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SPECIAL REPORT

Urotensin II evokes potent vasoconstriction in humans *in vivo*

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The peptide urotensin II (U II) evokes potent vasoconstriction in non-human primates. In human blood vessels studied *in vitro* variable effects of U II are reported; vasoconstriction, vasodilatation or no response. It is therefore of importance to determine the vascular effect of U II in humans *in vivo*. U II (0.1–300 pmol min^{-1}) was infused into the brachial artery of nine healthy volunteers. Changes in forearm blood flow (FBF) were determined by venous occlusion plethysmography. U II induced dose-dependent reduction in FBF. A threshold response was obtained by 1 pmol min^{-1}, and the highest dose of U II (300 pmol min^{-1}) reduced FBF by $31 \pm 4\%$ ($P < 0.01$). FBF returned to baseline values within 30 min. This study demonstrates that U II produces potent vasoconstriction in humans *in vivo*.

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Abbreviations: FBF, forearm blood flow; U II, urotensin II

Introduction Urotensin II (U II) is a cyclic peptide that was originally isolated from fish spinal cord (Pearson *et al*., 1980). Recently, the human form of U II (Coulouarn *et al*., 1998) and its receptor (Ames *et al*., 1999) were cloned. The U II precursor gene is expressed in the spinal cord and peripheral tissues (Coulouarn *et al*., 1998), and U II-like immunoreactivity has been demonstrated in both cardiac and vascular tissue (Ames *et al*., 1999). The receptor for U II is widely distributed in human tissue including human coronary arteries and skeletal muscle (Maguire *et al*., 2000).

Human U II was originally described to be the most potent mammalian vasoconstrictor identified so far (Ames *et al*., 1999). Intravenous administration of U II markedly increased total peripheral resistance in anaesthetized non-human primates. However, subsequent studies have indicated that both species and regional variations exist regarding the vascular actions of U II. In the rat U II evoked dose-dependent mesenteric and hindquarter vasodilatation (Gardiner *et al*., 2001). Furthermore, U II caused relaxation of rat isolated coronary arteries which was abolished by an inhibitor of nitric oxide synthase or endothelium removal (Bottrill *et al*., 2000). In human blood vessels studied *in vitro*, the effect has been variable. U II was 50 times more potent as a constrictor than endothelin-1 in coronary and mammary arteries, although 30% of the vessels did not respond to U II (Maguire *et al*., 2000). Stirrat *et al*. (2001) reported that U II evoked potent dilator effects in human pulmonary and abdominal resistance arteries. In a study on several different small isolated human arteries and veins, no constrictor or dilator effects of U II could be demonstrated, and therefore the importance of this peptide in cardiovascular regulation was questioned (Hillier *et al*., 2001).

There are no observations regarding the vascular effects of U II in humans under *in vivo* conditions, however. Based on the marked vasoconstrictor effects of U II previously described in non-human primates (Ames *et al*., 1999), it is of great importance to establish the vascular effects of U II in humans *in vivo*. In the present study, we therefore evaluated the effects of local administration of U II on forearm blood flow (FBF) in healthy subjects.

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Methods *Subjects* The study was performed on nine healthy, non-smoking males with a mean age of 24 ± 1 years. All subjects were normotensive (mean arterial pressure 89 ± 2 mmHg) and of normal height and weight (body mass index 22 ± 1). Informed consent was obtained from all subjects. The investigation was approved by the human ethics committee of the Karolinska Hospital.

Forearm blood flow studies The investigations were performed with the subjects in the supine position in a quiet laboratory with controlled temperature. The subjects were allowed a light breakfast without caffeine-containing drinks or alcohol on the day of the study. Heart rate was followed continuously from an ECG. A percutaneous catheter was inserted under local anaesthesia in the proximal direction into the brachial artery of the non-dominant arm for infusions. FBF was measured simultaneously in both arms by venous occlusion plethysmography (Pernow *et al*., 1991) using a mercury-in-silastic strain gauge around the widest part of the forearm. A cuff placed around each upper arm was inflated to 50 mmHg to induce venous occlusion for 10 s every 15 s during recordings of FBF. The circulation of the hands was occluded by inflating a wrist cuff to 30 mmHg above systolic blood pressure during the blood flow registrations. NaCl (0.9%) was infused into the brachial artery at a rate of 0.5 ml min^{-1} throughout the study. Basal FBF was determined during an additional infusion of saline for 2 min at a rate of 1 ml min^{-1}. A dose-response challenge to U II was then performed by infusing increasing doses (0.1, 1, 10, 100 and 300 pmol min^{-1}). Each dose was infused for 15 min at a rate of 1 ml min^{-1}.

Drugs U II (Clinalfa AG, Läufelfingen, Switzerland) was dissolved in 0.9% NaCl and thereafter stored frozen at $-80^{\circ}C$. On the day of the experiments U II was diluted to the proper concentrations in sterile 0.9% NaCl.

Calculations FBF was calculated as the mean of eight inflow recordings during 2 min. The ratio of flows in the infusion and non-infusion arms at each time point is expressed as percentage change from baseline. Data are also expressed as absolute flow in the infusion arm. All data are given as mean values and standard error of the mean (s.e.mean). Statistical differences were calculated using ANOVA followed by Dunnett's test. A $P<0.05$ was regarded as significant.

Results Administration of U II resulted in a dose-dependent significant reduction in FBF both when expressed in absolute values and per cent change (Figure 1). A threshold effect was achieved by 1 pmol min^{-1}. The maximum reduction in FBF obtained by 300 pmol min^{-1} was $31\pm4\%$ (Figure 1A). FBF had returned to pre-infusion levels 30 min after cessation of the highest dose (23 ± 4 ml min^{-1} 1000 ml^{-1} at 30 min after the infusion vs 24 ± 2 ml min^{-1} 1000 ml^{-1} before the infusions). There was no change in heart rate or FBF in the contralateral control arm during the infusions (19 ± 2 ml min^{-1} 1000 ml^{-1} pre-infusion vs 20 ± 3 ml min^{-1} 1000 ml^{-1} at 300 pmol min^{-1}).

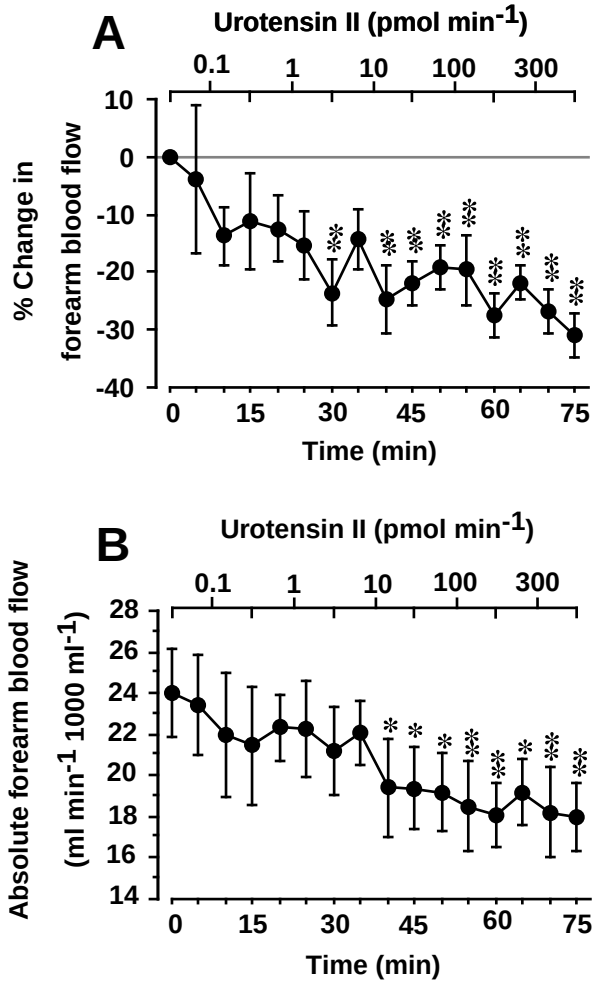

Figure 1 Effect of urotensin II on forearm blood flow. Values are expressed (A) as per cent change in flow and (B) in absolute blood flow. Data are given as mean values and s.e.means ($n=9$). Significant differences from pre-infusion values (Time 0) are shown; $^{*}P<0.05$, $^{**}P<0.01$.

Discussion The present study demonstrates that U II mediates potent vasoconstriction in the forearm of humans. This is in line with the findings of Ames *et al*. (1999) in the cynomolgus monkey, in which U II was a powerful vasoconstrictor. Previous studies of isolated human blood vessels have revealed variable results. The present results are in agreement with the potent vasoconstrictor response to U II in certain arterial preparations *in vitro* reported by Maguire *et al*. (2000). However, the results of the present study are in contrast to the findings of Hillier *et al*. (2001) who described that U II exerts no vasoconstrictor effect in several different isolated human arteries and veins. The inconsistent effects of U II observed under *in vitro* conditions emphasizes the importance of evaluating its vascular effects in the *in vivo* situation.

The reduction in FBF was dose-dependent with a threshold effect at 1 pmol min^{-1}. This demonstrates a high potency of U II as a vasoconstrictor *in vivo*. For comparison, endothelin-1, reduces FBF at a dose of 3–10 pmol min^{-1} under similar experimental conditions (Pernow *et al*., 1991). However, the magnitude of the constrictor response obtained with U II was smaller than that previously obtained with endothelin-1 which reduces FBF by approximately 60% at a similar dose range (Pernow *et al*., 1991). These findings are in agreement with those in isolated human arteries (Maguire *et al*., 2000). It is of course possible that higher doses of U II would result in larger response, but due to safety reasons higher doses which may cause systemic effects were not given. The doses given produced only local effects in the infused forearm since there were no changes in heart rate or blood flow in the contralateral control arm, demonstrating that no systemic effects were obtained. Furthermore, the unchanged blood flow in the control arm demonstrates the stability of the experimental model and that the reduction in FBF in the experimental arm was mediated by the U II infusion.

In conclusion, this study is the first to demonstrate a potent vasoconstrictor effect of U II in humans *in vivo*. This finding indicates that U II may be of importance for regulation of vascular tone in humans.

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