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Short communication

PINDOLOL DECREASES PLASMA ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS

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The β-adrenoceptor antagonist pindolol, given p.o. (10 mg/kg, once a day for 7 days) to 4 and 24 week old male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY), decreased plasma angiotensin-converting enzyme (ACE) activity only in young SHR. Kinetic studies indicated that the treatment reduced the ACE maximal velocity or the number of available ACE molecules. In vitro studies showed that pindolol had no direct effects on the activity of plasma ACE. A decrease in soluble (loosely bound) ACE activity was observed in the lung of young SHR treated with pindolol. The results suggest that pindolol affects the site of origin of plasma ACE. The susceptibility of young SHR to pindolol may indicate a probable role of β-adrenoceptors in the regulation of ACE activity in spontaneous (genetic) hypertension.

Plasma angiotensin-converting enzyme Pindolol Spontaneously hypertensive rats β-Adrenoceptors

1. Introduction

The renin-angiotensin system is controlled by a number of factors, among which β-adrenoceptors are well understood. In turn, the antihypertensive action of β-adrenoceptor antagonists may be partially due to antagonism of the renin-angiotensin system (Jackson and Campbell, 1980). Alterations of the renin-angiotensin system occur in spontaneously hypertensive rats (SHR). These animals show low angiotensin-converting enzyme (ACE) activity in plasma (Polsky-Cynkin et al., 1980). It has been postulated that plasma ACE originated in the lung, although endothelial cells in peripheral vessels could also make a contribution (Caldwell et al., 1976). Angiotensin II produced locally in arterial tissues by ACE may be important in maintaining vascular tone (Antonaccio and Kerwin, 1980).

We studied the effects of pindolol, a non-selective β-adrenoceptor antagonist and widely used antihypertensive drug, on plasma, lung and mesenteric artery ACE of young (4 week old, pre- or early hypertensive) and adult (24 week old) SHR and their normotensive controls, Wistar Kyoto rats (WKY).

2. Materials and methods

Pindolol was given orally for 7 days once a day at a dose of 10 mg/kg to male 4 and 24 week old SHR and WKY. Rats were decapitated 24 h after the last treatment and trunk blood was collected in heparinized tubes. ACE activity was measured by a radiochemical method (Rohrbach, 1978). The assay mixture (50 μl) contained 80 mM potassium phosphate buffer, pH 8.0, 300 mM NaCl, 0.8 mM p-chloromercuriphenylsulfonic acid and 5 mM [glycine-1-^{14}C]hippuryl-L-histidyl-L-leucine (3.5

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mCi/mmol, New England Nuclear, Boston, MA) as a substrate. Kinetic studies for plasma ACE were performed over six substrate concentrations ranging from 0.125 to 4 mM.

Lung tissue and mesenteric arteries were homogenized in 0.1 M potassium phosphate buffer pH 8.0 in glass-to-glass tissue grinders. The homogenate was centrifuged at $130000 \times g$ for 30 min, yielding a pellet and a supernatant. This supernatant was considered to be loosely bound (soluble) ACE containing fraction (F_1). The pellet was resuspended in the buffer containing 0.5% Triton X-100, ultrasonicated and freeze-thawed 3 times in order to solubilize particulate enzymes. The homogenate was centrifuged at $10000 \times g$ for 30 min; the supernatant was referred to as the F_2 fraction (particulate). Student's t-test was used to determine the statistical significance of differences between groups.

3. Results

Seven daily doses of pindolol decreased the heart weight in young SHR (500 ± 7 mg/100 g body weight in SHR-control and 436 ± 16 mg/100 g body weight in SHR-pindolol, $n = 5$, $P < 0.01$). Plasma ACE activity was lower in both young and adult SHR controls when compared to age-matched WKY. Seven daily doses of pindolol fur-

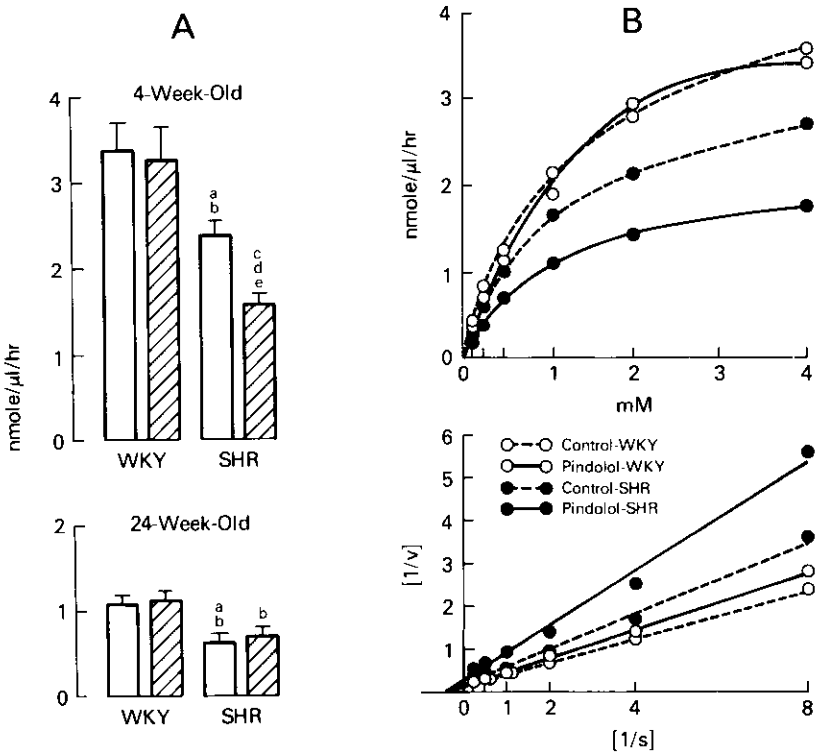

Fig. 1. Effect of pindolol on plasma ACE activity of spontaneously hypertensive rats (SHR) and age-matched Wistar Kyoto rats (WKY). (A) Results represent mean ± S.E.M. of groups of 5 rats, measured individually in duplicate. Substrate concentration was 5 mM. Open column: control rats; shaded column: pindolol-treated rats. [a] $P < 0.05$ vs. WKY-control; [b] $P < 0.05$ vs. WKY-pindolol; [c] $P < 0.01$ vs. SHR-control; [d] $P < 0.001$ vs. WKY-control; [e] $P < 0.01$ vs. WKY-control. (B) Michaelis-Menten plot and Lineweaver-Burk reciprocal plot for plasma ACE of 4 week old rats. Results represent means of duplicate samples, from one typical experiment. This experiment was replicated three times with similar results: K_m (mM): SHR-pindolol, 1.34 ± 0.08; SHR-control, 1.30 ± 0.05; WKY-pindolol, 1.40 ± 0.08; WKY-control, 1.42 ± 0.04. V_{max} (nmol/μl per h): SHR-pindolol, 2.04 ± 0.18 *,**; SHR-control, 3.18 ± 0.21 *; WKY-pindolol, 4.23 ± 0.23; WKY-control, 4.38 ± 0.32. * $P < 0.05$ vs. WKY-control, ** $P < 0.05$ vs. SHR-control.

TABLE 1

Effect of pindolol on lung and mesenteric artery ACE activity of young spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). Young (4 week old) rats were given pindolol (10 mg/kg p.o. once a day) for 7 days. The ACE activity (pmol/μg protein per h) was measured 24 h after the last treatment. Values represent mean ± S.E.M. of 5 duplicate determinations performed in individual animals.

	WKY-control	WKY-pindolol	SHR-control	SHR-pindolol
Lung				
F_1	59 ± 9	61 ± 6	40 ± 5	26 ± 6 [a,b]
F_2	635 ± 64	602 ± 83	424 ± 22 [a]	409 ± 22 [a]
Mesenteric artery				
F_1	5.1 ± 0.5	5.2 ± 0.6	4.6 ± 0.5	3.4 ± 0.6
F_2	42.0 ± 4.1	40.8 ± 5.6	26.3 ± 2.0 [a]	34.2 ± 5.8
Ratio of F_1 to $F_1 + F_2$				
Lung	0.089 ± 0.007	0.093 ± 0.006	0.086 ± 0.007	0.058 ± 0.010 [a,b,c]
Mesenteric artery	0.109 ± 0.010	0.115 ± 1.012	0.146 ± 0.025	0.092 ± 0.015

[a] $P < 0.05$ compared to WKY-control; [b] $P < 0.01$ compared to WKY-pindolol; [c] $P < 0.05$, compared to SHR-control.

ther decreased plasma ACE activity only in young, prehypertensive SHR (fig. 1A). This reduction in plasma ACE of SHR after pindolol did not appear to be related to a modification of enzyme affinity for the substrate, but to a decreased maximal velocity or number of available enzyme molecules (fig. 1B). In vitro studies showed that pindolol had no direct effects on ACE activity determined over pindolol concentrations ranging from 2×10^{-7} to 2×10^{-3} M (data not shown).

The ACE activity in the F_2 fraction (particulate) of lung in young control and pindolol-treated SHR was significantly lower than that in control WKY (table 1). In the F_1 fraction from lung, the ACE activity in pindolol-treated SHR was significantly decreased when compared to those of pindolol-treated WKY and control WKY. Conversely, in WKY, pindolol had no effects on ACE activity. In addition, when the ratio of ACE activity of F_1 to the combined activities of $F_1 + F_2$ was calculated, it was observed that pindolol significantly decreased the ratio of soluble to the combined soluble and particulate ACE activities only in young SHR. In addition, particulate ACE was low in mesenteric arteries of young control SHR when compared to control WKY (table 1).

4. Discussion

The alterations in plasma, lung and mesenteric artery ACE present in young SHR may be genetically determined and could be related to local alterations in angiotensin metabolism. The effects of pindolol on plasma ACE are only evident in young prehypertensive SHR. β-Adrenoceptor antagonists clearly reduce blood pressure in SHR only when treatment is started in the young animals (Richer et al., 1979). Thus, the effect of pindolol on plasma ACE of SHR could be related to its therapeutic effect.

Pindolol treatment may also affect the sites of origin of plasma ACE. The ACE ratio of F_1 (soluble) to the combined activities of $F_1 + F_2$ in lung is decreased by pindolol in young SHR, suggesting a possible role in the regulation of the ratio of soluble to particulate ACE. Pindolol could produce this effect directly, or indirectly through its suppressive effects on thyroid function. Particulate ACE is converted to the soluble form which is excreted from the endothelial cells into the blood (Friedland and Silberstein, 1983), a process regulated by a chymotrypsin-like esteroprotease, which in turn is induced by triiodothyronine (Silberstein et al., 1983).

It is of interest that the effects of pindolol occur only in young hypertensive animals. The susceptibility of young SHR to the effect of the β-adrenoceptor antagonist on ACE activity may be correlated with the increased peripheral sympathetic activity observed in these animals (Grobecker et al., 1975). As an alternative explanation, since β-adrenoceptors regulate membrane phospholipid

metabolism and determine the activity of membrane-bound enzymes, our results could be related to alterations in membrane phospholipid metabolism in this hypertensive model (Koutouzov et al., 1983).

References

Antonaccio, M.J. and L. Kerwin, 1980, Evidence for prejunctional inhibition of norepinephrine release by captopril in spontaneously hypertensive rats, European J. Pharmacol. 68, 209.

Caldwell, R.P.B., B.C. Seegal, K.C. Hsu, M. Das and R.L. Soffer, 1976, Angiotensin-converting enzyme: Vascular endothelial localization, Science 191, 1050.

Friedland, J. and E. Silberstein, 1983, Properties of soluble and particulate angiotensin-converting enzymes of rabbit lung, induced by macrophage and serum, Int. J. Biochem. 15, 1337.

Grobecker, H., M.F. Roizen, V. Weise, J.M. Saavedra and I.J. Kopin, 1975, Sympatoadrenal medullary activity in young hypertensive rats, Nature 258, 267.

Jackson, E.K. and W.B. Campbell, 1980, Inhibition of angiotensin AII potentiation of sympathetic nerve activity by beta-adrenergic antagonists, Hypertension 2, 90.

Koutouzou, S., P. Marche, A. Giard and P. Meyer, 1983, Altered turnover of polyphosphoinositides in the erythrocyte membrane of the spontaneously hypertensive rat, Hypertension 5, 409.

Polsky-Cynkin, R., S. Reichlin and B.L. Fanburg, 1980, Angiotensin-I-converting enzyme activity in the spontaneously hypertensive rats, Proc. Soc. Exp. Biol. Med. 164, 242.

Richer, C., N. Venturini-Souto and J.F. Giudicelli, 1979, β-Adrenoceptor blocking drugs, heart rate and genetic hypertension, Experientia 35, 655.

Rohrbach, M.S., 1978, [Glycine-1-^{14}C]: A substrate for the radiochemical assay of angiotensin-converting enzyme, Anal. Biochem. 15, 1337.

Silberstein, E., G.C. Schussler and J. Friedland, 1983, Elevated serum angiotensin-converting enzyme in hyperthyroidism, Am. J. Med. 75, 233.