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Steroidogenesis in Isolated Adrenal Cells of Rat

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ABSTRACT. A reliable and reproducible system for the isolation of rat adrenal cells was developed, using 0.25% trypsin for cell dispersion. The suspending cell in Krebs-Ringer bicarbonate buffer containing 0.2% glucose and 0.5% bovine serum albumin was incubated for 120 minutes at 37°C under 95% O_2 and 5% CO_2. Corticosterone production induced by synthetic $_{1-24}$ACTH showed a dose-related increase in decapsular cells. The precision of the inter-experiment of corticosterone production was 5.0% (average coefficients of variation).

Key words : Isolated adrenal cells — Steroidogenesis — Rat corticosterone

Adrenal cell preparations have been previously described by Kloppenborg *et al.*[1] and Sayers *et al.*[2] These preparations have used bacterial collagenase[1,3] and trypsin[2] as dispersing enzyme. Such preparations should allow easier entry of stimuli added to the incubation medium, and have theoretical advantages over tissue slices.

The present paper describes a method for preparing cells from the zona glomerulosa (capsular cells) or from the zona fasciculata-reticularis (decapsular cells), and demonstrates a stimulating effect of ACTH on steroidogenesis in the suspension of the decapsulated cells.

MATERIALS AND METHODS

Preparation of Adrenal Cell Suspensions (Table 1)

Male Wistar rat (150～300 g) were decapitated using a guillotione and the adrenals were removed immediately. The adrenals were collected in ice-cold saline. The loose of adherent connective and fat tissues were placed in a glass vial containing ice-cold Krebs-Ringer bicarbonate buffer to which 0.2% glucose and 0.5% bovine serum albumin had been added (KRBGA-buffer). The adrenal glands were separated into capsular and decapsular portions as previously described.[4] The bisected capsules and decapsules were cut with a razor blade into fine pieces. These pieces were transferred into a 20 ml beaker with 10 ml KRBGA-buffer containing 0.25% trypsin (1:250, Difco Laboratories, Michigan, U.S.A.), which was freshly prepared in each experiment. The cells were dispersed by mechanical stirring with a paddle rotating at 500 r.p.m. under 95% O_2 and 5% CO_2 atmosphere at 37°C. After dispersing for about 10 min, the supernatant was removed to plastic tubes with pasteur pipette, and 10 ml fresh buffer

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TABLE 1. Procedure for the preparation of rat adrenal isolated cells. KRBGA ; Krebs-Ringer bicarbonate buffer containing 0.2% glucose and 0.5% bovine serum albumin.

Rat ; Wistar male 150～300 g
↓
Decapitation
↓
Remove and collect of adrenals in ice-cold saline
↓
Separate into capsular and decapsular portions
↓
Digestion by 0.25% trypsin solution (KRBGA) at 37°C under 95% O_2 and 5% CO_2
↓
Centrifuge (100×g, 20 min, 4°C) ← repeat 2 times
↓
Resuspended cells in KRBGA with trypsin inhibitor
↓
Incubation → Cell count

with trypsin was replaced to the beaker, and then redispersed for 10 ml. In this way, a total of 50 ml of trypsin solution was used. The collected suspended cell was centrifuged at 100×g for 20 min at 4°C, and cell-free supernatant was discarded. Resuspended cells with 10 ml KRBGA-buffer containing 0.05% lima bean trypsin inhibitor (Sigma, St. Louis, MO, U.S.A.) were recentrifuged. This procedure was repeated two times. An aliquot of the cell suspension was mixed with an equal volume of 0.5% trypan blue, and the number of the variable cells was counted by Haemacytometer (Bürker-Türk).

Incubation of the Isolated Adrenal Cells

The volume of the suspension was adjusted by KRBGA-buffer, and a final incubation volume of cell suspension was 2 ml, in which 0.1 ml of adding agent (e.g. ACTH) was contained. All incubations were performed in 5 ml plastic tubes at 37°C in a shaking bath under 95% O_2 and CO_2. After incubation cell suspension was centrifuged at 100×g for ten minutes, and the supernatant was transferred to the tube and refrigerated for measuring the concentration of produced steroid. All glass instruments for the procedure of the cell preparation were siliconized.

Corticosterone Assay

The suspension fluid was diluted by distilled water and corticosterone concentration was measured by the radioimmunoassay as previously reported.[5] Corticosterone production was expressed as ng per 10^5 suspension cells.

RESULTS AND DISCUSSION

The Viability of Dispersed Cells

The viability of cells assessed by trypan blue staining was between 80 and 90%. The decapsulated cells were filled with lipid droplet in cytoplasma, and were larger than the cells from the capsulated preparation.

Time Period of Incubation

The effect of incubation time on steroidogenesis was presented in Figure 1. The corticosterone production of decapsular suspension cells increased time-relatedly for 240 minutes. As in most of the previous studies,[1-3,5] the incubation time was 120 minutes, incubation time 120 minutes was chosen in this study.

Effect of ACTH on Steroidogenesis of the Decapsular Cell Suspension

The response of corticosterone to ACTH was shown in Figure 2. Varying doses of ACTH (1.11×10^{-17}M~1.11×10^{-9}M) caused a dose-related increase of corticosterone production. The corticosterone production in 2 hr incubation was approximately 600 ng/10^5 cells at dosage of 1.11×10^{-9}M ACTH, and this value was similar to the results of other investigators.[6]

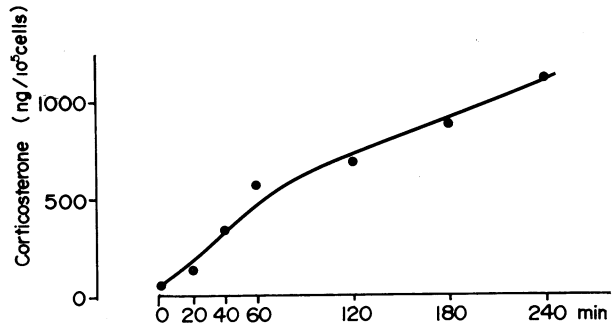

Fig. 1. The effect of incubation time on corticostrone production. The amount of added ACTH was 1.11×10^{-9}M.

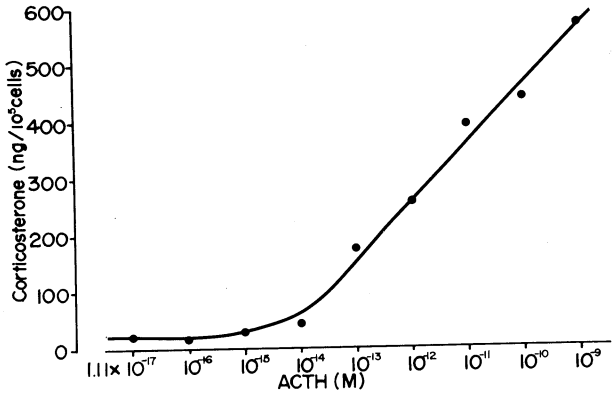

Fig. 2. The effects of ACTH (1.11×10^{-17}~1.11×10^{-9}M) on corticosterone production in adrenal decapsular cell. Incubation time : 120 min.

The Reliability of the Inter-experiment

The precision of the inter-experiment was shown in Table 2. An average coefficient of the inter-experiment precision was 3.5% and 6.4%, respectively, at the ACTH amount of 1.11×10^{-9}M and 1.11×10^{-10}M. These findings indicate that the isolated cell system is useful for study of adrenal function *in vitro*.

Table 2. The reliability of the inter-experiment (5 different experiments).

ACTH (M)	1.11×10^{-9}	1.11×10^{-10}
Corticosterone production ($ng/10^5$ cells)	607.2	344.4
	587.5	314.6
	514.6	439.0
	546.0	429.6
	642.0	457.6
Mean ± SEM	579.5±20.1	397.0±25.3
CV (%)		6.4

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