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Short communication

12-Hydroxyeicosatetraenoic acid directly potentiates angiotensin II-induced vascular contraction

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Abstract

We studied whether 12-hydroxyeicosatetraenoic acid (HETE) affected the angiotensin II-induced contractile response in isolated hamster aorta. After preincubation with 10 nM 12-HETE for 1, 3, 5, 10 and 30 min, the angiotensin II-induced contractions were increased to 101%, 109%, 114%, 109% and 98%, respectively. The optimum preincubation time for the maximum effect of 12-HETE was 5 min. Under these conditions, 12-HETE increased dose dependently the contraction induced by 10 nM angiotensin II, and the concentration needed for the maximum effect was 30 nM, which increased contraction to 118% compared to the control angiotensin II-induced contraction. We demonstrated clearly that 12-HETE directly potentiates the angiotensin II-induced contraction.

Keywords: 12-Hydroxyeicosatetraenoic acid; Angiotensin II; Hypertension; Vascular tissue

1. Introduction

Angiotensin II plays an important role in the regulation of blood pressure. It had been thought that angiotensin II is generated by the circulating renin–angiotensin system. However, biochemical and molecular biological techniques have allowed the detection of the major components of the system in various tissues such as brain and heart and vascular tissues (Unger et al., 1988; Lindpaintner et al., 1989). We have reported that both angiotensin converting enzyme activity and the angiotensin II level in vascular tissues were increased in the chronic stage of hypertensive models such as spontaneously hypertensive rats (SHR) and 2-kidney, one clip (2K1C) rats, while the renin and angiotensin converting enzyme activities in plasma and heart were apparently normal or low (Miyazaki et al., 1986; Okamura et al., 1986; Shiota et al., 1992). These results suggest that the increase of angiotensin II generated by angiotensin converting enzyme in vascular tissues plays a crucial role in the pathogenesis of hypertension. In fact, the blood pressure in these models as well as in human hypertension can be lowered by angiotensin converting enzyme inhibitors (Ryan et al., 1984; Norman et al., 1987).

It has been reported that 12-hydroxyeicosatetraenoic acid (HETE), which is generated from arachidonic acid via the 12-lipoxygenase pathway, is increased in SHR and 2K1C rats (Nozawa et al., 1990; Stern et al., 1993). Moreover, lipoxygenase inhibitors lowered the blood pressure in SHR and 2K1C rats but not in deoxycorticosterone acetate–salt hypertensive rats, the pathological mechanism of which is irrelevant to the renin–angiotensin system (Nozawa et al., 1990; Stern et al., 1993, 1996). Although it is recognized that 12-HETE mediates angiotensin II-induced intracellular Ca^{2+} transients in cultured vascular smooth muscle cells in rat (Saito et al., 1992), it is unclear whether or not 12-HETE directly potentiates angiotensin II-induced contraction in vessels.

In this study, we examined whether 12-HETE affected angiotensin II-induced contractile responses in isolated hamster vessels.

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2. Materials and methods

2.1. Vascular tissue

Twenty male hamsters weighing 150–160 g were purchased from Japan SLC (Shizuoka, Japan). All hamsters were housed at room temperature (22°C) with a 12 h light–dark cycle and had free access to food and water. The hamsters were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and killed by bleeding from the carotid artery. The experimental procedures for animals were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

2.2. Functional studies of vascular tissue

The hamster arteries were cut into helical strips, 10 mm in length and 1.0 mm in width. The artery strip was placed on a myograph under a resting tension of 0.8 g. The bathing medium was Tyrode's solution consisting of 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 12 mM $NaHCO_3$ and 5.7 mM glucose, pH 7.4 (Okunishi et al., 1993). The medium was maintained at 37°C and bubbled continuously with O_2/CO_2 (95:5). The strip was equilibrated 2 h before the experiments. The contractile response to 50 mM KCl was obtained first, and then the bathing medium was washed out. The medium was washed out twice for 15 min each time with fresh Tyrode's solution, and the preincubation was equilibrated for 30 min. The procedure for obtaining control angiotensin II (final concentration of 10 nM)-and norepinephrine (final concentration of 1 μM)-induced contractile responses was described previously, and these responses represent submaximum responses (Jin et al., 1997). Angiotensin II was added to the bathing medium. The step for the angiotensin II response was repeated twice, and the third angiotensin II response was regarded as the angiotensin II control response. After the response, the medium was washed out twice for 15 min, each time with fresh Tyrode's solution. 12-HETE (final concentration of 10 nM) was added and, after preincubation for 1, 3, 5, 10 or 30 min, the angiotensin II response was observed. The effects of 12-HETE (final concentration of 10 nM) were evaluated, using the contractile responses to norepinephrine. To study the concentrations of 12-HETE effective for angiotensin II-induced contraction, we observed the angiotensin II response after preincubation for 5 min with 1, 3, 10, 30 or 100 nM of 12-HETE.

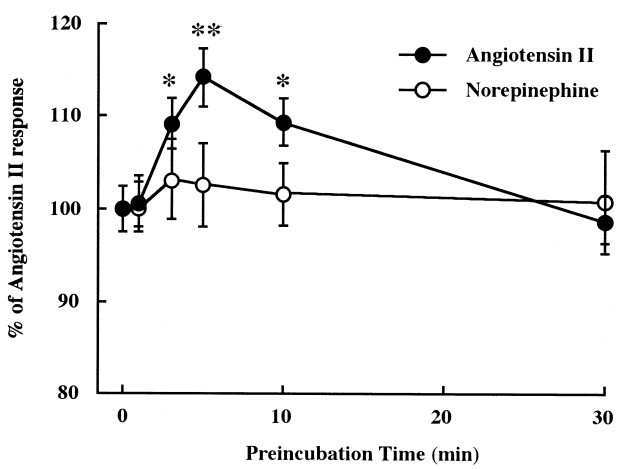

Fig. 1. Time-course for the effects of preincubation with 12-HETE on angiotensin II- and norepinephrine-induced contractile responses in isolated hamster vessels (angiotensin II-induced response, $n = 6$, norepinephrine-induced response, $n = 6$). Values are means ± S.E.M. $*$ $P < 0.05$, $**$ $P < 0.01$ vs. each control angiotensin II response.

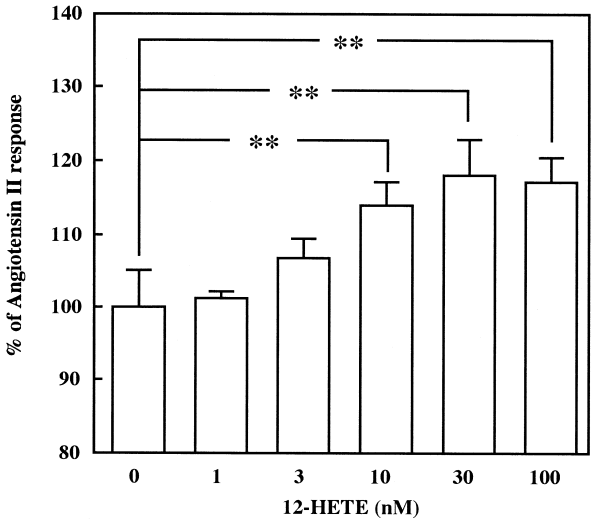

Fig. 2. Effects of 12-HETE on the Angiotensin II-induced contractile response in isolated hamster vessels ($n = 6$). Vertical bars represent means ± S.E.M. $**$ $P < 0.01$ vs. each control angiotensin II response.

2.3. Statistical methods

All results are presented as means ± S.E.M. Differences were considered significant when the P values were less than 0.05 with Duncan's multiple range test.

3. Results

The contractile response induced by 10 nM angiotensin II in isolated hamster aorta was 0.28 ± 0.02 g, and this control response was regarded as 100%. The percentage angiotensin II-induced contractile responses after preincubation with 12-HETE (10 nM) for 1, 3, 5, 10 and 30 min were 101%, 109%, 114%, 109% and 98%, respectively, the responses after preincubation for 3, 5 and 10 min being significantly potentiated compared with the control response (Fig. 1). The optimum preincubation time for the maximum effect of 12-HETE was 5 min. However, the

contractile responses induced by norepinephrine were not affected by 12-HETE (Fig. 1). After preincubation with 12-HETE concentrations of 1, 3, 10, 30 and 100 nM for 5 min, the contractions of 10 nM angiotensin II were increased to 101%, 107%, 114%, 118% and 117%, respectively, and the responses with 12-HETE concentrations of 10, 30 and 100 nM were significantly potentiated compared with the control response (Fig. 2).

4. Discussion

In the present study, we demonstrated for the first time that 12-HETE directly potentiated the angiotensin II-induced contractile response in isolated hamster vessels. The optimum preincubation time for the effect of 12-HETE was 5 min, while this effect was abolished with longer preincubation. 12-HETE is one of the products of arachidonic acid and it is well known that these products are usually unstable in tissues. Therefore, 12-HETE may be metabolized to inactive compounds. Although 12-HETE had no effect on the baseline response, it potentiated the angiotensin II-induced contractile response in a dose-dependent manner, and the concentration for the maximum effect was 30 nM. On the other hand, the norepinephrine-induced contractile response was not affected by 12-HETE. These results suggest that 12-HETE specifically potentiates angiotensin II-induced contraction. It is unclear why 12-HETE has different effects on contractions induced by angiotensin II and by norepinephrine. However, it is thought that different agonists of vascular contraction depend on different mechanisms. Norepinephrine-induced contraction depends on extracellular Ca^{2+} levels (Takuwa and Rasmussen, 1987; Bruschi et al., 1988), while the angiotensin II-induced contraction is sustained in a Ca^{2+}-free medium (Takuwa and Rasmussen, 1987). This suggests that mobilization of intracellular Ca^{2+} stores is primarily responsible for changes in intracellular Ca^{2+} levels. Angiotensin II activates phospholipase C in isolated vascular smooth muscle, resulting in the formation of inositol triphosphate and diacylglycerol, while 12-HETE potentiates the thrombin-induced production of inositol triphosphate and diacylglycerol (Setty et al., 1987; Friedlander et al., 1990). Recently, Sasaki et al. (1997) reported that the addition of 12-HETE increased angiotensin II-induced intracellular Ca^{2+} levels in cultured vascular smooth muscle cells in rat, but with a mechanism which is unclear. Therefore, 12-HETE may potentiate vascular contraction via the increase of intracellular Ca^{2+} levels by angiotensin II but not by norepinephrine, and the mechanism may depend on the interaction between 12-HETE and angiotensin II in signal transduction, with the production of diacylglicerol and inositol triphosphate, but not on Ca^{2+} channels.

The isolated hamster vessels used in this study were intact strips, not denuded strips. It is unclear whether or not, under these conditions, 12-HETE directly potentiates the angiotensin II-induced contractile responses because of the release of bioactive substances derived from endothelium. To avoid possible interference with responses to angiotensin II that affect biosynthesis and release some bioactive substances such as nitric oxide derivatives, prostanoids and endothelins, we examined the effects of 12-HETE on the angiotensin II-induced contraction after denuding the strips. We could thus observe the potentiation by 12-HETE of angiotensin II-induced contractile responses (data not shown). Although Sekiya et al. (1991) reported that, in bovine platelets, 12-HETE reduces the levels of thrombin-induced cAMP, which activates a vasodilator substance, prostacyclin, in vascular tissues, at least, an interaction of products via the cyclooxygenase pathway is not involved in the mechanism of potentiation by 12-HETE in the present study. The present study used 12(*S*)-HETE as 12-HETE, but 12-HETE is generally produced as stereoisomers by two different enzymes. Lipoxygenase and cytochrome *P*-450 produce 12(*S*)-HETE and 12(*R*)-HETE, respectively. Ma et al. (1991) reported that 12(*R*)-HETE reduces the internal diameter of isolated, perfused dog renal artery more strongly than does 12(*S*)-HETE. To find whether 12(*R*)-HETE potentiates the angiotensin II-induced contraction in vessels needs further study.

In vivo, the plasma 12-HETE concentration in Wistar–Kyoto (WKY) rats 12 weeks of age is about 16 nM, and that in SHR of the same age, when the blood pressure in SHR is found to be significantly elevated compared to that in WKY rats, is significantly increased (Sasaki et al., 1997). These findings suggest that up-regulated 12-HETE in the circulation may be involved in the pathogenesis of hypertension. Furthermore, 12-HETE production from platelets and aortic tissues is significantly increased in SHR compared to WKY rats, and inhibition by 12-lipoxygenase causes a decrease in blood pressure in SHR (Stern et al., 1996; Sasaki et al., 1997). In 2K1C renovascular hypertensive rats, a well-known model in which the development of hypertension is produced by an increase in the circulating renin–angiotensin system, lipoxygenase inhibitors prevent the development of hypertension (Nozawa et al., 1990). In human placental cotyledons, angiotensin II increases perfusion pressure and 12-HETE release, while lipoxygenase inhibitors reduce the angiotensin II-induced pressure increase (Kisch et al., 1997). However, the compounds used for lipoxygenase inhibition are non-specific 12-lipoxygenase inhibitors and may reduce various metabolites from unsaturated fatty acids including arachidonic acid. In any case, the hypotensive mechanism of lipoxygenase inhibitors has not yet been clarified. Our present results suggest that the 12-HETE level may directly regulate the functional role of angiotensin II and may be involved in the regulation of blood pressure.

In conclusion, the present study showed clearly that 12-HETE directly potentiates angiotensin II-induced con-

traction, and that the increase in 12-HETE may be involved in the pathogenesis of vascular diseases such as hypertension and vascular hypertrophy, in which angiotensin II formation plays a key role.

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