Specific Binding of [3 H]WB 4101, [3 H]Clonidine and [3 H]Dihydroalprenolol in Cerebral Cortical Membranes in Developing, Adult and Old Rats

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Several studies have demonstrated age-related changes of the content, uptake and release of noradrenaline (NA) in the brain of neonatal, developing and senescent rats (1–7). Presynaptic functions shown by such parameters develop well at the perinatal stage, followed by the increase with age and then decrease in senescent animals. With regard to the changes of adrenoceptors with age, the ontogeny of α_1 - and α_2 - (8, 9), β_1 - and β_2 -receptors (10) have been documented in the rat brain at the early postnatal stage. Little has been demonstrated concerning the comparison of age-related changes in α_1 -, α_2 - and β -receptors in the cerebral cortex. In addition to the studies on ontogenetic changes of binding of [3 H]WB 4101, [3 H]-clonidine and [3 H]dihydroalprenolol ([3 H]-DHA) to cerebral cortical membranes, we examined the binding of these ligands in 1-year- and 2-year-old animals as well. Ontogeny and aging in the interrelationship between presynaptic functions and adrenoceptors were also discussed.

Wistar rats of both sexes were used. After birth pups were maintained with their mother at 23°C under normal daylight conditions and weaned at 22 days. Crude mitochondrial P₂ fractions from the cerebral cortex of rats were dispersed with a Polytron® (setting No. 6) for 30 sec and centrifuged at 48,000×g for 20 min. Cold distilled water was added to the pellet and the suspensions were homogenized for 30 sec with a Polytron® (setting No 6). After centrifuging at 8,000×g for 20 min, the supernatant obtained was centrifuged at 48,000×g for 20 min. Resulting pellets were suspended in 50 mM Tris-HCl

buffer (pH 7.7). Experiments on binding of [3 H]WB 4101 and [3 H]clonidine were carried out by the methods of U'Prichard and Snyder (11). Reaction mixtures which were composed of 0.8 ml of membrane suspension (0.3–0.4 mg protein), 0.1 ml of displacer (100 μ M NA for [3 H]WB 4101 binding or 1 μ M clonidine for [3 H]clonidine binding) or buffer and 0.1 ml of a labeled ligand (0.05–1.5 nM [3 H]WB 4101 or 0.05–4.0 nM [3 H]clonidine) were incubated at 25°C for 30 min. For the [3 H]DHA binding assay, 0.8 ml of membrane suspension (0.3–0.4 mg protein), 0.1 ml of propranolol (final conc., 100 μ M) and 0.1 ml of [3 H]DHA (0.4–3.5 nM) was incubated at 23°C for 20 min according to the method of Bylund and Snyder (12). Bound radiolabeled ligands were separated by filtration on a Whatman GF/B filter, and the radioactivity was determined by a Packard Tri-Carb scintillation spectrometer. Specific binding of [3 H]WB 4101, [3 H]clonidine and [3 H]DHA was defined as the excess over blanks containing 100 μ M NA, 1 μ M clonidine and 100 μ M propranolol, respectively. The values of saturation constants were determined by Scatchard analysis (13). Proteins were determined by the methods of Lowry et al. (14). Statistical differences between two values at different stages were analyzed by Student's *t*-test.

Chemicals were obtained from the following sources: [3 H]WB 4101 (19.7 Ci/mmol, Amersham), [4- 3 H]clonidine hydrochloride (23.8 Ci/mmol, New England Nuclear), levo[propyl-1,2,3- 3 H]dihydroalprenolol hydrochloride (44.9 Ci/mmol, New

England Nuclear), NA hydrochloride (Nakarai Chemicals Co., Ltd.), clonidine hydrochloride (C. H. Boehringer Zohn), and propranolol hydrochloride (Sumitomo Chemical Co., Ltd.).

The changes of the B_{max} values of specific binding of [3H]WB 4101, [3H]clonidine and [3H]DHA were examined in cerebral cortical membranes of rats aged from 1 day to 2 years and are shown in Fig. 1. Specific bindings of [3H]WB 4101 and [3H]clonidine were detected on day 1, followed by a rapid increase until day 16 ([3H]clonidine binding) and day 20 ([3H]WB 4101 binding). The B_{max} value of [3H]WB 4101 binding in 1-year-old rats was significantly ($P < 0.05$) lower than the value at 30 days. In [3H]clonidine binding, the B_{max} at 70 days, 1 year and 2 years were also significantly low compared to that at 30 days. Although [3H]DHA binding was not detected on day 1, relatively low B_{max} values of [3H]DHA binding were observed on days 7 and 15, increasing rapidly between days 15 and 30. A subsequent decrease in [3H]DHA binding

was not found during aging. The K_D values ranged from 0.27 to 0.67 nM for [3H]WB 4101 binding, 0.27 to 1.49 nM for [3H]clonidine binding and 0.89 to 1.96 nM for [3H]DHA binding through aging.

The results presented here suggest that maturation of α -adrenoceptors is earlier than that of β -receptors in the cerebral cortex. It is interesting that a peak in the number of these three types of adrenoceptors occurs at around 30 days. It has been also reported that the number of [3H]spiperone binding sites reach a peak at 30 days in the rat striatum (15). Since the protein content continues to increase until day 45, followed by a plateau at the adult stage (2), developmental changes in adrenoceptor density can not be explained by those in the protein content in the brain of developing rats. The cerebral NA content gradually increases during development (Fig. 1 quoted from our previous findings (3)). The increase in the NA content as well as in the V_{max} values of [3H]NA uptake into synaptosomes until day 70 (3) suggest gradual development of

Fig. 1. Age-related changes of the B_{max} values of specific binding of [3H]WB 4101, [3H]clonidine and [3H]DHA in the cerebral cortical membranes of rats. The values were estimated by Scatchard analysis (13) for which the binding was carried out under the following concentration ranges of labeled ligands: 0.05–1.5 nM for [3H]WB 4101 binding, 0.05–4.0 nM for [3H]clonidine binding and 0.4–3.5 nM for [3H]DHA binding. Each point shows the mean $\pm$ S.E. of 3–4 independent experiments which were performed in triplicate, and the value adjacent to each point shows the K_D . * $P < 0.05$ and ** $P < 0.01$ vs. 30 days. The dotted line shows changes of NA content, which is quoted from our previous paper (3).

NA neurons at least until day 70. A difference in a developmental pattern between the presynaptic markers and adrenoceptor density indicates independent development of receptors from presynaptic functions. In another words, NA may not act on adrenoceptors as a trophic factor. The NA content in the hypothalamus and brain stem of senescent rats were reduced compared to that in 3-month-old rats (4), suggesting functional reduction of NA terminals during aging. The decrease in the number of adrenoceptors seems to be due to the loss of NA terminals. However, this is not the case since α_2 -receptors predominantly exist in post-synaptic sites in the cerebral cortex (16).

In conclusion, it is suggested that adrenoceptor, number reaches a peak around 30 days and that adrenoceptors develop independently from presynaptic functions. Moreover, a decrease in α -receptor number in contrast to non-significant changes in β -receptor number occurs during aging in rat cerebral cortex.

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