Studies on Responsiveness of Hepatoma Cells to Catecholamines
III. Difference between the Receptor-Adenylate Cyclase Regulating Systems in AH130 Cells and Cultured Normal Rat Liver Cells

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Abstract—The responsiveness to three β-adrenergic agonists, isoproterenol (IPN), epinephrine (Epi) and norepinephrine (NE) in AH130 cells was examined compared with that in normal rat liver cells which were cultured for 24 hr after collagenase digestion. As regards to the activation of adenylate cyclase in the cell homogenates, the relative affinity of the three agonists was in order of IPN > NE > Epi in AH130 cells and IPN > Epi > NE in cultured normal liver cells. While the efficacies of the three agonists were similar in cultured liver cells, those of NE and Epi were markedly lower than that of IPN in AH130 cells and were increased to the similar level of IPN by pretreatment with phentolamine, but not with prazosin. Clonidine inhibited the activation of adenylate cyclase by IPN in AH130 cells. When cells were preincubated with islet-activating protein (IAP), the activity of adenylate cyclase in the presence or absence of agonist in both cell lines increased. In IAP-treated AH130 cells, the efficacies of NE and Epi became close to that of IPN. Adenylate cyclase in IAP-treated AH130 cells was activated by GTP in a dose-dependent manner, but that in IAP-treated cultured liver cells was not. In the presence of IPN, biphasic (activatory and inhibitory) effects of GTP on the cyclase were observed, and the inhibitory phase was eliminated by the IAP-treatment in both cell lines. From these results, it is suggested that β-adrenoreceptors in AH130 cells have similar properties to β1-receptors and that adenylate cyclase was restricted by inhibitory guanine nucleotide regulatory protein which closely interacts with an inhibitory receptor such as the α2-adrenoreceptor in AH130 cells and interacts with a stimulatory receptor such as the β2-adrenoreceptor in cultured normal rat liver cells.

Because catecholamines and hormones exert their physiological activities on the cells through increases of intracellular cyclic AMP contents that are involved in the control of cell growth (1), it is of interest to study the responsiveness of the tumor cells to catecholamines in comparison with that of normal cells. It has been reported that the responsiveness to catecholamines or glucagon in ascites hepatoma cells was different from that in normal rat liver cells (2). We have previously reported that in the comparative studies on β-adrenergic responsiveness of rat ascites hepatoma cells and cultured normal rat liver cells, rat ascites hepatoma cells including AH130 cells possessed receptors with some different properties from those in normal rat liver cells and had some similar properties to mammalian β1-receptors (3, 4).

It is known that adenylate cyclase is regulated by two types of guanine nucleotide regulatory protein, one mediating activation and another mediating inhibition, and that the former links with a stimulatory receptor such as the β1-adrenoreceptor and the later links with an inhibitory receptor such as the α2-adrenoreceptor. The present paper deals with the difference between the adenylate
cyclase regulatory systems in rat ascites hepatoma AH130 cells and in cultured normal rat liver cells.

Materials and Methods

Drugs used: I-Isoproterenol hydrochloride (IPN), I-epinephrine bitartrate (Epi), I-norepinephrine hydrochloride (NE), clonidine hydrochloride (Sigma Chemicals), phenotolamine mesylate (Ciba Geigy) and guanosine triphosphate (GTP, P-L Biochemicals) were commercially purchased. Prazosin was kindly donated by Taito Pfizer, Tokyo, Japan. Islet-activating protein (IAP) was kindly given by Dr. Michio Ui of the Faculty of Pharmaceutical Sciences, Hokkaido University.

These drugs except IAP were dissolved in Tris-HCl buffer (pH 7.4) just before use. IAP, which was previously dissolved in 0.1 M potassium phosphate buffer (pH 7.0) containing 2 M urea and 1 mg/ml of bovine serum albumin, was added to the culture medium. AH130 cells and preparation of normal rat liver cells: Rat ascites hepatoma AH130 cells were maintained serially by the intraperitoneal passage at weekly intervals in female Donryu rats (weighing 100-150 g, 5 to 6 weeks-old, Shizuoka Laboratory Animal Center). AH130 cells were obtained from the abdominal cavity of the rat, washed with PBS and used for the following experiments. Normal rat liver cells were isolated from female Donryu rats (5 to 6 weeks-old) as described in the previous paper (3). The liver cells were cultured for 24 hr in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum. Hormones such as insulin and dexamethasone were not added to the culture medium.

Treatment with IAP: AH130 cells were suspended in Eagle's MEM containing 10% fetal calf serum (1×10^6 cells/ml MEM), and normal rat liver cells adhering and progressing on the plastic petri dishes were washed with Hanks' solution and changed to the fresh medium. These cells were treated with IAP for 3 hr (5, 6) at 37°C in a CO_2 incubator. As a control, IAP non-treated cells were added with 0.1 M potassium phosphate buffer (pH 7.0) containing 2 M urea and 1 mg/ml of bovine serum albumin in the same volume as the IAP solution. IAP-treated cells were washed twice with PBS. IAP-treated cells and non-treated cells were suspended in Tris-HCl buffer (pH 7.4) and then homogenized in a Teflon-glass homogenizer.

Adenylate cyclase assay: The adenylate cyclase activity of the cell homogenate in the presence or absence of adrenergic agents and GTP was determined according to the method described in the previous paper (3).

Assay of protein contents: Protein contents were determined by the method of Lowry et al. (7) with bovine serum albumin as the standard.

Results

Figure 1 shows the activation curves of adenylate cyclase in the homogenates of AH130 cells and cultured normal liver cells by varying concentrations of β-adrenergic agonists, IPN, Epi and NE. In cultured normal liver cells, the efficacies of the three β-agonists were similar. However, in AH130 cells, the efficacies of Epi and NE in AH130 cells were increased and approached to that of IPN (Table I), and the order of the relative affinities of the three agonists was just the same as that without phentolamine (Fig. 2B). Then, we carried out the following experiments using other α-adrenergic agents. Prazosin, an α1-selective antagonist, did not influence the activity of adenylate cyclase by NE in AH130 cells (Fig. 3). Clonidine, an α2-selective agonist, decreased IPN-stimulated activity of adenylate cyclase in a dose-dependent manner, and the extent was more remarkable in AH130 cells than in cultured
Fig. 1. Dose response curves of adenylate cyclase activity of I-IPN (○), I-Epi (●) and I-NE (△) in
AH130 cells (A) and cultured normal liver cells (B). Adenylate cyclase activity was assayed with 10^{-8}
M GTP in the homogenate of AH130 cells (A) and with 10^{-5} M GTP in the homogenate of cultured
normal rat liver cells (B). Each point indicates the mean of triplicate measurements in five experiments.

Concentration of agonist (M)

(A)  
100  
50  
0  
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 

AH130  
IPN  
NE  
Epi  

(B)  
100  
50  
0  
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 

Liver  
IPN  
Epi  
NE  

Fig. 2. Effect of phentolamine on the activation curves of adenylate cyclase by β-adrenergic agonists
in AH130 cells (A) and cultured normal liver cells (B). The cell homogenates were incubated with
10^{-4} M phentolamine for 10 min. Assays were done under the same condition as in Fig. 1. Each point
indicates the mean of triplicate measurements in three experiments. ○: I-IPN, ●: I-Epi, △: I-NE.

Concentration of agonist (M)

(A)  
100  
50  
0  
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 

AH130  
IPN  
NE  
Epi  

(B)  
100  
50  
0  
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 

Liver  
IPN  
Epi  
NE
Table 1. Activity of adenylate cyclase in the homogenates of AH130 cells and cultured normal rat liver cells

| Treatment            | AH130 cells (cAMP pmol/min/mg protein) | Liver cells (cAMP pmol/min/mg protein) |
|----------------------|----------------------------------------|----------------------------------------|
| Non-treated          | 11.62 (1.0)                            | 3.99 (1.0)                             |
| IPN                  | 49.32 (4.2)                            | 19.20 (4.8)                            |
| Phentolamine+IPN     | 53.71 (4.6)                            | 19.34 (4.8)                            |
| iAP                  | 30.27 (2.6)                            | 4.86 (1.2)                             |
| IAP+IPN              | 78.83 (6.8)                            | 57.92 (14.5)                           |

Values are the mean of triplicate measurements in 3 to 5 experiments. *Ratio of adenylate cyclase activity against non-treated activity. **Homogenates were preincubated with phentolamine (10^{-4} M) for 10 min before the addition of IPN (10^{-5} M). * Cells were pretreated with IAP (1 μg/ml) for 3 hr.

2A). Therefore, the difference between the efficacies of IPN and Epi or NE may be based on the function of Ni protein in AH130 cells. Effects of GTP on adenylate cyclase activity in IAP-treated cells were compared with those in non-treated cells in the presence or absence of IPN (Fig. 7). In the absence of IPN, adenylate cyclase in IAP-treated AH130 cells was activated by GTP (10^{-5} M) at a level about 3-fold that of IAP-non-treated cells, but IAP-treated cultured liver cells slightly responded to GTP (less than 1.5-fold) (Fig. 7 and Table 1). In the presence of IPN, biphasic (activatory and inhibitory) effects of GTP on adenylate cyclase were observed in AH130 cells and cultured normal liver cells. The inhibitory phase of GTP action was eliminated by pretreatment with IAP in both cell lines. In cultured liver cells, the treatment of cells with IAP evoked a marked potentiation of GTP activation of adenylate cyclase by IPN compared with the case in AH130 cells. Table 1 summarizes the activities of adenylate cyclase after several treatments in the presence of 10^{-5} M GTP. Phentolamine hardly changed the IPN-stimulated adenylate cyclase activity in AH130 cells and cultured liver cells. However, IAP enhanced the adenylate cyclase activity in the presence of IPN from 4.2-fold to 6.8-fold of the non-treated activity in AH130 cells and from 4.8-fold to 14.5-fold in cultured liver cells, while the toxin activated the non-treated activity 2.6-fold in AH130 cells, but caused only a small extent of activation in cultured liver cells.
Fig. 4. Effect of clonidine on IPN-stimulated adenylate cyclase activity in AH130 cells (A) and cultured normal rat liver cells (B). The cell homogenates were preincubated with clonidine for 10 min. The enzyme activities in AH130 cells and cultured normal liver cells were assayed with 10^{-6} M and 10^{-5} M of GTP, respectively, in the absence (○) or presence of 10^{-6} M IPN (●) or 10^{-5} M NE (▲). Each point indicates the mean of triplicate measurements in three experiments.

Fig. 5. Effect of IAP on NE-stimulated adenylate cyclase activity in AH130 cells (A) and cultured normal rat liver cells (B). Cells were preincubated with IAP at the indicated concentration for 3 hr. The cell homogenate was incubated with 10^{-6} M GTP in the absence (○) or presence (●) of NE. Concentration of NE was 10^{-6} M in AH130 cells and 10^{-4} M in cultured normal liver cells. Each point represents the mean of triplicate measurements.
Fig. 6. Activation curves of adenylate cyclase by 1-IPN (○), 1-Epi (●) and 1-NE (△) after preincubation with IAP in AH130 cells. Cells were incubated with IAP (1 μg/ml) for 3 hr. Adenylate cyclase activities in the cell homogenate were assayed with 10⁻⁶ M GTP. Each point indicates the mean of triplicate measurements in two experiments.

Discussion

We have previously reported differences between the β-adrenergic responsiveness of adenylate cyclases in cultured normal rat liver cells and rat ascites hepatoma cells; and these studies suggested that AH13 cells had a disorder in the interaction of β-adrenoceptors with adenylate cyclase (3), and AH130 cells possessed β₁-like receptors (4).

In this paper, the affinity of three adrenergic agonists (IPN, Epi and NE) was in the order of IPN > Epi > NE in cultured normal liver cells (Figs. 1B and 2B) and in the order of IPN > NE > Epi in AH130 cells (Figs. 1A, 2A and 6). Two major classes of adrenergic receptors, designated as α and β, mediated the action of catecholamines on target tissues. These receptors are subdivided into α₁-, α₂-, β₁- and β₂-subtypes according to pharmacological and radioligand binding studies. It is now accepted that the rank order of affinities for β₁-receptor is Epi = NE or Epi > NE, and that for β₁-receptor is Epi < NE.

Fig. 7. Effects of GTP on IPN-stimulated adenylate cyclase activity after the preincubation with or without IAP. Cells were preincubated in the presence (△, △) or absence (○, ●) of IAP (1 μg/ml). The cell homogenate was assayed in the presence (●, △) or absence (○, △) of IPN (10⁻⁶ M). Each point represents the mean of triplicate measurements.
Therefore, the present results suggest that β-receptors in AH130 cells have properties of β₁-like receptors, while those in cultured liver cells are β₂-receptors. This suggestion agrees with our previous evidence (4).

We also noticed that both the efficacies of NE and Epi were lower than that of IPN in AH130 cells, but the efficacies of the three agonists in cultured liver cells were similar to each other. In AH130 cells, the α-antagonist phentolamine potentiated the efficacies of NE and Epi to that of IPN (Fig. 2A), but the α₁-selective antagonist prazosin had no effect on the efficacies or potencies (Fig. 3). The reduction of the IPN-stimulated adenylate cyclase activity in AH130 cells by the α₂-selective antagonist clonidine was greater than that in cultured liver cells. IAP, which causes ADP-ribosylation of N protein and results in a loss of the function of N protein (8, 9), also increased the efficacies of NE and Epi to the similar level of IPN in AH130 cells (Fig. 6). These results may indicate that α₂-adrenergic receptors which interact with N protein greatly take part in the adenylate cyclase system in AH130 cells compared with that in cultured normal liver cells.

Receptors for adenylate cyclase are classified into two types, stimulatory receptors (Rs) and inhibitory receptors (Ri). Rs, including β₁- and β₂-adrenoceptors, mainly cause the activation of adenylate cyclase via Ns protein, whereas Ri, including α₂-adrenoceptors, mediate the inhibitory action against the enzyme via N protein (8). Recently, it has been shown that the stimulation of β-adrenergic receptors can activate not only Ns protein but also N protein: β-agonists increased the binding rate of a GTP analogue, GTP γS, to Ni protein and potentiated the GTPase activity of N protein (10). Biphasic effects of GTP on adenylate cyclase were observed when homogenates of AH130 cells and cultured normal liver cells were incubated with increasing concentrations of GTP in the presence of IPN. However, by pretreatment with IAP, the inhibitory phase caused by higher concentrations of GTP was eliminated in both cell lines (Fig. 7) as reported in rat adipocytes by Murayama and Ui (11). The enhancement by IAP of adenylate cyclase stimulated by IPN (10⁻⁵ M) and GTP (10⁻⁵ M) was more remarkable in cultured normal liver cells (from 4.8- to 14.5-fold) than in AH130 cells (from 4.2- to 6.8-fold) (Table 1). These results indicate that the stimulation of Rs by IPN evokes the function of N protein, and the interaction may work more efficiently in cultured liver cells than in AH130 cells.

On the other hand, IAP markedly increased the activity of adenylate cyclase of AH130 cells in the presence of high concentrations of GTP (10⁻⁶–10⁻⁴ M), while it hardly did so in cultured liver cells (Fig. 7, Table 1). In mouse 3T3 fibroblast (12, 13) and rat C6 glioma (5), the treatment of cells with IAP evoked potentiation of GTP activation of the membrane cyclase without significant potentiation of the superimposed effect of IPN, and this was similar to AH130 cells. In rat adipocytes (11), IAP did not enhance the activation of adenylate cyclase in the presence of GTP, but strikingly enhanced the stimulated activity by IPN, and this was similar to cultured normal liver cells.

Thus, the present study also indicates by using IAP that N proteins of AH130 cells may possess a different property or function from cultured normal rat liver cells. It has been reported that hydrolysis of GTP to GDP on N proteins occurred very slowly; this indicates that the activity of GTPase in the adenylate cyclase system is very small in normal metabolic systems. The difference of the function of N protein between AH130 cells and cultured normal rat liver cells may be based on their own GTPase activities.

The present study confirmed that AH130 cells had β₁-like receptors, and it suggested that α₂-adrenergic receptors interacting with N protein were present in the adenylate cyclase system of AH130 cells than in that of cultured normal rat liver cells. Further investigations on the determination of subtypes of adrenoceptors and on the adenylate cyclase system in both cell lines are in progress.

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