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Contribution of the tissue angiotensin converting enzyme to the antihypertensive effect of altiopril calcium (MC-838) in spontaneously hypertensive rats

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Abstract—The effect of a new orally active angiotensin converting enzyme (ACE) inhibitor, calcium (–)-*N*-[(S)-3-[(*N*-cyclohexylcarbonyl-D-alanyl)thio]-2-methylpropionyl]-L-prolinate (MC-838, altiopril calcium), on systemic blood pressure (SBP) and tissue ACE activity has been examined in conscious spontaneously hypertensive rats (SHRs). MC-838 (3 mg kg^{-1}) given orally to SHRs elicited a long-lasting hypotension lasting over 24 h. With the development of the hypotension, MC-838 significantly reduced ACE activity in the lung, kidney and aorta, but not in the brain and heart. Suppression of plasma ACE and rise of plasma renin activity occurred only transiently at an earlier stage.

MC-838 (altiopril calcium) is a new angiotensin converting enzyme (ACE) inhibitor (Sakai et al 1987), which is undergoing clinical study as a promising antihypertensive agent. When given orally in a single equi-potent hypotensive dose (3 mg kg^{-1}) to renal hypertensive rats and spontaneously hypertensive rats (SHRs), the antihypertensive effect of MC-838 was comparable to that of captopril in magnitude, but lasted much longer (Sakai et al 1987; Aono et al 1988). Although the precise mechanism responsible for the hypotension produced by MC-838 remains to be elucidated, several primary tissues can be presumed as its potential target sites (Cohen & Kurz 1982; Dzau 1986; Unger et al 1986; Campbell 1987).

The aim of the present study, therefore, was to evaluate the relative importance of individual tissues to the antihypertensive activity of MC-838 in SHRs.

Materials and methods

Antihypertensive activity. Male SHRs ca 350 g (20 weeks of age) from the Japanese Charles River Labs were used. Under pentobarbitone-Na anesthesia (50 mg kg^{-1} i.p.), the animals were surgically implanted, via the femoral artery into the abdominal aorta, with an indwelling polyethylene cannula to directly monitor systemic blood pressure (SBP) and heart rate (HR) (Sakai et al 1987). After surgery, each animal was individually caged and once a day the indwelling cannula was flushed with 0·1 mL of 0·9% saline solution by means of a syringe. Before SBP measurements, the rats were fasted overnight, and then the SBP was determined with a Nihon Kohden pressure transducer (MPU-0·5, Tokyo, Japan) and the HR with a Nihon Kohden heart rate counter (AT-600G) in unanaesthetized and unrestrained conditions. Each animal received MC-838 or 0·9% saline orally. Approximately 6 h after the dose of MC-838, the time at which the maximal decrease in SBP occurred, the rats were fed again. Recordings were made on a chart with a Watanabe linearcorder (WR-3101, Tokyo).

Determination of the plasma renin activity (PRA), plasma angiotensin converting enzyme (ACE) and tissue ACE activities. Seventy SHRs (20 weeks), fasted overnight, were divided into 14 groups (each n = 5). Blood and tissue samples for estimation of ACE activity were obtained after decapitation before, and at 0·5, 1, 2, 6, 12 and 24 h after the oral dose of 3 mg kg^{-1} MC-838 or 0·9% saline.

ACE activity. Blood was collected in a heparinized test tube, and an aliquot used immediately for an ACE assay of plasma. To make tissue ACE preparations, brain, heart, lung, kidney and aorta were removed immediately after death and cleaned of adhering connective tissues. They were weighed, chopped into small pieces, and suspended in an adequate volume of cold 0·12 M borate buffer (pH 8·3). The suspension was homogenized with a Potter-Elvehijem homogenizer (Omega Electric, Tokyo). Aliquots of the homogenate were transferred into test tubes and homogenized again with Polytron (PTA 10-35, Kinematica, Lucerne, Switzerland) for 1 min. The solution was filtered through gauze, and centrifuged for 10 min at 1300 *g*. The supernatant was assayed for ACE activity. Final concentrations of tissues in the supernatants were calculated as 20 mg mL^{-1} for lung, 25 mg mL^{-1} for aorta and 100 mg mL^{-1} for the other tissues. The time between tissue removal and incubation was always less than 1 h. The ACE activities were expressed as nmol min^{-1} mL^{-1} for plasma or nmol min^{-1} mg^{-1} wet weight for tissues.

ACE activity was determined spectrophotometrically with an ACE color kit (Fujirebio, Inc., Tokyo). Briefly, 0·05 mL samples were incubated at 37°C for 20 min with 0·5 mL of 0·12 M borate buffer (pH 8·3) containing 10 mM *p*-hydroxybenzoyl-glycyl-L-histidyl-l-leucine, 2·5 mM 4-aminoantipyrine, 3 u mL^{-1} hippuricase and 0·7 M sodium chloride. The reaction was stopped by the addition of 1·5 mL of 3 mM disodium EDTA and 0·2% Triton X-100. After extraction, 1·5 mL of 6·5 mM sodium metaperiodate was added, and the concentration of developed quinoneimine dye was quantitatively measured with a Beckman DU-8B spectrophotometer at its absorbance maximum at 505 nm. All assays were in duplicate.

Plasma renin activity. Blood samples were transferred to chilled vacutainer tubes containing disodium EDTA (3 mg/tube). Plasma was separated in a refrigerated centrifuge (1300 *g* for 10 min), and 0·1 mL of the samples were incubated at 37°C for 5 min in the presence of 8-hydroxyquinoline sulphate, pH 4·6. The formed angiotensin-I (A-I) was measured reproducibly with an A-I radioimmunoassay kit (Midori Juji, Tokyo). PRA is expressed as ng of A-I generated mL^{-1} of plasma h^{-1} of incubation.

Drugs used. Calcium(-)-*N*-[(S)-3-[(*N*-cyclohexylcarbonyl-D-alanyl)thio]-2-methylpropionyl]-L-prolinate, M.W. 417.55, $C_{19}H_{29}N_2O_5S \cdot 1/2Ca$ (MC-838, altiopril calcium) was synthesized in our organic chemistry laboratory (Tanaka et al 1985). MC-838, dissolved in or diluted with 0·9% saline solution, was introduced into the stomach in a volume of less than 1 mL.

Statistical analysis. Data are expressed as means ± s.e. Intergroup differences were analysed by Student's *t*-test. *P* values less than 0·05 were considered statistically significant.

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Results and discussion

A single oral dose of MC-838 (3 mg kg^{-1}) caused a large and long-lasting fall in SBP in SHRs (Fig. 1). No consistent changes in HR were observed (not shown). The time course of changes in PRA and plasma and tissue ACE activities after oral administration of 3 mg kg^{-1} MC-838 or 0·9% saline solution is depicted in Fig. 1. The SBP decrease induced by 3 mg kg^{-1} MC-838 attained

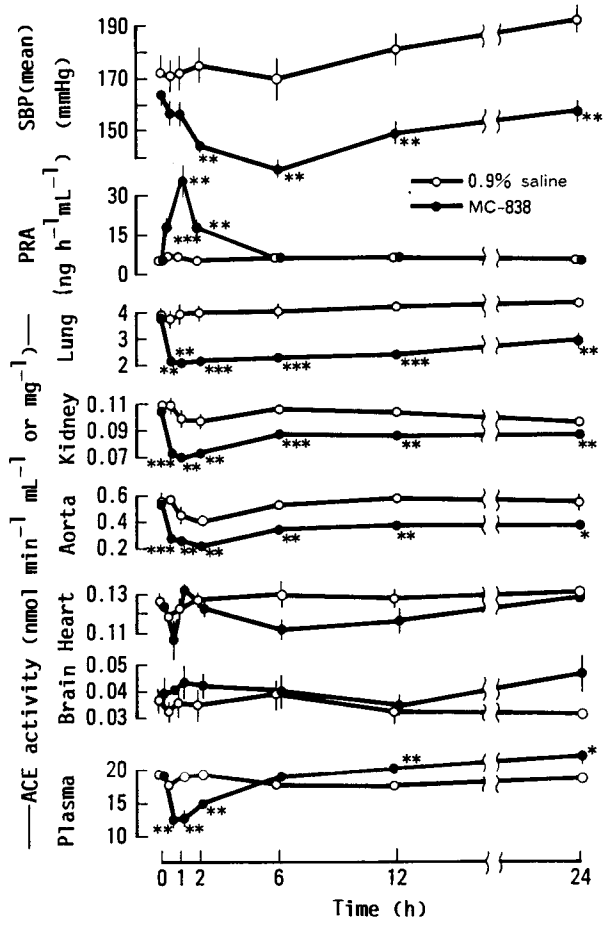

FIG. 1. Effects of 3 mg kg^{-1} MC-838 given orally on mean systemic blood pressure (SBP), plasma renin activity (PRA), plasma and tissue angiotensin converting enzyme (ACE) activities in spontaneously hypertensive rats. The ACE activities are expressed as nmol min^{-1} mL^{-1} for plasma or nmol min^{-1} mg^{-1} wet weight for tissues. Points are mean values ± s.e. for 5 rats at each time. $*P<0.05$, $**P<0.01$, $***P<0.001$ relative to the corresponding value from the 0·9% saline-treated groups.

a peak approximately 6 h after administration. A marked antihypertensive response was found even 24 h after treatment. Significant changes in PRA were observed for 2 h following the dose of MC-838. With the development of the sustained hypotension, marked changes of ACE activity in several tissues were noted, in addition to those in plasma ACE. In the lung, which had the highest basal ACE activity, the ACE activity was markedly inhibited by 3 mg kg^{-1} MC-838. Marked and persistent inhibition of ACE activity was also observed in the kidney and aorta, besides suppression of ACE activity in lungs. Although plasma ACE activity was significantly decreased for 2 h after the dose of MC-838, thereafter it gradually increased up to 24 h. This phenomenon remains to be evaluated.

It has been proposed that ACE inhibitors exert their beneficial cardiovascular actions not only by suppressing the activity of the plasma renin-angiotensin system (RAS), but also by reducing tissue RAS in various target organs of cardiovascular control, such as the brain, the lung, the kidney, the vascular wall and the heart (Cohen & Kurz 1982; Unger et al 1986; Campbell 1987). However, the importance of inhibition of tissue RAS in these target organs appears to be different, depending on the contribution of the respective organ to cardiovascular control. According to the present study, the ACE in three primary tissues, such as the lung, kidney and vascular wall, appears to play an important role in MC-838-induced hypotension. It should be noted that no inhibition of brain and heart ACE occurred at any time. As is well known, access of drugs to brain structures depends on their lipid solubility. Unger et al (1982) compared the effects of chronic oral treatment with the highly lipid soluble ACE inhibitor SA 446 and the structually related, but markedly less lipid-soluble compound, captopril, and found that the pressor response to angiotensin-I injected into the brain ventricle was significantly suppressed with SA 446, but not with captopril. MC-838, like captopril, is less lipid-soluble than SA 446.

Furthermore, a whole-body autoradiographic study of [^{14}C]MC-838 revealed that at 0·5, 1, 2, 4, 6, 12 and 24 h following the oral administration to SHRs, radioactivity in the brain was hardly detected (prepared for publication). Taking these points into consideration, it is conceivable that MC-838 (or the metabolites) may not easily cross the blood-brain barrier.

In summary, the present result confirms that MC-838 is an orally efficacious antihypertensive agent, and that the hypotensive effect may not be ascribable to a cardiac or central mechanism, but depends, to a major extent, on peripheral vasodilatation. Additionally, evidence from this paper suggests that, in SHRs, the vasodilatation is due to a fall in local vascular angiotensin-II synthesis rather than a fall in PRA, leading to a marked hypotension.

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