Effects of acupuncture on vasopressin-induced emesis in conscious dogs

Makoto Tatewaki,1,2 Carmen Strickland,1 Hiroyuki Fukuda,1 Daisuke Tsuchida,1 Etsuo Hoshino,2 Theodore N. Pappas,1 and Toku Takahashi1

1Department of Surgery, Duke University Medical Center and Veterans Affairs Medical Center, Durham, North Carolina; and 2Department of Internal Medicine, Japanese Foundation for Cancer Research, Tokyo, Japan

Submitted 26 May 2004; accepted in final form 21 September 2004

Table of Contents

ACUPUNCTURE HAS BEEN USED for various gastrointestinal (GI) conditions. A large body of clinical evidence suggests the effectiveness of acupuncture for suppressing nausea associated with chemotherapy, the postoperative state, and pregnancy. Acupuncture has also been used for a variety of other conditions, including postoperative ileus, achalasia, peptic ulcer disease, irritable bowel syndrome, functional dyspepsia (FD), diarrhea, constipation, and inflammatory bowel disease (12). Although acupuncture has been shown to be of significant clinical benefit, the mechanism of the effects of acupuncture still remains unclear.

The most commonly used acupuncture points in treating GI symptoms are the Zusanli point of the lower limbs [stomach-36 (ST36)] and the Neiguan point at the wrists [pericardium-6 (PC6)]. ST36 is located at the proximal one-fifth of cranialateral surface of the leg distal to the head of the Tibia in a depression between the muscles of the cranial Tibia and the long digital extensor. PC6 is located in the groove caudal to the flexor carpi radialis and cranial to the superficial digital flexor muscles, 3 cm proximal to the corpus.

Several clinical studies have suggested that acupuncture at PC6 is effective for treating and/or preventing emesis associated with surgery, chemotherapy, and motion sickness (1, 3, 13, 15, 16, 37, 40). However, it remains unclear whether the antiemetic effect of acupuncture at PC6 is due to the stimulation of gastric motility and/or the inhibition of activity of the vomiting center.

Emetic stimuli from the GI tract mainly involve the vagus nerve, which has two types of afferent fibers involved in the emetic response. Mechanoreceptors of the vagus are located in the muscular layer, and chemoreceptors of the vagus are located in the mucosal layers of the stomach (5). The chemoreceptor trigger zone (CTZ) is located in the area postrema. CTZ is principally activated by the vagus, phrenic, and spinal nerves, as well as by circulating chemicals. The vomiting center, located in the lateral reticular formation of the medulla oblongata, coordinates efferent impulses through the vagus, phrenic, and spinal nerves of the abdominal musculature during the action of vomiting (4, 41). Arginine vasopressin (AVP) is a posterior pituitary hormone that has been attributed to functions such as vascular regulation, osmoregulation, stress responses, renal regulation, and neuromodulation. Recent evidence implies that AVP plays a part in vomiting and nausea in humans and animals. The plasma AVP concentration is rapidly elevated in response to diverse emetic stimuli such as motion stimuli (27), apomorphine (33), and cisplatin (11, 17). Treatment with antagonists of the AVP receptor efficiently prevents motion sickness (8, 13, 15, 16, 37, 40). However, it remains unclear whether the antiemetic effect of acupuncture at PC6 is due to the stimulation of gastric motility and/or the inhibition of activity of the vomiting center.

Intravenous injection of AVP results in vomiting and retching in humans (27, 34) and dogs (6, 7). A low dose of exogenous AVP, which has no effect when given peripherally, causes an emetic response when given centrally (25). This suggests that the effects of AVP are mediated via the central nervous system.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Chen et al. (7) recently demonstrated that combined acupuncture at PC6 and ST36 significantly prevented vomiting and reduced the symptoms induced by vasopressin in dogs. The acupoint of ST36 has also been used widely for GI symptoms (12). However, it remains unclear whether acupuncture at ST36 itself has an antiemetic effect.

We studied whether vasopressin-induced emesis was antagonized by acupuncture at PC6 or ST36 in conscious dogs. To investigate the antiemetic effects of acupuncture on vasopressin-induced emesis, gastroduodenal motor activity and the number of retching and vomiting incidents were simultaneously recorded. It has been suggested that the analgesic effect of acupuncture is mediated via the opioid pathway because the antinociceptive effect of acupuncture has been shown to be antagonized by naloxone (12).

To investigate whether the antiemetic effects of EA are mediated via the central or peripheral opioid pathway, we studied the effects of naloxone (a central and peripheral opioid receptor antagonist) and naloxone methiodide (a peripheral opioid receptor antagonist).

MATERIALS AND METHODS

Preparation of animals. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All aspects of this research were approved by the Durham Veterans Affairs Medical Center (Durham, NC).

Seven mongrel dogs (five females and two males) weighing between 10 and 20 kg were used in this study. Food was withheld for 12 h before surgery. The dogs were induced with a single intravenous injection of 7 mg/kg body wt of propofol. General anesthesia was maintained by intratracheal inhalation of isoflurane and oxygen. The abdomen was prepared aseptically for a 10-cm ventral midline laparotomy. Four strain-gauge force transducers were implanted on the seromuscular layer to record circular muscle motor activity. Three transducers (8 × 14 mm, model F12IS, Star Medical, Tokyo, Japan) were sutured to the gastric body (at the level of the short gastric artery), the antrum (2–3 cm proximal to the pyloric ring), and the proximal duodenum (3–4 cm distal to the ring), respectively. One additional transducer (4 × 12 mm, model F12IS-P, Star Medical) was applied to the pyloric sphincter. A bundle of lead wires were routed through a subcutaneous tunnel in the costal flank. After the surgery, the lead wires were covered with a jacket protector. The dogs were allowed 2 wk to recover from the surgery.

Acupuncture and vasopressin infusion. Dogs were deprived of food for 12 h before the experiment. The lead wires were connected to a recording system (MacLab, ADInstruments, Colorado Springs, CO). Gastroduodenal motility was continuously monitored throughout the experiment. After phase III of the migrating motor complex cycle was finished, vasopressin infusion was started. Vasopressin was intravenously infused for 20 min at a dose of 0.1 U·kg\(^{-1}\)·min\(^{-1}\).

Electroacupuncture (EA, 1–30 Hz) at PC6 or ST36 was performed for 60 min before, during, and after the vasopressin infusion. EA at acupoints located on the back [bladder-21 (BL21)] were used as the location site for sham acupuncture. BL21 is located 1 cm lateral of the spinous process of the 12th thoracic vertebrae.

To investigate the antiemetic effects of EA on vasopressin-induced emesis, gastroduodenal motor activity and the number of retching and vomiting episodes were simultaneously recorded in conscious dogs. The frequency of vasopressin-induced retching and vomiting episodes induced by vasopressin was visually counted during the experiment. To study the reproducibility of the antiemetic effect of EA at PC6, the same experiments were repeated 1 mo later in each dog.

To investigate whether the opioid pathway was involved in EA-induced antinociceptive effects, naloxone (0.1 mg/kg, bolus plus 0.1 mg·kg\(^{-1}\)·h\(^{-1}\) iv) was administered before EA and the vasopressin infusions. Naloxone is able to cross the blood-brain barrier (BBB) and blocks both central and peripheral opioid receptors.

Fig. 1. Gastroduodenal motor responses and retching and vomiting induced by vasopressin. Vasopressin was administered after phase III of the migrating motor complex (MMC) cycle was completed. Intravenous administration of vasopressin (indicated by heavy line at top) induced retching (●) and vomiting (○). After the vasopressin infusion, retrograde peristaltic contractions (RPCs; indicated by arrows) were observed starting from the duodenum. Retching and vomiting were preceded by antral contractions. Subsequent episodes of retching and vomiting were again preceded by RPCs from the duodenum. The phasic contractions of the body (bottom) reflect the contractions of the diaphragm and abdominal muscles associated with retching and vomiting.
In contrast, naloxone methiodide does not cross the BBB and blocks only peripheral opioid receptors (42). To investigate whether peripheral opioid receptors were involved in EA-induced antiemetic effects, naloxone methiodide (0.5 mg/kg, bolus plus 0.5 mg·kg⁻¹·h⁻¹ iv) was administered before EA and the vasopressin infusion.

Vagal blockade by cooling. As shown in Fig. 1, immediately after the vasopressin infusion, phasic contractions were observed at the duodenum, which were orally migrating to the pylorus and antrum [retrograde peristaltic contractions (RPCs)]. To investigate the possible role of vasopressin in RPC mediation, bilateral vagal blockade was performed during vasopressin infusion in 7 different dogs. A: results without EA.

Fig. 2. Effects of electroacupuncture (EA; 10 Hz) on gastric motility induced by vasopressin. EA at pericardium-6 (PC6; B), but not at bladder-21 (BL21; C), significantly attenuated the RPCs as well as the frequency of retching (●) and vomiting (○) induced by vasopressin. The results were reproducible in 7 different dogs. A: results without EA.
four dogs. Bilateral cervical skin flaps were prepared for transient vagal nerve blockade. Under general anesthesia, bilateral skin incisions were made on the ventral neck. Bilateral vagosympathetic nerve trunks with surrounding connective and adipose tissues were isolated, and then a 5-cm portion was wrapped by the skin flap, as previously described (9, 23). The dogs were allowed to recover for 2 wk.

Transient vagal nerve blockade was initiated by circulating absolute ethanol at \(10^\circ\text{C}\) through U-shaped copper tubes hooked through the skin loops. The temperature of the wrapped skin was maintained at 4\(\circ\text{C}\). Nerve blockade was achieved within 15–20 min after the start of cooling and was characterized by a deep respiration and bilateral Horner’s syndrome, as previously reported (9, 23).

**Data and statistical analyses.** We calculated the area under the curve during 30 min using a computer-assisted system (MacLab, ADInstruments); this was expressed as a motility index. Values are shown as means \(\pm\) SE. Statistical analysis was performed by paired \(t\)-test or ANOVA. When significant differences were detected by ANOVA, differences between means were checked by Bonferroni’s method. \(P\) values of <0.05 were considered significant.

**Materials.** Naloxone and naloxone methiodide were obtained from Sigma (St. Louis, MO). Vasopressin was obtained from American Reagent (Shirley, NY).

**RESULTS**

Vasopressin was infused during the period of phase I. Immediately after the vasopressin infusion, phasic contractions were observed at the duodenum, which then orally migrated to the pylorus and antrum (RPCs). Retching and vomiting were frequently observed after RPCs induced by vasopressin in all dogs tested (Fig. 1). During and after the vasopressin infusion, the retching was observed 4.9 \(\pm\) 0.8 times and vomiting was observed 5.1 \(\pm\) 0.7 times in each dog (\(n = 7\)).

EA (10 Hz) at PC6 was performed before, during, and after the vasopressin infusion for 60 min. EA (10 Hz) at PC6 significantly reduced the number of episodes of retching (1.8 \(\pm\) 0.2 times) and vomiting (1.6 \(\pm\) 0.2 times), compared with results without EA (\(n = 7\), \(P < 0.01\), by Bonferroni) (Fig. 2B and Fig. 3). The latency time (15.5 \(\pm\) 0.8 min, \(n = 7\)) to induce retching and/or vomiting after the start of vasopressin infusion was not affected by EA at PC6.

Different frequencies (1, 3, 10, and 30 Hz) of EA at PC6 were also performed before, during, and after the vasopressin infusion for 60 min. EA (1–30 Hz) at PC6 decreased the number of retching and vomiting episodes induced by vasopressin in a frequency-dependent manner. The maximum effect was observed at 10 Hz, which showed 34.5 \(\pm\) 7.9\% (\(n = 7\)) inhibition of controls (Fig. 4).

In contrast, EA at BL21 (sham acupuncture) had no significant effects on the number of retching and vomiting episodes (Fig. 2C and Fig. 3). Similarly, EA at ST36 had no significant effects on the number of retching and vomiting episodes induced by vasopressin (Fig. 3).

EA at PC6 also suppressed the RPCs induced by vasopressin (Fig. 2). The motility index of RPCs of the duodenum induced by vasopressin was 1,142 \(\pm\) 571 g·min, which was significantly reduced to 57 \(\pm\) 14 g·min by EA at PC6 (\(n = 7\), \(P < 0.05\) by paired \(t\)-test). The motility index of RPCs of the antrum induced by vasopressin was 1,863 \(\pm\) 704 g·min, which was also significantly reduced to 219 \(\pm\) 74 g·min by EA at PC6 (\(n = 7\), \(P < 0.05\) by paired \(t\)-test).
There was a significant correlation observed between the motility index of RPCs of the duodenum and the number of retching and vomiting episodes induced by vasopressin (Fig. 5).

The same experiments were repeated 1 mo later in each dog. As shown in Fig. 6A, the vasopressin infusion similarly induced retching and vomiting in the same dog shown in Fig. 2. The antiemetic effects of EA were also observed (Fig. 6B).

Naloxone administration significantly increased the frequency of retching (8.9 ± 1.6 times) and vomiting episodes (7.2 ± 0.8 times) induced by vasopressin, compared with the number of episodes in controls (P < 0.05 by Bonferroni). When naloxone was given during EA and vasopressin infusion, the antiemetic effect of EA was no longer observed (Fig. 3 and Fig. 7A).

Naloxone methiodide had no effect on the number of retching and vomiting episodes induced by vasopressin, compared with the number of episodes in controls. In contrast to naloxone, naloxone methiodide had no inhibitory effects on the antiemetic effects of EA (Fig. 3 and Fig. 7B). Vagal blockade by cooling was started after phase III of the migrating motor complex cycle was finished. RPCs were no longer observed in response to vasopressin during vagal nerve blockade (Fig. 8). The number of retching (1.3 ± 0.3 times) and vomiting episodes (0.8 ± 0.3 times) in response to vasopressin was significantly reduced, compared with the number of episodes in controls (n = 4, P < 0.05 by paired t-test).

DISCUSSION

Clinical study has demonstrated that EA at PC6 significantly inhibited postoperative nausea and vomiting (19). Acupuncture at PC6 also decreased cisplatin-associated nausea and vomiting (14, 15, 21). EA stimulation reduced gastric tachyarrhythmia invection-induced motion sickness (24). EA stimulation significantly increased the percentage of regular slow waves, which was sustained in the recovery period in healthy volunteers. The increase of the regular slow-wave activity resulted from the normalization of gastric dysrhythmia. As a result, it is concluded that EA has a role in restoring the regularity of gastric myoelectrical activity (24). However, the mechanism of the antiemetic effect of acupuncture still remains obscure.

Our canine study demonstrated that EA at PC6 significantly antagonized vasopressin-induced retching and vomiting. Vasopressin is believed to act on the CTZ, which serves as a receptor site for chemical agents and may activate the vomiting center (28).

By administering morphine or naloxone systemically and intracerebroventricularly, Costello and Borison (10) demonstrated the opposing emetic and antiemetic actions of opioids in cats. This suggests that morphine has emetic effects as well as antiemetic effects.

The emetic effect of morphine is believed to be at a superficially located CTZ, whereas the antiemetic effect of morphine at the vomiting center is located more deeply in the medulla.
CTZ is one of the circumventricular organs of the brain, located outside of the BBB and the cerebrospinal fluid barrier. It can be activated by chemical stimuli received through the blood, as well as via the cerebrospinal fluid.

In contrast, the vomiting center is located beneath the solitary tract of the caudal brain stem. Both the emetic and antiemetic effects of morphine can be blocked by naloxone because naloxone can cross the BBB. The antiemetic effect of morphine cannot be blocked by naloxone methiodide because naloxone methiodide does not cross the BBB (42).

It has been demonstrated that a peripheral opioid receptor antagonist combined with morphine blocks apomorphine- and cisplatin-induced emesis in conscious dogs (18). Symptoms of motion sickness are made more severe by the administration of naloxone (2). Our present study also shows that the number of retching and vomiting episodes induced by vasopressin is increased by naloxone but not by naloxone methiodide. These observations suggest that central opioids may play a role in inhibiting the development of emesis. It remains unclear whether the antiemetic effect of central opioid is constitutive or specific to vasopressin.

Several researchers have demonstrated that acupuncture increases opioid levels in humans (12) and in animals (20, 22). We demonstrated that the antiemetic effect of EA at PC6 was antagonized by naloxone but not by naloxone methiodide. This suggests that the antiemetic effect of EA is mediated via the central opioid pathway. It is conceivable that released opioids by EA from the central nervous system play an antiemetic role.

Fig. 7. Effects of naloxone (A) and naloxone methiodide (B) on the antiemetic effect of EA at PC6. The antiemetic effect of EA at PC6 was abolished by naloxone (A) but not by naloxone methiodide (B). ⚪, Retching; ●, vomiting. Results were reproducible in seven different dogs.

Fig. 8. Effects of vagal blockade on vasopressin-induced RPCs. Vagal cooling was started after phase III of the MMC cycle was completed. Vagal blockade almost completely abolished vasopressin-induced RPCs. The frequency of retching (○) and vomiting (●) was also significantly reduced by vagal blockade. Results were reproducible in 4 different dogs.
at the vomiting center. In our in situ hybridization study (unpublished observations) shows that EA upregulates the gene expression of preproenkephalin at the periaqueductal gray in conscious rats. However, we cannot rule out another possibility that EA may interfere with the action of vasopressin on the vomiting center, independently of the opioid pathway.

After vasopressin infusion, RPCs were observed starting from the duodenum. Retching and vomiting were preceded by antral contractions. Subsequent episodes of retching and vomiting were again preceded by RPCs from the duodenum, as shown in Fig. 1.

Similar to our study, retrograde propagation of the spike activity was observed starting from the jejunum by intravenous infusion of dopamine in conscious dogs (30). Apomorphine infusion also caused retrograde giant contractions, which started from the mid-small intestine in conscious dogs (29, 36). Vomiting was observed, followed by strong antral contractions induced by apomorphine (36).

When dopamine infusion failed to change GI motility, retching and vomiting were no longer observed (30). Our vagal blockade study showed that once RPCs have disappeared the frequency of retching and vomiting was significantly attenuated, thus suggesting that the motility changes to RPCs in the GI tract may well be a prerequisite of subsequent occurrences of retching and vomiting.

The vomiting induced by vasopressin infusion was significantly reduced in vagotomized dogs compared with normal dogs (7). We proved that vagal blockade almost completely abolished RPCs and significantly attenuated the episodes of retching and vomiting induced by vasopressin. This suggests that the vagus nerve may have a key role in mediating emesis. Because our present procedure of vagal blockade does not distinguish between efferent and afferent mechanisms, it remains unclear whether the efferent or afferent vagal pathway is involved in mediating emesis induced by vasopressin.

Combined acupuncture at ST36 and PC6 increases the percentage of regular slow waves, resulting in the restoration of gastric dysrhythmia in healthy humans (31). Acupuncture at ST36 has been widely used for various GI symptoms. Although acupuncture at ST36 is well established to stimulate GI motility, it still remains unclear whether acupuncture at ST36 itself has an antiemetic effect.

The impact of acupuncture on upper GI motor activity seems to be variable and dependent on the acupoints used (32, 43). The different effects of EA between PC6 and ST36 on gastric myoelectrical activity have recently been demonstrated in healthy humans (38). EA at PC6 reduced peak dominant power (PDP) to 47% of baselines, whereas EA at ST36 increased PDP to 154% of baseline. Because PDP is associated with the amplitude of gastric contractions, it is suggested that EA at these two acupoints induces either an increase or a decrease in gastric motility (38).

Acupuncture at ST36 stimulates GI contraction via the somatotopasymathetic pathway (35, 39). However, it remains unclear whether acupuncture at PC6 affects GI motility. Our present study suggests that acupuncture at ST36 had no antiemetic effects.

A recent study has indicated that restraint stress causes a fivefold increase of the plasma AVP level in rats (26). Although nausea and vomiting are the common symptoms in patients with FD, it is still unknown whether increased AVP secretion induced by mental stress may mediate the nausea and vomiting in FD patients. Emesis observed in patients with FD may be treatable by acupuncture at PC6.

In conclusion, the frequency of vomiting and retching episodes induced by vasopressin was significantly reduced by acupuncture at PC6, but not by ST36, in conscious dogs. The antiemetic effect of acupuncture was antagonized by naloxone but not by naloxone methiodide. It is suggested that the antiemetic effect of acupuncture at PC6 is mediated via the central opioid pathway.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Gerald Olson, Mary Harris, and Rick Leary for skilled technical assistance at the Durham Veterans Affairs animal facility.

REFERENCES

11. Cubeddu LX, Lindley CM, Wetsel W, Carl PL, and Negro-Vilar A. Combined acupuncture at ST36 and PC6 increases the per-


