Pharmacological characterization of angiotensin-induced depolarizations of rat superior cervical ganglion *in vitro*

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1 The depolarizing responses to angiotensin II and angiotensin III of the rat superior cervical ganglion have been characterized *in vitro*, by the use of peptidase inhibitors, peptide and non-peptide antagonists and dithiothreitol (DTT).

2 Angiotensin II and III depolarized the ganglion in a concentration-related manner. Angiotensin II was approximately 30 fold more potent than angiotensin III.

3 The endopeptidase inhibitor, bacitracin, increased the potency of angiotensin II and III by approximately 4 and 20 fold respectively. The aminopeptidase inhibitor, amastatin, further increased the potency of angiotensin III (but not angiotensin II) by approximately 4 fold. In the presence of bacitracin and amastatin, angiotensin II and III were equipotent.

4 The peptide antagonist [Ile⁷]angiotensin III (0.01–0.3 μ M) produced a non-parallel rightward displacement of the angiotensin II concentration-response curve, with a suppression of the maximum response. The potency of [Ile⁷]angiotensin III was increased by bacitracin and amastatin.

5 The AT₁-selective non-peptide antagonist losartan (DuP 753; 0.03 and 0.1 μ M) produced a parallel rightward displacement of the angiotensin II concentration-response curve, with an apparent pK_B of 8.3 ± 0.1 . A higher concentration of losartan (0.3 μ M) depressed the maximum agonist response by $32 \pm 6.5\%$, possibly reflecting non-competitive behaviour of the antagonist. The potency of losartan was not influenced by bacitracin.

6 The AT₂-selective non-peptide antagonist, PD123177 (3 μ M) failed to antagonize the angiotensin II-induced depolarizations.

7 DTT (1 mM) produced a 22% reduction of the maximum response to angiotensin II.

8 We conclude that the angiotensin II-induced depolarizations of the rat superior cervical ganglion are mediated by angiotensin II receptors of the AT₁ subclass. The ability of peptidase inhibitors to modify the potency of peptide agonists and antagonists highlights the difficulties associated with the use of peptide agents to characterize angiotensin II receptors in this preparation.

Keywords: Angiotensin II; superior cervical ganglion; depolarization; peptide antagonists; non-peptide antagonists; angiotensin II receptor subtypes

Introduction

Angiotensin II is a potent stimulant of sympathetic ganglia, exerting its action directly on postganglionic cells (Reit, 1972; Dun *et al.*, 1978). The excitatory response to angiotensin II is associated with a slow depolarization, similar to that produced by muscarinic agonists (Brown *et al.*, 1980). Although Brown *et al.* (1980) have shown that this angiotensin II-induced depolarization can be antagonized by the peptide angiotensin II antagonist, [Ile⁷]angiotensin III, no further pharmacology of the effects of angiotensin II on ganglia has been reported.

Over the past years, there has been much speculation about the existence of multiple receptor subtypes for angiotensin II. Some of this evidence is based on differences in the relative potencies of angiotensin II and its heptapeptide metabolite, angiotensin III. In some tissues, angiotensin III is considerably weaker than angiotensin II (Moore *et al.*, 1976; Scanlon & Moore, 1988), while in other target organs, angiotensin III is equipotent to, or even more potent than its precursor peptide (Peach & Chiu, 1974; Jackson *et al.*, 1988; Carrithers *et al.*, 1990). In view of these observations, angiotensin receptors in different tissues have been separated according to agonist orders of potency (Peach, 1977). In addition, a number of peptide antagonist analogues of angiotensin II or III, such as [Sar¹, Ile⁸]angiotensin II and [Ile⁷]angiotensin III have been found to be more potent as antagonists of angiotensin II or III responses in some tissues than in others (Sarstedt *et al.*, 1975; Spielman *et al.*, 1976). However, recent studies with

non-peptide angiotensin II antagonists have provided more definitive evidence for the existence of angiotensin II receptor subtypes. Angiotensin II receptors have been divided into two subtypes, referred to as AT₁ and AT₂ (Bumpus *et al.*, 1991). Losartan (DuP 753) is a selective antagonist of the AT₁ subtype (alternatively known as type 1, AII-1, AII-B or AII α ; Chiu *et al.*, 1989a; Chang & Lotti, 1989; Whitebread *et al.*, 1989; Speth & Kim, 1990). PD123177, CGP42112A and [*p*-aminophenylalanine⁶]angiotensin II are selective for the AT₂ subtype (type 2, AII-2, AII-A or AII β) (Chiu *et al.*, 1989a; Whitebread *et al.*, 1989; Speth & Kim, 1990).

Additional support for this angiotensin II receptor classification has also been provided by the specific ability of the disulphide reducing agent, dithiothreitol (DTT) to inhibit the binding of radiolabelled ligands to AT₁ sites, while enhancing the binding to AT₂ sites (Whitebread *et al.*, 1989; Chiu *et al.*, 1989b). DTT also abolishes the contractile effects of angiotensin II in rabbit isolated aorta (Fleisch *et al.*, 1973), a tissue that expresses only AT₁ sites (de Gasparo *et al.*, 1990).

The purpose of the present experiments was to perform a detailed pharmacological study of the effects of angiotensin II and III on the isolated preparation of the rat superior cervical ganglion. We have investigated the effects of peptidase inhibitors, peptide and non-peptide antagonists as well as the disulphide reducing agent DTT on the agonist-induced depolarization responses.

Methods

Male Lister-Hooded rats, weighing 300–350 g were stunned by a blow to the head, decapitated and the superior cervical

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ganglia were dissected from the surrounding tissue. Each ganglion was desheathed under a dissecting microscope and arranged in a two-compartment chamber such that the ganglion cell body was isolated from the postganglionic trunk by a notched perspex barrier coated in silicone grease. Each ganglion rested on filter paper and was perfused with Krebs-Henseleit solution, pre-equilibrated with 5% CO₂ in O₂ and maintained at 37°C. The composition of the solution was (mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 2.5, MgSO₄ 1.6, NaHCO₃ 24.9 and glucose 5.6. Each compartment was perfused separately at a rate of 0.5 ml min⁻¹. Agonist drugs were added to the ganglion cell body compartment and potential differences across the two compartments were recorded with Ag/AgCl electrodes, one positioned in contact with each compartment. Up to six ganglion preparations were used in each experiment. For full details of tissue preparation and recording, see Ireland (1987).

To prevent tachyphylaxis, angiotensin II and angiotensin III were applied serially using an inter-dose interval of 20 min, each concentration remaining in contact with the tissue for 5 min. Only two concentration-response curves were determined on each preparation, the first curve always serving as a control for the second. Peptidase inhibitors or antagonists were perfused over the tissues for 30 min before construction of the second concentration-response curve. In each experiment, one preparation was perfused for the duration of the experiment without inhibitors or antagonists and served as a control. On this tissue, the first and second curves were always superimposable. To investigate the effect of antagonists in the presence of inhibitors, the inhibitors were applied before the first curve. Preliminary experiments indicated that tachyphylaxis develops rapidly to high doses of angiotensin II or III. As a result, it was not always possible to achieve the maximum of the agonist concentration-response curve. Thus, to measure agonist potency, the concentration of agonist required to produce a depolarization of 300 μ V was estimated by eye from individual concentration-response curves. This value is referred to as EC₃₀₀. A response of 300 μ V always lay on the linear part of the concentration-response curve. To measure antagonist potency, lateral displacements of the concentration-response curves were estimated by eye at the control EC₃₀₀ level, to give 'dose-ratios' between drug-treated and control curves. Dose-ratios, estimated from individual ganglion preparations were used to calculate the negative logarithm of the apparent dissociation constant (pK_B) for the antagonists using the equation, $pK_B = \log(\text{dose-ratio} - 1) - \log(\text{antagonist concentration})$. Results of antagonist experiments are shown graphically, normalised against the response achieved by the highest control agonist concentration used. This response was not necessarily the greatest that could be achieved. Statistical analyses used paired or unpaired Student's *t* test where appropriate, using a criterion for statistical significance of *P* < 0.05.

The drugs used were supplied from the following sources. Angiotensin II, angiotensin III, [Ile⁷]angiotensin III (Peninsula), bacitracin, amastatin, dithiothreitol (Sigma), losartan potassium (Du Pont Merck Pharmaceutical Co.) and PD123177 (1-(4-amino-3-methylphenyl) methyl-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazol[4,5-C]pyridine-6-carboxylic acid; synthesized in our laboratories).

Results

Angiotensin II and angiotensin III evoked concentration-dependent depolarization of the rat isolated superior cervical ganglion. The depolarization reached peak height within 4–5 min after the start of agonist perfusion and recovery to baseline was seen within 15 min of perfusing drug-free solution. The application of higher doses of angiotensin II or III was characterized by a rapid waning of the response as the depolarization approached its maximum. The potencies ($-\log EC_{300}$) of angiotensin II and III, were 7.2 ± 0.1 and 5.6 ± 0.2

respectively. Although depolarizing responses to angiotensin II and angiotensin III were not compared on any one tissue, angiotensin III was approximately 40 fold weaker than angiotensin II.

Effect of bacitracin and amastatin on agonist responses

The angiotensin II- and angiotensin III-induced depolarizing responses of the ganglia were potentiated by the application of bacitracin (1 mM). Bacitracin displaced the angiotensin II and III concentration-response curves to the left of the corresponding control curves (Figure 1a,b). The potencies of angiotensin II and angiotensin III in the presence of bacitracin were increased 3.6 fold and 24.1 fold respectively.

In the presence of bacitracin, the additional preincubation with amastatin (10 μ M) produced differential effects on the depolarizing responses to angiotensin II and angiotensin III. The potency of angiotensin II was not influenced by amastatin (Figure 2a), although the potency of angiotensin III was increased 4.6 fold (Figure 2b). In the presence of both bacitracin and amastatin, angiotensin II and angiotensin III were equipotent, with $-\log EC_{300}$ estimates of 8.2 ± 0.2 and 8.1 ± 0.1 respectively.

Effect of antagonists

To characterize the receptor mediating the ganglion depolarization produced by angiotensin II, we investigated the effects of [Ile⁷]angiotensin III, losartan, PD123177 and DTT on angiotensin II-induced depolarizations of the ganglia. Unless otherwise stated, all these studies were conducted in the presence of bacitracin (1 mM), to prevent the degradation of angiotensin II.

[Ile⁷]angiotensin III

Angiotensin II-induced depolarization of the ganglion was antagonized by [Ile⁷]angiotensin III. At concentrations of

Figure 1 Effect of bacitracin on depolarizing responses to angiotensin II (Ang II) (a) and angiotensin III (Ang III) (b). The symbols represent mean depolarizations (s.e. mean shown by vertical bars) in the absence of bacitracin (●) or during perfusion with 1 mM bacitracin (▽). *n* = 3 preparations.

Figure 2 Effect of amastatin on depolarizing responses to angiotensin II (Ang II) (a) and angiotensin III (Ang III) (b). The symbols represent mean depolarizations (s.e.mean shown by vertical bars) in the absence of amastatin (●) or during perfusion with 10 μM amastatin (▽). $n = 4$ preparations.

0.03–0.3 μM, the antagonist produced a non-parallel rightward displacement of the angiotensin II concentration-response curve, with suppression of the maximum response (Figure 3a). In view of these observations, the antagonist potency of [Ile⁷]angiotensin III was not measured quantitatively. The antagonist effect of [Ile⁷]angiotensin III was influenced by peptidase inhibitors. Thus, during the continued perfusion of amastatin (10 μM), in addition to bacitracin, [Ile⁷]angiotensin III (0.01–0.1 μM) produced greater rightward displacement of the agonist concentration-response curves than during the perfusion of bacitracin alone (Figure 3b). The suppression of the maximum response was also greater. Thus, in the presence of bacitracin (1 mM) alone, 0.1 μM [Ile⁷]angiotensin III reduced the agonist maximum response to $78.4 \pm 7.0\%$, whilst in the presence of bacitracin plus amastatin (10 μM), 0.1 μM [Ile⁷]angiotensin III reduced the agonist maximum response to $47.6 \pm 6.1\%$ ($P < 0.05$ versus bacitracin alone). Furthermore, in the absence of either bacitracin or amastatin, 0.1 μM [Ile⁷]angiotensin III failed to reduce significantly the maximum response to angiotensin II (Figure 3c).

Non-peptide antagonists

Angiotensin II-induced ganglion depolarization was also antagonized by the non-peptide antagonist losartan. Losartan (0.03 μM and 0.1 μM) produced an apparent rightward displacement of the agonist concentration-response curve (Figure 4a), although the maximum agonist response was reduced by $32 \pm 6.5\%$ in the presence of 0.3 μM losartan. The apparent dose-ratios for the two losartan concentrations (0.03 μM and 0.1 μM) producing parallel shifts of agonist concentration-response curves were 6 ± 1.5 and 23 ± 4.2 respectively, which yielded an apparent pK_B value of 8.3 ± 0.1 from the Gaddum equation (see above). In contrast to the results obtained with [Ile⁷]angiotensin III, the antagonist potency of losartan was not significantly influenced by peptidase inhibitors. Thus, the

Figure 3 Effect of [Ile⁷]angiotensin III on the concentration-response curve to angiotensin II (Ang II) on the rat isolated superior cervical ganglion. (a) During perfusion of bacitracin (1 mM). (b) During perfusion of bacitracin plus amastatin (10 μM). (c) No peptidase inhibitors. Control (●); [Ile⁷]angiotensin III 0.01 μM (○), 0.03 μM (▽), 0.1 μM (▼), 0.3 μM (□). Depolarizing responses are normalised around the close-to-maximum control agonist response (see Methods). $n = 3-6$ preparations.

apparent pK_B of losartan in the absence of bacitracin was 8.6 ± 0.1 (Figure 4b).

Preincubation of the ganglia with PD123177 failed to antagonize the angiotensin II-induced depolarization, at an antagonist concentration of 3 μM (data not shown).

Dithiothreitol

The disulphide reducing agent DTT produced complex effects on the angiotensin II-induced depolarization. The responses to lower concentrations of angiotensin II (3–10 nM) were not influenced by DTT (1 mM) although there was some suppression of the responses produced by the higher doses of angiotensin II. Maximum responses to angiotensin II were reduced by $22 \pm 5.9\%$ (data not shown).

Discussion

The most important finding of this study is that the angiotensin II-induced depolarization of the rat superior cervical ganglion can be antagonized by the AT₁ selective non-peptide angiotensin II antagonist, losartan. The antagonist effects of

Figure 4 Effect of losartan on the concentration-response curve to angiotensin II (Ang II) on the rat isolated superior cervical ganglion. (a) During perfusion of bacitracin (1 mM), (b) no bacitracin. Control (●); losartan 0.03 μ M (▽), 0.1 μ M (▼), 0.3 μ M (□). Depolarization responses are normalised around the close-to-maximum control agonist response (see Methods). $n = 3-5$ preparations.

losartan were concentration-dependent and appeared to be compatible with a competitive mode of action at low concentrations (0.03 μ M and 0.1 μ M), although maximum agonist responses were depressed at higher concentrations of losartan. This suggests other, non-competitive inhibitory effects of losartan. The pK_B estimate determined for losartan against the depolarization to angiotensin II is similar to that previously reported in other *in vitro* functional preparations (Wong *et al.*, 1990) and is also close to its $\log IC_{50}$ for displacement of angiotensin II binding from AT_1 binding sites in different tissues (Chiu *et al.*, 1989a; Criscione *et al.*, 1990; Michel & Barnes, 1991). These data indicate that the angiotensin II-induced depolarization of ganglion neurones is mediated by angiotensin II receptors of the AT_1 subclass. This is supported by the observation that the AT_2 selective antagonist, PD123177, failed to antagonize the depolarization, even at a concentration of 3 μ M, which is at least 30 fold higher than its IC_{50} for displacement of radiolabelled angiotensin II binding from AT_2 sites (Chiu *et al.*, 1989a; Michel & Barnes, 1991).

A further important observation from this study is the influence of peptidase inhibitors on the responses to angiotensin II and III and on the antagonist effects of [Ile⁷]angiotensin III. Our findings show that firstly, bacitracin increased the

potency of both angiotensin II, angiotensin III and [Ile⁷]angiotensin III and secondly, the potencies of angiotensin III and [Ile⁷]angiotensin III, but not angiotensin II were further increased by amastatin. The metabolism of angiotensin II is known to occur in part by the action of proline endopeptidase, which cleaves phenylalanine from the carboxyterminal (Daly *et al.*, 1985). The actions of proline endopeptidase are inhibited by bacitracin. Thus, in the present study increased potency of angiotensin II, angiotensin III and [Ile⁷]angiotensin III in the presence of bacitracin is not surprising, as all three peptides possess identical carboxyterminal amino acids. Sequential cleavage of amino acids from the aminoterminal end of angiotensin II also contributes to the inactivation of this peptide. Aminopeptidase-A cleaves aspartate from angiotensin II to yield angiotensin III, which can be subsequently cleaved by aminopeptidase-M to inactive fragments (Kugler, 1982). At the concentration used in the present study, amastatin is a potent inhibitor of both aminopeptidase-A and aminopeptidase-M (Powers & Harper, 1988). The potentiating effect of amastatin on angiotensin III and its antagonist analogue [Ile⁷]angiotensin III in the ganglion is likely to be due to the inhibition of aminopeptidase-M. It is unlikely that aminopeptidase-A plays a role in the metabolism of angiotensin II in this tissue, as amastatin failed to increase the potency of angiotensin II.

The observations with the peptidase inhibitors highlight the problems associated with the use of peptide agents in this tissue. The ability of bacitracin and amastatin to influence differentially the potencies of angiotensin II and angiotensin III highlights the difficulties of sub-classifying angiotensin II receptors by relative potencies of the naturally occurring peptides in different tissues (see Peach, 1977). In addition, variable degradation of peptide antagonists could explain the differences in the ability of peptide angiotensin II antagonists to inhibit various angiotensin II-mediated effects in different tissues (see Introduction). As expected, the non-peptide antagonists appear much more stable in this respect. This is highlighted by the lack of effect of bacitracin on the antagonist potency of losartan.

The disulphide reducing agent DTT failed to abolish the angiotensin II-induced depolarization of the ganglion. Although DTT inhibits the binding of radioligands to AT_1 sites, its ability to inhibit AT_1 receptor-mediated responses in functional studies is less consistent. DTT has been shown to abolish functional responses produced by AT_1 receptor activation in rabbit aorta and rat liver (Fleisch *et al.*, 1973; Gunther, 1984), although the effect of DTT in the present study is less apparent. Furthermore, Barnes *et al.* (1991) found that DTT did not inhibit AT_1 receptor-mediated contractions of rat uterus. It is possible that this apparent functional selectivity of DTT reflects differences in the AT_1 receptors. Alternatively, differential accessibility of DTT to AT_1 receptors in different tissues is a possible explanation.

In conclusion, we have provided pharmacological evidence to show that depolarization of rat superior cervical ganglion by angiotensin II is mediated through angiotensin II receptors of the AT_1 subclass. As such, this preparation provides a useful assay for the investigation of the actions of angiotensin II in neuronal tissue.

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References

- BARNES, J.C., BROWN, J.D., HAWCOCK, A.B. & MICHEL, A.D. (1991). Angiotensin II-induced contractions of the isolated preparation of rat uterus are mediated through the AT_1 receptor subtype. *Br. J. Pharmacol.*, **104**, 43P.
- BROWN, D.A., CONSTANTIN, A. & MARSH, S. (1980). Angiotensin mimics the action of muscarinic agonists on rat sympathetic neurones. *Brain Res.*, **193**, 614-619.
- BUMPUS, F.M., CATT, K.J., CHIU, A.T., DEGASPARO, M., GOOD-FRIEND, T., HUSAIN, A., PEACH, M.J., TAYLOR, D.G. & TIMMERMAN, P.B.M.W.M. (1991). Nomenclature for angiotensin receptors. *Hypertension*, **17**, 720-721.
- CARRITHERS, M.D., RAMAN, V.K., MASUDA, S. & WEYHENMEYER, J.A. (1990). Effect of angiotensin II and III on inositol polyphosphate production in differentiated NG108-15 hybrid cells. *Biochem. Biophys. Res. Commun.*, **167**, 1200-1205.
- CHANG, R.S.L. & LOTTI, V.J. (1989). Two distinct angiotensin II recep-

- tor binding sites in rat adrenal revealed by new selective non-peptide ligands. *Mol. Pharmacol.*, **29**, 347–351.
- CHIU, A.T., HERBLIN, W.F., MCCALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, J.A., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989a). Identification of angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.*, **165**, 196–203.
- CHIU, A.T., MCCALL, D.E., NGUYEN, T.T., CARINI, D.J., DUNCIA, J.V., HERBLIN, W.F., UYEDA, R.T., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989b). Discrimination of angiotensin II receptor subtypes by dithiothreitol. *Eur. J. Pharmacol.*, **170**, 117–118.
- CRISCIONE, L., THOMANN, H., WHITEBREAD, S., DE GASPARO, M., BUHLMAYER, P., HEROLD, P., OSTERMAYER, F. & KAMBER, B. (1990). Binding characteristics and vascular effects of various angiotensin II antagonists. *J. Cardiovasc. Pharmacol.*, **16** (suppl. 4), S56–S59.
- DALY, D.J., MASKREY, P., MANTLE, D. & PENNINGTON, R.J.T. (1985). Proline endopeptidase in human muscle. *Biochem. Soc. Trans.*, **13**, 1161–1162.
- DUN, N.J., NISHI, S. & KARCZMAR, A.G. (1978). An analysis of the effect of angiotensin II on mammalian ganglion cells. *J. Pharmacol. Exp. Ther.*, **204**, 669–675.
- FLEISCH, J.H., KRZAN, M.C. & TITUS, E. (1973). Pharmacologic receptor activity of rabbit aorta. Effect of dithiothreitol and N-ethylmaleimide. *Circulation Res.*, **33**, 284–290.
- DE GASPARO, M., WHITEBREAD, S., MELE, M., MOTANI, A.S., WHITCOMBE, P.J., RAMJOUÉ, H.-P. & KAMBER, B. (1990). Biochemical characterisation of two angiotensin II receptor subtypes in the rat. *J. Cardiovasc. Pharmacol.*, **16** (suppl. 4), S31–S35.
- GUNTHER, S. (1984). Characterization of angiotensin II receptor subtypes in rat liver. *J. Biol. Chem.*, **259**, 7622–7629.
- IRELAND, S.J. (1987). Origin of 5-hydroxytryptamine-induced hyperpolarisation of the rat superior cervical ganglion and vagus nerve. *Br. J. Pharmacol.*, **92**, 407–416.
- JACKSON, T.R., BLAIR, L.A.C., MARSHALL, J., GOEDERT, M. & HANLEY, M.R. (1988). The mas oncogene encodes an angiotensin receptor. *Nature*, **335**, 437–440.
- KUGLER, P. (1982). Amino-peptidase A is angiotensinase A II. Biochemical studies on aminopeptidase A and M in rat kidney homogenate. *Histochemistry*, **74**, 247–261.
- MICHEL, A.D. & BARNES, J.C. (1991). The effect of guanine-nucleotides on agonist interactions with angiotensin II receptor subtypes. *Br. J. Pharmacol.*, **102**, 205P.
- MOORE, A.F., HALL, M.M. & KHAIRALLAH, P.A. (1976). A comparison of the effects of angiotensin II and heptapeptide on smooth muscle (vascular and uterine). *Eur. J. Pharmacol.*, **39**, 101–107.
- PEACH, M.J. (1977). Renin angiotensin system: Biochemistry and mechanism of action. *Physiol. Rev.*, **57**, 313–353.
- PEACH, M.J. & CHIU, A.T. (1974). Stimulation and inhibition of aldosterone biosynthesis *in vitro* by angiotensin II and analogs. *Circ. Res.*, **34–35**, (suppl. 1), I7–I13.
- POWERS, J.C. & HARPER, J.W. (1988). Inhibitors of metalloproteases. In *Proteinase Inhibitors*. ed. Barrett, A.J. & Salvesen, G. pp. 219–298. Amsterdam, New York: Elsevier.
- REIT, E. (1972). Actions of angiotensin on the adrenal medulla and autonomic ganglia. *Fed. Proc.*, **31**, 1338–1343.
- SARSTEDT, C.A., VAUGHAN, E.D. & PEACH, M.J. (1975). Selective inhibition by des-1-Asp-8-Ile-Angiotensin II of the steroidogenic response to restricted sodium intake in the rat. *Circ. Res.*, **37**, 284–291.
- SCANLON, M.N. & MOORE, G.J. (1988). Classification of angiotensin receptors in rat isolated uterus, portal vein, and aorta with novel competitive antagonist sarasin. *Pharmacol.*, **37**, 137–147.
- SPETH, R.C. & KIM, K.H. (1990). Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, p-aminophenylalanine⁶ angiotensin II. *Biochem. Biophys. Res. Commun.*, **169**, 997–1006.
- SPIELMAN, W.S., DAVIS, J.O. & FREEMAN, R.H. (1976). Des-Asp-1-Angiotensin II: Possible role in mediating the renin-angiotensin response in the rat. *Proc. Soc. Exp. Biol. Med.*, **151**, 177–182.
- WHITEBREAD, S., MELE, M., KAMBER, B. & DE GASPARO, M. (1989). Preliminary biochemical characterisation of two angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.*, **163**, 284–291.
- WONG, P.C., PRICE, W.A., CHIU, A.T., CARINI, D.J., DUNCIA, J.V., JOHNSON, A.L., WEXLER, R.R. & TIMMERMANS, P.B.M.W.M. (1990). Nonpeptide angiotensin II receptor antagonists. Studies with EXP9270 and DuP 753. *Hypertension*, **15**, 823–834.

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