Vol. 139, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 29, 1986 Pages 46-51

EFFECT OF OSMOLALITY ON ANGIOTENSIN-STIMULATED ALDOSTERONE PRODUCTION BY PRIMARY CULTURES OF BOVINE ADRENAL GLOMERULOSA CELLS

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Received July 7, 1986

Studies were performed to examine the relationship between osmolality and aldosterone production using primary cultures of bovine adrenal glomerulosa cells. Cell monolayers were incubated under hypo- (234 mOsm), iso- (274 mOsm), or hyperosmotic (318 mOsm) conditions in the absence or presence of angiotensin II (10^{-12} M to 10^{-9} M). Although basal steroidogenesis was unaffected, angiotensin II-stimulated aldosterone production was inversely related to osmolality. Mannitol and NaCl were equally effective as osmotic particles. Thus, modulation of angiotensin II-stimulated aldosterone secretion produced *in vivo* by changes in plasma osmolality result, in part, from a direct effect on the glomerulosa cells.

In a variety of situations in which plasma osmolality and sodium concentration are altered, there is a marked disparity between plasma renin activity and aldosterone concentration (1-4). Such observations strongly suggest that these parameters modulate the renin-angiotensin-aldosterone system, but the means by which osmolality and sodium might exert their effects have not been well defined. Recent studies by Schneider *et al.* (4-6) demonstrated that the effect of osmolality was exerted, at least in part, directly at the level of the adrenal gland. Changes in osmolality, independent of changes in sodium and/or chloride concentration, markedly altered the aldosterone secretory response of the isolated canine adrenal gland to angiotensin II in an inverse manner. Results of studies using acutely dispersed, isolated glomerulosa cells, on the other hand, have failed to support a direct effect of osmolality and have also suggested that extracellular sodium and/or chloride concentration has little, if any, effect on aldosterone production (7-9). Thus, either the intra-adrenal action of osmolality is medi-

0006-291X/86 $1.50

ated indirectly via an effect on a non-glomerulosa component of the gland or the ability of dispersed glomerulosa cells to respond to osmolality is attenuated. To address these issues, we examined the effects of osmolality on glomerulosa cells that were allowed to recover from isolation procedures by maintenance in primary culture. The results obtained clearly demonstrate that changes in extracellular osmolality can affect aldosterone secretion by modifying the steroidogenic response of the glomerulosa cell to angiotensin II.

MATERIALS AND METHODS

Glomerulosa cells were isolated from bovine adrenal capsules by collagenase digestion and mechanical dispersion using methods previously described (10). Cells were plated in 12-well cluster dishes (Costar; Cambridge, MA) and grown to confluence in air at 37°C in Ham's F-12 medium containing HEPES (25 mM; pH 7.4), $NaHCO_3$ (4 mM), ascorbate (100 μM), α-tocopherol (1.2 μM), N_2ScO_3 (0.05 μM), butylated hydroxyanisole (50 μM), metyrapone (5 μM), penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (1 μg/ml) and horse serum (10%; v/v). Media were changed at 48-hour intervals. Confluence was generally achieved within five days. At confluence, the medium was replaced with a serum-free medium containing BSA (100 μg/ml), and the cells were maintained for an additional 20-24 h before being used in an experiment.

Cells were then preincubated for two 1-h periods in 2 ml of Krebs-Ringer/HEPES buffer (25 mM, pH 7.4) containing NaCl (116 mM), $NaHCO_3$ (4 mM), KCl (4 mM), $CaCl_2$ (1.25 mM), $MgCl_2$ (0.83 mM), Na_2HPO_4 (1.87 mM), glucose (5.6 mM), and BSA (100 μg/ml). Finally, cells were incubated in a Krebs Ringer/HEPES buffer under one of the following conditions: 1) low osmolality (233 ± 2 mOsm), 2) normal osmolality/NaCl (274 ± 2 mOsm), 3) normal osmolality/mannitol (275 ± 1 mOsm), 4) high osmolality/NaCl (318 ± 2 mOsm), or 5) high osmolality/mannitol (316 ± 2 mOsm). All experimental media were prepared from a low Na (120 mM), low osmolality (233 mOsm) buffer by the appropriate addition of NaCl and/or mannitol. Angiotensin II (^{5}Val-Angiotensin II; BACHEM) was added (10^{12} M - 10^{-9} M) to the media as indicated. Incubations were allowed to proceed for 2 h. The media were then collected and the aldosterone contents measured by direct radioimmunoassay (Diagnostic Products; Los Angeles, CA). Cell monolayers were rinsed twice with protein-free phosphate-buffered saline and dissolved in 0.5 N NaOH. Cell protein was measured by the method of Bradford (11).

RESULTS

When glomerulosa cells were incubated in an iso-osmotic (274 ± 2 mOsm) medium, aldosterone output increased from a basal rate of 0.7 ± 0.3 to 27.6 ± 4.5 ng/h/mg protein (a 40-fold increase) as the concentration of angiotensin II increased from 10^{12} M to 10^{-9} M (Figure 1).

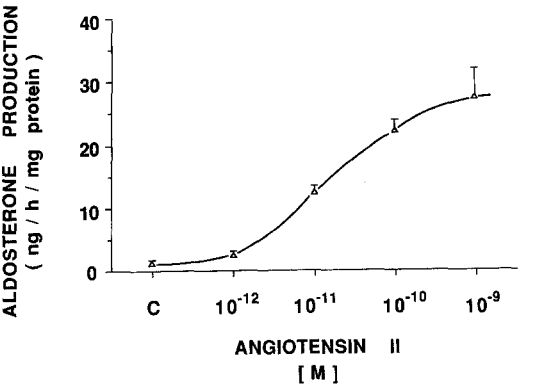

Figure 1. Effect of angiotensin II on aldosterone production by primary cultures of bovine glomerulosa cells. Cell monolayers were incubated in an iso-osmotic medium (274 mOsm) for 2 h in the presence of increasing concentrations of angiotensin II. Each point represents the mean and 1 SE of 4-6 determinations.

The interaction between osmolality and angiotensin II concentration is presented in Figure 2. Aldosterone production in response to maximal concentration (10^{-9} M) of angiotensin II was not affected by changes in osmolality. In contrast, the aldosterone secretory response to a submaximal concentration (10^{-11} M) of angiotensin II was potentiated when the osmolality of the medium was reduced (233 ± 2 mOsm) and was attenuated when osmolality was increased (318 ± 2 mOsm). Similarly, a reduction in osmolality markedly increased the amount of aldosterone produced in response to 10^{-12} M angiotensin II, a concentration that had no apparent steroidogenic effect

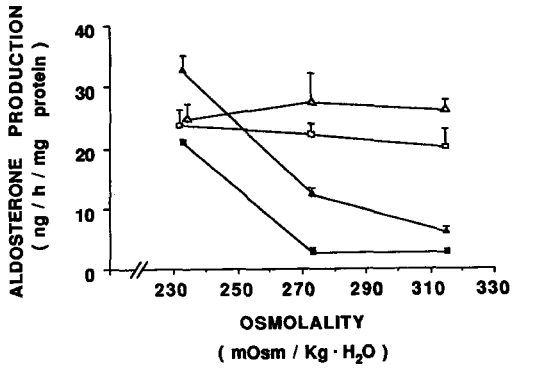

Figure 2. Effect of osmolality on angiotensin II-stimulated aldosterone production. Cells were incubated in the presence of 10^{-12} M (■), 10^{-11} M (▲), 10^{-10} M (□), and 10^{-9} M (△) angiotensin II under hypo- (233 mOsm), iso- (274 mOsm), or hyperosmotic (318 mOsm) conditions. Each point represents the mean and 1 SEM of 4-6 determinations.

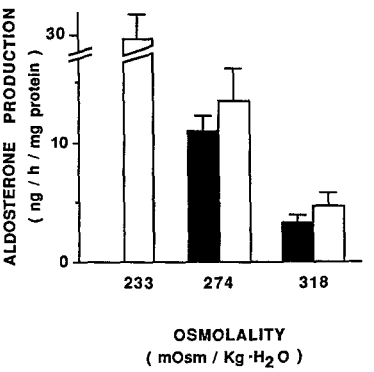

Figure 3. Effects of increasing osmolality on angiotensin-stimulated aldosterone production. Glomerulosa cells were incubated in the presence of 10^{-11} M angiotensin II in a hypo-osmotic medium and in media made iso-osmotic or hyperosmotic by the addition of either mannitol (solid bars) or NaCl (open bars). Each bar represents the mean and 1 SEM of 6 determinations.

when cells were incubated in an iso-osmotic medium. Changes in the osmolality of the incubation medium had no independent effect on aldosterone secretion (data not shown).

A comparison of the effects of increasing osmolality and/or sodium concentration on aldosterone production in response to sub-maxiumal concentration (10^{-11} M) of angiotensin II is presented in Figure 3. The enhanced rate of aldosterone production observed when cells were incubated in a hypo-osmotic medium was progressively decreased when the osmolality of the medium was increased by the addition of either mannitol or NaCl.

DISCUSSION

The present results provide the first demonstration that extracellular osmolality can directly modify the steroidogenic response of the glomerulosa cell to angiotensin II. In general, aldosterone production in response to the peptide was inversely related to the osmolality of the incubation medium. Likewise, the magnitude of the effect produced by a given change in osmolality was inversely related to the concentration of angiotensin II employed. For example, the ability of a reduction in extracellular osmolality to potentiate the steroidogenic response to angiotensin II was most dramatic

when the peptide was used at a concentration (10^{-12} M) that had no apparent effect on aldosterone production under iso-osmotic conditions. A similar reduction in osmolality, in contrast, did not enhance the production of aldosterone invoked by maximal concentrations of angiotensin II. An increase in osmolality from 274 mOsm to 318 mOsm did not significantly alter aldosterone production in response to a maximal concentration of angiotensin II, but it markedly attenuated the response to a submaximal concentration of the peptide. Thus, changes in osmolality, while having no apparent effect on basal aldosterone production or maximal steroidogenic capacity, markedly altered the sensitivity of the glomerulosa cell to angiotensin II.

These results are in contrast to those previously reported using acutely dispersed, isolated glomerulosa cells (7, 8). These investigators failed to observe an effect of osmolality on aldosterone output. The reason for this discrepancy is not readily apparent but may be related to differences in isolation/maintenance procedures and, consequently, to the responsiveness of the glomerulosa cell preparations or perhaps to the concentration of angiotensin II employed. The effects of osmolality on angiotensin-stimulated aldosterone production in cultured bovine glomerulosa cells noted here, however, are remarkably similar to those observed previously using the isolated, perfused canine adrenal gland (4-6). This similarity strongly suggests that the effects of osmolality observed using the perfused gland resulted from a direct effect on the zona glomerulosa. The possibility, however, that some other componenet of the adrenal gland (i.e., vascular or medullary) might also contribute to the effect of osmolality in vivo cannot be completely excluded. In any case, these results clearly demonstrate that the glomerulosa cell is remarkably sensitive to changes in extracellular osmolality, and they support the contention that plasma osmolality is an important modulator of angiotensin II-stimulated aldosterone production in vivo.

ACKNOWLEDGMENT

Supported in part by a Grant-in-Aid from the American Heart Association, Tennessee Affiliate, by USPHS Grant AM 33562, and by Biomedical Research Support Grant, UT - Memphis.

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