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Induction of tyrosine aminotransferase in isolated liver cells

Isolated liver cells are an attractive system for studying liver functions *in vitro* provided they exhibit normal cellular functions. Previous studies have shown that tyrosine aminotransferase (L-tyrosine–2-oxoglutarate aminotransferase, EC 2.6.1.5) was not induced by cortisol in isolated rat-liver cells and the lack of enzyme induction was attributed to the fact that such cells had damaged cell membranes. Recently, HOWARD *et al.*[2] have prepared isolated liver cells by incubating rat livers with a mixture of collagenase (EC 3.4.4.19) and hyaluronidase (EC 3.2.1.35 and 3.2.1.36). Such cells appear to have normal intact cellular membranes and have a high endogenous respiration rate[3]. Indeed, BURTON *et al.*[4] have shown that cells prepared by this method incorporate [^{14}C]leucine into the fatty acid synthetase complex. RAPPAPORT AND HOWZE[5,6] have prepared cell suspensions from mouse liver using sodium tetraphenylboron, a potassium chelating agent, as the dispersing agent. Recently, GERSCHENSON AND CASANELLO[7] have prepared isolated rat-liver cells by this method and have shown that such cells respond to insulin and glucagon. These data show that cells prepared by either method exhibit normal liver function. The purpose of this report is to demonstrate that isolated rat-liver cells prepared by either method are able to induce tyrosine aminotransferase in the presence of dexamethasone phosphate, a synthetic glucocorticoid. Previous studies by THOMPSON *et al.*[8] have shown that minimum deviation hepatoma cells may be induced by dexamethasone phosphate to form tyrosine aminotransferase.

Liver cells were prepared from rats obtained from the Holtzman Co. (Madison, Wisc.). Two procedures were used for the preparation of the liver cells. The first procedure was essentially the one described by HOWARD *et al.*[2,3] where the liver from 100–200-g rats was dispersed with a freshly prepared solution of 0.05% collagenase (Schwarz) and 0.10% hyaluronidase (Sigma) in Ca^{2+}-free Hanks salt solution. About 50% of the cells did not take up the vital stains, eosin Y and trypan blue, which is less than that reported by HOWARD *et al.*[2,3]. These cells had a good endogenous O_2 uptake which was linear for at least 2 h.

In the second procedure, sodium tetraphenylboron (Sigma) was used to disperse cells from the livers of 7–14-day-old rats according to the procedure described by GERSCHENSON AND CASANELLO[7] except that a Ca^{2+}- and glucose-free Hanks salt solution was used as the washing and incubation solution. In general, this procedure provided a better yield of isolated liver cells, and these cells had less tendency to reaggregate in solution than the cells prepared by the enzymatic method. However,

the tetraphenylboron method was not very effective in preparing cells from the larger rats (100–200 g) and did not aid in preparing more cells when added to the enzymatic solution. The liver cells prepared by the tetraphenylboron procedure took up the vital stains, whereas about 50% of the cells prepared by the enzymatic method did not take up the vital stain. This would suggest that the cells prepared by the tetraphenylboron procedure had a more permeable membrane.

The isolated liver cells were incubated at 37° with slow shaking in an atmosphere of O_2–CO_2 (95:5, v/v). Dexamethasone phosphate (Dex), a synthetic glucocorticoid was used in the hormonal induction experiments and was a gift from Dr. W. B. Gall of the Merck, Sharp and Dohme Research Laboratories. Liver cells were disrupted in a Sorvall Omnimixer (microattachment, 0°) at 10 000 rev./min, and the broken suspensions were centrifuged at 5000 × *g* and the supernatant solution was used for the enzymatic assays. Tyrosine aminotransferase was assayed according to the method described by LIN AND KNOX[9]. Cycloheximide was from Sigma Chemical Co. and actinomycin D was from Mann Biochemicals.

The data presented in Fig. 1 show that tyrosine aminotransferase is induced by 10 μM dexamethasone phosphate in the isolated liver cells prepared by the enzymatic method and that the extent of induction is less in the presence of actinomycin D and cycloheximide. In this experiment the inhibitors were added 1.5 h after the initial addition of Dex. Other experiments have shown similar patterns when the inhibitors are added at the same time as Dex. In 8 experiments there was a 2.4–7.5-fold induction (av. 4.4) of tyrosine aminotransferase which occurred between 3.5 and 7.0 h (av. 4.7). These results are similar to those obtained with whole animals[9] and hepatoma cells[7]. Both cycloheximide and actinomycin D inhibited the hormonal induction though at the concentration used the inhibition is not complete and indeed the results are somewhat variable. In 5 experiments the inhibition of cycloheximide ranged from 16 to 76% (av. 55) and that by actinomycin ranged from 22 to 55% (av. 32).

Fig. 1. Induction of tyrosine aminotransferase in isolated rat-liver cells prepared by the enzymatic method and the inhibition of induction by cycloheximide and actinomycin D. Total units represent the amount of enzyme present in cells prepared from 2 g of liver. The cells were prepared and incubated as described in the text. Each experimental point represents the cells from 0.1 g of liver suspended in 1 ml of incubation media. ○— — —○, control; ●—●, 10 μM dexamethasone phosphate added at zero time; □—□, 0.2 mM cycloheximide added after 1.5-h incubation with 10 μM Dex; ■—■, 0.5 μg actinomycin D per ml added after 1.5-h incubation with Dex.

Fig. 2. Induction of tyrosine aminotransferase in isolated rat-liver cells prepared by the tetraphenylboron method and the inhibition of induction by cycloheximide and actinomycin D. Total units represent the amount of enzyme present in cells prepared from 2 g of liver and each experimental point represents cells from 0.05 g liver suspended in 1 ml of incubation media (text). The other conditions and legends are identical to Fig. 1 except that the inhibitors were added at the same time as Dex.

The variability observed in the extent of induction and degree of inhibition may reflect differences in the preparation and population of the cells. Further experiments are necessary to clarify the effects of cycloheximide and actinomycin D.

The experiments presented in Fig. 2 are similar to those in Fig. 1 except that the liver cells were isolated by the tetraphenylboron technique. In this case, actinomycin D and cycloheximide were added at zero time, and both inhibitors were effective in preventing induction of tyrosine aminotransferase. Other experiments (9, from 7–18-day-old rats) have shown that the -fold induction varied from 1.3 to 4.6 (av. 3.0) in the presence of Dex. Also, the time to reach maximum induction was usually between 2-5 h (av. 3.0) which is somewhat earlier than the time observed with cells prepared by the enzymatic method. The experiment presented in Fig. 2 (tetraphenylboron) shows a larger induction time than the normal 3 h. The presence or absence of 10% calf serum had no apparent influence on the extent of induction and Medium 199 was as effective as Ca^{2+}- and glucose-free Hanks salt solution as the incubating medium.

These studies demonstrated that isolated liver cells may be induced to form tyrosine aminotransferase in the presence of dexamethasone phosphate, a synthetic glucocorticoid. The response in the liver cells isolated by the enzymatic cells is very similar to that observed with isolated hepatoma cells where it has been shown that the induction is due to enzyme–protein synthesis[10].

It would appear that the cells produced by the enzymatic method are somewhat superior to those obtained by the tetraphenylboron method, since the extent of induction was usually higher though enzyme induction occurs in both. The observation that cells prepared by the tetraphenylboron method take up vital stains would suggest that their membranes were different than the membranes of the cells prepared by the enzymatic method where the major portion of the cells did not take up the stains. Also, the protein inhibitors were more effective in the tetraphenylboron cells which may reflect differences in the cellular membranes.

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