Binding Characteristics of $^3$H-Dihydroalprenolol to $\beta$-Adrenergic Receptors of Rat Brain: Influence of Lectins

Hiroshi TSUCHIHASHI and Takafumi NAGATOMO
Department of Pharmacology, Niigata College of Pharmacy,
5829 Kamishinei-cho, Niigata 950-21, Japan
Accepted February 14, 1985

Abstract—The significance of the carbohydrate moieties of the $\beta$-adrenergic receptor molecule in the rat brain was examined using the radioligand binding assay method. Thus, this experiment was designed to assess the effects of lectins, concanavalin A (Con A), Phaseolus vulgaris agglutinin (PHA), and wheat germ agglutinin (WGA) on the affinity of the $\beta$-adrenoceptor. The rat brain was used and the $\beta$-adrenoceptor binding assay was carried out using $^3$H-dihydroalprenolol as a ligand. Con A and PHA significantly caused an increase in the values of the density of $\beta$-adrenoceptor ($B_{max}$) and a reduction in the values of the dissociation constant ($K_d$), but significant changes were not observed with WGA. These results strongly suggest that the carbohydrate moieties of the cell surface containing the $\beta$-adrenoceptor molecule may have a crucial role in the drug-receptor interaction, and they imply that the $\beta$-adrenoceptor molecule is a glycoprotein which contains N-linked carbohydrate chains.

In general, the carbohydrate moieties on the cell surface of the mammalian tissues play a crucial role in the various cellular functions, cell-to-cell recognition, cell aggregation, and drug-receptor interaction, etc. (1). Our previous report (2) demonstrated that the treatment of the rat heart membrane with neuraminidase resulted in good reproducibility of binding data and caused an increase in the binding sites for the $\beta$-adrenergic receptors using $^3$H-dihydroalprenolol as a ligand. In addition, we also showed by the binding assay method that the cationic and anionic charges on the cell surface of the rat brain were important for the drug-receptor interaction (3). Furthermore, it was suggested that the $\beta$-adrenoceptor molecules from hamster lung and rat erythrocytes were glycoproteins which contain complex type and/or high mannose type N-linked carbohydrate chains (4). Thus, the object of this study is to examine the significance of carbohydrate moieties by assessing the effects of lectins on the affinity of $\beta$-adrenoceptors, because lectins can obviously interact with the carbohydrates in the cell membrane.

Materials and Methods

Materials: $^3$H-dihydroalprenolol ($^3$H-DHA) (104.8 Ci/m mole) was purchased from New England Nuclear Corp. Albumin from bovine serum (BSA) was purchased from Sigma Chem. Co. Wheat germ agglutinin (WGA) and concanavalin A (Con A) were purchased from Miles Laboratories, Inc. Phytohemagglutinin type $\beta$ from Phaseolus vulgaris (PHA) was purchased from Serva Feinbiochemica GmbH & Co.

Hemagglutinability assay: The formalinization of the sheep erythrocytes was performed by the method of Butler (5). The agglutination assay was carried out by the following procedures. To each well of the Microtiter V-plate, 25 $\mu$l of lectin, 25 $\mu$l of 0.15 M NaCl, and 25 $\mu$l of 3.3% (v/v) cell suspension of formalinized erythrocytes were added, and then the plate was shaken vigorously for 10 min and left to stand for 90 min before the agglutination was evaluated. The end point of the agglutination was taken as the highest dilution of the lectin which
agglutinates erythrocytes. The specific agglutination activity was defined as the agglutination activity per mg of protein. The specific agglutination activities of Con A, PHA, and WGA were 320, 256 and 3200 units/mg protein, respectively.

The preparation of the membrane-enriched fraction: The membrane enriched fraction from the rat brain was prepared using the procedure described previously (3). Male Wistar rats weighing between 300–350 g were killed by a blow on the head. After removal of the brain, the cerebellum and cerebral cortex were minced with small scissors in 10 mM Tris-HCl containing 250 mM sucrose (pH 7.6) and were then homogenized using a glass homogenizer. The homogenate was filtered through 4 layers of gauze. The filtrate was centrifuged at 40,000 g for 30 min, and the resultant pellets were rinsed at one time and homogenized with a glass homogenizer using 20 ml of 75 mM Tris-HCl, 25 mM MgCl₂, pH 7.2. The membrane-enriched fraction prepared was stored at 4°C and used within 1 hr.

Binding assay: The β-adrenoceptor binding assay was carried out in duplicate with ³H-DHA in the presence (non-specific) and absence (total) of 100 nM dl-propranolol. For ³H-DHA binding, 0.25 ml of membrane suspension (about 0.25 mg) was incubated with shaking for 30 min at 23°C with 1.2 nM of ³H-DHA and different concentrations of various lectins in a total volume of 0.5 ml containing 60 mM Tris-HCl, 20 mM MgCl₂ (pH 7.2). The Scatchard analyses were carried out in duplicate with various concentrations of ³H-DHA in the presence and absence of 10 units of lectins per ml. At the end of the incubation period, the incubation medium was immediately filtered through a GF/C glass fiber filter using an improved method (3), which was continuously filtered, washed, and air-dried. The filter was added to 5 ml of Tt76 scintillation fluid. The difference in mean values between total and non-specific binding was taken as the specific binding. All binding assays were performed within 3 hr from the removal of the brain from rat. Protein was determined by the method of Lowry et al. (6). Significant differences were analyzed using Student's t-test.

Results

The data presented in Figs. 1–3 show the effect of increasing concentrations, which are shown by the agglutination activities (units) plotted as the abscissa of Con A (Fig. 1), PHA (Fig. 2) and WGA (Fig. 3) on the number of β-adrenoceptor binding sites. The binding of ³H-DHA to brain membrane was increased 20–40% and 60–100% when the membranes were incubated with over 20 units of PHA and Con A per mg of membrane protein, respectively, whereas, the addition of up to 800 µg of BSA per mg of membrane

![Fig. 1. Effects of Con A on β-adrenoceptor binding. The data represent the mean±S.E. of duplicate determinations from four separate experiments. Total (——), specific (—○—) and non-specific (—▲—) binding. *P<0.05, **P<0.02 or ***P<0.01 vs. without Con A.](image1)

![Fig. 2. Effects of PHA on β-adrenoceptor binding. The data represent the mean±S.E. of duplicate determinations from four separate experiments. Total (——), specific (—○—) and non-specific (—▲—) binding. *P<0.05 or **P<0.02 vs. control.](image2)
The protein did not have any effect (Fig. 4).

Table 1 shows the results of the influence of the addition of the lectins to the β-adrenoceptor obtained by Scatchard analysis.

PHA and Con A significantly caused the increase (44.5 and 54.6%, respectively) in the density of β-adrenoceptor (B_m) and the decrease (34.7 and 46.2%, respectively) in the values of the dissociation constant (K_d). The values of the Hill coefficient with or without lectins were equal to unity. These results suggest that in this β-adrenoceptor binding, with or without lectins, there is neither negative or positive cooperativity nor multiple binding sites.

As it is well known, the cell surface of the mammalian tissues contain carbohydrate moieties in glycolipids, glycoproteins, and glycosaminoglycans. The lectins may bind to these carbohydrate moieties and manifest the agglutinability. Con A specifically binds to mannose-containing carbohydrates in which more than one mannose residue has C-3, C-4 and C-6 unsubstituted (7). Especially, Con A interacts with serum-type glycoproteins which contain high mannose type N-linked carbohydrate chains (8, 9).
PHA binds to carbohydrate moieties containing C-3 and C-4 hydroxyl groups with the galactose configuration (10), and it specifically binds to complex-type N-linked carbohydrate chains of glycoproteins (8, 9, 11). WGA binds to carbohydrate moieties containing N-acetyl glucosamine (12) and sialic acid (13) and interacts with N-linked and/or O-linked carbohydrate chains (8, 12, 13). Stiles and coworkers suggested that β-adrenoceptor molecules were glycoproteins which contained complex and/or high mannose type N-linked carbohydrate chains (4). These authors also showed that the mammalian β-adrenoceptor molecules from hamster lung and rat erythrocytes were deglycosylated by the treatment of endoglycosidase F, which was capable of removing both complex and high mannose type N-linked carbohydrate chains, and agarose-conjugated Con A or WGA was bound to these β-adrenoceptors (4). In this study, PHA and Con A significantly caused an increase in the value of $B_{\text{max}}$ and a reduction in the value of $K_d$. These facts imply that the β-adrenoceptor molecule is a glycoprotein which contains at least high mannose type and/or complex type N-linked carbohydrate chains, and they strongly suggest that the N-linked carbohydrate chains bound to PHA and Con A in the cell surface containing β-adrenoceptor molecules play a crucial role in the drug-receptor interaction.

Stiles and coworkers also suggested that WGA-conjugated agarose bound to β-adrenoceptors from rat erythrocytes and hamster lung (4). In the present study, the addition of WGA caused no changes in the values of $B_{\text{max}}$ and $K_d$. These results imply that O-linked carbohydrate chains are independent of the drug-receptor interaction if the β-adrenoceptor molecule contains the carbohydrate chains and that the N-linked carbohydrate chains which are capable of binding to WGA are also independent of the drug-receptor interaction, thus suggesting that the PHA- and Con A-binding moieties of the carbohydrate chains may be N-linked hybrid-type chains.

Lectins also have been shown to increase the membrane fluidity (14, 15) and receptor half-life (16). We have not yet studied the relationship between the increase in the density of β-adrenoceptors and these phenomena. However, there are increasing evidences to indicate that lectins inhibit the densitization of β-adrenergic receptors in frog erythrocytes (17), nicotinic receptors of rat adrenal medulla (18) and glutamate receptors of locust skeletal muscle (19). Chuang et al. reported that Con A could virtually restore the number of membrane-bound β-adrenoceptors to the control value in isoproterenol-treated cells, thus indicating that the effect of Con A was mediated through binding to cell surface glycoproteins (17). These authors, therefore, suggested that Con A failed to inhibit the internalization of receptors recognition sites during densitization of β-adrenoceptor. Furthermore, it was suggested that the β-adrenoceptor molecules were glycoproteins which contain N-linked carbohydrate chains (4) as described above. The results reported here, therefore, may suggest that lectins can bind to N-linked carbohydrate chains in the β-adrenoceptors of rat brain and that β-adrenoceptor-bound lectins resulted in the changes in the conformation and in the affinity of drugs to β-adrenoceptors.

Acknowledgement: This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References
1 Sharon, N.: Complex Carbohydrates. Addison-Wesley Publishing Co. Inc., London (1975)
2 Nagatomo, T. and Sasaki, M.: Effects of neuraminidase and deoxyribonuclease on the β-adrenergic receptors in rat heart. Japan. J. Pharmacol. 33, 481-484 (1983)
3 Tsuchihashi, H. and Nagatomo, T.: Influence of polymeric effectors on the β-adrenergic receptor binding of rat brain. Japan. J. Pharmacol. Supp. 36, 102P (1984)
4 Stiles, G.L., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J.: Mammalian β-adrenergic receptors. Distinct glycoprotein populations containing high mannose or complex type carbohydrate chains. J. Biol. Chem. 259, 8655-8663 (1984)
5 Butler, W.T.: Hemagglutination studies with formalinized erythrocytes. Effects of bis-diazobenzidine and tannic acid treatment on sensitization by soluble antigen. J. Immunol. 90,
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
7 Goldstein, I.J., Hollerman, C.E. and Smith, E.E.: Protein-carbohydrate interaction. II. Inhibition studies on the interaction on concanavalin A with polysaccharides. Biochemistry 4, 876–883 (1965)
8 Fukuda, M. and Osawa, T.: Isolation and characterization of a glycoprotein from human group O erythrocyte membrane. J. Biol. Chem. 248, 5100–5105 (1973)
9 Irimura, T., Kawaguchi, T., Terao, T. and Osawa, T.: Carbohydrate-binding specificity of the so-called galactose-specific phytohemagglutinins. Carbohydr. Res. 39, 317–327 (1975)
10 Ohtani, K., Shibata, S. and Misaki, A.: Purification and characterization of Tora-bean (Phaseolus vulgaris) lectins. J. Biochem. 87, 407–416 (1980)
11 Kornfeld, R. and Kornfeld, S.: Structure of a phytohemagglutinin receptor site from human erythrocytes. J. Biol. Chem. 245, 2536–2545 (1970)
12 Allen, A.K., Neuberger, A. and Sharon, N.: Purification, composition, and specificity of wheat-germ agglutinin. Biochem. J. 131, 155–162 (1973)
13 Goldstein, I.J., Hammurstrom, S. and Sundbald, G.: Precipitation and carbohydrate-binding specificity studies on wheat germ agglutinin. Biochim. Biophys. Acta 405, 53–61 (1975)
14 Beppu, M., Terao, T. and Osawa, T.: Covalently cross-linked monovalent, divalent, and trivalent derivatives of concanavalin A. J. Biochem. 85, 1275–1287 (1979)
15 Ito, Y., Yoshimoto, R., Irimura, T., Setaka, M., Shimizu, H. and Osawa, T.: Concanavalin A-induced increase in the membrane fluidity of chicken erythrocytes. J. Biochem. 86, 1807–1815 (1979)
16 Prives, J., Hoffman, L., Tarrab-Hazdai, R., Fuchs, S. and Amsterdam, A.: Ligand induced changes in stability and distribution of acetylcholine receptors on surface membranes of muscle cells. Life Sci. 24, 1713–1718 (1979)
17 Chung, D.-M., Kinner, W.J., Farber, L. and Costa, E.: A biochemical study of receptor internalization during β-adrenergic receptor desensitization in frog erythrocytes. Mol. Pharmacol. 18, 348–355 (1980)
18 Kirpekar, S.M. and Prat, J.C.: Blockade of desensitization of nicotinic receptors of the cat adrenal medulla by concanavalin A. Br. J. Pharmacol. 62, 549–552 (1978)
19 Mathers, D.A. and Usherwood, P.N.R.: Concanavalin A blocks desensitization of glutamate receptors on insect muscle fibres. Nature 259, 409–411 (1976)