Neural contribution to the effect of glucagon-like peptide-1-(7—36) amide on arterial blood pressure in rats

JOSE´ MANUEL BARRAGÁN,1 JOHN ENG,2 RAQUEL RODRÍGUEZ,3 AND ENRIQUE BLÁZQUEZ3

1Department of Biochemistry and Molecular Biology, University of Salamanca, 37007 Salamanca, Spain; 2Department of Medicine, Veterans Affairs Medical Center, Bronx, New York 10468; and 3Department of Biochemistry and Molecular Biology, Faculty of Medicine, Complutense University, 28040 Madrid, Spain

Barragán, J osé Manuel, John Eng, Raquel Rodríguez, and Enrique Blázquez. Neural contribution to the effect of glucagon-like peptide-1-(7—36) amide on arterial blood pressure in rats. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E784–E791, 1999.—This study was designed to determine the contribution of the central nervous system (CNS) to the effects of glucagon-like peptide-1-(7—36) amide (tGLP-1) on arterial blood pressure and heart rate in rats. Accordingly, intracerebroventricular administration of the peptide produced an increase in cardiovascular parameters, which was blocked by previous administration of exendin-(9—39) through the same route, but not when it was intravenously injected. Intravenous administration of tGLP-1 produced a significant increase in arterial blood pressure and heart rate, which was blocked by the previous intracerebroventricular or intravenous administration of exendin-(9—39). Bilateral vagotomy blocked the stimulating effect of intracerebroventricular tGLP-1 administration on arterial blood pressure and heart rate. Also, bilateral vagotomy prevented the blocking effect of intracerebroventricular but not of intravenous exendin-(9—39) on cardiovascular parameters after intravenous administration of tGLP-1. These findings suggest that the action of tGLP-1 on cardiovascular parameters is under a dual control generated in the CNS and in peripheral structures and that the neural information emerging in the brain is transmitted to the periphery through the vagus nerve.

cardiovascular parameters; neural control; vagal mediation

GLUCAGON-CONTAINING (glicentin and oxyntomodulin) and glucagon-like peptides [GLP-1-(1—37), GLP-1-(7—37), GLP-1-(7—36) amide (tGLP-1), and GLP-2] are components of the proglucagon gene (4), which gives rise to an mRNA transcript that is identical in sequence (20) in the pancreas, intestine, and brain, although posttranslational processing of the precursor yields different products in these organs. In the L cells of the gut, glucagon is predominantly processed to glicentin, oxyntomodulin, GLP-1, and GLP-2, and truncated and amidated forms of GLP-1 are produced by further enzymatic processing. In the brain, the processing of proglucagon resembles that of the intestine (18).

GLP-1-(1—37) has low biological activity, and the other component of the COOH-terminal portion of mammalian proglucagon, or GLP-2, is considered to be a stimulator of small bowel epithelial proliferation (9). However, the truncated forms of GLP-1 such as GLP-1-(7—37) amide and tGLP-1 are very active molecules, acting on both peripheral tissues and the central nervous system (CNS). tGLP-1 is released after meals and is a powerful stimulus for glucose-dependent insulin secretion (19). It also inhibits gastric acid secretion and gastric emptying in normal humans (39). Also, a role for tGLP-1 in pulmonary surfactant secretion by type II pneumocytes (5), and also in arterial blood pressure and heart rate (2, 3), has been described in the rat. In calves, tGLP-1 administered intravenously increased heart rate but had no effect on arterial blood pressure (11), whereas in humans the subcutaneous injection of tGLP-1 increased both arterial blood pressure and heart rate (12).

Cloning and functional expression of GLP-1 receptors (33) in pancreatic islets have been reported. In addition, specific high-affinity binding sites have been characterized in rat insulinoma cells (14), gastric glands (35), lung (28), brain (6, 7, 17, 36), and adipocyte rat membranes (37). There is experimental evidence that tGLP-1 and its own receptors are actually synthesized in the same brain regions, which leads to a better understanding of the actions of this peptide in the CNS, such as the selective release of neurotransmitters from different brain nuclei (21) after perfusion with tGLP-1 and the inhibitory effect on food and drinking intakes (1, 22, 32, 34) after the central administration of the peptide. In addition, the colocalization of GLP-1 receptors with glucokinase and GLUT-2 (1, 22) in the same neurons supports the idea that these cells may play an important role in glucose sensing in the brain.

Besides the actions of the glucagon-related peptides cited previously, the cardiovascular effects have also been defined. Glucagon has positive inotropic and chronotropic effects (31); it affects regional blood circulation and also produces a slight but significant increase in arterial blood pressure (25). GLP-2 has no effect and GLP-1-(1—37) produces a moderate increase in arterial blood pressure, whereas tGLP-1 induces a concentration-dependent increase in systolic and diastolic blood pressure and heart rate (2, 3). The action of tGLP-1 on these parameters seems to be mediated through its own receptors, because it was tested after the intravenous administration of exendin-4 as an agonist and exendin-(9—39) as an antagonist of that peptide (3). It is noteworthy that tGLP-1 and its own receptors have been found in significant amounts in the nucleus tractus solitarius (16, 18, 36), which is involved...
in central control of cardiovascular function. Thus the possibility that the effects of tGlp-1 on cardiovascular parameters may be induced through a central mechanism should be taken into account. Here we report the action of tGlp-1 on arterial blood pressure and heart rate when this peptide was administered intravenously or intracerebroventricularly. We also describe the contribution of the vagus nerve to this process.

**Materials and Methods**

Materials. Synthetic human GLP-1(7—36) amide was obtained from Peninsula Laboratories (St. Helens, UK). Exendin-9—39 was prepared as previously reported (27). This peptide was produced on a solid-phase support of polycrylic (PAL) resin utilizing activated N-(9-fluorenylmethoxy carbonyl) amino acids on a Milligen 9050 peptide synthesizer (Milligen, Burlington, MA) and was purified by preparative HPLC.

Experimental animals. Male Sprague-Dawley rats weighing 250–300 g were housed under standard conditions of lighting (12:12-h light-dark cycle) and at a temperature of 21°C, with free access to food and water.

Surgical procedures. For intracerebroventricular administration of the peptides, polyethylene cannulas aimed at the third ventricle were implanted stereotaxically in the rats. After being anesthetized, rats were positioned in a stereotaxic head frame, the shaved scalp was incised, the periosteum was removed, and the skull was exposed. Holes were drilled into the frontal and parietal bones to receive one of three stainless-steel anchoring screws. With the use of bregma as reference, the cannula was lowered through a burr hole above the third ventricle and positioned according to the stereotaxic coordinates of the atlas of Paxinos and Watson (26) (7.7 interaural, 0.5 mm lateral right, and 4.5 mm deep). The cannula was a thin-wall tubing that was fixed to the skull and to the anchoring screws with dental cement. The presence of the cannula in the third ventricle was checked postmortem, after the injection of methylene blue.

A group of rats were bilaterally vagotomized at the cervical region, after dissection of the nerves from the carotid arteries. Bilateral vagotomy was carried out 1—2 h before experiments were done. This surgical procedure did not cause lung congestion, edema, or death in the rats used in our experiments. To determine cardiovascular parameters, rats were anesthetized with urethan (250 mg/kg ip) and then placed upside down on a homeothermic blanket system, where temperature was maintained at 37.0 ± 0.2°C by means of a rectal probe. After induction of anesthesia, the trachea was cannulated with a polyethylene tube and the animal was allowed to breathe room air spontaneously. After this procedure had been completed, the right common artery and the right jugular vein were cannulated for continuous recording of arterial blood pressure and heart rate and for intravenous injection of drugs, respectively. The cannula inserted into the right common artery was connected through a Druck Prdr 75 transducer to a Lectromed recorder apparatus.

Experimental protocols. After surgical preparation, arterial blood pressure was recorded and the animals were allowed to stabilize for 60 min. At the end of this period, recording of cardiovascular parameters was started and control values were obtained over a further 30-min period. Groups of 6–10 animals were used for each treatment.

At the end of the control period, 0.9% NaCl or tGlp-1 was injected intravenously through the jugular vein or intracerebroventricularly into the third ventricle. The total injection volume for the jugular vein was 0.2–0.3 ml, and the total volume injected into the third ventricle was 5 µl. After the injection, cardiovascular parameters were recorded for a further 30-min period. The action of exendin-9—39 was investigated by jugular administration of 2,500 ng of the peptide 5 min before intravenous or intracerebroventricular administration of 100 ng of tGlp-1.

Experimental recordings. During the experiments, arterial blood pressure was recorded via a physiological pressure transducer (Druch Prdr 75). A parallel output was taken from the preamplifier stage of the polygraph, passed via a Lectromed apparatus with thermosensitive millimetric paper, and then appropriate calibration values for systolic, diastolic, and mean arterial blood pressure (mmHg) and heart rate (beats/min) were obtained.

Statistical analysis. Results are expressed as means ± SE from groups of 6–10 rats. Because arterial blood pressure and heart rate values remained stable after 0.9% NaCl administration, statistical comparisons were performed in each animal between time 0 and each period of time after administration of the peptides. In these cases, statistical significance was assessed for P < 0.05 with Student’s t-test. ANOVA with the Newman-Keuls test was used when statistical comparisons were done with the data obtained from two different groups of animals. The percent variation of mean arterial blood pressure or heart rate was calculated as follows:

\[
\text{% variation} = \frac{\text{postinjection value} - \text{preinjection value}}{\text{preinjection value}} \times 100
\]

**Results**

Effects of intracerebroventricular administration of tGlp-1 on arterial blood pressure and heart rate. To determine the effects of tGlp-1 on arterial blood pressure and heart rate, this peptide was administered either intravenously or intracerebroventricularly at doses of 10, 50, and 100 ng. The effects obtained were compared with those observed after the administration of 0.9% NaCl (control values). After it had been seen that arterial blood pressure and heart rate values remained unchanged from the control values after the administration of 0.9% NaCl in one group of rats (Fig. 1), the mean of the control period values of each individual animal was used for statistical comparisons of the data between time 0 and each time point after the peptide administration.

As previously reported (2), intravenous administration of 10, 50, and 100 ng of tGlp-1 produced an increase in mean arterial blood pressure and heart rate (data not shown). Also, when the results were plotted as the percentage of variation of mean arterial blood pressure and heart rate (beats/min) after intracerebroventricular administration of 10, 50, and 100 ng of tGlp-1 (Fig. 1, A and B), an increase in the values was observed. In the same way, after intravenous or intracerebroventricular injections of tGlp-1, an increase in both systolic and diastolic blood pressure was obtained (Fig. 2), compared with the results found in control rats. The route used for the administration of the peptide may influence its effects on cardiovascular parameters (Table 1). Thus the intravenous administration of tGlp-1 produced a greater maximum percent change of mean arterial blood pressure and heart rate, compared with the data obtained after intracerebroven-
tricular injection of the peptide, whereas central administration of tGLP-1 resulted in a greater time at maximum percent change and time to 50% maximum response than when it was injected peripherally (Table 1).

Blocking effect of exendin-(9—39) on the action of tGLP-1 on arterial blood pressure and heart rate.

Having observed that intracerebroventricular administration of tGLP-1 has a potent stimulating effect on arterial blood pressure and heart rate, we next tested the antagonist effect of exendin-(9—39) on the same parameters. First, the effect of exendin-(9—39) alone on arterial blood pressure and heart rate was studied. Intracerebroventricular administration of 2,500 ng of exendin-(9—39) alone did not produce changes in cardiovascular parameters. To ensure that the action of tGLP-1 on arterial blood pressure and heart rate was indeed a direct effect, 100 ng of the peptide were either intravenously or intracerebroventricularly injected 5 min after the administration of 25-fold the dose of exendin-(9—39) through one route or the other, respectively. Intracerebroventricular administration of tGLP-1 produced an increase in both arterial blood pressure and heart rate (Fig. 3, A and B), but when the rats were pretreated with 2,500 ng of exendin-(9—39) 5 min before intracerebroventricular injection of 100 ng of tGLP-1, no effects of the latter peptide on either cardiovascular parameter were observed. As reported previously (3), intravenous administration of 100 ng of tGLP-1 produced a significant increase in arterial blood pressure and heart rate (Fig. 3, A and B).

Table 1. Maximum change, time at maximum change, and time to 50% maximum response of mean arterial blood pressure and heart rate after intravenous or intracerebroventricular administration of tGLP-1 in rats

<table>
<thead>
<tr>
<th></th>
<th>Time at Maximum %Change, min</th>
<th>Time to 50% Maximum Response, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>17.7±3.1</td>
<td>1</td>
</tr>
<tr>
<td>Intracerebroventricular</td>
<td>12.3±2.1</td>
<td>6</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>9.2±1.4</td>
<td>2</td>
</tr>
<tr>
<td>Intracerebroventricular</td>
<td>6.5±3.2</td>
<td>12</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–9 rats. Values shown in columns time at maximum %change and time to 50% maximum response were based on data used for column maximum %change. tGLP-1, glucagon-like peptide-1-(7-36) amide; tGLP-1 dosage, 100 ng.
pressure and heart rate, but these effects were completely blocked by previous intravenous administration of 2,500 ng of exendin-(9—39).

To determine whether the effect of tGLP-1 on the cardiovascular parameters was due to a central or peripheral mechanism of action, this peptide was administered either intracerebroventricularly or intravenously, and in each case exendin-(9—39) was injected through the opposite route. As shown in Fig. 4, intravenous administration of 100 ng of tGLP-1 to intact rats produced the already known increase in mean arterial blood pressure, but this effect disappeared in bilaterally vagotomized animals. Interestingly, bilateral vagotomy did not prevent the stimulating action of intravenous tGLP-1 on mean arterial blood pressure (Fig. 7, A and B), although this effect was lower in vagotomized than in intact rats. Also, bilateral vagotomy abolished the blocking effect of intracerebroventricular exendin-(9—39) on mean arterial blood pressure after intravenous administration of tGLP-1 (Fig. 8, A and B).

**Effect of tGLP-1 on mean arterial blood pressure and heart rate in bilateral vagotomized rats.** In an attempt to determine whether the signal generated by the tGLP-1 on the CNS was transmitted from the brain to the periphery by the vagus nerve, the effect of this peptide on cardiovascular parameters was studied in bilaterally vagotomized rats. As expected, mean arterial blood pressure (sham-operated: 58.1 ± 2.3 and vagotomized: 68.0 ± 1.7) and heart rate (sham-operated 381.0 ± 9.0 and vagotomized: 436.7 ± 10.1) increased significantly (P < 0.001) in vagotomized rats before tGLP-1 treatment. As shown in Fig. 6, intracerebroventricular administration of 100 ng of tGLP-1 to intact rats produced the already known increase in mean arterial blood pressure, but this effect disappeared in bilaterally vagotomized animals. Interestingly, bilateral vagotomy did not prevent the stimulating action of intravenous tGLP-1 on mean arterial blood pressure (Fig. 7, A and B), although this effect was lower in vagotomized than in intact rats. Also, bilateral vagotomy abolished the blocking effect of intracerebroventricular exendin-(9—39) on mean arterial blood pressure after intravenous administration of tGLP-1 (Fig. 8, A and B).
DISCUSSION

Previous studies carried out in rats (2) have shown that peripheral administration of tGLP-1 induces an increase in systolic and diastolic blood pressure and heart rate. Glucagon and other glucagon-related peptides were less effective when the same parameters were considered. The action of tGLP-1 on arterial blood pressure and heart rate seems to be mediated through its own receptors because exendin-4 acts as an agonist and exendin-(9—39) blocks the effects of that peptide on cardiovascular parameters (3). Because both tGLP-1 and its receptors have been found in significant amounts in the nucleus tractus solitarius (15, 16, 30, 36), which is involved in the central control of cardiovascular function (8), the possibility exists that exogenously administered tGLP-1 alters cardiovascular parameters through a central mechanism. The area postrema is also significantly involved in cardiovascular regulation (13) and has significant amounts of GLP-1 receptors (24), suggesting that the action of tGLP-1 on arterial blood pressure would be the consequence of previous binding of this peptide to the nucleus tractus solitarius and area postrema. Accordingly, experiments were designed in an attempt to elucidate the possible control of the CNS on the effect of tGLP-1 on arterial blood pressure and heart rate. Here we report that intracerebroventricular administration of tGLP-1 produces an increase in systolic and diastolic blood pressure and heart rate, these effects being blocked by previous injection of exendin-(9—39). It is noteworthy to mention that the potency and timing of tGLP-1 effects were different depending on the route of its administration. Thus intravenous injection of the peptide produces a more potent and rapid response on cardiovascular parameters then when it was intracerebroventricularly administered, whereas central injection of tGLP-1 induced a more prolonged time of action. Further studies are needed for a better understanding of these differences, although the possibility of a greater dilution of the peptide in the blood circulation, a slower degradation of tGLP-1 in the CNS, and/or a different number of GLP-1 receptors on peripheral or central locations should be kept in mind. Our results suggest a specific role of the CNS on the stimulating effect of tGLP-1 on cardiovascular parameters. It should be considered that the hypothalamus and brain stem are both sites of highest tGLP-1 content and of high GLP-1 receptor densities and that the nucleus of the solitary tract is involved in cardiorespiratory regulation and in

Fig. 5. Effect of icv administration of tGLP-1 on mean arterial blood pressure (A) and heart rate (B) in rats pretreated with exendin-(9—39) administered either iv or icv. Animals were injected with 2,500 ng of exendin-(9—39) either iv (•) or icv (○), 5 min before the icv administration of 100 ng of tGLP-1 (○). Results are means ± SE; n = 6–8 rats. ANOVA with Newman-Keuls test was used for statistical comparisons between data obtained after icv administration of tGLP-1 and those determined either after iv or icv injection of exendin-(9—39). *P < 0.01.

Fig. 6. Effect of icv administration of tGLP-1 on arterial blood pressure (A) and heart rate (B) in rats with bilateral vagotomy. Animals with (○) or without (●) bilateral vagotomy at cervical region were injected in third ventricle with 100 ng of tGLP-1. Results are means ± SE; n = 6–8 rats. ANOVA with Newman-Keuls test was used for statistical comparisons between data obtained in 2 experimental groups. *P < 0.01.
metabolic homeostasis. Also, microinjection of neuropeptide Y into the caudal nucleus of the solitary tract produces significant dose-related reductions in mean arterial blood pressure, pulse pressure, and respiratory minute volume (10). This peptide antagonizes the effects of tGLP-1 on cardiovascular parameters as well as food and drink intake.

Exendins are a group of peptides isolated from Helodermatidae venoms, with structural and functional analogies with tGLP-1, in which exendin-4 works as an agonist and exendin-(9—39) as an antagonist of the truncated forms of GLP-1. These facts open the possibility for the use of this peptide as a tool to test the role of tGLP-1 in physiological states.

In an attempt to know whether the action of tGLP-1 on cardiovascular parameters was due to a peripheral and/or central effect, the peptide was injected either intravenously or intracerebroventricularly, whereas the antagonist exendin-(9—39) was administered through the same or the other route. With this procedure, we found that previous intracerebroventricular administration of exendin-(9—39) prevents the stimulating action of intravenous injection of tGLP-1 on arterial blood pressure, indicating the contribution of the CNS to this process. By contrast, when injected into the peripheral blood circulation, exendin-(9—39) did not antagonize intracerebroventricular administration of tGLP-1. These results suggest that exendin-(9—39) cannot cross the blood-brain barrier or that the circulating amounts of this peptide are not sufficient to block the action of intracerebroventricularly administered tGLP-1.

A key finding of our experiments is that intracerebroventricular exendin-(9—39) blocks the effects of intravenously administered tGLP-1. This result can be explained in terms of a “leakage” of the antagonist peptide to the periphery. This view, however, is inconsistent with our finding that intracerebroventricular tGLP-1 has no physiological effect in bilaterally vagotomized animals, and more so because bilateral vagotomy prevents the central antagonism blockade of peripheral agonist activity. Interestingly, peripheral administration of tGLP-1 into the jugular vein of bilaterally vagotomized rats produced a significant increase in mean arterial blood pressure, suggesting that this peptide may act through two pathways, one under parasympathetic influence and the other one generated at the periphery. This second option points to the possibility that the presence of GLP-1 receptors in the heart (38) might play a role in the stimulating effect of the peptide on cardiovascular parameters, as well as open the question of whether or not there are GLP-1 receptors in vascular beds or in adrenal medulla. Also, the effects of the central sympathetic outflow on the
GLP-1 system should be considered. However, the effects of tGLP-1 on arterial blood pressure and heart rate are not mediated by catecholamines through either α- or β-adrenergic receptors (2). Earlier studies indicated that GLP-1 is a potent insulin secretagogue (19) acting directly on pancreatic β-cells, but later on it was reported that other extrapancreatic biological effects of this peptide are mediated via the brain rather than in peripheral tissues (21, 22, 32, 34). These actions include the effects of tGLP-1 on the release of neurotransmitters from selective brain nuclei (21), the inhibition of gastric acid secretion and motility (39), and control of food and water intake (22, 32, 34). Here, we also report a role of the CNS in the effect of tGLP-1 increasing arterial blood pressure and heart rate and also that the signal arising in the brain is transmitted by the vagus nerve to the periphery. Other regulatory peptides, such as neuropeptide Y and pancreatic polypeptide, produce some of their effects by mediation of the parasympathetic nervous system. Thus bilateral subdiaphragmatic vagotomy prevents both basal and glucose-induced hyperinsulinaemia of rats chronically intracerebroventricularray treated with neuropeptide Y (29). Taking as an example pancreatic polypeptide, this peptide enters the brain through the blood-brain barrier-free area postrema and adjacent subpostrema area and then binds to the dorsal vagal motor nucleus and nucleus of the solitary tract. The binding of pancreatic polypeptide to these nuclei inhibits vagal inputs to the gastrointestinal system (40). Because tGLP-1 enters the brain by binding to GLP-1 receptors located in the area postrema and the subfornical organ (24), it has been suggested (15) that this peptide, as well as pancreatic polypeptide, may play a similar role in a putative gut-brain axis. Also, tGLP-1 inhibits gastric acid secretion, gastric emptying, and pancreatic enzyme secretion (39). These effects of the peptide are not found in vagotomized subjects, suggesting a centrally mediated effect (23).

A central effect of tGLP-1 on cardiovascular parameters may be induced by the peptide synthesized either in the gut and/or the brain. Peripheral blood-borne tGLP-1 might enter the brain by binding to blood-brain barrier-free organs, such as the area postrema and subfornical organ (24), or might be transported into the brain by the choroid plexus, which has a high density of GLP-1 receptors (1). In addition, the tGLP-1 released from the brain may also play a physiological role. Gut-derived tGLP-1 and brain-derived tGLP-1 are structurally identical, and both may interact with GLP-1 receptors in the central nervous system. These receptors are identical to its counterparts located peripherally. GLP-1 receptor cDNA from human (38) and rat (1) brain has been cloned and sequenced, and the deduced amino acid sequences are the same as the sequence found in pancreatic islets.

In summary, our findings indicate that the stimulating effect of tGLP-1 on arterial blood pressure and heart rate in the rat is under dual control emerging from the CNS and from peripheral structures. It seems that the neural activity generated in this process is transmitted from the brain to the periphery through the vagus nerve, while the presence of GLP-1 receptors in peripheral cardiovascular sites may be useful for the extraparasymathetic influence of tGLP-1 on arterial blood pressure and heart rate.

This work was supported by grants from the Direccio General de Investigación Científica y Técnica, and Fondo de Investigaciones Sanitarias, Spain.

Address for reprint requests and other correspondence: E. Blázquez, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain.

Received 21 January 1999; accepted in final form 9 July 1999.

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