DEVELOPMENTAL CHANGE OF BETA-ADRENERGIC RECEPTORS IN RAT BRAIN

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Accepted November 24, 1979

Abstract—The developmental change of beta-adrenergic receptors in rat whole brain, except the cerebellum, was studied by binding assays with \((-\)\) 3H-dihydroalprenolol. The synaptic membrane fraction, prepared from 2-day-old and 6-week-old rat brains, had binding sites for \((-\)\) 3H-dihydroalprenolol, which seemed to represent physiological beta-adrenoceptors. The maximal binding capacity and the dissociation constant for \((-\)\) 3H-dihydroalprenolol of the synaptic membrane fraction did not vary with the age of the rats, but the yield of synaptic membrane fraction from the brain of 2-day-old rats was much less. Therefore, the total number of binding sites was less in 2-day-old rats. In addition, there was a significant difference in the inhibitory effects of 1-isoproterenol on binding of \((-\)\) 3H-dihydroalprenolol to the synaptic membrane fractions in the 2-day as compared to the tissues from the 6-week-old animals. The developmental changes in the number and nature of beta-adrenoceptors may result in expression of catecholamine-sensitive adenylate cyclase activity.

There have been several reports on developmental changes of the catecholaminergic system in the brain and developmental changes in the response of adenylate cyclase to catecholamine have also been reported (1-4). No significant increase in cAMP accumulation in response to catecholamines was observed during the first few days after birth and the response was seen to develop between 9 and 13 days postnatally. Moreover, the basal activity of adenylate cyclase was found in rat brain from the time of birth (2). We studied the beta-adrenoceptors in the brains of 2-day-old and 6-week-old animals to obtain information on development of the catecholamine response.

MATERIALS AND METHODS

Synaptic membrane fraction (SMF) was prepared from the whole brain, except the cerebellum of Sprague-Dawley rats, by a modification of the method of Whittaker et al. (5). The basic reaction mixture consisted of 50 mM Tris-HCl buffer (pH 7.4), 100 mM KCl and 50-150 \(\mu\)g synaptic membrane protein. The mixture was preincubated for 15 min at 37°C and then the reaction was started by adding \((-\)\) 3H-dihydroalprenolol (DHA) (50.6 Ci/m mole, New England Nuclear). Test agents were added to the reaction mixture before preincubation. After incubation at 37°C for 5 min, the mixture was rapidly cooled to 0°C, filtered through 0.45 \(\mu\)m Millipore filter HA type and the filter was washed with 25 ml of ice-cold incubation buffer. The bound \((-\)\) 3H-DHA remaining on the filter was
counted in a liquid scintillation counter. Specific binding was defined as the difference between the total counts on the filter in the absence and presence of $10^{-4}$ M unlabeled (−)-alprenolol. Protein was determined by the method of Lowry et al. (6).

RESULTS

The specific binding was linearly related to protein concentration in the range of 50 to 150 µg, in all assay systems. The binding reached equilibrium at 3 min, at which time the addition of unlabelled alprenolol ($10^{-4}$ M) resulted in a rapid dissociation of bound (−)$^3$H-DHA with a half time of about 30 sec. The displacement of (−)$^3$H-DHA (1 nM) by non-radioactive various agents was then studied. The order of potency of the displacement was 1-isoproterenol>1-adrenaline=1-noradrenaline. Dopamine, tyramine and serotonin were inactive at concentrations up to $100 \mu$M. The specific binding of (−)$^3$H-DHA to the SMF from rat brains was saturable. Thus, specific binding of (−)$^3$H-DHA to the SMF from rat brains was rapid, reversible, of high affinity and saturable. As in various other tissues (7-10), the binding sites of (−)$^3$H-DHA in the brain may be physiological beta-adrenoceptors. A Scatchard plot obtained from the saturation curve is shown in Fig. 1. The values of $K_n$ of $^3$H-DHA for the binding sites in the 2-day-old and 6-week-old rat SMF were $3.6 \times 10^{-8}$ M and $3.4 \times 10^{-8}$ M, respectively. This indicates there was no significant difference in the binding affinity of the sites for $^3$H(−)DHA. The means±s.e. for four experiments of the binding sites of the 2-day-old and 6-week-old rat SMF were 1.4±0.2 and 1.2±0.05 pmole per mg protein, respectively. Thus, the number of binding sites for (−)$^3$H-DHA in the two SMF preparations were similar, but the yield of SMF from brains of 2-day-old animals was much less than that from the 6-week-old rats. Therefore, the density of binding sites in the brain of 2-day-old animals was less than that of 6-week-old animals (Table 1). The ratio of the density of the binding sites in the brain of 2-day-old rats to that of 6-week-old rats is smaller than the ratio observed by Harden et al. (1). This discrepancy may be the result of different preparations; Harden et al. studied the P₂ fraction of cerebral

![Scatchard plot for (−)$^3$H-DHA binding with neonatal and adult rat synaptic membrane fraction. Results are means from four separate experiments; s.e. all <15%.](image-url)
cortex (1), whereas we studied SMF of whole brain except the cerebellum (11). Accordingly, the discrepancy may be due to a different recovery of SMF at the process of purification from P2 fractions between brains from 2-day-old and 6-week-old rats and due to a different time course of the synaptogenesis between cerebral cortex and subcortical areas. The KD value observed here was slightly higher than the value reported by Alexander et al. (7). Such may also be due to a difference between the preparations; Alexander et al. used a P2 fraction from the cerebral cortex. In addition, there is the possibility that the other components, except SMF, have an effect on the binding affinity of the sites for 3H(-)DHA in the rat brain.

The inhibitions of 0.8 nM (-)3H-DHA binding by agonist and antagonist were also tested. The values of IC50 for dl-propranolol in brains from 2-day-old and 6-week-old animals were similar, being approximately 1.0×10⁻⁸ M and 0.7×10⁻⁸ M, respectively. However, 1-isoproterenol inhibited the (-)3H-DHA binding to a greater extent in 6-week-old rat SMF than in the 2-day-old rat SMF, the IC50 values for 1-isoproterenol being 1.1×10⁻⁶ M and 3.0×10⁻⁵ M, respectively (Fig. 2). This difference may indicate a developmental change in the affinity of 1-isoproterenol to beta-adrenoceptors, but direct binding studies using radioactive beta-adrenergic agonists are necessary to determine the interaction of beta-adrenoceptors with beta-adrenergic agonists.

### DISCUSSION

In studies on rat cerebral cortex, Harden et al. (1), reported that the affinity of 1-isoproterenol for beta-adrenoceptors was similar in animals of 10 to 100 days of age.
In 2-day-old rats, there was significant basal and NaF-stimulated adenylate cyclase activity in homogenates of cerebral cortex, but neither norepinephrine nor a combination of norepinephrine and adenosine produced increases in the cyclic AMP level in slices of the cerebral cortex. Between 9 and 13 days after birth, norepinephrine elevating the cyclic AMP level, and the accumulation of cyclic AMP by 1-isoproterenol in slices of cerebral cortex of 13-day-old rats was blocked by propranolol, but not by phentolamine (2). It was suggested that beta-adrenoceptors are not yet efficiently coupled with adenylate cyclase in the brain of 2-day-old rats. This difference between our findings and those of Harden et al. (1) on developmental change in the inhibitory effect of agonist on antagonists binding may be due to the difference in age of the rats examined, and this may indicate a change in the state of the receptors during development. A possible explanation of the change is that beta-adrenoceptors and adenylate cyclase are not coupled efficiently in rat brain for several days after the birth.

The coupling of beta-adrenoceptors and adenylate cyclase has been studied in S49 lymphoma cells (12). In wide type S49 cells, 1-isoproterenol increased the level of cyclic AMP, while in variant cells, adrenergic agonists did not stimulate the enzyme activity, although the variant cells contained both the enzyme and receptors. These findings suggest that coupling between receptors and enzyme was inefficient in these cells. 1-Isoproterenol inhibited 125I-iodehydroxybenzylpindolol (IHYP) binding to the cell membranes of wild type S49 cells at a significantly lower concentration in the absence of guanosine triphosphate (GTP). However, there was no difference in the affinities of 125I-IHYP binding of the two preparations.

Sulfhydryl reagents such as N-ethylmaleimide (NEM) block receptor desensitization (13) and seem to lower the efficiency of coupling of beta-adrenoceptors to the enzyme (5). In frog erythrocyte membranes (13), 5 mM NEM caused a reduction in the affinity of beta-adrenergic agonist for beta-receptors, but had no observable effect on (—)3H-DHA binding. Similar results on the effect of NEM were obtained in studies on S49 lymphoma cells (14). Thus, it seems likely that the affinity of beta-adrenergic agonist for beta-adrenoceptor is lower in uncoupled systems; i.e. S49 lymphoma cells variant type and immature brains, than in efficiently coupled systems. We speculate from this work that developmental changes in the number and nature of beta-adrenoceptors result in change in the expression of catecholamine sensitive adenylate cyclase activity in rat brain during development.

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

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