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Atypical angiotensin II receptors coupled to phosphoinositide turnover/calcium signalling in catfish hepatocytes

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Abstract

In catfish (*Ictalurus punctatus*) hepatocytes angiotensin II induced an immediate increase in cytosolic Ca^{2+} concentration. Other angiotensin analogues also induced this effect including: human angiotensin II, fish angiotensin II, human angiotensin III, human angiotensin I, fish angiotensin I and saralasin. CGP 42112A induced a very small effect at the highest concentration tested and angiotensin IV was without effect. Angiotensin II also increased the resynthesis of phosphatidylinositol and the production of IP_3. These physiological effects were not blocked by losartan (AT_1-selective antagonist) or PD 123177 (AT_2-selective antagonist).

[^{125}I]Angiotensin II bound to liver plasma membranes in a saturable fashion with high affinity (K_D 2.7 nM) and a B_{max} of 185 fmol/mg of protein. Binding competition experiments showed the following order of potency: human angiotensin II = fish angiotensin II > human angiotensin III ≥ human angiotensin I = fish angiotensin I. These sites were insensitive to losartan or PD 123177.

The data indicate that the angiotensin II receptors expressed in catfish hepatocytes are coupled to the phosphoinositide turnover/calcium mobilization signal transduction pathway and are atypical receptors, i.e., pharmacologically distinct from mammalian AT_1 and AT_2 receptors.

Keywords: Angiotensin II receptor; Hepatocyte; Inositol trisphosphate; Calcium signalling

1. Introduction

Angiotensin II (AII) is a multifunctional octapeptide hormone that regulates a plethora of physiological actions by interacting with cells surface receptors [1,2]. In mammalian tissues, at least two types of angiotensin II receptors (AT_1 and AT_2) have been identified by pharmacological and molecular biological approaches [2–5]. Both receptors belong to the seven-transmembrane domains superfamily of G protein-coupled receptors and differ in their affinities and selectivity for synthetic non-peptide antagonists [3–5]. Thus, AT_1 receptors have high affinity for losartan and very low affinity for PD123177 whereas AT_2 receptors display the opposite order of affinities for these nonpeptide antagonists [3–5]

AT_1 receptors mediate most of the best known actions of AII and are coupled to the phosphoinositide turnover/ calcium mobilization signal transduction process [2,6,7]. Depending on the cell type, these receptors can also be coupled to other effector systems such as phospholipases A_2 and D, as well as voltage dependent Ca^{2+} channels, and to inhibition of adenylyl cyclase [7,8]. The signal transduction

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mechanism utilized by AT_2 receptors is not yet completely understood. However, it has been proposed these receptors regulate the activity of tyrosine phosphatases [9,10].

There is a third group of heterogeneous angiotensin receptors that share one common characteristic: that is, they have little affinity for either AT_1 or AT_2 receptor nonpeptide antagonist. This group of receptors, named atypical receptors [5], have been described in some tissues of non-mammalian vertebrates, including amphibians [11–14] and birds [15,16]. Other atypical receptors, such as those observed in differentiated mouse neuroblastoma cells [17], aortic canine tissue [18], and in an epithelial cell line derived from the rat small intestine [19], have in common a much reduced affinity for the nonpeptide antagonists.

In rat liver cells, AII activates phosphorylase *a* activity via the phosphoinositide turnover/calcium mobilization signal transduction pathway through AT_1 receptors [6,7]; these receptors are also coupled, in an inhibitory fashion, to adenylyl cyclase [7]. During our studies on the α_1-adrenoceptors expressed in the liver of different species [20,21], we observed that AII increases intracellular calcium in catfish hepatocytes. Previous studies have shown the existence of AII receptors in teleost liver cells [22–25]. Janssens et al. [22] showed that AII decreases glucose release in carp liver, although the signal transduction and the type(s) of receptor(s) involved were not defined. Cobb and Brown [23] detected AII binding to rainbow trout tissues by autoradiography. Marsigliante and co-workers [24,25], identified two AII receptors expressed by the eel (*Anguilla anguilla*); one of them, was recognized by a monoclonal antibody raised against mammalian AT_1 receptors [24,25]. We characterized the AII receptors of catfish *(Ictalurus punctatus)* liver cells; our data indicate that these teleost liver cells express atypical receptors which are coupled to the phosphoinositide turnover/calcium mobilization signal transduction process.

2. Materials and methods

Human angiotensin I, human AII, human angiotensin III, fish AII ([Asn^1, Val^5]AII), goosefish angiotensin I ([Asn^1, Val^5]agiotensin I), saralasin ([Sar^1, Ala^8]AII), HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) and bacitracin were obtained from Sigma Chemical Co. Indo-1/AM was from Molecular Probes, CGP 42112A from Research Biochemicals International and bovine serum albumin (fraction V) from Armour. Inositol-1,4,5-trisphosphate radioreceptor assay kits, human [^{125}I-Tyr^4]AII (2200 Ci/mmol) and [^{32}P]Pi (carrier free) were from New England Nuclear. Losartan (DUP 753) and PD 123177 were generously provided by The DuPont Merck Pharmaceutical Company.

Adult catfish, *Ictalurus punctatus*, weighing 200–300 g were purchased from a local dealer and maintained in Centro Acuícola 'La Paz' (water temperature 25 ± 5°C). Animals were transported to the laboratory 4–5 days before experimentation; animals were placed in tanks containing 150 l of dechlorinated tap water at room temperature (23–25°C) that was continuously aerated.

Hepatocytes were isolated by liver retrograde perfusion with collagenase [26] and were incubated in the following buffer: 136 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4$, 5 mM $NaHCO_3$, 0.33 mM Na_2HPO_4, 10 mM HEPES, pH was adjusted to 7.63 [27]. All incubations were performed at 20°C [21].

Intracellular calcium ($[Ca^{2+}]_I$) and IP_3 determinations: $[Ca^{2+}]_I$ was determined as described [21]. In brief, hepatic cells were loaded with 10 μM indo-1/AM for 30 min at room temperature and washed with the same buffer mentioned above. Excitation and emission wavelengths of 340 and 410 nm were employed. The fluorimeter (AMINCO-Bowman Series 2 Luminescence Spectrometer) was fitted with a magnetic stirrer.

Production of IP_3 was carried out according to Fabbri et al. [28] with minor modifications. In brief, cell suspensions (1 ml containing 50 mg of cells wet weight) were incubated at 20°C in presence of AII or other agonists; antagonists were added 5 min before the agonist. Reactions were stopped at 30 s or the indicated times by adding ice-cold perchloric acid (6% final concentration). Acidified samples were vortexed and kept on ice for 1 h before centrifugation at $2000 \times g$ for 15 min. Supernatants were removed and neutralized with 1.5 M KOH/75 mM Hepes; 100 μl of these neutralized supernatants were used for quantification, employing a commercial IP_3 radioreceptor assay kit.

Phosphatidylinositol (PI) labeling: PI labeling was performed by incubating the cells for 60 min in buffer without sodium phosphate and supplemented with 10 μCi/ml of [^{32}P]Pi. At the end of the incubation lipids were extracted with chloroform/methanol (2:1) and phospholipids separated by one-dimensional thin-layer chromatography [29].

Membrane preparation and [^{125}I]AII binding assays: Liver membranes were isolated by the discontinuous sucrose density gradient method of Neville [30]. Human [^{125}I]AII binding was performed as described [31]. In brief, catfish liver membranes (50 μg protein) were incubated in a total volume of 100 μl of buffer containing: 100 mM NaCl, 10 mM $MgCl_2$, 0.2% bovine serum albumin, 0.1 mg/ml bacitracin, 20 mM Tris, pH 7.5. Incubations were in a water bath shaker at 12°C, after 60 min 3 ml of ice-cold buffer were added to the membrane suspension; the membranes were immediately filtered on GF/C filters and washed three times (3 ml each time) with the same buffer. Filters were presoaked in 0.3% polyethylenimine to decrease nonspecific binding to the filter. Saturation experiments were performed using concentrations in the range of 0.5–150 nM [^{125}I]AII and binding competition studies with 0.3–0.5 nM radioactive ligand (15–20% receptor occupation). Nonspecific binding was evaluated in the presence of 1 μM AII; specific binding represented 90–95% of the total binding at the K_D. Binding saturation and competition data were analyzed using the RADLIG program (Biosoft-Elsevier). K_i values were calculated according to Cheng and Prussoff [32].

3. Results

In catfish hepatocytes 1 μM human AII induced an almost immediate increase in $[Ca^{2+}]_I$ that returned very slowly to near basal levels (Fig. 1). AII also increased $[Ca^{2+}]_I$ in cells incubated in buffer without calcium and containing 25 μM EGTA (data not shown). At lower concentrations of human angiotensin II (1 pM to 1 nM) $[Ca^{2+}]_I$ increased in an oscillatory pattern, as described for α_1-adrenergic agents [21]. The effects of human AII, and those of the different analogues tested, were dose-dependent (Fig. 2). It can be observed that fish AII was as potent and as effective as human AII to increase

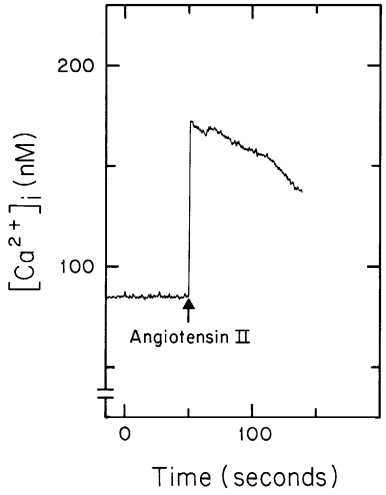

Fig. 1. Effect of angiotensin II on $[Ca^{2+}]_I$. Cells were equilibrated in the fluorimeter and 1 μM angiotensin II was added. A representative experiment, of nine performed with different cell preparations, is presented.

$[Ca^{2+}]_i$ and reached a maximum of 2.5- to 3-fold over basal, the EC_{50} values for both of these agents were $\approx$ 1 nM. Human angiotensin III was also as

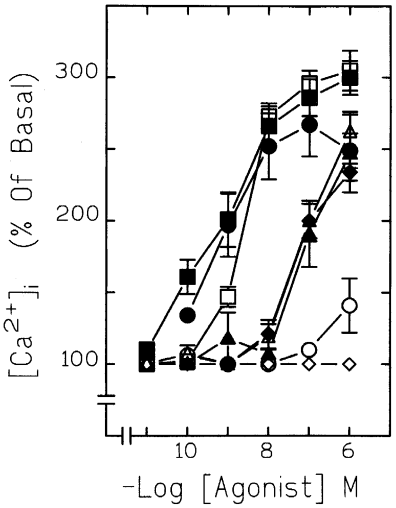

Fig. 2. Effect of angiotensin II and angiotensin analogues on $[Ca^{2+}]_I$. Cells were incubated in the presence of different concentrations of human angiotensin II (filled circles), fish angiotensin II (filled squares), human angiotensin III (open squares), saralasin (filled diamonds), human angiotensin I (filled triangles), fish angiotensin I (open triangles), CGP 42112A (open circles), or angiotensin IV (open diamonds). Results are expressed as percentage of basal $[Ca^{2+}]_I$, which was 111 ± 5 nM. Plotted are the means and vertical lines represent the S.E.M. of 6–8 experiments performed with different cell preparations.

effective as the two AII analogues but it was slightly, but consistently, less potent ($EC_{50} \approx 3$ nM). Saralasin, an AII analogue, that in some systems behaves as an antagonist/ partial agonist, clearly induced an almost full $[Ca^{2+}]_I$ response at the highest concentration tested (1 μM); similar effects were observed with the human and fish forms of angiotensin I (Fig. 2). CGP 42112A, a peptide analogue of AII, behaved as a weak agonist, inducing a very small effect at the highest concentration tested (1 μM) (Fig. 2). Recent studies have shown novel binding sites for the C-terminal 3–8 hexapeptide fragment of AII, named angiotensin IV binding sites [33]. We studied the effect of this fragment but angiotensin IV did not induce any measurable effect on $[Ca^{2+}]_I$ in isolated fish hepatocytes at the concentrations tested (Fig. 2).

In order to determine the type of AII receptor involved in this effect we studied the effect of the non-peptide antagonists losartan (AT_1-selective) and PD123177 (AT_2-selective). None of these agents affected basal $[Ca^{2+}]_I$ by themselves (data not shown). As shown in Fig. 3 the effects of 1 μM fish AII or 1 μM human AII on $[Ca^{2+}]_I$ were not blocked by either losartan (10 μM) or PD 123177 (10 μM) (Fig. 3). In fact neither of these nonpeptide antagonists were able to shift the dose-response curves for these AII forms (data not shown).

In order to get further insights, phosphoinositide

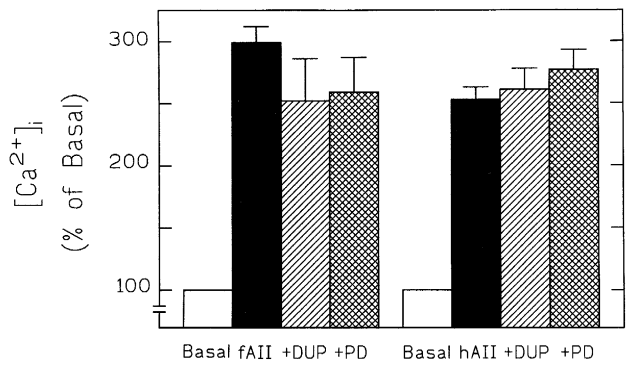

Fig. 3. Effects of angiotensin II and angiotensin II antagonist on $[Ca^{2+}]_I$. Cells were incubated in the absence (Basal) or presence of 1 μM fish angiotensin II (fAII), or 1 μM human angiotensin II (hAII) alone, or with 10 μM DUP (+DUP) or 10 μM PD123177 (+PD). Results are expressed as percentage of basal $[Ca^{2+}]_I$, which was 90 ± 9 nM. Plotted are the means and vertical lines represent the S.E.M. of 6–8 experiments performed with different cell preparations.

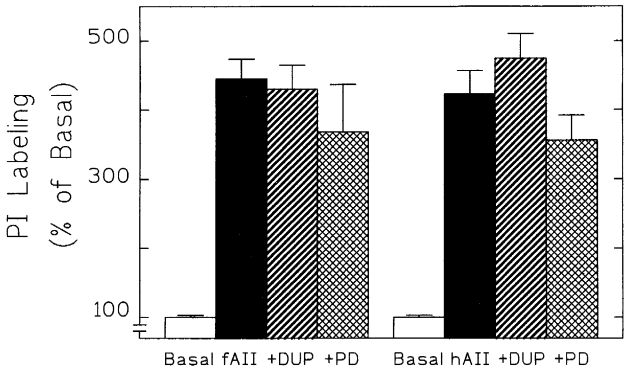

Fig. 4. Effect of angiotensin II and angiotensin II antagonist on phosphatidylinositol (PI) labeling. Cells were incubated in the absence (Basal) or presence of 1 μM fish angiotensin II (fAII), or 1 μM human angiotensin II (hAII) alone, or with 10 μM DUP (+DUP) or 10 μM PD123177 (+PD). Results are expressed as percentage of basal labeling which was 153 ± 6 cpm/100 mg cells wet weight. Plotted are the means and vertical lines represent the S.E.M. of 5 experiments performed with different cell preparations.

turnover was studied. PI labeling (resynthesis) is an event secondary to phosphatidylinositol 4,5-bisphosphate hydrolysis. It can be observed in Fig. 4 that both 1 μM human AII and 1 μM fish AII increased PI labeling $\approx$ 4.5-fold and that these effects were not blocked by losartan or PD123177, confirming the previous results. No effect of the AII forms, on the labeling of other phospholipids, was observed (data not shown). The antagonists themselves did not alter the basal labeling of PI (data nor shown).

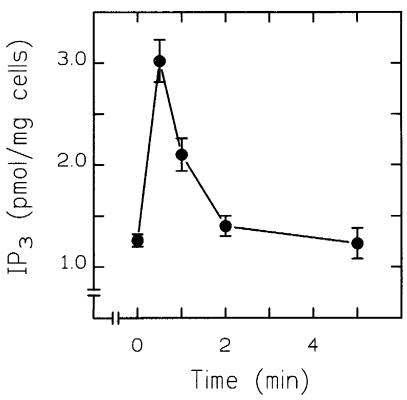

Fig. 5. Time-course of the effect of angiotensin II on inositol-1,4,5-trisphosphate (IP_3) production. Cells were incubated for the time indicated with 1 μM angiotensin II. Plotted are the means and vertical lines represent the S.E.M. of 4 experiments performed with different cell preparations.

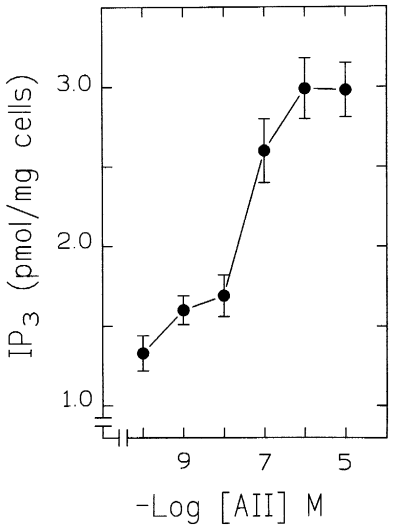

Fig. 6. Effect of angiotensin II on inositol-1,4,5-trisphosphate (IP_3) production. Cells were incubated in the presence of different concentrations of angiotensin II. Plotted are the means and vertical lines represent the S.E.M. of 4 experiments performed with different cell preparations

The effects of human AII on IP_3 production was quantified in liver cells. AII (1 μM) induced an almost immediate increase in IP_3 production (2.5–3-fold over basal level) that reached its maximum 30 seconds after its addition to the cells; this effect also decreased rather rapidly reaching near basal levels,

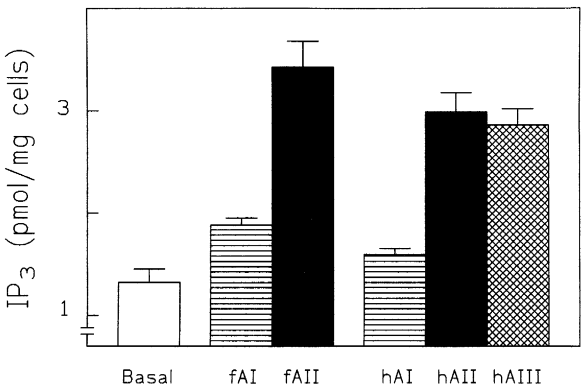

Fig. 7. Effect of angiotensin II and angiotensin analogues on inositol-1,4,5-trisphosphate (IP_3) production. Cells were incubated in the absence (Basal) or presence of 1 μM fish angiotensin I (fAI), 1 μM fish angiotensin II (fAII), 1 μM human angiotensin I (hAI), 1 μM human angiotensin II (hAII), or 1 μM human angiotensin III (hAIII). Plotted are the means and vertical lines represent the S.E.M. of 4 experiments performed with different cell preparations.

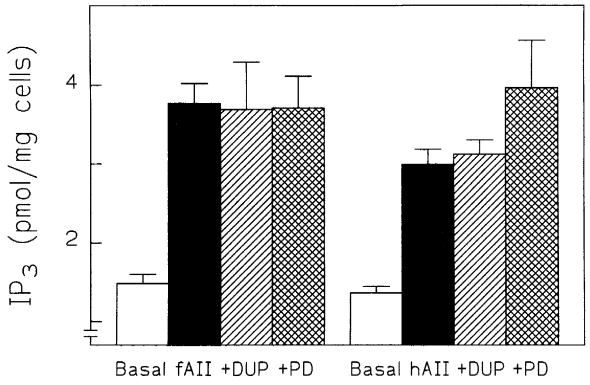

Fig. 8. Effect of angiotensin II and angiotensin II antagonist on inositol-1,4,5-trisphosphate (IP_3) production. Cells were incubated in the absence (Basal) or presence of 1 μM fish angiotensin II (fAII), or 1 μM human angiotensin II (hAII) alone, or with 10 μM DUP (+DUP) or 10 μM PD123177 (+PD). Plotted are the means and vertical lines represent the S.E.M. of 4 experiments performed with different cell preparations.

approximately 2–5 min after hormone addition (Fig. 5). The effect of human AII was dose-dependent with an EC_{50} of $\approx$ 40 nM (Fig. 6).

The effect of AII analogues on IP_3 production was evaluated and the data shown in Fig. 7. The agents were tested at a concentration of 1 μM and the magnitude of the effect observed was: fish AII $\geq$ human AII = human angiotensin III > fish angiotensin I $\geq$ human angiotensin I. The effects of human or fish AII on IP_3 production were not blocked by the angiotensin II antagonists, losartan and PD123177 (Fig. 8). The antagonists by themselves

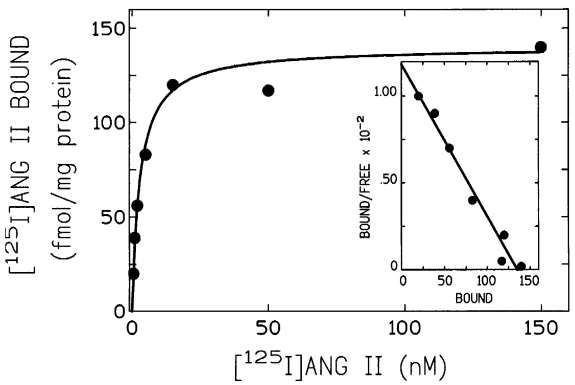

Fig. 9. [^{125}I]Angiotensin II saturation isotherm using fish liver membranes. Plotted is the specific binding and the Rosenthal analysis (insert). The figure is representative of five experiments using different membrane preparations.

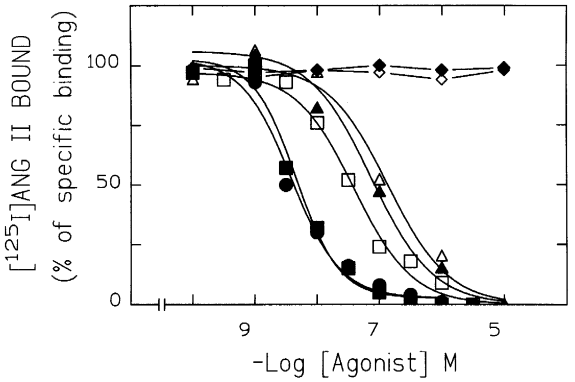

Fig. 10. Binding competition experiments using fish liver membranes. The following agonist were employed: human angiotensin II (filled circles), fish angiotensin II (filled squares), human angiotensin III (open squares), human angiotensin I (filled triangles), fish angiotensin I (open triangles), DUP753 (filled diamonds), and PD123177 (open diamonds). The figure is representative of 3–6 experiments using different membrane preparations.

were without effect on this parameter (data not shown).

Radioligand binding studies using human [^{125}I]AII were performed to further assess the pharmacological properties of the AII receptor present in catfish hepatocytes. Human [^{125}I]AII bound to liver plasma membranes in a saturable fashion with high affinity (K_D 2.7 ± 0.5 nM) and a B_{max} of 185 ± 41 fmol/mg of protein was detected (results are the means $\pm$ S.E.M. of 4 determinations using different membrane preparations; a representative saturation isotherm is presented in Fig. 9). Binding competition experiments (Fig. 10) indicated the following order of potency: human AII = fish AII > human angiotensin III = human angiotensin I = fish angiotensin I (Table 1). In contrast, the non-peptide antagonist, losartan or PD123177, did not compete for the [^{125}I]AII binding sites present in catfish liver membranes (Fig. 10 and Table 1).

Table 1
Parameters derived from the [^{125}I]angiotensin II binding competition curves

Agent	K_i (nM)	Slope
Human angiotensin II (4)	3.6 ± 0.4	0.83 ± 0.18
Fish angiotensin II (4)	5.7 ± 1.3	0.96 ± 0.20
Human angiotensin III (3)	62.0 ± 13.0	0.87 ± 0.03
Human angiotensin I (6)	79.0 ± 24.0	1.09 ± 0.13
Fish angiotensin I (4)	97.0 ± 14.0	0.73 ± 0.10
Losartan (4)	$< 10\,000$	–
PD123177 (4)	$< 10\,000$	–

Results are the mean $\pm$ S.E.M. with the number of determinations using different membrane preparations in parentheses.

4. Discussion

The present data indicate that AII receptors are expressed in catfish hepatic cells and modulate calcium mobilization. This response is observed in cells incubated in buffer without Ca^{2+} and containing EGTA which indicated that such increases in $[Ca^{2+}]_I$ are due to the release of this cation from intracellular stores. Therefore, we determined phosphoinositide turnover. Our data clearly indicate that AII induced the production of the second messenger IP_3 and a secondary resynthesis of PI. These data indicate that the AII receptors present in catfish hepatocytes activate the phosphoinositide turnover/ calcium mobilization signal transduction process as it has been observed for AT_1 receptors [2,5–8].

The AII EC_{50} for IP_3 production (≈ 40 nM) is bigger than that observed in the $[Ca^{2+}]_I$ studies (≈ 1 nM) which suggests that the former is not subject to amplification and that, therefore, this effect is more closely related to receptor activation. It is clear that 10 nM AII induced a near maximal $[Ca^{2+}]_I$ response whereas this concentration of the peptide stimulated IP_3 production only to a relatively small extent. These data indicate that, although the density of AII sites detected is not very big, there is some receptor reserve for the $[Ca^{2+}]_I$ response.

The actions elicited by activation of the AII receptor present in fish hepatocytes are insensitive to the well known nonpeptide AII receptor antagonists losartan and PD 123177. Furthermore, the binding competition experiments showed that such receptors have negligible affinity for these AII nonpeptide antagonists. These data suggest that the AII receptors expressed in fish hepatocytes should be considered among the atypical receptors such as those observed in amphibians [11–14] and birds [15,16].

AII is an octapeptide whose sequence, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, is present in human and most mammals. This sequence has been conserved during evolution and the amino acid sequences determined to date indicate great similarity among verte-

brates angiotensin II isoforms. In teleost fish only two changes have been observed: $Asp^1 \rightarrow Asn$ and $Ile^5 \rightarrow Val$ [34]. These changes in sequence, between fish and human angiotensins, do not seem to be critical for their biological activity in catfish hepatocytes since no major differences in the functional responses or in binding affinity were observed. This is consistent with structure-activity studies which have indicated that the carboxyl-terminus of the molecule and the three aromatic side groups (Try, His and Phe), play the major role in determining activity [35].

Like the mammalian AII receptors, the catfish receptor bound AII analogues with nanomolar affinity. However, binding competition assay demonstrated some differences with the cloned mammalian receptors where it has been observed that AII is slightly more potent than angiotensin III at AT_1 receptors ($\approx$ 4-fold) [16], AII and angiotensin III are equipotent at AT_2 receptors [36,37] and angiotensin I has much lower affinity for both subtypes of mammalian receptors [16,36,37]. In our studies with the catfish AII receptor, AII was approximately 17-fold more potent than angiotensin III and 27-fold more potent than angiotensin I.

Xenopus laevis express atypical receptors with low affinity for losartan and PD123177 [11–14]. There are some differences in affinities for AII analogues, between the *X. laevis* atypical AII receptor and the catfish receptor. *X. laevis* myocardial receptors have very high affinity for AI and AII [12]. Nevertheless, these amphibian AII receptors are functionally coupled to the phosphoinositide turnover signal transduction pathway that leads to mobilization of intracellular Ca^{2+} stores [12,13], in a fashion similar to the catfish receptor. Interestingly, the atypical AII receptors expressed in Pekin duck adrenal [16] have affinities for AII analogues similar to those of the catfish receptors.

The atypical AII receptors detected in mouse neuroblastoma [17] and rat intestinal epithelial cells [19] markedly differ from those of catfish liver. The binding sites in neuroblastoma cells have high affinity for AII (7 nM) but very low affinity for angiotensin III [17]. The order of potency observed in rat intestinal cells was very different to that of the present study, i.e., angiotensin I > AII $\gg$ angiotensin III [19].

Interestingly, mutational analysis of the AT_1 receptor has shown that the binding site of non-peptide antagonist is distinct from the site at which peptides bind to the receptor [38,39]. This has been confirmed, in a very elegant study, in which the residues required for losartan binding in the mammalian receptor were exchanged for the corresponding aminoacids in the *Xenopus* receptor to generate a mutant amphibian receptor that bound losartan with the same affinity as the rat AT_1 receptor [40].

In summary, our data clearly indicate that AII receptors are present in catfish liver and that they modulate $[Ca^{2+}]_I$ and phosphoinositide turnover. These receptors have pharmacological characteristics distinct from mammalian AT_1 and AT_2 receptors, and should be considered among the atypical AII receptors.

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References

[1] Jackson, E.K. and Garrison, J.C. (1996) Goodman and Gilman's The Pharmacological Basis of Therapeutics, in: Hardman, J.G., Limbird, L.E., Molinoff, P.B. Ruddpm, R.W. and Gilman, A.G. (Eds.), pp. 733–758, McGraw-Hill, New York.

[2] Griendling, K.K., Lassègue, B. and Alexander, R.W. (1996) Annu. Rev. Pharmacol. Toxicol. 36, 281–306.

[3] 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. and Timmermans, P.B.M.W.M. (1989) Biochem. Biophys. Res. Commun. 165, 196–203.

[4] Timmermans, P.B.M.W.M., Wong, P.C., Chiu, A.T., Herblin, W.F., Benfield, P., Carini, D.J., Lee, R.J., Wexler, R.R., Saye, J.A.M. and Smith, R.D. (1993) Pharmacol. Rev. 45, 205–251.

[5] De Gasparo, M., Husain, A., Alexander, W., Catt, K.J., Chiu, A.T., Drew, M., Goodfriend, T., Harding, J.W., In-

agami T. and Timmermans, P.B.M.W.M. (1995) Hypertension 25, 924–927.
[6] García-Sáinz, J.A. and Macías-Silva, M. (1990) Biochem. Biophys. Res. Commun. 172, 780–785.
[7] Bauer, P.H., Chiu, A.T. and Garrison, J.C. (1991) Mol. Pharmacol. 39, 579–585.
[8] Inagami, T., Mizukoshi, M. and Guo, D.F. (1994) Angiotensin receptors, in: Saavedra, J.M. and Timmermans, P.B.M.W.M. (Eds.), pp. 1–15, Plenum Press, New York..
[9] Kambayashi, Y., Bardhan, S., Takahashi, K., Tsuzuki, S. and Inui, H.J. (1993) J. Biol. Chem. 268, 24543–24546.
[10] Bottari, S.P., King, I.N., Riechin, S., Dahlstroem, I., Lydon, N. and de Gasparo, M. (1992) Biochem. Biophys. Res. Commun. 183, 206–211.
[11] Ji, H., Sandberg, K. and Catt, K.J. (1991) Mol. Pharmacol. 39, 120–123.
[12] Sandberg, K., Ji, H., Millan, M.A. and Catt, K.J. (1991) FEBS Lett. 284, 281–284.
[13] Ji, H., Sandberg, K., Zhang, Y. and Catt, K.J. (1993) Biochem. Biophys. Res. Commun. 194, 756–762.
[14] Bergsma, D.J., Ellis, C., Nuthulaganti, P.R., Nambi, P., Scaife, K., Kumar, C. and Aiyar, N. (1993) Mol. Pharmacol. 44, 277–284.
[15] Nishimura, H., Walker, E., Patton, C.M., Madison, A.B., Chiu, A.T. and Keiser, J. (1994) Am. J. Physiol. 267, R1174–R1181.
[16] Murphy, T.J., Nakamura, Y., Takeuchi, K. and Alexander, W. (1993) Mol. Pharmacol. 44, 1–7.
[17] Chaki, S. and Inagami, T. (1992) Biochem. Biophys. Res. Commun. 182, 388–394.
[18] Burns, L., Clark, K.L., Bradley, J., Robertson, M.J. and Clark, A.J.L. (1994) FEBS Lett. 343, 146–150.
[19] Smith, R.D. (1995) FEBS. Lett. 373, 199–202.
[20] García-Sáinz, J.A. and Macías-Silva, M. (1995) Pharmacol. Commun. 6, 53–60.
[21] García-Sáinz, J.A., Olivares-Reyes, J.A., Macías-Silva, M. and Villalobos-Molina, R. (1995) Gen. Comp. Endocrinol. 97, 117–120.
[22] Janssens, P.A. and Lowrey, P. (1987) Am. J. Physiol. 252, R653–R660.
[23] Cobb, Ch.S. and Brown, A.J. (1992) Comp. Biochem. Physiol 162, 197–202.
[24] Marsigliante, S., Verri, T., Barker, S., Jimenez, E., Vinson, G.P. and Storelli, C (1994) J. Mol. Endocrinol. 12, 61–69.
[25] Marsigliante, S., Musculi, A., Vilely, S., Nicolardi, G.L., Ingrosso, V., Ciardo, V., Zonno, G.P., Vinson, M., Ho, M. and Storelli, C J. (1996) Mol. Endocrinol. 16, 45–56.
[26] Moon, T.W., Walsh, P.J. and Mommsen, T.P. (1985) Can. J. Fish. Aquat. Sci. 42, 1771–1782.
[27] Moon, T.W., Capuzzo, A., Puviani, A.C., Ottolenghi, C. and Fabbri, E. (1993) Am. J. Physiol. 264, E735–E740.
[28] Fabbri, E., Gambarotta, A. and Moon, T.W. (1995) Gen. Comp. Endocrinol. 99, 114–124.
[29] García-Sáinz, J.A. and Fain, J.N. (1980) Biochem. J. 186, 781–789.
[30] Neville, J. (1968) Biochim. Biophys. Acta. 154, 540–552.
[31] García-Sáinz, J.A. and Olivares-Reyes, J.A. (1995) Peptides 16, 1203–1207.
[32] Cheng, Y.C. and Prusoff, W.H. (1973) Biochem. Pharmacol. 22, 3099–3108.
[33] Swanson G.N., Hanesworth, J.M., Sardinia, M.F., Coleman, J.K., Wright, J.W., Hall, K.I., Miller-Wing, A.V., Stobb, J.W., Cook, V.I., Harding, E.C.E. and Harding, J.W. (1992) Regul. Pept. 40, 409–419.
[34] Takei, Y., Hasegawa, Y., Watanabe, T.X., Nakajima, K. and Hazon, N.A. (1993) J. Endocrinol. 139, 281–285.
[35] Peach, M.J. (1979) Kidney Intl. 15, S3–S6.
[36] Kambayashi, Y., Bardhan, S., Takahashi, K., Tsuzuki, S., Inui, H., Hamakubo, T. and Inagami, T. (1993) J. Biol. Chem. 268, 24543–24546.
[37] Mukoyama, M., Nakajima, M., Horiuchi, M., Sasamura, H., Pratt, R.E. and Dzau, V.J. (1993) J. Biol. Chem. 268, 24539–24542.
[38] Schambye, H.T., Hjorth, S.A., Bergsma, D.J., Sathe, G. and Schwartz, T.W. (1994) Proc. Natl. Acad. Sci. USA 91, 7046–7050.
[39] Ji, H., Leung, M., Zhang, Y., Catt, K.J. and Sandberg, K. (1994) J. Biol. Chem. 269, 16533–16536.
[40] Ji, H., Zheng, W., Catt, K.J. and Sandberg, K. (1995) Proc. Natl. Acad. Sci. USA 92, 9240–9244.