Vasoconstricting Effect of Angiotensin II in Human Hand Veins: Influence of Aging, Diabetes Mellitus and Hypertension

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We examined human hand veins to determine whether vasoconstricting response to angiotensin II (Ang II) and noradrenaline (NA) was influenced by aging or such diseases as diabetes mellitus (DM) and hypertension (HT). Twenty healthy male subjects (20–73 years), and 8 male patients with non-insulin-dependent DM and 8 male patients with essential HT were included in this study. A constant dose (50 ng/min) of Ang II or increasing dose (2–256 ng/min) of NA was infused into the dorsal hand vein and its diameter was measured using a linear variable differential transformer. The constant infusion of Ang II caused rapid desensitization or tachyphylaxis. The vasoconstriction by Ang II in the 8 elderly subjects (58 to 73 years) was significantly (p < 0.05) larger than that in the 8 young subjects (20 to 36 years) from 6 to 18 min after the start of the infusion (after 6 min: 63.6 ± 11.6 (mean ± SD)% vs. 39.9 ± 20.8%, 12 min: 34.0 ± 11.9% vs. 12.0 ± 12.0%). However, the vasoconstriction by Ang II in the patients with DM or HT was not significantly different from that in the 9 age-matched control subjects. No significant difference in vasoconstrictor response to NA was observed between the young and elderly subjects, nor between the control subjects and the patients with DM or HT. These findings indicated that vasoconstrictor response to Ang II might be greater in the elderly but might not be influenced by DM nor HT. (Hypertens Res 2002; 25: 683–688)

Key Words: angiotensin II, tachyphylaxis, aging, diabetes mellitus, hypertension

Introduction

The renin-angiotensin system plays an important role in cardiovascular homeostasis. Angiotensin II (Ang II) is one of the most potent vasoconstrictors, but it is known to lead to development of tachyphylaxis (rapid desensitization) (1, 2). It is therefore thought that Ang II contributes to balancing vascular tone, although the physiological and pathological significance of tachyphylaxis remain to be clarified. The vasoconstrictor response to Ang II that produces tachyphylaxis has been studied mainly by using isolated preparations of vascular and non-vascular smooth muscle, and there have been few investigations into the influence of aging and such diseases as hypertension (HT) and diabetes mellitus (DM), which are main risk factors of cardiovascular events, on this phenomenon.

By using the dorsal hand vein method (3), which is a sensitive and reproducible means of assessing the effect of drugs on peripheral veins, the influence of aging or of these diseases on the effects of vasoactive agents such as α- and β-adrenoceptor agonists has been investigated (4–8). The vascular effect of Ang II has also been studied using this method (9–11); however, to our knowledge, it is unknown whether the effect of Ang II on hand veins is influenced by HT, DM, or aging.

Unlike other vasoconstrictors such as noradrenaline (NA), Ang II does not cause typical dose-dependent vasoconstriction, and it has been clearly demonstrated that tachyphylaxis to Ang II occurs in the dorsal hand veins in humans (9–11). We previously showed that continuous infusion of Ang II causes transient vasoconstriction; that is, vasoconstriction by 50 ng/min Ang II peaked at 3 min after the start of infusion and thereafter attenuated, whereas NA is known to cause constant vasoconstriction (11).

In the present study, we used human hand veins to investi-
Table 1. Characteristics of the Study Population (Mean with Range)

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 9)</th>
<th>HT patients (n = 8)</th>
<th>DM patients (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 (40–68)</td>
<td>56 (43–69)</td>
<td>55 (42–66)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65 (61–73)</td>
<td>64 (57–70)</td>
<td>66 (57–79)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128 (108–140)</td>
<td>165 (152–182)</td>
<td>127 (112–154)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82 (66–90)</td>
<td>82 (84–98)</td>
<td>83 (68–88)</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>96 (86–107)</td>
<td>98 (90–111)</td>
<td>157 (122–175)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>188 (152–213)</td>
<td>201 (176–230)</td>
<td>192 (168–225)</td>
</tr>
</tbody>
</table>

Methods

Subjects

Twenty healthy male subjects with blood pressure less than 150/90 mmHg and normal serum blood sugar level, 8 male NIDDM patients with blood pressure less than 150/90 mmHg (42 to 66 years old; mean, 55 years), and 8 male essential HT patients with normal serum blood sugar level (43 to 69 years old; mean, 56 years) were included in this study. To determine the influence of aging, we chose 8 younger subjects ranging in age from 20 to 36 years (mean: 26 years) with body weight ranging from 58 to 80 kg (mean: 67 kg) and 8 older subjects ranging in age from 58 to 73 (mean: 65 years) with body weight ranging from 52 to 73 kg (mean: 64 kg) from the 20 healthy subjects. To evaluate the influence of the diseases, we also chose 9 age-matched healthy subjects (40 to 68 years old; mean, 55 years) from the 20 healthy subjects as controls. There was 1 smoker each in the young and elderly subjects, 3 in the control subjects, 3 in the DM patients and 1 in the HT patients, but none of them smoked in the 12 h before the study.

The baseline characteristics of the patients and the control subjects are shown in Table 1. The HT patients (disease history, 4 to 20 years) and the DM patients (6 to 17 years) did not have symptoms of angina pectoris, claudication, or cerebro-vascular ischemia, but the DM patients had at least one of the following complications, although the symptoms were mild: retinopathy, nephropathy and neuropathy.

Neither the patients nor the healthy controls took any medications that could influence vascular reactivity, such as anti-hypertensive drugs, non-steroidal anti-inflammatory drugs, etc., for at least 5 days before the study, although 6 of the patients with DM continued treatment with glibenclamide (2.5–7.5 mg/day) and/or voglibose (0.4–0.6 mg/day), an α-glucosidase inhibitor, during the study.

This study was approved by the Ethics Committee of Jichi Medical School. All the control subjects and the patients gave their informed consent to participate in this study.

Dorsal Hand Vein Technique

The dorsal hand vein technique was applied as described previously (3). A linear variable differential transformer (LVDT) (Schaevitz type 025 MHR; Schaevitz, Pennsauken, USA) was used. The LVDT has a freely movable core that rests over the center of the vein to be studied, and there is a linear relationship between the vertical movement of the core and changes in the output voltage. During the study, the subjects rested in the supine position, with one arm placed on a support sloping upwards at an angle of about 30 degrees from the horizontal to induce emptying of the vein. A 25-gauge needle was inserted into a dorsal hand vein and continuous infusion of physiological saline was started (0.4 ml/min) with an infusion pump (Terufusion STC-525; Terumo, Tokyo, Japan).

The position of the core was recorded before and after inflation of a sphygmomanometer cuff to 45 mmHg for 1 min on the same arm. The difference between the two positions of the core caused by the drug infusion gave a measure of the diameter changes of the vein under the constant and standardized occlusive pressure. The baseline diameter during saline infusion with the cuff inflated was defined as 0% venoconstriction and the recording with the cuff not inflated was defined as 100% venoconstriction. The constriction of the hand vein induced by the drug infusion was expressed as a percentage of the change in hand vein diameter.

Protocol

Subjects were asked to refrain from caffeine-containing and alcoholic beverages for more than 12 h before the study. The studies started about 4 h after a light lunch and were performed in a room with a controlled ambient temperature of 25–27°C. This study was performed on 2 separate occasions with a 1- to 3-week interval between. On the first study day, NA (Nor-Adrenaline®; Sankyo, Tokyo, Japan) was infused at a starting dose of 1 ng/min (0.012 nmol/l/min), which was doubled every 4 min until a maximum of 256 ng/min (1.5 nmol/l/min) was reached. The cuff was inflated during the last 1 min of the 4 min infusion period for each dose. On the second day of the study, a constant dose (50 ng or 0.048 nmol/l/min) of human recombinant Ang II (Delivert®; Toa Eiyo, Tokyo, Japan) was infused for 22 min and the cuff was inflated for 1 min every 3 min. Based on our previous study (11), we chose 50 ng/min for the dose of continuous infusion of Ang II, since this dose induces evident tachyphylactic re-
sponse but causes negligible change in blood pressure or pulse rate. During the drug infusion, blood pressure and pulse rate were monitored by a semiautomated sphygmomanometer (BP103iII; Nihon Colin, Komaki, Japan) on the opposite arm.

Statistical Analysis

Data are expressed as the means $\pm$ SD. Statistical analysis was performed by Student’s unpaired t-test to compare the differences between the young and elderly subjects, and by analysis of variance (ANOVA) to determine the differences among the patients with HT and DM and the control subjects, and the changes of blood pressure and pulse rate during the drug infusion. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

In all the healthy subject and patient groups, infusion of Ang II at a dose of 50 ng/min resulted in rapid desensitization or tachyphylaxis; venoconstriction by Ang II reached a peak at 3 min after the initiation of infusion and then gradually declined (Figs. 1, 2).

Venoconstriction by constant Ang II infusion in the elderly subjects was significantly larger than that in the young subjects from 6 to 18 min after the start of the infusion (Fig. 1). The venodilation occurring from 3 to 9 min after the start
of the Ang II infusion was significantly \( (p < 0.05) \) larger in the young than in the elderly subjects (44.2 ± 11.2% vs. 32.0 ± 14.8%).

However, venoconstriction by Ang II in the HT or DM patients was not significantly different from that in the control subjects at all the observation points (Fig. 2). Venoconstriction by the Ang II infusion of these control subjects and patients with HT and DM was not significantly different from that of the elderly subjects, whereas it was significantly larger (by ANOVA) compared to that of the young subjects at 9 and 12 min after the infusion.

Blood pressure and pulse rate did not change significantly by the Ang II infusion in any of the groups (Table 2).

The venoconstrictor response to NA in the young subjects was not significantly different from that in the elderly subjects (Fig. 3). The response in the patients with DM or HT was also not significantly different from that in the control subjects (Fig. 4). There were no significant changes in blood pressure or pulse rate during the NA infusion.

**Discussion**

Because aging and such diseases as DM and HT are risk factors for cardiovascular events, it is interesting to investigate their influences on vascular responsiveness to vasoactive agents. The dorsal hand vein method (3) is a useful way to investigate the effects of vasoactive agents in humans, although the responsiveness of the vein to an agent does not necessarily represent that of arteries. Using this method, the effect of an agent on venoconstrictor response can be detected without affecting the systemic hemodynamics, because the measurement is made within the vicinity of the infusion site, allowing the volume of the agent to be small.

Although Ang II plays an important role in the regulation of blood flow in various vascular beds, studies concerning the effect of Ang II on blood vessels in humans are limited, compared with those in animals or isolated preparations. In studies on humans using the dorsal hand vein method, it has been reported that Ang II produces a dose-dependent vasoconstriction with a good reproducibility (10, 11). The venoconstrictor effect of Ang II is, however, smaller than that of noradrenaline, and the dose-response curves of Ang II do not show the typical sigmoid-shaped pattern, suggesting the development of tachyphylaxis (10, 11). In our previous study (11), in which venoconstrictor responses to increasing doses (2–256 ng/min) of Ang II and noradrenaline were examined (each dose was infused for 4 min), the maximal venoconstriction by Ang II, which was obtained at a dose of between 12 and 128 ng/min, was 43.8 ± 12.2%, whereas that by noradrenaline was 93.1 ± 4.7% at the highest dose. This phenomenon was shown more prominently by a constant infusion of Ang II into the dorsal hand vein at a dose of 50 ng/min, which did not change either the systemic blood pressure or the pulse rate. The venoconstriction by Ang II reached a peak at 3 min after the start of infusion and declined thereafter, whereas that of NA induced constant venoconstriction (11).

In the present study, we used this method to investigate the influence of aging, DM and HT on the venoconstrictor response to Ang II that produces tachyphylaxis, a characteristic phenomenon of this agent. We showed that venoconstriction by Ang II infusion in the elderly subjects was larger than that in the young subjects from 6 to 18 min after the start of the infusion. The venodilation occurring from 3 to 9 min after the Ang II infusion was larger in the young than in the elderly subjects, indicating that tachyphylaxis to Ang II is attenuated by aging. On the other hand, the Ang II-induced venoconstriction of the patients was similar to that of the age-matched control subjects, indicating that essential
HT and non-insulin dependent DM have only a minimal influence on this phenomenon.

However, the Ang II-induced venoconstriction of these control subjects and patients with HT and DM was not significantly different from that of the elderly subjects, whereas it was significantly larger compared to that of the young subjects. This might be because the patients were rather old; mean age of the patients with HT and DM was 56 and 55, respectively. Therefore, to elucidate the influence of HT and DM more clearly, further studies including younger patients will be needed. The patients included in this study had diseases of mild to moderate severity. It is unknown whether the severity or duration of the disease influences the effects of Ang II observed in this study, and further studies will be needed to elucidate this.

There have been several studies that examined the influence of aging or disease on the tachyphylactic response to Ang II using animal models (12–14). However, the results of these studies are conflicting, and are not necessarily consistent with those of the present study.

Human studies concerning the influence of aging or disease on vasoconstrictor response to Ang II are limited. Although the reactivity of the veins to Ang II is not the same as that of arteries, the results of the following papers are consistent with those of the present study. Baan Jr et al. (15) reported that forearm vasoconstrictor response to Ang II is not changed as a result of hypertension. Duggan et al. (16) reported that blood pressure increase in response to Ang II infusion is greater in elderly than in the young human subjects.

As possible mechanisms of tachyphylaxis to Ang II, alternations at the receptor sites have been proposed, including internalization of the receptor (17) and changes in receptor affinity (18). In addition, vasodilator prostaglandins might be involved in the development of this Ang II induced phenomenon (11, 19). Aging might influence these mechanisms, resulting in the attenuation of Ang II-induced tachyphylaxis by aging.

The present study showed that noradrenaline induced typical dose-dependent venoconstriction, and this response was not altered by aging, HT or DM. These findings indicate that tachyphylaxis to α-adrenoceptor stimulation does not occur and the vasoconstricting response to α-adrenoceptor stimulation is influenced neither by aging, HT nor DM. These findings are consistent with the previous studies (4–6) using the dorsal hand vein method. By contrast, the β-adrenoceptor-mediated venodilating effect in HT or DM patients is reported to be reduced as compared with that in control subjects (7, 8), and it is also reduced by aging (5).

The physiological and pathological significance of the tachyphylaxis to Ang II has not been clarified. But, because Ang II is one of the most potent vasoconstrictors, the development of tachyphylaxis is thought to contribute to the balancing of vascular tone. Disturbance in cardiovascular homeostasis is associated with aging, and the decrease in tachyphylactic response to Ang II might, at least in part, contribute to this disturbance. To this Ang II related phenomenon, DM and HT might be minimal contributors, although several studies (20–22) indicate that blocking the Ang II receptor assists in the protection against cardiovascular complications.

The reactivity of the veins is not necessarily the same as that of the arteries, and there might be considerable regional and species differences in the reactivity of blood vessels. However, based on the present findings, we think that the increase in the vasoconstrictor response and the attenuation of tachyphylactic response to Ang II is included in the age-related changes in the vasoreactivity. Further studies will be needed to determine whether the present findings are related with the pathogenesis of cardiovascular diseases.

References


