Effects of angiotensin II antagonists on the contractile and hydrosmotic effect of AT II and AT III in the toad (*Bufo arenarum*)

Abstract The effects of peptide and non-peptide angiotensin II receptor antagonists on the responses to angiotensin II were examined using aortic rings and skin isolated from the toad. The contractile responses of aortic rings to (Ala-Pro-Gly) angiotensin II were inhibited by the angiotensin II analogue Leu8 angiotensin II, with a pA2 value of 7.6. Similarly, the concentration response curve for (Ala-Pro-Gly) angiotensin II was displaced to the right by the specific angiotensin receptor subtype antagonist DuP 753, with a pA2 value of 6.0. In contrast, the angiotensin receptor subtype 2 antagonists PD 123177 and CGP 42112A did not modify the contractile response to (Ala-Pro-Gly) angiotensin II. None of the antagonists was able to alter the contractile response to norepinephrine. Both Leu8 angiotensin II (10^{-8} \text{ mol} \cdot \text{L}^{-1}) and DuP 753 (10^{-6} \text{ mol} \cdot \text{L}^{-1}) partially inhibited angiotensin III-induced contractions in toad aorta. Angiotensin III, in turn, exhibited lower activity than [Asn1-Val5] angiotensin II in this preparation, its molar potency ratio being 0.293. Previous work from this laboratory reported that osmotic water permeability in the skin of the toad *Bufo arenarum* was increased by angiotensin II, the effect being blocked by the peptide antagonist Leu8 angiotensin II. The hydromotic response to [Asn1-Val5] angiotensin II (10^{-7} \text{ mol} \cdot \text{L}^{-1}) was significantly inhibited by DuP 753 (10^{-6} and 5 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}), whereas the response was not inhibited by a tenfold higher concentration of either PD 123177 or CGP 42112A. DuP 753 (10^{-6} \text{ mol} \cdot \text{L}^{-1}) also inhibited the hydromotic response to angiotensin III (10^{-7} \text{ mol} \cdot \text{L}^{-1}). These results suggest that receptors for angiotensin II present in isolated toad aorta and skin exhibit pharmacological features similar to those characterized as angiotensin subtype 1 in mammalian tissues.

Key words Angiotensin II · Angiotensin II receptor subtypes · Contractility · Osmotic water permeability · Toad aorta and skin

Abbreviations $AT_{1}$ angiotensin receptor subtype 1 · $AT_{2}$ angiotensin receptor subtype 2 · $AT_{II}$ angiotensin II · $AT_{III}$ angiotensin III · CDRC cumulative doseresponse curve(s) · NE norepinephrine · SCC short-circuit current

Introduction

Availability of new AT II antagonists, the non-peptide antagonists DuP 753 and PD 123177 and the peptide antagonist CGP 42112A, has made it possible to identify different subtypes of AT II receptor in tissues of mammalian origin (Chiu et al. 1990; Timmermans et al. 1991). Thus, $AT_{1}$ binds DuP 753, whereas $AT_{2}$ binds both PD 123319 and CGP 42112A but does not bind DuP 753 (Chiu et al. 1990). $AT_{1}$ is the predominant receptor in blood vessels, liver, kidney, lung and nervous system; it mediates the principal known effects of AT II. $AT_{2}$ is found in adrenal medulla (where it constitutes 80% of the receptors), uterus, myocardium and brain, its function being as yet unknown. $AT_{1}$ and $AT_{2}$ differ also in other respects: $AT_{1}$ exhibits sensitivity to sulphhydryl reducing agents, and are coupled to G proteins (Bottari et al. 1991). $AT_{1}$ has been better studied by expression-cloning techniques, and further subclassified into $AT_{1A}$ receptors, found in rat vascular and kidney tissues (Iwai et al. 1991), and $AT_{1B}$ receptors reported to exist in the adrenal gland of the rat (Iwai and Inagami 1992).
More recently, the AT₃ subtype has been identified in a cell line derived from mouse neuroblastoma. This receptor is not blocked by either AT₁- or AT₂-specific inhibitors (Chaki and Inagami 1992).

Receptor subtypes differ also in their affinity for AT II and AT III. AT₁ exhibits greater affinity for AT II than for AT III, whereas both agonists are similarly effective on AT₂ (Bumpus et al. 1991). AT₃, in contrast, is characterized by a lack of binding of AT III.

Although the renin-angiotensin system in amphibians is well established (Wilson 1984) and specific binding sites for AT II have been localized in kidney, adrenal gland and central nervous system of different species (Kloas and Hanke 1992a, b, c), AT I receptors are not found in other tissues like the adrenal tissue of *Xenopus laevis* (Kloas and Hanke 1992d) and the kidney and adrenal gland of *Ambystoma mexicanum* (Kloas and Hanke 1993). However, attempts to characterize receptor subtypes in these vertebrates have been limited. In *Xenopus laevis* (Ji et al. 1991; Sandberg et al. 1991), the receptors in cardiac membranes were reported to be pharmacologically different from either AT₁ or AT₂. However, ovarian AT II receptors are functionally similar to mammalian AT₁ but are not blocked by the specific AT₁ antagonist DuP 753 (Sandberg et al. 1990). In previous studies we have demonstrated that AT II exerts a potent contractile effect on aortic rings of the toad *Bufo arenarum*, which is partially inhibited by different peptide AT II antagonists (Peral de Bruno et al. 1988) and by agents blocking extracellular calcium (Peral de Bruno and Covielio 1992). Increases in SCC and osmotic water permeability (*P*<sub>o</sub>) induced by AT II in isolated toad skin were also inhibited by the AT II peptide antagonist Leu<sup>8</sup> AT II (Covielio et al. 1974), whereas AT III exhibited lower potency compared to AT II (Covielio et al. 1978). The present studies were carried out in an attempt to characterize the action of specific antagonists to AT₁ and AT₂ on the contractile and hydrosomatic effects of AT II in *Bufo arenarum*. No prior data were found regarding the existence of AT II receptor subtypes in this species.

### Material and methods

**Animals**

*Bufo arenarum* of either sex were used. The animals were pithed and their aorta and skin were removed.

**Aortic contractility**

Long sections (5 mm) were cut from the initial portion of the abdominal aorta and mounted as rings by suspending them between two stainless steel hooks. One of the hooks was fixed to the bottom of an isolated organ bath, and the other was attached to an isometric tension transducer (Gould UC2-USA). Care was exercised to prevent damage to the endothelium. Smooth muscle contractions were recorded in an Acromat recorder (RM 302-Argentina). The solution bathing the aortic rings contained (in mmol l<sup>-1</sup>): NaCl, 80; KCl, 3; CaCl₂, 1; NaHCO₃, 24; NaH₂PO₄, 0.1; glucose, 1; 220 mosmol kg<sup>-1</sup> H₂O, and was maintained at 24°C and bubbled with a mixture of 95% O₂ and 5% CO₂. The pH of the solution was 7.4. A resting tension of 1.5 g was applied, and the tissue allowed to rest during a 90-min equilibration period, during which the bathing fluid was changed every 15 min and the tension adjusted each time to 1.5 g. The reactivity of the tissue was assessed at the beginning and end of the experiment by brief exposures to 80 mmol l<sup>-1</sup>-<sup>1</sup> KCl solution, after which the preparation was rinsed with fresh bathing solution three times at 15-min intervals, thus allowing it to return to baseline tension. The bathing solution was changed at least six times at 15-min intervals between successive doses of AT II or NE throughout the experiment. The tissue was exposed to NE (10<sup>-8</sup> mol l<sup>-1</sup>) prior to AT II stimulation, this treatment ruling out tachyphylaxis to AT II as previously demonstrated (Peral de Bruno et al. 1988). When used, AT II antagonists were added to the bath 5 min prior to the addition of the agonist. CDRC to AT II were constructed according to the technique of van Rossum (1963). The pA<sub>2</sub> values (−log *K*<sub>p</sub>) for Leu<sup>8</sup> AT II and DuP 753 against (Ala-Pro-Gly) AT II in toad aorta were calculated using the technique of Arunlakshana and Schild (1959); the dose-ratio produced by the blocker (i.e. the ratio of concentrations of agonist giving an equal response in the presence and absence of the antagonist measured at the ED₅₀) being determined for various concentrations of the antagonist. The responses to different agonists were expressed as increases in tension (mg) over the resting basal level, adjusted to 1.5 g.

### Experimental protocols

**Effect of AT II antagonists on the contractile response to (Ala-Pro-Gly) AT II**

The effect of Leu<sup>8</sup> AT II on the vascular response to AT II was examined in arteries pretreated with the antagonist before addition of the agonist. A control CDRC to AT II (5·10<sup>-9</sup> to 10<sup>-6</sup> mol l<sup>-1</sup>) was performed and dose-response relationships were established. After rinsing the preparation, a second AT II CDRC (same concentrations) was generated in the presence of Leu<sup>8</sup> AT II (1·10<sup>-7</sup> mol l<sup>-1</sup>). After this, the preparation was rinsed during a period of 60 min and allowed to return again to its basal level. At this point, a third AT II CDRC was performed once more in the presence of Leu<sup>8</sup> AT II (5·10<sup>-7</sup> mol l<sup>-1</sup>). Preliminary studies showed that repeated responses to AT II could be obtained without tachyphylaxis. These experiments were repeated with lower concentrations of Leu<sup>8</sup> AT II: 5·10<sup>-8</sup> and 10<sup>-7</sup> mol l<sup>-1</sup>. A similar protocol was used to assess the effects of specific antagonists to AT II receptor subtypes, substituting DuP 753 or CGP 42112A for Leu<sup>8</sup> AT II. For DuP 753, each aortic ring was exposed to several concentrations of the inhibitor (10<sup>-6</sup>, 5·10<sup>-6</sup> and 10<sup>-5</sup> mol l<sup>-1</sup>), whereas CGP 42112A was used at a concentration of 10<sup>-6</sup> mol l<sup>-1</sup>. Experiments with PD 123177 were performed in arteries previously stimulated with AT II 10<sup>-7</sup> mol l<sup>-1</sup>, which were rinsed and stimulated once again with AT II in the same concentration in the presence of the antagonist (10<sup>-6</sup> mol l<sup>-1</sup>).

**Effects of AT II antagonists on the contractile response to AT III**

The effect of AT II antagonists (Leu<sup>8</sup> AT II and DuP 753) on the contractile response to AT III was examined as described above. Potency ratios (on a molar basis) of AT III against (Ala-Pro-Gly) AT II and [Asn<sup>2</sup>-Val<sup>5</sup>] AT II were calculated according to the method used by Watanabe et al. (1977). A dose of angiotensin eliciting 50% of the maximal response was determined for each CDRC.