Pharmacological characterization of the contractile responses to angiotensin analogues in guinea-pig isolated longitudinal muscle of small intestine

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1 The contractile responses to angiotensin II, angiotensin III and two synthetic analogues, [Lys²]angiotensin II and [Sar¹]angiotensin II, in the guinea-pig isolated longitudinal muscle preparation of small intestine have been characterized *in vitro*.

2 Tachyphylaxis to the angiotensin analogues was reduced by use of a Krebs-Henseleit solution containing a raised (sub-contractile) concentration of potassium (11.2 mM). Under these conditions, reproducible cumulative concentration-response curves to all agonists were established. The pD₂ estimates for angiotensin II, [Lys²]angiotensin II, angiotensin III and [Sar¹]angiotensin II were 9.15 ± 0.14, 7.42 ± 0.06, 7.69 ± 0.18 and 9.50 ± 0.15 respectively and the maximum responses achieved were not significantly different.

3 The contractile responses to angiotensin II, angiotensin III and [Sar¹]angiotensin II were reduced by greater than 80% by tetrodotoxin (TTX; 0.1 µM). However, the responses to [Lys²]angiotensin II were reduced by only 63 ± 5%. Atropine (0.1 µM) also reduced the responses to angiotensin II, angiotensin III and [Lys²]angiotensin II, although its effect was less than that produced by TTX. Furthermore, while responses to these agonists were not significantly modified by the NK₁ receptor antagonist (±)-CP-96,345 (30 nM) alone, the combined pre-incubation with both atropine and (±)-CP-96,345 reduced maximum agonist responses to a level not significantly different from those produced by TTX.

4 Indirect and direct contractile responses to angiotensin II and [Lys²]angiotensin II (in the presence of TTX) respectively were characterized by use of the selective AT₁ receptor antagonist, losartan and the AT₂ receptor antagonist, PD123177. Losartan produced parallel rightward displacement of the concentration-response curve to angiotensin II and [Lys²]angiotensin II, with an estimated pK_B of 8.56 (8.42–8.68) and 9.18 (8.63–9.50) respectively. The AT₂ receptor antagonist, PD123177 (3 µM) failed to modify the contractile responses to either angiotensin II or [Lys²]angiotensin II.

5 We conclude that two populations of angiotensin II receptors exist in the guinea-pig longitudinal muscle of small intestine, one located neuronally mediating the release of both acetylcholine and substance P and the other located on the smooth muscle mediating direct contractile responses. The neuronal component provides the major contribution to the agonist responses. Both receptor populations are of the AT₁ receptor subtype.

Keywords: Angiotensin; guinea-pig small intestine; losartan; angiotensin II receptor subtypes

Introduction

Early studies have shown that angiotensin II is a potent spasmogen of guinea-pig isolated ileum, acting via the release of acetylcholine and via direct stimulation of the smooth muscle (Robertson & Rubin, 1962; Godfraind *et al.*, 1966). However, these early observations have not been substantiated by others. Paiva *et al.* (1976) demonstrated that acetylcholine was not involved in the response to angiotensin II. In addition, while it has been shown that some indirectly acting spasmogens contract the guinea-pig ileum via the release of both acetylcholine and substance P (Buchheit *et al.*, 1985; Guard & Watson, 1987; Lucaites *et al.*, 1991), no detailed study has investigated the involvement of substance P in the response to angiotensin. Thus, further studies to characterize the nature of the contractile response to angiotensin II in this tissue are warranted.

Studies investigating the actions of angiotensin II in various isolated tissues are additionally complicated because of the susceptibility to tachyphylaxis (Paiva *et al.*, 1974; Shimuta *et al.*, 1989). As a consequence, antagonist studies have produced very limited quantitative data (Wong *et al.*, 1990). However, Miasiro *et al.* (1983) demonstrated that a synthetic analogue of angiotensin II, [Lys²]angiotensin II does not induce tachyphylaxis, compared to angiotensin II itself or

[Sar¹]angiotensin II. Thus, [Lys²]angiotensin II may provide a useful tool for characterization studies in tissues in which tachyphylaxis occurs readily. An additional way to reduce tachyphylaxis to angiotensin II may be to increase the potassium concentration in the bathing solution. This method has successfully prevented tachyphylaxis to the angiotensin II response in rat isolated femoral arteries (Juul *et al.*, 1987) and in the rat isolated uterus (Freer, 1975).

The subdivision of angiotensin II receptors into two subtypes, AT₁ and AT₂ is now well established (Bumpus *et al.*, 1991). Losartan (DuP 753) is a selective antagonist at the AT₁ subtype, while PD123177 is selective for the AT₂ subtype (Chiu *et al.*, 1989). Thus, the purpose of the present study was to characterize the contractile responses to a range of angiotensin analogues, angiotensin II, angiotensin III, [Lys²]angiotensin II and [Sar¹]angiotensin II in the guinea-pig isolated longitudinal muscle of small intestine (ileum and jejunum), a high potassium Krebs-Henseleit solution being used to reduce tachyphylaxis. To investigate whether the agonist responses are mediated by receptors on the smooth muscle or via the release of neurotransmitter, we have evaluated the actions of tetrodotoxin (TTX), atropine and the NK₁ receptor antagonist, (±)-CP-96,345 (Snider *et al.*, 1991). Also, using the non-peptide angiotensin II receptor antagonists, losartan and PD123177, we have characterized the angiotensin II receptor subtype involved.

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Methods

Male Dunkin-Hartley guinea-pigs weighing 200–250 g were killed by cervical dislocation. A 12–15 cm length of small intestine was removed proximal to the terminal 10 cm, and divided into 6 equal lengths. Each piece was placed on a 1 ml glass pipette and the longitudinal muscle layer with adhering myenteric plexus was separated from the circular muscle and mucosa by rubbing with a cotton bud. The muscle preparations were then mounted in 5 ml organ baths under 0.5 g tension.

Experiments were carried out in modified Krebs-Henseleit solution of composition (mM): NaCl 112.7, KCl 10, MgSO₄ 1.6, NaHCO₃ 24.9, KH₂PO₄ 1.2, glucose 5.6 and CaCl₂ 1.2 aerated with 5% CO₂ in oxygen, maintained at 37°C. Responses were recorded isotonicity on Bioscience T3 transducers. At the start of each experiment, increasing concentrations of [Lys²]angiotensin II were applied in a cumulative manner, ensuring that each concentration had achieved a maximal response before the addition of the next. Repeated cumulative concentration-response curves were constructed at 60 min intervals. The use of [Lys²]angiotensin II as a 'priming' agonist helped to stabilize angiotensin responses throughout the course of each experiment. With this procedure, up to 6 cumulative agonist concentration-response curves could be constructed reproducibly on any one tissue.

In the first series of experiments, responses to [Lys²]angiotensin II, angiotensin II, angiotensin III and [Sar¹]angiotensin II were compared. After stable responses to [Lys²]angiotensin II were established, cumulative concentration-response curves to angiotensin II, angiotensin III and [Sar¹]angiotensin II were constructed. In each experiment, one tissue receiving only [Lys²]angiotensin II served as a control. Agonist potency was expressed as pD₂, the – log of the effective concentration inducing 50% of the maximum response (EC₅₀). EC₅₀ values were measured graphically. Maximum responses (relative to [Lys²]angiotensin II) were also measured.

The effects of TTX, atropine and (±)-CP-96,345 against concentration-response curves to all agonists were then investigated. In these experiments, [Lys²]angiotensin II was again used as the 'priming' agonist until stable responses were achieved. Concentration-response curves to the test agonist without and 45 min following TTX (0.1 µM), atropine (0.1 µM), (±)-CP-96,345 (30 nM) or atropine plus (±)-CP-96,345 were then constructed. A control tissue receiving the test agonist alone was also included in each experiment.

In view of the residual response to [Lys²]angiotensin II (but not angiotensin II) remaining in the presence of TTX, the antagonist actions of losartan (1–100 nM) and PD123177 (3 µM) were investigated against both [Lys²]angiotensin II (in the presence of TTX, 0.1 µM) and angiotensin II. The antagonist preincubation time was 45 min for all experiments. To measure antagonist potency, parallel lateral displacements of the concentration-response curves were estimated by eye at the control EC₅₀ level, to give 'dose ratios' between drug-treated and control curves. Potency was expressed as the negative logarithm of the apparent dissociation constant (pK_B) obtained from the equation, $pK_B = \log(\text{dose ratio} - 1) - \log(\text{antagonist concentration})$. Where three or more concentrations of antagonist were used, Schild analysis (Arunlakshana & Schild, 1959) was applied and if the slope of the linear regression was not significantly different from unity, a pK_B estimate was obtained using a slope constrained to 1. Statistical analyses used Student's *t* test, with a criterion for statistical significance of $P < 0.05$.

The drugs used were supplied from the following sources: angiotensin II, angiotensin III, [Sar¹]angiotensin II (Peninsula laboratories), [Lys²]angiotensin II (Peptide & Protein Research consultants, Washington Singer Laboratories, University of Exeter, Perry Road, Exeter), losartan potassium (Du Pont Merck Pharmaceutical Co.), atropine sulphate, tetrodotoxin, (Sigma), PD123177 (1-(4-amino-3-methylphenyl)methyl-5-(diphenyl-acetyl)-4,5,6,7-tetrahydro-1H-imidazol

[4,5-C] pyridine-6-carboxylic acid) and (±)-CP-96,345 (*cis*-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo [2.2.2] octan-3-amine) (synthesized in our laboratories). All drugs were dissolved as the base in distilled water and diluted in Krebs-Henseleit solution.

Results

Effect of agonists

Cumulative concentration-response curves to angiotensin II, angiotensin III and [Sar¹]angiotensin II, compared to [Lys²]angiotensin II are shown in Figure 1. The curves of all agonists were parallel and maximum responses achieved were not significantly different. E_{max} values relative to [Lys²]angiotensin II (100%) were 99 ± 5% (angiotensin II), 99 ± 2% (angiotensin III) and 95 ± 5% ([Sar¹]angiotensin II). The mean (± s.e.mean) pD₂ estimates for angiotensin II, [Lys²]angiotensin II, angiotensin III and [Sar¹]angiotensin II were 9.15 ± 0.14, 7.42 ± 0.06, 7.69 ± 0.18 and 9.50 ± 0.15 respectively.

Effect of tetrodotoxin, atropine and (±)-CP-96,345

Table 1 shows the effect of TTX, atropine and (±)-CP-96,345 on responses to angiotensin II, [Lys²]angiotensin II, angiotensin III and [Sar¹]angiotensin II. The effect of TTX, atropine and (±)-CP-96,345 on the responses to angiotensin II are also expressed graphically in Figure 2. Preincubation with TTX (0.1 µM) markedly attenuated the responses to all agonists. Maximum responses to angiotensin II, angiotensin III and [Sar¹]angiotensin II were reduced by greater than 80%. However, there was a much greater residual response remaining to [Lys²]angiotensin II in the presence of TTX, and the maximum response was only reduced by 63 ± 5% (Table 1). TTX (0.1 µM) abolished responses to the indirectly acting nicotine receptor agonist, dimethylphenylpiperazinium (DMPP) (data not shown). Atropine also substantially reduced the maximum responses to angiotensin II, angiotensin III and [Lys²]angiotensin II, although less effectively than TTX (Table 1, Figure 2b). The concentration of atropine used in these experiments (0.1 µM) also abolished responses to DMPP (data not shown). The effects of the NK₁ receptor antagonist, (±)-CP-96,345 against responses to angiotensin II, angiotensin III and [Lys²]angiotensin II were evaluated alone or in combination with atropine. The concentration of (±)-CP-96,345 used was 30 nM, which has previously been shown to displace the concentration-response curve to the NK₁ receptor agonist substance P methylester by approximately 30 fold in the guinea-pig ileum (Beresford *et al.*, 1991). Concentrations of (±)-CP-96,345 greater than 30 nM could not be used because of antagonist activity at L-type calcium channels occurring at higher concentrations (Schmidt *et al.*, 1992). Preincubation of (±)-CP-96,345 alone reduced the maximum agonist responses by less than 10% (Table 1, Figure 2c). However, in combination with atropine (0.1 µM), maximum agonist responses achieved were much less than following preincubation with either (±)-CP-96,345 or atropine alone. Furthermore, maximum responses achieved under these conditions were not significantly different from those achieved in the presence of TTX (Table 1, Figure 2d).

Effects of antagonists

To characterize the angiotensin receptor mediating the indirect (TTX-sensitive) contractile responses, angiotensin II was used as the agonist, as responses to angiotensin II were almost abolished by TTX (see above). The effect of losartan on responses to angiotensin II are shown in Figure 3. Losar-

tan produced parallel, concentration-dependent rightward displacements of the angiotensin II concentration-response curve (Figure 3a). A total of 4 concentrations of losartan was used for Schild analysis and the Schild plot constructed from these data had a gradient of 1.03 (not significantly different from unity) (Figure 3b). The pK_B estimate (95% confidence

limits) was 8.56 (8.42–8.68).

To characterize the angiotensin II receptor mediating the direct contractile responses, $[Lys^2]$ angiotensin II was used as the agonist and all experiments were conducted in the presence of TTX (0.1 μM). Under these conditions, losartan displaced the concentration-response curve to $[Lys^2]$ angiotensin II to the right in a parallel and concentration-dependent manner (Figure 4). However, we encountered difficulties obtaining dose-ratio shifts greater than 10, because of the low potency of $[Lys^2]$ angiotensin II and the limitation of not being able to increase the agonist concentration high enough to re-establish agonist responses. Despite this, concentrations of 1 and 3 nM yielded an apparent pK_B estimate (95% confidence limits) of 9.16 (8.63–9.50) ($n = 7$). There was a significant difference ($P < 0.05$) between the pK_B estimate of losartan against $[Lys^2]$ angiotensin II and that obtained with angiotensin II as the agonist.

The AT_2 receptor antagonist, PD123177 (3 μM) had no significant effect on the concentration-response curve to angiotensin II or $[Lys^2]$ angiotensin II in the presence of TTX (data not shown).

Discussion

The occurrence of tachyphylaxis to angiotensin II in many isolated tissues has often limited detailed pharmacological investigations of the actions of this peptide *in vitro*. In the present study, using guinea-pig small intestine, we have shown that, in the presence of a raised concentration of potassium, just below that required to increase baseline contractile tone, cumulative concentration-response curves to $[Lys^2]$ angiotensin II, angiotensin II, angiotensin III and $[Sar^1]$ angiotensin II can be established. Comparison of the contractile responses to $[Lys^2]$ angiotensin II, angiotensin II, angiotensin III and $[Sar^1]$ angiotensin II revealed that the slopes of the concentration-response curves were not significantly different. Furthermore, there was no difference in the maximal contractile responses achieved. These observations suggest that there was little evidence for any marked and progressive desensitization to the agonists upon repeated application. Previous studies have shown that $[Sar^1]$ angiotensin II and angiotensin II in particular, induce tachyphylaxis in guinea-pig ileum very readily and near-maximal responses to these agonists have failed to induce consistent contractile responses upon subsequent application (Miasiro *et al.*, 1983; Shimuta *et al.*, 1989). Although the precise mechanisms by which increased potassium concentration can reduce tachyphylaxis in this and in other preparations (Freer, 1975; Juul *et al.*, 1987) are not fully understood, this method has allowed us to characterise successfully the contractile responses to different angiotensin analogues.

Angiotensin II and $[Sar^1]$ angiotensin II were the most potent agonists, angiotensin III and $[Lys^2]$ angiotensin II being approximately 30–100 fold weaker. While some of these data are comparable with previous studies in guinea-pig ileum (Shimuta *et al.*, 1989), other studies have shown angiotensin II and angiotensin III to be less potent (Robertson *et al.*, 1992; Schinke *et al.*, 1991). Furthermore, differences in the relative potencies of angiotensin II and angiotensin III in different tissues are also evident. In some tissues, angiotensin II and angiotensin III have been shown to be equipotent (Peach & Chiu, 1974; Jackson *et al.*, 1988). While differences in agonist orders of potency led to early speculation about the possible heterogeneity of angiotensin receptors (Peach 1977), it is now well established that potencies of some angiotensin peptide agonists can be markedly increased by inhibitors of peptidase enzymes (Robertson *et al.*, 1992; Hawcock *et al.*, 1992). Thus, from these observations as well as differences in the tachyphylactic properties of different peptides in different tissues, the use of agonist orders of potency to characterize angiotensin II receptors is limited.

Figure 1 Cumulative concentration-response curves to $[Lys^2]$ angiotensin II, angiotensin II, angiotensin III and $[Sar^1]$ angiotensin II in the guinea-pig isolated longitudinal muscle preparation of small intestine. Agonist responses were compared to $[Lys^2]$ angiotensin II (●) on the same tissue. Open symbols represent responses to (a) angiotensin II, (b) angiotensin III and (c) $[Sar^1]$ angiotensin II. All responses are expressed as mean ($\pm$ s.e.mean) values, as percentage of the maximum control response (E_{max}) to $[Lys^2]$ angiotensin II ($n = 4-6$).

Table 1 Effect of tetrodotoxin (TTX), atropine and (±)-CP-96,345 on the maximum responses to [Lys²]angiotensin II, angiotensin II, angiotensin III and [Sar¹]angiotensin II in guinea-pig isolated longitudinal muscle preparation of small intestine

Agonist	TTX (0.1 μM)	Atropine (30 nM)	(±)-CP-96,345 (30 nM)	Atropine plus (±)-CP-96,345
[Lys ²]angiotensin II	63 ± 5	33 ± 4	9 ± 1	72 ± 6
Angiotensin II	88 ± 3	60 ± 7	6 ± 4	84 ± 2
Angiotensin III	80 ± 4	49 ± 13	10 ± 6	84 ± 4
[Sar ¹]angiotensin II	81 ± 5	ND	ND	ND

Values are expressed as mean (± s.e.mean) % reduction of control E_{max} . ($n = 4-6$). ND, not determined.

Figure 2 Effect of tetrodotoxin (TTX), atropine, (±)-CP-96,345 and atropine plus (±)-CP-96,345 on contractile responses to angiotensin II in the guinea-pig isolated longitudinal muscle preparation of small intestine. The filled symbols represent control responses to angiotensin II. The open symbols represent responses to angiotensin II in the presence of (a) TTX (0.1 μM), (b) atropine (0.1 μM), (c) (±)-CP-96,345 (30 nM) and (d) atropine plus (±)-CP-96,345. All responses are expressed as mean (± s.e.mean) values, as a percentage of the maximum control response (E_{max}) to angiotensin II ($n = 4-6$).

A more important observation revealed by the use of different agonists in the present studies is the possibility that different mechanisms of action may exist. Experiments using TTX, atropine and the tachykinin NK_1 receptor antagonist (±)-CP-96,345 have shown that the contractile responses to all angiotensin agonists are mediated in part via the release both of acetylcholine and a tachykinin, probably substance P, from the myenteric plexus. The ability of atropine and (±)-CP-96,345, applied in combination, to reduce all agonist responses to a level not significantly different from that measured in the presence of TTX indicates that acetylcholine and substance P are the two major neurotransmitters which mediate the indirect responses in this tissue. Furthermore, in contrast to angiotensin II, angiotensin III and [Sar¹]angiotensin II, a significant proportion of the response to [Lys²]angiotensin II was insensitive to the actions of TTX. As this residual response could not be inhibited further by atropine or (±)-CP-96,345 (data not shown), it is unlikely that [Lys²]angiotensin II acts to release acetylcholine or substance P by direct depolarization of nerve terminals. Thus, the most likely explanation is a direct effect via receptors on the smooth muscle. With regard to the indirect responses, it is interesting that (±)-CP-96,345, in the absence of atropine, produced very little reduction of the contractions produced

Figure 3 Effect of losartan on contractile responses to angiotensin II in the guinea-pig isolated longitudinal muscle preparation of small intestine. In (a) the filled symbols represent control responses to angiotensin II. The open symbols represent responses to angiotensin II in the presence of losartan 10 nM (○) or 30 nM (Δ). All responses are expressed as mean (± s.e.mean) values, as a percentage of the maximum control response (E_{max}) to angiotensin II ($n = 3$). (b) Shows the Schild plot, using 4 concentrations of losartan (2 determinations at 3 or 100 nM and 3 determinations at 10 or 30 nM).

by all agonists. As the concentration of (±)-CP-96,345 used has been shown to produce a 30 fold displacement of the concentration-response curve to an NK_1 receptor agonist in

Figure 4 Effect of losartan on contractile responses to [Lys²]angiotensin II (in the presence of tetrodotoxin 0.1 μ M) in the guinea-pig isolated longitudinal muscle preparation of small intestine. The filled symbols represent control responses to angiotensin II. The open symbols represent responses to angiotensin II in the presence of losartan 1 nM (∇), or 3 nM ($\square$). All responses are expressed as mean ($\pm$ s.e.mean) values, as a percentage of the maximum control response ($E_{\max}$) to [Lys²]angiotensin II ($n = 3-4$).

the guinea-pig isolated ileum preparation (Beresford *et al.*, 1991), it is unlikely that the lack of effect of this non-peptide antagonist alone is due to insufficient antagonism at NK₁ receptors. We would prefer to conclude that the lack of effect of ($\pm$)-CP-96,345 alone more probably relates to the relatively minor role of substance P compared to acetylcholine in mediating contractile responses to these angiotensin analogues under normal conditions and only when the actions of acetylcholine are inhibited can substance P exert its contractile effect. However, while the inhibition of L-type calcium channels by higher concentrations of ($\pm$)-CP-96,345 (Schmidt *et al.*, 1992) limits extensive use of this antagonist, further studies using a wider concentration range of more specific non-peptide NK₁ receptor antagonists such as CP-99,994 (McLean *et al.*, 1992) could be used to substantiate these findings.

Assuming that the TTX-sensitive and insensitive components of the agonist responses are due to activation of angiotensin receptors located neurally and on the smooth

muscle respectively, we attempted to characterize both populations of receptors using the AT₁ selective antagonist, losartan and the AT₂ selective antagonist, PD123177. Angiotensin II was the agonist used to characterize the neuronal receptors, and [Lys²]angiotensin II, in the presence of TTX was used to study the receptors on the smooth muscle. The AT₁ receptor antagonist losartan produced concentration-related parallel displacements of angiotensin II curves, yielding a pK_B estimate of 8.56. The potency of losartan is close to that previously measured against responses to angiotensin II in other tissues (Barnes *et al.*, 1991; Hawcock *et al.*, 1992; Robertson *et al.*, 1992). Furthermore, these findings agree with previous studies showing AT₁ receptor-mediated contractile responses to angiotensin II in guinea-pig (Wong *et al.*, 1990) and rat ileum (Schinke *et al.*, 1991). The lack of effect of the AT₂ receptor selective antagonist, PD123177 confirms that the neuronal responses to angiotensin II are mediated by the AT₁ receptor. Experiments conducted in the presence of TTX, using [Lys²]angiotensin II as the agonist confirmed that receptors on the smooth muscle were also of the AT₁ subtype. Losartan, but not PD123177, antagonized responses to [Lys²]angiotensin II. It is interesting that losartan was approximately 4 fold more potent in antagonizing responses to [Lys²]angiotensin II on the smooth muscle than those mediated neurally by angiotensin II. The significance of this is not yet clear. However, in view of the recent evidence from cloning studies highlighting the existence of more than one losartan-sensitive angiotensin receptor (Iwai *et al.*, 1992; Sandberg *et al.*, 1992), it is tempting to speculate that this difference may relate to agonist actions at different angiotensin receptor subtypes. Sandberg *et al.* (1992) have shown a similar difference in the affinity of losartan for displacement of angiotensin binding to the two cloned receptor subtypes. Furthermore, a distinction between neuronal and smooth muscle receptors of other neuropeptides in the guinea-pig ileum has been previously established (see Laufer *et al.*, 1988 and references cited therein). However, further work is required to substantiate this possibility.

In conclusion, we have demonstrated that in the presence of raised potassium concentration in the bathing medium, it is possible to construct cumulative concentration-response curves to angiotensin II and related agonist analogues. Under these conditions, two populations of angiotensin receptors have been shown to exist. One receptor population is located neurally and mediates release of acetylcholine and substance P. The other is located on the smooth muscle and mediates direct contraction. Both of these receptor populations appear to be of the AT₁ receptor subtype.

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References

- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14**, 48–58.
- 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 AT₁ receptor subtype. *Br. J. Pharmacol.*, **104**, 43P.
- BERESFORD, I.J.M., BIRCH, P.J., HAGAN, R.M. & IRELAND, S.J. (1991). Investigation into species variants in tachykinin NK₁ receptors by use of the non-peptide antagonist, ($\pm$)-CP-96,345. *Br. J. Pharmacol.*, **104**, 292–293.
- BUCHHEIT, K.H., ENGEL, G., MUTSCHLER, E. & RICHARDSON, B. (1985). A study of the contractile effects of 5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **329**, 36–41.
- BUMPUS, F.M., CATT, K.J., CHIU, A.T., DE GASPARO, M., GOODFRIEND, T., HUSAIN, A., PEACH, M.J., TAYLOR, D.G. & TIMMERMANS, P.B.M.W.M. (1991). Nomenclature for angiotensin receptors. *Hypertension*, **17**, 720–721.
- CHIU, A.T., HERBLIN, W.F., MCCALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, J.V., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989). Identification of angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.*, **165**, 196–203.
- FREER, R.J. (1975). Calcium and angiotensin tachyphylaxis in rat uterine smooth muscle. *Am. J. Physiol.*, **228**, 1423–1430.
- GODFRAIND, T., KABA, A. & POLSTER, P. (1966). Specific antagonism to the direct and indirect action of angiotensin on isolated guinea-pig ileum. *Br. J. Pharmacol. Chemother.*, **28**, 93–104.
- GUARD, S. & WATSON, S.P. (1987). Evidence for neurokinin-3 receptor-mediated tachykinin release in the guinea-pig ileum. *Eur. J. Pharmacol.*, **144**, 409–412.
- HAWCOCK, A.B., BARNES, J.C. & MICHEL, A.D. (1992). Pharmacological characterisation of angiotensin-induced depolarisations of rat isolated superior cervical ganglion *in vitro*. *Br. J. Pharmacol.*, **105**, 686–690.

- IWAI, N. & INAGAMI, T. (1992). Identification of 2 subtypes in the rat type 1 angiotensin II receptor. *FEBS Lett.*, **298**, 257–260.
- 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.
- JUUL, B., AALKJAER, C. & MULVANY, M.J. (1987). Responses of femoral resistance vessels to angiotensin *in vitro*. *Eur. J. Pharmacol.*, **135**, 61–68.
- LAUFER, R., GILON, C., CHOREV, M. & SELINGER, Z. (1988). Desensitisation with a selective agonist discriminates between multiple tachykinin receptors. *J. Pharmacol. Exp. Ther.*, **245**, 639–643.
- LUCAITES, V.L., MENDELSON, L.G., MASON, N.R. & COHEN, M.L. (1991). CCK-8, CCK-4 and gastrin-induced contractions in guinea-pig ileum: evidence for differential release of acetylcholine and substance P by CCK-A and CCK-B receptors. *J. Pharmacol. Exp. Ther.*, **256**, 695–703.
- MCLEAN, S., GANONG, A., SEYMOUR, P.A., SNIDER, R.M., DESAI, M.C., ROSEN, T., BRYCE, D.K., LONGO, K.P., REYNOLDS, L.S., ROBINSON, G., SCHMIDT, A.W., SIOK, C. & HEYM, J. (1992). Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin NK₁ receptor. *Regul. Pept.*, (Suppl 1), S120.
- MIASIRO, N., OSHIRO, M.E.M., PAIVA, T.B. & PAIVA, A.C.M. (1983). Role of the two N-terminal residues of angiotensin II in the production of tachyphylaxis. *Eur. J. Pharmacol.*, **87**, 397–406.
- PAIVA, T.B., JULIANO, L., NOUAILHETAS, V.L.A. & PAIVA, A.C.M. (1974). The effect of pH on tachyphylaxis to angiotensin peptides in the isolated guinea-pig ileum and rat uterus. *Eur. J. Pharmacol.*, **25**, 191–196.
- PAIVA, T.B., MENDES, G.B., ABOULAFIA, J. & PAIVA, A.C.M. (1976). Evidence against cholinergic mediation of the effect of angiotensin II on the guinea-pig ileum. *Pflügers Arch.*, **365**, 129–133.
- 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 analogues. *Circ. Res.*, **34–35**, (suppl 1), 17–113.
- ROBERTSON, M.J., CUNEOOSAMY, M.P. & CLARK, K.L. (1992). Effect of peptidase inhibition on angiotensin receptor agonist and antagonist potency in rabbit isolated thoracic aorta. *Br. J. Pharmacol.*, **106**, 166–172.
- ROBERTSON, P.A. & RUBIN, D. (1962). Stimulation of intestinal nervous elements by angiotensin. *Br. J. Pharmacol. Chemother.*, **19**, 5–12.
- SANDBERG, K., JI, H., CLARK, A.J.L., SHAPIRA, H. & CATT, K.J. (1992). Cloning and expression of a novel angiotensin II receptor subtype. *J. Biol. Chem.*, **267**, 9455–9458.
- SCHINKE, M., DOODS, H.N., GANTEN, D., WIENEN, W. & ENTZEROTH, M. (1991). Characterisation of rat intestinal angiotensin II receptors. *Eur. J. Pharmacol.*, **204**, 165–170.
- SCHMIDT, A.W., MCLEAN, S. & HEYM, J. (1992). The substance P receptor antagonist CP-96,345 interacts with Ca²⁺ channels. *Eur. J. Pharmacol.*, **215**, 351–352.
- SHIMUTA, S.I., NAKAIE, C.R., PAIVA, A.C.M., CORDOPATIS, P. & THEODOROPoulos, D. (1989). Changes in residues 1 and 4 of angiotensin II affect differently its agonist and tachyphylactic properties. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 309–313.
- SNIDER, R.M., CONSTANTINE, J.W., LOWE, J.A., LONGO, K.P., LEBEL, W.S., WOODY, H.A., DROZDA, S.E., DESAI, M.C., VINICK, F.J., SPENCER, R.W. & HESS, H.-J. (1991). A potent nonpeptide antagonist of the substance P (NK₁) receptor. *Science*, **251**, 435–437.
- WONG, P.C., PRICE, W.A., CHIU, A.T., CARINI, D.J., DUNCIA, J.V., JOHNSON, A.L., WEXLER, R.R. & TIMMERMAN, 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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