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ANGIOTENSIN II EXERTS ITS EFFECT ON ALDOSTERONE PRODUCTION AND POTASSIUM PERMEABILITY THROUGH RECEPTOR SUBTYPE AT_1 IN RAT ADRENAL GLOMERULOSA CELLS

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Abstract—The stimulatory effect of angiotensin II (AT) on the accumulation of inositol phosphates and on aldosterone production is abolished by the AT_1 selective receptor antagonist DuP753 while PD123177, an AT_2 antagonist, is ineffective. Similarly, a depolarizing effect of AT (inhibition of $K^+/^{86}Rb$ efflux) is prevented by DuP753. While mediators derived from phospholipase C activation have a central role in the stimulation of aldosterone production by AT, the effect of AT on K^+ permeability is mimicked neither by elevation of cytoplasmic $[Ca^{2+}]$ by ionomycin nor by kinase C activation with phorbol ester. Our results suggest that AT stimulates phospholipase C and the subsequent steroid production by glomerulosa cells through AT_1 receptors. In addition some events induced by the activation of AT_1 receptors may not be mediated by the activation of phospholipase C.

Two different binding sites of angiotensin II (AT‡) were identified recently with highly specific nonpeptide antagonists in rat adrenal cortex [1–3] and in other tissues [1, 2, 4]. Only one subtype (AT_1) was found in rabbit adrenal and liver [5], showing the possibility of tissue- and species-specific distribution of these receptor subclasses. There are few data on the messenger mechanisms associated with the two AT receptor subtypes. In liver cells only DuP753 (an antagonist of receptor subtype AT_1) inhibits the formation of inositol phosphates and activation of phosphorylase *a* in AT-stimulated cells [5, 6]. These data show that ligand binding to AT_1 receptors activates phospholipase C and induces a Ca^{2+} signal. The role of receptor subtype AT_2 has not been elucidated.

The response to AT is also induced by activation of phospholipase C in adrenal glomerulosa cells (for review see Refs 7 and 8). However, it is not known whether AT_1 receptors are involved in this response. We also examined the action of AT on the K^+ permeability of glomerulosa cells to elucidate whether the same AT receptor subtype and the same intracellular mediators are responsible for the effect of AT on aldosterone production and on K^+ permeability.

MATERIALS AND METHODS

Angiotensin II (Ile^5-angiotensin II) was obtained from Serva (Heidelberg, F.R.G.), TPA from the Sigma Chemical Co. (St Louis, MO, U.S.A.), ionomycin from Calbiochem (Luzern, Switzerland) and nonpeptide AT receptor antagonists were synthesized at DuPont (Wilmington, DE, U.S.A.). Nitrocellulose membrane filter (pore size 3 μm) was from Schleicher and Schüll (Feldbach ZH, Switzerland), *myo*-[2-^{3}H]inositol (16.6 Ci/mmol) from DuPont (Boston, MA, U.S.A.). and [^{86}Rb]-RbCl (0.5 Ci/g) from Izinta (Budapest, Hungary).

Rat adrenal glomerulosa cells were prepared from capsular tissues of Wistar rats (200–300 g) using collagenase and mechanical dispersion as described previously [9].

In the aldosterone experiments adrenal glomerulosa cells derived from male rats were preincubated for 3 hr in a mixture of Krebs–Ringer–bicarbonate–glucose solution and Medium 199 (2:1 v/v). The composition of Krebs–Ringer solution was modified to give the following final concentrations in mM: Na, 145; K, 3.6; Ca, 1.2 and Mg, 0.5; human serum albumin (fraction V), 2 g/L. The pH was kept at 7.4 under a mixtue of 95% O_2 + 5% CO_2 (37°). Then the cells were washed and incubated (about 10^5 cells/600 μL) for 60 min under identical conditions. Aldosterone content of the supernatant (after pelleting the cells) was measured by radioimmunoassay [10]. Mean basal aldosterone production was 0.89 ± 0.28 pmol/10^5 cells × hr (N = 4).

Experiments on the formation of inositol phosphates were performed as described previously [11]. Labeling period was followed by a 15-min stimulation in the presence of LiCl (10 mM). Inositol phosphates were separated by anion exchange chromatography [12].

The K^+ permeability of the cells was examined by measuring ^{86}Rb efflux from isotope preloaded glomerulosa cells. Cells derived from female rats (about 10^5) were preincubated in the presence of a

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‡ Abbreviations: AT, angiotensin II; TPA, Arg-AVP, tetradecanoyl phorbol acetate; vasopressin.

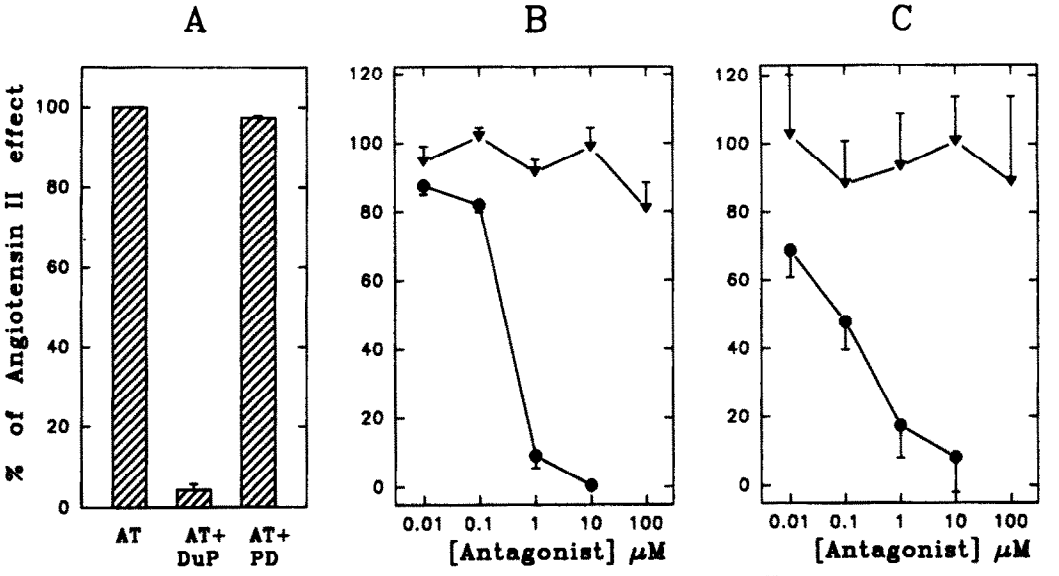

Fig. 1. Effect of selective nonpeptide AT receptor antagonists on AT-induced [^{3}H]inositol trisphosphate accumulation (A), on AT-stimulated aldosterone production (B) and on AT-induced inhibition of ^{86}Rb efflux (C). Cells were stimulated with AT (25 nM) in the presence or absence of DuP753 (●) or PD123177 (▼). Means ± SEM are shown (A, N = 3; B, N = 4; C, N = 5).

tracer amount of ^{86}RbCl (0.2–0.5 μCi/100 μL) for 70 min at 37°. Here, the medium was buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (20 mM) instead of bicarbonate. Isotope efflux was induced by a 15-fold dilution with isotope free medium containing stimulatory substances or solvent. Incubation was usually terminated 15 min after the onset of stimulation by vacuum filtration through nitrocellulose membrane filters. The activity of ^{86}Rb on the filter was measured by liquid scintillation counting.

RESULTS AND DISCUSSION

Maximally effective concentration of AT (25 nM) induced a 6.0 ± 0.5-fold increase in the accumulation of [^{3}H]inositol trisphosphates in glomerulosa cells (N = 3). This effect of AT was almost completely abolished by the AT_1-specific receptor antagonist DuP753 (10 μM) while the AT_2-specific PD123177 (10 μM) had a negligible effect only (Fig. 1A). The same concentration of AT induced a 14.7 ± 5.2-fold stimulation of aldosterone production (N = 4). DuP753 dose-dependently inhibited the effect of AT (IC_{50} approx. 250 μM) while PD123177 did not modify the effect of AT up to 10 μM (Fig. 1B). Neither Dup753 nor PD123177 exerted any significant effect on aldosterone production by control cells or cells stimulated with 8.4 mM K^+ (data not shown). These data show that the stimulatory effect of AT on phospholipase C and on steroid production is mediated by the AT_1 receptor subtype.

A dual effect of AT was reported previously on the potassium permeability of bovine glomerulosa cells [13]. An initial increase was followed by a sustained reduction of potassium permeability. The initial increase was attributed to Ca^{2+}-activated K^+ efflux. In fact, we also found that Ca ionophores enhanced ^{86}Rb efflux from rat adrenal glomerulosa cells (the residual ^{86}Rb content after 5 min efflux was 80.6 ± 5.9% of control in the presence of 10 μM ionomycin, $P < 0.05$, N = 4). However, the Ca-mobilizing AT (25 nM) did not induce an initial increase and evoked sustained inhibition only of ^{86}Rb efflux in our experiments (Fig. 2A). Therefore, it may be assumed that in AT-stimulated rat glomerulosa cells Ca^{2+}-activated K^+ efflux is masked by an unknown mechanism leading to reduced K^+ permeability. Conversely, the reduction of K^+ permeability may not be attributed to the Ca^{2+} signal. Considering that cytoplasmic Ca^{2+} exerts a mediating role in AT-induced steroid production, the idea arose that AT-evoked Ca^{2+} signal and inhibition of K^+ efflux are initiated by different receptor subtypes. To test this presumption, we have examined the effect of AT antagonists also on K^+ permeability. AT-induced inhibition of ^{86}Rb efflux (5 min) was influenced by DuP753 and PD123177 in the same manner as aldosterone production (Fig. 1C).

V_1-type AVP receptors in glomerulosa cells are coupled to the Ca signalling pathway [14]. Like AT, AVP exerted a significant inhibitory effect on ^{86}Rb efflux (Fig. 2B, $P < 0.02$, N = 4). (The small effect of AVP as compared to AT may be attributed to the low density of V_1 receptors [14].) The role of protein kinase C is unlikely to mediate the effect of AT or AVP on K^+ permeability, since TPA that activates protein kinase C under similar conditions [15] was found to be completely ineffective (Fig. 2B). Thus, the effects of protein kinase C on K^+ permeability seem to be different in bovine [16] and rat glomerulosa cells.

The present data suggest that in rat adrenal glomerulosa cells the AT_1 subtype of angiotensin receptors is coupled to Ca mobilization and thereby

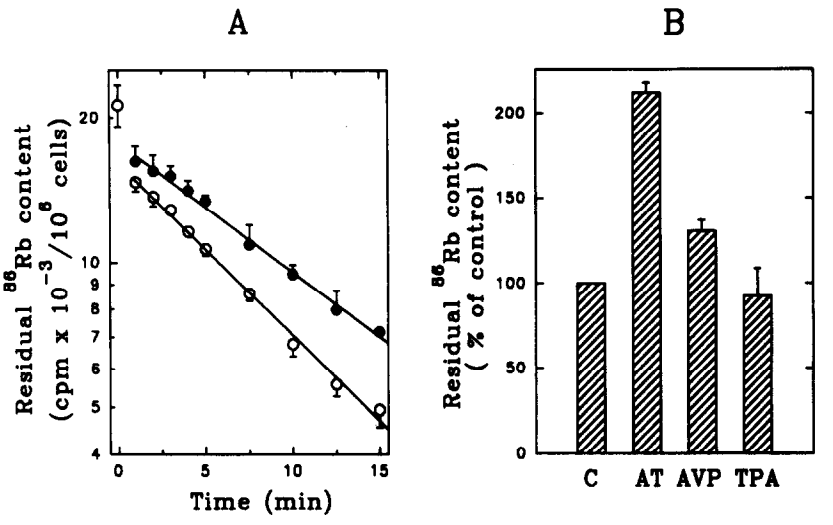

Fig. 2. Effect of AT, AVP and TPA on ^{86}Rb efflux. (A) Time-course of ^{86}Rb efflux from unstimulated (○) and AT (25 nM) treated cells (●). Fifteen-fold dilution of prelabelled cells with isotope free medium was done at 0 min. Residual ^{86}Rb content of the cells is shown (means ± SEM, N = 3). (B) Residual ^{86}Rb content of the cells after 15 min efflux in the presence of AT (25 nM), AVP (100 nM) or TPA (50 nM). Results were expressed as percentages of residual ^{86}Rb content of control cells (means ± SEM, N = 4–5).

to steroid production. The decrease in K^+ permeability may participate in AT induced depolarization [17, 18] of the cells. This effect is also mediated by AT_1 receptors. Our results also indicate that known messengers generated through activation of phospholipase C have no role in the reduction of K^+ permeability since the Ca ionophore ionomycin increases K^+ permeability whereas phorbol ester, an activator of kinase C, fails to affect K^+ permeability. These observations raise the possibility that binding of AT to AT_1 receptors, in addition to its known effects, activates a hitherto unrecognized transducing mechanism. Further experiments are required to elucidate whether the unidentified modulator of potassium channels is a G-protein coupled to the AT_1 receptor or is generated in a later step of the Ca signalling pathway.

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