Developmental Difference in Noradrenaline and 3-Methoxy-4-Hydroxyphenylethyleneglycol Sulfate (MHPG-SO₄) Levels in Rat whole Brain

YASUKO, KOHNO, MASATOSHI TANAKA, RYOICHI NAKAGAWA,
YOSHISHIGE IDA, KENICHIRO IIMORI AND NOBUYUKI NAGASAKI

Department of Pharmacology, Kurume University School of Medicine, Kurume, 830 Japan

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Developmental characteristics of noradrenergic neurons in animal brains have been studied on noradrenaline (NA) level, NA uptake, and enzyme activities involved in transmitter amine synthesis and degradation (Karki et al., 1962; Coyle and Axelrod, 1972; Lamprecht and Coyle, 1972; Nomura et al., 1976). Only limited biochemical information exists, however, on the functional development of noradrenergic neurons. It has been suggested that the brain level of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄), the major metabolite of endogenous NA in the rat brain (Schanberg et al., 1968 a, b), is a measure of the functional activity of central noradrenergic neurons (Sugden and Eccleston, 1971; Korf et al., 1973; Braestrup and Nielsen, 1976; Stone, 1976; Kohno et al., 1981). In order to investigate the relationship between postnatal concentrations of NA and its utilization, we examined levels of NA and MHPG-SO₄ in whole brains of rats ranging in age from birth to 17 weeks.

Male Wistar rats (ages detailed in Fig. 1) were used. The pregnant rats were kept in individual cages, and the time of birth of the pups was noted within 15 hrs. The rats within 24 hrs after birth was designated as the birth. The rats were weaned

on the 22 nd day after birth.

The animals were sacrificed by decapitation, and the whole brain (minus olfactory bulb, cerebellum, and pineal body) was rapidly dissected into two hemispheres, frozen on solid CO₂, and stored at -45°C until assayed. The levels of NA and MHPG-SO₄ in the brain were determined fluorometrically by the method of Kohno et al. (1979) using a single hemisphere from rats between the ages of 2 and 17 weeks and a whole brain from rats between the ages of birth and 1 week. The assay was performed twice using 6 samples for each age group.

The postnatal changes in body weights and wet weights of the rat brains are indicated in Fig. 1. The body weight continued to increase gradually throughout the 17 weeks, while the brain weight increased rapidly during the first 2 weeks after birth and reached almost adult weight after 5 weeks. The mature brain weight was reached 10 weeks after birth.

Fig. 2 shows the postnatal changes in NA and MHPG-SO₄ levels during the 17 weeks. NA levels increased from 122 ng/g wet weight at birth to 580 ng/g at 17 weeks of age. During the first week after birth the amine level did not increase appreciably (from 22 % at birth to only 28 % at 1

Fig. 1. Changes in body weights and brain weights in developing rats.

Body weights are shown as the mean $\pm$ S.E.M. of 12 rats (birth and 1 week of age) and of 6 rats (2-17 weeks of age). Brain weights are shown as the mean $\pm$ S.E.M. of wet weight of whole brain (minus olfactory bulb, cerebellum and pineal gland). The sample number at each age is the same for both body weight and whole brain weight determinations.

week, when indicated as percentage of the level at 17 weeks). By 3 to 5 weeks the amine level had increased from 60% to 65% and reached adult level by 10 weeks.

The MHPG-SO₄ level in the whole brain was already detectable at birth, and increased markedly from 40 ng/g to 123 ng/g during the first week. At 5 weeks it had increased to the peak value of 158 ng/g and reached adult level at 10 to 17 weeks.

The present study revealed postnatal development of MHPG-SO₄ and transmitter amine NA levels in whole rat brains. The results obtained from both hemispheres

are highly reproducible. The present finding of postnatal development of NA shows good agreement with previously reported results (Karki et al., 1962; Coyle and Axelrod, 1972; Perkins and Moore, 1973; Morris et al., 1980).

In earlier studies the brain level of MHPG-SO₄ has been measured to investigate changes in NA release in the adult rats. The developmental aspect of the metabolite has not been examined. The present study demonstrated that the MHPG-SO₄ level in whole rat brain is present even at birth and remarkable elevation of the metabolite occurs during the

Fig. 2. Postnatal development of noradrenaline (NA) and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄) levels in rat whole brain.

Each point is represented as the mean $\pm$ S. E. M. of 6 samples.

first week of life. These data support previous findings suggesting that functional development of noradrenergic neurons occurs within a few days of birth (Perkins and Moor, 1973; Kellogg and Wennerström, 1974; Nomura and Segawa, 1979; Morris et al., 1980).

Despite a gradual increase in NA, the maturation of MHPG-SO₄ level occurred approximately 3 to 4 weeks after birth, peaked at 5 weeks, and then regressed slightly to adult level. Both NA and MHPG-SO₄ levels reached a plateau at 10-17 weeks of age. These results suggest that releasable NA develops in the early postnatal period, and storage pool NA develops by 10 weeks. Synthesis and re-release of NA then approaches equilibrium by 10 to 17 weeks.

The rate of increase of MHPG-SO₄ during the first few weeks after birth was

greater than not only that of NA as indicated in this study, but also those of tyrosine hydroxylase, dopadecarboxylase, dopamine- β -hydroxylase (Coyle and Axelrod, 1972; Lamprecht and Coyle, 1972) and monoamine oxydase activities (Parvez and Parvez, 1980). Therefore, it is unlikely that brain MHPG-SO₄ of neonatal rat is derived from mainly intraneuronal degradation because of poor storage function as observed by Karki et al. (1962). Furthermore, the relation between development of NA and MHPG-SO₄ is quite similar to that of the amine and α_1 and α_2 -adrenoceptors (Morris et al., 1980). In the serotonergic system, 5-hydroxy-indoleacetic acid, the major metabolite of serotonin, was also found to reach the mature level prior to the amine (Baker and Quay, 1969).

The present preliminary study of the

whole rat brain suggests that MHPG-SO₄ level indicates NA utilization in the neonatal brain, as well as adult brain. For confirmation of this hypothesis, we are currently investigating whether MHPG-SO₄ levels in neonatal rat brain reflect changes in NA turnover caused by pharmacological and physiological treatments.

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