

Angiotensin-(1-7) increases osmotic water permeability in isolated toad skin

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Abstract

Angiotensin-(1-7) (Ang-(1-7)) increased osmotic water permeability in the isolated toad skin, a tissue with functional properties similar to those of the distal mammalian nephron. Concentrations of 0.1 to 10 μM were effective, with a peak at 20 min. This effect was similar in magnitude to that of frog skin angiotensin II (Ang II) and oxytocin but lower than that of human Ang II and arginine-vasotocin. The AT_2 angiotensin receptor antagonist PD 123319 (1.0 μM) fully inhibited the response to 0.1 μM Ang-(1-7) but had no effect on the response to Ang II at the same concentration. The specific receptor antagonist of Ang-(1-7), A-779, was ineffective in blocking the response to Ang-(1-7) and to frog skin Ang II. The AT_1 receptor subtype antagonist losartan, which blocked the response to frog skin Ang II, was ineffective in blocking the response to Ang-(1-7). The present results support the view of an antidiuretic action of Ang-(1-7) in the mammalian nephron.

Key words

- Angiotensin-(1-7)
- Toad skin
- Osmotic water permeability
- Receptor subtypes

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Introduction

Angiotensin-(1-7) (Ang-(1-7)) is a biologically active peptide of the renin-angiotensin system produced from angiotensin I by an independent pathway of the angiotensin-converting enzyme with cellular functions that differ from angiotensin II (Ang II) (1,2). Several renal actions of Ang-(1-7) have been reported, indicating that the peptide has a role in hydromineral balance. In water-loaded rats it produces marked antidiuresis (3) by a direct effect on inner medullary collecting ducts (4). This effect is not blocked by the V_2 receptor antagonist (4) but is blocked by the specific antagonist A-779 (5,6) and losartan (7). Ang-(1-7) may play a role during dehydration and hemorrhage in rats (8). In the rat proximal straight tubules a

biphasic effect on fluid absorption blocked by losartan has been reported (9), as well as an inhibitory effect on sodium fluxes in proximal tubule cells (10). On the other hand, diuretic and natriuretic effects *in vivo* (11) and in the isolated rat kidney (12) have been found which may involve PGI_2 release (13).

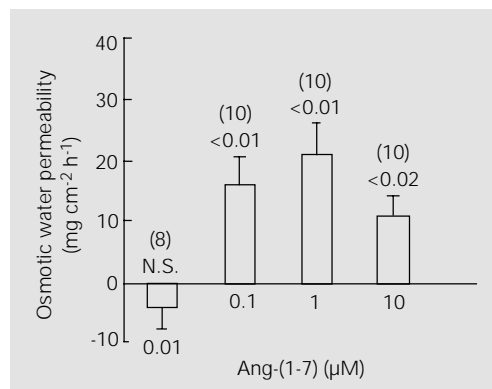
Amphibian skin is a tissue with functional properties similar to those of the distal mammalian nephron (14). In isolated toad skin Ang II increases osmotic water permeability (Posm) (15) through a common receptor with antidiuretic hormone (16-19). Confirming this effect, a primary antidiuretic action of Ang II was demonstrated in the dog kidney (20). In order to obtain further information about the antidiuretic effect of Ang-(1-7) we measured Posm in isolated toad skin and compared its response to that ob-

tained with other Ang II and neurohypophysial peptides (oxytocin and arginine-vasotocin).

Material and Methods

Toads (*Bufo arenarum*) hydrated overnight were used. After pithing, the ventral pelvic skin was dissected and divided into two symmetrical parts which were mounted (dermal surface outward) over the bottom of plastic tubes (surface area 1.77 cm²) and immersed in a bath of 10 ml of aerated Ringer solution. The inner epidermal surface was bathed with the same solution diluted 1/5 in distilled water and Posm was measured gravimetrically by weighing the tube on a scale (Mettler H10) to the nearest 0.1 mg. After an equilibration period of 1 h in which the solutions were changed twice, one or two control periods of 20 min were allowed to elapse, followed by an experimental one in which the Posm response to the hormone added to the dermal bath was measured. In some experiments, an antagonist was added to the paired half-skin 10 min before the addition of the hormone (Ang II) to both halves. In another experiment the preparation was weighed during several periods of 20 min in order to determine the time course of the response. Results are reported as mg cm⁻² h⁻¹ and the hormonal effect as the mean and standard error (SEM) of the difference between the experimental period and the previous control one. The

Figure 1 - Effect of different Ang-(1-7) concentrations on osmotic water permeability in isolated toad skin. The number of experiments is indicated in parentheses. N.S. Not significant; the P values are given above the columns. In this and following graphs the mean effect and its standard error (SEM) were obtained from the difference between the experimental period and the preceding control one and the Student t-test for paired samples was applied. One-way ANOVA followed by the Duncan test demonstrated that the mean effects of different Ang-(1-7) concentrations producing significant increases in osmotic water permeability did not differ from one another.



Ringer solution contained 90 mM NaCl, 2 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂, 25 mM Tris-HCl buffer, and 5 mM glucose, pH 7.4, 220 mOsmol/kg H₂O.

Drugs

Ang-(1-7) (Asp-Arg-Val-Tyr-Ile-His-Pro) was synthesized by Dr. Clara Peña, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad Nacional de Buenos Aires, Argentina, and also obtained from Bachem (Torrance, CA, USA); frog skin Ang II (Ala-Pro-Gly [Ile³,Val⁵] angiotensin II), human Ang II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), oxytocin and arginine-vasotocin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); losartan (DuP 753) was a gift from Dr. Ronald D. Smith (DuPont Merck, Wilmington, DE, USA); PD 123319 (P-186) was obtained from Research Biochemicals Int. (Natick, MA, USA); A-779 (Asp-Arg-Val-Tyr-Ile-His-D-Ala) was a gift from Professor Robson A.S. Santos (Belo Horizonte, MG, Brazil).

Statistical analysis

Results are reported as the mean ± SEM and the Student *t*-test for paired samples was used for statistical analysis. When more than two means were compared, one-way ANOVA was performed, followed by the Newman-Keuls test. The level of significance was set at P<0.05.

Results

Ang-(1-7) significantly increased Posm over basal flow at concentrations of 0.1 to 10 µM (Figure 1). The higher concentration (10 µM) elicited a lower response that was not statistically different from 1 µM. Ang-(1-7) obtained from two different sources did not differ in the response elicited (data not shown). Figure 2 comparatively shows the response to different angiotensins and neu-

rohypophyseal hormones (oxytocin and arginine-vasotocin). Arginine-vasotocin, the antidiuretic neurohypophyseal hormone of Amphibia, was by far more potent in increasing Posm. The time course of the response to Ang-(1-7) showed a peak at 20 min after addition of the hormone to the dermal bathing solution (Figure 3A,B). The response of the skins varied widely. Figure 3B shows the response of a highly reactive skin. A-779 (1 μM), a specific antagonist of Ang-(1-7), had no effect on the response of toad skin to 0.1 μM Ang-(1-7) (Figure 4) and to 0.1 μM frog skin Ang II (Figure 5). However, the Posm response to Ang-(1-7) was completely blocked by the AT₂ antagonist PD 123319 (Figure 6). The AT₁ antagonist losartan (Figure 7) was unable to block the response to Ang-(1-7). The antagonists used (A-779, PD 123319 and losartan) had no effect on basal osmotic water permeability (data not shown).

Discussion

Ang-(1-7) significantly increased toad skin Posm at concentrations of 0.1 to 10 μM with a peak at 20 min similar to that observed with Ang II (15). There was a wide variability in the response between animals with non-responsive skins and others showing a clear response (Figure 3B). Human Ang II was more potent than Ang-(1-7), whose effect was similar to that of frog skin Ang II, an angiotensin peptide found in the skin of the frog *Crinia georgiana* (21). Arginine-vasotocin, the naturally neurohypophyseal peptide existing in Amphibia, had the highest effect. Doses of 10 μM Ang-(1-7) elicited responses of lower magnitude, although without being statistically significant. This may be attributed to the release of inhibitory substances such as prostaglandins at these concentrations since PGE₁ inhibited the effect of Ang II on toad skin Posm (22). No effect of Ang-(1-7) on Posm was found in the isolated toad bladder (data not shown).

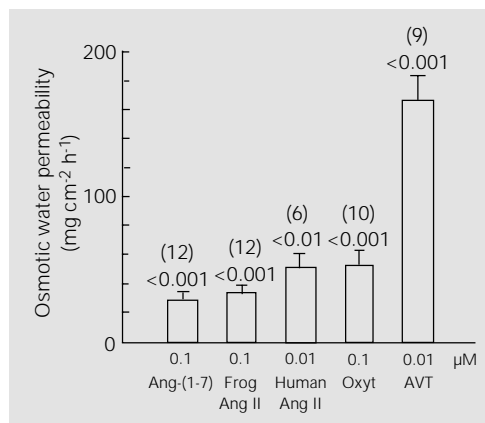


Figure 2 - Comparative effects of different angiotensins (Ang) and neurohypophyseal hormones on osmotic water permeability in isolated toad skin. Data are reported as means \pm SEM. The numbers in parentheses indicate the number of experiments and the P values are given above the columns. One-way ANOVA followed by the Newman-Keuls test demonstrated that the effect of Ang-(1-7) was significantly lower than that of human Ang II, oxytocin and arginine-vasotocin but did not differ from that of frog Ang II. Oxyt, Oxytocin; AVT, arginine-vasotocin.

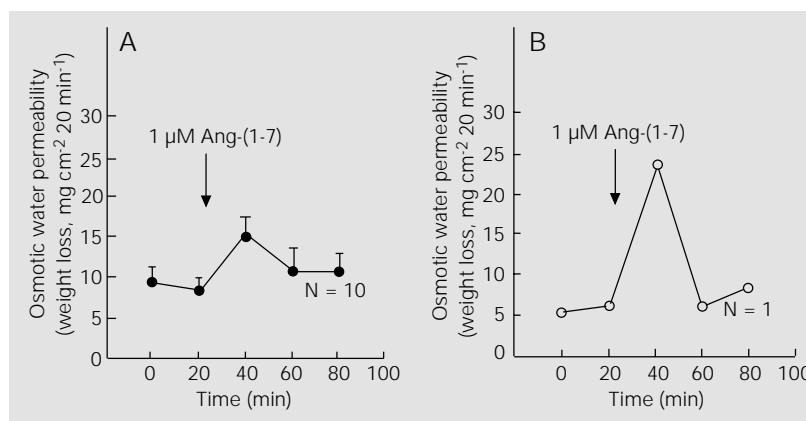


Figure 3 - Time course of the response to Ang-(1-7). A, Mean \pm SEM for 10 experiments; B shows the response of a single reactive skin. The maximal responses occurred 20 min after the addition of 1.0 μM Ang-(1-7) to the dermal surface of the skins.

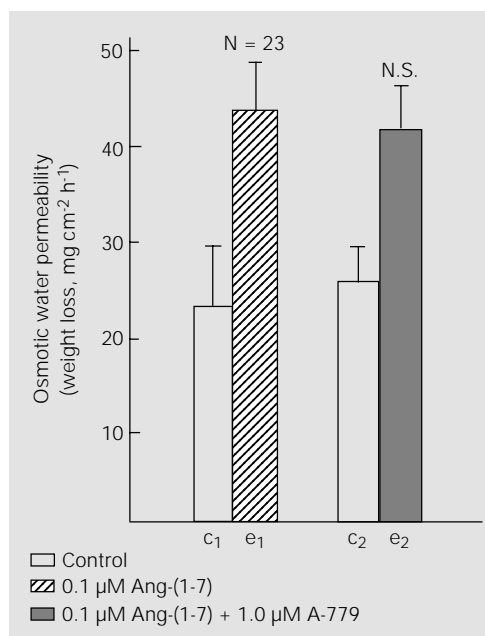


Figure 4 - Lack of effect of the specific Ang-(1-7) antagonist (A-779) on osmotic water permeability stimulated with Ang-(1-7). Data are reported as means \pm SEM. One-way ANOVA followed by the Newman-Keuls test showed no significant differences (N.S.) between e₁ and e₂ or c₁ and c₂. In this and the following figures c₁ and e₁ are the control and experimental periods of one skin half and c₂ and e₂ the control and experimental periods of the other paired half.

Figure 5 - Lack of effect of the specific Ang-(1-7) antagonist (A-779) on osmotic water permeability stimulated with frog skin Ang II. Data are reported as means \pm SEM. One-way ANOVA followed by the Newman-Keuls test showed no significant differences (N.S.) between e_1 and e_2 or c_1 and c_2 .

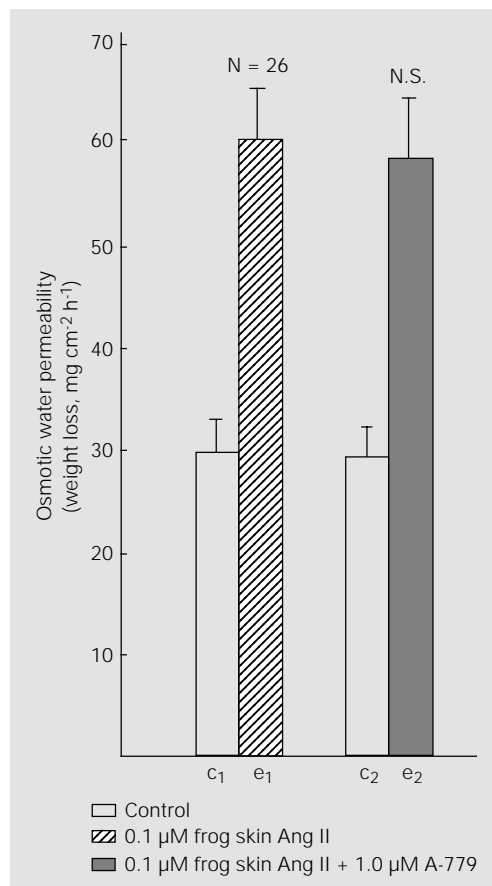
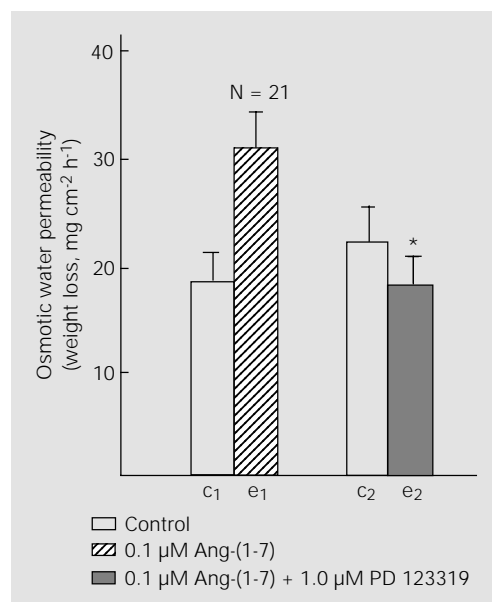


Figure 6 - Inhibition of the response of toad skin to Ang-(1-7) by the AT₂ receptor antagonist PD 123319. Data are reported as means \pm SEM. One-way ANOVA followed by the Newman-Keuls test showed a significant difference (* $P < 0.01$) between the experimental periods e_1 and e_2 . There was no significant difference between c_1 and c_2 .



The response of toad skin to Ang II is mediated by an AT₁ receptor subtype since the effect of Ang II on Posm is blocked by losartan but not by PD 123319 (17). The fact that antidiuretic hormone antagonists blocked the stimulating effect of Ang II on Posm (18) and that losartan blocked the Posm response to antidiuretic hormone in toad skin (19) supports the view of a common receptor for Ang II and antidiuretic hormone in toad skin. In the present experiments, we unexpectedly obtained a complete inhibition of the Posm response to Ang-(1-7) by PD 123319 whereas A-779, considered to be a specific antagonist of Ang-(1-7) (5,6), had no effect. Losartan was also unable to block the response to Ang-(1-7). A-779 completely blocked the antidiuretic effect of Ang-(1-7) on water-loaded rats, suggesting the existence of a non-AT₁ angiotensin receptor that is recognized by losartan (7). Our results using PD 123319 showed that Ang-(1-7) increased Posm mainly through an AT₂ receptor which in this case was not recognized by losartan, an AT₁ receptor subtype antagonist. Our data concerning toad skin did not show a specific receptor for Ang-(1-7), in contrast to the ventrolateral medulla of rats (23,24), porcine coronary artery rings (25) and bovine aortic endothelial cells (26).

Blockade of the Ang-(1-7) receptor by PD 123319 and losartan has been described in studies on the release of [³H] norepinephrine from rat atria (27), suggesting that Ang-(1-7) may act on receptor subtypes 1 and 2. Also, in dog coronary arteries the release of nitrites by Ang-(1-7) was reduced by losartan and PD 123319 (28), as also was the relaxing effect on precontracted pig coronary arteries in the presence of bradykinin (29). In C6 glioma cells the effect of Ang-(1-7) on prostaglandin synthesis was mediated by subtype 1 angiotensin receptors (30).

Ang II may be converted to Ang-(1-7) by carboxypeptidases and prolylcarboxypeptidase (31). If this were the case in our preparation, PD 123319, the specific antagonist of

the AT₂ receptor subtype which blocks the effect of Ang-(1-7), would also block at least partially the effect of Ang II on osmotic water permeability in toad skin, but this inhibition was not observed.

The results obtained here with toad skin, a tissue with functional properties similar to those of the distal mammalian nephron, support the antidiuretic effect of Ang-(1-7) observed in the rat (3) and the increase in hydraulic conductivity of mammalian inner medullary collecting tubules (4). We demonstrated for the first time the effect of an angiotensin on Posm in isolated toad skin mediated by an AT₂ receptor subtype which is not recognized by losartan.

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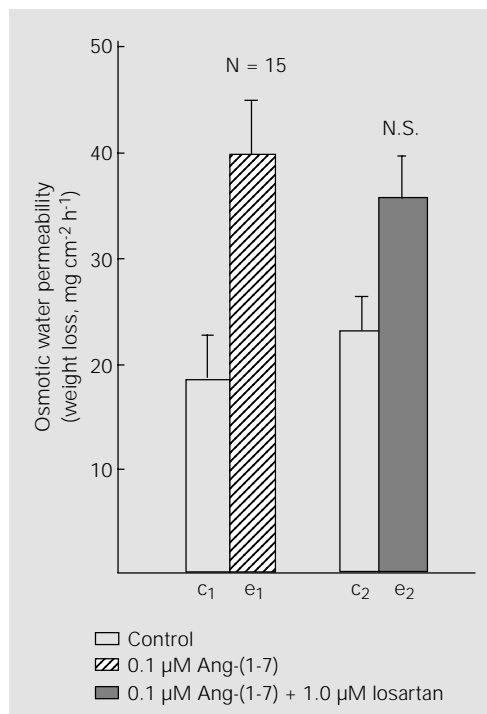


Figure 7 - Lack of effect of the specific antagonist of the AT₁ receptor losartan on osmotic water permeability stimulated with Ang-(1-7). Data are reported as means \pm SEM. One-way ANOVA followed by the Newman-Keuls test showed no significant differences (N.S.) between e₁ and e₂ or c₁ and c₂.

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