Effects of lithium on angiotensin-stimulated phosphatidylinositol turnover and aldosterone production in adrenal glomerulosa cells: a possible causal relationship

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Turnover of ^{32}P-labelled phosphatidylinositol (PI) was examined in isolated adrenal glomerulosa cells. Increased incorporation of [^{32}P]phosphate into PI in response to angiotensin II was completely prevented by Li^+. A simultaneous accumulation of ^{32}P activity in phosphatidic acid (PA) was also observed. Angiotensin II increased the breakdown of PI despite the presence of Li^+. These results suggest that Li is a suitable tool to interrupt the accelerated PI cycle in angiotensin-stimulated cells. Aldosterone production of superfused cells was inhibited by Li^+ when the cells were stimulated with angiotensin II. On the other hand, Li^+ did not inhibit the aldosterone response of the cells to ACTH, a hormone which acts via cyclic AMP and does not enhance PI turnover in these cells. On the basis of these results, we assume that the inhibitory effect of Li^+ on aldosterone production is related to its effect on PI turnover.

Phosphatidylinositol metabolism *Lithium* *Aldosterone* *Angiotensin II* *Corticotropin* myo-*Inositol*

1. INTRODUCTION

Enhanced breakdown and resynthesis of inositol phospholipids is a characteristic response to those hormones and neurotransmitters which act via changes in cytosolic calcium ion concentration [1–4]. This phenomenon is generally regarded as a mechanism responsible for calcium-gating and therefore as a trigger of the events which lead to the specific response of the cell. It was pointed out in [5] that Li^+ may be an appropriate tool to investigate the importance of accelerated phosphoinositide cycle in cell activation. It was shown that Li^+ inhibited the activity of the enzyme *myo*-inositol-1-phosphatase [5–7]. Accordingly, Li^+ may prevent the formation of inositol necessary for the synthesis of PI, and may lead to the accumulation of inositol-1-phosphate in the stimulated cell. However, it has not yet been checked how Li^+ influences the turnover of PI.

Angiotensin II, a peptide hormone which generates calcium signal, activates PI turnover in several tissues [8,9]. In adrenal glomerulosa cells PI turnover is similarly enhanced by angiotensin II [10–13] while it is not influenced by other physiological stimuli such as ACTH and potassium [12,14]. This study was undertaken to investigate the effects of Li^+ both on PI cycle and on aldosterone production in isolated rat glomerulosa cells. It was found that Li^+ interrupted the accelerated PI cycle and inhibited the increased aldosterone production of the angiotensin-stimulated cells. On the other hand, Li^+ did not influence the effect of ACTH on aldosterone production suggesting an inhibition by Li^+ of the specific mechanism(s) involved in the action of angiotensin II.

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Abbreviations: PI, phosphatidylinositol; PA, phosphatidic acid; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; ACTH, adrenocorticotropin

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2. MATERIALS AND METHODS

2.1. *Preparation and incubation of isolated glomerulosa cells*

Isolated glomerulosa cells were prepared from the adrenal capsular tissue of male Sprague-Dawley (CFY) rats (250–350 g) by means of digestion with collagenase as in [15]. Cell yield was 2×10^5 cells per rat, on average. The cells were incubated in a mixture of modified Krebs-Ringer-glucose solution and Medium 199 (Wellcome, Beckenham, Kent, England) (2:1, v/v) containing 2 g/l human serum albumin (fraction V) and buffered with 20 mM Hepes (pH 7.4). Concentration of potassium and calcium was 3.6 and 1.8 mM, respectively.

2.2. *Effect of Li^+ on the incorporation of ^{32}P into different phospholipids*

Cells equivalent to one pair of adrenals were incubated in 100 μl incubating medium in the presence or absence of 10 mM LiCl for 30 min at 37°C. Angiotensin II (asp^1, ileu^5-angiotensin II, Serva, Heidelberg, FRG) (25 nM) or solvent and [^{32}P]phosphate (750 MBq/l) were then added and the incubation was continued for a further 60 min. Phospholipids were immediately extracted [16] and separated by thin-layer chromatography [17]. Radioactivity in PI, PA, PC, PE and PS were determined by liquid scintillation counting.

2.3. *Effect of angiotensin II on the breakdown of PI*

Isolated glomerulosa cells were loaded with [^{32}P]phosphate (3 GBq/l) for 1 h at 37°C, washed and incubated without radioactive phosphate for a further 150 min. LiCl (10 mM) was added and the incubation was continued for 30 min. At this time (taken as 0 min, fig.2) the cell suspension was divided into two tubes, one contained angiotensin II (25 nM) and the other served as control. The incubation was continued and samples containing cells equivalent to one pair of adrenals were withdrawn at the times indicated (fig.2). Radioactivity of different phospholipids were measured as described above.

2.4. *Aldosterone production by superfused glomerulosa cells*

Isolated glomerulosa cells were mixed with preswollen Bio-Gel P-2 (Mesh < 400, Bio-Rad Laboratories, Richmond, CA) and 800 μl of the mixture, containing about 10^6 cells, were transferred to plastic columns (i.d., 7 mm) kept at 37°C. The cells were superfused with 8 ml incubation medium/h. The effluent was collected at 0°C, its aldosterone content was measured by radioimmunoassay as in [15]. The cells were stimulated with angiotensin II (2.5 nM) or with ACTH (Synacthen, CIBA-Geigy, Basel, Switzerland) (10 nM) after a control superfusion period of 1 h. Lithium and inositol when added were present throughout the whole superfusion period.

3. RESULTS AND DISCUSSION

PI turnover of isolated glomerulosa cells was examined in the absence or presence of 25 nM angiotensin II, a concentration evoking maximal aldosterone production in our laboratory. To attain maximal inhibition of *myo*-inositol-1-phosphatase, we applied Li^+ in a concentration of 10 mM [5]. As shown in fig.1, Li^+ completely blocked the angiotensin-induced increase in the incorporation of [^{32}P]phosphate into PI. The striking accumulation of ^{32}P activity in PA indicates that the hormone stimulated the breakdown of phosphoinositides also under such conditions. This

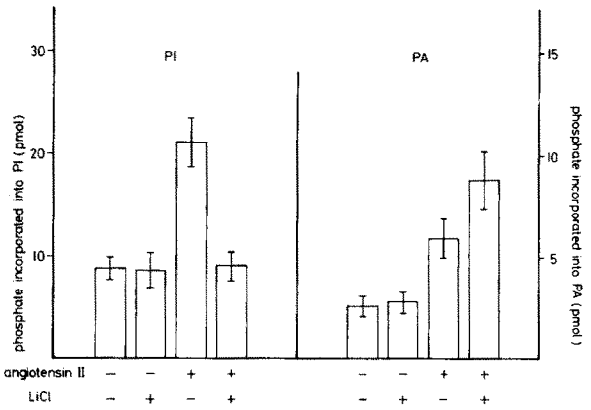

Fig.1. Effect of angiotensin II (25 nM) on the incorporation of [^{32}P]phosphate into phosphatidylinositol (PI) (left panel) and into phosphatidic acid (PA) (right panel) in isolated glomerulosa cells. LiCl (10 mM) was added 30 min before the labelling period. Values are expressed in pmol/10^5 cells. Means ± SE of 6 separate experiments are given, each carried out in duplicates.

latter assumption was checked in a further experiment. It was demonstrated that angiotensin II evoked a rapid depletion of ^{32}P activity in the prelabelled PI pool of glomerulosa cells also in the presence of Li^+ (fig.2). Neither the basal incorporation of [^{32}P]phosphate into PI and PA (fig.2), nor the labelling of other phospholipids such as PC, PE and PS were significantly influenced by Li^+.

These results are compatible with earlier findings [5–7] that Li^+ blocks the conversion of *myo*-inositol-1-phosphate into *myo*-inositol. Therefore we tried to reverse the effects of Li^+ on PI synthesis by the addition of exogenous *myo*-inositol. The angiotensin-induced incorporation of [^{32}P]-phosphate into PI was restored only partially even by high concentrations of *myo*-inositol (10 mM) added simultaneously with Li^+. A 1.68 ± 0.14-fold stimulation in the incorporation of [^{32}P]phosphate into PI was observed in contrast to the 2.19 ± 0.14-fold one, elicited by angiotensin II alone ($\bar{X}$ ± SE, n = 5). A corresponding reduction in the labelling of PA was also observed (not shown). The failure of exogenous *myo*-inositol to overcome completely the effect of Li^+ may be explained by a limited availability of this substance for the PI synthesising mechanism. If, for example, *myo*-inositol is phosphorylated while crossing the plasma membrane, its conversion to *myo*-inositol will also be inhibited by Li^+.

The consequences of the interruption of PI turnover by Li^+ was studied on aldosterone production. Hormone production by isolated glomerulosa cells was examined in a continuous-flow (superfusion) system. The cells were stimulated with angiotensin II in a submaximally effective concentration (2.5 nM). As shown by a representative curve in fig.3a, the aldosterone response was inhibited by Li^+. The cumulative production of aldosterone during the first hour of stimulation was reduced by 51.8–4.0% (mean ± SE, n = 5, p < 0.001). Similar inhibition was found when the

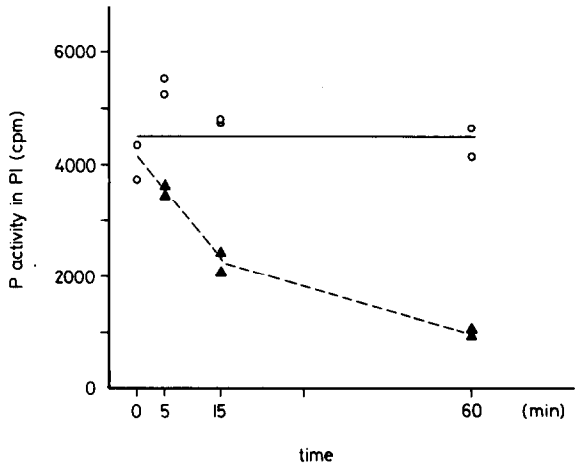

Fig.2. Effect of angiotensin II (25 nM), in the presence of Li^+ (10 mM), on the [^{32}P]phosphate content of prelabelled phosphatidylinositol (PI) pool in isolated glomerulosa cells. Control (—○—), angiotensin II (--▲--).

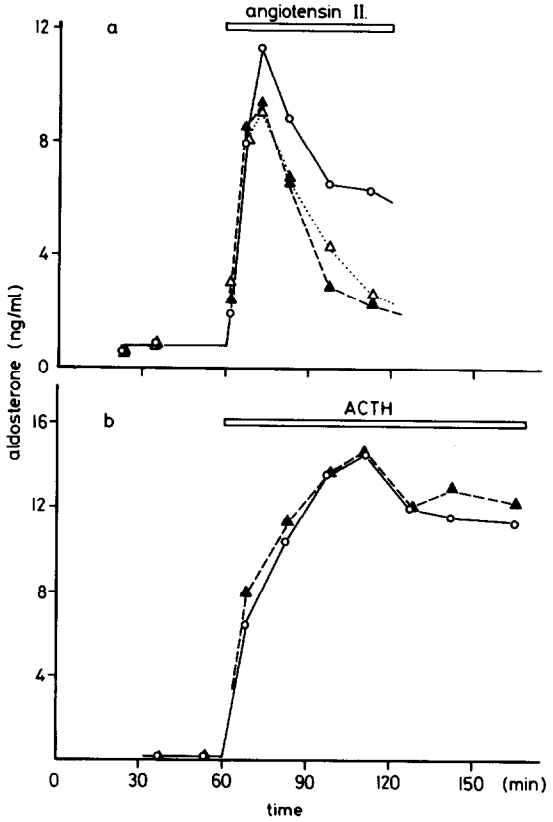

Fig.3. Effect of Li^+ (10 mM) on aldosterone production by isolated glomerulosa cells stimulated with 2.5 nM angiotensin II (panel a) or 10 nM ACTH (panel b). Addition of the stimuli is indicated by horizontal lines. Lithium was present (--▲--) or absent (—○—) during the whole experiment. *myo*-Inositol (10 mM) was administered simultaneously with Li (. . .Δ. . .). Representative curves are shown from 4 (panel a) and 2 (panel b) similar experiments.

cells were stimulated with 25 nM angiotensin II (not shown). Addition of *myo*-inositol (10 mM) failed to affect the response of the Li^+ treated cells to angiotensin II (fig.3a). To test whether Li^+ inhibited aldosterone production by inhibiting PI synthesis or by a non-specific mechanism, we examined its effect on aldosterone production of glomerulosa cells stimulated with ACTH, a hormone acting via cAMP. Here, ACTH (3×10^{-12}–3×10^{-7} M) did not enhance [^{32}P]phosphate incorporation into PI and PA in these cells (not shown). It is demonstrated in fig.3b that Li^+ did not interfere with the stimulating effect of ACTH on aldosterone production. In these experiments ACTH was also added in a submaximally effective concentration (10 nM).

These results show that interruption of the PI cycle results in the reduction of the secretory response of glomerulosa cells to a Ca mobilizing agent. Angiotensin-induced PI hydrolysis together with an inhibited PI resynthesis in Li^+-treated glomerulosa cells may lead to the decrease in the membrane pool of PI. In fact, inactivation of Ca-gating due to the decreased membrane pool of PI was demonstrated in the serotonin stimulated blowfly salivary gland [18]. However, this is probably not the sole mechanism since a partial restoration of PI synthesis by exogenous *myo*-inositol was not followed by an increase in the aldosterone response. The accumulation of inositol-phosphates which cannot be eliminated by the addition of *myo*-inositol may be responsible for the decreased responsiveness of the Li^+-treated cells.

Experiments are in progress which examine the consequences of the inhibition of PI synthesis by Li^+ on the metabolism of polyphosphoinositides which are supposed to be the primary substrate hydrolysed following activation of calcium-mobilizing receptors [4,19].

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