Age Related Changes in the Inhibitory Effect of Clonidine on High K+-Evoked Noradrenaline Release from Brain Slices of Rats

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The effects of clonidine on a high K+-induced release of noradrenaline (NA) were studied in 70-d- and 2-year-old rats. Treatment with 0.1 and 1.0 μM clonidine significantly (p < 0.01) inhibited 20 mm KCl-evoked L-[3H]NA release from cerebral cortical slices in 70-d-old rats. The amounts of high K+-evoked L-[3H]NA release were markedly decreased at 2 years, compared to that at 70 d. In addition, the inhibitory effects of clonidine on high K+-evoked L-[3H]NA release were no longer observed in 2-year-old animals. These results suggested that presynaptic functions, including the modulation of NA release by α2-adrenoceptors, could become low in the brains of the 2-year-old rats.

Keywords — noradrenaline release; presynaptic α2-adrenoceptor; clonidine; aging

Introduction

The aging of central monoamine containing neurons including noradrenaline (NA), has been discussed from aspects of age-related changes in the monoamine content, biosynthetic and degradation enzymes and receptor binding.1,2) [3H]Dopamine accumulation and release from striatal slices do not seem to differ in young, mature and senescent rats.3) Several pharmacological and neurochemical findings regarding developmental changes in the locomotor effect of clonidine,4) specific [3H]clonidine binding to brain membranes5) and the inhibitory effect of clonidine on a depolarization-evoked NA release from brain slices6) have suggested age-related changes in functions of α2-adrenoceptors in rat brain. Although the density and affinity of [3H]clonidine binding do not change in rats between day 70 and year 2, guanosine triphosphate (GTP) binding and/or coupling activity of inhibitory GTP-binding proteins to adenylate cyclase decrease in cerebral cortical membranes during aging.5) However, it has never been investigated whether autoreceptor function of central α2-adrenoceptors changes with age. The present study was designed to clarify whether the functions of presynaptic α2-adrenoceptors modulating NA release change in the cerebral cortex of rats during aging.

Materials and Methods

Male Wistar rats, both 70-d-old and 2-year-old, were used. The animals were maintained at 23 °C under a 12 h light/12 h dark cycle. The animals were decapitated, the whole brains were removed and the cerebral cortexes were dissected over ice. Slices (300 × 300 × 300 μm, 20 mg wet weight) preloaded with L-[3H]NA6,7) were suspended in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.2, 126.5 mM NaCl, 2.4 mM KCl, 0.83 mM MgCl2, 1.1 mM CaCl2, 0.5 mM Na2SO4, 2.75 mM NaHCO3, 0.5 mM KH2PO4 and 5.9 mM glucose) and bubbled with 95% O2-5% CO2. Slices were then incubated for 10 min at 37 °C to release endogenous amines. The slices were washed twice with Krebs-Ringer solution containing 10 μM ni-alamide, a monoamine oxidase inhibitor, and 20 μl of 1 μM L-[3H]NA (final concentration, 20 nM) and the mixtures were incubated at 37 °C for 15 min. Release experiments were carried out by the superfusion method previously described.6,7) The test drugs were perfused for 10 min. The
release of L-[3H]NA evoked by each drug was estimated by subtracting the estimated amount of spontaneous release from the amount of total radioactive amine release during the perfusion of the drug. In the experiment for examining the effects of clonidine, clonidine was perfused 5 min before and during the perfusion of KCl. The amount of the total uptake of L-[3H]NA is referred to as the sum of the total release and the residual on the filter. The statistical significance of differences between control and test values was analyzed using the Student’s t-test.

The drugs used were: Levo-[ring-2,5,6-3H]noradrenaline (48.4 Ci/mmol) obtained from New England Nuclear and clonidine purchased from C.H. Boehringer Sohn.

Results and Discussion

The amount of L-[3H]NA uptake into the slices was significantly (p < 0.01) reduced at 2 years compared to that at 70 d (Table I), suggesting either a decrease of uptake capacity per nerve terminal, or a loss of functional nerve terminals with increasing age. A high K+ concentration (20 mM KCl) caused a significant enhancement in L-[3H]NA release (Table I) in a Ca^{2+}-dependent manner (data not shown), indicating that depolarization-evoked NA release is from nerve terminals. At 2 years, the amount of high K+ enhanced release was markedly reduced, being approximately one third of the release in 70-d-old animals (Table I). The reduction did not seem to be due to a decrease in the amount of uptake, since the degree of reduction in the K+ evoked release was much more than that in the uptake during aging. The NA nerve terminals probably become insensitive to depolarization at the senescent stage, although details of the effect of aging on the mechanism of excitation-secretion coupling are not obvious. The finding that spontaneous NA release was significantly high at 2 years compared to that at 70 d may show the mechanical fragility of senescent synaptosomes during the present experiments.

As shown in Table II, the treatment with clonidine at concentrations of both 0.1 and 1.0 µM resulted in a significant (p < 0.01) reduction in the 20 mM KCl-enhanced release at 70 d. α2-

### Table I. The Active Uptake and Release of L-[3H]NA in Cerebral Cortical Slices of Adult and Senescent Rats

<table>
<thead>
<tr>
<th>Age</th>
<th>L-[3H]NA uptake (fmol/mg wet weight/20 min)</th>
<th>L-[3H]NA release (% of total uptake)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spontaneous release</td>
</tr>
<tr>
<td>70-d-old</td>
<td>21.5±1.4 (14)</td>
<td>3.52±0.24 (17)</td>
</tr>
<tr>
<td>2-year-old</td>
<td>16.0±1.0 b) (10)</td>
<td>4.96±0.11 a)</td>
</tr>
</tbody>
</table>

The amount of the uptake was calculated as the sum of the radioactivity of the total release and of residual in slices. The amount of the release was calculated as the sum of release/10 min. The number of experiments is shown in parenthesis. Significance, a) p < 0.05 and b) p < 0.01 vs. adult.

### Table II. Effects of Clonidine on a 20 mM K+ Induced Release of L-[3H]NA from Cerebral Cortical Slices in Adult and Senescent Rats

<table>
<thead>
<tr>
<th>KCl (mM)</th>
<th>Clonidine (µM)</th>
<th>Enhanced L-[3H]NA release (% of total uptake)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>70-d-old</td>
</tr>
<tr>
<td>20</td>
<td>—</td>
<td>18.29±1.18 (10)</td>
</tr>
<tr>
<td>20</td>
<td>0.1</td>
<td>9.22±1.71 b) (6)</td>
</tr>
<tr>
<td>20</td>
<td>1.0</td>
<td>8.11±1.49 b) (5)</td>
</tr>
</tbody>
</table>

Release experiments were carried out by the superfusion method as described in Materials and Methods. Each drug was perfused for 10 min. Clonidine was perfused for 5 min before and during the perfusion of KCl. The number of experiments is shown in parenthesis. Each value represents the mean ± S.E.M. Significance, a) P < 0.01 vs. adult; b) p < 0.01 vs. 20 mM KCl alone.
Adrenoceptors located on presynaptic terminals probably modulate NA release as shown previously. It is of interest that clonidine at 0.1 and 1.0 μM did not exert any effects on the depolarization-evoked NA release in slices obtained from 2-year-old animals. The autoreceptor function of presynaptic α2-receptors was lost at 2 years. Since the inhibitory effects of clonidine on NA release were not observed in preparations pretreated with pertussis toxin, the presynaptic α2-receptor-inhibitory GTP-binding protein (G tyres mesenger (adenosine 3',5'-cyclic monophosphate (cyclic AMP) generating) system involved in the regulation of NA release ceases to function in the senescent brain. In fact, signal transduction between G1 and adenylate cyclase has been known to decrease with age.

From our recent findings indicating that stimulatory effects of isoproterenol, NaF and forskolin on NA release are reduced in senescent slices, it is likely that the β-adrenoceptor-stimulatory GTP-binding protein (Gβ0-adenylate cyclase system involved in the enhancement of NA release functionally reduces with age as well. It is also important to notice that oxotremorine is not able to inhibit a high K+-induced [3H]acetylcholine release from both striatal and hippocampal slices of senescent rats in contrast to the effect at the adult stage.

In conclusion, several functions of NA nerve terminals such as NA uptake capacity, NA release responsible for depolarization and regulation by presynaptic adrenoceptor-effector mechanisms underlying NA release seem to become less effective in the central nervous system with increasing age.

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References