Effects of glucagon-like peptide-1(7–36) amide on neurohypophysial and cardiovascular functions under hypo- or normotensive hypovolaemia in the rat

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Abstract

To date, glucagon-like peptide 1(7–36) amide (tGLP-1) has been found to affect the neurohypophysial and cardiovascular functions in normotensive and normovolaemic rats. The aim of the present study was to investigate possible effects of tGLP-1 on the mean arterial blood pressure and the release of vasopressin and oxytocin under conditions of blood volume depletion in the rat. In the first series of experiments, the animals were injected i.p. with either 0·15 M saline or 30% polyethylene glycol (PEG). PEG caused an 18% reduction of blood volume 1 h after injection. No significant changes in the mean arterial blood pressure were found in either normo- or hypovolaemic rats during the experiment. tGLP-1 injected i.c.v. at a dose of 1 µg/5 µl 1 h after the i.p. injection increased similarly the arterial blood pressure in normo- and hypovolaemic rats. The plasma vasopressin/oxytocin concentrations were markedly elevated in hypovolaemic animals and tGLP-1 further augmented the release of both hormones. In the second study, hypovolaemia was induced by double blood withdrawal. The haemorrhage resulted in a marked decrease of the mean arterial blood pressure and in the elevated plasma vasopressin/oxytocin concentrations. tGLP-1 injected immediately after the second blood withdrawal increased the arterial blood pressure. In parallel, tGLP-1 enhanced significantly vasopressin and oxytocin secretion when compared with haemorrhaged, saline-injected rats. The results of this study indicate that tGLP-1 may affect the arterial blood pressure and the secretion of neurohypophysial hormones under pathological conditions brought about by blood volume depletion. Journal of Endocrinology (2002) 172, 303–310

Introduction

Hypovolaemia and hypotension are known to be powerful stimuli increasing the release of arginine vasopressin (AVP) and oxytocin (OXY) in the rat (Schiltz et al. 1997, Kadekaro et al. 1998). The reduction of blood volume is mainly detected by cardiac stretch (volume) receptors whilst the reduction of blood pressure affects the activity of arterial baroreceptors (Renaud 1996, Share 1996, Thrasher & Keil 2000). The information is then transmitted to the nucleus of the solitary tract (NST) and to other, yet not fully recognised, structures involved in the control of the hypothalamo–neurohypophysial complex (Smith et al. 1995, Renaud 1996, Share 1996). As a result, the electrophysiological activity of vasopressinergic and oxytocinergic neurons changes in a manner characteristic for each of these neuronal systems (Poulain & Wakerley 1982).

The new pattern of activity depends on the altered synaptic transmission within the hypothalamo–neurohypophysial complex. Numerous neurotransmitters and neuromodulators have been shown to influence the function of magnocellular hypothalamic neurons under conditions of hypovolaemia (Yokoi et al. 1996, Kadekaro et al. 1998, Ueta et al. 1998, Yamaguchi et al. 1998). In this respect, it is of interest to investigate possible effects of another brain peptide, glucagon-like peptide 1(7–36) amide (tGLP-1), postulated to be a neuromodulator of the autonomic nervous system, on the hypovolaemia-induced release of neurohypophysial hormones.

tGLP-1 was found in cell bodies localised in some brain structures including the NST as well as in nerve fibres projecting to the paraventricular nuclei (PVN) and supraoptic nuclei (SON) (Jin et al. 1988). Within nerve terminals, tGLP-1 was detected in the synaptosome fraction, thus implying its role as a neurotransmitter or neuromodulator (Kreymann et al. 1989). Moreover, tGLP-1 binding sites or mRNA for tGLP-1 receptor were found to be widely distributed in the rat brain. For example, the hypothalamic magnocellular neurons express mRNA for tGLP-1 receptor (Navarro et al. 1996, Shughrue et al. 1996, Merchenthaler et al. 1999, Zueco et al. 1999). Specific tGLP-1 binding sites were also demonstrated in the rat neurohypophysis (Göke et al.)
Materials and Methods

Animals

The experiments were carried out on male Wistar rats (250–350 g). They were kept on a 14 h light:10 h darkness cycle. Food and water were freely available.

Experimental procedure

Urethane-anaesthetised (1·2 g/kg body weight (BW), i.p. injection) rats were fitted with heparinised polyethylene cannulae (outer diameter 0·80 mm, inner diameter 0·50 mm; Critchley Electrical Ltd, Silverwater B.C., Australia) inserted in both femoral arteries and the femoral vein as well as with an i.c.v. cannula implanted in the lateral brain ventricle. One arterial cannula was connected to a BP-1 blood pressure monitor via a BLPR transducer (World Precision Instruments, Berlin, Germany) to monitor continuously the blood pressure.

Each experiment started after an equilibration period, when blood pressure was found to be stable. Then the animals under sustained urethane anaesthesia were subjected to the experimental procedure which resulted finally in non-hypotensive or hypotensive hypovolaemia (see below).

Series 1: effects of tGLP-1 on the hormonal and cardiovascular response to non-hypotensive hypovolaemia

Non-hypotensive hypovolaemia was induced by polyethylene glycol (PEG) treatment. Systemically injected PEG was proved to reduce the blood volume without affecting significantly the arterial blood pressure and the plasma osmolality. Moreover, the release of both neurohypophysial hormones was shown to be enhanced markedly under PEG-induced hypovolaemia (Stricker & Verbalis 1986, Forsling et al. 1991).

At the beginning of the experiment, a 1 ml blood sample was taken from the femoral artery. This sample was immediately replaced with an equal volume of sterile isotonic saline infused into the femoral vein. Twenty minutes later, 30% (w/v) PEG (molecular mass 3350 Da; Sigma Aldrich, Poznan, Poland) dissolved in 0·15 M NaCl or saline alone (in control animals) was injected i.p. in a dose of 20 ml/kg BW. The blood pressure was recorded every 10 min over a 70 min period after PEG injection. One hour after PEG injection, 1 µg tGLP-1 (Calbiochem, Unimarket, Poznan, Poland) dissolved in 5 µl isotonic saline or the vehicle alone was infused i.c.v. over 1 min. The second blood sample was taken 10 min later. The tGLP-1 dose and the time of sampling were selected on the basis of previous experiments (Bojanowska & Stempniak 2000). The second sample was replaced with an equal volume of saline containing cells from the first sample. Further analyses were performed as described below.

Series 2: effects of tGLP-1 on the hormonal and cardiovascular response to hypotensive hypovolaemia

Hypotensive hypovolaemia was induced by double blood withdrawal. Two successive blood samples of 10 ml/kg BW each were taken from the femoral artery; the second sample was obtained 10 min after the first. Then 1 µg tGLP-1, dissolved in 5 µl isotonic saline, or vehicle alone was injected i.c.v. The third blood sample of 1 ml was taken 10 min later. This sample, however, was immediately replaced with the appropriate volume of saline containing blood cells from the previous sample. The control, i.e. non-haemorrhaged rats, were subjected to similar procedures except that blood samples were immediately replaced with the same volume of saline containing resuspended blood cells; the first sample was replaced with saline only. The mean arterial blood pressure was recorded every 5 min throughout the experiment. At the end of each experiment, further analyses were performed as described below.

Analyses

Blood samples obtained during experiments were immediately centrifuged at 3000 r.p.m. (550 g) for 3 min at 4 °C; the plasma was separated and kept at −20 °C until assayed. The blood cells were resuspended in the appropriate volume of isotonic saline and, when necessary, reinjected to the animal. Haematocrit and plasma osmolality were also determined in all blood samples. The latter analysis was performed by freezing-point depression by means of a semimicro-osmometer (Knauer GmBH, Berlin, Germany).

At the end of each experiment the position of the i.c.v. cannula was verified by injection of 5 µl 5% (w/v) Evans blue solution into the ventricular system. Simultaneously, the blood volume was determined in all animals. Each rat was injected i.v. with 50 µl 5% Evans blue solution. The animals were decapitated 10 min later. The trunk blood was collected into heparinised tubes, centrifuged, and the blood plasma used for analysis. The plasma concentrations of the dye were estimated using a SPEKOL spectrophotometer (Carl Zeiss, Jena, Germany) at 625 nm. After
decapitation, the neurohypophysis was isolated and homogenised in 0.5% (v/v) acetic acid.

**RIAs**

AVP and OXY were extracted from plasma using C18 Sep-Pak Plus cartridges (Waters Co., Milford, MA, USA). The recoveries of synthetic AVP and OXY added to plasma samples during extraction were 70%; the hormone levels were not corrected for recovery. The hormone concentrations were measured as described previously (Lewandowska et al. 1995, Bojanowska et al. 1999). OXY and AVP antisera were raised by Dr M Orlowska-Majdak (Department of Physiology, Medical University of Lodz, Poland). Cross-reactivity with AVP for OXY antibodies was 1.1% and cross-reaction with OXY for AVP antibodies was less than 1%. The intra-assay coefficients of variation for AVP and OXY assay were 4 and 5.5% respectively. In each series of experiments, samples were tested in a single assay to avoid interassay variability.

**Statistical analysis**

Results are expressed as means ± S.E.M. of six to eight animals. The effects of tGLP-1 on the plasma hormone level, mean arterial blood pressure, plasma osmolality, and haematocrit in normo- or hypovolaemic rats over the time course of the experiment were estimated using three-way ANOVA followed by the post-hoc least significant difference (LSD) test. The neurohypophysial response to tGLP-1 and PEG or haemorrhage was analysed using two-way ANOVA and an LSD test for multiple comparisons (Statistica, StatSoft, Krakow, Poland) with \( P < 0.05 \) considered to be significant.

**Results**

**Series 1: tGLP-1 and PEG-induced hypovolaemia**

The PEG treatment reduced the blood volume by 18%. No significant differences between experimental groups were found as to the plasma osmolality within a 70 min period after i.p. saline or PEG injection.

The effect of tGLP-1 on the arterial blood pressure in normo- and hypotensive rats is shown in Fig. 1. The reduction of blood volume did not affect significantly blood pressure 1 h after PEG injection. tGLP-1 increased markedly the mean arterial blood pressure in both normo- and hypovolaemic rats as compared with the '0' time in each group. The blood pressure measured in normovolaemic, tGLP-1-treated rats at the 70th min of the experiment (i.e. 10 min after i.c.v. injection) was also significantly higher than the value obtained in the normovolaemic, saline-injected group (\( P < 0.003 \)) at the same time. Similar results were obtained when the comparisons were made between PEG-treated animals injected with tGLP-1 or saline (\( P < 0.001 \)). On the other hand, blood pressure after tGLP-1 injection did not differ significantly in normo- and hypovolaemic animals.

Figure 2 shows the effects of tGLP-1 on the release of neurohypophysial hormones under normotensive
hypovolaemia. PEG-induced hypovolaemia markedly enhanced the release of AVP in saline-injected rats. tGLP-1 significantly increased the plasma AVP level in normovolaemic animals when compared with the initial values in this group as well as with the hormone concentration in saline-injected, normovolaemic controls ($P<0.001$). On the other hand, the plasma hormone concentration in hypovolaemic tGLP-1-treated rats was markedly higher than in hypovolaemic, saline-injected animals at the end of the experiment (Fig. 2A). tGLP-1 enhanced markedly ($P<0.02$) the OXY release in normovolaemic rats and increased significantly ($P<0.001$) the hormone secretion previously enhanced by the reduction of blood volume (Fig. 2B).

As shown in Fig. 3, the AVP and OXY content in the neurohypophysis was significantly decreased in PEG-treated rats when compared with normovolaemic controls. tGLP-1 did not further modify the neurohypophysial hormone storage.

**Series 2: tGLP-1 and haemorrhage-induced hypovolaemia**

The effects of blood withdrawal on plasma osmolality, haematocrit and blood volume are shown in Table 1. The double bleeding caused 22% reduction of the blood volume as compared with non-haemorrhaged controls. The plasma osmolality in both normo- and hypovolaemic rats did not change significantly during the experiment. On the other hand, ANOVA showed a significant ($P<0.01$) effect of the time course × blood volume interactions on the haematocrit index. Haematocrit measured in the last blood sample in non-haemorrhaged rats did not differ significantly from the initial value. In haemorrhaged animals, however, haematocrit decreased markedly after bleeding (as compared with the initial value) in both saline- and tGLP-1-injected rats in both groups.

The effects of tGLP-1 on the arterial blood pressure in haemorrhaged animals are shown in Fig. 4. The blood pressure in haemorrhaged rats injected i.c.v. with saline decreased significantly after bleeding whilst blood pressure was stable in the respective non-haemorrhaged controls during the experiment. When, however, tGLP-1 was injected immediately after the second blood withdrawal, blood pressure tended to rise over a 10 min period after...
On the other hand, blood pressure in non-haemorrhaged rats injected with tGLP-1 was markedly \( (P < 0.02) \) higher than the control value in saline-injected rats 10 min after the i.c.v. injection as well as than the initial value in the same group (normovolaemic, tGLP-1-treated rats).

Figure 5 shows the effects of tGLP-1 on neurohypophysial hormone release under hypotensive hypovolaemia. Both the AVP and OXY release were significantly augmented in haemorrhaged rats 10 min after the second bleeding. tGLP-1 injected after the second blood withdrawal markedly increased the plasma level of both hormones as compared with the initial values in this group and the respective values in hypovolaemic controls at the same time \( (P < 0.001) \) and \( P < 0.01 \) for AVP and OXY respectively. In normovolaemic rats, tGLP-1 also increased the plasma AVP/OXY concentrations, the respective values being, however, significantly \( (P < 0.001) \) lower than the hormone level in haemorrhaged, tGLP-1-injected rats.

The neurohypophysial levels of AVP and OXY are summarised in Table 2. The neurohypophysial AVP/OXY storage was not affected markedly by either blood volume depletion or drug treatment. In particular, the neurohypophysial hormone content in all groups did not differ significantly from the control values in non-haemorrhaged, saline-injected rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma osmolality (mOsm/kg H(_2)O)</th>
<th>Haematocrit (%)</th>
<th>Blood volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Non-haemorrhaged, saline-injected</td>
<td>287 ± 4 (7)</td>
<td>280 ± 2 (7)</td>
<td>284 ± 7 (6)</td>
</tr>
<tr>
<td>Non-haemorrhaged, tGLP-1-injected</td>
<td>289 ± 4 (6)</td>
<td>293 ± 6 (6)</td>
<td>293 ± 5 (6)</td>
</tr>
<tr>
<td>Haemorrhaged, saline-injected</td>
<td>280 ± 4 (8)</td>
<td>289 ± 4 (8)</td>
<td>285 ± 3 (8)</td>
</tr>
<tr>
<td>Haemorrhaged, tGLP-1-injected</td>
<td>281 ± 2 (8)</td>
<td>288 ± 5 (8)</td>
<td>288 ± 5 (8)</td>
</tr>
</tbody>
</table>

\( a P < 0.001, b P < 0.01 \) (as compared with the initial value in each group).

drug injection. On the other hand, blood pressure in non-haemorrhaged rats injected with tGLP-1 was markedly \( (P < 0.02) \) higher than the control value in saline-injected rats 10 min after the i.c.v. injection as well as than the initial value in the same group (normovolaemic, tGLP-1-treated rats).

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![Figure 4](https://www.endocrinology.org)
Discussion

The results of the present study indicate that tGLP-1 may affect blood pressure and neurohypophysial hormone release under pathological conditions brought about by blood volume depletion in the rat.

In order to produce hypovolaemia, two experimental procedures were employed in this study. The PEG injection reduced the blood volume without any marked changes in the arterial blood pressure. On the contrary, in the second series of experiments, two subsequent blood withdrawals resulted not only in diminished blood volume but also in decreased arterial blood pressure. The degree of the blood volume reduction was comparable in both series of experiments (i.e. ~20%).

Series 1: tGLP-1 and PEG-induced hypovolaemia

The PEG-induced hypovolaemia is rather moderate and develops gradually in time (Stricker & Verbalis 1986). It seems that, under these conditions, the compensatory mechanisms are able to maintain blood pressure at a relatively stable level in spite of the disturbed volaemic status. This could account for a similar cardiovascular response to tGLP-1 occurring in both normo- and hypovolaemic rats. On the other hand, the release of AVP and OXY was markedly increased under normotensive hypovolaemia and tGLP-1 additionally enhanced the secretion of both hormones in hypovolaemic rats. On the other hand, the release of AVP and OXY was markedly increased under normotensive hypovolaemia and tGLP-1 additionally enhanced the secretion of both hormones in hypovolaemic rats. The latter is a key observation in our study since, to date, tGLP-1 has been found to modulate the secretion of neurohypophysial hormones under basal conditions only (Larsen et al. 1997, Bojanowska & Stempniak 2000). It is also noteworthy that the hormonal response to tGLP-1 in PEG-treated animals was markedly augmented when compared with the hormone secretion in tGLP-1-injected normovolaemic rats. This indicates that the stimulatory effects of hypovolaemia and tGLP-1 on the neurohypophysial hormone release are additive. Hence, these findings suggest that tGLP-1 seems to be a relatively powerful stimulus for vasopressinergic and oxytocinergic neurons under conditions of hypovolaemia.

Table 2 Neurohypophysial arginine vasopressin (AVP) and oxytocin (OXY) content (means ± s.e.m, number of animals in parentheses) in haemorrhaged (two subsequent blood withdrawals of 10 ml/kg BW each) and non-haemorrhaged rats injected i.c.v. with tGLP-1

<table>
<thead>
<tr>
<th>Group</th>
<th>AVP (ng)</th>
<th>OXY (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-haemorrhaged, saline-injected</td>
<td>1736 ± 210 (7)</td>
<td>1763 ± 108 (6)</td>
</tr>
<tr>
<td>Non-haemorrhaged, tGLP-1-injected</td>
<td>1608 ± 175 (8)</td>
<td>1684 ± 190 (6)</td>
</tr>
<tr>
<td>Haemorrhaged, saline-injected</td>
<td>1501 ± 336 (7)</td>
<td>1497 ± 188 (7)</td>
</tr>
<tr>
<td>Haemorrhaged, tGLP-1-injected</td>
<td>1537 ± 126 (8)</td>
<td>1725 ± 129 (7)</td>
</tr>
</tbody>
</table>
On the contrary, the neurohypophysial AVP/OXY content was decreased in hypovolaemic rats; tGLP-1 did not alter the hormone storage regardless of the volaemic status of the animals. Possibly, the bolus injection of tGLP-1 produced only a short-term hormone output, which was too weak to cause significant changes in the neurohypophysial AVP/OXY content. A previous report (Bojanowska & Stempniak 2000) showed that the effect of tGLP-1 on the AVP/OXY release is transient and disappears as soon as 15 min after the injection. On the other hand, the gradual blood volume reduction may lead to the noticeable depletion of neurohypophysial hormone stores as found in this study.

Series 2: tGLP-1 and haemorrhage-induced hypovolaemia

The double blood withdrawal resulted in a marked fall in the mean arterial blood pressure accompanied by an elevated AVP/OXY level in the blood plasma. In contrast, the neurohypophysial hormone content was not altered by either bleeding or the bolus tGLP-1 injection. Apparently, the release of both hormones was not enhanced enough to affect significantly the AVP/OXY stores in the neurohypophysis during a relatively short time after the blood withdrawal or the peptide injection.

In control (saline-injected) rats, haemorrhage caused an acute hypotensive response followed by a moderate rise of the arterial blood pressure after the blood withdrawal. It seems that the initial decrease in blood pressure is a result of either suppressed sympathetic or enhanced parasympathetic activity (Ullman 2000). tGLP-1 not only accelerated the compensatory response immediately after the second bleeding but also increased blood pressure to the level comparable with the initial value. Because AVP per se is known to be a powerful vasoconstrictor, the enhanced hormone secretion, additionally induced by tGLP-1, could also be considered as an important compensatory mechanism involved in the rise of blood pressure in haemorrhaged animals (Szczepan’ska-Sadowska 1996). Interestingly, blood pressure in haemorrhaged, saline-injected rats decreased markedly in spite of the enhanced vasopressor response. This suggests a primary role of tGLP-1 for regulation of blood pressure in blood-depleted rats under conditions of this experiment. An earlier report (Barragán et al. 1999) showed that the vagus nerve mediates the effect of centrally injected tGLP-1 on the arterial blood pressure. Moreover, the vagal tone is known to be altered under hypovolaemia. In the present study, however, tGLP-1 was found to produce similar, stimulatory effects as to blood pressure in normo- and hypotensive rats regardless of whether hypovolaemia was gradual or acute. Therefore, we hypothesise that tGLP-1 can influence equally both the basal and altered parasympathetic activity in terms of the control of arterial blood pressure. This effect is likely to be mediated by the NST and/or the area postrema, both of them being known not only to be involved in the control of blood pressure under haemorrhage (Badoer et al. 1992) but also to contain mRNA for tGLP-1 receptor (Merchenthaler et al. 1999). I.c.v. injected GLP-1 was also demonstrated to activate selectively neurons of these structures (Van Dijk et al. 1996).

Moreover, tGLP-1 increased the AVP/OXY release previously augmented by haemorrhage as it did under PEG-induced hypovolaemia. Since the blood volume depletion is known to enhance the electrophysiological (Poulain & Wakerley 1982), gene and secretory (Fenelon et al. 1993, Shoji et al. 1993) activities of magnocellular neurons, tGLP-1 is likely to influence each of these functions. For example, tGLP-1 was found to induce the release of some excitatory amino acid neurotransmitters in the hypothalamus (Calvo et al. 1995). Moreover, centrally injected GLP-1 was shown to influence the gene activity in discrete brain structures (Van Dijk et al. 1996), some of them (e.g. the area postrema) being known to affect the hypothalamo–neurohypophysial system. It is also noteworthy that acute hypovolaemia is a stressful stimulus that leads to enhanced adrenocorticotropic hormone secretion (Lilly et al. 2000) and interoceptive stress has been shown to activate tGLP-1 neurons in the brain (Rinaman 1999). This may suggest a possible effect of tGLP-1 in the maintenance of body homeostasis under haemorrhage. Therefore, further experiments need to be aimed at establishing the possible involvement of endogenous tGLP-1 in the regulation of blood pressure and neurohypophysial hormone secretion under hypovolaemia.

Together, the present findings indicate that tGLP-1 may play a role in the adaptive mechanisms involved in the cardiovascular and hormonal response to both normo- and hypotensive hypovolaemia. Probably, tGLP-1 exerts its action through modulation of the neural activity including the function of vasopressinergic and oxytocinergic neurons.

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References


