Inhibition of the haemodynamic effects of angiotensin II in conscious rats by AT₂-receptor antagonists given after the AT₁-receptor antagonist, EXP 3174

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1 Conscious, Long Evans rats ($n = 10$), chronically instrumented for the measurement of regional haemodynamics, were studied on 3 consecutive experimental days to assess responses to angiotensin II (AII) (125 pmol kg^{-1} , i.v.) and noradrenaline (1 nmol kg^{-1} , i.v.) in the absence and presence of the AT₂-receptor antagonist, PD 123319 (10 mg kg^{-1} , i.v.) (day 1), the AT₁-receptor antagonist, EXP 3174 (1 mg kg^{-1} , i.v.) (day 2), and PD 123319 (10 mg kg^{-1} , i.v.) given 24 h after EXP 3174 (day 3).

2 In naive rats (day 1), PD 123319 did not antagonize the haemodynamic effects of AII or noradrenaline. EXP 3174 (day 2) caused a marked, prolonged blockade of the haemodynamic effects of AII but not those of noradrenaline. Twenty four h after administration of EXP 3174 (day 3) there was still significant attenuation of the haemodynamic effects of AII. However, administration of PD 123319 at this time caused a further inhibition (lasting 1 h) of the effects of AII but not those of noradrenaline.

3 An identical 3 day protocol was used in a separate group of rats ($n = 6$) in which the AT₂-receptor antagonist, PD 123177, was given instead of PD 123319, and the results were essentially the same, i.e., PD 123177 significantly attenuated the haemodynamic effects of AII but only when given 24 h after EXP 3174.

4 In a separate group of rats ($n = 4$), a low dose of EXP 3174 ($60 \mu\text{g kg}^{-1}$ i.v.) was given to naive rats in order to simulate the degree of inhibition of the effects of AII seen after administration of AT₂-receptor antagonists in animals pretreated with EXP 3174. This low dose of EXP 3174 did not produce a sustained inhibition of the effects of AII and the time course of recovery of AII responses was similar to that seen with PD 123319 or PD 123177 given after the high dose of EXP 3174.

5 The apparent inhibition of the effects of AII by the AT₂-receptor antagonists, PD 123319 and PD 123177, when these were administered 24 h after the AT₁-receptor antagonist, EXP 3174, may have been due to the functional activation of AT₂-receptors and/or loss of AT₂-receptor antagonist selectivity, and/or the displacement of nonspecifically bound EXP 3174 by AT₂-receptor antagonists. While the latter explanation seems the most likely, these results raise the possibility that nonpeptide, AII-receptor antagonists that act at both AT₁- and AT₂-receptors may have therapeutic advantages over selective AT₁-receptor antagonists.

Keywords: Angiotensin II; EXP 3174; PD 123319; PD 123177; conscious rats; haemodynamic effects

Introduction

Recently, the development of several nonpeptide angiotensin II (AII) antagonists, and their application in radioreceptor assays has provided convincing evidence for the existence of two subtypes of the AII receptor (Chiu *et al.*, 1989; Whitebread *et al.*, 1989). Based on studies using nonpeptide antagonists such as DuP 753 (Losartan) (Chiu *et al.*, 1990; Wong *et al.*, 1990b), PD 123177 (Chiu *et al.*, 1989; Wong *et al.*, 1990a) and PD 123319 (Dudley *et al.*, 1990), a new receptor classification for AII has been proposed (Bumpus *et al.*, 1991). The AII receptor subtype sensitive to either DuP 753, or its active metabolite, EXP 3174 (Wong *et al.*, 1990c), has been designated AT₁, while the receptor subtype sensitive to either PD 123319 or PD 123177 has been designated AT₂.

DuP 753 is approximately 10,000 fold more selective for the AT₁-, than the AT₂-receptor, while PD 123177 displays about a 3500 fold selectivity for AT₂-receptors (Timmermans *et al.*, 1991). AT₁- and AT₂-receptors are widely distributed in the brain and peripheral tissues; however, the majority of AII receptors in vascular tissue and in central cardiovascular control regions sensitive to AII are of the AT₁ subtype (Whitebread *et al.*, 1989; Gehlert *et al.*, 1991). Furthermore, all the cardiovascular and fluid homeostatic effects of AII

appear to be mediated through stimulation of AT₁-receptors. As yet, there has been no functional role identified for the AT₂-receptor (Dudley *et al.*, 1990; Wong *et al.*, 1990a), although a novel signal transduction mechanism has recently been identified (Bottari *et al.*, 1992).

The possibility exists that AT₂-receptors are clearance receptors, analogous to those for atrial natriuretic peptide (Maack *et al.*, 1987). However, Wong *et al.* (1990a) found that pretreatment with AT₂-receptor antagonists did not augment responses to AII acutely, so this possibility seems unlikely. Furthermore, the ability of AT₁-receptor antagonists to abolish all known effects of AII indicates that AT₂-receptors are not involved in the effects of AII under normal, acute circumstances. Nevertheless, considering the long duration of action of peripherally-administered AT₁-receptor antagonists it seemed feasible that in the period following administration of AT₁-receptor antagonists when responses to AII were recovering, a functional role for AT₂-receptors might be discernible. To assess this possibility, we gave the non-competitive AT₁-receptor antagonist, EXP 3174 (Wong *et al.*, 1990c) to conscious rats, and determined whether or not the haemodynamic effects of AII were sensitive to the subsequent administration of the AT₂-receptor antagonists, PD 123319 or PD 123177. A preliminary account of this study has been published (Widdop *et al.*, 1992).

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Methods

Male, Long Evans rats (350 to 450 g) were anaesthetized (sodium methohexitone, 60 mg kg⁻¹, i.p. supplemented as required) and had pulsed Doppler flow probes implanted to monitor changes in renal, mesenteric and hindquarters blood flows (Gardiner *et al.*, 1990a). At least 7 days later, animals were briefly anaesthetized (sodium methohexitone, 40 mg kg⁻¹, i.p.) and had 3 catheters implanted in the right jugular vein and a catheter inserted into the distal aorta, via the caudal artery. After a further 24 h recovery, experiments were begun in conscious, unrestrained animals with free access to food and water.

Continuous recordings were made of mean and phasic intra-arterial blood pressures and instantaneous heart rate, and the mean and phasic renal, mesenteric and hindquarters Doppler shift signals with a modified (Gardiner *et al.*, 1990b) pulsed Doppler system (Crystal Biotech, Holliston, U.S.A.). Regional vascular conductances were calculated by dividing the appropriate mean Doppler shift by mean arterial blood pressure (Gardiner *et al.*, 1990a).

Experiment 1

This experiment lasted for 3 days. On day 1, animals ($n = 8$) received randomized bolus i.v. injections of AII (125 pmol kg⁻¹) and noradrenaline (1 nmol kg⁻¹) at least 1 h before, and at approximately 15 min and 1 and 2 h after PD 123319 (10 mg kg⁻¹). On day 2, bolus i.v. injections of AII and noradrenaline were given at least 1 h before and at approximately 15 min, and 1, 2 and 6 h after administration of EXP 3174 (1 mg kg⁻¹). On day 3, AII and noradrenaline were given at least 1 h before (i.e. 24 h after EXP 3174) and at approximately 15 min, and 1 and 2 h after PD 123319 (10 mg kg⁻¹).

To avoid any possible interaction arising from the repeated administration of PD 123319, only the latter 2 days of the above protocol were run in 2 additional animals. Since these data provided essentially identical results to the full, 3 day protocol, these data have been included for days 2 and 3 (i.e. $n = 10$).

In 6 of these animals, EXP 3174 (1 mg kg⁻¹) was given 2 h after PD 123319 (10 mg kg⁻¹) on day 3 and responses to AII were assessed 15 min later.

Experiment 2

The protocol for this experiment was identical to Experiment 1 except that animals ($n = 6$) received PD 123177 (10 mg kg⁻¹) instead of PD 123319, to ensure the results with these AT₂-receptor antagonists were similar.

Experiment 3

It became apparent in Experiments 1 and 2 that both AT₂-receptor antagonists, PD 123319 and PD 123177, attenuated the haemodynamic effects of AII (by 40–60%), but only when tested 24 h after the administration of the AT₁-receptor antagonist EXP 3174 (see Results). Moreover, under these circumstances, the effects of PD 123319 and PD 123177 were relatively short-lasting (see Results). Therefore, in order to assess the time course of recovery from the effects of a dose of EXP 3174 that inhibited responses to AII to an extent similar to that seen after administration of AT₂-receptor antagonists in animals pretreated with EXP 3174, naive rats ($n = 4$) were given a lower dose of EXP 3174 (60 µg kg⁻¹) and the responses to AII (125 pmol kg⁻¹ i.v.) were assessed before and 15 min, and 1 and 2 h after this compound.

The 60 µg kg⁻¹ dose of EXP 3174 was determined from pilot experiments, and agrees well with the IC₅₀ value of EXP 3174 against the pressor effects of AII in conscious normotensive rats studied by Wong *et al.* (1990c).

Data analysis

The effects of the nonpeptide antagonists against either AII or noradrenaline on each experimental day were subjected to an initial analysis of variance (ANOVA) with repeated measures using a commercially available statistical package (CLR ANOVA on a Macintosh SE). Additionally, the effects of antagonists relative to baseline were assessed in a similar manner. A P value < 0.05 was taken as significant.

Drugs

Angiotensin II (Bachem), noradrenaline bitartrate (Sigma), PD 123177 (1-[(4-amino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazol[4,5-c]pyridine-6-carboxylic acid), and PD 123319 ((S)-1-[[4-(di-methylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazol[4,5-c]pyridine-6-carboxylic acid) were dissolved in isotonic saline, while EXP 3174 ((2-n-butyl-4-chloro-1-[(2'-1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid) was dissolved in equal parts of 5% NaHCO₃ and 5% dextrose. EXP 3174 and PD 123177 were obtained from Dr R. Smith (DuPont, U.S.A.) and PD 123319 was obtained from Dr J. Keiser (Parke-Davis, U.S.A.). AII and the nonpeptide antagonists were injected via separate i.v. catheters and flushed in with 0.1 ml saline (that being the catheter dead space).

Figure 1 Cardiovascular responses to angiotensin II (AII) (125 pmol kg⁻¹, i.v.) before and 15 min and 1 and 2 h after administration of the AT₂-receptor antagonist, PD 123319 (10 mg kg⁻¹, i.v.) in conscious, Long Evans rats ($n = 8$) on the first experimental day. Values are mean with s.e.mean shown by vertical lines.

Results

Experiment 1

Day 1 AII (125 pmol kg⁻¹, i.v.) caused marked pressor and bradycardic responses, accompanied by pronounced reductions in renal and mesenteric flow, but smaller changes in hindquarters flow (see Figures 1 and 2). Hence, there were marked reductions in renal and mesenteric conductance, as well as a small reduction in hindquarters conductance. This haemodynamic pattern of marked renal and mesenteric vasoconstriction, with a negligible hindquarters effect in response to AII has been reported previously from our laboratory (Gardiner *et al.*, 1988).

Noradrenaline (1 nmol kg⁻¹, i.v.) evoked changes similar to, but less marked, than those seen with AII; they consisted of increases in blood pressure (34 ± 2 mmHg) and decreases in heart rate (−55 ± 8 beats min⁻¹), renal (−35 ± 4%), mesenteric (−65 ± 2%) and hindquarters (−5 ± 5%) flows, and decreases in renal (−50 ± 3%), mesenteric (−73 ± 2%) and hindquarters (−27 ± 3%) conductances.

PD 123319 (10 mg kg⁻¹, i.v., *n* = 8), itself, caused a transient tachycardia (at 1 min, 29 ± 7 beats min⁻¹, *P* < 0.05), together with transient increases in renal flow (at 1 min, 10 ± 2%, *P* < 0.01), mesenteric flow (1–2 min, maximum = 15 ± 2%, *P* < 0.01), renal conductance (at 1 min, 11 ± 2%, *P* < 0.01), and mesenteric conductance (1–3 min, maximum = 15 ± 2%, *P* < 0.05). PD 123319 did not significantly alter the haemodynamic effects of AII (Figure 1) or noradrenaline (data not shown), when these were tested over the following 2 h.

Day 2 The control haemodynamic effects of AII (and noradrenaline) were similar to those obtained on day 1

(Figure 3). EXP 3174 (1 mg kg⁻¹ i.v., *n* = 10), itself, caused a modest change in haemodynamic status, with the maximum effects occurring within 15 min after injection; there was a small fall in blood pressure (4–15 min, maximum = −7 ± 2 mmHg, *P* < 0.01) and increases in heart rate (1–15 min, maximum = 46 ± 11 beats min⁻¹, *P* < 0.01), renal flow (1–15 min, maximum = 17 ± 5%, *P* < 0.01), mesenteric flow (1–15 min, maximum = 15 ± 4%, *P* < 0.05), renal conductance (1–15 min, maximum = 22 ± 6%, *P* < 0.01), mesenteric conductance (1–15 min, maximum = 22 ± 5%, *P* < 0.01) and hindquarters conductance (at 15 min, 13 ± 7%, *P* < 0.05).

The effects of AII were abolished when it was administered 15 min after EXP 3174, and even 6 h after the latter compound the effects of AII were still substantially inhibited (Figures 2 and 3). However, hindquarters flow was increased by AII to a similar extent throughout the 6 h period. As a consequence, when the pressor response to AII was abolished by EXP 3174, this resulted in an increase in hindquarters conductance. In contrast to AII, the effects of noradrenaline were not significantly altered by EXP 3174 (data not shown).

Day 3 Twenty four h after the administration of EXP 3174, the haemodynamic effects of AII were still significantly attenuated compared with the effects of AII given before EXP 3174 (Figure 4). Administration of PD 123319 (10 mg kg⁻¹, i.v., *n* = 10) at this time again caused transient increases in heart rate (1–3 min, maximum = 68 ± 1 beats min⁻¹, *P* < 0.01), renal flow (1–4 min, maximum = 10 ± 2%, *P* < 0.05) and mesenteric flow (1–2 min, maximum = 13 ± 3%, *P* < 0.05). Additionally, there was a small, but significant, pressor response (1–2 min, maximum = 6 ± 2 mmHg, *P* < 0.05), which was not seen on day 1.

Fifteen min after the administration of PD 123319 on day

Figure 2 Cardiovascular responses to angiotensin II (AII) (125 pmol kg⁻¹, i.v.) in a conscious Long Evans rat before and approximately 20 min after the AT₁-receptor antagonist, EXP 3174 (1 mg kg⁻¹, i.v.), on the second experimental day.

Figure 3 Cardiovascular responses to angiotensin II (AII) (125 pmol kg^{-1} , i.v.) on the first experimental day (d1) (open columns) and on the second experimental day (d2) (solid columns), and then at the times indicated after administration of the AT_1 -receptor antagonist, EXP 3174 (1 mg kg^{-1} , i.v.) on day 2 in conscious, Long Evans rats ($n = 10$). Values are mean with s.e.mean shown by vertical lines; * $P < 0.05$, ** $P < 0.01$ versus day 2 control value (ANOVA).

3, the pressor effect of AII was significantly attenuated relative to the control response of that day. Furthermore, the reductions in renal and mesenteric flows and conductances evoked by AII were attenuated 15 and 60 min after PD 123319 (Figure 4). However, 2 h after PD 123319, the effects of AII were not significantly different from the control responses of that day (Figure 4). In addition, the effects of noradrenaline were not significantly altered by PD 123319 at any time (data not shown).

EXP 3174 (1 mg kg^{-1} , i.v.), given 2 h after the administration of PD 123319 on day 3, abolished the response to AII when tested 15 min later ($n = 6$) (Figure 4).

Experiment 2

Day 1 PD 123177 (10 mg kg^{-1} , i.v., $n = 4$), itself, caused no significant changes in baseline haemodynamic variables, except for a significant tachycardia (1–3 min, maximum = $49 \pm 5 \text{ beats min}^{-1}$, $P < 0.01$). Moreover, PD 123177 had no significant effect on the haemodynamic responses to either AII or noradrenaline when tested 15 min, and 1 and 2 h later (data not shown).

Figure 4 Cardiovascular responses to angiotensin II (AII) (125 pmol kg^{-1} , i.v.) on the second experimental day (d2) (open columns) and 24 h after EXP 3174, i.e., on the third experimental day (d3) (solid columns), and then at the times indicated after administration of the AT_2 -receptor antagonist, PD 123319 (10 mg kg^{-1} , i.v. $n = 10$) on day 3. Finally, EXP 3174 (1 mg kg^{-1} , i.v., $n = 6$) was given approximately 2 h after PD 123319 and the response to AII was tested 15 min later. Values are mean with s.e.mean shown by vertical lines.

†Between the 2 left-hand histograms indicate significant differences between day 2 and day 3 ($P < 0.01$); asterisks over subsequent histograms (* $P < 0.05$; ** $P < 0.01$) show significant differences versus day 3 control values (ANOVA).

Day 2 EXP 3174 (1 mg kg^{-1} , i.v., $n = 6$) abolished the effects of AII but not noradrenaline, as described in Experiment 1 (data not shown).

Day 3 Twenty four h after EXP 3174, the response to AII was still significantly attenuated, as observed in Experiment 1 (Figure 6). PD 123177 (10 mg kg^{-1} , i.v., $n = 6$), itself, caused a transient tachycardia (1–3 min, maximum = $32 \pm 5 \text{ beats min}^{-1}$, $P < 0.01$) and transient increases in mesenteric flow (at 1 min, $13 \pm 3\%$, $P < 0.01$) and conductance (1–5 min, maximum = $17 \pm 3\%$, $P < 0.01$); all other variables did not change significantly from baseline.

Following the administration of PD 123177, responses to AII were inhibited in a manner similar to that already described for PD 123319 (Figures 5 and 6). Responses to noradrenaline were unaffected by PD 123177 (data not shown).

Figure 5 Cardiovascular responses to angiotensin II (AII) (125 pmol kg^{-1} , i.v.) on day 3, in the same, conscious, Long Evans rat as shown in Figure 2. AII was tested before and at the times indicated after the AT₂-receptor antagonist, PD 123177 (10 mg kg^{-1} , i.v.).

EXP 3174 (1 mg kg^{-1} , i.v.), given 2 h after PD 123177 abolished all responses to AII when this was given 15 min later ($n = 3$).

Experiment 3

EXP 3174 ($60 \mu\text{g kg}^{-1}$, i.v., $n = 4$) caused an increase in renal flow (1–15 min, maximum = $23 \pm 4\%$, $P < 0.01$) and conductance (1–15 min, maximum = $27 \pm 6\%$, $P < 0.01$) and a decrease in hindquarters flow (at 10 min, $-13 \pm 3\%$, $P < 0.05$); all other variables did not change significantly from baseline.

The haemodynamic effects of AII were significantly attenuated (by approximately 40 to 60%) 15 min after the administration of EXP 3174 ($60 \mu\text{g kg}^{-1}$, i.v., $n = 4$) in naive rats (Figure 7). The degree of inhibition of the effects of AII caused by this dose of EXP 3174 was similar to that observed in Experiments 1 and 2, when AII was given 15 min after either PD 123319 or PD 123177 on day 3, i.e. 24 h after administration of EXP 3174 (1 mg kg^{-1}). Furthermore, 2 h after EXP 3174 ($60 \mu\text{g kg}^{-1}$), the mesenteric responses to AII had fully recovered, while the AII-induced pressor response and reductions in renal flow and conductance were still significantly impaired (Figure 7).

Discussion

The major finding from the present study is that the selective, AT₂-receptor antagonists, PD 123319 and PD 123177, attenuated the regional haemodynamic effects of AII, but only after the prior administration of the AT₁-receptor antagonist, EXP 3174. This finding represents the first demonstration of any effects of AT₂-receptor antagonists on

cardiovascular responses to AII. Moreover, the fact that the haemodynamic responses to noradrenaline were unaffected by any of the treatment schedules also indicates that the AT₂-receptor antagonists were not causing a non-specific reduction in cardiovascular reactivity.

We can think of 3 possible explanations of these observations: (1) There was up-regulation of AT₂-receptors following administration of EXP 3174; (2) Following EXP 3174, the relative selectivity of the AT₂-receptor antagonists was lost; (3) There was displacement of non-specific binding of EXP 3174 (to tissue and plasma proteins) by both PD 123319 and PD 123177, sufficient to cause blockade of functional AT₁-receptors.

Since the present work did not provide unequivocal evidence for one or other of these possibilities, we shall consider the pros and cons for each.

(1) Functional activation of AT₂-receptors

It is conceivable that, following the administration of EXP 3174, there was an up-regulation of AT₂-receptors and/or an enhanced coupling of AT₂-receptors to effector mechanisms. Although the AT₁-receptor usually predominates in vascular smooth muscle (Whitebread *et al.*, 1989; Chang & Lotti, 1991) it is feasible that an increase in AT₂-receptor number occurred in our experimental conditions. However, a simple increase in receptor number would not necessarily produce the results seen, since, even in the adrenal medulla, which contains approximately 90% AT₂-receptors, AII-induced adrenaline release is mediated via AT₁-receptors (Wong *et al.*, 1990a). Therefore, any up-regulation of AT₂ receptors would probably need to be accompanied by a functional coupling of this binding site to post-receptor effector mechanisms. Interestingly, it has recently been reported that differentiation of

Figure 6 Cardiovascular responses to angiotensin II (AII) (125 pmol kg^{-1} , i.v.) on the second experimental day (d 2) (open columns) and 24 h after EXP 3174, i.e. on day 3 (d 3) (solid columns), and then at the times indicated after administration of the AT_2 -receptor antagonist, PD 123177 (10 mg kg^{-1} , i.v.) in conscious, Long Evans rats ($n = 6$). Values are mean with s.e.mean shown by vertical lines.

†Between the 2 left-hand histograms indicate significant differences between day 2 and day 3 ($P < 0.01$); asterisks over subsequent histograms (** $P < 0.01$) show significant differences versus day 3 control values (ANOVA).

NG-108-15 cells results in a change from predominantly AT_1 - to AT_2 -receptor expression, as soon as 2 days later, although a functional response mediated through the AT_2 -receptor was not identified (Bryson *et al.*, 1992). In contrast, AT_2 -receptors in rat adrenal glomerulosa and PC12W cells stimulate protein tyrosine phosphatase activity which inhibits particulate guanylate cyclase activity (Bottari *et al.*, 1992). However, it remains to be determined if this occurs in vascular smooth muscle.

In the present study, EXP 3174 was also given on day 3, i.e. after the administration of either of the AT_2 -receptor antagonists, at a time when the effects of AII were back to control (i.e. day 3) levels. Since EXP 3174 abolished the effects of AII at this stage, it appears that, if up-regulated AT_2 -receptors had become functionally coupled, these effects were expressed via an EXP 3174-sensitive mechanism.

Figure 7 Cardiovascular responses to angiotensin II (AII) (125 pmol kg^{-1} , i.v.) before and 15 min and 1 and 2 h after administration of a low dose of EXP 3174 ($60 \text{ } \mu\text{g kg}^{-1}$, i.v.) in conscious, Long Evans rats ($n = 4$). Values are mean with s.e.mean shown by vertical lines.

* $P < 0.05$; ** $P < 0.01$ versus control value (ANOVA).

(2) Loss of AT_2 -receptor antagonist selectivity following EXP 3174

It is possible that exposure to EXP 3174 induced a conformational change in the AT_1 -binding site which then allowed AT_2 -receptor ligands to bind to it. In this context, it is notable that the kinetics of the AT_1 -receptor appear to be quite complex, since Wong & Timmermans (1991) found that a competitive AT_1 -receptor antagonist reversed the reduced maximum response which occurred with an insurmountable AT_1 -receptor antagonist. However, when PD 123177 was added to the rabbit aorta *in vitro* in the presence of DuP 753, it failed to cause any further blockade of the AII-induced contraction of this tissue (Wong *et al.*, 1990a).

The present results might be explained by EXP 3174 reducing the number of available AT_1 -receptors to a level where a non-selective antagonism of both AT_1 - and AT_2 -receptors, by either PD 123177 or PD 123319, was revealed. This proposal would only be tenable if there was normally a large AT_1 -receptor reserve, such that AII would occupy only a small proportion of the available AT_1 -receptors to evoke its maximum response. Thus, under normal conditions, any apparent 'non-selectivity' of the AT_2 -receptor antagonists would

not be observed. However, while the importance of the relationship between receptor occupancy and function is well recognised for adrenoceptor subtypes (see Kenakin, 1984; Ruffolo *et al.*, 1991), there is no information for AII receptor subtypes in this context.

(3) Displacement of bound EXP 3174 by AT₂-receptor antagonists

Compounds such as EXP 3174 are highly bound to plasma protein (Chiu *et al.*, 1991). Therefore, in the present work, it is feasible that PD 123319 and PD 123177 displaced previously administered EXP 3174 that was still persistently bound to plasma and/or tissue proteins, and the EXP 3174 then inhibited the effects of AII. If this were so, we reasoned that it should be possible to mimic the effects of the AT₂-receptor antagonists (given 24 h after EXP 3174) by administering a lower dose of EXP 3174 (60 µg kg⁻¹) to naive rats. This dose of EXP 3174, itself, caused hyperaemic renal vasodilatation, while other baseline haemodynamics were not altered. It is difficult to compare these changes with the effects of PD 123319 or PD 123177, given 24 h after the high dose of EXP 3174, since the AT₂-receptor antagonists also exerted some influences on baseline cardiovascular status in naive rats. Nevertheless, selective renal vasodilatation, as occurred with the lower dose of EXP 3174 in naive rats, was not seen with either AT₂-receptor antagonist, given 24 h after the high dose of EXP 3174. However, the degree and time course of inhibition of the effects of AII evoked by the lower dose of EXP 3174 were very similar to the changes seen with the AT₂-receptor antagonists in animals previously exposed to the high dose of EXP 3174. Hence, these findings are, in part, consistent with displacement of bound EXP 3174 by the AT₂-receptor antagonists being responsible for their effects.

It would be interesting to test the selective peptide AT₂-receptor antagonist, CGP 42112A (Whitebread *et al.*, 1989) to determine whether or not a compound structurally dissimilar to either PD 123319 or PD 123177 was an effective displacing agent. Other nonselective peptide antagonists, i.e. analogues of AII, would not be suitable since not only are they partial agonists, but they also have functional antagonistic activity in their own right.

In preliminary experiments ($n = 2$; Widdop *et al.*, unpublished results) we have given PD 123177 at a dose of 100 mg kg⁻¹ (i.e. 10 fold higher than here), 24 h after EXP 3174, and have found almost total loss of responses to AII.

But this could simply have been due to displacement of a greater amount of EXP 3174 by the higher dose of PD 123177. Against this, there is the additional preliminary finding ($n = 2$; Widdop *et al.*, unpublished results) that PD 123177 (100 mg kg⁻¹) given 48 h after EXP 3174 (1 mg kg⁻¹), when responses to AII were not impaired, caused substantial inhibition of the effects of AII. It seems unlikely this could have been due to displacement of non-specifically bound EXP 3174, unless the latter was present in the absence of any functional signs of antagonism of AII-mediated effects. While this line of investigation may be worth following, additional experiments were not possible due to shortage of AT₂-receptor antagonists.

The fact that PD 123177 is without effect on AII-induced contractions of the rabbit aorta pretreated with DuP 753 *in vitro* (Wong *et al.*, 1990a), could be taken as evidence that the phenomena described here do not occur in the absence of plasma proteins. However, it should be noted those were acute experiments, and that rabbit tissues contain few vascular AT₂-binding sites compared with rat tissues (Chang & Lotti, 1991). Moreover, DuP 753 and EXP 3174 show different behaviour as AT₁-receptor antagonists (Chiu *et al.*, 1990; Wong *et al.*, 1990b,c).

In conclusion, we have shown that the AT₂-selective ligands PD 123319 and PD 123177 caused significant AII antagonism, but only when given 24 h after the AT₁-receptor antagonist, EXP 3174. While we have offered several explanations for these results, there is no unequivocal evidence to support one or other of the hypotheses. However, on balance, the displacement of non-specifically bound EXP 3174 by the AT₂-receptor antagonists seems the most likely explanation.

Regardless of the exact mechanism involved, these novel findings should be borne in mind if EXP 3174, or the precursor compound, DuP 753, are used in the clinical setting. The present results also raise the possibility that nonpeptide, AII-receptor antagonists that act at both AT₁- and AT₂-receptors may have therapeutic advantages over those that are selective for AT₁-receptors, assuming the latter compounds find a clinical application.

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