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Characteristic β-adrenergic receptors in a rat ascites hepatoma cell line (AH130)

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The β-adrenergic receptors are functionally coupled to adenylate cyclase and subdivided into β_1- and β_2-subtypes according to their different affinities for several adrenergic ligands. In the normal mammalian liver, it has been reported that the functional adrenergic receptor is the β_2-subtype [1, 2]. Uncontrolled tumor cell growth may result from some malignant transformation accompanied by changes in the hormonal membrane receptors and the cAMP production system [3, 4].

Lacombe *et al.* [5] had suggested that there is a switchover of adrenergic receptors from the normal β_2-type to β_1-type in Zajdela hepatoma, which was derived from rat liver treated with dimethylaminoazobenzene, judging from the change in the relative potency of various β-agonists in adenylate cyclase activation. We have studied the adrenergic responsiveness of AH130 cells, an ordinary cell line among a series of rat ascites hepatoma cell lines that were induced in the liver by dimethylaminoazobenzene treatment and established as transplantable tumors [6], and indicated that the cells hardly respond to β_2-adrenergic agonists in adenylate cyclase activation [7]. We have also reported that AH130 cells have many β-adrenergic receptors and α-adrenergic receptors dominated by α_2-like receptors [8]. In this paper, we identified the qualitative and quantitative differences of β-adrenergic receptors in AH130 cells from those in normal rat hepatocytes.

Materials and methods

Cells. AH130 cells were maintained serially by intraperitoneal passage at weekly intervals in female Donryu rats (5–7 weeks old, Shizuoka Laboratory Animal Center, Hamamatsu, Japan) and used 7 days after the cell inoculation. Normal rat hepatocytes were isolated from female Donryu rats by collagenase digestion *in situ* by the method of Berry and Friend [9].

Adenylate cyclase assay. It has been reported for liver and hepatoma cells that adrenergic responsiveness in the plasma membrane was lower than that in the cell homogenate [10, 11]. Therefore, the cell homogenates were used in this assay. A reaction mixture contained 2 mM ATP, 8 mM phosphoenolpyruvate, 20 μg/ml pyruvate kinase, 10 mM theophylline, 5 mM $MgCl_2$, 1 mM EDTA, 10 μM GTP, and various concentrations of a β-adrenergic agent, *l*-isoproterenol (IPN), *l*-epinephrine (Epi), *l*-norepinephrine (NE, Sigma Chemicals Co., St Louis, MO), *dl*-salbutamol (Sankyo Co., Tokyo, Japan), or *dl*-turobuterol (Hokuriku Seiyaku Co., Katsuyama, Japan), in the absence or presence of 100 μM *dl*-phentolamine (Ciba-Geigy Co., Summit, NJ) in 30 mM Tris–HCl buffer (pH 7.4); this was incubated for 5 min at 37°. After the incubation, the reaction was started by the addition of cell homogenate and continued for 10 min at 37°. The cAMP formed was measured by radioimmunoassay using a cAMP assay kit (Yamasa Shoyu, Choshi, Japan). K_a values for β-adrenergic agents were determined as the concentration necessary for half-maximum activation of adenylate cyclase. Relative efficacies were calculated from the maximum activation by each agent compared to IPN.

Binding assay. Membranes (50–150 μg protein/tube) of AH130 cells and hepatocytes prepared by Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) sedimentation [12] were incubated with various concentrations of [^{125}I]iodocyanopindolol ([^{125}I]ICYP, 2000 Ci/mmol, Amersham, U.K.) in the absence or presence of 10 μM *dl*-propranolol (Sigma) in a total volume of 0.25 ml of 42 mM Tris–HCl buffer (pH 7.5) containing 15 mM $MgCl_2$, for 45 min at 37°. The reaction was then stopped by the addition of 3 ml of Tris buffer and immediate vacuum filtration of the samples on a GF/C filter (Whatman, Maidstone, U.K.). Non-specific binding at the K_d value of ICYP in the presence of propranolol was about 8.1% and 1.6% of the total binding in hepatocytes and AH130 cells, respectively. To measure the potency of *dl*-propranolol, *dl*-metroprolol (Ciba-Geigy), and *dl*-salbutamol in inhibiting ICYP binding, samples were incubated with various concentrations of each agent as described above. The concentration of [^{125}I]ICYP was 50 pM for normal rat hepatocytes or 100 pM for AH130 cells, which was close to the K_d of ICYP for each cell membrane (61.2 pM for hepatocytes and 121.6 pM for AH130 cells [8]). The K_d values for [^{125}I]ICYP binding were calculated by Scatchard analysis. K_i values for the inhibition of ICYP binding by

Table 1. Effects of adrenergic agents on adenylate cyclase activity in homogenates of normal rat hepatocytes and AH130 cells

Agent	Hepatocytes		AH130 cells	
	K_a (μM)	Efficacy (IPN = 1.00)	K_a (μM)	Efficacy (IPN = 1.00)
Isoproterenol	0.44 ± 0.05	1.00	0.078 ± 0.004	1.00
Epinephrine	1.26 ± 0.06	1.03 ± 0.04	4.64 ± 0.16	0.51 ± 0.11
Norepinephrine	11.0 ± 0.3	0.92 ± 0.02	1.05 ± 0.04	0.56 ± 0.03
Salbutamol	3.32 ± 0.35	0.60 ± 0.02	ND	ND
Turobuterol	5.46 ± 0.72	0.43 ± 0.03	ND	ND
In the presence of 100 μM phentolamine				
Isoproterenol	0.17 ± 0.01	1.00	0.023 ± 0.003	1.00
Epinephrine	0.99 ± 0.07	0.99 ± 0.01	0.65 ± 0.02	1.06 ± 0.06
Norepinephrine	3.42 ± 0.19	0.97 ± 0.04	0.21 ± 0.01	1.01 ± 0.01

The results were the mean ± SE of at least three separate experiments done in triplicate. ND: not detectable.

Table 2. Dissociation constants (K_i) of adrenergic agents for [^{125}I]ICYP binding in the membranes of normal rat hepatocytes and AH130 cells

Agent	K_i (μM)	
	Hepatocytes	AH130 cells
Propranolol	0.007 ± 0.001	0.066 ± 0.011
Metoprolol	3.28 ± 0.30	2.02 ± 0.61
Salbutamol	1.93 ± 0.12	146 ± 14
	56.1 ± 1.1	

The results were the mean ± SE of at least five separate experiments done in duplicate.

agents were calculated by the method of Cheng and Prusoff [13].

Protein contents were measured by the method of Lowry *et al.* [14].

Results and discussion

As shown in Table 1, the responsiveness of adenylate cyclase in the homogenate of AH130 cells to β-adrenergic agents was quite different from those of normal rat hepatocytes. While the relative efficacies of IPN, EPi, and NE were similar in normal rat hepatocytes, in AH130 cells the efficacies of Epi and NE were significantly lower than that of IPN. Efficacies of Epi and NE in AH130 cells were increased by treatment with the α-antagonist phentolamine. This suggests that the inhibitory α_2-adrenergic receptors are associated with the adenylate cyclase system in these hepatoma cells. Increased potencies of these β-agonists in both cell types were also observed in the presence of phentolamine. The altered interaction between the inhibitory α_2-receptors and the guanine nucleotide binding regulatory proteins after blockade by the antagonist may influence the affinity of these agonists for the stimulatory β-receptors. We have described the switch from dominance of α_1- to α_2-adrenergic receptors in the AH130 cell membrane [8]. Moreover, we observed here the unresponsiveness to β_2-selective agonists and the higher potency of NE than that of Epi in AH130 cells. The adenylate cyclase of normal rat hepatocytes responded to β_2-agonists and was activated by IPN, Epi, and NE, in the order of potency. It is now accepted that the rank order of potency for the β_1-receptor is IPN > NE ≥ Epi, and that for the β_2-receptor is IPN > Epi > NE [15, 16]. These results suggest that the β-adrenergic receptor of AH130 cells has β_1-type property similar to that of Zajdela hepatoma cells as described by Lacombe *et al.* [5].

To further analyse the subtype of β-adrenergic receptors as [^{125}I]ICYP binding sites in each membrane competition experiments were conducted. The inhibition of specific ICYP binding by propranolol and metoprolol in these membranes resulted in linear Hofstee plots with Hill coefficients near 1.0 and gave a K_i value for each antagonist. The inhibition by salbutamol in normal rat hepatocytes made a curvilinear Hofstee plot, which was shifted to a linear plot with low affinity displacement by addition of GTP (data not shown), but in AH130 cell membranes, this resulted in a linear Hofstee plot regardless of GTP. This indicates that salbutamol is an agonist at the ICYP binding sites in hepatocytes; in contrast it is an antagonist, probably β_1-type, in AH130 cells, because salbutamol is a β_2-partial agonist. The dissociation constants (K_i) of β-adrenergic agents are summarized in Table 2. The K_i value of the β_1-selective agent metoprolol for AH130 cells was lower than that for normal rat hepatocytes; in contrast the K_i value of salbutamol for AH130 cells was much higher than both high and low K_i values of the agent for hepatocytes. Although propranolol is a non-selective β-antagonist, the K_i value for AH130 cells was about one order of magnitude larger than that for normal rat hepatocytes. The results presented in this paper indicate that the receptor of normal rat hepatocytes shows the properties of the β_2-subtype as reported by other investigators [1], but that of AH130 cells has properties distinct from that of hepatocytes and similar to the β_1-subtype. Our previous paper showed the lack of α_1-receptors and the dominance of α_2-like receptors in AH130 cells [8]. The replacement of the population of the adrenergic receptor-subtype may be associated with the neoplastic change in the hepatocellular membrane.

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