The dorsal vagal complex as a site for cocaine- and amphetamine-regulated transcript peptide to suppress gastric emptying

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Smedh, Ulrika, and Timothy H. Moran. The dorsal vagal complex as a site for cocaine- and amphetamine-regulated transcript peptide to suppress gastric emptying. Am J Physiol Regul Integr Comp Physiol 291: R124–R130, 2006. First published February 2, 2006; doi:10.1152/ajpregu.00234.2004.—Cocaine- and amphetamine-regulated transcript-derived peptides (CARTp) and corticotropin-releasing factor (CRF) alter feeding and gastrointestinal function after central administration, and the gastric inhibitory effects are mediated through CRF. We hypothesized that dorsal hindbrain effects of CARTp on gastric emptying are mediated by the vagus nerve and that the dorsal vagal complex (DVC) is a site of action for the gastric inhibitory effects of both CARTp and CRF. Rats were equipped with chronic intragastric fistulas and guide cannulae aimed at the fourth ventricle or the DVC. Fourth intracerebroventricular CARTp-induced suppression of 12 ml glucose (12.5%) gastric emptying during fill was blocked by subdiaphragmatic vagotomy. To establish whether the DVC may be a site of action for CARTp and/or CRF, intraparenchymal microinjections of 0.25 μl of CARTp (0.1 and 0.5 μg) and CRF (5 and 10 pmol) were administered in the DVC. Each dose, previously shown to be ineffective after fourth intracerebroventricular administration, suppressed gastric emptying during gastric fill vs. vehicle, but neither peptide changed gastric secretion volume or gastric acidity. The results indicate that the DVC is a target site for CRF and CARTp to inhibit gastric emptying and that the vagus mediates dorsal hindbrain effects of CARTp on gastric motor function.

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Surgery

For surgical procedures, the rats were anesthetized with a 4:3 mixture of ketamine (100 mg/ml; Phoenix Pharmaceutical, St. Joseph, MO)-xylazine (20 mg/ml, Phoenix Pharmaceutical), injected intramuscularly. The dose was 1 ml/kg body wt. Chronic gastric fistulas were implanted in the ventral forestomach, as previously described (9, 20). For the first experiment, subdiaphragmatic vagotomies or sham surgeries were carried out in combination with gastric fistula implantation. The vagal trunks and their major branches were identified on the esophagus. The vagal trunks were cut proximal to the bifurcations of the hepatic and celiac branches. To prevent any potential nerve regeneration, all vagal branches were cleared from the lower end of the esophagus and cut a second time. 10 mm distal to the proximal section of the vagal trunks, and the nerve tissue intersections were removed. Finally, the esophagus was carefully inspected under a dissecting microscope to ensure that no intact vagal nerve fibers remained. Sham surgeries were performed in a similar way; however, no nerve cuts were performed. The wound was closed, and the animals were allowed 9 days of recovery before implantation of fourth intracerebroventricular, or DVC, guide cannulas.

The procedure for stereotaxic implantation of chronic, 27-gauge guide cannulas, aimed at the fourth ventricle or the DVC, was performed as previously described for fourth ventricle intracerebroventricular and raphe nucleus injections (8, 20). The 10-mm guide reached, but did not penetrate, the dura mater. For fourth ventricle intracerebroventricular placements, the coordinates were 2.6 mm anterior to the occipital crest in the midline. For the intraparenchymal placements, the guide coordinates were 1.60 mm anterior to the occipital crest and 0.60 mm lateral to the mid sagittal line, aiming at the right DVC. The animals were allowed another 6–7 days of recovery before the beginning of habituation training sessions.

Procedure

The experiments were carried out every third day to avoid possible carryover effects of the peptides. The animals experienced each set of conditions once, in a balanced, crossover design. The testing was carried out between 2:00 and 5:30 PM, with each animal tested at approximately the same time point each day. The animals were habituated to the experimental procedure during four once-daily training sessions in which they underwent the entire testing protocol but received no central injections. The gastric fistulas were opened 60 min before testing, intragastric contents were carefully evacuated with water per lavage, and the animals were subsequently placed in Plexiglas test cages with wire mesh floors. Just before testing, the fistulas were again opened, and a silicone tube connected to a syringe mounted on a Harvard Pump 22 (Harvard Instruments, Natick, MA) was attached. An intragastric infusion (1.0 ml/min) of 12.5% glucose was delivered during 12 min, and, immediately after infusion offset, the silicone tube was clamped and cut closely to the opening of the fistula. The remaining intragastric glucose solute was rapidly aspirated, either at the termination of the infusion or 30 min later, and the volume of the collected sample was measured. The stomachs were then rinsed with 5.0 ml distilled water to collect any solute still remaining intragastrically.

The glucose concentrations of the collected samples and of the rinse returns were determined with a glucose oxidase kit (Trinder; Sigma-Aldrich). The samples were analyzed in duplicate, and absorbances were determined at a wavelength of 505 nm on a Bausch and Lomb Spectronic 20 spectrophotometer. The amount of solute emptied and the gastric secretion volumes were calculated from the known gastric volumes delivered, the volumes recovered, and the gastric secretion volumes were calculated from the known Lomb Spectronic 20 spectrophotometer. The amount of solute em-}

Central Drug Injections

For fourth ventricle intracerebroventricular injections, a Gilmont microinjector attached to a 32-gauge injection cannula via a polyethylene (PE)-20 tube was used. The animals were gently restrained by hand, the injection cannula was inserted through the guide and into the fourth ventricle, and drug or vehicle (3 µl) was injected fourth icv over 1 min. The injector was left in place for another 30 s to reduce the risk of backwash. After the injection needle was removed, a new obturator was inserted in the guide. After the last experimental testing session, the animals were anesthetized, and 3 µl India ink were injected in the fourth ventricle. The animals were killed by decapitation, and the brains were removed, frozen, and sectioned. The site of injection was confirmed by inspection of the dye location in the fourth ventricle. Data from one animal in experiment 1 were excluded from the study since the dye was found in the cerebellum and not in the fourth ventricle.

For brain stem microinfusions, a Harvard Pump 11 with a 25-µl Hamilton syringe connected to a 32-gauge injection needle via a PE-20 tube was used. The length of the injector was chosen based on a previous pilot experiment where a series of dye injections in the DVC was performed with cannulas of different lengths, using the same coordinates and size of rats as in the present experiment. The injector used here was 17.0 mm long, and thus extended 7.0 mm from the surface of the cerebellum into the brain stem. The animals were gently restrained by hand, the injection needle was inserted through the guide and into the brain stem, and drug or vehicle (0.25 µl/min) was infused over 60 s. The accuracy of the volumes delivered with this system, commonly used for microdialysis, is ±1%, and the reproducibility is ±0.1% at this Hamilton syringe size and flow rate, as determined by the manufacturer (Harvard Apparatus). After drug delivery, the injector was left in place for 30 s to reduce any risk of backwash. The infusion needle was finally removed, and a new obturator was inserted in the guide. After the last experimental testing session, the animals were anesthetized, and 0.25 µl of India ink were injected using the same Harvard Pump 11 injection system. The animals were killed by decapitation, and the brains were removed, frozen, and sectioned. After staining with cresyl violet, microscopic inspection of the sections was performed, and the location of each ink trace was recorded on a brain stem atlas sheet. Presence of India ink was found in the dorsal motor nucleus of the vagus and/or overlying ventral NTS (Fig. 2). In one animal, the ink trace extended more ventrally in the brain stem as well (data not shown). The data collected from this rat was excluded from the study.

Drugs

Synthetic CART-(55–102) peptide (rat; American Peptide, Sunnyvale, CA) and CRF (rat and human; Sigma Aldrich) were dissolved in saline, separated into aliquots, and frozen (–20°C). Fresh aliquots were thawed on each experimental day before injections, and any excess was discarded. Pentagastrin (Sigma-Aldrich) was dissolved in saline.

Experimental Design

Experiment 1. This experiment was designed to establish whether the vagus nerve participates in mediating the effects of fourth icv CARTp on gastric emptying during and after fill. In addition, the
effects of fourth icv CARTp on the two phases of gastric emptying controls, during and after gastric fill (9), were evaluated. Before testing (15 min), vagotomized and sham-operated rats received a fourth icv of 1.0 μg (190 pmol) CARTp, a dose previously shown effective to suppress emptying after fourth icv (23). At time 0, the animals were given a 12-min intragastric infusion of glucose (1.0 ml/min). Gastric samples were either collected immediately after the glucose infusion to reflect gastric emptying during gastric fill or 30 min later to reflect emptying in the postfill period. Experiment 2. The effects of CARTp and CRF injected in the DVC on gastric emptying of glucose during gastric fill, gastric secretion volume, and gastric acidity were examined. The following two doses of CARTp were administered: an 0.1-μg (19 pmol) dose previously shown not to significantly affect emptying when delivered fourth icv and 0.5 μg (95 pmol) CARTp, which causes suppression of gastric emptying when given fourth icv. A low dose of CRF (5 pmol), which is without effect when injected in the fourth ventricle, was administered in the DVC to determine whether CRF acts in the DVC to inhibit gastric emptying. In addition, a high dose of CRF (10 pmol) previously shown to cause suppression of gastric emptying during fill after fourth icv administration; see Ref. 20) was used as a positive control for the DVC injections. Each drug, or saline as a vehicle, was microinfused 10 min before the onset of the intragastric infusion (1 ml/min) of glucose. Gastric samples were collected immediately after intragastric infusion offset to reflect emptying and secretion during gastric fill. Because third- and fourth-ventricular infusions of CARTp have previously been shown to result in behavioral changes, including movement-induced tremors and flattened body posture, we observed the rats after the DVC CARTp injections and noted the occurrence of any behavioral changes.

Experiment 3. As a control experiment for the sensitivity of the gastric acid secretion paradigm, pentagastrin (10 μg/kg) or saline as a control was injected (1 ml/kg ip) 15 min before intragastric infusion onset. A 12-ml glucose (12.5%) infusion was administered at 1.0 ml/min. The stomach was evacuated at infusion offset, and the gastric contents were analyzed for gastric acidity, as described above.

Statistical Analysis

Results from experiment 1 were analyzed with a three-way mixed-design ANOVA using solute emptied as the dependent measure [group (intact/vagotomy) × drug treatment (CARTp/vehicle) × sample latency (12, 42 min)]. Post hoc Newman-Keuls’s test was used.

For experiment 2, the effects of CRF and CARTp were analyzed separately, with repeated-measures ANOVA, using the same control condition. Post hoc comparisons were performed with Dunnet’s test. We considered P < 0.05 as significant.

Results from experiment 3 were analyzed using a paired, two-tailed Student’s t-test.

RESULTS

Experiment 1

Figure 1 shows the effect of CARTp on gastric emptying during and after gastric fill in intact (n = 7) and vagotomized (n = 9) rats. The ANOVA revealed main effects of fourth ventricle intracerebroventricular CARTp [F(1,14) = 102.846, P < 0.001] and sample latency [12 and 42 min after infusion onset; F(1,14) = 23.001, P < 0.001] and of vagotomy [F(1,14) = 4.878, P < 0.05]. There were no significant two-way interactions (vagotomy × sample latency; vagotomy × CARTp treatment; sample latency × CARTp treatment), but there was a significant three-way interaction [vagotomy × sample latency × CARTp treatment, F(1,14) = 9.708, P < 0.01]. Post hoc Newman-Keuls’s test showed that injection of 1 μg CARTp in the fourth ventricle significantly suppressed gastric emptying compared with vehicle in intact rats at the 12-min time point (P < 0.01). CARTp was without effect at the 42-min time point compared with intact vehicle-treated animals [not significant (NS)], indicating that the
CARTp-induced suppression of during-fill emptying was followed by a relative increase in the postfill period emptying rate. Gastric emptying was significantly increased in response to vehicle during gastric fill (12-min time point) in vagotomized animals compared with sham-operated animals ($P < 0.001$). There was no effect of CARTp on gastric emptying at the 12-min time point in vagotomized rats (NS), indicating that vagotomy interrupted the CARTp-induced suppression of gastric emptying during gastric fill. Finally, gastric emptying in the postfill period was decreased in the vagotomized CARTp-treated group ($P < 0.05$) compared with the vagotomized vehicle-treated group ($P < 0.05$), which indicates that, after vagotomy, CARTp caused a suppression of emptying in the postfill period.

Experiment 2

Repeated-measures ANOVA revealed a significant effect of DVC CARTp on gastric emptying during fill [$F(2,10) = 20.06$, $P < 0.001$]. As shown in Fig. 3A, post hoc Dunnett’s test indicated a significant effect of 0.1 and 0.5 μg CARTp on gastric emptying compared with vehicle. The repeated-measures ANOVAs for the secretion volume (Fig. 3B) and the gastric acidity results (Fig. 3C) were not significant [$F(2,10) = 0.6536$ and $F(2,10) = 0.4247$; $P > 0.05$, respectively]. No behavioral effects, such as flat posture or tremors, in response to CARTp injections in the DVC were noticed in any of the animals immediately after injections and up to 40 min thereafter. Analysis of the effects of DVC CRF treatment on gastric emptying revealed a significant effect of CRF treatment [$F(2,10) = 14.05$, $P < 0.01$]. As shown in Fig. 4A, post hoc Dunnett’s test showed a significant suppression of gastric emptying during gastric fill ($P < 0.01$) in response to both doses of CRF. CRF did not cause any changes in gastric secretion volume (Fig. 4B) or gastric acidity (Fig. 4C), since the repeated-measures ANOVAs for the gastric secretion volume and the gastric acidity conditions were not significant [$F(2,10) = 3.428$ and $F(2,10) = 0.656$; $P > 0.05$, respectively].

In experiment 3, Student’s $t$-test showed that gastric acidity was increased from $2.63 \pm 0.36$ to $7.96 \pm 1.91 \mu$Eq/ml in response to pentagastrin (Fig. 5).

DISCUSSION

Although the receptors for CARTp are yet to be described, the distribution of CARTp in vagal structures, such as the NTS, nodose ganglion, and area postrema suggests roles for both the DVC and the vagus nerve in mediating the ability of CARTp to modulate gastrointestinal physiology. Consistent with this view, a previous study by Okumura et al. (17) demonstrated a role for the vagus to mediate central nervous CARTp inhibition of gastric acid secretion. Our present results extend that finding to demonstrate that the vagus also mediates CARTp-induced suppression of gastric emptying during gastric fill and that the DVC may be a target for CARTp and CRF-induced effects on gastric emptying.

Our first experiment provides an important characterization of CARTp-induced inhibition of gastric emptying. First, we showed that the ability of fourth icv CARTp to suppress gastric emptying was limited to the during-fill period. CARTp produced a significant suppression of emptying during gastric fill (Fig. 1 and Ref. 23), but the effect of CARTp was eliminated in the postfill period. In fact, postprandial emptying was relatively increased after CARTp treatment such that at the 30-min postfill time point, emptying was the same with and without CARTp administration. The mechanism underlying this rebound increase in gastric emptying after CARTp-induced, during-fill gastric emptying-suppression is not fully clear. The controls of emptying during and after the period of gastric fill differ. Postfill emptying depends on caloric, postpyloric feedback (15). The CARTp-induced reduction in within-fill gastric emptying produces less of a caloric, postpyloric signal, which, in turn, may account for the postfill acceleration in gastric emptying after CARTp administration. Such a rebound acceleration after a during-fill gastric emptying suppression is similar to what has been previously demonstrated for the somatostatin agonist octreotide (21). In response to subcutaneous octreotide administration, which potently suppresses emptying during gastric fill, there is a rapid rebound increase in...
the emptying rate that occurs immediately after the cessation of

gastric fill regardless of the duration of the intragastric infusion
so that cumulative emptying, which includes the during- and

postfill periods, is unaffected (21). That CARTp and octreotide
should produce similar emptying dynamics is not surprising.

Previous work has suggested that CARTp-induced gastric
emptying inhibition is CRF dependent (23) and that CRF’s
gastric inhibitory actions are mediated through somatostatin
(20–22). Thus somatostatin may be an additional downstream
mediator of the gastric inhibitory actions of CARTp.

Results from the second experiment demonstrate that the
DVC is a potential site of action for fourth icv CARTp. Direct
DVC injections of CARTp at doses that do not affect gastric
emptying when administered in the fourth ventricle signifi-
cantly suppressed during-fill gastric emptying. This is consis-
tent with prior reports demonstrating the presence of CARTp in
the vagus nerve (12) and in the nodose ganglion (2, 12), the
NTS, as well as the area postrema (4, 12). This finding is also
consistent with the results of experiment 1 demonstrating the
disruption of hindbrain CARTp effects on gastric emptying by
vagotomy (Fig. 1). Recently, we showed that the controls for

fourth icv CARTp-induced suppression of gastric emptying,
but not of sucrose ingestion, are separable, since the former
but not the latter is blocked by pretreatment with a CRF
antagonist (23). Given a recent report by Zheng et al. (27)
where fourth icv CARTp was shown to suppress sucrose
intake, but microinjection of CARTp in the DVC was
without effect, our results from experiment 2 showing sig-
nificant effects on gastric emptying suppression after local
DVC injection suggest that the vagally associated CARTp
(2, 4, 12) is involved in strictly controlling gastrointestinal
functions.

The DMX-NTS complex is the final common path for vagal
transmission. Results from experiment 2 showing a significant
suppression of gastric emptying after CARTp microinjection
in the DVC also demonstrated a similar action for CRF. Earlier
studies on central actions of CRF and CARTp in the rat showed
that both peptides act via vagal pathways to affect gastric acid
secretion (5, 17, 24), and centrally acting CRF was shown to
inhibit gastric emptying in part via a vagal mechanism (2, 25).
Furthermore, CRF (20) and CARTp (23) cause suppression of
gastric emptying when delivered into the fourth ventricle,
suggesting an action on receptors within the dorsal hindbrain.
The present data establish that the DVC may not only relay the
effects of CARTp or CRF on gastric emptying suppression,
elicited in other hindbrain areas, but importantly, that the DVC
may be a site of action for CARTp and for CRF to control
gastric emptying. The finding that CARTp and CRF act in the
DVC to inhibit gastric emptying does not, of course, exclude
other brain stem substrates as additional candidate target sites
after intraventricular or intracisternal delivery. Other areas in
the hindbrain that express receptors for these peptides and that
project to the DVC may be simultaneously targeted after
intraventricular injection of CARTp and/or CRF.

In the present study, we did not detect any changes in gastric
acidity or volume of gastric secretions in the during-fill period
in response to either CARTp (Fig. 3, B and C) or CRF (Fig. 4,
B and C) administered in the DVC. These data are similar to
our previous findings after fourth icv CARTp (23) and CRF
(20). The previous observation that the rate of gastric secretion
is higher during, than after, gastric fill (9) in the similar
paradigm would suggest that any diminution of secretion
would have been easy to detect. This lack of effect on secretion
volume and gastric acidity contrasts to findings from previous
studies of the intraventricular effects of CARTp (17) and CRF
(5, 14, 24) where gastric acid secretion and secretion volume
were significantly reduced. An explanation for the contradict-
ing results may be that agents delivered intracisternally or

![Fig. 4. Effects of corticotropin-releasing factor (CRF) injected in the DVC. CRF (5 and 10 pmol) caused suppression of gastric emptying of glucose during gastric fill (A). CRF failed to affect gastric secretion volume (B) or gastric acidity (C). **P < 0.01 (n = 6).](http://ajpregu.physiology.org/)

![Fig. 5. Effect of ip pentagastrin on gastric acidity during gastric fill. Gastric acidity during gastric fill was significantly increased in response to 10 μg/kg pentagastrin. *P < 0.05 (n = 7).](http://ajpregu.physiology.org/)
intracerebroventricularly are more likely to access and target multiple putative receptor substrates as well, than after local DVC microinfusion. This would suggest that, whereas the DVC appears to be a target for CRF and CARTp to suppress gastric emptying, it may not be a direct site of action for these peptides to cause inhibition of gastric acid secretion or changes in gastric secretion volume. Another explanation for failure to detect changes in acid output and secretion volume after DVC injection of CRF or CARTp in the present study is difference in testing paradigms. Okumura et al. (17), in examining the effects of CARTp on acid secretion, used a model where the pylorus was held closed with a cuff and sampled for 4 h, as opposed to the present study where the pylorus remained unoccluded and samples were collected over 12 min. Most previous studies on CRF-induced inhibition of gastric acid secretion have typically been performed in pylorus-ligated rats where samples were collected after 1–2 h (14, 24). To validate the sensitivity of our method, we confirmed in experiment 3 that changes in acidity are detected over a shorter period (12 min), as indicated by the increase in acidity in response to a typical dose of pentagastrin (Fig. 5). This is consistent with previous studies on histamine effects in the guinea pig, in a similar testing paradigm (1). In this context, it should be emphasized that our testing paradigm assesses gastric emptying of glucose and gastric acid secretion during gastric fill in a manner that resembles the short-term food intake situation, i.e., glucose is infused intragastrically in a volume and rate that are similar to what rats would normally ingest from a drinking spout (10). Importantly, basal gastric emptying during fill after intragastric infusion of glucose does not differ from emptying rates that are observed after ingestion of the same stimulus (9–11). Our finding that no suppression of gastric secretion volume or acid secretion by either CRF or CARTp injected in the DVC could be detected during gastric fill may therefore infer that the acid suppression observed over 4 h by Okumura et al. (17) may not be of physiological relevance during ongoing food ingestion.

In summary, dorsal hindbrain CARTp suppresses gastric emptying during gastric fill, but not after gastric fill, via a vagal pathway. In addition, CARTp and CRF each act in the DVC to inhibit gastric emptying without changing gastric acid secretion or gastric secretion volume.

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