Effect of eprosartan on catecholamines and peripheral haemodynamics in subjects with insulin-induced hypoglycaemia

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ABSTRACT

ANG II (angiotensin II) facilitates catecholamine release from the adrenal medulla and neuronal NE (noradrenaline) release. Since animal experiments point to specific sympatho-inhibitory properties of the AT1 (ANG II type 1)-receptor blocker EPRO (eprosartan), the primary aim of this study was to clarify if EPRO inhibits sympathetic activity in humans as determined by the effect of EPRO on insulin-induced catecholamine release. Sixteen healthy male volunteers were randomized in a double-blind cross-over study to receive a single dose of EPRO (600 mg) compared with placebo, followed by insulin-induced hypoglycaemia [0.15 IU (international unit)/kg of body weight; intravenous bolus] on two study days 1 week apart. From baseline to the end of hypoglycaemia (170 min), the sympatho-adrenal reactivity was mapped by invasive continuous blood pressure monitoring and repeated measurements of FBF (forearm blood flow), arterial and venous concentrations of glucose, catecholamines [EPI (adrenaline) and NE (noradrenaline)], renin, ANG II and aldosterone. EPRO induced an 8–10-fold increase in plasma renin and ANG II concentrations compared with placebo. Plasma glucose decreased equally during placebo and EPRO from baseline 5.9 mmol/l to 1.9 mmol/l and 2.1 mmol/l respectively, inducing a 17-fold increase in arterial EPI concentration at peak. The AUC (area under the curve) during hypoglycaemia for arterial EPI concentrations was 314 ± 48 nmol·min·l⁻¹ in placebo compared with 254 ± 26 nmol·min·l⁻¹ following EPRO treatment (P = 0.14). EPRO attenuated the corresponding AUC for the EPI-induced pulse pressure response (4670 ± 219 mmHg·min in EPRO compared with 5004 ± 266 mmHg·min in placebo; P = 0.02). Moreover, EPRO caused a less pronounced increase in FBF compared with placebo (402 ± 30 compared with 479 ± 46 ml·100 g⁻¹ of body weight; P = 0.04). Musculocutaneous NE release was not affected by EPRO and the AUC for NE release was 51.69 ± 15.5 pmol·min⁻¹·100 g⁻¹ of body weight in placebo compared with 39.35 ± 18.2 pmol·min⁻¹·100 g⁻¹ of body weight after EPRO treatment (P = 0.57). In conclusion, EPRO did not significantly inhibit sympathetic reactivity compared with placebo; however, it blunted the haemodynamic responses elicited by the sympatho-adrenal stimulation which only tended to be attenuated by this drug.

INTRODUCTION

Apart from its vasodilator and antiproliferative effects, it appears that the capacity of ACE (angiotensin-converting enzyme)-inhibitors to reduce enhanced SNS (sympathetic nervous system) activity is an important feature of these drugs [1]. There is evidence that this may also be the case with AT1 [ANG II (angiotensin II)]

Key words: adrenaline, angiotensin II, haemodynamics, hypoglycaemia, insulin, receptor blocker.

Abbreviations: ACE, angiotensin-converting enzyme; ALDO, aldosterone; ANG II, angiotensin II; AT1, ANG II type 1; AUC, area under the curve; BP, blood pressure; DBP, diastolic BP; EPI, adrenaline; EPRO, eprosartan; FBF, forearm blood flow; FVR, forearm vascular resistance; IU, international unit; MAP, mean arterial pressure; NE, noradrenaline; PPR, pulse pressure response; PRC, plasma renin concentration; RAS, renin–angiotensin system; RSA, regional musculocutaneous sympathetic activity; SBP, systolic BP; SNS, sympathetic nervous system.

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type 1]-receptor blockers [2], although evidence from human studies is contradictory [3,4]. In light of the importance of enhanced sympathetic activity in determining BP (blood pressure), neurohormonal activity, cell death, remodelling and arrhythmia, it appears rational to attenuate this activity: a principle widely employed by the use of β-blockers. ANG II, through interaction with specific membrane-bound receptors (AT1, AT2 and AT3), stimulates and facilitates SNS activity at different levels. Thus ANG II facilitates the release of EPI (adrenaline) from the adrenal medulla, increases the release of NE (noradrenaline) from prejunctional sympathetic nerve endings, blocks NE uptake and enhances NE synthesis [5,6].

The sympatho-adrenal system is a functional branch of the SNS, where stimulation of the adrenal medulla induces primarily EPI and, to a minor extent, NE secretion. The best in vivo approach to stimulating this part of the SNS is insulin-induced hypoglycaemia, which produces a markedly increased EPI release and a modest increase of circulating NE. Employing this model, our group has shown in both humans [7,8] and rats [9] that ACE inhibition, but not AT1-receptor blockade with losartan, attenuates EPI release. On the contrary, losartan tends to increase circulating NE levels in rats [10]. It has been shown that NE release facilitated by ANG II is mediated by the AT1 receptor [11]. Animal studies indicate that the AT1-receptor antagonist EPRO (eprorsartan), in contrast with other AT1-receptor antagonists, possesses specific presynaptic AT1-receptor-blocking abilities [12]. The mechanism of this putative important feature of EPRO is unknown.

Episodic hypoglycaemia with subsequent sympatho-adrenal activation is frequent in patients with diabetes [13]. Diabetes and related diseases such as hypertension, ischaemic heart disease and congestive heart failure are often treated with drugs inhibiting the RAS (renin–angiotensin system). Since sympathetic and sympatho-adrenal reactivity is a major concern in these conditions, it is important to characterize further the putative sympatho-inhibitory effect of EPRO in humans. In the present study, we utilized the clinically relevant model of episodic hypoglycaemia and evaluated the effect of oral EPRO on systemic EPI and NE concentrations, regional NE release and haemodynamic responses in healthy volunteers.

METHODS

Sixteen healthy male volunteers (20–35 years of age) participated in the study. All had normal body weight (body mass index range, 19–25 kg/m²) and a normal level of physical training activity. On the evening before each study day, the subjects received 40 mg of furosemide orally to obtain a mild degree of sodium depletion, i.e. homoeostatic dependency on RAS. The subjects abstained from food, smoking, coffee and tea from midnight before the study days. On the morning of the two study days, a light identical standardized meal was served at the research facility. The protocol was approved by the County Ethical Committee, and written informed consent was obtained from all participants.

Study protocol

After arrival at 08:00 hours, a light standardized meal was served. At 08:20 hours, the subjects were placed in the supine position and stayed supine, but awake, throughout. Ambient room temperatures were between 24–27 °C. Between 08:25 and 08:50 hours, the subject was equipped on his right arm with a mercury-in-Silastic strain gauge and FBF (forearm blood flow) measurements (ECSR; DE Hokanson, Washington, U.S.A.) were determined by venous occlusion plethysmography. FBF (ml · 100 g⁻¹ of tissue · min⁻¹) was determined as an average of five measurements separated by a few seconds. Cannulas were inserted into the radial artery and the median cubital vein of the left arm. Invasively measured BP and HR (heart rate) were recorded continuously throughout the study. The arterial lumen was connected to a pressure transducer and analogue signals were A/D converted and fed into a computer at 1000 Hz using software tailored for this study and written in Labview® (National Instruments; www.ni.com). SBP and DBP (systolic and diastolic BP respectively) were recorded as the zeniths and nadirs respectively, of the pressure curve. Because of the well-known pulse pressure-increasing effect of EPI, PPR (pulse pressure response) was calculated from SBP and DBP. MAP (mean arterial pressure) was determined by integrating the pressure curve over time. At 09:30 hours, the subjects received a tablet of EPRO (600 mg) or placebo orally with a glass of tap water. The medication was administrated in a double-blind randomized placebo-controlled cross-over fashion. At 11:00 hours, insulin [0.15 IU (international unit)/kg of body weight (bolus)] was injected into the venous catheter. During the following 85 min, arterial and venous blood samples for determination of catecholamine and arterial glucose concentrations were obtained at baseline, before insulin injection and at the time points indicated in the Figures. Blood samples for determination of plasma levels of renin, ANG II and ALDO (aldosterone) were drawn before EPRO/placebo, before insulin and during hypoglycaemia (see Table 1). FVR (forearm vascular resistance) was calculated as: FVR(t) = MAP(t)/FBF(t). An estimate of RSA (regional musculoscutaneous sympathetic activity) in the forearm was calculated as the difference between outflow of NE and inflow of NE corrected for the fractional extraction. The calculation is based on the assumption of equal relative extractions of EPI and NE in the forearm. Thus EPI, which is not produced in these tissues, serves as a marker of catecholamine extraction according to the following...
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$$\text{RSA} = ([\text{NE}]_{\text{venin}} - ([\text{NE}]_{\text{artery}} \times [\text{EPI}]_{\text{venin}}/[\text{EPI}]_{\text{artery}})) \times \text{plasma flow}$$

where plasma flow = FBF × (1 − haematocrit)

**Analytical methods**

Glucose concentration was determined immediately after plasma sampling on a model 6517 Glucose Analyser II (Beckmann Instruments, Fullerton, CA, U.S.A.). Catecholamine analyses were based on HPLC separation of radioenzymically labelled catecholamines [15,16]. PRC (plasma renin concentration) was determined by using the principle of antibody trapping [17], as modified by Millar et al. [18]. ANG II and ALDO plasma levels were measured by RIA. ANG II was measured according to Kappelgaard et al. [19], with the use of Sep-Pak C_{18} (Millipore Waters) for plasma extraction. ALDO plasma concentrations were analysed using a commercial kit (DSL-8600; Diagnostic Systems Laboratories, Webster, TX, U.S.A.). All blood volumes drawn were immediately replaced by isotonic saline.

**Statistics**

The data are presented as means ± S.E.M. Mean values for each time point were compared between the two treatments using two-sided Student $t$ tests for paired data. Summary statistics for repeated measurements were done by calculating the area under the plasma concentration versus time curve (AUC) according to the trapezoidal rule for each individual, followed by comparison of the distributions with a two-sided Student $t$ test for paired observations. RSA data were evaluated further by ANOVA for repeated measurements. Significance level was $\alpha = 0.05$.

**RESULTS**

As shown in Figure 1(A), plasma glucose declined rapidly to a nadir (placebo, 1.9 ± 0.5 mmol/l; EPRO, 2.1 ± 0.2 mmol/l) after 20 min, followed by a steady increase. This pattern was identical between the treatment groups ($P_{(AUC)} = 0.22$).

PRC and ANG II and ALDO levels are shown in Table 1. The substantial increases in PRC and ANG II level and the decline in ALDO level during EPRO treatment suggested effective blockade of juxtaglomerular and adrenal cortical AT₁ receptors.

**Arterial catecholamines**

The arterial plasma concentrations of catecholamines are shown in Figures 1(B) and 1(D). After insulin injection and subsequent hypoglycaemia, arterial EPI increased rapidly and peaked after 40 min with a 17-fold increase during placebo and a 14-fold increase during EPRO. The aggregate measure of the total EPI response AUC_{EPI,Placebo} was 19% lower than AUC_{EPI,Placebo}, but this was not statistically significant ($P = 0.15$; Table 2). NE increased quickly to peak after 40 min. The mean estimates of arterial plasma NE were higher at all time points for EPRO than placebo. The aggregate measurements of the total NE response AUC_{NE,Placebo} compared with AUC_{NE,Placebo} did not differ ($P = 0.27$).
Table 1  PRG and plasma concentrations of ANG II and ALDO during EPRO and placebo
Values are means ± S.E.M. Baseline t = −5 min. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with placebo.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PRG (mU/l)</th>
<th>ANG II (pmol/l)</th>
<th>ALDO (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPRO</td>
<td>Placebo</td>
<td>EPRO</td>
</tr>
<tr>
<td>−5</td>
<td>46.8 ± 7.4</td>
<td>50.7 ± 8.5</td>
<td>18.6 ± 2.5</td>
</tr>
<tr>
<td>85</td>
<td>185.8 ± 43.1**</td>
<td>40.4 ± 7.8</td>
<td>69.4 ± 17.1</td>
</tr>
<tr>
<td>120</td>
<td>333.0 ± 63.2**</td>
<td>30.1 ± 7.6</td>
<td>108.9 ± 17.9**</td>
</tr>
<tr>
<td>170</td>
<td>423.2 ± 66.2***</td>
<td>34.6 ± 6.0</td>
<td>157.6 ± 19.4**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>ANG II (pmol/l)</th>
<th>ALDO (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPRO</td>
<td>Placebo</td>
</tr>
<tr>
<td>−5</td>
<td>18.6 ± 2.5</td>
<td>223.2 ± 41.7</td>
</tr>
<tr>
<td>85</td>
<td>69.4 ± 18.4*</td>
<td>146.5 ± 39.9</td>
</tr>
<tr>
<td>120</td>
<td>108.9 ± 17.9**</td>
<td>128.1 ± 28.6</td>
</tr>
<tr>
<td>170</td>
<td>157.6 ± 19.4**</td>
<td>289.2 ± 30.1*</td>
</tr>
</tbody>
</table>

Table 2  AUCs for arterial and venous catecholamines, forearm NE release, HR, MAP, PPR, FBF and FVR after placebo and EPRO during insulin-induced hypoglycaemia
Values are means ± S.E.M. P value is compared with placebo.

<table>
<thead>
<tr>
<th>AUC</th>
<th>Placebo</th>
<th>EPRO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial catecholamines (nmol · min · l⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>314 ± 48</td>
<td>254 ± 26</td>
<td>0.147</td>
</tr>
<tr>
<td>NE</td>
<td>143 ± 8.6</td>
<td>161 ± 12</td>
<td>0.278</td>
</tr>
<tr>
<td>Venous catecholamines (nmol · min · l⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>252 ± 36</td>
<td>212 ± 25</td>
<td>0.165</td>
</tr>
<tr>
<td>NE</td>
<td>135 ± 6.8</td>
<td>148 ± 11</td>
<td>0.378</td>
</tr>
<tr>
<td>Forearm NE release (pmol · min⁻¹ · 100 g⁻¹ of body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>51.7 ± 16</td>
<td>39.4 ± 18</td>
<td>0.571</td>
</tr>
<tr>
<td>NE</td>
<td>5854 ± 219</td>
<td>5877 ± 202</td>
<td>0.763</td>
</tr>
<tr>
<td>PPR (mmHg · min⁻¹)</td>
<td>5835 ± 174</td>
<td>5487 ± 168</td>
<td>0.118</td>
</tr>
<tr>
<td>MAP (mmHg · min⁻¹)</td>
<td>5004 ± 266</td>
<td>4670 ± 219</td>
<td>0.016</td>
</tr>
<tr>
<td>FBF (ml · min⁻¹ · 100 g⁻¹ of body weight)</td>
<td>479 ± 46</td>
<td>402 ± 30</td>
<td>0.043</td>
</tr>
<tr>
<td>FVR (mmHg · 100 g⁻¹ of body weight · min⁻² · ml⁻¹)</td>
<td>1364 ± 161</td>
<td>1354 ± 122</td>
<td>0.940</td>
</tr>
</tbody>
</table>

Venous catecholamines
The venous plasma concentrations of catecholamines are shown in Figures 1(C) and 1(E). EPI and NE increased rapidly from 110 min and peaked at 120–130 min. AUCs (Table 2) were not statistically different (EPI, P = 0.17; NE, P = 0.38).

Regional forearm NE spillover
RSA (Figure 1F and Table 2) revealed no overall difference between EPRO and placebo [P(AUC) = 0.57]. Since the NE spillover rate during hypoglycaemia from a visual impression appeared transiently blunted by EPRO, we compared NE spillover during EPRO and placebo in a one-way ANOVA for repeated measurements. Evidently, the overall change over time was highly significant (P = 0.007); however, there was no significant interaction between drugs over time (P = 0.45).

Haemodynamics
The time course of PPR, MAP and HR are shown in Figure 2 and Table 2. SBP tended to be lowered by EPRO compared with placebo [P(AUC) = 0.052], but DBP (results not shown) and MAP were not affected by EPRO compared with placebo [P(AUC) = 0.21 and P(AUC) = 0.12 respectively]. PPRs were attenuated significantly (7%) with EPRO compared with placebo [P(AUC) = 0.02].

DISCUSSION
The key findings of the present study are two-fold. First, AT1-receptor blockade with a single dose of EPRO did not significantly attenuate the EPI response to insulin-induced hypoglycaemia. Furthermore, the surrogate of
Figure 2  Time course of haemodynamic parameters during placebo and EPRO treatment before and after insulin-induced hypoglycaemia

Values are means ± S.E.M. * P < 0.05 compared with placebo. Forearm Vasc. Resist, FVR.

RSA, i.e. forearm NE spillover, was not affected by AT₁-receptor blockade. Secondly, however, FBF as well as PPR to the hypoglycaemia-induced EPI release were significantly blunted (16% and 7% respectively) by EPRO. This indicates that, although the present data show no significant effect on the EPI response in terms of arterial EPI concentrations, the observed tendency to blunt the response is compatible with the significant functional haemodynamic effects observed during EPRO treatment.

This interpretation is supported by the typical adrenergic pattern of the observed findings of FBF and PPR. As shown in Figure 2 after administration of EPRO, but before insulin, FVR decreased and FBF increased significantly after EPRO only, as expected from the vasodilatory effect of AT₁-receptor blockade. However, during the EPI surge from 100 min, the FBF (EPRO) curve crossed over the corresponding placebo curve and stayed lowered throughout. Noting that EPI is a highly potent agonist of the vasodilatory β₂ receptors, the above findings might be explained by an apparently blunted EPI stimulation of the forearm vasculature during EPRO compared with placebo. The same mechanism might pertain to the observed inhibition of PPR during EPRO treatment. Another, more simple, explanation of the decreased FBF during EPRO, however, might be that, although EPRO tends to decrease the resistance to blood flow in the forearm, the systemic BP decreases even more over time and thereby decreases the peripheral blood flow. However, since the driving BP (MAP) was not significantly decreased by EPRO, our data do not substantiate that hypothesis.

To our knowledge this is the first study investigating the effect of EPRO on sympatho-adrenal reactivity in healthy humans. In a comparable study employing insulin-induced hypoglycaemia, we found that AT₁-receptor blockade with losartan did not affect the EPI response or FBF [7], or PPR (R. Wörck, H. Ibsen, E. Frandsen and H. Dige-Petersen, unpublished work). Since the present findings point to a functionally relevant impairment in the EPI response to hypoglycaemia, pharmacodynamic differences between AT₁-receptor blockers might exist at least in the current experimental setting. The present study, however, evidently does not elucidate the physiological mechanisms behind our observations. Hypotheses have been put forward discussing subgroups of AT₁ receptors with different binding characteristics and that the putative sympatho-inhibitory effects of EPRO may be linked to the unique non-biphenyl structure of EPRO [20–22], which might confer important differences in receptor affinity and selectivity [12]. Different relative access/affinities to AT₂ and AT₁ receptors on adrenal medullary cells [23,24] might account for putative differences between AT₁-receptor blockers. The interaction between adrenergic and angiotensinergic signal transduction was complicated further by recent in vitro and in vivo findings by Barki-Harrington et al. [25]. They demonstrated that the G-protein-coupled AT₁ receptors and β-adrenoceptors interact at the downstream (intracellular) level, such that pharmacological blockade of either receptor induces functional uncoupling of the reciprocal receptor, so
called ‘trans-inhibition’. In the current context, this might explain why the EPRO-induced (non-significant) reduction in EPI release from the adrenal medulla in spite of unchanged peripheral sympathetic activity, through EPRO-induced trans-inhibition of adrenergic β2-mediated vasodilatation, translates into significant reductions of FBF and PPR after all.

With respect to forearm sympato-inhibition, our finding in vivo that EPRO does not inhibit peripheral NE release agrees to some extent with recent results from Heusser et al. [4], who found no inhibitory effects of EPRO on baseline or stress-induced hormonal and haemodynamic parameters in young males. Several studies in vitro and in the pithed rat model have pointed to a specific sympato-inhibitory potential of EPRO compared with other AT1-receptor blockers [3,26]. However, this could not be supported by more recent findings by Dendorfer et al. [27].

The present study design has some methodological reservations. The current model is optimized for mapping EPI release and haemodynamics; however, it should be pointed out that the model is not ideal for detailed study of RSA. First, the hypoglycaemic stimulus primarily targets the adrenal medulla and thus EPI release and, to a lesser extent, NE release. Secondly, the calculated estimate of NE spillover is probably inferior to the calculated ‘NE appearance rate’ during changes of FBF [28]. Thirdly, the assumption of equal relative extractions of EPI and NE in the forearm may be hampered by differently and oppositely directed neuronal and extra-neuronal uptake (EPI compared with NE) kinetics [29]. Furthermore, EPI may recirculate after neuronal uptake in the forearm and thus contribute to an underestimation of regional NE release. Thus the gold standard requires tritiated NE as the exogenous marker. The vasodilatory effect of insulin is well known in hyperinsulinaemic clamp studies [30]. The present study, however, showed no signs of insulin-mediated vasodilatation. Furosemide was administered to prevent instances of suppressed PRC. Baseline PRC and ANG II, EPI and NE levels on the study day were in the normal range and thus not activated by furosemide priming.

**Conclusions**

In the acute single-dose regimen, the AT1-receptor antagonist EPRO did not significantly attenuate sympathetic reactivity as determined by the EPI response during transient insulin-induced hypoglycaemia in young healthy males. However, EPRO inhibited haemodynamic responses, an aggregate functional measure of whole body reactivity to hypoglycaemia. This contrasts with earlier findings with the AT1-receptor antagonist losartan, which had no haemodynamic effects. EPRO showed no inhibitory effects on whole body or regional NE release in this model.

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**REFERENCES**


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