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COMMENTARY

Endothelial AT_2-receptors: chicken or egg?

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In this issue of the journal Katada & Majima (2002) convincingly report that the activation of angiotensin II AT_2-receptors on endothelial cells of perfused mesenteric arteries of the rat causes a dilatation, to which the local production of bradykinin and the resulting stimulation of kinin B_2-receptors contribute greatly (Katada & Majima, 2002). Earlier observations had demonstrated that disruption of the AT_2 receptor gene leads to hypertension and hyperresponsiveness to the vasoconstrictor effects of angiotensin II (Ichiki *et al*., 1995; Hein *et al*., 1995; Siragy *et al*., 1999; Tanaka *et al*., 1999). That angiotensin II can lead to the production of bradykinin which in turn releases endothelium-derived relaxing factors has already been suggested (Wiemer *et al*., 1993; Seyedi *et al*., 1995; Jalowy *et al*., 1998; Gohlke *et al*., 1998; Liu *et al*., 1997; Henrion *et al*., 2001). However, Katada & Majima, by the appropriate use of genetically kininogen-deficient rats and of inhibitors of kallikreins, as well as the measurement of the production of bradykinin, provide an elegant demonstration of the concept. Hence, their study reinforces considerably the observation that overexpression of AT_2-receptors leads to an increased production of bradykinin (Tsutsumi *et al*., 1999). Taken in conjunction with the available information, the results of Katada & Majima add significantly to the hypothesis that AT_2-receptors contribute to the regulation of vasomotor tone, and thus of arterial blood pressure, by offsetting, in an endothelium-dependent, kinin-dependent fashion the vasoconstrictor properties of the powerful peptide angiotensin II.

Before jumping to the conclusion that a novel important physiological role for angiotensin II and the second subtype of its receptors has been discovered, the question, as always, has to be addressed: where would the angiotensin II come from? In the *in vitro* experiments reported by Katada & Majima, angiotensin II was purchased and applied exogenously. In the *in vivo* situation, angiotensin II could reach the endothelial cells from the luminal side, presumably after transformation of circulating angiotensin I by converting enzyme conveniently located at the endothelial cell membrane. Alternatively, angiotensin II could be produced in the blood vessel wall itself through a local renin-angiotensin pathway and vascular chymases. In both cases, the activated peptide would switch on the local vascular kallikrein-kinin system, the existence of which has been established beyond doubt (Schmaier *et al*., 1988; 1999; Gardes *et al*., 1990; Oza *et al*., 1990; Van Iwaarden *et al*., 1998; Nolly *et al*., 1992; Mombouli & Vanhoutte, 1991; 1992; Madeddu *et al*., 1993; Seyedi *et al*., 1995; Okamoto *et al*., 1998; Wolf *et al*., 1999; Yayama *et al*., 1998; Sakakibara *et al*., 1998; Sasaguri *et al*., 1999; Bergaya *et al*., 2001a; Dedio *et al*., 2001). The locally produced bradykinin in turn activates B_2-kinin receptors on the endothelial cells leading to the release, towards the underlying vascular smooth muscle, of nitric oxide, endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (e.g. Cherry *et al*., 1982; Vanhoutte *et al*., 1993; Nakashima *et al*., 1993). The studies reported by Katada & Majima do not really permit dissection of the relative contribution of those three endothelial mediators, as the authors only used indomethacin to block cyclooxygenases, but not inhibitors of NO synthase (to prevent the formation of nitric oxide) or the mixture apamin plus charybdotoxin (to inhibit that of EDHF). The relatively modest effect of even 10 μM of indomethacin, and its independency of flow rate, imply that prostacyclin is not a major contributor to the response to angiotensin II. Indeed, at 10 μM, the question can be raised of the selectivity for cyclooxygenases of the effect(s) of indomethacin. Therefore, it may be premature to conclude, as Katada & Majima do, that the release of bradykinin and prostacyclin occur in parallel, rather than in sequence.

If locally produced angiotensin II, through stimulation of AT_2-receptors, activates the local vascular kallikrein-kinin system, as is strongly suggested by the experiments of Katada & Majima, the next obvious question is what could set the generation of angiotensin II in motion? The answer may well come from another elegant study, reported so far in abstract form (Bergaya *et al*., 2001b). These authors, using wild type and kallikrein-gene knockout mice, demonstrate that AT_2-, but not AT_1-receptors antagonists inhibit in part flow-dependent dilatation in the wild type, an effect that is not observed in the knockout animals. AT_2 and B_2-kinin antagonists had a comparable, non-additive effect. Hence, the authors conclude that AT_2-receptors contribute to the bradykinin-dependent component of the endothelium-dependent response to shear stress, which underlies flow-dependent dilatation. Their experiments thus indicate that angiotensin II and bradykinin act in sequence rather than synergistically, but do not permit to conclude which of the two peptides comes first in the chain of events following an increase in shear stress. In turn, the experiments by Katada & Majima may provide the answer since they imply that the formation of angiotensin II must precede that of bradykinin. A role of AT_2-receptors in flow-dependent vasodilatation has already been suggested (Matrougai *et al*., 1999). That locally produced bradykinin contributes to flow-dependent dilatation has been demonstrated not only in animal blood vessels, but also in the human coronary and peripheral circulation (Mombouli & Vanhoutte, 1992; Groves *et al*., 1995; Hornig *et al*., 1997; Bergaya *et al*., 2001a; Meneton *et al*., 2001).

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Can the observations of Katada on Majima, truly be used to suggest a central role of AT_2-receptor activation in flow-dependent dilatation at this stage? Certainly, the effect of AT_2-receptors is variable, and its importance seems of greater magnitude in the rat (Katada & Majima, 2002) than in mice (Bergaya *et al*., 2001b) but the same can be argued, even in humans, for the bradykinin-dependent component of flow-dependent vasodilatation that appears more pronounced in the coronary than in the femoral circulation (Groves *et al*., 1995; Hornig *et al*., 1997). If shear stress were to generate an ubiquitous production of vascular angiotensin II, one would expect AT_1-antagonists to cause acute relaxations in perfused blood vessels, or to augment flow-dependent vasodilatation. This has not been observed so far (Mombouli & Vanhoutte, 1991; Matrougui *et al*., 1999; Bergaya *et al*., 2001b). One should not exclude the possibility that endogenously produced bradykinin (the release of which increases with increased flow rates, as demonstrated by Katada & Majima) and exogenously added angiotensin II may interact with a common binding site on the endothelial cells, that shares pharmacological properties with both AT_2 and B_2-kinin receptors. After all, the two peptides also share common binding sites on converting enzyme. Such common binding site could be upregulated by shear stress, or modulated in an allosteric fashion by occupancy of the AT_1-receptors. Alternatively if endothelial cells are endowed with both AT_1 and AT_2 receptors, the occupancy of the AT_1-side may be required before the AT_2-activation can result in release of endothelial mediators, which would explain why high concentration of angiotensin II increase the production of nitric oxide (Boulanger *et al*., 1995; Caputo *et al*., 1995). Whatever the cellular mechanism involved, the findings of Katada & Majima throw a new light on the pharmacology of angiotensin II AT_1-receptor blockers, and if they can be extrapolated to the human vasculature, may help to understand why these drugs share many pharmacological and therapeutic properties with converting enzyme inhibitors.

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