Interactive effects of growth hormone and oestrogen on vascular responses in hypophysectomised female rats

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Abstract
Objective: Growth hormone (GH) and oestrogen (E2) are associated with beneficial effects on the cardiovascular system and it is therefore of great interest to study their interactive effects on haemodynamics and vascular function.

Design and Methods: Female hypophysectomised (Hx) rats were treated for seven days with GH, E2 or a combination of the hormones. Systolic blood pressure (SBP), heart rate (HR) and plasma insulin-like growth factor-I (IGF-I) were measured. Contractile properties and endothelial function were studied in isolated resistance arteries using the wire-myograph technique.

Results: Hypophysectomy, per se, caused a fall in SBP and HR, while vascular adrenergic reactivity (sensitivity to applied noradrenaline) was enhanced. Impaired acetylcholine-induced relaxation and basal release of nitric oxide, suggests endothelial dysfunction after Hx. After supplementation with GH, SBP remained low while HR increased towards the control level. GH increased plasma IGF-I, but had no effect on vascular contractility or endothelial responses. E2 replacement resulted in blunted plasma IGF-I, while the vascular adrenergic and serotonergic responses were reinforced. Endothelial function was not improved after E2 treatment. When GH and E2 were given in combination, the GH-induced increase in body weight, plasma IGF-I levels and HR were counteracted by E2. Moreover, the anticipated reinforcement of the vascular serotonergic response by E2 was reduced. Neither E2 nor GH+E2 affected SBP.

Conclusions: The results suggest that GH and E2 might have interactive effects on haemodynamic and metabolic parameters, but not on the contractility or endothelial function of resistance arteries, in Hx female rats.

European Journal of Endocrinology 146 267–274

Introduction
The cardiovascular system is controlled by a large number of different hormones. Two of these hormones, growth hormone (GH) and oestrogen (E2), are associated with beneficial effects on both central haemodynamics and peripheral vascular function. GH has been shown to improve endothelial function and vascular reactivity (1–3) and to increase heart rate (HR) and nitric oxide (NO) formation (4). E2 has been documented to prevent atherosclerosis (5, 6) and to modulate endothelial function (7–10), NO synthesis and blood flow (11, 12).

As both these hormones show beneficial effects on the cardiovascular system, it is of great interest to study their interactive effects. Interaction between GH and E2 has previously been reported from other organ systems (13, 14). E2 has been suggested to increase the sensitivity of the GH receptor and the expression of GH binding proteins e.g. in hepatocyte-, osteosarcoma- and osteoblast-like cells (15–17).

The aim of this study was to explore the combined treatment of GH and E2 and their effects on cardiovascular function, compared with single hormone therapy. We treated hypophysectomised (Hx) female rats for seven days with GH, E2 or a combination of GH and E2, measuring systolic blood pressure (SBP) and HR using the tail-cuff technique. Vascular contractile properties (adrenergic, serotonergic and potassium-induced responses) and endothelial function (NO synthesis and acetylcholine (ACh) responses) were measured using the wire-myograph technique.
Materials and methods

Animals

Female Wistar rats, 7 weeks old and weighing approximately 180 g (M&B, Ejby, Denmark), were used in this study. The protocol conformed to guidelines on the conduct of animal experiments issued by the Swedish National Board for Laboratory Animals and was approved by the Ethics Committee for Animal Experiments at Göteborg University. Six intact rats served as controls. Twenty rats underwent hypophysectomy at M&B one week prior to arrival. These rats were randomly divided into four experimental groups: hypophysectomised rats (Hx, n = 5), Hx rats treated with bovine growth hormone (GH, 1 mg/kg/day, n = 5) (18), Hx rats treated with β-oestradiol-3-benzoate (E2, 10 mg/kg/day, n = 5) and Hx rats treated with both bovine growth hormone (1 mg/kg/day) and β-oestradiol-3-benzoate (10 mg/kg/day) (GH+E2, n = 5). All Hx rats received physiological levels of glucocorticoids (cortisol phosphate, 400 μg/kg/day) (18) and thyroxine (10 μg/kg/day) (19). The rats were injected s.c. every 12 h during seven days. After seven days of treatment, plasma insulin-like growth factor I (IGF-I) and uterus weights were measured in order to establish the effects of the different hormonal treatments. The highest uterus weights were found in the E2 and GH+E2 groups, however only the E2 group reached significance compared with control (P < 0.01, data not shown). The uterus weights of the GH+E2 group were significantly increased compared with the GH group (P < 0.05, data not shown).

Experimental procedures

Body weight (BW) was measured one week after Hx (at arrival), before treatment (two weeks later) and after seven days of treatment immediately prior to killing. SBP and HR were measured before and after treatment by the tail-cuff technique (Nacro BioSystems, Houston, TX, USA).

After seven days of treatment, the rats were killed by decapitation. Segments (approx. 2 mm long) of small arteries (internal diameter 229 ± 8 μm) were taken from the mesenteric bed and mounted in a Multi Myograph 610M (Danish Myo Technology, Aarhus, Denmark) for recordings of their isometric wall tension to ACh and by repeating the NA and 5-HT contraction–response protocols after 15 min incubation with 100 μmol/l N[ω]-nitro-l-arginine (L-NNA). ACh was administered cumulatively (10^-9 – 10^-6 mol/l) on precontracted vessels (NA, maximal contraction), increasing the concentration in log steps every 2 min.

Cocaine (1 μmol/l) and propranolol (1 μmol/l) were added to the bath before NA was applied to avoid any neuronal uptake or β-adrenergic effect of NA.

Solutions and drugs

The composition (in mmol/l) of the physiological salt solution (PSS) was NaCl 119, NaHCO3 25, glucose 5.5, KCl 4.7, CaCl2 2.5, KH2PO4 1.8, MgSO4 1.17, ethylenediaminetetraacetic acid (EDTA) 0.026.

Oestrogen was dissolved in sesame oil. GH was dissolved in 1.6% glycerol, 0.02% Na azide and 0.05 mol/l phosphate buffer, pH 8.6. Thyroxine and glucocorticoids were dissolved in NaCl. Stock solutions of applied drugs were dissolved in distilled water and diluted in PSS. Solutions were equilibrated with 5% CO2 in air (pH 7.4) and bath temperature was maintained at 37°C.

The following drugs were used: acetylcholine (Sigma, St Louis, MO, USA), β-oestradiol-3-benzoate (ICN Biomedicals Inc, Aurora, Ohio, USA), cocaine (Sigma), glucocorticoids (cortisol phosphate, Solucortef; Upjohn, Puurs, Belgium), growth hormone (bovine, a gift from American Cyanamid Corp, Princeton, NJ, USA), N[ω]-nitro-l-arginine (L-NNA, Sigma,), l-thyroxine (Nycomed, Oslo, Norway), noradrenaline ([l]-arterenol, Sigma), propranolol (Sigma) and serotonin (5-hydroxytryptamine, Sigma).

Measurement of plasma IGF-I

After seven days of treatment, plasma samples were collected in all groups. Plasma IGF-I was analysed using a commercial IGF-I RIA kit (Mediagnost, Reutlingen, Germany).

Statistics

SBP, HR and BW were analysed by two-way ANOVA using Bonferroni as a post-hoc test. One-way ANOVA followed by Bonferroni as a post-hoc test was used to analyse plasma IGF-I and uterus weight/100 g BW. Concentration–response relations were analysed by non-linear regression (Graph Pad Systems, San Diego, CA, USA). The curves were fitted to the individual concentration twofold every 2 min. Cumulative concentration–response relationships of KCl (range 15 – 125 mmol/l) were obtained by applying salt solution where parts of the NaCl were replaced by equimolar amounts of KCl, thus increasing the concentration by 1.5 every 2 min.

Endothelial function was evaluated by the response to ACh and by repeating the NA and 5-HT contraction–response protocols after 15 min incubation with 100 μmol/l N[ω]-nitro-l-arginine (L-NNA). ACh was administered cumulatively (10^-9 – 10^-6 mol/l) on precontracted vessels (NA, maximal contraction), increasing the concentration in log steps every 2 min.

Cocaine (1 μmol/l) and propranolol (1 μmol/l) were added to the bath before NA was applied to avoid any neuronal uptake or β-adrenergic effect of NA.
concentration–response data based on the relationship
\[ E = E_{\text{max}} A^p \left( \frac{A}{E_{\text{CP}50}} + 1 \right)^{-1}, \]
where \( E \) is the response obtained for a given concentration \( A \), \( E_{\text{max}} \) the maximally attainable response, \( E_{\text{CP}50} \) the concentration required for half maximal effect, whereas exponent \( P \) represents the slope of the relationship (Hill-coefficient).

Statistical analysis was performed by one-way ANOVA followed by Neuman-Keuls multiple comparison test. Student’s t-test for paired comparisons was used when appropriate. A probability level of less than 0.05 is regarded as significant. Values are given as means±s.e.m.

Results

Body weight (Table 1)

Hypophysectomy per se induced a marked loss in BW. The BW of the Hx group did not change significantly over time, indicating a complete hypophysectomy. After seven days of hormonal treatment, there was a significant increase in BW in the GH-treated group compared with Hx rats \((P < 0.05)\), while E2 treatment alone demonstrated a significant reduction in BW compared with Hx rats \((P < 0.05)\). No weight increase was observed in rats treated with both GH and E2.

Plasma IGF-I

Plasma IGF-I was measured in all groups after seven days of treatment. The Hx group demonstrated a statistically significant decrease in total plasma IGF-I to approx. 18% of the control levels \((125±10 \text{ ng/ml vs controls } 705±61, \ P < 0.05)\). After supplementation with GH, the plasma IGF-I content increased in Hx rats to 91% of the control levels \((634±53 \text{ ng/ml, } P < 0.05 \text{ vs Hx})\). In the group treated with E2 alone, a non-significant reduction of IGF-I compared with the Hx group was noted \((58±14 \text{ ng/ml})\). However, when GH and E2 were given in combination, the plasma level of IGF-I was increased compared with both the Hx (2.5 times) and E2 (5.5 times) groups while it was significantly reduced (0.5 times) compared with the GH group \((\text{GH+E2 } 318±46 \text{ ng/ml, } P < 0.05 \text{ vs all treated groups})\).

Systolic blood pressure and heart rate (Table 1)

SBP and HR were measured prior to treatment and after seven days of treatment. Both parameters were decreased in Hx rats compared with controls \((P < 0.05)\). The SBP was not significantly changed after treatment with GH, E2 or GH+E2. Treatment with GH normalised HR, while GH in combination with E2 had no significant influence on HR, suggesting that E2 influences the chronotrophic effect of GH.
Noradrenaline response (Fig. 1, Table 2)

Hypophysectomy per se caused a marked increase in vascular adrenergic sensitivity (EC₅₀) compared with control rats, demonstrated by a leftward shift of the concentration–response relationship to applied NA. Neither GH, nor the combination GH+E₂ significantly influenced the adrenergic response of the Hx rats. E₂ treatment alone seemed to further increase the sensitivity to NA. The maximal vascular contraction (Eₘₐₓ) was not significantly affected in any of the treated groups.

Serotonin (5-HT) response (Fig. 2, Table 2)

Hypophysectomy per se did not significantly influence the vascular serotonergic sensitivity (EC₅₀) or maximal contraction (Eₘₐₓ), although the dose–response relationship to applied 5-HT was shifted to the left. Treatment with GH or GH+E₂ had no further effect on the vasoconstrictor action of 5-HT. After E₂ treatment alone, however, there was a significant increase in serotonergic response (EC₅₀) compared with controls, suggesting influence of oestrogen on the serotonergic vascular reactivity.

Potassium response (Fig. 3)

The vascular contractile responses to unspecific depolarisation, induced by applying increasing concentrations of potassium salt, did not differ significantly between the groups.

Endothelial response (Table 2, Fig. 4)

NO release from the endothelium was tested by performing NA and 5-HT dose–response relationships after incubation with the NO-synthase inhibitor L-NNA. The response curves of the control arteries were significantly shifted to the left during L-NNA exposure (Table 2), indicating a basal NO release during contraction. However, the contractile response was not significantly affected by L-NNA after hypophysectomy, suggesting an attenuated basal release of NO. None of the treatments, GH, E₂ or GH+E₂, restored NO production during vasoconstriction.

Table 2 Effects of treatment on maximal response (Max) and sensitivity (Log EC₅₀) to noradrenaline (NA) and serotonin (5-HT). The NO-synthase inhibitor, L-NNA, was used to study any release of NO from the endothelium. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>NA Max (mN)</th>
<th>NA Log EC₅₀ (mol/l)</th>
<th>NA+L-NNA Max (mN)</th>
<th>NA+L-NNA Log EC₅₀ (mol/l)</th>
<th>5-HT Max (mN)</th>
<th>5-HT Log EC₅₀ (mol/l)</th>
<th>5-HT+L-NNA Max (mN)</th>
<th>5-HT+L-NNA Log EC₅₀ (mol/l)</th>
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<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>3.77±0.25</td>
<td>-5.89±0.07</td>
<td>-6.15±0.11†</td>
<td>-6.14±0.03</td>
<td>3.29±0.29</td>
<td>-6.14±0.03</td>
<td>-6.77±0.04†††</td>
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</tr>
<tr>
<td>Hx</td>
<td>5</td>
<td>3.45±0.28</td>
<td>-6.23±0.09†</td>
<td>-6.48±0.28</td>
<td>-6.49±0.10†</td>
<td>2.96±0.15</td>
<td>-6.49±0.10†</td>
<td>-6.57±0.07</td>
<td></td>
</tr>
<tr>
<td>Hx+GH</td>
<td>5</td>
<td>3.25±0.34</td>
<td>-6.18±0.10†</td>
<td>-6.23±0.11†</td>
<td>-6.23±0.13†</td>
<td>2.76±0.37</td>
<td>-6.23±0.13†</td>
<td>-6.30±0.07†</td>
<td></td>
</tr>
<tr>
<td>Hx+E₂</td>
<td>5</td>
<td>3.24±0.32</td>
<td>-6.48±0.09***</td>
<td>-6.53±0.20</td>
<td>-6.58±0.08*</td>
<td>2.81±0.44</td>
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<tr>
<td>Hx+GH+E₂</td>
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<td>2.29±0.25</td>
<td>-6.45±0.14</td>
<td>-6.44±0.10</td>
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</table>

*P < 0.05 and ***P < 0.001 for comparison between control rats and hormone-treated groups (one-way ANOVA for multiple comparisons); †P < 0.05 and †††P < 0.001 for comparison between non-incubated and L-NNA-incubated vessels in the same treatment group (Student’s t-test for paired comparisons).
ACh added to the bath resulted in a dose-dependent relaxation of precontracted arteries, which was reduced after hypophysectomy (Fig. 4). ACh (0.1 μmol/l) almost fully relaxed the control arteries (90%), while it only reduced the maximal contraction by 50% in Hx arteries. The area under the curves (AUC) of the Hx arteries was significantly different from that of controls ($P < 0.01$), assessing impaired endothelial function after hypophysectomy. The response to ACh was not improved after treatment with GH, E2 or the combination of GH+E2 (AUC, $P < 0.05$ vs control for all groups).

**Discussion**

The present study demonstrates that removal of the pituitary gland in female rats results in decreased central haemodynamics (e.g. SBP and HR) and BW together with increased peripheral vascular reactivity and impaired NO release, despite replacement with thyroxine and glucocorticoids. After supplementation with GH, SBP was unaffected, while HR increased to the level of the control rats. Furthermore, GH replacement increased plasma IGF-I levels and BW, but did not affect either the Hx-induced increase in adrenergic sensitivity or the impaired endothelial function in resistance arteries. Treatment with E2 to Hx rats resulted in blunted plasma IGF-I response and reinforcement of the adrenergic and serotonergic vascular responses, while it had no effect on endothelial function. When GH and E2 were given in combination, the expected GH-induced increases in BW, plasma IGF-I and HR were counteracted by E2. Moreover, the E2-induced slight increase in vascular 5-HT sensitivity was neutralised when E2 and GH were combined.

These findings suggest interactive effects of GH and E2 for such physiological parameters as central haemodynamics, BW and plasma IGF-I concentrations. Furthermore, in small mesenteric arteries the possibility...
of an interaction between E₂ and GH was implied for the serotonergic tension development. No apparent interactive effect on endothelial dysfunction was seen. Further studies on other vascular beds are needed to fully elucidate the vascular interaction between GH and sex hormones.

Impaired cardiovascular response after Hx is a well-known phenomenon in both the experimental situation and in patients with pituitary hormone deficiency. The lack of glucocorticoids, thyroxine, GH and/or vasopressin has been put forward to explain these haemodynamic effects. In the present study, hypophysectomy of female rats resulted in decreased SBP and HR. Since all rats were supplemented with thyroxine and glucocorticoids, these hormones are probably not sufficient for maintaining haemodynamic functions. Furthermore, seven days of supplementation with GH, E₂, GH+E₂ had no increased effect on SBP, while GH alone normalised HR. The latter is in accordance with clinical studies on patients with GH-deficiency treated with GH for six months and up to one year, where an increase in HR has been observed (4). Interestingly, when GH was combined with E₂, the increase in HR, which was shown after GH treatment alone, did not appear. This may suggest that GH and E₂ have interactive effects on central haemodynamic functions.

In the present study, plasma IGF-I was measured in all rats. The plasma IGF-I was substantially decreased in Hx rats, indicating complete hypophysectomy. After supplementation with GH, the plasma level of IGF-I increased to a similar range as in control rats, demonstrating a complete supplementation of GH in these rats. Treatment with E₂ resulted in reduction of BW together with a non-significant decrease in plasma IGF-I, compared with Hx rats. Furthermore, the expected GH-induced increase in IGF-I was reduced when GH and E₂ were given in combination, and no increase in BW was observed. It is well known that oestrogen can increase metabolic rate, which may explain the reduced BW seen in both E₂ and GH+E₂ rats. The effect on IGF-I could also be involved in the reduced BW. A limitation of the present study is the high and probably supra-physiological dose of oestrogen. However, several studies have shown that pharmacological levels of oestrogens are needed to obtain significant haemodynamic effects (21).

Hypophysectomy per se causes increased sensitivity to NA in mesenteric resistance arteries. A decrease in the release of catecholamines and in adrenal catecholamine-synthesising enzymes (22, 23) as well as increased vascular NA sensitivity (24, 25), have been described after Hx. An upregulation of vascular adrenoceptors after Hx may be responsible for the increase in vascular adrenergic reactivity seen in the current study (26). Growth hormone replacement did not modify the adrenergic response in mesenteric small arteries. Other studies, from two different vascular beds in Hx male rats, have demonstrated a decrease in NA sensitivity after GH supplementation (24, 27). However, those results might reflect β-adrenergic effects of GH, since in the present study, propranolol was present during all NA experiments avoiding any influence of E₂ on β-adrenergic relaxation (28). Oestrogen treatment has been shown to increase both the α-adrenergic receptor affinity (29) and the α-1 adrenoceptor population (30) in different vascular beds. In the present study, the vascular sensitivity to NA was reinforced by E₂ supplementation, especially in the lower agonist concentration range, supporting the above hypothesis of an increase in α-adrenergic receptor affinity and/or population.

Abnormalities in the serotonergic system may play an important role in the pathophysiology of various cardiovascular diseases (31). Our model of Hx female rats showed no significant changes in serotonergic vascular reaction compared with intact female rats. Neither GH nor GH+E₂ further influenced the serotonergic response. Interestingly, E₂ treatment to Hx rats seemed to have similar effects on the sensitivity to
5-HT as was shown by applied NA. E2 has been shown to change vascular 5-HT reactivity in other experimental studies (32) but this has not been studied in Hx rats before. Neither Hx nor hormonal replacement demonstrated any effects on potassium-induced smooth muscle contraction compared with control. This suggests that the increased agonist-induced contractile responses after Hx and E2 treatment are specific responses, rather than unspecific increases in smooth muscle contractility.

The observed parallel shifts in the NA and 5-HT dose–response relations in arteries from control rats during L-NNA incubation, suggests a basal NO release in mesenteric resistance arteries (33). After hypophysectomy, however, the basal NO release was abolished, demonstrated by unaltered responses to NA and 5-HT during L-NNA incubation. Surprisingly, none of the treatments, GH, E2 or the combination of the two, could improve the release of NO during contraction. Lack of effect on endothelial function by E2 and GH was also the case when endothelial function was defined as the response to applied ACh. This finding is not in accordance with previous studies on endothelial-mediated effects of E2 and GH (4, 8–11). However, this is the first study on the effects of E2 supplementation in arteries from Hx female rats. Our data suggest that endothelial dysfunction in resistance arteries seen after hypophysectomy, including impaired NO synthesis, is not restored by the present hormonal replacement protocols.

In summary, GH supplementation to Hx female rats increased BW and HR, but when GH-treatment was combined with E2, both of these effects were abolished. Small peripheral arteries from Hx female rats demonstrated endothelial dysfunction together with increased adrenergic reactivity. GH treatment alone had no effect on either contractile or endothelial responses. E2 replacement alone seemed to reinforce both adrenergic and serotonergic vascular reactivity, but there was no evidence that E2 could improve the endothelial function after hypophysectomy. When GH and E2 were combined, the expected E2-induced increase in serotonergic vascular reactivity was not seen, and the combination treatment did not improve endothelial function. We can conclude that the effects of combined GH and E2 treatment to Hx female rats indicate interactive effects on central haemodynamic and metabolic parameters while there is no evidence that the combination improves peripheral vascular function.

Acknowledgements

This study was supported by grants from the Swedish Society of Medicine (98-02-0474), the Foundation of Adlerbert (the Royal Society of Arts and Sciences in Göteborg), the Swedish Hypertension Society, Swedish Medical Research Council (to LH, 12605), the Foundation of Sannäs for Cardiovascular Research (Göteborg Medical Society) and the Foundations of Wilhelm and Martina Lundgren.

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Received 1 June 2001
Accepted 31 October 2001