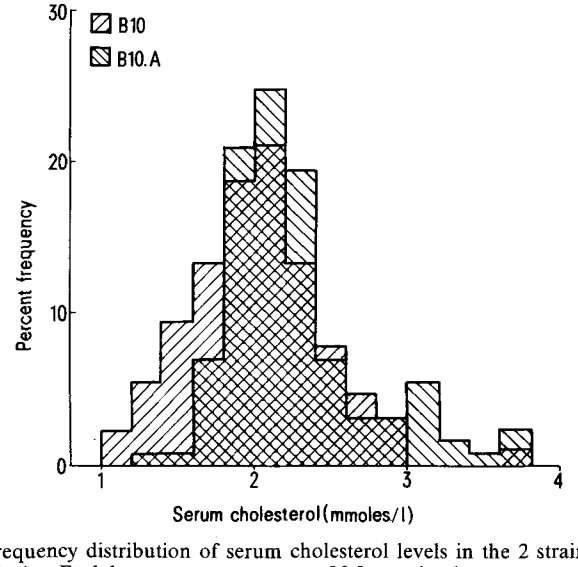
compatibility region where the B10 strain is haplotype H-2^b, and the B10. A strain haplotype H-2^a. Thus inherited differences between these 2 strains are likely to be determined, directly or indirectly, by genes in the major histocompatibility complex. We observed that the mean serum cholesterol level of female B10.A mice in our colony was significantly higher than that of female B10 mice. A similar result was noted in mice purchased from Olac Laboratories. The results described here are pooled results from mice of both colonies.

Mice were caged under identical conditions in the same room and fed the same diet (CRM diet, Labsure) for at least 1 month prior to death. To minimize age related differences in serum cholesterol, in every experiment a B10 mouse was paired with a B10.A mouse of the same age in weeks. No mouse tested was younger than 8, or older than 16 weeks. Mice were killed by rapid asphyxia with CO_2. Blood was removed by cardiac puncture, serum separated, and cholesterol determined, using an enzymatic oxidation method[7] by Mrs S. Duff and Miss K. Rowan of our clinical chemistry group.

Mean serum cholesterol of 128 female B10 mice was 1.96 mmoles/l; of 129 female B10.A mice 2.22 mmoles/l. Median values were 1.94 mmoles/l and 2.09 mmoles/l. The mode of the 2 groups was similar. The difference in mean serum cholesterol levels arose largely because the B10.A population distribution was significantly skewed (coefficient of skewness = 1.12). There were a disproportionate number of high serum cholesterol levels in the B10.A population. Since there was a significant deviation from the normal distribution, a non-parametric test (the Mann-Whitney test) was used to compare populations. Using this test, the difference in serum cholesterol levels was very highly significant ($p < 0.0001$). The figure shows a histogram of this data.



Frequency distribution of serum cholesterol levels in the 2 strains of mice. Each bar represents a range of 0.2 mmoles/l.

The effect of strain difference on serum cholesterol levels was confined to female mice. Male mice had considerably higher serum cholesterol levels than female but there was no difference between the B10 and B10.A strains (mean values 2.85 mmoles/l and 2.76 mmoles/l respectively).

Changes in blood levels of sex hormones, or changes in adrenal cortex function, can both affect cholesterol levels. The strains B10 and B10.A do show significant differences in both plasma testosterone levels and testosterone binding capacity[8], however we observed a significant difference in serum cholesterol levels only in female and not in male mice. Differences in cortisol binding protein associated with the H-2^a/H-2^b haplotype difference have also been reported[9], and in our study adrenal weights were higher in the B10.A strain, both in females and in males, though the difference was larger in the female sex. Mean values of weight of each adrenal pair were; in our B10 females 3.29 mg (SE = 0.07 mg), in our B10.A females 3.62 mg (SE = 0.07 mg). Statistically, this difference was highly significant. Adrenal weight distributions were sufficiently close to the normal distribution to use an unpaired t test, which gave $p < 0.001$.

Our results suggest that it may be worthwhile to include serum cholesterol measurements in clinical studies of the relationship between HLA type and coronary artery disease.

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Action of ACTH, cAMP and cytochalasin B on steroid production by Y-1 mouse adrenal tumor cells in culture[1,2]

J.J. Mrotek, P.F. Hall[3] and I. Lacko

Department of Biological Sciences, North Texas State University, Denton (Texas 76203, USA), 31 March 1982

Summary. Continued incubation of Y-1 mouse adrenal tumor cells with adrenocorticotrophic hormone (ACTH) or with dibutyryl-3′,5′-cyclic adenosine monophosphate (dbcAMP) resulted in an initial increase and a subsequent decrease in steroidogenesis. ACTH- or dbcAMP-stimulated steroidogenesis was inhibited by cytochalasin B (CB) to approximately the same extent during the entire period of incubation; CB inhibition of the ACTH response was reversible.

Recently, Nolin[4] suggested that rat adrenal steroidogenesis may be stimulated when ACTH is internalized. Gonadotropins are internalized by an endocytotic[5] process which may require microfilament activity[6]. Both endocytosis and microfilament function have been reported to be inhibited by CB[6-8]. Since ACTH-controlled steroid synthesis involves changes in the thin filaments[9] and is inhibited by CB[10-14], it was suggested that CB may prevent thin filaments from

participating in the internalization of ACTH into the adrenal cell[15]. The present study was conducted to evaluate the possibility that CB may inhibit steroidogenesis by preventing the internalization of ACTH.

Materials and methods. Y-1 mouse adrenal tumor cells[16] were maintained using methods reported previously[10,11,17]. At the start of the experimental incubation we replaced the serum-containing (SC) medium with nonserum-containing (NSC) medium in order to remove steroid-binding serum proteins which attach to culture dishes[18] and interfere with the assay for steroids. 2 successive 0.5-h incubations with the NSC medium cleared the system of serum proteins permitting measurement of subsequent steroid synthesis upon the addition of fresh NCS medium at 0.5-h intervals. The NSC medium containd: 1% (v/v) ethanol/dimethyl sulfoxide (E/DMSO; 1:1, v/v) and 10 mU ACTH, 2 mM dbcAMP or 10 µl saline. Another series of NSC mediums containing 10^{-5}M CB and these same agents was prepared using a concentrated stock solution of CB dissolved in E/DMSO; DMSO was stored at 4 °C to retard deterioration. The steroids from each incubation period were extracted from the medium with ether (1:5, v/v; twice) and assayed fluorometrically[19]. All experiments were performed in duplicate.

Results. During the 1st 0.5-h incubation, steroid production increased 8- or 14-fold with ACTH or dbcAMP, respectively; thereafter, the incremental rate of steroid synthesis declined either immediately (with ACTH) or following a 1.5-h delay (with dbcAMP) (figs 1 and 2). The temporal steroid response of the cells during continuous treatment with ACTH/CB or with dbcAMP/CB paralleled that of the ACTH-treated cells. However, the rate of steroid production only reached 60-90% of that obtained in response to ACTH, or dbcAMP alone, for each successive 0.5-h period (figs 1 and 2). In an attempt to reverse CB inhibition the ACTH/CB medium used for 1 set of cell cultures was replaced with the ACTH medium at the 2nd h of incubation. The subsequent rate of ACTH-stimulated steroid production by the previously ACTH/CB-treated cells was similar to the rate measured for cells incubated continuously with ACTH (fig. 1). Control or CB-treated cells produced relatively small amounts of steroid (figs 1 and 2). To compare our results with data obtained when SC incubation medium is changed less frequently[12,14,20] our data was expressed as cumulative steroid production. Cumulative plots of steroid production (figs 3 and 4) reveal that steroid synthesis was not linear, regardless of treatment.

Discussion. If ACTH acts only after internalization, as suggested[4,15], it presumably enters the cell bound to site on the membrane; the number of sites thus available for binding should diminish during continuous incubation in the presence of the hormone. A continuing decline in steroid secretion by ACTH-treated cells (fig. 1) is consistent with the concept of internalization. Since stimulation by dbcAMP also decreased with time after 1.5 h (fig. 2), the continuously diminishing response to dbcAMP argues for mechanisms involving either endocytosis or saturation phenomena. Consistent with this last possibility are studies which indicate that cAMP is actively transported into the Y-1 cell[21]. While CB inhibited ACTH-stimulated steroid production, it also prevented exogenous dbcAMP from fully stimulating steroid production; consequently, CB does not appear to be preventing endocytosis. The present results are consistent with the earlier suggestion[11] that ACTH initiates steroidogenesis by stimulating microfilament-mediated transport of cholesterol into adrenal mitochondria, a suggestion based on the effects of CB on microfilaments[7-9] and on inhibiting steroidogenesis[10,11].

The decline in rate of production of steroids by the adrenal cells continuously exposed to ACTH or dbcAMP is analo-

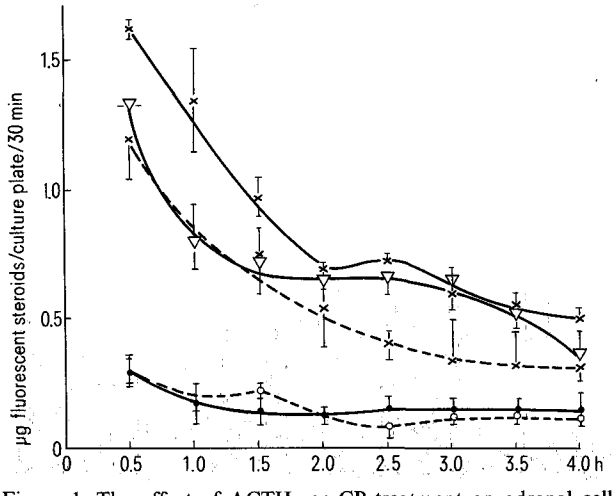

Figure 1. The effect of ACTH- or CB-treatment on adrenal cell steroid production when the incubation medium was frequently exchanged for fresh medium containing the same treatment. In 3 different sets of plates fresh control medium (●——●), medium containing ACTH (×——×) or CB-containing medium (○---○) was exchanged for incubated medium every 0.5 h. 2 sets of plates were initially incubated with ACTH plus CB-containing medium (△——△, ×---×) for 4 0.5-h periods. After this initial period, medium in 1 set of plates was replaced with medium containing ACTH alone (△——△); ACTH plus CB-containing medium (x---x) was not replaced on the 2nd set of plates. The incubation of both sets of plates was continued for 4 additional 0.5-h periods. 2 experiments with 2 replicates per treatment were performed; values shown are mean ± SE.

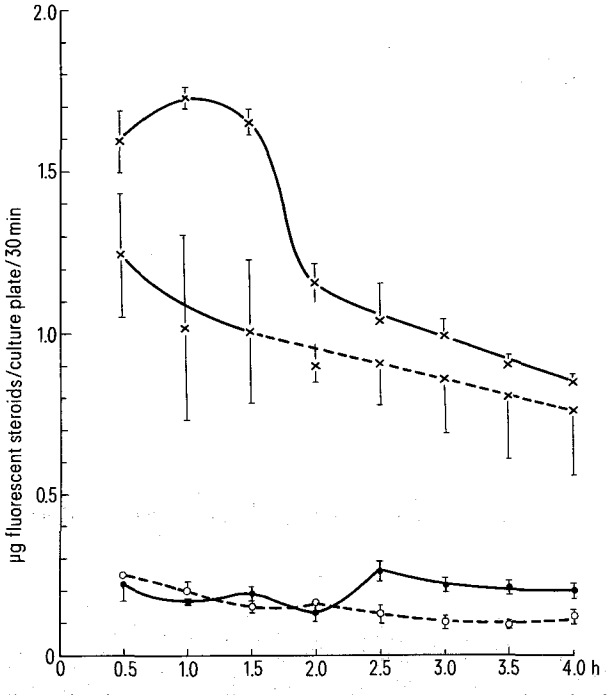

Figure 2. The effect of dbcAMP- or CB-treatment on adrenal cell steroid production when the incubation medium was frequently exchanged for fresh medium containing the same treatment. Fresh control medium (●——●) or media containing dbcAMP (×——×), dbcAMP plus CB (×---×), or CB (○---○) were exchanged for incubated medium every 0.5 h. 2 experiments with 2 replicates per treatment were performed; values shown are mean ± SE.

gous to that demonstrated in quartered rat adrenals continuously infused with ACTH or cAMP[22], and it is similar to that observed in cultured Y-1 adrenal tumor cell cytoplasts[23]. Decline in this response does not appear to result from cell death, since no more than 20% of the cells were stained with the live-dead stain, trypan blue, at the end of an experiment. Other explanations for declining steroid production by adrenal cells continuously incubated in the presence of ACTH or dbcAMP include differences in the length of time cells are continuously cultured[24], the absence of serums in the experimental incubation method[25] and the accumulation of metabolic end-products[26].

After clonal strains of mouse adrenal tumor cells were continuously cultured for 6 months or more, the ability to respond to 2 h of stimulation was lost if the cells were incubated in media without serum proteins[24]. The cells in the present study responded to stimulation, in spite of being incubated in NSC medium; they were from the 50th passage. Clones of these cells from the 70th passage (approximately 1-year-old) exhibited the same response to stimulation. For this reason it seems unlikely that the passage number influenced the temporal response of our cells when they were stimulated while being incubated in NSC medium.

Cultured Y-1 mouse adrenal tumor cells were used by several laboratories to evaluate the effects of ACTH, dbcAMP and CB on steroid synthesis[10-12,14,17,20,23,24]. In these studies some investigators[12,14,20] incubated the cells continuously in the same SC medium, while others exchanged incubated NSC medium[10,11], or SC medium[23], at close intervals (such as 0.5 h). To facilitate comparison of our data with that obtained from cells continuously incubated with ACTH or dbcAMP[12,14,20], we expressed our data as cumulative steroid production (figs 3 and 4). The decreasing rate of steroid production by stimulated cells incubated in frequently changed NSC medium contrasts with the linear accumulation of steroids observed with stimulated cells incubated in unchanged SC medium. Although long term culture of cells in NSC medium depletes the cells of serum-derived growth factors[25] and cholesterol-containing lipoproteins[12], it is not clear what the effects of short term culture are in NSC medium. Steroids appear to bind to serum proteins attached to culture dishes and interfere with steroid assays[18], it was, therefore, not convenient to use SC medium during the relatively short term incubations of this study. Because of the NSC medium we used, it is difficult to compare the nonlinear cumulative steroid production of our study with the linear production obtained by others[12,14,20]. However, studies using enucleated, but functioning, Y-1 cells, SC medium and frequent medium changes also report a decreasing rate of steroid production[23]. The latter studies[23] suggest that the presence

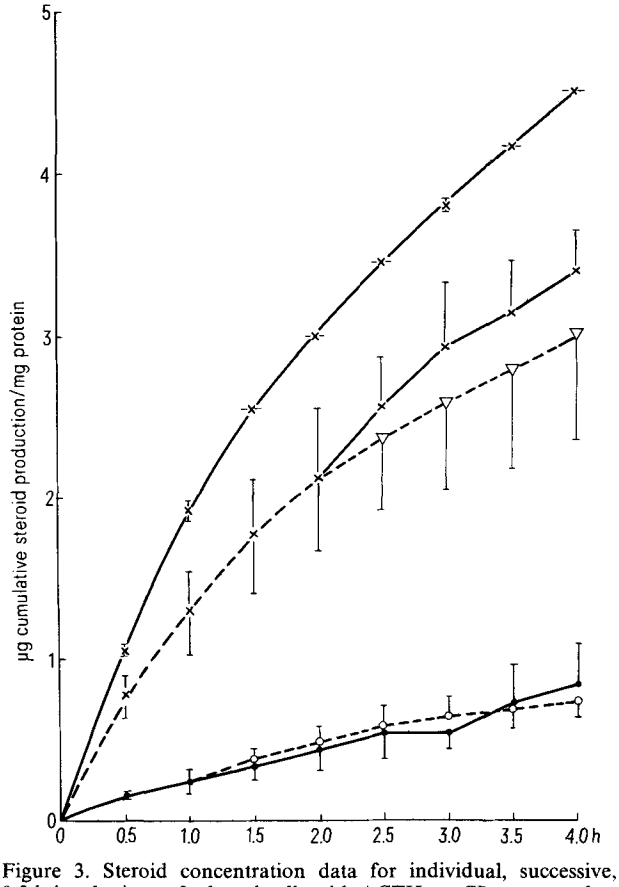

Figure 3. Steroid concentration data for individual, successive, 0.5 h incubations of adrenal cells with ACTH, or CB, expressed as cumulative steroid production. In 3 different sets of plates fresh control medium (●——●), medium containing ACTH (×—×) or CB-containing medium (○---○) was exchanged for incubated medium every 0.5 h. 2 sets of plates were initially incubated with ACTH plus CB-containing medium (△——△, ×---×) for 4 0.5-h periods. After this initial period, medium in 1 set of plates was replaced with medium containing ACTH alone (△——△), ACTH plus CB-containing medium (×---×) was not replaced on the 2nd set of plates. The incubation of both sets of plates was continued for 4 additional 0.5-h periods. The amount of steroid produced in response to a particular treatment during a 0.5-h incubation was added to the total produced in response to the treatment during preceding 0.5 h incubations. 2 experiments with 2 replicates per treatment were performed; values shown are mean ± SE.

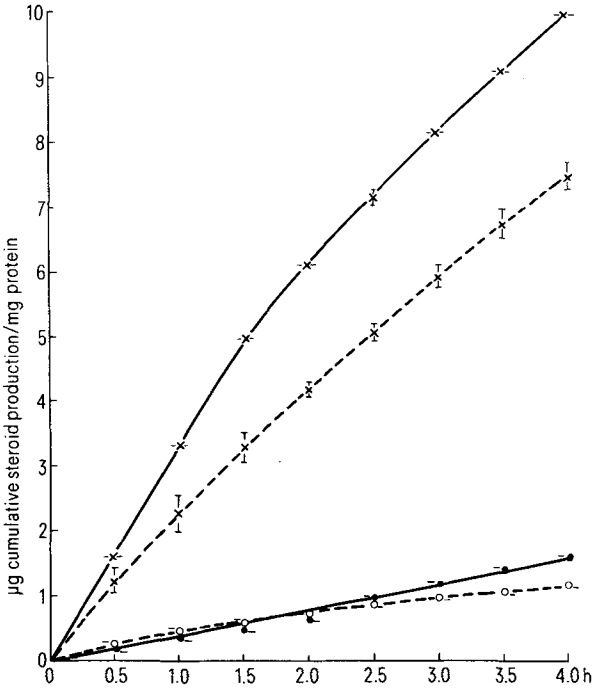

Figure 4. Steroid concentration data for individual, successive, 0.5 h incubations of adrenal cells with dbcAMP, or CB, expressed as cumulative steroid production. Fresh control medium (●——●) or media containing dbcAMP (×—×), dbcAMP plus CB (×---×), or CB (○---○) were exchanged for incubated medium every 0.5 h. The amount of steroid produced in response to a particular treatment during a 0.5-h incubation was added to the total produced in response to the treatment during preceding 0.5 h incubations. 2 experiments with 2 replicates per treatment were performed; values shown are mean ± SE.

or absence of serum proteins may not explain the nonlinear steroid production we observed during short term incubations.

The incubation of cells with fresh solutions of ACTH or dbcAMP in NSC medium may minimize the exposure of the cells to accumulating metabolic products, including acids, proteins, and steroids. The necessity for this precaution was indicated by the observations that ACTH-stimulated steroidogenesis diminished upon exposure of isolated rat and bovine adrenal cells to physiological concentrations of exogenous corticosterone or cortisol[26]. It seems unlikely that the nonlinear steroid production in our study resulted from an effect of secreted steroids on the response of the cells to stimulation. Because stimulated cells incubated for long periods in SC medium[12,20] may be exposed to increasing concentrations of steroids[26], further studies are required in order to determine an optimum experimental design that maximizes linear steroid production in response to continuous stimulation, but minimizes exposure to accumulating metabolic products.

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Effect of 3,5,3′-tri-iodothyronine on cellular growth and oxygen consumption in neonatal rat brain

Rema R. Rajan and S.S. Katyare

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay-400085 (India), 18 September 1981

Summary. The effect of thyroid deficiency and treatment with tri-iodothyronine (T_3) on oxygen consumption by neonatal (6-day-old and 15-day-old) rat brain was examined using glutamate, β-hydroxybutyrate, succinate and ascorbate + TMPD as substrates. The respiration rates decreased significantly in 15-day-old hypothyroid pups. Treatment of normal and of hypothyroid pups with T_3 resulted in a significant increase in the respiration rate at both ages. Respiration rates with glucose as the substrate were not affected under these conditions.

The role of thyroid hormones in regulation of cell formation and maturation during the postnatal development of the brain in rats is well recognized[1-3]. Their role in the regulation of oxygen consumption in this tissue, however, is not clearly understood. Barker[4] reported that while oxygen consumption in tissues such as liver, diaphragm, kidney, heart, salivary gland and pancreas was significantly stimulated by treatment with thyroid hormones, brain, spleen and testis did not respond to this treatment in the adult rat. Reiss et al.[5], in their subsequent studies, were able to show that treatment with thyroid hormones could effectively bring about a stimulation of oxygen consumption in neonatal rat brain. In more recent studies, however, Schwartz and Oppenheimer[6] were unable to confirm these findings and concluded that oxygen consumption in the brain is not responsive to thyroid hormone treatment even in the neonatal rats.

Since the reports on thyroid hormone effect on oxygen consumption in the brain seem to be contradictory, we decided to re-examine this problem by studying the effect of tri-iodothyronine (T_3) on neonatal rats on the 3rd and 11th days after birth. Control and hypothyroid pups were used for these studies and substrates such as glutamate, β-hydroxybutyrate, succinate and ascorbate + TMPD, which are directly metabolized by the electron transport system were used in addition to glucose, which was the only respiratory substrate tested by Reiss et al.[5] and Schwartz and Oppenheimer[6]. These studies have clearly shown that in normal as well as hypothyroid neonatal rats, treatment with T_3 significantly enhances oxygen consumption rates; oxygen consumption with glucose as a substrate, however, was not influenced under these conditions indicating that possibly glucose may not be an adequate substrate for such studies.

Materials and methods. Adult female rats (175 g b.wt) were mated and the morning that spermatozoa were detected in the vaginal tract was considered as day 1 of pregnancy. The control animals had access to food and water ad libitum. In order to produce hypothyroidism, the pregnant females were placed on a diet containing 0.3% propylthiouracil