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# Role of adenosine $A_1$ and $A_2$ receptors in the regulation of aldosterone production in rat adrenal glands

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*Summary.* To investigate the roles of adenosine $A_1$ and $A_2$ receptors in the regulation of aldosterone production, we examined the effects of adenosine and adenosine agonists ($N^6$-cyclohexyl adenosine; selective adenosine $A_1$ receptor agonist and 5'-N-ethylcarboxamine adenosine; selective adenosine $A_2$ receptor agonist) on aldosterone and cyclic AMP production in rat adrenal capsular cells. Neither adenosine nor 5'-N-ethylcarboxamine adenosine caused significant effects on basal aldosterone or cyclic AMP production. Also, adenosine ($10^{-3}$ M) showed no consistent effects on aldosterone and cyclic AMP production induced by ACTH. On the other hand, $N^6$-cyclohexyl adenosine exhibited a significant inhibition of basal aldosterone and cyclic AMP production at doses of $10^{-4}$ M and $10^{-3}$ M; furthermore, $10^{-3}$ M $N^6$-cyclohexyl adenosine inhibited aldosterone and cyclic AMP production stimulated by ACTH. These results suggest that adenosine $A_1$ receptors are coupled to and inhibit adenylate cyclase and may be involved in the inhibition of aldosterone production.
*Key words.* Aldosterone; cyclic AMP; adenosine; adenosine $A_1$ receptor; adenosine $A_2$ receptor.

Adenosine has been shown to have a variety of biological activities including effects on hemodynamics, sympathetic nerve activity, renin and hormonal secretions[1-5]. However, the role of adenosine in the regulation of adrenal steroidogenesis is still controversial. Kowal and Fiedler[6], using monolayer cultures of adrenal cells and primary cultures of adrenal tumor cells, reported that adenosine stimulated adrenal steroidogenesis. Wolff and Cook[7] found that adenosine increased steroidogenesis and adenylate cyclase activity in adrenal tumor cells. Adenosine was also reported to cause a significant increase in cortiocosterone secretion in in situ isolated perfused rat adrenal preparations[8]. On the other hand, in other studies adenosine was shown to have no direct effect on adrenal steroidogenesis in vivo or in vitro[9,10]. Furthermore, Shima[11] observed that adenosine and its analog showed an inhibitory effect on ACTH-stimulated steroidogenesis and adenylate cyclase activity without affecting basal steroid production or adenylate cyclase activity in rat adrenal gland. With regard to the effect of adenosine on aldosterone production, Shima[11] reported that adenosine inhibited $ACTH^{1-24}$-stimulated aldosterone production without affecting basal aldosterone production in dispersed rat adrenal cells. Hinson et al.[8], however, indicated that adenosine did not exhibit a consistent effect on aldosterone secretion in perfused rat adrenal gland or aldosterone production in dispersed rat adrenal cells.

Recently, two functionally distinct adenosine receptors that stimulate or inhibit plasma membrane adenylate cyclase have been identified and reviewed[12]. These two subclasses of adenosine receptors are designated $A_1$ and $A_2$. A variety of adenosine analogues have been introduced in recent years. $N^6$-cyclohexyl adenosine (CHA) and 5'-N-ethylcarboxamine adenosine (NECA) are relatively selective agonists for $A_1$ and $A_2$ receptors, respectively. The present study was undertaken in an attempt to evaluate the effects of adenosine, and adenosine $A_1$ and $A_2$ receptor agonists (CHA and NECA), on aldosterone and cyclic AMP production in rat adrenal capsular cells. In addition, the effects of adenosine and CHA on ACTH-stimulated aldosterone production and ACTH-induced cyclic AMP production were also examined.

## *Materials and methods*

Adenosine, CHA, NECA and bovine serum albumin (BSA) were purchased from Sigma Chemical (St. Louis, MO, USA). $ACTH^{1-24}$ was obtained from the Protein Research Foundation (Osaka, Japan). Medium 199 was from GIBCO Laboratories (Grand Island, NY, USA)

and crude collagenase was purchased from Funakoshi Pharmaceuticals (Tokyo, Japan).

*Preparations of adrenal capsular cells.* Sprague-Dawley female rats (200–220 g), maintained on regular rat chow, were used in each study. Adrenal capsular cells were prepared by a modification of the procedures of Fredlund et al.[13] and of Hanings et al.[14]. In brief, after the animals had been decapitated the adrenal glands were removed and separated into the capsular and decapsular portions. The capsular portion was minced into small pieces and incubated with crude collagenase (2 mg/ml Medium 199) for 50 min at 37 °C and then dispersed with an Eppendorf pipette. Collagenase-dispersed capsular cells (mainly zona glomerulosa cells) were washed several times with fresh Medium 199 and used for the experiments without preincubation.

*Effects on aldosterone production.* The capsular cell suspensions were incubated in Medium 199 containing BSA (2 mg/ml) with various amounts ($10^{-6}$–$10^{-3}$ M) of either adenosine, CHA or NECA for 2 h at 37 °C under 95 % $O_2$-5 % $CO_2$. To examine the effects of adenosine or CHA on ACTH-induced aldosterone production, the capsular cell suspensions were incubated with two concentrations ($10^{-10}$ and $10^{-9}$ M) of $ACTH^{1-24}$ in the presence or absence of $10^{-3}$ M adenosine or CHA under the same conditions.

*Effects on cyclic AMP production.* The capsular cell suspensions were incubated in Medium 199 containing BSA (2 mg/ml) with individual concentrations ($10^{-6}$–$10^{-3}$ M) of adenosine, CHA and NECA for 1 h at 37 °C. The cell suspensions were also incubated with three concentrations ($10^{-8}$–$10^{-6}$ M) of $ACTH^{1-24}$ in the presence or absence of $10^{-3}$ M of adenosine or CHA for 1 h at 37 °C. After incubation the cell suspensions were boiled in a water bath for 10 min and then centrifuged. The supernatant was used for the measurement of cyclic AMP.

*Measurement of aldosterone and cyclic AMP.* Aldosterone released into the incubation medium was measured directly by radioimmunoassay as described previously[15]. For cyclic AMP determination, samples were succinylated and then measured using a radioimmunoassay kit (Yamasa Shoyu, K. K., Chiba, Japan). The intraassay and interassay coefficients of variation were 5.2 % and 8.6 %, respectively.

*Statistical analysis.* Results are expressed as the mean ± SEM. Statistical significance was assessed by Dunnett's procedure for multiple comparison with a standard, and the data of variables within concentrations were analyzed by the non-paired t-test. A p value less than 0.05 was considered to be significant.

## Results

*Effects on basal production of aldosterone and cyclic AMP.* As shown in figure 1, adenosine and NECA did not elicit any significant change in aldosterone production. CHA showed a significant and concentration-dependent inhibition of aldosterone production (from the control value of 26.7 ± 2.3 ng/$10^5$ cells to the maximum inhibition of 12.8 ± 1.0 at $10^{-3}$ M of CHA, $p < 0.01$). Also, CHA caused a significant and concentration-dependent inhibition of cyclic AMP production ($p < 0.05$ at $10^{-4}$ M and $p < 0.01$ at $10^{-3}$ M, fig. 1), whereas neither adenosine nor NECA had any significant effect on cyclic AMP production.

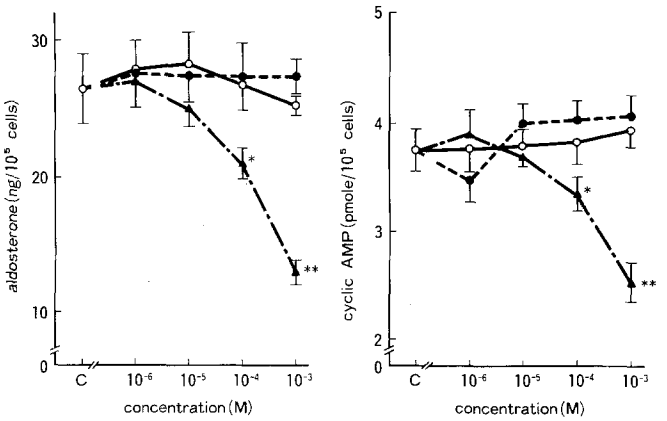


Figure 1. Effects of adenosine, NECA and CHA on aldosterone (left panel) and cyclic AMP (right panel) production. Comparison was made within treatments. o——o, adenosine; ●– – –●, NECA; ▲——▲, CHA. C, control; n = 6, *p < 0.05, **p < 0.01 vs C.

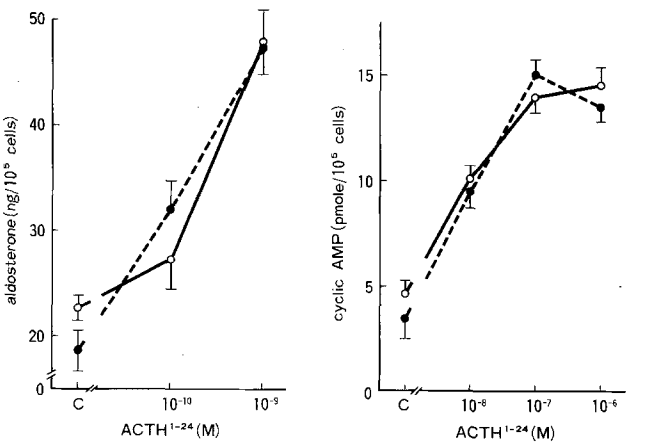


Figure 2. Adenosine had no significant effect on $ACTH^{1-24}$-induced aldosterone (left panel) and cyclic AMP (right panel) production. Comparison was made within concentrations. o——o, without adenosine; ●– – –●, with adenosine ($10^{-3}$ M); C, control; n = 6.

*Effects on aldosterone and cyclic AMP production induced by $ACTH^{1-24}$.* $ACTH^{1-24}$ produced a significant and concentration-dependent increase in aldosterone and cyclic AMP production. Although aldosterone and cyclic AMP responses to $ACTH^{1-24}$ were not significantly affected by $10^{-3}$ M of adenosine (fig. 2), CHA ($10^{-3}$ M) markedly inhibited both aldosterone and cyclic AMP production stimulated by $ACTH^{1-24}$ (fig. 3).

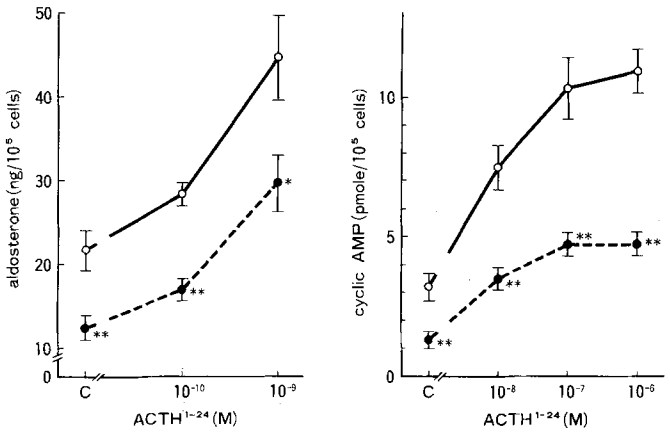


Figure 3. Effects of CHA on aldosterone (left panel) and cyclic AMP (right panel) production stimulated by $ACTH^{1-24}$. Comparison was made within concentrations. o——o, without CHA; ●- - - -●, with CHA ($10^{-3}$ M); C, control; n = 6, *p < 0.05, **p < 0.01 vs without CHA.

*Discussion*

The available data on adenosine and adrenal steroidogenesis are conflicting. The fact that there are two subclasses of membrane receptors for adenosine ($A_1$ and $A_2$), and the differences in the materials and methods used in these studies, may explain the different results. Activation of $A_1$ receptors may cause a reaction which is opposed by activation of $A_2$ receptors, since adenylate cyclase activity is inhibited by activation of the $A_1$ receptor, and stimulated by activation of the $A_2$ receptors.

In the present study, the effects on aldosterone and cyclic AMP production of relatively selective $A_1$ and $A_2$ adenosine receptors agonists (CHA and NECA), and adenosine itself, were examined in rat dispersed adrenal capsular cells. CHA significantly inhibited not only aldosterone production but also cyclic AMP production in adrenal capsular cells, while adenosine and NECA had no significant effects on aldosterone or cyclic AMP production. CHA is the $N^6$-substituted compound of adenosine and the most potent adenosine $A_1$ receptor agonist. Trost and Stock [16] reported that the effects of adenosine were augmented by the structure of the $N^6$-substituent of adenosine in isolated rat adipocytes. Therefore, it is possible that the inhibitory effect of CHA on cyclic AMP and aldosterone production is more potent than that of adenosine in rat adrenal glomerulosa cells. It is reported that in adipocytes, fibroblasts and heart, brain and nervous system cells, adenosine $A_1$ receptors have relatively high affinities towards adenosine, in the nanomolar range, and $A_2$ receptors have relatively low affinities towards adenosine, in the micromolar range [16–20]. In our study, relatively high concentrations of CHA were necessary to inhibit cyclic AMP and aldosterone production. Thus it is unlikely that CHA has any physiological significance. However, it also seems unlikely that this inhibitory effect of CHA is due to a direct toxic effect on the capsular cells, since the aldosterone response to $ACTH^{1-24}$ is well maintained even after 2 h of preincubation with CHA (data not shown).

It is well recognized that cyclic AMP is the intracellular mediator of the steroidogenic action of ACTH [21]. However, we used relatively high concentrations of $ACTH^{1-24}$ for cyclic AMP study, since a low dose of $ACTH^{1-24}$ ($10^{-10}$ M) stimulated significant aldosterone production without causing detectable changes in cyclic AMP production (data not shown). These results are in agreement with previous studies of the effect of ACTH on steroidogenesis [22–24]. It is hard to explain this discrepancy between concentrations stimulating steroidogenesis and those stimulating cyclic AMP production. The phosphatidylinositol system is also an intracellular messenger for endocrine substances; however, to our knowledge there is no report as to the effect of ACTH on phosphatidylinositol turnover. One possible explanation is that even a small increase in cyclic AMP production is sufficient to induce steroidogenesis.

In this study receptor binding was not examined; however, judging from the biological responses, it is indicated that adenosine $A_1$ receptors are coupled to and inhibit adenylate cyclase and are involved in the inhibition of aldosterone production. The fact that adenosine and NECA have no significant effects on cyclic AMP and aldosterone production suggests that the membranes of rat adrenal glomerulosa cells have relatively low affinities toward adenosine and NECA.

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