Angiotensin II inhibits K^+-induced Ca^{2+} signal generation in rat adrenal glomerulosa cells

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The Ca^{2+}-mobilizing hormone angiotensin II (AII) dose-dependently inhibited the K^+-induced sustained increase of cytoplasmic Ca^{2+} concentration in adrenal glomerulosa cells and caused a rapid decrease of cytoplasmic Ca^{2+} when added to cells already stimulated with K^+. These effects of AII on the K^+-induced Ca^{2+} signal were mimicked, although less effectively, by other Ca^{2+}-mobilizing agonists such as [Arg^8]vasopressin (AVP) and thapsigargin. Phorbol esters did not show such effects, nor did corticotropin (ACTH), a secretagogue acting via cyclic AMP. The K^+-stimulated initial $^{45}Ca^{2+}$ uptake, a measure of Ca^{2+} entry into glomerulosa cells, was also prevented by AII pretreatment, and was inhibited by AVP, but not by ACTH. The stimulatory effect of K^+ on aldosterone production, however, was not inhibited by AII, and the AII-induced aldosterone production was further increased by increasing K^+. These data indicate that AII is able to inhibit static increases in cytoplasmic Ca^{2+} by inhibiting Ca^{2+} entry through voltage-sensitive Ca^{2+} channels and, possibly, by activating Ca^{2+} extrusion from the cells. It is also concluded that the Ca^{2+} signal evoked by AII is very efficient in stimulating hormone secretion, and the secretory response of the cells becomes more sensitive to any further increase of Ca^{2+} entry through voltage-sensitive Ca^{2+} channels.

INTRODUCTION

Angiotensin II (AII) and increasing concentrations of K^+ are major regulators of the secretion of aldosterone by the adrenal zona glomerulosa. Both stimuli are believed to trigger increased hormone production by generating a Ca^{2+} signal in the cytoplasm. The rapid rise in cytoplasmic Ca^{2+} concentration evoked by K^+ is due to increased Ca^{2+} entry from the extracellular fluid through voltage-sensitive Ca^{2+} channels (VSCC) that are already activated by small depolarizations (see [1] for review). AII, on the other hand, stimulates the release of Ca^{2+} from intracellular Ca^{2+} stores, owing to the very rapid formation of the Ca^{2+}-mobilizing messenger Ins(1,4,5)P_3 [2–4]. AII also stimulates the entry of Ca^{2+} into glomerulosa cells, although the mechanism of this Ca^{2+} entry is still a matter of debate [5–7]. The stimulatory effect of AII on aldosterone production is inhibited by inhibitors of VSCC, such as nifedipine or nitrendipine [7–9], and could be enhanced by the dihydropyridine derivative Ca^{2+}-channel agonist BAY-K 8644 [8,10], suggesting that VSCC are involved in Ca^{2+}-signal generation. On the other hand, the stimulated entry of $^{45}Ca^{2+}$ [7] (but see [11]), as well as the cytoplasmic Ca^{2+} increase [12] in AII-treated glomerulosa cells, cannot be prevented by the dihydropyridine antagonist nifedipine. Electrophysiological studies on the effects of AII on Ca^{2+} currents are also conflicting. AII was found to inhibit an inward Ca^{2+} current in rat glomerulosa cells [13], whereas in other reports the peptide increased the activity of T-type Ca^{2+}-channels in bovine glomerulosa cells [14] or the activity of L-type channels in an adrenocortical cell line [15].

In the present study we examined the effects of AII on the cytoplasmic Ca^{2+} signal that was generated by the activation of VSCC by K^+-induced depolarization. We also studied how the interaction between these two stimuli, both generating a cytoplasmic Ca^{2+} signal, affects the hormone production by rat adrenal glomerulosa cells.

METHODS

Superfusion of isolated rat adrenal glomerulosa cells

Rat adrenal glomerulosa cells were prepared from the adrenal capsular tissue of male Wistar rats (250–350 g) by collagenase digestion and mechanical dispersion as described previously [7]. Isolated cells (about 10^6 cells/column) were mixed with 600 μl of pre-swollen Bio-Gel P10 resin and loaded on to small columns prepared from 2.0 ml syringes. Cells on the column were superfused at a flow rate of 8.0 ml/h with a 2:1 (v/v) mixture of modified Krebs–Ringer bicarbonate–glucose solution and Medium 199 (concentrations of Na^+, K^+ and Ca^{2+} in mM were 145, 3.6 and 1.2 respectively) supplemented with human serum albumin (2 g/l) as detailed elsewhere [16]. The effluent was collected on ice, and its aldosterone concentration was measured by radioimmunoassay [16].

Measurements of cytoplasmic Ca^{2+} concentration and initial $^{45}Ca^{2+}$ uptake

Cytoplasmic Ca^{2+} concentration was measured by the fluorescent Ca^{2+} probe fura-2 as detailed elsewhere [17]. A previously described method [7] was used for the measurements of initial (60 s) uptake of $^{45}Ca^{2+}$ into glomerulosa cells.

Statistics

Results are shown as means ± S.E.M. for the superfusion experiments, where aldosterone responses were expressed as fold stimulation in each of the experiments relating aldosterone concentrations of the effluent to those of averaged basal, pre-

Abbreviations used: AII, angiotensin II; ACTH, adrenocorticotropin; AVP, [arginine^8]vasopressin; VSCC, voltage-sensitive Ca^{2+} channels; PMA, phorbol 12-myristate 13-acetate; PdBu, phorbol 12,13-dibutyrate.

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stimulated, values of the respective columns. Representative traces are shown from the cytoplasmic Ca^{2+} measurements, each repeated at least three times with different cell preparations. Means ± S.E.M. are shown for the $^{45}Ca^{2+}$-uptake experiments, each carried out in triplicate.

Materials

Materials used for cell isolation and for the incubations are described elsewhere [7]. The acetoxymethyl ester (AM) and the free acid of fura-2 were obtained from Calbiochem (San Diego, CA, U.S.A.). AII ([Ile^5]AII), ACTH (Synacthen) and vasopressin ([Arg^8]vasopressin, AVP) were from Serva (Heidelberg, Germany), CIBA–GEIGY (Basle, Switzerland) and Bachem (Torrance, CA, U.S.A.) respectively. BAY-K 8644 was obtained from Bayer (Leverkusen, Germany). The $^{45}Ca^{2+}$ was purchased from Izinta (Budapest, Hungary). Both phorbol esters (PMA and PdBu) were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals used were of analytical grade.

RESULTS

Effects of K^+ and AII on cytoplasmic Ca^{2+} concentration

The cytoplasmic Ca^{2+} concentration increased very rapidly in response to stimulation with either K^+ or AII in rat adrenal glomerulosa cells. Whereas the Ca^{2+} response to 18 mM-K^+ was characterized by a sustained increase (Fig. 1*a*), the response to 30 nM-AII was composed of an early spike increase and a subsequent plateau phase that was only slightly higher than the baseline (Fig. 1*b*). When the same dose of K^+ (18 mM) was added after a high dose of AII (30 nM), there was only a small transient increase observed in the Ca^{2+} concentration (Fig. 1*b*). Interesting enough, the Ca^{2+}-channel agonist BAY-K 8644 (100 nM) was able to increase cytoplasmic Ca^{2+} further under these conditions (Fig. 1*b*).

Changing the order of additions produced similar results; the K^+-evoked increase in cytoplasmic Ca^{2+} started to decrease slowly as a low dose (300 pM) of AII was added, and was rapidly antagonized by the subsequent addition of a higher dose (30 nM) of AII (Fig. 1*a*).

Decreasing the dose of AII to levels as low as 100 pM (where the increase in cytoplasmic Ca^{2+} was mostly undetectable in our system) also interfered with K^+-induced Ca^{2+}-signal generation. Although the early increase in cytoplasmic Ca^{2+} evoked by 18 mM-K^+ was unchanged, this increase was only transient, and cytoplasmic Ca^{2+} levels returned to a lower steady-state value. Both the height of the K^+-induced Ca^{2+} peak and the plateau value were decreased when higher concentrations of AII were applied before K^+ (Fig. 2). The rate of decline at which cytoplasmic Ca^{2+} approached the lower steady-state level (after the K^+-induced initial Ca^{2+} spike) was higher as the time between the additions of AII (300 pM) and 18 mM-K^+ was increased (results not shown). Since the kinetics of aldosterone secretion could only be studied in an extended time scale (see below), in two additional experiments the ability of AII to inhibit the K^+-induced Ca^{2+} response was tested after 1 h stimulation with K^+, as was the difference between the K^+-induced Ca^{2+} response in control cells versus cells treated with AII for 1 h. Although there was a considerable dye leakage during these incubations, AII (30 nM) clearly decreased the cytoplasmic Ca^{2+} in cells that had been stimulated by K^+ (8.4 mM or 18 mM), and the Ca^{2+} response to 18 mM-K^+ was inhibited in cells stimulated by AII (30 nM) (results not shown).

We also tested whether activation of protein kinase C is responsible for the observed effects of AII. Concentrations of phorbol esters (PMA and PdBu, 10–250 nM) that were effective in inducing translocation of the enzyme in these cells [18] failed to mimic the effects of AII on the K^+-induced cytoplasmic Ca^{2+} response (Fig. 3*a*). Similarly, the cyclic AMP-generating hormone ACTH (10–300 nM) had no effect on the Ca^{2+} signal generated by K^+ (Fig. 3*b*). On the other hand, other Ca^{2+}-mobilizing stimuli such as AVP or thapsigargin were found to have similar effects as lower doses of AII (Fig. 3*c*).

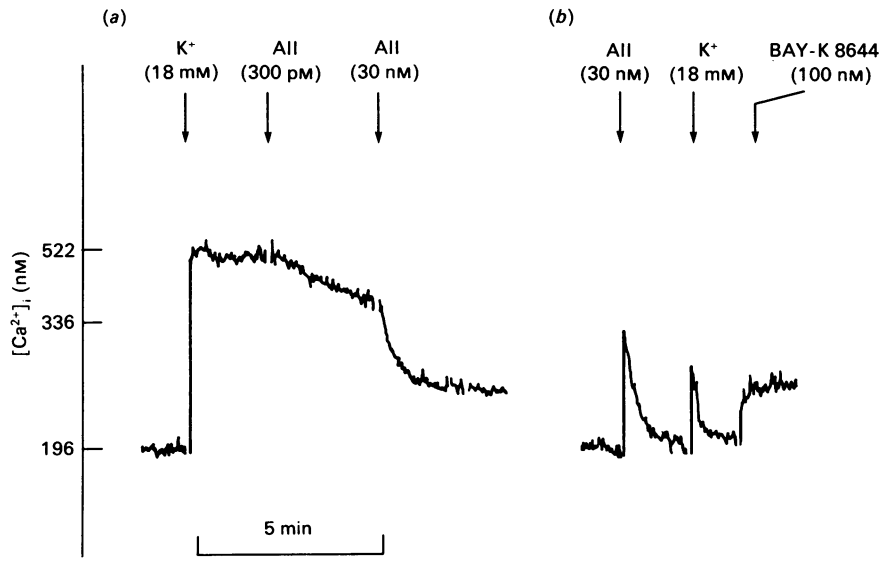

Fig. 1. Effect of AII on the K^+-induced increase of cytoplasmic Ca^{2+} concentration in rat adrenal glomerulosa cells

Isolated rat adrenal glomerulosa cells were loaded with 0.2 μM fura-2/AM, and the fluorescence of about 2×10^6 cells incubated in 2 ml volume was monitored as described in the Methods section. Additions of stimuli at the indicated final concentrations are shown by the arrows (for clarity, the horizontal lines caused by the closure and opening of the shutter were removed from the traces). The ordinate with the calculated intracellular concentrations of Ca^{2+} ($[Ca^{2+}]_i$) refers to both panels (*a*) and (*b*). Similar results were obtained with at least ten different cell preparations.

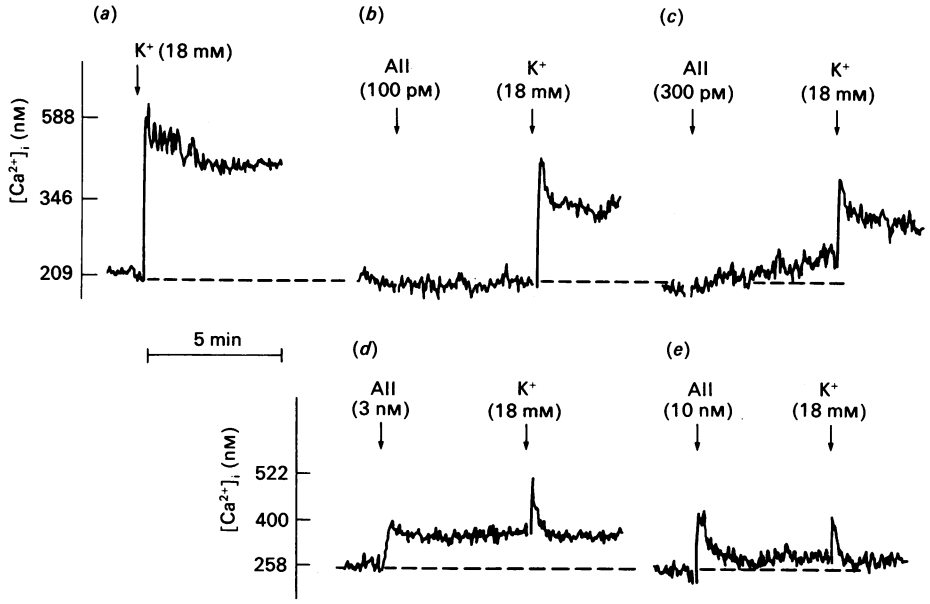

Fig. 2. Effect of a 5 min stimulation with increasing dose of AII on the cytoplasmic Ca^{2+} increase evoked by 18 mM-K^+

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of isolated rat adrenal glomerulosa cells was monitored with the fluorescent Ca^{2+} probe fura-2 as described in the legend to Fig. 1. Results from a representative experiment are shown, and were repeated four times with different cell preparations.

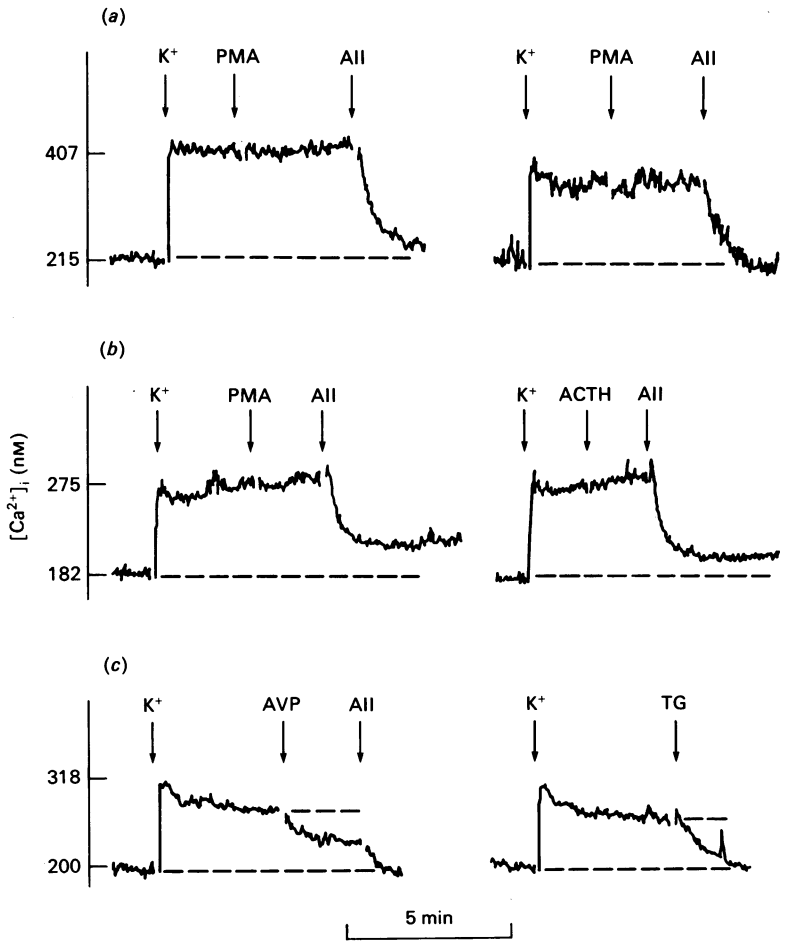

Fig. 3. Effect of PMA, ACTH, AVP and thapsigargin on the cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) response to 18 mM-K^+ in rat adrenal glomerulosa cells

Panels (*a*), (*b*) and (*c*) show traces from three different experiments. The final concentrations of stimuli were: 100 nM- and 250 nM-PMA in the left and right panels in (*a*), 25 nM-PMA and 100 nM-ACTH in panel (*b*), and 1 μM-AVP and 1 μM-thapsigargin (TG) in panel (*c*). The concentration of AII was 10 nM in all traces. Similar results were obtained in two to five other cell preparations.

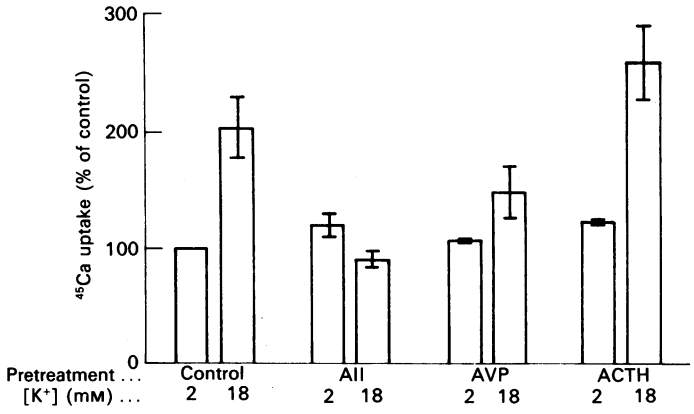

Fig. 4. Effect of 10 min stimulation with different stimuli on the K^+-stimulated 60 s $^{45}Ca^{2+}$ uptake by rat adrenal glomerulosa cells

Isolated rat adrenal glomerulosa cells were incubated with control medium or stimulated with AII (25 nM), AVP (1 μM) or ACTH (300 nM) for 10 min. $^{45}Ca^{2+}$ was then added for 60 s either with K^+ (to a final concentration of 18 mM) or leaving the concentration of K^+ unchanged (2 mM). The cell-associated radioactivity was measured by vacuum filtration as detailed in the Methods section. Results are expressed as percentages of basal $^{45}Ca^{2+}$ uptake by the cells ('control'). Results are means ± S.E.M. of three experiments, each carried out in triplicate.

Effects of different hormonal stimuli on the stimulation of $^{45}Ca^{2+}$ uptake by K^+

In subsequent experiments we examined the ability of AII and other secretagogues to interfere with the stimulatory effect of K^+ on Ca^{2+} entry. The initial 60 s $^{45}Ca^{2+}$ uptake was used as a measure of the rate of Ca^{2+} entry into adrenal glomerulosa cells. As shown previously [7], K^+ (18 mM) increased the initial 60 s uptake of $^{45}Ca^{2+}$, indicating the opening of VSCC. However, when cells were treated with AII (25 nM) for 10 min, the subsequent addition of K^+ (18 mM) failed to increase the uptake of $^{45}Ca^{2+}$. A similar but smaller inhibitory effect was seen when AVP (1 μM) was used instead of AII (Fig. 4). The effect of ACTH (300 nM), if any, was stimulatory on K^+-induced $^{45}Ca^{2+}$ entry (Fig. 4). In two separate experiments, PMA (10–100 nM) failed to inhibit the K^+-evoked increase of $^{45}Ca^{2+}$ uptake (results not shown).

Effects of AII and K^+ on steroid production by superfused adrenal glomerulosa cells

In light of the observation that AII inhibited the Ca^{2+} signal generated by K^+, it was important to know if this was reflected in the secretory response of the cells. In these superfusion experiments we used the most effective concentration of K^+ (8.4 mM) to stimulate aldosterone production in combination with 30 nM-AII. When K^+ was added first and AII was administered 1 h later, no inhibition was observed in the aldosterone response as compared with that evoked by K^+ itself (Fig. 5*a*). This finding indicates that, in spite of the decreasing Ca^{2+} levels, the stimulation of hormone production was maintained. Similar results were obtained with 18 mM-K^+, to which the aldosterone-secretory response was only transient, and 30 nM-AII rapidly stimulated the production of aldosterone, preventing its decay (T. Balla, P. Várnai, Zs. Holló & A. Spät, unpublished work). When the additions were reversed, i.e. K^+ (8.4 mM final concn.) was added to cells that had been stimulated with 30 nM-AII for 1 h, the aldosterone production by the cells was increased above the values that are characteristic for the secretory response to this concentration of AII (Fig. 5*b*). These latter results also indicate that elevated levels of K^+ have a stimulatory effect on the hormone production by AII-stimulated glomerulosa cells, although the K^+-induced increase in cytoplasmic Ca^{2+} is diminished and transient and the enhancement of Ca^{2+} entry is completely abolished.

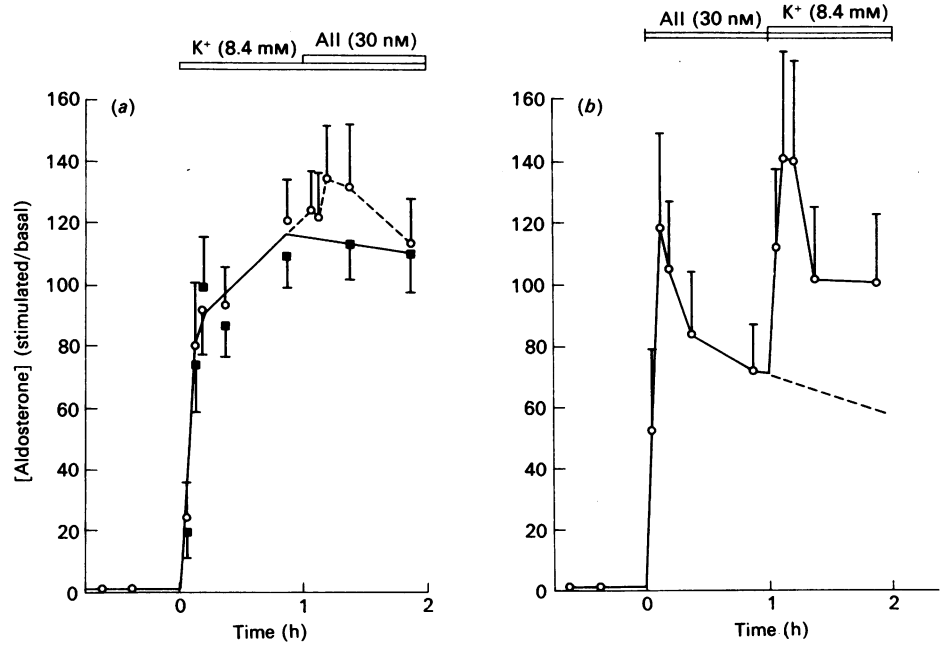

Fig. 5. Effect of AII (30 nM) and K^+ (8.4 mM) and their combination on the aldosterone-secretory response of rat adrenal glomerulosa cells

The hormone production by isolated glomerulosa cells was monitored in a cell superfusion system as described in the Methods section. Results are normalized to the averaged pre-stimulation values of aldosterone concentrations. The additions of stimuli are indicated by the horizontal bars. Means ± S.E.M. of five (*a*) or four (*b*) experiments are shown: ■, 8.4 mM-K^+; ○, 8.4 mM-K^+ + 30 nM-AII.

DISCUSSION

In the present studies we found that the Ca^{2+}-mobilizing hormone AII, which is known to enhance Ca^{2+} entry into glomerulosa cells (see [1] for review), is able to inhibit the cytoplasmic Ca^{2+} increase induced by K^+ and also prevents the increased rate of $^{45}Ca^{2+}$ entry in K^+-stimulated rat adrenal glomerulosa cells. This phenomenon was previously noticed in bovine adrenal glomerulosa cells, where AII administered after K^+ was able to decrease the elevated cytoplasmic Ca^{2+} levels [19]. Also, more recently, Ca^{2+}-mobilizing hormones (but not phorbol esters) were found to decrease rapidly the elevated cytoplasmic Ca^{2+} concentrations evoked by the compound 2,5-di-(t-butyl)-1,4-benzohydroquinone in hepatocytes, owing to stimulation of a Ca^{2+}-extrusion mechanism [20]. Similarly, stimulation of M_3 muscarinic receptors inhibited voltage-dependent Ca^{2+} influx in small-cell lung carcinoma, an effect not attributed to protein kinase C activation [21].

The negative interaction between AII and the K^+-induced increase of cytoplasmic Ca^{2+} can already be detected at AII concentrations as low as 100 pM, indicating that this phenomenon is not caused by a channel inactivation as part of desensitization owing to high hormone levels. It is difficult to decide from our data whether AII activates a mechanism responsible for Ca^{2+} extrusion from the cell, or whether it leads to inhibition or rapid inactivation of the VSCC that are activated by K^+. Since high doses of AII (above 10 nM) prevented both the K^+-induced enhancement of $^{45}Ca^{2+}$ uptake and the increase in cytoplasmic Ca^{2+} concentration, we suggest that there is an inhibition of Ca^{2+} entry through these VSCC. This conclusion is consistent with the finding that AII inhibited a Ca^{2+} current activated by depolarization in rat adrenal glomerulosa cells [13]. Also, repeated or prolonged addition of the Ca^{2+}-mobilizing agonist gonadotropin-releasing hormone was shown to inhibit L-type channels in pituitary gonadotrophs [22]. On the other hand, applied at lower (sub-nanomolar) concentration, AII could also enhance the inactivation of a Ca^{2+}-entry mechanism or activate Ca^{2+} extrusion, thereby determining the steady-state cytoplasmic Ca^{2+} concentration. Activation of Ca^{2+} efflux by AII in adrenal glomerulosa cells has been described previously [23,24], together with the ability of the hormone to decrease total or exchangeable Ca^{2+} content of the cells [23,25,26].

Phorbol esters and other activators of protein kinase C have been shown to inhibit depolarization-induced cytoplasmic Ca^{2+} increases [27,28] as well as voltage-dependent Ca^{2+} currents [29,30]. In the present study, phorbol esters did not mimic the inhibitory effects of AII on K^+-induced Ca^{2+}-signal generation. ACTH, a stimulus mainly acting via the cyclic AMP messenger system, but reportedly stimulating Ca^{2+} entry [11] (an effect also seen in the present study), failed to decrease the effect of K^+ on both cytoplasmic Ca^{2+} and $^{45}Ca^{2+}$ responses to K^+. On the other hand, other Ca^{2+}-mobilizing stimuli such as AVP or thapsigargin were able to mimic the effects of AII. The question is still to be answered of whether this means that mobilization of intracellular Ca^{2+} at special intracellular loci and/or emptying of the $InsP_3$-sensitive Ca^{2+} pool is important in this respect.

It is important to note that the decrease in cytoplasmic Ca^{2+} concentrations, evoked by AII in K^+-stimulated cells, were not accompanied by decreased hormone production. Since a close correlation between cytoplasmic Ca^{2+} increase and stimulation of aldosterone production in K^+-stimulated cells (up to 9 mM-K^+) was described (cf. [17]), this suggests that in the presence of AII the seemingly decreased Ca^{2+} signal is still fully effective in stimulating hormone secretion. Also important is the observation that K^+ is able further to increase aldosterone production of cells stimulated with AII, in spite of its failure to cause a detectable sustained increase in cytoplasmic Ca^{2+}. These data together suggest that the Ca^{2+} signal generated by the Ca^{2+}-mobilizing AII is very efficient to stimulate secretion, in spite of the overall decrease in 'average' cytoplasmic Ca^{2+} concentration as detected by fura-2 in cell suspension. A similar conclusion was reached previously [19] by using a different approach, where it was concluded that, in contrast with K^+ stimulation, during AII action the increased aldosterone production, although Ca^{2+}-sensitive, does not require a sustained substantial elevation of cytoplasmic Ca^{2+} concentration.

In summary, the present study describes an inhibitory action of a Ca^{2+}-mobilizing hormone on the Ca^{2+} signal generated by K^+-induced depolarization. This indicates that, in addition to their known stimulatory effect on Ca^{2+} entry, these agonists might inhibit Ca^{2+} entry through voltage-sensitive Ca^{2+} channels. It is also shown that during their action the secretory mechanism becomes much more sensitive to intracellular Ca^{2+}.

The skilful technical assistance of Miss Ágnes Ribár and Mrs. Zsuzsa Legeza is gratefully acknowledged. Thapsigargin was kindly provided by Dr. S. Brøgger Christensen (Royal Danish School of Pharmacy, Copenhagen, Denmark). We thank Professor György Gárdos (National Institute of Haematology and Blood Transfusion, Budapest, Hungary) for the access to the Hitachi fluorimeter. The aldosterone antibody was a gift from NIAMD (Bethesda, MD, U.S.A.).

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Received 29 March 1990/6 August 1990; accepted 27 September 1990