CCKB-receptor subtype (Harro and Vasar, 1991; Singh et al., 1991). Bradwejn et al. (1992) has shown that the anxiogenic effects of increasing i.v. doses of CCK4 in patients with panic disorders showed a strong relationship to the increases in HR and diastolic blood pressure experienced in the same patients. The central or peripheral origin of the cardiovascular activity of CCK in animals is uncertain. No definitive study demonstrating the partitioning of CCK into the brain for central activity after i.v. administrations has been reported. However, high immuno-reactive concentrations of the endogenous peptide have been found in regions of the brain associated with cardiovascular control such as the nucleus tractus solitarius (NTS) and area postrema (AP) (Newton and Maley, 1985; Howes et al., 1989). These regions can also be indirectly affected by stimulation of CCK at peripheral vagal afferent fibers leading into this area of the brain (Koyama et al., 1990). This hypoth-

Fig. 3. Dose-response curves for the CCK4 receptor antagonist, devazepide (♀), and the CCKB receptor antagonists L-365,260 (●), CP-212,454 (○), CI-988 (▲), CP-310,713 (♦) to antagonize CCK4-induced decreases in HR in the guinea pig. Data are expressed as percentage of control maximum response to CCK4 and each point represents the mean (±S.E.M.) of not less than four guinea pigs.

Fig. 4. Dose-response curves for the CCK4 receptor antagonist, devazepide (♀), and the CCKB receptor antagonists L-365,260 (●), CP-212,454 (○), CI-988 (▲), CP-310,713 (♦) to antagonize CCK4-induced increases in MAP in the guinea pig. Data are expressed as percentage of control maximum response to CCK4 and each point represents the mean (±S.E.M.) of not less than four guinea pigs.

Fig. 5. Effect of the CCKB antagonist on heart rate changes in the conscious dog. Top panel. Effect of 50 mg/kg of orally administered CP-212,454 (●) or 4 ml/kg vehicle (□) on basal heart rate before CCK4 challenges. Bottom panel. Percentage change in maximal heart rate response to CCK4 after CP-212,454 (●) or vehicle (□). Each point represents the mean of at least four dogs.
esis is supported by experiments where i.v. injection of CCK has been shown to stimulate gene expression of Fos-like protein, an indicator of neural stimuli, not only in the nucleus tractus solitarius and area postrema, but also in the ventrolateral medulla that receives projections from the nucleus tractus solitarius (Luckman, 1992).

Although the cardiovascular activity of different CCK peptides has been known for some time, the association to a particular receptor is just beginning to be addressed. In 1991, Mei and Han described that intrathecal CCK₈ can antagonize the hypotension induced by μ and κ opioid agonists. The antagonist activity was mediated by the CCK₄ receptor subtype (Mei and Han, 1993), as evidenced by the 20- to 40-fold greater potency of the CCK₄ receptor antagonist, L-365,260, over the CCK₃ receptor antagonist, devazepide, to block the effect of CCK₄. More recently, Gaw et al. (1995) concluded that the cardiovascular effects of CCK₈S could be attributed to CCK₄-mediated activity. This conclusion was supported by data that showed bolus i.v. doses of CCK₈S in the pithed rat produced increases in the MAP and decreases HR. These effects curiously were only partially attenuated with devazepide but not at all with L-365,260 or CI-988. However, in the same report when CCK₄ was used, similar increases in MAP were produced and were unaffected by treatment with devazepide, L-365,260 or phentolamine. Because CCK₈S has almost equal affinity to both the CCK₄ and CCK₈ receptors (see table 1), the unaccounted for activity observed after devazepide with CCK₈S may be due to the remaining CCK₄ activity. Gaw et al. (1995) also concluded that because neither devazepide nor L-365,260 blocked the increase in MAP induced by CCK₄, these effects were not mediated by either CCK₈ or CCK₄ receptors. Our data suggest that even though L-365,260 has affinity for CCK₈ in vitro, it is a relatively weak antagonist for CCK₈-mediated activity in all three of our functional assays. In the binding assay, the rank order for potency at displacing 125I-BH-CCK₈S binding for the CCK₈ receptor was CP-310,713 > CI-988 > CP-212,454 > L-365,260 > devazepide. The order of potency was closely followed for inhibiting pentagastrin-stimulated PI hydrolysis in rat pancreatoma cells and inhibition of pentagastrin-induced acid secretion in the rat model that also followed the observed activity in the guinea pig cardiovascular system.

The contribution of CCK to endogenous tone of the cardiovascular system appears to be negligible under normal circumstances. Figures 7 and 8 show that when 1.0 μM/kg of CP-212,454 is given as a bolus i.v. dose that concentrations are

![Fig. 6. Effect of the CCK₈ antagonist on mean arterial blood pressure changes in the conscious dog. Top panel, Effect of 50 mg/kg of orally administered CP-212,454 (■) or 4 ml/kg vehicle (○) on basal mean arterial blood pressure before CCK₄ challenges. Bottom panel, Percentage change in maximal mean arterial pressure increase to CCK₄ after CP-212,454 (■) or vehicle (□). Each point represents the mean (±S.E.M.) of at least four dogs.](image-url)

![Fig. 7. Effect of the CCK₈ antagonist on HR changes in the conscious guinea pig. Top panel, Effect of 0.1 μM/kg (▼), 1.0 μM/kg (▲), 10 μM/kg (●) or 3 ml/kg vehicle (□) of CP-212,454 (i.v.) on HR changes induced by CCK₄ (20 μg/kg, i.v.) challenges. Bottom panel, Percentage change in maximal heart rate decrease to CCK₄ after 0.1 μM/kg (▲), 1.0 μM/kg (▲), 10 μM/kg (●) or 3 ml/kg vehicle (□) of CP-212,454 (i.v.). Each point represents the mean (±S.E.M.) of at least five guinea pigs.](image-url)
sufficient to antagonize the transient increases in HR and MAP for at least 135 min yet no changes were observed in the basal values at each time period before CCK4 challenges. At the highest dose of CP-212,454, there was a significant decrease in basal HR with no concomitant drop in MAP. The increase in basal HR may be more directly involved in modulating cardiovascular responses not influenced by the sympathetic system (Gaw et al., 1995). Therefore these effects were previously indistinguishable from the nonspecific agonist, CCK$_{8S}$, but can be separated using the selective, peripheral CCK$_B$ agonist, CCK$_4$.

Acknowledgments

The authors thank Ms. Laura Ringer and Roxanne Winslow for their invaluable technical assistance with these studies.

References


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