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Angiotensin II-induced responses in vascular smooth muscle cells: inhibition by non-peptide receptor antagonists

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Abstract

The present study investigates the effect of angiotensin II and LR-B/081 (-methyl 2-[[4-butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl) [1,1'-biphenyl]-4-yl] methyl]-1(6*H*)-pyrimidinyl] methyl]-3-thiophenecarboxylate), a novel non-peptide angiotensin II receptor antagonist, on both early and late responses in rat vascular smooth muscle cells. Angiotensin II induced a rapid and transient elevation of inositol trisphosphate intracellular levels, triggered the release of both prostaglandin E_2 and prostaglandin I_2 ($EC_{50} = 21 \pm 3$ and 16 ± 2 nM, respectively), and, in long-term studies, increased leucine and thymidine incorporation. All angiotensin II effects were antagonized by LR-B/081 and losartan, the reference non-peptide angiotensin AT_1-selective receptor antagonist, whereas they were unaffected by PD123177 (1-(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine carboxylic acid), a non-peptide angiotensin AT_2-selective receptor antagonist. LR-B/081 displayed a much higher potency than losartan in inhibiting angiotensin II-induced prostaglandin E_2 ($IC_{50} = 0.15 \pm 0.02$ and 39 ± 9 nM, respectively) and prostaglandin I_2 release ($IC_{50} = 0.18 \pm 0.04$ and 134 ± 40 nM, respectively) and was also more potent in blocking the increase in protein synthesis ($IC_{50} = 242 \pm 119$ nM and 1221 ± 687 nM, respectively). Moreover, LR-B/081 and losartan blocked the response to angiotensin III but failed to inhibit the prostaglandin release stimulated by vasopressin or the mitogenic effect of serum. LR-B/081 and losartan were devoid of intrinsinc agonistic properties in the experimental conditions employed. The present results describe LR-B/081 as a novel, highly specific and potent, non-peptide angiotensin AT_1-selective receptor antagonist, that is capable of blocking angiotensin II-proliferative responses, which may be of relevance for cardiovascular diseases.

Keywords: LR-B/081; Angiotensin II; Smooth muscle cell; Inositol trisphosphate; Prostaglandin; Hypertrophy

1. Introduction

Vascular smooth muscle cells are important in the pathophysiology of both hypertension and atherosclerosis. Angiotensin II, which may be also generated by a local renin angiotensin system, promotes, in addition to the well-known vasoconstrictor effects, the growth of vascular smooth muscle cells, leading to structural changes of the vessel wall (Chiu et al., 1991; Rosenberg, 1993). Indeed, angiotensin II, through stimulation of membrane receptors, has been shown to induce protein and DNA synthesis in cultured human and rat aortic vascular smooth muscle cells and, under certain conditions, to increase cell proliferation and extracellular matrix formation (Bunkenburg et al., 1992; Scott-Burden et al., 1990; Koh et al., 1994).

The newly developed non-peptide angiotensin II receptor antagonists have been useful tools in differentiating angiotensin II receptors into two pharmacologically distinct subclasses: the angiotensin AT_1 receptor subtype having high affinity for losartan (DuP 753) and the angiotensin AT_2 receptor subtype showing low affinity for losartan and high affinity for PD123177 (Timmermans et al., 1992, 1993). Angiotensin II and angiotensin III do not distinguish between subtypes but

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paraminophenylalanine[6] angiotensin II binds preferentially to the angiotensin AT_2 receptor (Speth and Kim, 1990). The peculiar behavior of angiotensin II-(3–8) led to the identification of a new binding site in guinea pig and bovine brain, named the angiotensin AT_4 receptor (Harding et al., 1992). The angiotensin AT_1 receptor subtype mediates the effects commonly associated with angiotensin II, while the function of the angiotensin AT_2 receptors has not been defined. Vascular smooth muscle cells predominantly express the angiotensin AT_1 subtype, which mediates cellular hypertrophy and proliferation as well as vasoconstriction (Timmermans et al., 1992, 1993).

Three main transduction pathways are involved in the cellular response to angiotensin II: (1) stimulation of a phosphatidylinositol-specific phospholipase C, (2) activation of L-type voltage-dependent Ca^{2+} channels, and (3) inhibition of adenyl cyclase activity. The rat angiotensin AT_1 receptor can independently couple with all these three signals (Ohnishi et al., 1992). Moreover, angiotensin II may activate phospholipase A_2, leading to the release of arachidonic acid which is then metabolized into prostaglandins. Angiotensin II stimulates the release of prostaglandins, mainly prostaglandin I_2 and prostaglandin E_2, in a variety of tissues including components of the vasculature, such as endothelial cells (Jaiswal 1992; Jaiswal et al., 1992) and vascular smooth muscle cells (Jaiswal et al., 1993). These vasodilator prostanoids are supposed to act as modulators of angiotensin II-induced vasoconstriction (Jackson and Herzer, 1993). Several reports describe the inhibitory effect of losartan on angiotensin II-stimulated second messenger systems in various models. In particular, the prostaglandin release induced by angiotensin II in C6 glioma cells (Jaiswal et al., 1991a) and in rat and human glomerular mesangial cells is blocked by losartan (Chansel et al., 1992).

The aim of the present study was to determine the action of angiotensin peptides on inositol trisphosphate and prostaglandin release and on protein and DNA synthesis in rat vascular smooth muscle cells, and to compare the ability of losartan and LR-B/081 (-methyl 2-[[4-butyl-2-methyl-6-oxo-5-[[2′-(1*H*-tetrazol-5-yl) [1,1′-biphenyl]-4-yl] methyl]-1(6*H*)-pyrimidinyl] methyl]-3-thiophenecarboxylate), a novel non-peptide angiotensin II receptor antagonist, to inhibit the angiotensin II-induced responses in these cells.

2. Material and methods

2.1. Materials

6-keto-Prostaglandin $F_{1\alpha}$ and prostaglandin E_2 were obtained from Biomol (PA, USA), angiotensin II-(3–8) and *p*-aminophenylalanine[6] angiotensin II from Bachem (Bubendorf, Switzerland). The prostaglandin E_2 antiserum was purchased from UBI (Upstate Biotechnology, Lake Placid, NY, USA). Angiotensin II, angiotensin III, arachidonic acid, vasopressin, the Ca^{2+} ionophore A23187 (6*S*-[6α(2*S**,3*S**),8β(*R**),9β, 11α]-5-(methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-yl]methyl]-4-benzoxazolecarboxylic acid), bovine insulin, activated charcoal and 6-keto-prostaglandin $F_{1\alpha}$ antiserum were obtained from Sigma (St. Louis, MO, USA). Dextran T-70 was from Pharmacia Biotech (Uppsala, Sweden). Culture medium and supplements were from Gibco (Grand Island, NY, USA), except fetal bovine serum which was from Hyclone (Logan, UT, USA). [^{3}H]Prostaglandin E_2 (140–170 Ci/mmol), [^{3}H]6-keto-prostaglandin $F_{1\alpha}$ (120–180 Ci/mmol), [^{3}H]leucine (120–190 Ci/mmol), [^{3}H]thymidine (40–60 Ci/mmol) and the inositol trisphosphate assay system kit were purchased from Amersham (Buckingamshire, UK). PD123177 (1-(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]-pyridine carboxylic acid) was synthetized by Cookson Chemicals, Southampton, UK. Losartan and EXP3174 ((2-*n*-butyl-4-chloro-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid) were kindly provided by Dr. Pancras C. Wong (DuPont, Wilmington, DE, USA). Vascular smooth muscle cells, isolated from the thoracic aorta of male Sprague-Dawley rats, were kindly provided by Dr. M. Ziche, Department of Pharmacology, University of Florence, Italy.

2.2. Cell culture

Cells were cultured in Dulbecco's modified Eagle's medium (DMEM), low glucose, supplemented with 15% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and fungizone (2.5 μg/ml). Vascular smooth muscle cells were grown until slightly confluent at 37°C in a humidified atmosphere of 5% CO_2-95% air. For subcultures, cells were dissociated with trypsin (0.25% trypsin, 0.5 mM EDTA in saline) and the resulting cell suspension was diluted 6-fold before seeding. For experiments, cells between passage 3 and 10 were used.

2.3. Measurement of inositol 1,4,5-trisphosphate

Inositol 1,4,5-trisphosphate production was quantified using a procedure previously described (Koh et al., 1989). Vascular smooth muscle cells were plated into 60 mm Petri dishes and grown to confluency. Then, the cells were washed twice with serum-free medium and after being preincubated for 30 min with the vehicle or the antagonist, angiotensin II or vehicle was added. The incubation was stopped at the indicated time by rapidly aspirating the medium and adding 2 ml of

ice-cold 4% perchloric acid. The cells were kept on ice for 20 min, then the perchloric acid extract was removed and centrifuged at 2000 × *g* for 20 min at 4°C to precipitate residual denaturated proteins. The supernatant was titrated to pH 7.5 with 10 N KOH and kept on ice for 60 min. After removal of insoluble $KClO_4$ by centrifugation, inositol 1,4,5-trisphosphate was measured by a commercially available radioreceptor assay kit.

2.4. Measurement of prostaglandin release

Confluent cell cultures were washed twice with DMEM containing 1% fetal bovine serum and preincubated in the same medium for 30 min at 37°C in the presence or absence of the antagonist. Then angiotensins were added and the cells were incubated for 60 min. At the end of the incubation period, the medium was removed and stored at −80°C until assay. The cells were scraped off and homogenized in Tris 10 mM pH 7.4 buffer and an aliquot was used for protein determination, using the Bio-Rad protein assay according to the method of Bradford (1976). The amounts of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ (a stable metabolite of prostaglandin I_2) released into the culture medium were determined by radioimmunoassay. Briefly, 100 μl of sample or standard was mixed with 100 μl of tracer and 100 μl of the appropriate dilution of antiserum. The tubes were incubated overnight at 4°C, then bound tracer was separated from free tracer by addition of 1% dextran-coated charcoal (1 ml), followed by centrifugation for 15 min at 3000 rpm (4°C). The radioactivity present in the supernatant was determined by liquid scintillation counting. The amount of prostaglandin present in each sample was calculated using a computer program for radioimmunoassay analysis (Riasmart, Packard Instruments) and reported as ng/mg cell protein. The cross-reactivity of the anti-6-keto-prostaglandin $F_{1\alpha}$ antibody was < 1% for prostaglandin $A_{1(2)}$, prostaglandin $B_{1(2)}$, < 4% for prostaglandin E_2. The cross-reactivity of the anti-prostaglandin E_2 antibody was < 0.1% for prostaglandin $A_{1(2)}$, prostaglandin $B_{1(2)}$, < 2% for prostaglandin $F_{1\alpha}$.

2.5. Measurement of protein synthesis

Protein synthesis was measured by the incorporation of [^{3}H]leucine in confluent serum-deprived cells, grown in 24-multiwell plates. Confluent cells were washed twice with DMEM containing 0.1% fetal bovine serum and incubated in this medium for 48 h. At the end of the serum-deprivation period, the cells were cultured in leucine-deficient medium, in the absence or presence of the antagonist. After a 30-min preincubation period, angiotensin II was added and the cells were labelled with [^{3}H]leucine (1 μCi/ml). After 18 h, the cells were washed twice with phosphate-buffered saline and incubated in 10% trichloroacetic acid at 4°C for 30 min. Thereafter, the trichloroacetic acid-insoluble material was washed once with 10% trichloroacetic acid and twice with absolute ethanol, and then extracted with 0.1 N NaOH. The radioactivity present in the NaOH extract was determined by liquid scintillation counting.

2.6. Measurement of DNA synthesis

The cells were plated in 24-well culture plates and grown until confluent. DNA synthesis was measured by the incorporation of [^{3}H]thymidine in confluent, serum-deprived cells. Confluent cells were washed twice with DMEM containing 0.1% fetal bovine serum and incubated in this medium for 48 h. At the end of this period, the cells were cultured in fresh medium plus 5 μg/ml of insulin, in the absence or in the presence of the antagonists. After a 30-min preincubation, angiotensins or fetal bovine serum (10%) was added and the cells were labelled with [^{3}H]thymidine (3 μCi/ml, 1.5 μM). The incorporation of tritiated thymidine was stopped after a 24-h incubation. The cells were washed twice with phosphate-buffered saline and incubated at 4°C for 30 min in 10% trichloroacetic acid. Thereafter, the trichloroacetic acid-insoluble material was washed twice with absolute ethanol and the precipitated material was extracted with 0.1 N NaOH. The radioactivity was measured by liquid scintillation counting.

2.7. Data analysis

Data are expressed as means ± S.E.M. of at least three experiments performed in triplicate. Results were compared by unpaired Student's *t*-test, using the computer program Instat for MacIntosh (Graph Pad, San Diego, CA, USA). Differences were considered to be significant at $P < 0.05$. Dose-response curves were analysed by the program MacAllfit for MacIntosh (Consorzio Mario Negri) in order to determine EC_{50} and IC_{50} values.

3. Results

3.1. Angiotensin II-induced inositol trisphosphate production

As shown in Fig. 1A, addition of angiotensin II (1 μM) to vascular smooth muscle cells resulted in a transient increase in the intracellular level of inositol trisphosphate. The maximal increase of inositol trisphosphate concentration (from 78 ± 30, basal condi-

tions, to 373 ± 78, stimulated conditions, pmol/dish) was found at 30 s incubation, after which the response slowly faded. The basal level of inositol trisphosphate measured in control non-stimulated conditions remained practically constant throughout the experiment. A 30-s incubation time was chosen for testing the antagonists. LR-B/081 and losartan (angiotensin AT_1-selective receptor antagonists) and PD123177 (angiotensin AT_2-selective receptor antagonist) did not significantly modify inositol trisphosphate formation by themselves. However, pre-incubation of the cells with LR-B/081 or losartan (1 μM) abolished the angiotensin II-induced elevation of inositol trisphosphate (Fig. 1B). By contrast, PD123177 (1 μM) was unable to inhibit the effect of angiotensin II.

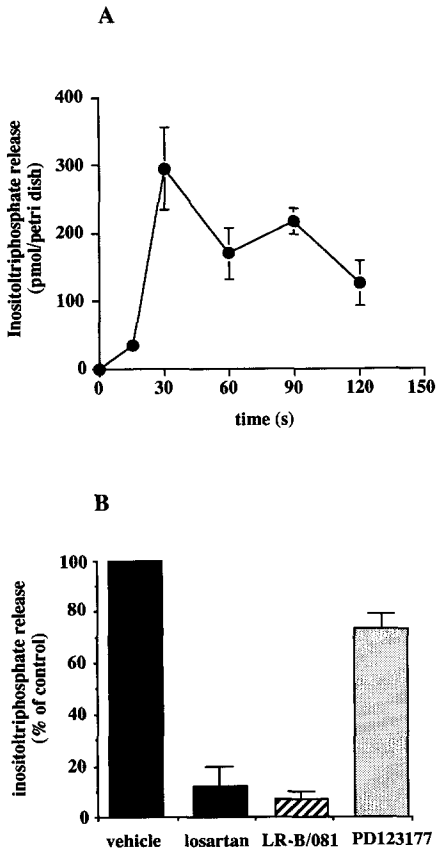

Fig. 1. Angiotensin II-induced formation of inositol 1,4,5-trisphosphate in vascular smooth muscle cells. (A) Time course of inositol 1,4,5-trisphosphate formation. Confluent vascular smooth muscle cells were preincubated in DMEM for 30 min, then 1 μM angiotensin II (stimulated release) or vehicle (spontaneous release) was added and the reaction was stopped at the indicated time. The amount of inositol trisphosphate produced was measured as described in Materials and methods. Data are expressed as net angiotensin II-stimulated inositol trisphosphate production (pmol/Petri dish). (B) Effect of various compounds on the angiotensin II-induced inositol 1,4,5-trisphosphate production. Confluent vascular smooth muscle cells were preincubated for 30 min in the presence of vehicle (control closed bar), or 1 μM losartan (dark grey bar), LR-B/081 (hatched bar) or PD123177 (light grey bar). Angiotensin II (1 μM) or vehicle was then added and the reaction was stopped after 30 s. The amount of inositol trisphosphate produced was determined as described in Materials and methods. Data are expressed as percentage of the net control response to angiotensin II.

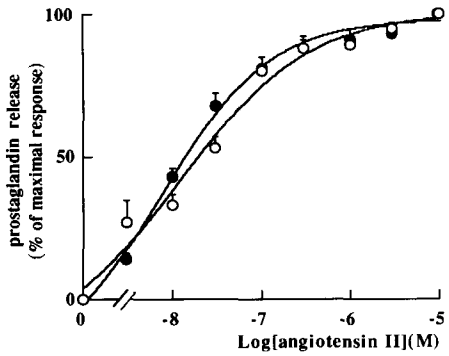

Fig. 2. Concentration dependency of the angiotensin II-induced prostaglandin E_2 and prostaglandin I_2 release from vascular smooth muscle cells. Confluent vascular smooth muscle cells were incubated in DMEM containing 1% fetal bovine serum for 30 min. Thereafter, cells were exposed to angiotensin II, at concentrations ranging from 3×10^{-9} to 10^{-5} M, and incubated for 60 min. The amounts of prostaglandin E_2 (open circles) and prostaglandin I_2 (solid circles) released into the culture medium were determined by radioimmunoassay. For each prostaglandin, data are expressed as percentage of the maximal angiotensin II-induced response.

3.2. *Angiotensin II-induced prostaglandin E_2 / prostaglandin I_2 release*

Under basal non-stimulated conditions vascular smooth muscle cells secreted both prostaglandin E_2 and prostaglandin I_2 (2 ± 1 and 9 ± 2 ng/mg protein, respectively) and the release of both prostaglandins was maximally stimulated by the addition of 1 μM angiotensin II up to 11 ± 2 ng/mg protein for prostaglandin E_2 and up to 52 ± 9 ng/mg protein for prostaglandin I_2. The effect of angiotensin II was comparable to that of 0.1 μM vasopressin (data not shown).

The angiotensin II-stimulated production of prostaglandin E_2 and prostaglandin I_2 was concentration dependent with superimposable dose-response curves (EC_{50} = 21 ± 3 and 16 ± 2 nM, for prostaglandin E_2 and prostaglandin I_2) (Fig. 2).

In order to characterize the receptor involved in angiotensin II-induced prostaglandin release, selective angiotensin II receptor antagonists were used. The basal release of prostaglandin E_2 or prostaglandin I_2 was not affected by pretreatment of the cells with either LR-B/081, losartan, EXP3174 (the active metabolite of losartan) or PD123177 when used at concentrations up to 10^{-5} M (data not shown). Conversely, LR-B/081 and losartan antagonized in a dose-dependent manner the prostaglandin E_2 and prostaglandin I_2 release stimulated by angiotensin II, as shown in Fig. 3A and 3B. LR-B/081 appeared more potent than losartan, displaying a pattern of inhibition

similar to that of EXP3174. The respective IC_{50} values for LR-B/081, losartan, and EXP3174 were 0.15 ± 0.02, 39 ± 9, 0.15 ± 0.02 nM, in the case of prostaglandin E_2 release, and 0.18 ± 0.04, 134 ± 40, 0.18 ± 0.03, in the case of prostaglandin I_2 release.

In contrast, PD123177 even at the highest concentration (1 μM) was unable to inhibit the angiotensin II-induced prostaglandin release. Among the other angiotensins tested (p-aminophenylalanine^6 angiotensin II, angiotensin II-(3–8) and angiotensin III), only angiotensin III efficaciously stimulated the release of prostaglandins, with a maximal response at 1 μM that was $76 \pm 6\%$ that of angiotensin II ($EC_{50} = 50 \pm 13$ and 49 ± 11 nM for prostaglandin E_2 and prostaglandin I_2 release, respectively). The antagonists behaved as they did against angiotensin II. In fact, LR-B/081,

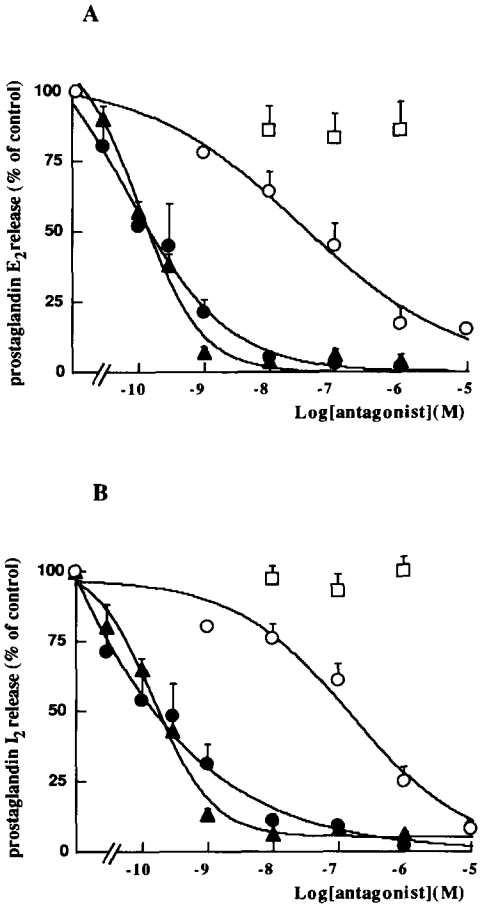

Fig. 3. Effect of various compounds on angiotensin II-induced prostaglandin E_2 and prostaglandin I_2 release from vascular smooth muscle cells. Confluent vascular smooth muscle cells were incubated for 30 min in DMEM containing 1% fetal bovine serum in the absence (vehicle) or in the presence of losartan (open circles), LR-B/081 (solid circles), EXP3174 (closed triangles) or PD123177 (open squares) at the indicated concentrations. Angiotensin II, 1 μM, or vehicle was then added and the incubation was continued for 60 min. The amounts of prostaglandin E_2 (3A) and prostaglandin I_2 (3B) released into the culture medium were determined by radioimmunoassay. Data are expressed as percentage of angiotensin II-induced prostaglandin release in control, vehicle-treated cells.

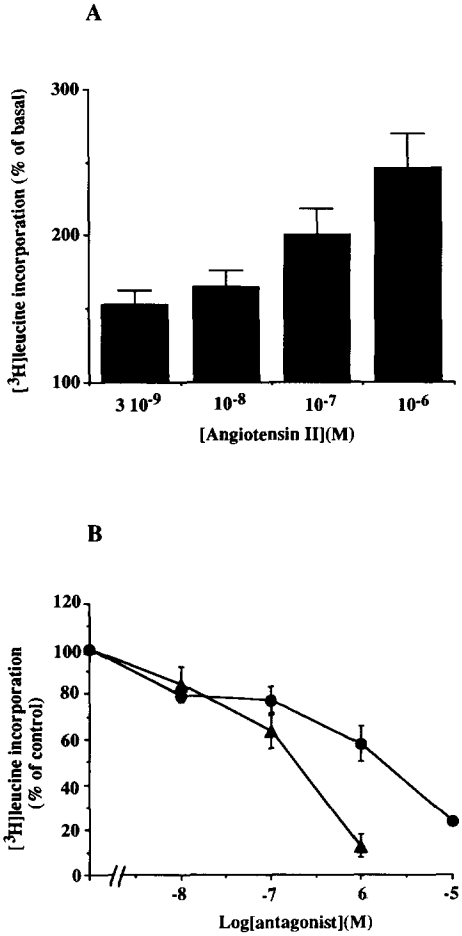

Fig. 4. Effect of angiotensin II on protein synthesis in vascular smooth muscle cells. (A) Angiotensin II dose-response curve. Confluent, serum-deprived, vascular smooth muscle cells were incubated for 18 h with vehicle or increasing concentrations of angiotensin II and [^{3}H]leucine. Protein synthesis was determined as described in Materials and methods. Data are expressed in percentages of basal non-stimulated leucine incorporation. (B) Effect of losartan and LR-B/081 on angiotensin II-induced protein synthesis. The cells were preincubated in the absence (vehicle) or the presence of increasing concentrations of losartan (closed circles) or LR-B/081 (closed triangles) for 30 min. Angiotensin II or vehicle was then added and the cells were labelled for 18 h with [^{3}H]leucine. Data are expressed as percentage of the angiotensin II response in control, vehicle-treated cells.

EXP 3174 and losartan dose dependently inhibited angiotensin III-induced prostaglandin release whereas PD123177 was again completely ineffective. For prostaglandin I_2 release, IC_{50} values for LR-B/081, EXP3174 and losartan were 2 ± 1, 0.39 ± 0.06 and 26 ± 11 nM, respectively. All the antagonists (up to 10^{-5} M) failed to affect the response to vasopressin (0.1 μM) (data not shown).

3.3. Angiotensin II-induced protein synthesis

Fig. 4 shows the effect of angiotensin II on leucine incorporation into proteins in vascular smooth muscle

cells. The addition of angiotensin II to confluent serum-deprived cells dose dependently enhanced the incorporation of [^{3}H]leucine into proteins, reaching a value of 246 ± 23% of basal at 1 μM (Fig. 4A). This effect was dose dependently prevented by preincubation of the cells with LR-B/081 and losartan (10^{-9} to 10^{-5} M) (Fig. 4B). Again, LR-B/081 displayed a higher potency than losartan (IC_{50} = 242 ± 119 nM and 1221 ± 687 nM, respectively), 80–90% of the angiotensin II response being blocked at 1 μM. LR-B/081 and losartan (up to 10^{-5} M) did not affect the basal leucine incorporation.

3.4. Angiotensin II-induced DNA synthesis

Angiotensin II enhanced, in a concentration-dependent manner, the incorporation of [^{3}H]thymidine in confluent serum-deprived vascular smooth muscle cells, the incorporation being increased to 154 ± 6% of the control value at 1 μM. At the concentrations used, LR-B/081 and losartan per se lacked any effect on DNA synthesis but inhibited dose dependently the incorporation of [^{3}H]thymidine induced by angiotensin II (1 μM), both antagonists displaying a similar pattern of inhibition (Fig. 5A). In addition, LR-B/081 and losartan (at 0.1 and 1 μM) abolished the response to angiotensin III, which, unique among the other angiotensins tested, stimulated DNA synthesis to the same extent as angiotensin II (144 ± 4% of basal at 1 μM). Fetal bovine serum (10%) stimulated thymidine incorporation to 253 ± 10% of control and this mitogenic effect was not significantly affected by preincubation of the cells with the antagonists (Fig. 5B). PD123177, 10^{-5} M, did not show any significant effect on angiotensin II/angiotensin III-stimulated DNA synthesis.

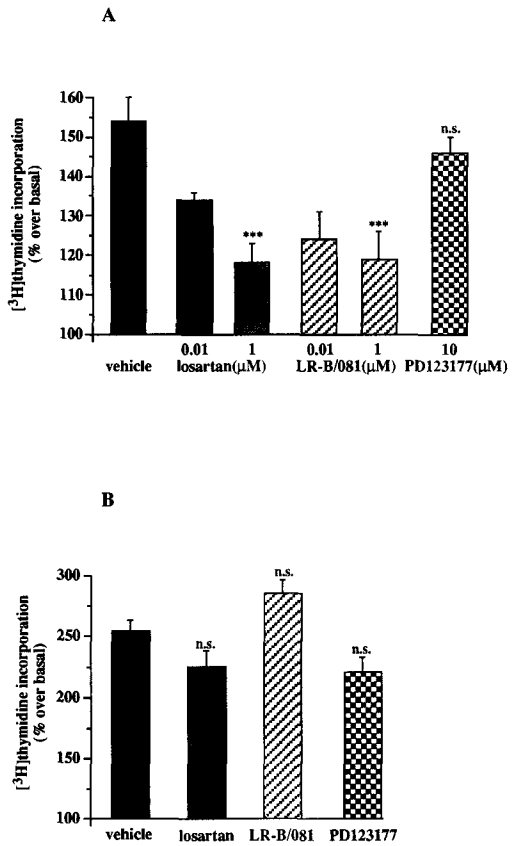

Fig. 5. Effect of various compounds on angiotensin II-(5A) and serum-(5B) induced DNA synthesis in vascular smooth muscle cells. (A) Confluent, serum-deprived cells were preincubated in the absence (vehicle, black bar) or the presence of the indicated concentrations of losartan (grey bar), LR-B/081 (hatched bar) or PD123177 (dotted bar) for 30 min. Angiotensin II or vehicle was then added and the cells were labelled for 24 h with [^{3}H]thymidine. DNA synthesis was determined as described in Materials and methods. Data are expressed as percentage of basal non-stimulated conditions. (B) The cells were preincubated in the absence (vehicle, black bar) or the presence of 1 μM losartan (grey bar), LR-B/081 (hatched bar) or PD123177 (dotted bar) for 30 min. Fetal bovine serum (10%) or vehicle was then added and the cells were labelled for 24 h with [^{3}H]thymidine. Data are expressed as percentage of basal non-stimulated conditions.

4. Discussion

The present study describes the effects of angiotensin peptides and angiotensin II receptor antagonists on both short-term, inositol trisphosphate and prostaglandin production, and long-term, protein and DNA synthesis, cellular responses in rat vascular smooth muscle cells. In short-term studies, angiotensin II induced a transient increase in inositol trisphosphate production and triggered in a concentration-dependent manner the release of both prostaglandin E_2/prostaglandin I_2. In long-term studies, angiotensin II was found to increase both leucine and thymidine incorporation as a measure of cell hypertrophy. The results show also that LR-B/081 and losartan, the non-peptide angiotensin AT_1-selective receptor antagonist used as reference compound, antagonize both the short-term and the long-term cellular effects of angiotensin II, while PD123177, a non-peptide angiotensin AT_2-selective receptor antagonist, was ineffective in both studies. Although it was suggested that the angiotensin AT_2 receptor could be expressed in vascular smooth muscle cells cultured in particular conditions (Kambayashi et al., 1993), our data clearly indicate that the angiotensin AT_1 receptor mediates all the effects of angiotensin II. These results are in agreement with previous reports showing that angiotensin II-induced inositol trisphosphate production, protein and DNA synthesis are blocked by angiotensin AT_1 receptor antagonists in various models including vascular smooth muscle cells (Timmermans et al., 1993; Herbert et al., 1994). The predominance of angiotensin AT_1 receptors in our cell preparation was also confirmed by the observation that

p-aminophenylalanine[6] angiotensin II and angiotensin II-(3–8), two angiotensins capable of discriminating between the angiotensin AT_2 (Speth and Kim, 1990) and angiotensin AT_4 (Harding et al., 1992) subtypes, respectively, were weak agonists for inducing prostaglandin release and DNA synthesis and that only angiotensin III shared agonistic properties similar to those of angiotensin II.

Using this angiotensin AT_1 receptor cell preparation, we characterized LR-B/081 in terms of potency and selectivity. LR-B/081 displayed a 2–3 orders of magnitude higher potency than losartan, while it was approximately equipotent with EXP3174 in inhibiting angiotensin II-induced prostaglandin E_2 and prostaglandin I_2 release. Moreover, LR-B/081 was found to be more potent than losartan in blocking the angiotensin II-induced increase in protein synthesis. A higher potency relative to losartan has been reported for EXP3174 in antagonizing the angiotensin II-induced protein synthesis in rat vascular smooth muscle cells (Sachinidis et al., 1993) and in human mesangial cells (Chansel et al., 1992). In the latter case, EXP 3174 was also 300-fold more potent than losartan in suppressing angiotensin-induced prostaglandin E_2 production. LR-B/081 and losartan dose dependently reduced also the increase in DNA synthesis, but in this case the compounds showed similar potency. These results are in agreement with previous studies reporting that concentrations of losartan similar to the ones used in our work antagonized angiotensin II-induced responses in various cellular models, i.e., intracellular Ca^{2+} increase, inositol phosphate formation and thymidine incorporation (Timmermans et al., 1993).

LR-B/081 and losartan were devoid of intrinsinc agonistic properties in the experimental conditions used. This observation is important since losartan has been reported to produce a concentration-dependent increase in prostaglandin I_2 release in human astrocytes and C6 glioma cells (Jaiswal et al., 1991b; Tallant et al., 1991b). Moreover, LR-B/081 and losartan failed to inhibit the prostaglandin release stimulated by vasopressin or the mitogenic effect of fetal bovine serum, thus demonstrating their specificity of action and suggesting that their inhibitory effects result solely from blockade of the angiotensin AT_1 receptors and not of other pathways.

The fact that LR-B/081 is able, as the other angiotensin AT_1 receptor antagonists, to inhibit angiotensin II- and angiotensin III-stimulated DNA synthesis might be important since vascular cell growth may contribute to the development of hypertension. Prostaglandins also play an important role in modulating the action of angiotensin II. Prostaglandin I_2 infusion in rats is known to inhibit angiotensin II-induced changes in mean arterial blood pressure, total peripheral resistance and renal and mesenteric vascular resistances (Jackson and Herzer, 1993). Prostaglandin E_2 and prostaglandin I_2 are released by vascular smooth muscle cells prepared from Wistar-Kyoto rats and spontaneously hypertensive rats, but in the latter case, basal and angiotensin II-stimulated prostaglandin I_2 production is reduced (Jaiswal et al., 1993). The inhibition of vasodilator prostaglandin release by the antihypertensive angiotensin II antagonists does not seem paradoxical if one considers that when the vasoconstrictor effects of angiotensin II are blunted by antagonists, endogenous compensatory mechanisms must also be inhibited to avoid a potentially harmful hypotensive effect.

The present results, obtained using a cellular preparation, are in good agreement with the results obtained both in receptor binding experiments (Renzetti et al., 1995, in preparation) and in functional in vivo pharmacological studies (Cirillo et al., 1995, in press), showing the high specificity and potency of LR-B/081 as an angiotensin AT_1 receptor antagonist and as an antihypertensive agent. Taking together, the data presented in this study describe LR-B/081 as a novel non-peptide angiotensin AT_1-selective receptor antagonist that is capable of blocking proliferative responses to angiotensin II, which may be of relevance for cardiovascular diseases.

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