Potassium and Angiotensin II Increase the Concentrations of Phosphatidic Acid, Phosphatidylinositol, and Polyphosphoinositides in Rat Adrenal Capsules In Vitro

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ABSTRACT We examined the effects of K^+ and angiotensin II, the major regulators of aldosterone secretion, on phospholipid metabolism during incubation of glomerulosa-rich, adrenal capsules. Addition of increasing amounts of K^+ and angiotensin II to the incubation media elicited progressive increases in corticosterone production and capsular concentrations of phosphatidic acid, phosphatidyl-inositol, and polyphosphoinositides. These effects are similar to those previously reported for ACTH in the whole adrenal cortex. A common mechanism, i.e., activation of the phosphatidate-polyphosphoinositide cycle, may be operative in the action of steroidogenic agents in their target tissues.

INTRODUCTION

We have recently reported that ACTH increases adrenal polyphosphoinositides (1), and these phospholipids, by virtue of their polyphosphorylated head group, enhance cholesterol side-chain cleavage (2), the rate-limiting step in glucocorticoid production (3). We then obtained evidence strongly suggesting that this phospholipid effect may be responsible for mediating ACTH effects on steroidogenesis (4). Furthermore, the increase in polyphosphoinositides appeared to be due to an ACTH-induced increase, via cyclic (c)AMP, in phosphatidic acid; a labile protein was required for the latter, as well as for increases in other phospholipids in the phosphatidate-polyphosphoinositide cycle (5) and steroidogenesis (6–8).

Because steroidogenesis is probably regulated by similar processes in most steroid-forming tissues, we determined the effects of angiotensin II and K^+ on phospholipid metabolism in adrenal (glomerulosa-rich) capsules. Our results demonstrate that these major regulators of aldosterone secretion (9, 10) elicit virtually the same phospholipid effect as ACTH, viz., increases in net concentrations of phosphatidic acid, phosphatidylinositol, and polyphosphoinositides.

METHODS

Capsular and decapsulated (interior) portions of adrenals from 250-g male Holtzmann rats were prepared as described by Williams et al. (11) and Albano et al. (12). In each experiment, adrenal capsules or decapsulated adrenals from each of eight rats were bisected and evenly distributed to four flasks containing 2 ml of Krebs-Ringer bicarbonate buffer supplemented with 10 mM glucose and 4% bovine serum albumin (KRBGA).[1] The K^+ concentration of KRBGA was 3.6 mM in angiotensin experiments, and 2, 3.6, 5.9, or 8.4 mM in K^+ experiments (12). After a 90-min preincubation at 37°C under 95% O_2 + 5% CO_2, media were replaced with fresh KRBGA having the same K^+ concentration. The flasks were returned to incubation for 60 min with or without increasing amounts of 5-valine angiotensin II (Sigma Chemical Co., St. Louis, Mo.). After incubation, media were decanted and analyzed for corticosterone by the acid-fluorescence method (13). Tissue phospholipid concentrations were determined by thin-layer chromatography (solvent-system B) and phosphorus quantitation, as described (1, 4, 5).

RESULTS

As shown in Fig. 1, the presence of increasing amounts of K^+ in the incubation media was attended by progressive increases in corticosterone production and capsular concentrations of phosphatidic acid, phosphatidylinositol, diphosphoinositide, and triphosphoinositide. In contrast, the concentrations of phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine were not significantly influenced by the medium K^+ concentration (results not shown).

Addition of increasing amounts of angiotensin II to

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[1] *Abbreviation used in this paper*: KRBGA, Krebs-Ringer bicarbonate buffer supplemented with 10 mM glucose and 4% bovine serum albumin.

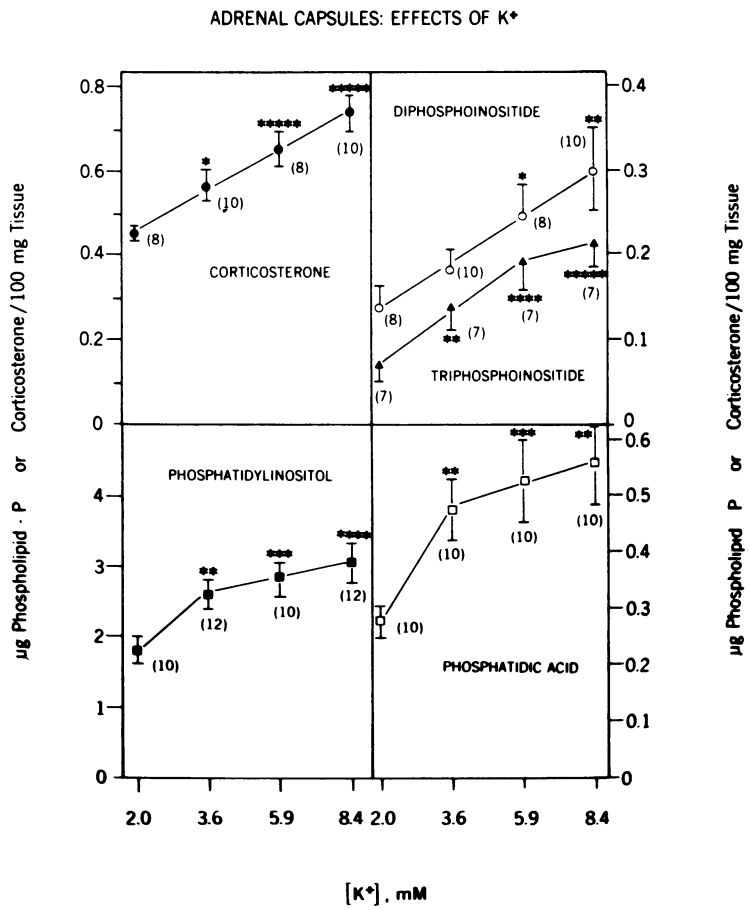

FIGURE 1 Adrenal capsules: effects of K^+. Dose effects of K^+ on cortiosterone production (●) and adrenal capsular concentrations of diphosphoinositide (○), triphosphoinositide (▲), phosphatidylinositol (■), and phosphatidic acid (□). Mean values±SEM are shown by bars with the number of determinations in parentheses. *P* values shown in the figure were determined by standard *t* test comparison with the 2 mM control: *, <0.05; **, <0.025; ***, <0.01; ****, <0.005; *****, <0.001.

the incubation media also elicited progressive increases in corticosterone production (Fig. 2). This too was accompanied by progressive increases in phosphatidylinositol, triphosphoinositide, diphosphoinositide, and phosphatidic acid. As with K^+, other phospholipid concentrations were not affected appreciably by angiotensin II.

Whereas capsular corticosterone and phospholipids were regularly increased by K^+ and angiotensin II, these treatments evoked only slight (21 and 52%, respectively) increases in costicosterone production and no significant changes in phospholipid concentrations in decapsulated adrenals (results not shown). These results indicate that K^+ and angiotensin II, at the concentrations used, elicit steroidogenic and phospholipidic responses primarily in the zona glomerulosa, and have little or no effect in the zona fasiculata-reticularis.

DISCUSSION

It is apparent that both K^+ and angiotensin II evoke phospholipid effects in the zona glomerulosa that are similar to those of ACTH in the zona fasciculata-reticularis. In the latter case, we have presented strong evidence suggesting a causal or mediatory relationship between ACTH-induced phospholipids and subsequent glucocorticoid production (1, 2, 4, 5). By analogy, it seems likely that a similar relationship exists in the regulation of corticosterone and aldosterone production by K^+ and angiotensin II in the zona glomerulosa. This would not exclude the participation of other control mechanisms in the steroidogenic process in these tissues, e.g., generation of free cholesterol substrate for the side-chain cleavage reaction (14, 15). However, the cycloheximide-insensitive increase in free cholesterol may not, itself, be sufficient

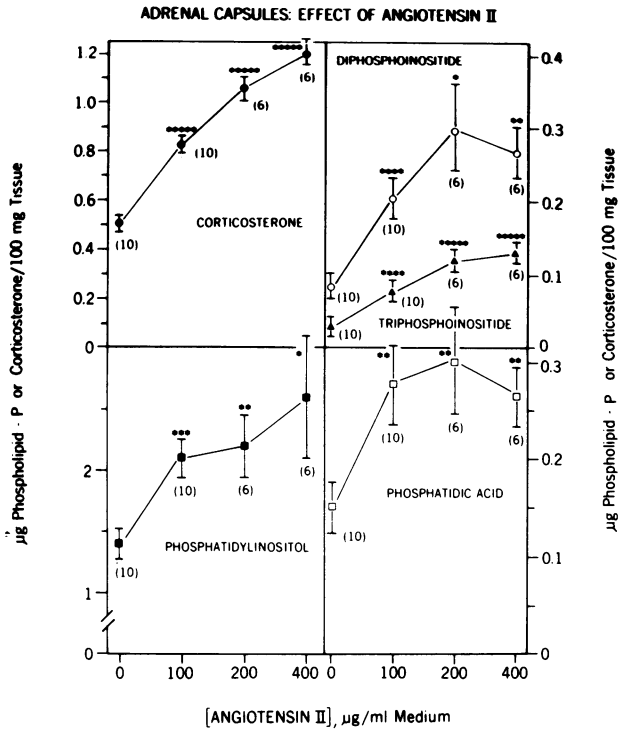

FIGURE 2 Adrenal capsules: effect of angiotensin II. Dose effects of angiotensin II on corticosterone production and adrenal capsular phospholipid concentrations. *P* values shown in the figure were determined by standard *t* test comparison with the control. For meaning of asterisks, see Fig. 1.

to increase cholesterol side-chain cleavage (16–18), and another cycloheximide-sensitive factor (possibly polyphosphorylated phospholipids [19]) is apparently required to effect interaction of cholesterol with cytochrome P-450 (20–22).

In the zona fasciculata-reticularis, the phospholipid effects of ACTH appear to be due to previous increases in cAMP (4, 5). The latter, by a cycloheximide-sensitive process, also increases phosphatidic acid, phosphatidylinositol, polyphosphoinositides, and phosphatidylglycerol, which is also derived from CDP ~ diaglycerol via phosphatidylglycerol phosphate (note: the latter has a polyphosphorylated head group and is therefore potentially steroidogenic [2, 19]). Unlike the fasciculata, however, the role of cAMP in the action of K^+ and angiotensin II in the zona glomerulosa is uncertain. Although higher doses of K^+ and angiotensin II may increase cAMP in adrenal capsules (12), Fujita et al. (23) have reported that fully effective doses of angiotensin II and K^+ are not associated with increases in total or receptor-bound cAMP in glomerulosa-cell preparations. Since we observed definite increases in phospholipids with the lowest effective dose of angiotensin II (100 μg/ml) in the capsular system, and since cAMP increases are not observed at this angiotensin II dosage (12), it may be asked whether the observed phospholipid effects are due to subtle increases in cAMP that defy measurement, or to factors other than cAMP. Further studies will be required to resolve this important question.

The present and previous (1, 2, 4, 5) findings suggest that most or all steroidogenic substances may operate via a common final-effector system, viz., stimulation of the phosphatidate-polyphosphoinositide-polyglycerophospholipid pathway. This system is rapidly inducible, rapidly reversible, and dramatically responsive to stimulation (1, 2, 4, 5): it is thus ideally suited as a regulator of minute-to-minute changes in steroidogenesis. The mechanism whereby this phospholipid pathway is activated by ACTH, angiotensin II, and K^+ is presently unknown, but recent findings (unpublished) in our laboratory suggest that ACTH enhances phosphatidic acid synthesis from 1,2-diacylglycerol and ATP. Obviously, the 1,2-diacylglycerol is derived from sources other than phosphatidylinositol, and the putative labile protein seems to be required for the conversion of 1,2-diacylglycerol and ATP to phosphatidic acid. Possible sources of 1,2-diacylglycerol include phosphatidylcholine and triglycerides, but the former seems unlikely since it, too, is eventually increased by ACTH (24). Should triglyceride prove to be the source, the formulated sequence would be: stimulator → cAMP or other second messenger → triglyceride → 1,2-diacylglycerol → phosphatidic acid → other phospholipids → cholesterol side-chain cleavage.

This simple scheme has considerable appeal, not only as a mechanistic explanation for steroidogenesis, but also because of its potential for explaining other actions of trophic agents, e.g., regulation of membrane-associated enzymes, and stimulation of membrane synthesis and subsequent triggering of cell hypertrophy. Clearly, the validity and importance of this scheme must be tested further.

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