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Effect of different endothelin receptor antagonists and of the novel non-peptide antagonist Ro 46–2005 on endothelin levels in rat plasma

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The goal of our study was to evaluate and compare the effects of receptor blockade with different endothelin (ET) receptor antagonists on plasma concentrations of ET-1, big ET-1 and ET-3 in conscious rats. Ro 46–2005 (10 mg/kg, i.v.), a novel non-peptide antagonist of both ET_A and ET_B receptors, increased the concentrations of ET-1 in plasma to 200 ± 13% of basal levels ($P < 0.001$). This effect was dose- and time-dependent and reached a maximum at 15 min. Ro 46–2005 had no effect on plasma concentrations of big ET-1 and only a minor effect on those of ET-3. In contrast to Ro 46–2005, the selective peptide ET_A antagonists BQ-123 and FR-139317 had no effect on plasma ET-1 concentrations. The increase in plasma ET-1 concentrations by Ro 46–2005 was most likely not due to de novo synthesis, since big ET-1 levels were not increased and peak levels were reached early after compound injection, but perhaps to displacement of ET-1 from the ET_B receptors.

Endothelin; Endothelin receptor; Endothelin receptor blockade; Hypertension

1. INTRODUCTION

The endothelins (ET-1, ET-2 and ET-3) are a family of locally acting 21-amino acid peptides with potent vasoconstrictor activities [1,2]. The three mature peptides, ET-1, ET-2 and ET-3 are derived by enzymatic cleavage of their respective precursor polypeptides, the big endothelins, at position 21 by a putative 'endothelin converting enzyme' [3]. ET-1, ET-2 and ET-3 differ with respect to their binding affinities towards the two described endothelin receptor subtypes ET_A and ET_B [4,5]. The peptides ET-1, big ET-1 and ET-3 are present in low concentrations in plasma [6,7]. So far it is unclear whether the endothelin detected in plasma has physiological functions by its own [8] or whether the endothelin plasma concentrations reflect only a spillover of endothelin which did not bind to tissue receptor sites [9]. The goal of our study was to evaluate and compare the effects of ET-receptor blockade with Ro 46–2005, a novel non-peptide endothelin receptor antagonist which binds to both ET_A and ET_B receptors [10] and with BQ-123 [11] and FR-139317 [12], two peptidic ET_A selective receptor antagonists, on the plasma concentrations of endothelins.

2. MATERIALS AND METHODS

The ^{125}I-labeled endothelins were purchased from Anawa (Wangen, Switzerland). BQ-123 was synthesized by A. Trzeciak at F.Hoffmann-La Roche Ltd. (Basel, Switzerland) and FR-139317 by Neosystem S.A. (Strasbourg, France). Ro 46–2005 was dissolved in 0.9% (w/v) NaCl. BQ-123 and FR-139317 were solved in 0.9% (w/v) NaCl containing 30% (v/v) DMSO.

Wistar rats were instrumented under evipan anesthesia (100 mg/kg, i.p.) with catheters inserted in one carotid artery and one jugular vein. Three hours later either the antagonists or solvent were administered by intravenous bolus injection. Arterial blood samples (1 ml, heparinized) were taken 5 min before and up to 240 min after intravenous injection. Blood samples were chilled immediately to 4°C, plasma was separated and stored at −20°C until endothelin measurement. Immunoreactive ET-1 and big ET-1 were measured after SepPak extraction by specific radio immunoassays as described recently [6] except that the detergent Tween-20 was omitted in the assay buffer system. Immunoreactive ET-3 was measured as described for ET-1 [6] using the ET-3 specific rabbit antiserum RAS 6911 at a final dilution of 1:30,000 (Peninsula Laboratories, Merseyside, England).

Plasma concentrations of immunoreactive endothelins were normalized to the basal levels measured 5 min before intravenous injection of either compound or solvent. Data are given as mean ± S.E.M. Statistical differences were analyzed by unpaired two-tailed Student's t-test. A P value < 0.05 was considered significant.

3. RESULTS

Normal concentrations of immunoreactive ET-1, big ET-1 and ET-3 were 20.0 ± 0.4 pg/ml, 182.5 ± 3.6 pg/ml and 35.6 ± 1.2 pg/ml of plasma, respectively ($n = 45$ per parameter, respectively). Ro 46–2005 increased plasma concentrations of immunoreactive ET-1 in a dose- and time-dependent manner. The ET-1 plasma concentrations were maximally elevated at 15 min and remained significantly increased up to 240 min after compound injection (Fig. 1). At 10 mg/kg (i.v.) Ro 46–2005 increased plasma concentrations of ET-1 to 200 ± 13% of basal levels (Fig. 2). The ET-1 plasma concentrations did not reach a plateau at this concentration. The

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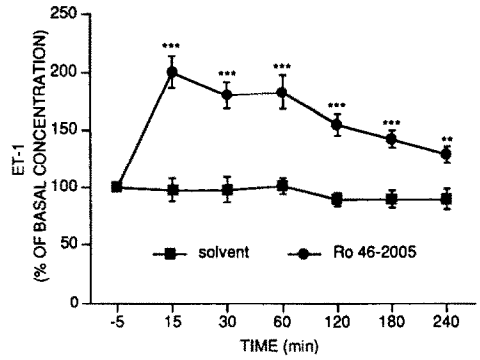

Fig. 1. Time dependency of the changes in ET-1 plasma concentrations after i.v. administration of either solvent or 10 mg/kg Ro 46–2005. ET-1 concentrations are expressed as percent of basal concentrations. $n = 9$; $^{**}P < 0.005$, $^{***}P < 0.001$.

plasma concentrations of ET-3 were also slightly elevated by Ro 46–2005 (Fig. 3). In contrast, the plasma concentration of big ET-1 was not affected in the presence of Ro 46–2005 (Fig. 4). Surprisingly, the selective peptide ET_A antagonists BQ-123 and FR-139317 had no effect on the concentrations of ET-1 in plasma (Table I).

4. DISCUSSION

Our study shows that Ro 46–2005 increases plasma levels of ET-1 and to a lesser extent those of ET-3. A similar effect on circulating angiotensin II levels has been described for the non peptide angiotensin II receptor antagonist losartan [13,14]. The intravenous doses of Ro 46–2005 (3 and 10 mg/kg) are effective pharmacological doses [10] and led to a two-fold increase in circulating ET-1 concentrations. Comparatively, blockade of angiotensin II (AT 1) receptors by pharmacological doses of losartan led to a 17-fold increase in angiotensin II levels [13]. The latter increase was most likely due to increased plasma renin activity and positive feedback on the synthesis of angiotensin II ([13] and refs. therein). In the case of increased plasma ET-1 concentrations by Ro 46–2005 (i) big ET-1 concentrations were not increased, and (ii) peak elevations were already reached at an early time point after compound administration. This suggests that the increase in ET-1 concentrations by Ro 46–2005 was not due to de novo synthesis. In contrast such an early burst could be due to release of ET-1 from storage compartments or from ET receptors.

In order to evaluate the receptor subtypes potentially involved in this ET increase, we used the selective ET_A receptor antagonists BQ123 and FR 139317 at pharmacologically active doses. No increase of plasma ET-1 and ET-3 (data not shown) concentrations was observed, suggesting that Ro 46–2005, a mixed antagonist, increases ET levels via an action on ET_B receptors. This seemed to be confirmed by the observation that also ET-3 plasma levels were elevated by Ro 46–2005, although to a much lesser extent as compared to ET-1. This suggests that ET is displaced from the ET_B receptors of the endothelial cell layer, exposed to the luminal side of the vascular system. Thus, the elevated ET plasma levels would be a direct consequence of the com-

Table I

Concentrations of immunoreactive ET-1 15 min after i.v. bolus injection of BQ-123 of FR-139317

	ET-1 (percent of basal concentrations)	
	Solvent	Compound
BQ-123 (10 mg/kg i.v.)	101.1 ± 8.1	107.4 ± 5.7
FR-139317 (10 mg/kg i.v.)	109.5 ± 15.2	113.4 ± 15.0

Values are expressed as percent of basal ET-1 concentrations ($n = 6$, respectively).

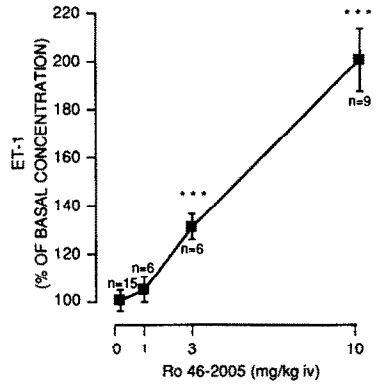

Fig. 2. Dose dependency of the effect of Ro 46–2005 on plasma ET-1 concentrations 15 min after compound administration. ET-1 concentrations are expressed as percent of basal concentrations. $^{***}P < 0.001$.

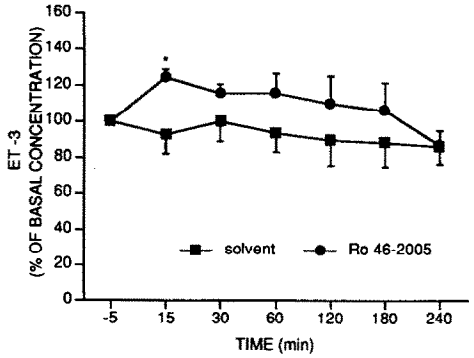

Fig. 3. Relative changes of ET-3 plasma concentrations after i.v. administration of either solvent or 10 mg/kg Ro 46–2005. ET-1 concentrations are expressed as percent of basal concentrations. $n = 6$, $^{*}P < 0.05$.

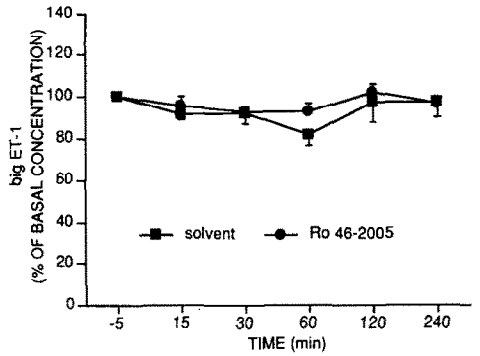

Fig. 4. Relative changes of big ET-1 plasma concentrations after i.v. administration of either solvent or 10 mg/kg Ro 46–2005. ET-1 concentrations are expressed as percent of basal concentrations. $n = 6$.

petitive interaction of Ro 46–2005 with the luminal, e.g. exposed, ET_B receptors. This would also indicate basal occupation of ET_B receptors by ET-1. The ET_A selective antagonists BQ-123 and FR-139317 could not increase ET-1 plasma concentrations, either because ET_A antagonism does not lead to ET-1 release, or because the ET-1 release is delayed. From the measurements performed in this study we cannot exclude that the ET_A selective antagonists also produce elevated plasma ET-1 levels at a later time point, i.e. after passing the endothelial cell layer which carries predominantly ET_B receptors [15], and interacting with ET_A receptors on smooth muscle cells.

The increase of circulating ET-1 concentrations by Ro 46–2005 might have physiological consequences. Recently, it could be demonstrated that even circulating ET-1 concentrations could increase ET_B dependent c-*fos* expression (Personnel communication: Dr. H. Tabuchi, Nippon Roche Research Center, Kamakura, Japan). The fact that Ro 46–2005 induces a mixed blockade of all ET receptors known so far explains the total absence of functional consequences of this increase.

Finally, this increase of ET-1 plasma concentrations may be a useful indicator of receptor blockade in future clinical trials.

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REFERENCES

[1] Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. (1988) Nature 332, 411–415.
[2] Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K. and Masaki, T. (1989) Proc. Natl. Acad. Sci. USA 86, 2863–2867.
[3] Matsumura, Y., Ikegawa, R., Takaoka, M. and Morimoto, S. (1990) Biochem. Biophys. Res. Commun. 167, 203–210.
[4] Arai, H., Hori, S., Aramori, I., Ohkubo, H. and Nakamichi, S. (1990) Nature 348, 730–732.
[5] Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K. and Masaki, T. (1990) Nature 348, 732–735.
[6] Löffler, B.-M., Jacot-Guillarmod, H. and Maire, J.-P. (1992) Biochem. Int. 27, 755–761.
[7] Miyauchi, T., Suzuki, N., Kurihara, T., Yamaguchi, I., Sugishita, Y., Matsumoto, H., Goto, K. and Masaki, T. (1991) Biochem. Biophys. Res. Commun. 178, 276–281.
[8] Yang, Z., Richard, V., von Segesser, L., Bauer, E., Stulz, P., Turina, M. and Lüscher, T. (1990) Circulation 82, 188–195.
[9] Löffler, B.-M., Roux, S., Kalina, B., Clozel, M. and Clozel, J.-P. (1993) J. Mol. Cell. Cardiol. 25, 407–416.
[10] Clozel, M., Breu, V., Burri, K., Cassal, J.-M., Fischli, W., Gray, G.A., Hirth, G., Löffler, B.-M., Müller, M., Neidhart, W. and Ramuz, H. (1993) Nature, in press.
[11] Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K., Nishikibe, M. and Yano, M. (1992) Life Sci. 50, 247–255.
[12] Fukami, N., Neya, M., Hemmi, K. and Tanaka, H. (1992) Synthesis and structure activity relationship of endothelin receptor antagonist FR139317. 13th Medicinal Chem. Symp. Tokyo 1992, Abstract P59: 118.
[13] Goldberg, M.R., Tanaka, W., Barchowsky, A., Bradsteet, T.E., McCrea, J., Lo, M., McWilliams Jr., E.J. and Bjornsson, T.D. (1993) Hypertension 21, 704–713.
[14] Vèniant, M., Clozel, J.-P., Hess, P. and Fischli, W. (1992) Hypertension 19, 255–262.
[15] Saijonmaa, O., Nyman, T., Fyhrquist, F. (1992) Biochem. Biophys. Res. Commun. 188, 286–291.