Gastric motor and cardiovascular effects of insulin in dorsal vagal complex of the rat

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Krownicki, Zbigniew K., Nicole A. Nathan, and Pamela J. Hornby. Gastric motor and cardiovascular effects of insulin in dorsal vagal complex of the rat. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G964–G972, 1998.—Insulin-binding sites exist in the lower brain stem of the rat, raising the possibility that the circulating hormone may have cardiovascular and gastric effects at this site. Therefore, we investigated the autonomic effects of applying rat insulin to the surface of the dorsal medulla (0.3 and 3 µU/rat) or microinjecting it into the dorsal vagal complex (DVC) (0.1–10 nU/site) in anesthetized rats. Application of rat insulin to the surface (3 µU/rat) and its microinjection into the DVC (1 and 10 nU/site) both evoked marked, albeit transient, increases in intragastric pressure, pyloric and greater curvature contractile activity, and blood pressure. Much higher doses of human (100 mU) and porcine insulin (3 mU) were needed to evoke modest changes in gastric motor and cardiovascular function when applied to the surface of the dorsal medulla. In addition, a 1,000-fold higher dose of porcine insulin (10 µU) in the DVC was not enough to mimic the autonomic effects of rat insulin microinjected into the same site. The excitatory gastric motor effects of rat insulin in the lower brain stem were abolished by vagotomy, whereas spinal cord transection blocked insulin-evoked increases in blood pressure. To test whether the gastric motor effects of rat insulin in the lower brain stem were caused by potential contamination with pancreatic polypeptide, we microinjected rat pancreatic polypeptide into the DVC at a single dose of 2 pmol. Only a modest increase in intragastric pressure in response to the hormone was observed. Thus it is likely that insulin, through its action in the lower brain stem, may be implicated in the pathogenesis of gastrointestinal and cardiovascular complications in hyperinsulinemia. In addition, species variations in the amino acid sequence of insulin may affect its biological activity in the brain of different species.

gastric motility; intragastric pressure; blood pressure

There is considerable evidence that the blood glucose concentration influences gastric motility. In contrast, relatively little is known about a possible regulatory effect of insulin on gastric motor function. In general, both stimulation (31, 36) and inhibition (18, 21) of gastric motility by insulin in experimental animals have been reported. Unfortunately, studies dealing with the effect of insulin in vivo are hampered by the fact that the resulting hypoglycemia has effects of its own. Thus it is difficult to assess the effects of insulin on autonomic control of gastrointestinal motility, because hypoglycemia may change vagal and sympathetic outflow to the gastrointestinal tract (27). Direct effects of insulin on the stomach have been reported. Specifically, porcine insulin augmented peptide-stimulated bombesin and gastrin release from the perfused isolated rat stomach (47). Therefore, insulin may modulate the action and release of putative peptidergic neurotransmitters of the rat intrinsic nervous system. However, the mechanisms behind the gastric motor effects of insulin remain unclear. Our working hypothesis is that one site of action of insulin to increase gastrointestinal motility may be the dorsal vagal complex (DVC). The DVC comprises the dorsal motor nucleus of vagus, where some cardiac and most gastrointestinal preganglionic neurons originate from, and the nucleus of solitary tract, where baroreceptor and gastrointestinal afferents terminate. There is considerable evidence that the DVC is a potential site of action for circulating hormones that regulate gastrointestinal function (43). These hormones can gain access to the DVC, because the caudal nucleus of solitary tract has fenestrated capillaries and enlarged perivascular spaces that permit entry of serum proteins (6) and there exists a close anatomic association of the DVC to the area postrema, a circumventricular organ (11). However, to date no one has investigated the effects of insulin on gastrointestinal function in the DVC, which is surprising in view of the fact that insulin receptors have been localized in the DVC (53, 55) and the neighboring area postrema (54).

There also exists a growing body of evidence linking circulating insulin levels and increased blood pressure over a wide range of insulin levels and in a variety of clinical conditions. Chronic intravenous insulin infusion in the rat, in the absence of hypoglycemia, causes an increase in blood pressure (5, 20). Moreover, chronic exogenous hyperinsulinemia accelerates the time course of the development of hypertension in genetically predisposed spontaneously hypertensive rats (7, 58). Despite its pressor effects in some animal models, supraphysiological insulin concentrations are generally required to get similar changes in human studies. Nonetheless, a recent study (34) reports that finger systolic blood pressure increases in response to physiological concentrations of insulin. In addition, previous experimental studies (25, 28), using heterogeneous insulin, have provided contradictory results on the cardiovascular effects of insulin in the DVC.

In an attempt to clarify this confusion and to investigate gastric motor effects of insulin in the lower brain stem, we used rat insulin (1R1s) as well as human and porcine hormone for local application to the surface of the dorsal medulla and for microinjection into the DVC.

MATERIALS AND METHODS

General methods. Male Sprague-Dawley rats (200–295 g) purchased from Charles River Laboratories (Wilmington, MA) were used in all experiments. The rats were maintained in a temperature-controlled environment and on a 12:12-h
light-dark cycle with free access to food and water, except the night preceding the experiment. At this time, the animals were deprived of food (18–20 h) but allowed ad libitum access to water. All procedures performed on the animals were approved by the Louisiana State University Medical Center Institutional Animal Care and Use Committee.

Animals were initially anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg) intramuscularly, and separate indwelling catheters were placed in the left femoral artery and vein. Afterward, α-chloralose (80 mg/kg) was administered intravenously, and a tracheotomy was performed to ease respiration. If needed, the animal was connected to a small animal respirator (Kent Scientific, Litchfield, CT). Urethan (600 mg/kg iv) or xylazine (2.5 mg/kg iv) was used as necessary to maintain full surgical anesthesia in the presence of α-chloralose.

After laparotomy, an intraluminal latex balloon was inserted into the stomach for continuous recording of intragastric pressure (IGP). Two strain gauges, mounted on the surface of the stomach, were used for monitoring smooth muscle contractile activity. One of them was oriented to the circular smooth muscle in the pyloric region and used to record pyloric contractile activity (PCA). The second one was placed along the longitudinal muscle of the greater curvature and allowed us to record greater curvature contractile activity (GCCA; Ref. 24). Heart rate (HR) was monitored by a tachograph triggered by the arterial pressure pulse from the left femoral artery (model 7P4H, Grass Instruments, Quincy, MA). Rectal temperature was maintained between 37.0°C and 37.5°C with radiant heat.

Applications to surface of dorsal medulla. The animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and after a limited occipital craniotomy, 5–10 µl aliquots of insulin solutions and vehicle were applied under visual control to the surface of the dorsal medulla with the Hamilton microsyringe. A small piece of filtration paper, cut to size, was used to keep the solution at the surface.

Microinjection technique. Animals were mounted in the stereotaxic frame and five or seven-barreled micropipettes (30–40 µm total external tip diameter), prepared from glass capillaries (Dagan, Minneapolis, MN), were lowered into the right DVC (0.7 mm rostral to the obex, 0.5 mm lateral to the midline, and 0.45–0.5 mm down from the surface). Minor adjustments to these coordinates for the final injections were made by identifying the most sensitive location for gastric excitatory effects of l-glutamate (7.5 nmol), as described in detail previously (24). After location of the gastric sites, the order in which insulin solutions (0.1, 1, and 10 nU) and vehicle were microinjected was randomized throughout experiments. The injections were delivered in a volume of 30 nl for ~15 s (30 psi), using a pneumatic pico-pump (model PV830, World Precision Instruments, New Haven, CT), with 30-min intervals between injections. At the end of each series of microinjections, 1% Pontamine sky blue was injected into the same site in a volume of 30 nl and a bolus injection of pentobarbital sodium (80 mg/kg) was used to terminate the experiments. Then the animals were perfused transcardially with saline and subsequent 4% paraformaldehyde in PBS, and their brains were removed and fixed in 4% paraformaldehyde with 20% sucrose. Frozen sections of the brain stem (40–50 µm) were cut and stained with neutral red to determine placement of the micropipette tip in the DVC by a Nikon Labophot microscope.

Drugs. rIns (biological activity, 28.8 U/mg: Novo Nordisk, Copenhagen, Denmark) was purchased from Linco Research (St. Louis, MO). Porcine insulin (biological activity, 26 U/mg) was obtained from Sigma Chemical (St. Louis, MO). Rat pancreatic polypeptide (rPP) was obtained from Bachem California (Torrance, CA). All these peptides were dissolved in 0.9% saline with 0.05% BSA and stored at −80°C before being used in the experiments. Human insulin of recombinant DNA origin (Humulin R, 100 U/ml; Eli Lilly, Indianapolis, IN) was diluted with 0.9% saline with 0.05% BSA to the desired concentration immediately before use. The dose of rPP (2 pmol/site), based on the results of McTigue and co-workers (30), was chosen because of marked gastric motor effects of rIns in the DVC at doses of 1 and 10 nU/site, which equals ~0.005 and ~0.05 fmol of the hormone/site, respectively.

Experimental design and treatment. The experiments were started 1.5 h after the surgical instrumentation was completed. No further manipulations were performed until stable baseline gastric and cardiovascular recordings were obtained. This was generally 2.5 h after the initial anesthetic dose. Application of agents to the dorsal surface of the medulla or microinjections into the DVC were performed for the subsequent 2–2.5 h, at which time experiments were terminated. In 21 rats, vehicle and the different types of insulin were applied to the surface of the dorsal medulla. Specifically, nine rats were treated with rIns (0.3 and 3 µU), five with porcine (3 mU), and seven with human insulin (100 µU). Six rats in which rIns was applied to the surface of the dorsal medulla were also used for microinjections into the DVC. A total of 17 rats were used for microinjections into the DVC. Specifically, 10 rats were used for the rIns dose-response (0.1–10 nU/site) study, three rats were microinjected with porcine insulin at a single dose of 3 µU/site, and four rats were used for microinjections of rPP into the DVC. Applications to the surface and microinjections of insulin into the DVC were both performed with at least 15-min intervals between treatments. Eight of the rats from the dose-response study for rIns were used for investigating the vagus nerve involvement in the motor and cardiovascular effects of rIns in the lower brain stem. Bilateral vagotomy was performed at the medullary level. Briefly, the vagus nerves were carefully separated from the left and right common carotid arteries, silk snare were loosely placed around them, and then vagotomy was achieved by avulsion. Transection of the cervical spinal cord was performed at the level of the medullospinal transition region in three animals. The complete interruption of spinal afferents was assured by excision of 0.5 cm of the spinal cord. As a rule, vagotomy or spinal cord transection was performed after typical responses to the hormone were obtained, followed by repeat application or microinjection of rIns (the micropipette was left in the site) 30–60 min later.

Data analysis. Data were analyzed as described in detail previously (24). Briefly, the area of the response in IGP for each treatment was calculated using a computer-based imaging system. Contractile activity of the pyloric circular muscle (PCA) and the greater curvature longitudinal muscle (GCCA) was calculated as minute motility index (MMI) (37). In addition, peak changes in intragastric pressure were also calculated. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the pulse pressure. The changes in MAP and HR are expressed as differences between the baseline and the peak or nadir response after injection.

Statistical methods. The differences between groups were assessed by one-way or one-way repeated measures ANOVA followed by the Student-Newman-Keuls multiple comparisons test as well as by paired t-test. P < 0.05 was considered to be statistically significant.
RESULTS

Gastric motor and cardiovascular effects of insulin applied to surface of dorsal medulla. First, we applied rIns at two different doses of 0.3 and 3 µU (in a volume of 5 µl) as well as porcine insulin (3 µU) and recombinant human insulin (100 mU) to the surface of the dorsal medulla (immediately above the DVC). rIns, at both doses applied, increased PCA (Fig. 1A). The PCA changes in response to rIns at a dose of 0.3 µU started 3.2 ± 0.8 min after application and lasted for 4.5 ± 1.2 min. The higher (3 µU) dose of rIns evoked PCA changes at 2.0 ± 0.8 min, which lasted for 4.6 ± 0.8 min. The higher dose of rIns (3 µU) was required to increase IGP (both peak response and area of the response) and GCCA (Fig. 1A), as well as MAP (Fig. 1B). The changes in HR were not significantly different from vehicle application (Fig. 1B). The time course of onset and duration of the responses was as follows. For IGP, the changes started at 1.7 ± 0.7 min and lasted for 5.7 ± 1.6 min. For GCCA, the changes appeared at 2.0 ± 0.7 min after rIns application to the surface and lasted for 5.2 ± 1.7 min. Similarly, MAP increased at 1.3 ± 0.2 min after application of rIns to the surface of the dorsal medulla and returned to baseline within the next 6.0 ± 2.1 min. The excitatory gastric motor responses to rIns applied to the surface of the dorsal medulla at a dose of 3 µU were completely abolished by bilateral vagotomy at midcervical level (Table 1).

Trace recordings from a representative experiment in which rIns, at a dose of 3 µU, was applied to the surface of the dorsal medulla, are shown in Fig. 2A. An increase in IGP (peak change, 7.5 cmH₂O; area of the response, 3.35 cm²) occurred 30 s after application and returned to baseline within 13 min. Increases in PCA (ΔMMI was 6.0) and GCCA (ΔMMI was 5.5) were noted in the same animal. The application of rIns also evoked a gradually developing bradycardia in this particular example (nadir change was −80 beats/min (bpm) and a biphasic change in MAP that involved a transient depressor response (nadir was −10 mmHg) followed by a prolonged pressor response (peak change was 30 mmHg).

Porcine and human insulin, applied to the surface of the dorsal medulla at doses of 3 and 100 mU, respectively, evoked modest changes in gastric motor and cardiovascular function (Table 2).

Gastric motor and cardiovascular effects of insulin in DVC. To determine the medullary site for gastric motor and cardiovascular effects of insulin in the lower brain stem, rIns (0.1, 1 and 10 nU/site) and porcine insulin (10 µU/site) were microinjected into the DVC in a total number of 13 rats, while gastric motor and cardiovascular function was recorded. At the higher two doses of rIns (1 and 10 nU/site), there were significant increases in IGP (both peak and the total area of the response) and GCCA (Fig. 3A), as well as in MAP (Fig. 3B). The increases in PCA (Fig. 3A) and HR (Fig. 3B) attained statistical significance only after rIns at a dose of 10 nU/site. Porcine insulin, microinjected into the DVC of three rats at a single dose of 10 µU/site, did not significantly change gastric motor or cardiovascular function (Table 2). Microinjection sites in the lower brain stem for rIns are shown in Fig. 4, which illustrates that the pipette tip was within or on the ventral border of the dorsal motor nucleus of vagus.

A tracing recording from one experiment in which rIns was microinjected into the DVC at a dose of 10 nU/site is shown in Fig. 2B. An increase in IGP (peak change, 13.5 cmH₂O; area of the response, 4.06 cm²) occurred immediately after injection and returned to baseline within 8 min after that. Increases in PCA (ΔMMI was 9) and GCCA (ΔMMI was 3.5) as well as in HR (peak change, 30 bpm) were observed in this animal. In this particular animal, no changes in MAP in response to rIns in the DVC were observed.

To ascertain the anatomic specificity of the cardiovascular and gastric responses to rIns in the DVC, we microinjected the hormone (10 nU/site) into the brain stem medulla outside the DVC (coordinates: 0.5 mm
rostral to the obex, 1.5 mm lateral from the midline, and 0.7 mm down from the surface; Ref. 22) in two animals. Microinjection of rIns into this site resulted in no discernible changes in gastric motor or cardiovascular function. However, typical gastric motor and cardiovascular responses were noted when rIns was microinjected into the DVC of the same animals.

rIns (10 nU/site)-evoked increases in gastric tone and motility after its microinjection into the DVC were abolished by bilateral vagotomy at the midcervical level (Table 1). The increase in MAP (10 ± 3 mmHg, n = 3) in response to rIns (10 nU/site) in the DVC was completely blocked by spinal cord transection (0 ± 0 mmHg, n = 3).

Gastric motor and cardiovascular effects of rPP in DVC. We were concerned that gastric motor and cardiovascular effects of rIns could be due to a potential contamination with rPP in the commercially available preparation. Therefore, rPP was microinjected into the DVC of four rats at a single dose of 2 pmol/site. This resulted in a small, but statistically significant, increase in the area of the response IGP (Table 3). However, there were no other changes in gastric motor or cardiovascular function.

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**Table 1. Effect of bilateral cervical vagotomy on changes in IGP, PCA, and GCCA in response to rIns**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRIGP, cmH2O</th>
<th>ARIGP, cm²</th>
<th>PCA, MMI</th>
<th>GCCA, MMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.0 ± 0.0 (3)</td>
<td>0.0 ± 0.0 (3)</td>
<td>−0.3 ± 0.3 (2)</td>
<td>0.0 ± 0.0 (3)</td>
</tr>
<tr>
<td>rIns before BCV</td>
<td>4.7 ± 0.6* (3)</td>
<td>0.1 ± 0.1* (3)</td>
<td>2.5 ± 0.5* (2)</td>
<td>1.7 ± 0.7 (3)</td>
</tr>
<tr>
<td>rIns after BCV</td>
<td>0.3 ± 0.4t (3)</td>
<td>0.1 ± 0.1t (3)</td>
<td>0.0 ± 0.0t (2)</td>
<td>0.0 ± 0.0 (3)</td>
</tr>
<tr>
<td>DVC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.1 ± 0.1 (5)</td>
<td>0.0 ± 0.0 (5)</td>
<td>0.1 ± 0.1 (4)</td>
<td>0.0 ± 0.0 (5)</td>
</tr>
<tr>
<td>rIns before BCV</td>
<td>11.0 ± 1.0* (5)</td>
<td>2.2 ± 0.5* (5)</td>
<td>6.4 ± 1.2* (4)</td>
<td>3.5 ± 0.6* (5)</td>
</tr>
<tr>
<td>rIns after BCV</td>
<td>−0.1 ± 0.4t (5)</td>
<td>0.0 ± 0.1t (5)</td>
<td>0.5 ± 0.4t (4)</td>
<td>0.0 ± 0.0t (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals and is given in parentheses. Rat insulin (rIns) was applied to the surface of the dorsal medulla (3 µU/rat) or microinjected into the dorsal vagal complex (DVC) (10 nU). IGP, intragastric pressure. PRIGP, peak response IGP. ARIGP, area of response IGP. PCA, pyloric contractile activity. GCCA, greater curvature contractile activity. BCV, bilateral cervical vagotomy. MMI, minute motility index. *Statistically significant (P < 0.05) compared with corresponding mean for vehicle microinjection. †Statistically significant (P < 0.05) compared with corresponding mean for rIns microinjection before BCV.
DISCUSSION

Our study demonstrates for the first time that rIns may elicit excitatory gastric motor responses by acting in the lower brain stem and that the DVC is the most likely medullary site for these effects. In addition, the gastric and hypertensive responses elicited by insulin appear to be species selective, in that rIns evoked much greater autonomic effects than either porcine or human insulin. Gastric motor and cardiovascular effects of rIns in the DVC are unlikely to be related to insulin-induced hypoglycemia, because these effects appear immediately after microinjection of insulin into this site. Moreover, serum glucose levels do not change after microinjection of insulin into the DVC of cats (25). This is important because the increase in gastric motor activity in response to peripherally administered insulin is usually attributed to vagal stimulation due to insulin-induced hypoglycemia (4). However, peripherally administered insulin has been used successfully to increase radiation-induced delays in gastric emptying in the rat and no simple correlation between the hypoglycemic action of insulin and its effect on gastric emptying was found (16). It has been suggested previously (10) that insulin receptors in the brain may initiate insulin-induced gastric acid secretion and mo-

Table 2. Effect of pIns and hIns on IGP, PCA, GCCA, HR, and MAP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRIGP, cmH2O</th>
<th>ARIGP, cm²</th>
<th>PCA, MMI</th>
<th>GCCA, MMI</th>
<th>HR, bpm</th>
<th>MAP, mmHg</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>pIns</td>
<td>1.1 ± 0.3*</td>
<td>0.2 ± 0.1*</td>
<td>0.4 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>-17 ± 6*</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>hIns</td>
<td>1.5 ± 0.3*</td>
<td>0.2 ± 0.1*</td>
<td>1.2 ± 0.4*</td>
<td>0.2 ± 0.2</td>
<td>-7 ± 3</td>
<td>9 ± 2*</td>
</tr>
<tr>
<td>Vehicle</td>
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<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>pIns</td>
<td>1.0 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>-13 ± 3</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Porcine (3 mU/rat; pIns; n = 5) and human insulin (100 mU/rat; hIns; n = 6) were applied to the surface of the dorsal medulla. pIns (10 µU/site; n = 3) was also microinjected into the DVC. HR, heart rate. MAP, mean arterial pressure. bpm, Beats/min. *Statistically significant (P < 0.05) compared with corresponding mean for vehicle microinjection.

Fig. 3. Gastric motor and cardiovascular effects of vehicle or rIns (0.1, 1, and 10 nU) microinjected into the DVC. A: changes in IGP, PCA, and GCCA. B: changes in HR and MAP. No. of animals is indicated either below or above each bar. *Statistically significant (P < 0.05) compared with effect of vehicle.

Fig. 4. The locations of the micropipette tips (●) after microinjection of rIns (10 nU/site) are transposed onto the right DVC, while nuclei are labeled on the left DVC. Sections are drawn by using a drawing tube attachment to a Nikon Labophot microscope and are arranged from most caudal at the top (0.7 mm rostral to the obex) to most rostral at the bottom (1.0 mm rostral to the obex). AP, area postrema; cc, central canal; DMV, dorsal motor nucleus of vagus; NTS, nucleus of solitary tract; TS, solitary tract; XII, hypoglossal nucleus.
itor activity in the rat, and the present study supports this idea.

In general, peripherally administered insulin is initially associated with gastric motor inhibition. For example, in the dog, there is an inhibition of gastric motility within 20 min after peripherally administered insulin (18, 52). This response is not abolished by vagotomy (18), atropine (18, 52), or adrenergic blockade (18). However, initial decreases in gastric motility are invariably followed by increases to levels above the control (52) or increases in the rhythmic rate of gastric contractions (31). The tachygastria depended on intact vagal innervation of the stomach (31). In a different study in the dog, the augmentation of antral contractile activity was accompanied by relaxation of the proximal stomach, so gastric emptying in response to insulin increased only slightly (40). All these data suggest that insulin can be associated with increases in gastric motility. Perhaps the time required for circulating insulin to reach its receptors in the lower brain stem may account for the delayed increase in gastric motor activity after peripheral administration of insulin.

rPP has been reported (30) to markedly increase gastric motility when microinjected into the DVC of the rat at doses from 0.004 fmol to 4 pmol. Therefore, to test whether the observed gastric motor effects of rIns in the lower brain stem were caused by potential contamination of the extract with rPP, we microinjected rPP into the DVC at a dose of 2 pmol/site. A modest increase in the area of the response of intragastric pressure of about one-fifth the response to rIns at a dose of 1 nU/site was observed after microinjection of rPP. Therefore, the increase in IGP and gastric motility in response to rIns cannot be accounted for by rPP contamination of the rIns preparation. We were surprised that rPP at a dose of 2 pmol did not more dramatically increase gastric motility. We cannot explain the observed differences in the gastric motor responses to rPP in the DVC of the same species between our study and that by McTigue et al. (30). However, one potential difference in the protocols is the use of dexamethasone pretreatment by McTigue et al. (30). This glucocorticoid has been shown to decrease the relaxing effect of vasoactive intestinal polypeptide on isolated intestinal smooth muscle cells (41). Possible glucocorticoid-induced attenuation of nonadrenergic, noncholinergic vagal gastric inhibition may mean that there was an enhanced sensitivity to rPP in the DVC to provoke gastric excitation in the study by McTigue and colleagues (30). Differences in the strain of the animals (Long-Evans vs. Sprague-Dawley rats) used and anesthesia might also contribute to the discrepancies between the studies.

In our experiments, rIns, applied to the dorsal surface of the medulla or microinjected into the DVC, increased blood pressure. On the other hand, porcine insulin did not alter blood pressure or HR in the DVC and evoked slight bradycardia when applied to the dorsal surface of the medulla. These data are consistent with the lack of effects of porcine insulin on blood pressure and HR in the nucleus tractus solitarius of urethan-anesthetized rats (28). Microinjection of human insulin into the dorsal motor nucleus of vagus in α-chloralose and urethan-anesthetized cats (at a dose as high as 2 mU/site) also did not affect the resting HR and MAP (25). In the present study, the very high dose of human insulin (100 mU), applied to the dorsal surface of the medulla, evoked only a modest increase in MAP, which was approximately one-half the size of the hypertensive response evoked by rIns. However, other studies conducted in the rat with insulin from different species, have provided mixed results as to the cardiovascular effects of the hormone in the brain. Intracerebroventricular administration of heterogeneous insulin in various species has been reported to produce hypotensive and bradycardic responses (32, 46), to enhance reflex tachycardia (35), or to have no effect on blood pressure and HR at all (25). Based on the results of our study using three different types of insulin, the most likely explanation for these contradictory results is that they may be caused by different relative affinities of insulin from different species to its receptors in the rat or cat brain. This assumption is based on the available data that rat and cat insulin differ substantially from other mammalian insulin (13, 17) and rIns is difficult to assay with commonly available insulin antibodies. Moreover, insulin receptors are known to be heterogeneous in structure and function (19). In addition, the biological activity of mammalian insulin has been measured by standard mouse convulsion (hypoglycemia related) or blood glucose assays (49), which do not address the binding of insulin to brain receptors. Consequently, although no differences between binding of human or porcine insulin to human brain insulin receptors have been found (22), the receptor affinity constant of human insulin was found to be significantly lower in comparison to porcine insulin in porcine brain tissues (45). The issue of species differences in the case of the other pancreatic hormone, rPP, has also been addressed recently by McTigue and co-workers (29). Specifically, intracerebroventricular injections of rPP in nonanesthetized rats produced a significant increase in the gastrointestinal transit, whereas bovine PP yielded no effects, even at high doses. In conclusion, our experiments with rat, porcine, and human insulin, applied to the surface of the dorsal

### Table 3. Effect of rPP microinjected into DVC on IGP, PCA, GCCA, HR, and MAP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRIGP, cmH₂O</th>
<th>ARIGP, cm²</th>
<th>PCA, MMI</th>
<th>GCCA, MMI</th>
<th>HR, bpm</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>3 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>rPP</td>
<td>1.0 ± 0.4</td>
<td>0.1 ± 0.0*</td>
<td>1.6 ± 0.9</td>
<td>1.0 ± 1.0</td>
<td>8 ± 3</td>
<td>5 ± 2</td>
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</tbody>
</table>

Values are means ± SE; n = 4. rPP, rat pancreatic polypeptide. *Statistically significant (P < 0.05) compared with corresponding mean for vehicle microinjection.
medulla or microinjected into the DVC of the rat, clearly indicate that insulin from the same species should be used in studies investigating the brain effects of the hormone.

The most likely origin of insulin in the DVC is from the peripheral circulation, since the hormone of pancreatic origin has been considered to be the main source for insulin in the brain (48). The transport of plasma insulin to the central nervous system is mediated by a saturable mechanism consistent with insulin binding to blood-brain barrier insulin receptors and subsequent transcytosis through microvessel endothelial cells (3). In addition, rat cerebrospinal fluid normally contains 0.2–0.4 µU/ml of endogenous insulin, which constitutes 1.7–2.8% of the plasma level, and systemic treatment with exogenous insulin elevates the cerebrospinal fluid insulin levels substantially (39). However, since the caudal nucleus of solitary tract has fenestrated capillaries and enlarged perivascular spaces that permit entry of serum proteins (6) and there exists close anatomic association of the DVC to the area postrema, a circumventricular organ (11), it is not necessary for plasma insulin to cross the blood-brain barrier to affect the DVC neurons. The presence of insulin-binding sites in the area postrema and the DVC has been well documented (53–55).

An endogenous source for insulin in the DVC may also arise from the brain. This is because a small portion of insulin in the brain may be derived from its own synthesis (44). The latter finding indicates a possible neurotransmitter or neuromodulator role for insulin in the brain. Morphological mapping studies of insulin immunoreactivity (2) or insulin mRNA by in situ hybridization (57) revealed that insulin is present in cell bodies of the paraventricular hypothalamic nucleus, which directly projects to the DVC and controls gastric function (42).

It could be argued that the effects of insulin in the DVC on gastric motor and cardiovascular function could be, at least in part, due to changes in brain metabolism. However, whether or not glucose utilization in the brain is insulin dependent is still a controversial issue. The metabolic changes in response to insulin have been studied in different brain regions and both a noticeable suppression of cerebral glucose metabolism (12) and a lack of alteration in glucose transport, glucose oxidation, and glycogen synthesis (14, 48) were found. A recent communication by Leloup and co-workers (26) reveals the presence of the insulin-sensitive glucose transporter in the rat medulla oblongata. Therefore, at the present time, we cannot exclude the possibility that insulin effects in the lower brain stem are related to its effect on glucose metabolism in this region.

Finally, there exists a close relationship between insulin and endothelin-1, produced by the endothelium, brain, and gastrointestinal tract. Specifically, insulin stimulates production and secretion of endothelin-1 from endothelial cells in vitro (15), elevates endothelin-1 plasma levels in vivo (38), and stimulates its receptor-mediated action (8). Peripherally administered endothelin-1 increases intragastric pressure, gastric smooth muscle contractile activity, and MAP in the rat (23). Because combined α1- and β-adrenergic receptor blockade did not prevent hypertension induced by peripherally administered insulin (20), we hypothesize that the hypertensive effect of insulin may be mediated by a direct effect of endothelin-1 on the blood vessels.

What is the potential clinical significance of our observations? First, insulin is widely used in the treatment of diabetes mellitus and its overdose or intensive insulin therapy may lead to hyperinsulinemia. Second, there also exists a reactive hyperinsulinemia, which may occur in obesity, impaired glucose tolerance, or noninsulin-dependent diabetes mellitus, as well as in obese, nondiabetic patients with hypertension. Achieving near-normal glucose levels in patients with noninsulin-dependent diabetes mellitus requires a mean total insulin dose of as much as 100 U/day with serum insulin concentrations in the range of 65–142 µU/ml to overcome the existing insulin resistance (9). The latter may be severe when obesity also exists. Despite the origin of insulin, the resulting hyperinsulinemia is usually associated with abnormal gastric motor function and hypertension. So far, the mechanisms behind these disorders have not been fully elucidated.

In patients with noninsulin-dependent diabetes mellitus of less than 2 years duration and normal autonomic function without any symptoms of gastrointestinal dysfunction, gastric emptying is usually accelerated (50). Acceleration of gastric emptying may be also found in patients with insulin-dependent diabetes (33) as well as in obese subjects (56). An increase in gastric contractility cannot be simply associated with accelerated gastric emptying. However, it is possible that insulin, through its action on the DVC neurons, may not only increase gastric motility, but also increase gastric emptying. However, further studies investigating the effect of centrally administered insulin on gastric emptying are needed to better address the practical significance of our observations.

Because peripherally administered insulin has a vasodepressor action, which is offset by a pressor effect, it has been suggested that the development of hypertension in diabetic patients with insulin resistance and hyperinsulinemia may be due to a relative imbalance between the depressor and pressor effects of insulin in favor of the latter (1). Similarly, prolonged hyperinsulinemia in over-treated patients with insulin-dependent diabetes may contribute to the development of hypertension (51). Therefore, we hypothesize that the DVC may be involved in the development of cardiovascular disease in hyperinsulinemia.

In summary, our study indicates the DVC to be a lower brain stem site in which insulin evokes potent gastric motor and cardiovascular effects. These findings may be used as a basis for future studies on the mechanisms behind gastrointestinal and cardiovascular complications in hyperinsulinemia.
REFERENCES


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