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Angiotensin-stimulated production of inositol trisphosphate isomers and rapid metabolism through inositol 4-monophosphate in adrenal glomerulosa cells

(angiotensin II/inositolphospholipid turnover/inositol monophosphate isomers/calcium mobilization/aldosterone secretion)

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ABSTRACT **The production and metabolism of inositol phosphates in rat adrenal glomerulosa cells prelabeled with [^{3}H]inositol and stimulated with angiotensin II were analyzed by high-performance anion-exchange chromatography. Exposure to angiotensin II was accompanied by a rapid and substantial decrease in the phospholipid precursor, phosphatidylinositol (PtdIns) 4,5-bisphosphate with only a slight and transient increase in the level of the biologically active product, inositol 1,4,5-trisphosphate (Ins-1,4,5-P_3), to a peak at about 5 sec. Inositol 1,3,4-trisphosphate (Ins-1,3,4-P_3), the putative metabolite of Ins-1,4,5-P_3, was also formed rapidly and maintained an elevated steady-state level during stimulation by angiotensin II. Inositol 1,4-bisphosphate (Ins-1,4-P_2) exhibited a simultaneous and prominent increase that could not be accounted for solely by direct breakdown of PtdIns 4-phosphate, indicating that large amounts of Ins-1,4,5-P_3 must also have been produced and metabolized. The rapid formation of a substantial amount of inositol 4-monophosphate (Ins-4-*P*), with no significant change in the level of inositol 1-monophosphate (Ins-1-*P*) during the first minute of stimulation, was a notable feature of the glomerulosa cell response to angiotensin II. These observations indicate (*i*) that PtdIns-4,5-P_2 catabolism in the angiotensin-stimulated glomerulosa cell initially proceeds via Ins-1,4,5-P_3 through Ins-1,3,4-P_3 and Ins-1,4-P_2 to form Ins-4-*P* rather than Ins-1-*P* and (*ii*) that direct hydrolysis of PtdIns by phospholipase C does not occur during the initial phase of angiotensin action. In glomerulosa cells stimulated by angiotensin II in the presence of Li^+, the progressive accumulation of both Ins-4-*P*, and after a short lag period, Ins-1-*P* indicated that dephosphorylation of both isomers was inhibited by Li^+. The increase of Ins-*P* isomers in the presence of Li^+ was associated with increased and progressive accumulation of Ins-1,4-P_2 and Ins-1,3,4-P_3 but not of Ins-1,4,5-P_3. These data demonstrate that sustained and massive breakdown of PtdIns phosphates begins within seconds during cell activation by angiotensin II. The Ca^{2+}-mobilizing metabolite, Ins-1,4,5-P_3, is rapidly converted to Ins-1,3,4-P_3 and degraded through Ins-1,4-P_2 and Ins-4-*P*, in contrast to the previous view that conversion to Ins-1-*P* is the major route of PtdIns 4,5-bisphosphate metabolism.**

The action of angiotensin II upon aldosterone production in the adrenal gland is exerted through receptor-mediated hydrolysis of inositol phospholipids and increased cytosolic Ca^{2+} concentration (1–5). Such ligand-induced turnover of PtdIns phosphates is a common feature of the transmembrane signaling mechanism during activation of target cells by Ca^{2+}-dependent hormones and other stimuli (6, 7). The most important event in this process is the cleavage by phospholipase C of phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P_2) to provide inositol 1,4,5-trisphosphate (Ins-1,4,5-P_3) and 1,2-diacyl-*sn*-glycerol, both of which act as intracellular second messengers to mediate hormone action (6, 8). The ability of Ins-1,4,5-P_3 to release Ca^{2+} from a nonmitochondrial compartment, presumably the endoplasmic reticulum (9, 10), is probably a major determinant of rapid increases in cytoplasmic Ca^{2+} concentration. Since the concomitant product of PtdIns-4,5-P_2 hydrolysis, diacylglycerol, is a potent activator of protein kinase C (11), temporal or quantitative interactions between the two branches of the Ca^{2+} messenger system have been proposed (3, 12).

Recent studies on the metabolism of inositol phospholipids have revealed new aspects of inositol polyphosphate formation and metabolism in ligand-activated cells. The first indication of a more complex route of inositol phosphate metabolism was the finding of another isomer of inositol trisphosphate, identified as Ins-1,3,4-P_3, during cholinergic stimulation of the parotid gland (13). This isomer, in addition to the biologically active product, Ins-1,4,5-P_3, is also present in hepatocytes and dimethyl sulfoxide-differentiated HL-60 cells (14). The origin of the 1,3,4-isomer is still uncertain, although recent evidence suggests that it is produced from Ins-1,4,5-P_3 through conversion to inositol-1,3,4,5-tetrakisphosphate (Ins-1,3,4,5-P_4) and subsequent 5-dephosphorylation (15, 16). Also, the 1,2-cyclic form of Ins-1,4,5-P_3 has been shown to be formed from PtdIns-4,5-P_2 by a phospholipase C isolated from the sheep seminal vesicle (17). In view of these findings and of the recent identification of individual isomers among the inositol monophosphates (18, 19), there is a need for high-resolution analysis of the intermediates formed during inositol phospholipid metabolism.

During studies on the stimulation of aldosterone production by adrenal cells in response to angiotensin II, we analyzed by high-performance anion-exchange chromatography the major metabolites formed during inositolphospholipid breakdown. The early events of inositol phospholipid metabolism were studied in adrenal zona glomerulosa cells, in which acceleration of PtdIns-4,5-P_2 turnover is a primary step in the mechanism through which angiotensin II acts to regulate the rate of aldosterone secretion (1–3).

MATERIALS AND METHODS

Materials. *myo*-[^{3}H]Inositol, phosphatidyl [^{3}H]inositol ([^{3}H]-PtdIns), phosphatidyl[^{3}H]inositol 4-monophosphate ([^{3}H]PtdIns-4-*P*), [^{3}H]PtdIns-4,5-P_2, [^{3}H]inositol 1,4-

Abbreviations: Ins-1,4,5-P_3: inositol 1,4,5-trisphosphate; Ins-1,3,4-P_3: inositol 1,3,4-trisphosphate; Ins-1,3,4,5-P_4: inositol 1,3,4,5-tetrakisphosphate; Ins-1,4-P_2: inositol 1,4-bisphosphate; Ins-1-*P*: inositol 1-monophosphate; Ins-4-*P*: inositol 4-monophosphate; PtdIns: phosphatidylinositol; PtdIns-4-*P*: phosphatidylinositol 4-phosphate; PtdIns-4,5-P_2: phosphatidylinositol 4,5-bisphosphate.

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bisphosphate ([^{3}H]Ins-1,4-P_2), [^{3}H]Ins-1,4,5-P_3, and [^{3}H]ATP were purchased from New England Nuclear; [^{14}C]inositol 1-monophosphate ([^{14}C]Ins-1-*P*), from Amersham; *myo*-inositol trisphosphate, from Sigma; and [Ile^5]angiotensin II from Peninsula Laboratories (Belmont, CA). All other chemicals were of HPLC or analytical grade. Inositol-free medium 199 containing 3.6 mM K^+ and 25 mM Hepes (pH 7.4) was prepared by the National Institutes of Health Media Unit and supplemented with 1 g of bovine serum albumin per liter (M199).

Preparation and Incubation of Rat Adrenal Glomerulosa Cells. Glomerulosa cells were prepared from the adrenal capsules of male Sprague–Dawley rats by mechanical dispersion of the collagenase-digested tissue as described (20). After the cells were washed, about 3×10^7 were resuspended in 2 ml of M199 containing *myo*-[^{3}H]inositol (175 μCi/ml, 60 Ci/mmol; 1 Ci = 37 GBq). The cells were incubated for 3 hr at 37°C, then diluted with 4 vol of the same medium without *myo*-[^{3}H]inositol, and centrifuged at $150 \times g$ for 10 min. After a further hour of incubation at 37°C in M199, the cells were again centrifuged, taken up in the same medium, and dispensed to give about 1.6×10^6 cells per 200 μl. When LiCl (10 mM) was included, it was added after 30 min of the 60-min incubation. After the cells were dispensed, they were kept at 37°C for 10 min; then 5 μl of a solution of angiotensin II (final concentration, 10 nM) or medium was added with continuous agitation for the times indicated.

Preparation of Samples for HPLC. Incubations were terminated by addition of 200 μl of ice-cold 20% (wt/vol) CCl_3COOH and immediate freezing in liquid nitrogen. After the samples were thawed, 10 μg of Ins-1,4,5-P_3 was added, and the samples were kept on ice for 15 min. After centrifugation at $4000 \times g$ for 20 min, the precipitate was washed with 200 μl of 5% CCl_3COOH, and the pooled supernatants were washed four times with 1.5 ml of diethyl ether and freeze-dried. For HPLC analysis, samples were dissolved in water, neutralized with KOH, and applied to the column by an automatic injector system. The recovery of exogenously added [^{3}H]Ins-1,4,5-P_3 was >80% under these conditions.

Separation and Identification of Inositol Phosphates by HPLC. Inositol phosphates were resolved by strong anion-exchange chromatography on a 4.6 × 250 mm Adsorbosphere (5 μm) column (Alltech/Applied Science, Deerfield, IL). Elution was performed with a linear gradient of 0–0.7 M $NH_4H_2PO_4$ (pH 3.35) from 5 to 75 min at a flow rate of 1.0 ml/min. The column was washed with water (1.5 ml/min) for 10 min between application of samples. The radioactivity of the effluent was continuously measured by an in-line radioactive flow detector (Flo-One Beta IC, Radiomatic, Tampa, FL), which performed automatic peak integration using an internal MicroMate computer and program software version 3#.002.11100. The counting efficiency was 38% and was not significantly influenced by the increasing concentration of $NH_4H_2PO_4$.

Identification of the respective peaks of inositol phosphates was based on comigration with radiolabeled standard compounds. In addition to the commercially available standards, we prepared glycerophosphoinositol and its phosphorylated derivatives from the corresponding tritiated lipids by quantitative alkaline deacylation (21). A mixture of Ins-1-*P* and inositol 4-monophosphate (Ins-4-*P*) was obtained by alkaline hydrolysis (22) of [^{3}H]Ins-1,4-P_2. The position of Ins-4-*P* was also determined by following the enzymatic hydrolysis of [^{32}P]Ins-1,4,5-P_3 prepared from [γ-^{32}P]ATP-labeled erythrocyte ghosts (23), with bovine adrenal membranes as the source of the enzymes. Because of the absence of labeling with phosphate at the 1-position, the only labeled monophosphate formed under these conditions is Ins-4-*P*, as shown in liver membranes by Storey *et al.* (24). The Ins-4-*P* peak from glomerulosa cells was found to be homogenous when rerun on a weak anion-exchange column (Partisil-NH_2, Alltech/Applied Science).

Extraction and Separation of Inositol Phospholipids. The CCl_3COOH pellet was extracted, and the phospholipids were analyzed by TLC as described (2). After autoradiography of the TLC plates for 10 days at −70°C using Kodak XAR-5 film, the regions corresponding to the PtdIns phosphates and PtdIns were cut out and analyzed by liquid scintillation spectrometry. Elution of the lipids was enhanced by addition of 200 μl of 1 M HCl to PtdIns-4,5-P_2 and PtdIns-4-*P* and 200 μl of H_2O to PtdIns and *lyso*-PtdIns.

RESULTS

HPLC Analysis of Inositol Phosphates. The typical HPLC elution profiles of one control and two stimulated samples obtained from adrenal glomerulosa cells prelabeled with [^{3}H]inositol are shown in Fig. 1. This HPLC method provided a complete separation of the major inositol phosphates and their isomers. Of particular interest was the presence of two isomers of Ins-*P*: the first eluted was Ins-1-*P*, and the second was identified as Ins-4-*P*. The possible appearance of Ins-2-*P* comigrating with Ins-4-*P* as a result of acidic hydrolysis of 1,2-cyclic Ins-*P* was also considered. However, we did not detect any of the cyclic forms of inositol phosphates using neutral extraction conditions, while still observing a rapid increase in the putative Ins-4-*P* peak eluting after Ins-1-*P* (data not shown).

Two forms of Ins-P_3 were also distinguished, the 1,4,5-isomer and an immediately preceding peak that was consistently detected only in extracts from hormone-stimulated cells. Based on its slightly shorter retention time and its comigration with the [^{3}H]ATP standard, this peak was identified as the compound characterized by Irvine *et al.* (13)

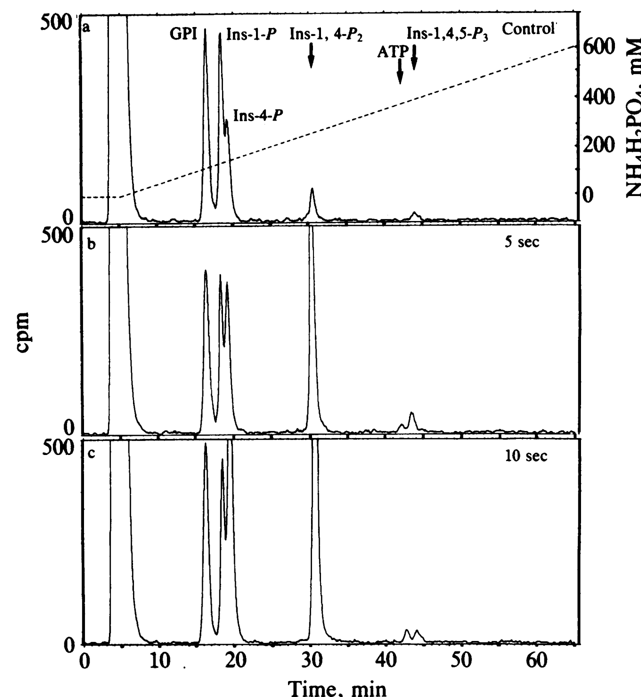

FIG. 1. Typical HPLC elution profile of inositol phosphates obtained from one control (*a*) and two stimulated (*b* and *c*) samples of adrenal glomerulosa cells. Isolated glomerulosa cells prelabeled with [^{3}H]inositol were stimulated with 10 nM angiotensin II for 5 sec (*b*) or 10 sec (*c*). Inositol phosphates from the cell extracts were separated by high-performance anion-exchange chromatography as detailed. The gradient of $NH_4H_2PO_4$ (---) used for elution and the positions of the reference standards are shown in *a*. ATP indicates the position of Ins-1,3,4-P_3. GPI: glycerophosphoinositol.

as Ins-1,3,4-P_3. A negative finding of significance was the observation that extended gradient elution did not reveal additional peaks corresponding to higher inositol phosphates, either in control or angiotensin-stimulated glomerulosa cells.

Rapid Kinetics of Inositol Polyphosphate Metabolism in Stimulated Glomerulosa Cells. Addition of angiotensin II to glomerulosa cells was followed immediately by a marked increase in the formation of Ins-1,4-P_2, which was associated with only a slight and transient elevation of Ins-1,4,5-P_3 and the appearance of a peak of Ins-1,3,4-P_3. The most prominent feature of the early inositol phosphate response was the rapid increase of Ins-4-*P* in the absence of a change in Ins-1-*P*, the expected product of inositol polyphosphate metabolism. Since Ins-4-*P* can be derived only from the hydrolytic products of PtdIns phosphates, its marked increase within 5 sec after angiotensin stimulation indicates that the formation and degradation of inositol polyphosphates must be extremely rapid (Fig. 1).

Further experiments were performed to clarify the time course of inositol phosphate metabolism and to examine the effects of Li^+ on the levels of the respective metabolites. The earliest time point that could be examined was between 2 and 3 sec (for simplicity designated as 2.5 sec) after stimulation by angiotensin II, and the responses were followed up to 20 min.

As shown in Fig. 2*a*, both Ins-1,4,5-P_3 and Ins-1,3,4-P_3 increased rapidly upon stimulation. The increase in the level of Ins-1,4,5-P_3 was only transient, showing a 2-fold increase ($P < 0.05$, $n = 3$) at about 5 sec. On the other hand, Ins-1,3,4-P_3 increased progressively during the first 60 sec and remained elevated for the 20-min period. In contrast with the minor changes in the biologically active isomer, Ins-1,4,5-P_3, there was an extremely large increase in the level of its proximal metabolite, Ins-1,4-P_2. The peak increase of almost 10-fold in Ins-1,4-P_2 occurred at 10 sec and was followed by a decline to an elevated steady-state level that was maintained throughout the 20-min incubation period (Fig. 3*a*).

Rapid changes in the level of Ins-4-*P*, but not of Ins-1-*P*, were observed in the first minute of stimulation. The increase in Ins-4-*P* was already apparent at 2.5 sec and reached its maximum at about 30 sec, followed by a steady-state high level throughout the 20-min period. In contrast, Ins-1-*P* was moderately increased only after 2.5 min of stimulation by angiotensin II (Fig. 4*a*).

In view of the small amount of Ins-1,4,5-P_3 detected by HPLC during stimulation by angiotensin II, the breakdown of PtdIns-4,5-P_2 and PtdIns-4-*P* was also determined in each experiment. There was rapid and substantial hydrolysis of both inositol polyphosphate precursors in angiotensin II-stimulated cells during the first 60 sec of incubation (Fig. 5).

Effects of Lithium Ions. In cells stimulated with angiotensin II, only the 1,3,4-isomer of Ins-P_3 continued to accumulate in the presence of Li^+. In contrast to the major increase in Ins-1,3,4-P_3, there was no significant effect of Li^+ on the levels of Ins-1,4,5-P_3 during the first minute of hormonal stimulation. At later times there was sometimes a minor elevation above the control levels (Fig. 2*b*), although this was not consistently observed in additional experiments. The basal level of Ins-1,4-P_2 was only slightly increased (about 2-fold) in the presence of Li^+, but stimulation with angiotensin II caused a rapid increase and continuous accumulation of this metabolite (Fig. 3*b*).

Li^+ caused major increases in the basal levels of both Ins-*P* isomers. Against this high basal activity, changes in Ins-4-*P* during angiotensin stimulation were not apparent for up to 30 sec, but thereafter Ins-4-*P* increased progressively to extremely high levels throughout the 20-min period. Accumu-

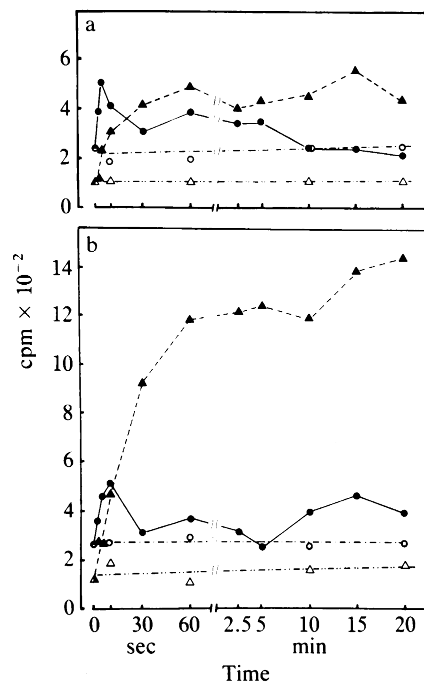

FIG. 2. Changes in the levels of Ins-1,4,5-P_3 (●) and of Ins-1,3,4-P_3 (▲) in angiotensin II-stimulated adrenal glomerulosa cells as a function of time. Isolated glomerulosa cells were prelabeled with [^{3}H]inositol and divided into two parts (*a* and *b*), one of which (*b*) was treated with 10 mM LiCl for 40 min before hormone addition. Angiotensin II (10 nM) was then added for the times indicated, and the Ins-P_3 isomers were analyzed by HPLC. ○, △, Respective controls without hormone addition. Due to the numbers of cells required, the time courses from 0–60 sec and from 1–20 min were derived from single determinations in separate experiments in which the basal and stimulated activities of all metabolites were almost identical. Two additional experiments of the same kind were performed with essentially identical results. In similar experiments performed at single time points, the average deviation of replicates from the mean was 13% for both Ins-P_3 isomers, 8% for Ins-P_2 isomers, and 5% for the Ins-*P* isomers.

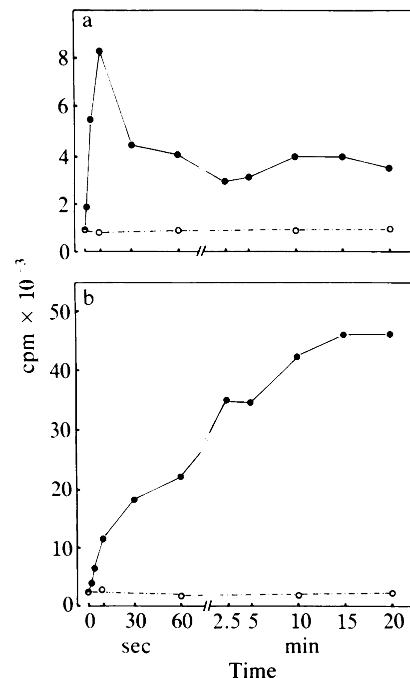

FIG. 3. Changes in the level of Ins-1,4-P_2 (●) in angiotensin-stimulated adrenal glomerulosa cells as a function of time. Samples are from the same experiments described in the legend to Fig. 2. (*a*) No LiCl. (*b*) 10 mM LiCl. ○, Control without hormone addition. Note the difference in the scales of the ordinates in *a* and *b*.

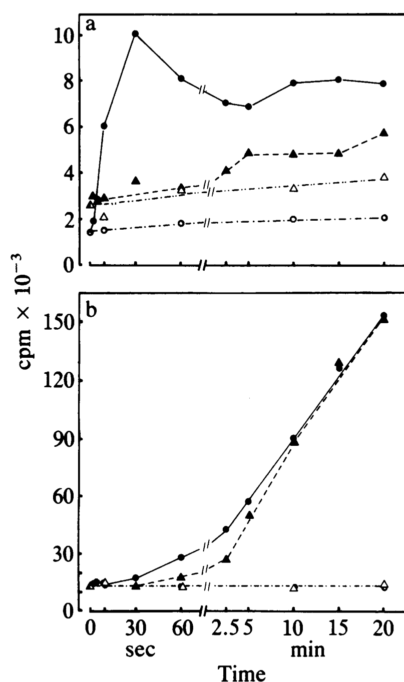

FIG. 4. Changes in the levels of Ins-4-P (●) and Ins-1-P (▲) in angiotensin-stimulated adrenal glomerulosa cells as a function of time. Samples are from the same experiments described in the legend to Fig. 2. (*a*) No LiCl. (*b*) 10 mM LiCl. ○, △, Respective controls without hormone addition. Note the difference in the scales of the ordinates in *a* and *b*.

lation of Ins-1-P was also prominent in Li^+-treated cells, and although its increase was delayed in comparison with the Ins-4-P isomer, the levels of the two monophosphates were similar between 5 and 20 min of stimulation by angiotensin II (Fig. 4*b*).

DISCUSSION

The acceleration of inositolphospholipid turnover is a major component of the transmembrane signaling mechanism when cells are stimulated by Ca^{2+}-dependent hormones such as angiotensin II (25). The primary link between inositolphospholipid breakdown and Ca^{2+} mobilization is believed to be Ins-1,4,5-P_3, which has been shown to release Ca^{2+} from an intracellular pool (9, 10) that appears to be located in the endoplasmic reticulum, possibly closely adjacent to the plasma membrane (26). After its generation by phospholipase C-catalyzed breakdown of PtdIns-4,5-P_2, the active 1,4,5-isomer of Ins-P_3 binds to high-affinity intracellular receptors (23, 27) to mobilize stored Ca^{2+} and is rapidly degraded by the sequential actions of specific phosphatases. The phosphate in the 5-position is first removed to produce Ins-1,4-P_2, which is further degraded to form Ins-P isomers and finally inositol (24, 28). However, Ins-1,4-P_2 and Ins-1-P could also be produced by the actions of phospholipase C on PtdIns-4-P and PtdIns, respectively. Thus, it may be difficult to decide whether changes in the levels of these metabolites solely reflect degradation of Ins-1,4,5-P_3 or whether phospholipase C activity is not restricted to PtdIns-4,5-P_2 (cf. ref. 29).

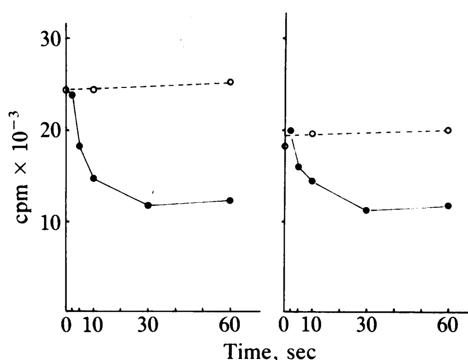

FIG. 5. Breakdown of PtdIns-4,5-P_2 (*Left*) and PtdIns-4-P (*Right*) in angiotensin-stimulated adrenal glomerulosa cells. Samples are from the same experiment shown in Figs. 2–4. Lipids were extracted from the denatured cell pellet and separated by TLC (2). The positions of the lipids were identified by autoradiography, the areas corresponding to individual lipids were cut out, and their radioactivities were determined in a scintillation counter. ●, With hormone addition; ○, control without hormone addition.

In the present study, we observed extremely rapid changes in the levels of all inositol phosphates in adrenal glomerulosa cells during stimulation by angiotensin II. There was no significant time delay in the appearance of Ins-1,4-P_2 and Ins-1,3,4-P_3 when compared to the increase in Ins-1,4,5-P_3, and even Ins-4-P was significantly increased at the earliest time point (2.5 sec) of stimulation. In contrast to the extremely large increases in its putative metabolites, the increase of Ins-1,4,5-P_3 never exceeded 3-fold, despite attempts to minimize its degradation by stopping the reaction as rapidly as possible. Comparison of the radioactivities of the several inositol phosphates indicates that large amounts of Ins-1,4,5-P_3 must be produced and converted to Ins-1,4-P_2 and Ins-4-P in the first few seconds of stimulation. The rapid and substantial decrease of PtdIns-4,5-P_2 also suggests that considerable amounts of Ins-1,4,5-P_3 had been generated and served as the major source of the high levels of Ins-1,4-P_2. However, the concomitant and less-marked decrease of PtdIns-4-P could reflect its hydrolysis by phospholipase C to form Ins-1,4-P_2 as well as its rapid conversion to PtdIns-4,5-P_2. The low level and small increase in Ins-1,4,5-P_3 may reflect such rapid and extensive hydrolysis of the active isomer that its degradation continues for a brief period after addition of CCl_3COOH to terminate the incubations.

The rapid increase of Ins-4-P in the absence of a significant change of Ins-1-P during the first minute of stimulation suggests that sequential conversion to Ins-1,4-P_2 and Ins-4-P is the preferred pathway for Ins-1,4,5-P_3 degradation in the glomerulosa cell. An important corollary to be drawn from these results is that PtdIns is not cleaved by phospholipase C during the first minute of stimulation by angiotensin II. Whether the subsequent increase in Ins-1-P formation after 2.5 min reflects the direct breakdown of PtdIns is not yet certain. In angiotensin-stimulated vascular smooth muscle cells, biphasic production of diacylglycerol has been attributed to a shift in phospholipase C activity from PtdIns phosphates toward PtdIns (30). The delayed increase in the level of Ins-1-P in the present study is compatible with such a "late" activation of PtdIns hydrolysis, but the alternative route of its production via degradation of the rising amounts of Ins-1,4-P_2 cannot be excluded.

The inhibition of inositol-1-phosphatase by Li^+ (31) has been widely used as a maneuver to amplify ligand-induced changes in inositol phosphate production. In the present study, Ins-4-P as well as Ins-1-P accumulated in the presence of Li^+, suggesting that dephosphorylation of both Ins-P isomers is inhibited. This observation contrasts with the preliminary report (32) that degradation of inositol polyphosphates through Ins-4-P in hepatocytes is not sensitive to inhibition by Li^+. In a recent report, accumulation of Ins-4-P in human platelets was augmented by Li^+ during stimulation by vasopressin but not by thrombin (19). Another conclusion to be drawn from the progressive accumulation of Ins-4-P in the presence of Li^+ during the 20-min period of stimulation is that PtdIns phosphates are continuously degraded during angiotensin II action.

The large increase in the level of Ins-1,4-P_2 in the presence of Li^+ could be simply a consequence of the build-up of both Ins-1-*P* and Ins-4-*P*, although a direct inhibitory effect of Li^+ on the specific phosphatases is also possible. Providing that the formation of Ins-1,4-P_2 is not enhanced by Li^+, comparison of its levels measured with and without Li^+ may give an indication of its rate of degradation in the stimulated glomerulosa cells.

There was little (if any) effect of Li^+ on the level of Ins-1,4,5-P_3 in the angiotensin-stimulated cells. The minor and transient rise in Ins-1,4,5-P_3, in association with the high level of Ins-1,4-P_2, indicates that removal of the active Ca^{2+}-mobilizing isomer must be both rapid and highly effective. Such a mechanism may be of importance not only to terminate the initiating step of the biological response but also to ensure that the intracellular milieu is only briefly exposed to the potentially toxic effects of increased cytosolic Ca^{2+} concentrations.

It is also clear from the present results that, as in pancreatic acinar cells (14), accumulation of Ins-1,3,4-P_3 rather than the biologically active isomer could be responsible for the increased levels of Ins-P_3 observed in many tissues when stimulated in the presence of Li^+ and analyzed by Dowex ion-exchange chromatography. The immediate appearance of Ins-1,3,4-P_3 without a significant lag time, together with the absence of detectable higher inositol phosphates, indicates that, if Ins-1,3,4,5-P_4 is an intermediate in the conversion of Ins-1,4,5-P_3 to Ins-1,3,4-P_3 as recently suggested (15, 16), its turnover must be extremely rapid with a level that is not demonstrable under the present labeling conditions.

In summary, we have demonstrated that angiotensin II elicits extremely rapid changes in inositolphospholipid metabolism in adrenal glomerulosa cells (Fig. 6). The production and degradation of Ins-1,4,5-P_3 appear to be so fast and effective that its true intracellular level cannot be measured accurately. This biologically active compound is immediately and almost completely metabolized even in the presence of Li^+, when the levels of all other metabolites are substantially increased. The selective increase in the level of Ins-4-*P* over Ins-1-*P* in the early phase of cell activation suggests that Ins-4-*P* rather than Ins-1-*P* is the major metabolite of inositolphospholipid hydrolysis. The continuous accumulation of Ins-4-*P* in the presence of Li^+ in angiotensin-stimulated cells indicates that the 4-phosphatase pathway is also inhibited by Li^+ and that sustained hydrolysis of inositolphospholipid is a concomitant of angiotensin II receptor activation in the zona glomerulosa cell.

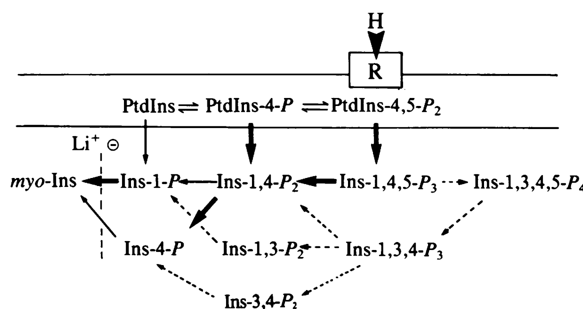

FIG. 6. Production and metabolism of inositol phosphates during activation of phospholipase C by angiotensin II in adrenal glomerulosa cells. Similar metabolic pathways are involved in the disposition of inositol phosphates formed during hormone–receptor interactions in many Ca^{2+}-dependent target cells. Bold arrows show major pathways of production or elimination of the individual metabolites, and dashed arrows indicate recently defined pathways of inositol polyphosphate metabolism (15, 16).

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