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INFLUENCES OF SODIUM, OUABAIN AND TRICYCLIC ANTIDEPRESSANT DRUGS ON L-[³H]NOREPINEPHRINE UPTAKE INTO SYNAPTOSOMAL FRACTIONS OF DEVELOPING RAT BRAIN

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The central noradrenergic neuron has a high affinity uptake mechanism of L-[³H]norepinephrine (L-[³H]NE) which is saturable, sodium dependent and ouabain sensitive (1-3). The high affinity uptake mechanism of L-[³H]NE appears first at 18 days of gestation in the rat brain (4, 5). Although the innervation of both noradrenergic and serotonergic neurons progress in the rat brain with increasing age, no developmental difference in affinity of L-[³H]NE and [³H]5-hydroxytryptamine ([³H]5-HT) to brain synaptosomes has been demonstrated (4, 5, 6, 7). With regard to the high affinity [³H]5-HT uptake system, sodium dependence was lower in the neonatal synaptosomes than in the adult (6). On the other hand, two antidepressant drugs, desmethylinipramine (DMI) and imipramine (IM) did not show significant difference in those inhibitory potencies on [³H]5-HT uptake at any developmental stage, suggesting that [³H]5-HT uptake might be specific for 5-HT neuron also in the neonatal brain (6).

To gain insight into the sodium dependence and specificity of the high affinity L-[³H]NE transport mechanism in the neonatal brain, we investigated the influence of sodium, ouabain, DMI and IM on the L-[³H]NE uptake into synaptosomal fractions of the developing rat brain.

The uptake experiment was carried out by the method previously reported (6). Wistar rats of both sexes and at various ages were sacrificed by cervical decapitation and the brain was homogenized in 9 vol. of ice-cold 0.32 M sucrose bubbled with 95% O₂-5% CO₂ for 10 min using a Teflon pestle. The crude mitochondrial P₂ fraction prepared by the technique

of Segawa and Kuruma (8) was suspended in modified Krebs-Ringer solution (pH 7.4) or isotonic solution (pH 7.4) replaced with sucrose instead of sodium chloride in modified Krebs-Ringer solution. The normal modified Krebs-Ringer solution was composed of 118 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl_2 , 0.5 mM MgCl_2 , 25.0 mM NaHCO_3 and 11.1 mM glucose, further containing 2.0×10^{-5} M pheniprazine (a monoamine oxidase inhibitor) and 0.2% ascorbic acid. Both suspensions (0.4–0.8 mg protein/ml) bubbled with 95% O_2 –5% CO_2 for 10 min were divided into 1.6 ml units, each of which was transferred into 10 ml polyethylene tubes, and 0.2 ml of 10 mM ouabain (final concentration: 1 mM) or saline solution was added. All of these procedures were performed at 4°C. After pre-incubation for 10 min at 37°C, 0.2 ml of 0.5 $\mu\text{Ci/ml}$ L-[^3H]NE (final concentration: 4.8×10^{-8} M) was added and a further incubation was carried out at 37°C for 10 min. The S_1 fraction from the developing brain was used in the experiments examining the effects of tricyclic anti-depressants. The whole brain except the cerebellum was homogenized in 4 vol. of ice-cold 0.32 M sucrose. The homogenate was centrifuged at $900 \times g$ for 10 min. The supernatant (S_1 fraction) was diluted to 5 times with modified Krebs-Ringer solution containing pheniprazine (2×10^{-5} M) and 0.2% of ascorbic acid and then bubbled with 95% O_2 –5% CO_2 . Aliquots (1.6 ml) of this suspension were distributed into 10 ml polyethylene tubes. DMI or IM (0.2 ml) of 10^{-5} M was added to the polyethylene tubes at 4°C and the reaction mixture incubated at 37°C for 10 min. Then 0.2 ml of L-[^3H]NE (final concentration: 4.8×10^{-8} M) was added and incubation was continued for another 10 min. At the end of the incubation period, 6 ml of the cold saline were added to the reaction mixture and the preparation was centrifuged at $11,500 \times g$ for 20 min at 4°C after which the supernatant fluid was decanted. Both procedures of washing of crude synaptosomal pellets and of transferring crude synaptosomal pellets into Bray's solution were performed by the method of Nomura and Segawa (9). Radioactivity was determined by Packard Tri-Carb liquid scintillation counter. Counting efficiency was 20–25%. The thin layer chromatographical analysis showed preliminarily that above 90% was unmetabolized L-[^3H]NE in the total radioactivity in crude synaptosomal pellets. The protein was estimated by the Folin phenol reagent method of Lowry et al. (10).

The drugs used were: L-[^3H]NE (8.8 Ci/mmol, Radiochemical Centre, Amersham), DMI hydrochloride (Daiichi Pharmaceutical Co. Ltd., Tokyo), IM hydrochloride (Fujisawa Pharmaceutical Co. Ltd., Osaka) and ouabain (g-strophanthin, Merck, Germany).

Neonatal synaptosomal fractions showed the temperature dependent L-[^3H]NE uptake (Fig. 1). But the active uptake, indicated by subtracting the uptake at 4°C from the uptake at 37°C, in the neonate was lower than that in the adult (Fig. 1). In addition, ouabain (0.1 mM) inhibited the active uptake more strongly in the adult (58.4%) than in the neonate (35.0%). In both the neonate and the adult, ouabain was less effective in the lower sodium solutions. Such can be explained by the fact that the terminals do not acquire much sodium when the external sodium is low, even though the sodium pump is inhibited. Although 15 mM sodium was effective in activating the NE transport system, the effect of 133 mM sodium was significantly stronger than of 15 mM at both stages. Since a kinetic study on

the L-[³H]NE uptake into the neonatal synaptosomal fractions showed the same value of K_m as seen in adult synaptosomal fractions at lower concentrations (10^{-8} M— 5×10^{-8} M) of L-[³H]NE in suspending medium (6), it seems that neonatal synaptosomes already have a sodium dependent, high affinity L-[³H]NE uptake system.

It has been demonstrated that DMI is more effective in inhibiting NE accumulation

FIG. 1. Comparison of the effects of temperature, sodium chloride and ouabain on the L-[³H]norepinephrine uptake between at the neonate and the adult brain. The crude mitochondrial P_2 fraction prepared from the brain of a 4 day old rat and an adult animal was suspended in modified Krebs-Ringer solution. Aliquots (1.6 ml) of this solution were incubated with 0.2 ml of ouabain or saline for 10 min at 37°C. Then 0.2 ml of L-[³H]norepinephrine solution (final concentration: 4.8×10^{-8} M) was added, and incubation at 4°C or 37°C was continued for 10 min. Each column represents the mean value with S.E.M. of six to nine determinations.

FIG. 2. Developmental change in the inhibitory potencies of desmethylinipramine and imipramine on the L-[³H]norepinephrine uptake. S_1 fraction prepared from the brain from rats of various ages was diluted to five times with modified Krebs-Ringer solution (pH 7.4). Aliquots of 1.6 ml portions were incubated with 0.2 ml of desmethylinipramine or imipramine solution (final concentration of both antidepressant drugs: 10^{-6} M) for 10 min at 37°C. Then 0.2 ml of L-[³H]norepinephrine solution (final concentration: 4.8×10^{-8} M) was added, and further incubation was carried out for 10 min at 37°C. The ordinate is expressed as the percentage of the control uptake and each point represents the mean value with S.E.M. of six to nine determinations.

than either chlorimipramine or IM (11, 12, 13). Fig. 2 shows the developmental change of the inhibitory potencies of DMI and IM on L-[³H]NE uptake. There were no significant differences in the inhibitory effect of DMI at any developmental stages examined. This is the same result as that reported by Coyle and Axelrod (4). Furthermore, IM showed a tendency toward a decrease in inhibition on L-[³H]NE uptake, suggesting that the neonatal synaptosomes could accumulate L-[³H]NE as well as [³H]5-HT (6) with the same specificity as seen with adult synaptosomes.

Our findings support the idea that the L-[³H]NE transport mechanism of the central NE neuron is highly specific and sodium dependent in the neonate as well as at the adult.

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