Access of peripherally administered DuP 753 to rat brain angiotensin II receptors

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The *in vivo* access of the nonpeptide angiotensin II (Ang II) antagonist, DuP 753 (10 mg kg⁻¹, i.v.), to Ang II receptors of rat brain was investigated by *in vitro* autoradiography with [¹²⁵I]-[Sar¹, Ile⁸] Ang II as a ligand. DuP 753 markedly inhibited the binding to sites which contain exclusively AT₁ receptors both outside and within the blood brain barrier, such as the circumventricular organs, paraventricular hypothalamic nucleus, median preoptic nucleus and nucleus of the solitary tract. However, binding to other nuclei containing AT₂ receptors was not significantly inhibited. These results demonstrate that DuP 753 and/or its active metabolite readily cross the blood brain barrier *in vivo* and selectively inhibit binding to AT₁ receptors in specific brain nuclei.

Keywords: Rat brain; angiotensin II; receptor subtypes; antagonist

Introduction Angiotensin II (Ang II) plays an important role in maintaining fluid and electrolyte homeostasis and cardiovascular functions by both peripheral and central actions. Ang II receptor binding sites, Ang II-responsive neurones and Ang II containing nuclei have been demonstrated at sites within and outside the blood brain barrier (Mendelsohn *et al.*, 1990). Recently, two distinct subtypes of Ang II receptors, AT₁ and AT₂, have been identified by use of new nonpeptide Ang II antagonists (Chiu *et al.*, 1989). The distribution of these Ang II receptor subtypes in rat brain has been described by *in vitro* autoradiography (Gehlert *et al.*, 1990; Wamsley *et al.*, 1990; Song *et al.*, 1991), and anxiolytic and cognitive-enhancing actions of the AT₁ antagonist, DuP 753, have been reported (Barnes *et al.*, 1990a,b) suggesting that the drug has central actions. However, it is not known whether these compounds have access to Ang II receptor sites within the blood brain barrier *in vivo*. The previously used peptide Ang II receptor antagonists do not penetrate the blood brain barrier, and only have access to the circumventricular organs. Similarly, the angiotensin converting enzyme inhibitors do not readily penetrate the blood brain barrier except after chronic administration of high doses (Sakaguchi *et al.*, 1988). We have therefore examined the effect of peripherally administered DuP 753 on Ang II receptor occupancy in rat brain using *in vitro* autoradiography.

Methods Four adult male Sprague-Dawley rats were injected with DuP 753 (2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl) methyl] imidazole) via the tail vein at a dose of 10 mg kg⁻¹, and four other animals received vehicle; 60 min after administration of DuP 753, rats were killed by decapitation. The brains were removed, snap frozen in isopentane-dry ice (−40°C) and stored at −80°C. Sections (20 µm) were cut on cryostat at −18°C, dehydrated overnight under reduced pressure at 4°C, and stored at −80°C in sealed container with silica gel until use.

The methods for *in vitro* autoradiographic localization of Ang II receptors with [¹²⁵I]-[Sar¹, Ile⁸] Ang II have been described previously (Song *et al.*, 1991). Briefly, sections were incubated in 10 mM sodium phosphate (pH 7.4) containing 150 mM NaCl, 5 mM Na₂EDTA, 0.2% bovine serum albumin (BSA) and 0.4 mM bacitracin with 0.2 µCi ml⁻¹ (90 pM) of [¹²⁵I]-[Sar¹, Ile⁸] Ang II. Non-specific binding was determined in the presence of 1 µM unlabelled Ang II. After incubation, the sections were washed with ice-cold buffer, loaded into X-ray cassettes and exposed to Agfascope CR3B X-ray film

for 2 weeks. A set of ¹²⁵I radioactivity standards was included. After exposure, the films were processed in a Kodak RPX-OMAT automatic developer and the optical density was quantitated with an MCID image analysis system (Imaging Research Inc., Ontario, Canada).

Results Ang II receptor binding to the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (10), paratrigeminal nucleus (Pa5), and medial nucleus of the inferior olive (IOM) in a control rat is shown in Figure 1a. *In vivo* administration of DuP 753 resulted in a marked decrease of binding to the nucleus of the solitary tract (29 ± 5%, compared to control), the dorsal motor nucleus of the vagus (31 ± 6%), and the paratrigeminal nucleus (35 ± 9%) (Figure 1b). These are sites where AT₁ receptors occur predominantly (Song *et al.*, 1991). Ang II receptor binding in the circumventricular organs, the subfornical organ, vascular organ of the lamina terminalis, median eminence and area postrema, are also inhibited by more than 50% in rats treated with DuP 753. In addition, binding to many brain structures which contain predominantly AT₁ receptors and are within the blood brain barrier, such as the median preoptic nucleus, amygdala, lateral parabrachial nucleus, spinal trigeminal nucleus, bed nucleus of the stria terminalis, and fusiform nucleus, were also markedly inhibited. By contrast, Ang II receptor binding to sites where AT₂ receptors occur predominantly, such as the medial nucleus of the inferior olive (5 ± 1%, Figure 1b), lateral septal nucleus, many nuclei in the thalamus, subthalamic nucleus (10 ± 2%), superior colliculus (11 ± 3%), locus coeruleus were not influenced significantly by prior administration of DuP 753. These results are consistent with our previous *in vitro* autoradiography studies in which the drug was added to the incubation medium (Song *et al.*, 1991).

Discussion The central actions of Ang II include stimulation of drinking and salt appetite, control of the autonomic nervous system, and regulation of both anterior and posterior pituitary hormone secretion. Using *in vitro* autoradiography, we (Song *et al.*, 1991) and others (Gehlert *et al.*, 1990; Wamsley *et al.*, 1990) have demonstrated that many brain nuclei involved in these functions contain predominantly AT₁ receptors, as determined in the presence of an excess concentration (10 µM) of the AT₂ antagonist, PD 123177. The present study demonstrates that peripherally administered DuP 753 blocks AT₁ receptor binding, not only to the circumventricular organs which are outside the blood brain barrier, but also at sites within the blood brain barrier, such as the median preoptic nucleus, hypothalamic paraventricular nucleus, lateral parabrachial nucleus, spinal trigeminal nucleus, bed

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Figure 1 Angiotensin II (Ang II) receptor binding at the level of the nucleus of the solitary tract of rat medulla oblongata: (a) shows binding in a control rat; (b) in a rat previously treated with DuP 753 10 mg kg^{-1} , i.v.; (c) shows non-specific binding in a control rat, and (d) shows non-specific binding in a DuP 753-treated rat. White represents high density of binding while black indicates background levels. Abbreviations are: NTS, nucleus of the solitary tract; 10, dorsal motor nucleus of the vagus; Pa5, paratrigebral nucleus; IOM, medial nucleus of the inferior olive. Calibration bar: 0.5 cm.

nucleus of the stria terminalis and nucleus of the solitary tract. No difference was observed in inhibition between the sites within and outside the blood brain barrier. By contrast, the effect of DuP 753 administered *in vivo* on AT_2 sites was very weak and related to the ratio of colocalization of AT_1 and AT_2 receptors in each site (Song *et al.*, 1991). For instance, in the subthalamic nucleus, AT_2 receptors account for 80%, and AT_1 receptors for 20% of total binding and here the extent of inhibition by *in vivo* DuP 753 was 10% compared with 50% inhibition on AT_1 receptors in the same sections. The central effect of DuP 753 is therefore highly selective for AT_1 sites.

Although DuP 753 does not cross the blood brain barrier after oral administration of single dose of the drug (Wong *et al.*, 1990a), the inhibition of Ang II receptor binding in rat brain by intravenously administered DuP 753 strongly sug-

gests that, unlike peptide antagonists or converting enzyme inhibitors, DuP 753 and/or its active metabolites can readily cross the blood brain barrier and exert potent and selective inhibition of AT_1 receptors outside or within the blood brain barrier. Whether this effect is mediated by DuP 753 or its active metabolites, such as EXP 3174 (Wong *et al.*, 1990b), remains to be further investigated; however, the current results are relevant to predicting and understanding the central actions of these new classes of Ang II antagonists.

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References

- BARNES, N.M., COSTALL, B., KELLY, M.E., MURPHY, D.A. & NAYLOR, R.J. (1990a). Anxiolytic-like action of DuP 753, a non-peptide angiotensin II receptor antagonist. *Neuroreport*, **1**, 20–21.
- BARNES, N.M., COSTALL, B., KELLY, M.E., MURPHY, D.A. & NAYLOR, R.J. (1990b). Anxiolytic-like action of DuP 753, a non-peptide angiotensin II receptor antagonist. *Neuroreport*, **1**, 239–242.
- CHIU, A.T., HERBIN, W.F., MACALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, R., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.I. & TIMMERMANS, P.B.M.W.M. (1989). Identification of angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.*, **165**, 196–203.
- GEHLERT, D.R., GACJENHEIMER, S.L., REEL, J.K., LIN, H.S. & STEINBERG, M.I. (1990). Non-peptide angiotensin II receptor antagonists discriminate subtypes of ^{125}I -angiotensin II binding sites in the rat brain. *Eur. J. Pharmacol.*, **187**, 123–126.
- MENDELSON, F.A.O., ALLEN, A.M., CHAI, S.Y., MCKINLEY, M.J., OLDFIELD, B.J. & PAXINOS, G. (1990). The brain angiotensin system. Insight from mapping its components. *Trends Endocrinol. Metab.*, **2**, 189–198.
- SAKAGUCHI, K., CHAI, S.Y., JACKSON, B., JOHNSON, C.I. & MENDELSON, F.A.O. (1988). Differential angiotensin-converting enzyme inhibition in brain after oral administration of perindopril demonstrated by quantitative *in vitro* autoradiography. *Neuroendocrinol.*, **48**, 223–228.
- SONG, K., ALLEN, A.M., PAXINOS, G. & MENDELSON, F.A.O. (1991). Angiotensin II receptor subtypes in rat brain. *Clin. Exp. Pharmacol. Physiol.*, **18**, 93–96.
- WAMSLEY, J.K., HERBLIN, W.F., ALBURGES, M.E. & HUNT, M. (1990). Evidence for the presence of angiotensin II-type I receptors in brain. *Brain Res. Bull.*, **25**, 397–400.
- WONG, P.C., PRICE, W.A., CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1990a). Nonpeptide angiotensin II receptor antagonists. VIII. Characterization of functional antagonism displayed by DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, **252**, 719–725.
- WONG, P.C., PRICE, W.A., CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1990b). Nonpeptide angiotensin II receptor antagonists. XI. Pharmacology of EXP3174: An active metabolite of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, **255**, 211–217.

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