Effects of ACTH and Calcium on Cyclic AMP Production and Steroid Output by the Zona Glomerulosa of the Adrenal Cortex

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Synopsis

Effects of ACTH and calcium on cyclic AMP production and steroid output by the zona glomerulosa (the capsular fraction) from the rat adrenal cortex have been studied. Although high concentrations of extracellular calcium potentiated the stimulatory action of ACTH on cyclic AMP and aldosterone output, tetracaine or verapamil inhibited aldosterone output but not cyclic AMP production during ACTH-stimulation. Lanthanum reduced both aldosterone and cyclic AMP accumulation induced by ACTH. These results suggest that an extracellular calcium would be essential in stimulating the capsular steroidogenesis without involvement of the cyclic AMP system.

Materials and Methods

ACTH₁₋₂₄ (corticotropin 1-24, tetracosa peptide) and cyclic AMP were generously donated by Daiichi Seiyaku, Tokyo. Tetracaine, verapamil and X537A were also gifts of Kyorin Pharmac., Tokyo, Eisai Co. Ltd. and Nippon Roche Res. CTR., Kamakura,

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Japan. After removal of the decapsulated fraction (the zona fasciculata-reticularis) from the adrenal of adult male rats of Donryu strains (Nippon rats Co. Ltd., Tokyo) weighing 200 to 300 g, fifty to hundred mg of the capsular fractions (the zona glomerulosa) were preincubated in Krebs Ringer bicarbonate buffer solution (pH 7.4), containing 2 mg/ml of glucose and 1 mg/ml of bovine serum albumin (KRBG buffer), gassed with 95% O₂-5% CO₂ at 37°C for 40 min. Subsequently the tissues were transferred to fresh medium with various agents for further incubations. For calcium-free incubation, tissues were initially incubated for 40 min in KRB buffer without calcium, containing 0.1 mM EGTA, then transferred to identical fresh medium for the final 15 min- and 90 min-incubation. After the incubation, tissues were homogenized in 5% trichloroacetic acid at 0°C and centrifuged. Supernatants were ether-extracted and then lyophilized. Cyclic AMP levels were estimated by a competitive binding assay (Gilman, 1970), using a protein from rabbit skeletal muscle.

Corticosteroids in the medium after 90 min-incubation were extracted and purified by the procedure described previously (Shima et al., 1970; Shima et al., 1968). The determination of corticosterone concentration was made by H₂SO₄-induced fluorescence (Shima and Pincus, 1969). Aldosterone concentration was determined by a radioimmunoassay (CEA-IRE-SORIN-Kit) (Kono et al., 1976).

Results

30 to 1500 nM of ACTH produced cyclic AMP within 15-min incubation, then was followed by a fall to basal levels at 90-min incubation. In contrast a great increase in aldosterone levels was shown at 90-min incubation (Fig. 1).

Both the minimum and the maximum effective doses of ACTH in turns of cyclic AMP and aldosterone accumulation appear to be the same (Fig. 2). The half maximal effect of ACTH was achieved at 1.0 × 10⁻⁹ M for cyclic AMP and 1.2 × 10⁻⁹ M for steroid output. Incubation of the capsular fraction in the calcium-free medium with EGTA resulted in a marked accumulation of cyclic AMP, associated with lower aldosterone output than basal levels (Fig. 3 and 4). Raising the concentrations of calcium from 0 to 12.7 mM in the medium increased both levels of cyclic AMP and aldosterone (Fig.
Fig. 2. Effect of ACTH on steroid output and cyclic AMP levels in the capsular fraction of rat adrenals. Fifty to one hundred mg of tissue was incubated in 2 to 4 ml of KRB buffer 30 min, then transferred to fresh medium for the final 15 min incubation (for tissue cyclic AMP levels) or 90 min incubation (for steroid output) with various concentrations of ACTH as indicated. Each result represents the mean ± standard error (brackets) of four to five separate experiments.

Fig. 4. Effect of ACTH and various agents on cyclic AMP levels (15 min incubation) and steroid output (90 min incubation) in the capsular fraction of rat adrenals. Conditions of incubation were identical with the legend Fig. 1. The concentrations of agents added to the medium were 1.0×10⁻⁸ M of ACTH, 10⁻³ M of tetracaine, 5×10⁻⁶ M of verapamil, 10⁻³ M of lanthanum, and 5×10⁻⁶ M of X537A. In the experiments for lanthanum, phosphate was omitted from the medium and replaced with Tris., 0.326 gm/l, pH 7.2 to avoid precipitation of lanthanum phosphate. Each result represents the mean ± standard error of four separate experiments.
Fig. 3. Effect of calcium on steroid output and cyclic AMP levels in the capsular fraction of rat adrenals. Tissues were initially incubated for 40 min in KRBG buffer without calcium, containing 0.1 mM EGTA, then transferred to a fresh buffer with calcium concentrations ranging 0 to 12.7 mM for the final 15 min (for tissue cyclic AMP levels) and 90 min (for steroid production) incubation. The concentrations of agents added to the medium were $1.0 \times 10^{-8}$ M of ACTH, $10^{-3}$ M tetracaine and $5 \times 10^{-6}$ M of X537A. Each result represents the mean ± standard error (brackets) of four separated vessels.

3). At a concentration of 25.4 mM calcium became inhibitory. The calcium-induced accumulation of cyclic AMP and aldosterone was further enhanced by the addition of ACTH (Fig. 3). Tetracaine was inhibitory for aldosterone output induced by calcium or/and ACTH, but not for cyclic AMP production (Fig. 3). Lanthanum was inhibitory for both the aldosterone and cyclic AMP accumulation either unstimulated or stimulated by ACTH (Fig. 4). A calcium ionophore, X537A stimulated aldosterone output only in the calcium-free incubation medium. In the presence of calcium in the medium, X537A was always inhibitory for steroidogenesis even in the ACTH-
stimulation (Fig. 4). Cyclic AMP accumulation induced by high concentrations of calcium was abolished by X537A (Fig. 3). Meanwhile, ACTH-stimulated cyclic AMP production was not prevented by the calcium ionophore (Fig. 4).

Discussion

The previous study on the capsular fraction of the rat adrenal gland (Shima et al., 1976; Shima et al., 1978) indicated that the cyclic AMP system was not involved in the steroidogenic stimulation by angiotensin II. The finding that low concentrations of ACTH stimulate aldosterone output in a typical concentration-dependent manner corresponding with a rise in cyclic AMP appears to prove cyclic AMP to be the obligatory intermediate of ACTH action on aldosterone output by the capsular fraction (Fig. 2). This is, however, unlikely to be the case. Stimulation of ACTH on aldosterone output was completely inhibited by tetracaine or verapamil, while elevated cyclic AMP levels by ACTH were still maintained even in the presence of tetracaine or verapamil (Fig. 3 and 4). There was an apparent dissociation between capsular steroid output and cyclic AMP production during ACTH stimulation. Raising calcium concentrations in the medium increased cyclic AMP accumulation as seen in the decapsulated fraction (Shima et al., 1979), suggesting a stimulatory action of external calcium on aldosterone in the membrane independently of ACTH receptors. It is also of interest that unlike the decapsulated fraction, high concentrations of calcium itself were stimulatory to the capsular steroidogenesis (Fig. 3). This suggests that external calcium is more easily available for steroidogenesis by the glomerulosa cells than the fasciculata-reticularis cells of the adrenal cortex.

Aldosterone production by the capsular fraction, susceptible to tetracaine or verapamil (Fig. 3 and 4) was quite different from corticosterone production by the decapsulated fraction, where these calcium antagonists were without effect (Shima et al., 1979). Steroidogenesis by the capsular fraction stimulated by ACTH might be in the process of calcium influx sensitive to tetracaine (Blaustein and Goldman, 1966; Feinstein and Paimre, 1967; Kwant and Seeman, 1969) or verapamil (Watanabe and Besch, 1974; Devis et al., 1975; Shapiro et al., 1977), and distinct from the cyclic AMP system. Moreover, in the capsular fraction the activation of receptors of ACTH would interact with the calcium channels to increase intracellular calcium in the different manner from the ACTH action on the decapsulated fraction. Lanthanum was also inhibitory to aldosterone output by the capsular fraction either unstimulated or stimulated by ACTH. These results again confirm the previous finding (Shima et al., 1976; Shima et al., 1978) that steroidogenesis by the capsular fraction is highly dependent on calcium.

Meanwhile, calcium would interact with ACTH receptors linked to adenylate cyclase to increase cyclic AMP levels, which are separated from the process of the calcium current coupled to steroidogenesis. Tetracaine, verapamil or lanthanum completely inhibited steroidogenesis by the capsular fraction (Fig. 3 and 4). As to cyclic AMP system, tetracaine or verapamil was also without effect on ACTH-elevated cyclic AMP levels as observed in the decapsulated fraction. Lanthanum, which is inhibitory to cyclic AMP system in the decapsulated fraction (Browitz, 1972; Haksar et al., 1976), also inhibited ACTH-induced cyclic AMP accumulation in the capsular fraction (Fig. 4).

The previous experiment (Shima et al., 1978) demonstrated that the capsular cyclic AMP levels were increased by calcium-deprivation by EGTA treatment. The present
experiment also confirmed that an increase in cyclic AMP by calcium-deprivation occurred not in the decapsulated fraction (Shima et al., 1979) but only in the capsular. No increase in the medullary cyclic AMP of the rat adrenal gland was also observed by omission of calcium from the incubation medium (Shima et al., 1977). Rubin et al. (1972) have reported on an increase in the whole adrenal cyclic AMP accumulation by removal of calcium from the medium. The present findings that increasing concentrations of calcium are stimulatory to cyclic AMP system (Shima et al., 1976; Shima et al., 1979; 1978) (Fig. 3) are not compatible with the hypothesis that the mechanism of cyclic AMP system stimulated by calcium-deprivation is similar to that of ACTH (Rubin et al., 1972). Increased cyclic AMP levels by EGTA-treatment could be partly explained to be due to the decrease in cyclic AMP metabolism including cyclic nucleotides-phosphodiesterase activities (Levin and Weiss, 1976). Extremely higher activities of cyclic AMP-phosphodiesterase have been found in the capsular than in the decapsulated fraction (Guidotti and Costa, 1974; Gallant et al., 1974; Shima et al., 1978). Tetracaine was markedly effective in inhibiting cyclic AMP-phosphodiesterase activities of the capsular fraction (Shima et al., 1978). ACTH would interact with extracellular calcium in stimulating the cyclic AMP system of the capsular fraction which might not be linked to aldosterone production. Alternatively, ACTH receptors would act on giving access of calcium to a calcium binding activator site which coupled to the adenylate cyclase in the capsular membrane.

Meanwhile, steroidogenesis by the capsular fraction might be stimulated by ACTH exclusively through the process of the calcium channels, without involvement of cyclic AMP system. Unlike the decapsulated fraction, the effects of X537A were observed to be inhibitory for ACTH- and/or calcium-induced aldosterone production (Fig. 3 and 4). The decrease in aldosterone output seen even in the medium as low as 0.1 mM calcium with X537A could explain an excess of intracellular calcium, which in turn inhibited steroidogenesis. An increase in aldosterone in calcium free medium with X537A would be interpreted as the stimulation of membrane-bound or stored calcium released by the calcium ionophore (Vale and Carvalho, 1975), which might be sufficient for steroidogenic stimulation (Fig. 3). Moreover, the capsular steroidogenesis might be more sensitive to intracellular calcium concentrations than the decapsulated gland.

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References


