To date, glucagon-like peptide–1 (7-36) amide (tGLP-1) has been found to enhance the vasopressin and oxytocin secretion in vivo but not in vitro (i.e., when the isolated neurointermediate lobe of the pituitary was used for experiments). The goal of this study was to investigate whether tGLP-1 can influence the function of the hypothalamo-neurohypophysial complex in vitro. Also, the effect of a tGLP-1 agonist, exendin-4, and antagonist, exendin-(9-39), on the release of vasopressin/oxytocin from the isolated rat hypothalamo-neurohypophysial complex was tested. tGLP-1 enhanced the basal but not the potassium-stimulated release of vasopressin and oxytocin from the hypothalamo-neurohypophysial complex. On the other hand, tGLP-1 failed to affect the release of both hormones from the isolated neurointermediate lobe. The tGLP-1 agonist increased the secretion of oxytocin and vasopressin from the hypothalamo-neurohypophysial system whilst the tGLP-1 antagonist completely abolished the stimulatory effect of tGLP-1 on the secretion of both hormones. It is concluded that tGLP-1 affects the function of vasopressin- and oxytocinergic neurones through specific hypothalamic receptors.

Key words: GLP-1, vasopressin, oxytocin, exendin-4, exendin-(9-39), neurointermediate lobe, hypothalamo-neurohypophysial complex

INTRODUCTION

Glucagon-like peptide-1 (7—36) amide (tGLP-1) is a peptide derived from a precursor molecule pre-pro-glucagon which has been found in the central nervous system (1) as well as in the pancreatic A-cells and intestinal L-cells (2). As a result of posttranslational processing of the pre-pro-glucagon molecule, a number of bioactive peptides, including tGLP-1, are produced in a tissue-specific manner (2). Various cleavage products of this precursor peptide were also found in numerous brain structures (3). For example, tGLP-1 was shown to be a major product of pre-pro-glucagon posttranslational processing in the hypothalamus (4). Moreover, tGLP-1 receptors or mRNA for tGLP-1 receptor are widely distributed in the brain. It is of interest that mRNA for
tGLP-1 receptor was identified within the magnocellular vasopressin- and oxytocinergic neurones (1, 5, 6), suggesting that the peptide may be involved in the control of the vasopressin/oxytocin secretion. Specific binding sites for tGLP-1 were also found in the neurohypophysis (7, 8). It is also noteworthy that exendin-4, a specific tGLP-1 agonist (9), and tGLP-1 were shown to bind to the same brain regions (10).

A growing body of evidence has been accumulated that tGLP-1 is involved in the regulation of numerous autonomic functions including eating and drinking behaviour (11—14), cardiovascular activity (15), stress response (16, 17), secretion of some pituitary (18—20) and pancreatic (21) hormones. Moreover, exendin-4 was found to mimic effects produced by tGLP-1 on feeding behaviour (22) and cardiovascular system (23). On the other hand, exendin-(9-39), a tGLP-1 receptor antagonist (9), produced effects opposite to those of tGLP-1 on food intake (24) and suppressed the stimulatory effect of tGLP-1 on feeding behaviour as well as on the blood pressure (15, 23, 25).

To date, tGLP-1 has been found to stimulate the vasopressin release in the rat when injected intravenously (20) or intracerebroventriculaily (20, 26) whilst it failed to affect the vasopressin release from the isolated neurointermediate pituitary lobe (6, 8). These findings suggest that tGLP-1 influences the function of the neurohypophysis indirectly through some extrahypophysial receptors. However, it is difficult to conclude from the in vivo studies whether tGLP-1 can affect directly the function of magnocellular hypothalamic nuclei. Therefore, the aim of the present study was to investigate the possible action of tGLP-1 on vasopressin/oxytocin secretion from the isolated rat hypothalamo-neurohypophysial complex. To test the specificity of tGLP-1 action, the effects of a tGLP-1 receptor agonist and antagonist on the neurohypophysial function were also examined.

MATERIALS AND METHODS

Animals

Male Wistar rats (250—350 g) were used as donors of the hypothalamo-neurohypophysial system. The animals were kept in a 14: 10 light-dark cycle at 20—22°C. Food and water were given ad libitum.

Drugs

tGLP-1, exendin-4, and exendin-(9-39) were purchased from Sigma. All drugs were dissolved in distilled water to obtain the appropriate stock solutions i.e., 100 µg /455 µl for tGLP-1, 100 µg /360 µl for exendin-4, and 100 µg /90 µl for exendin-(9-39). The aliquots of stock solutions were kept at –20°C. The
final dilutions were prepared just before use. Then, 15 µl of the respective solution was added, when necessary, to a vial containing 1 ml of the Krebs-Ringer fluid (KRF). The concentrations of tGLP-1, exendin-4 and exendin-(9—39) in respective final solutions are mentioned below.

**Experimental protocol**

After decapitation the brain and the pituitary with the stalk still attached (except experiments of Series II) were carefully removed from the skull. Then a block of tissue containing the hypothalamus with intact axonal projections to the neurohypophysis was isolated according to Gregg and Sladek (27). In Series II, only the isolated neurointermediate lobe of the pituitary was used for experiments. The explant (or the pituitary) was placed immediately in a vial with 1 ml of the KRF containing (mmol/l): NaCl — 120, KCl — 5, CaCl₂ — 2.6, KH₂PO₄ — 1.2, MgSO₄ — 0.7, NaHCO₃ — 22.5, glucose — 10 as well as 1 g/l bovine serum albumin and 0.1 g/l ascorbic acid (pH = 7.4—7.6; osmolality = 285—300 mOsm/kg H₂O). The medium was continuously gassed with carbogen (95% O₂ and 5% CO₂) and maintained at 37°C. The incubation fluid was changed every 20 min. Each study started after a 60-minute equilibration period.

**Series I**

In the first series of experiments the effect of tGLP-1 on the basal and potassium-stimulated vasopressin/oxytocin release was studied. The explants were incubated successively in: (1) the normal KRF (B1); (2) the modified KRF containing the excess (40 mmol/l) of potassium chloride (S1; the osmolality was kept stable owing to the appropriate reduction of NaCl concentration); (3) the incubation fluid as (1) alone or with 1—1,000 nmol/l tGLP-1 (B2), and (4) the KRF as (2) alone or with tGLP-1 in the same concentrations (S2). Incubation in each medium proceeded for 20 min. In between the incubation period No. 2 and 3, the explants were washed in the normal medium and these samples were discarded.

**Series II**

In the second series of experiments the effect of tGLP-1 on the vasopressin/oxytocin release from the isolated neurointermediate lobe was studied. The experimental protocol was similar to that of Series I. tGLP-1 concentrations used (100 or 1,000 nmol/l) were selected on the basis of the results from Series I.
Series III

In the third series, the effect of a specific tGLP-1 agonist, exendin-4, on the basal as well as potassium-evoked vasopressin/oxytocin release was studied. The experimental protocol was similar to that described above (Series I) except that either tGLP-1 or exendin-4 at a concentration of 100 nmol/l was used as the incubation medium. The exendin-4 concentration was as same as the lowest tGLP-1 dose found to produce a significant effect on the neurohypophysial hormone output in the first series of experiments.

Series IV

In this series, the effect of a specific tGLP-1 antagonist, exendin-(9-39), on the basal and tGLP-1-stimulated vasopressin/oxytocin secretion was tested. After an equilibration period, the explants were incubated for 20 min in one of the following media: (1) the normal KRF; (2) the KRF containing 100 nmol/l tGLP-1; (3) the KRF containing 5,000 nmol/l exendin-(9-39); (4) the KRF containing both 100 nmol/l tGLP-1 and 5,000 nmol/l exendin-(9-39). The viability of all explants was tested at the end of the experiment by the incubation in the KRF enriched with 40 mmol/l KCl.

RIA

The vasopressin and oxytocin concentrations in the medium samples were measured by radioimmunoassay (28). Anti-oxytocin and anti-vasopressin antibodies were raised by Dr. Monika Orłowska-Majdak (Department of Physiology, Medical University of Lodz). Cross reactivity with arginine vasopressin (AVP) for oxytocin antibodies was 1.1% and cross-reaction with oxytocin for AVP antibodies was less than 1%.

Statistical analysis

Statistical significances were estimated by one-way ANOVA followed by the least significant difference (LSD) test (Statistica, StatSoft, Poland). All the data are expressed as means ± S.E.M. and p values less than 0.05 were considered as significant.

RESULTS

The potassium-stimulated vasopressin and oxytocin release from the hypothalano-neurohypophysial complex (as calculated from all experiments of the first, second and third series; period S1) was markedly higher than the
basal secretion of both hormones (period B1). The hormonal response of the hypothalamo-neurohypophysial complex to the excess of potassium in the fourth series of experiments was comparable with the potassium-stimulated vasopressin/oxytocin output found in other series (Table I), thus suggesting that the incubation procedure did not disturb the viability of the explants.

The effect of tGLP-1 on the neurohypophysial hormone secretion in Series I, II, and III was estimated using B2/B1 (basal release) and S2/S1 (stimulated release) ratios for each explant.

Table 1. Basal (period B1) and K⁺-stimulated (period S1) release of neurohypophysial hormones from the isolated rat hypothalamo-neurohypophysial complex (Series I and III) or the separated neurointermediate lobe (Series II) as well as final, potassium-evoked secretion of these hormones in Series IV (pg per tube; mean ± SEM; number of animals in parentheses).

<table>
<thead>
<tr>
<th>Series</th>
<th>Vasopressin</th>
<th>Oxytocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal release (series I)</td>
<td>5.4 ± 0.5 (n = 61)</td>
<td>2.5 ± 0.2 (n = 63)</td>
</tr>
<tr>
<td>Stimulated release (series I)</td>
<td>26.6 ± 2.3 (n = 61)</td>
<td>16.8 ± 1.8 (n = 63)</td>
</tr>
<tr>
<td>Basal release (series II)</td>
<td>5.8 ± 1.1 (n = 22)</td>
<td>2.7 ± 0.4 (n = 23)</td>
</tr>
<tr>
<td>Stimulated release (series II)</td>
<td>21.1 ± 2.9 (n = 22)</td>
<td>21.1 ± 3.8 (n = 23)</td>
</tr>
<tr>
<td>Basal release (series III)</td>
<td>9.7 ± 1.8 (n = 32)</td>
<td>3.7 ± 1.1 (n = 27)</td>
</tr>
<tr>
<td>Stimulated release (series III)</td>
<td>54.2 ± 3.6 (n = 32)</td>
<td>48.6 ± 6.1 (n = 27)</td>
</tr>
<tr>
<td>Stimulated release (series IV)</td>
<td>42.1 ± 4.4 (n = 36)</td>
<td>36.4 ± 4.9 (n = 33)</td>
</tr>
</tbody>
</table>

Series I. Effects of tGLP-1 on the release of vasopressin/oxytocin from the hypothalamo-neurohypophysial complex

ANOVA showed a significant (p < 0.001) effect of tGLP-1 on the basal vasopressin release. The further analysis demonstrated that tGLP-1 at a concentration range of 10—1,000 nmol/l markedly enhanced the vasopressin release when compared with the control group (Fig. 1). In contrast, ANOVA showed no significant dose-dependent effects of tGLP-1 on the potassium-stimulated hormone secretion.
ANOVA indicated that there is a significant (p < 0.05) tGLP-1-dependent effect on the basal oxytocin release. The post-hoc analysis showed that tGLP-1 in a concentration of 100 and 1,000 nmol/l enhanced markedly the oxytocin secretion whilst the lower tGLP-1 concentrations used failed to affect significantly the hormone release (Fig. 1). Again, ANOVA showed no significant dose-dependent effects of tGLP-1 on the potassium-stimulated oxytocin release.

**Series II. Effects of tGLP-1 on the release of vasopressin/oxytocin from the isolated neurointermediate lobe**

tGLP-1 did not affect significantly either the basal or the potassium-stimulated release of vasopressin and oxytocin from the separated neurointermediate lobe at any concentration used.
Series III. Exendin-4 and the release of neurohypophysial hormones

As indicated ANOVA, the drug treatment significantly affected the basal release of vasopressin (p < 0.02) and oxytocin (p < 0.01). Both tGLP-1 and its agonist, exendin-4, increased similarly the basal vasopressin/oxytocin secretion as compared with the respective controls (Fig. 2). On the other hand, neither of the drugs used affected significantly the K⁺-stimulated hormone output.

Series IV. Interaction of tGLP-1 – exendin-(9-39) and the release of neurohypophysial hormones

ANOVA showed a significant effect of the drug treatment on the basal release of vasopressin and oxytocin (p < 0.05 and p < 0.01, respectively).
particular, tGLP-1 increased markedly the secretion of both hormones whilst a tGLP-1 antagonist, exendin-(9-39), completely blocked this stimulatory effect. On the other hand, exendin-(9-39) alone did not affect the release of either vasopressin or oxytocin (Fig. 3).

**Fig. 3.** Effects of 100 nmol tGLP-1 and 5,000 nmol exendin-(9-39) (EXE-(9-39)) on the basal vasopressin (AVP) and oxytocin (OXY) release from the isolated rat hypothalamo-neurohypophysial complex. All the comparisons were made with respect to the control explants incubated in the normal Krebs-Ringer fluid (nKRF). Each bar represents mean SEM; figures in bars indicate the number of animals in each group. *p < 0.02; **p < 0.01.

**DISCUSSION**

The present study has demonstrated that tGLP-1 increases the basal release of vasopressin and oxytocin from the isolated hypothalamo-neurohypophysial complex but not from the separated neurointermediate lobe of the pituitary. Moreover, the stimulatory effect of tGLP-1 was found to be similar to that induced by a tGLP-1 agonist and was blocked by a tGLP-1 antagonist.
To date, tGLP-1 has been shown to stimulate the secretion of neurohypophysial hormones when injected in vivo (20, 26). By contrast, tGLP-1 administered in vitro was found to exert only a poor stimulatory effect on the oxytocin release (6) or to fail to affect the secretion of both neurohypophysial hormones (8). The latter finding is in concert with results of this study, where no effect of the peptide on neurohypophysial hormone secretion from the isolated neurointermediate lobe could be shown. tGLP-1, however, increased the release of both neurohypophysial hormones when the hypothalamo-neurohypophysial complex with intact axonal projections to the neurohypophysis was used for experiments. Therefore, it seems, that tGLP-1 acts primarily on the perikarya of hypothalamic neurones rather than on the axonal terminals in the neurohypophysis.

Indeed, tGLP-1 binding sites or mRNA for tGLP-1 receptor were identified in vasopressin- and oxytocinergic neurones in magnocellular hypothalamic nuclei (1) as well as in the human and rat neurohypophysis (7, 8). However, the functional relevance of neurohypophysial receptors has not been confirmed. Namely, tGLP-1 could not be shown to change the intracellular cAMP concentration in the isolated neurointermediate lobe of the pituitary (8). cAMP is known to be a second messenger for some peptide neuromodulators (e.g., pituitary adenylate cyclase activating polypeptide, vasoactive intestinal polypeptide) that stimulate the release of neurohypophysial hormones (29, 30). Moreover, tGLP-1-induced insulin secretion from the pancreas (31) as well as luteinizing hormone-releasing hormone (LH-RH) secretion from LH-RH-containing neurones (19) was proved to be associated with the enhanced cAMP production. The fact that tGLP-1 did not change either the cAMP level or the hormone secretion from the isolated neurointermediate lobe suggests that binding of tGLP-1 to its neurohypophysial receptors did not alter the function of the neurohypophysis. On the other hand, some recent findings (32) suggest that tGLP-1 receptors in the pancreas may be different from those present in other tissues. If this is indeed the case, a second messenger other than cAMP could be coupled with tGLP-1 receptors in the hypothalamo-neurohypophysial system. At the present stage of knowledge, however, the functional significance of neurohypophysial tGLP-1 receptors is unclear.

A similar phenomenon was reported as to angiotensin type 2 receptors which were demonstrated to be present in the posterior pituitary lobe but were not found to display angiotensin binding activity (33) nor to be involved in the control of vasopressin output from the hypothalamo-neurohypophysial complex (34).

Intracerebroventricular injection of tGLP-1 was shown to increase gene activity, as reflected by c-fos expression, in magnocellular nuclei (26). This was associated with the augmented neurohypophysial hormone release (20, 26), an event occurred also in the present study, when tGLP-1 could interact not
only with neurohypophysial but also with hypothalamic binding sites. Hence, these findings may indicate that hypothalamic tGLP-1 receptors play indeed a major role in the regulation of vasopressin/oxytocin release.

Using a tGLP-1 agonist and antagonist we also demonstrated that the effect produced by tGLP-1 on the vasopressin/oxytocin secretion was mediated by specific tGLP-1 receptors. Exendin-4, a peptide isolated from the Heloderma suspectum venom, was previously shown to bind to tGLP-1 binding sites in the brain (10) and to mimic tGLP-1 action on their functions. On the other hand, exendin-(9-39), a truncated form of exendin-4, was shown to prevent the actions of tGLP-1. Moreover, tGLP-1, exendin-4, and exendin-(9-39) were found to bind in vitro to cloned islet tGLP-1 receptor (35), tGLP-1 receptor in hypothalamic neuronal cells (19) and insulin-secreting pancreatic B cells (9). On the other hand, the peptides structurally related to tGLP-1 (pituitary adenylate cyclase activating polypeptide, vasoactive intestinal polypeptide, glucagon) could not be shown to bind to tGLP-1 receptors in hypothalamic neurones (19) thus suggesting that the binding was specific. Furthermore, in pancreatic B cells, exendin-4, like tGLP-1, was demonstrated to increase cAMP level whilst exendin-(9-30) was shown to block tGLP-1-evoked cAMP production (35). Hence, these drugs are believed to be, respectively, a tGLP-1 receptor agonist and antagonist.

A similar hormonal response to tGLP-1 and its agonist, found in this study, is consistent with observations as to the effects of both drugs on other physiological parameters, e.g., the blood pressure (23) as well as insulin secretion in vivo and in vitro (36). In these studies, tGLP-1 and exendin-4, used in the same concentrations, showed similar potency with respect to the effects investigated. Moreover, other authors reported that exendin-4 exhibits even higher potency than tGLP-1 as to the effect on food and water intake (37) or the blood glucose concentration (38). Therefore, in the present study, the exendin-4 concentration employed was as same as the lowest tGLP-1 dose previously (Series I) found to produce a significant effect on the vasopressin/oxytocin release in vitro.

On the other hand, the tGLP-1 antagonist completely abolished the response to tGLP-1 without affecting the basal vasopressin/oxytocin secretion. The ratio of exendin-(9-39) : tGLP-1 concentrations used in this study was comparable with that employed in the in vivo studies, where exendin-(9-39) was demonstrated to antagonize the effects produced by tGLP-1 (15, 25, 39). Again, the results of the present work are in concert with other reports where a similar, inhibitory effect of exendin-(9-39) on tGLP-1-induced changes in other physiological functions was found. For example, exendin-(9-39) abolished the effect of tGLP-1 on the blood pressure (15, 23), colonic motility (39) or food and water intake (11).
It is concluded that tGLP-1 stimulates the secretion of vasopressin and oxytocin from the hypothalamo-neurohypophysial system in vitro through specific hypothalamic tGLP-1 receptors.

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