Locomotor Network Maturation Is Transiently Delayed in the MAOA-Deficient Mouse

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Cazalets, Jean-René, Marie Gardette, and Gérard Hilaire. Locomotor network maturation is transiently delayed in the MAOA-deficient mouse. J. Neurophysiol. 83: 2468–2470, 2000. In vivo and in vitro experiments were performed in control (C3H) and monoamine oxidase A (MAOA)-deficient (Tg8) neonatal mice to determine whether MAOA deficiency affected spinal locomotor network maturation. Comparing the swimming behaviors at birth in C3H mice with those in Tg8 mice revealed a delayed role for the hindlimbs in Tg8 swimming, even though adult swimming behavior was acquired at postnatal day 14 (P14) in both strains. Analyzing the locomotor network activity in vitro showed that serotonin (5-HT) induced and modulated locomotor-like discharges in hindlimb ventral roots of C3H but not Tg8 neonates. The Tg8 network began, however, to be affected by 5-HT at P11. Thus both in vivo and in vitro results argue for a transient delay of locomotor network maturation in the Tg8 strain.

INTRODUCTION

Serotonin (5-HT) is a widely distributed neuromodulator that has a role in numerous functions in adulthood (Vogt 1982) and that modulates rhythmic activities of the locomotor (Cazalets et al. 1992; Sqalli-Houssaini et al. 1993) and the respiratory (Hilaire and Duron 1999) networks at birth. Moreover, as suggested by its early expression and its role in the control of cell division, differentiation, growth, and synaptogenesis (Lauder 1993; Lauder and Krebs 1978; Levitt et al. 1997; Whitaker-Azmitia 1991), 5-HT may affect CNS maturation (Cases et al. 1996; Chubakov et al. 1986; Mooney et al. 1998; Yan et al. 1997a,b). Therefore, besides its role in modulating the locomotor network, 5-HT might also play a role in its maturation, as suggested by the transient difficulties in swimming abilities observed at birth in rats prenatally treated with para-chlorophenylalanine (Nakajima et al. 1998).

In the MAOA-deficient (Tg8) neonatal mouse, which was created from the C3H/HeJ (C3H) strain by disrupting the gene encoding monoamine oxidase A (MAOA), the enzyme that degrades 5-HT, the lack of MAOA activity results in 5-HT levels 10 times larger than those in C3H neonates (Cases et al. 1995; Lajard et al. 1999). To determine whether MAOA deficiency affects locomotor network maturation, we used in vivo and in vitro approaches to compare the locomotor activity produced by Tg8 neonates with that produced by C3H neonates. Both results suggest a transient delay in the maturation of the Tg8 locomotor network, which provides increasing evidence for a possible role of 5-HT in mammalian CNS maturation.

METHODS

In the in vivo experiments, the swimming behavior of 35 C3H and 24 Tg8 pups was observed daily from postnatal day 0 to postnatal day 7 (P0–P7). Each pup was gently immersed in a warmth-regulated water bath (37 ± 1°C) for a 30 s trial during which its motor activity was observed and classified in one of three swimming patterns, H0, H1, or H2, depending on whether it used 0, 1, or 2 hindlimbs for swimming (see RESULTS). The observer did not know which strain was being observed. In addition, seven Tg8 and six C3H mice from two different litters of each strain were again tested from P13 to P15. Results were analyzed with statistical software SYSTAT in multidimensional contingency tables as the occurrence of the H0, H1, and H2 swimming patterns versus the strains and the classes of age. The multidimensional contingency tables were treated as log-linear models (Fienberg 1980).

The in vitro experiments were performed as reported previously for newborn rats (Cazalets et al. 1992; Sqalli-Houssaini et al. 1993). Newborn mice (5 C3H and 5 Tg8 pups at P0–P5 and 3 Tg8 pups at P11) were decapitated under ether anesthesia and their spinal cords were dissected and cut at the level of T8. The motor output from the lumbar ventral roots was recorded, amplified, and stored. Up to three spinal cords were pooled in the same experimental chamber so that simultaneous recordings could be performed. The spinal cords were continuously superfused with oxygenated saline into which drugs could be added (Sigma Chemicals). The mean period (± SE) of ventral root rhythmic activity was estimated from 60 cycles, and differences between the means were taken as significant at P < 0.05 (unpaired t-test).

RESULTS

Rodents are immature right after birth and exhibit a very poor motor repertoire. Locomotor activity can be observed, however, when postural constraints are removed [Cazalets et al. 1990 (swimming experiments); McEwen et al. 1997 (air-stepping experiments)]. Behavioral data were collected in vivo using mouse swimming activity as a criterion of locomotor network maturation. Figure 1 shows that swimming pattern development is different in C3H and Tg8 strains. At birth (P0–P1), most of the Tg8 pups presented the H0 pattern (no rhythmic hindlimb movements); they either floated motionlessly or with smooth uncoordinated leg movements, or they presented alternated movements of the forelimbs while their hindlimbs were extended backward. In the same age class, 23% of the C3H pups already presented the H1 pattern; they showed alternated rhythmic forelimb movements while one hindlimb...
(and only one) moved in synchrony with the contralateral forelimb. The second hindlimb was maintained extended backward. In the P2–P3 age class, 75% of the Tg8 pups still presented the H0 pattern whereas 46% of the C3H pups were displaying the H1 pattern and 24% already showed the H2 pattern, wherein they swam with all four legs and presented rhythmic and alternating movements of their forelimbs and hindlimbs. In the P4–P5 age class, 21% of the Tg8 neonates still exhibited the H0 pattern, 36% acquired the H1 pattern, and 43% acquired the H2 pattern. Most of the C3H pups (85%), however, already displayed the H2 pattern. By P6–P7, almost all the pups of the two strains showed the H2 pattern. Finally, the adult swimming pattern, wherein alternating rhythmic hindlimb movements are evident while immobile forelimbs are extended forward, was present by P14 in both strains. To swim, rats and mice first use only their forelimbs, then both forelimbs and hindlimbs, and finally only hindlimbs. In C3H and Tg8 pups, statistical treatment revealed that acquisition of the swimming pattern was age- and strain-dependent (P < 0.001); Tg8 pups were less able to use their hindlimbs for swimming than C3H pups up to P6–P7.

In vitro experiments were carried out on spinal cord preparations similar to the one used to analyze locomotor-like activity in neonatal rats (Cazalets et al. 1992; Squalli-Houssaini et al. 1993). As in rats, the mouse isolated locomotor network was quiescent when the spinal cord was superfused with normal saline (no activity in ventral roots), but could be activated by adding drugs to the saline, which led to rhythmic motor command of the hindlimbs (Fig. 2). In C3H preparations (n = 5), 5-HT (5 × 10^{-5} M) induced alternating rhythmic bursts of action potentials in the left and right ventral roots (Fig. 2A1; mean burst period ± SE, 7.1 ± 0.5 s). In contrast, 5-HT (from 10^{-6} to 10^{-3} M) never induced rhythmic or tonic activity in Tg8 preparations from P1 to P7 (n = 5) (Fig. 2B1). To show that the lack of activity in the Tg8 spinal cord during 5-HT bath application was not caused by a general disruption of all neuronal activity, we tested the action of other transmitters. N-methyl-d, l-aspartate (NMA, 2 × 10^{-5} M), an N-methyl-d-aspartate (NMDA) receptor agonist known to induce locomotor-like activity in the newborn rat (Cazalets et al. 1992), also induced rhythmic bursting in all the tested C3H preparations (Fig. 2A2; n = 5) and in three-fifths of the Tg8 preparations (Fig. 2B2). In the two remaining Tg8 preparations, NMA only induced tonic discharges in ventral roots. The burst period of the NMA-induced activity, although irregular, was significantly shorter in C3H (1.6 ± 0.6 s) than in Tg8 preparations (2.6 ± 0.1 s). In C3H mouse (Fig. 2A3), as in rat preparations (Squalli-Houssaini et al. 1993), adding 5-HT (5 × 10^{-5} M) to the NMA-containing saline modified and improved the motor rhythm. The burst period of the NMA-induced activity was significantly lengthened (2.5 ± 0.1 s, n = 5, Fig. 2A3). In contrast, in three Tg8 preparations, adding 5-HT had no effect over NMA alone (Fig. 2B3, 2.4 ± 0.1 s). Because MAOA...
deficiency affects both noradrenaline and 5-HT levels, we tested noradrenaline effects. In both Tg8 (n = 5) and C3H (n = 5) preparations, noradrenaline (10^{-5} M) (Fig. 2, A4–B4) induced slow rhythmic discharges in ventral roots. 5-HT (5 \times 10^{-5}) was applied in three Tg8 preparations at P11 and induced ventral root tonic discharges in each preparation. The response was observed only once at the first trial, however, and after washout with normal saline could not be elicited again. This suggests that at least a partial recovery occurred with age for the 5-HT response in Tg8. Because of the viability of the preparation at this age, however, it could not be concluded if recovery was complete or not.

**DISCUSSION**

Both in vivo and in vitro results suggest that MAOA deficiency transiently delays locomotor network maturation, although it cannot be unambiguously established that the the same neuronal changes underlie the perturbations observed in vivo and in vitro. Tg8 neonates swim without their hindlimbs for a longer period than C3H neonates, but both strains acquire the adult hindlimb swimming pattern by P14. The in vitro study performed in parallel may partly explain the delayed onset of hindlimb use in Tg8 swimming. Whereas 5-HT induced and modulated locomotor-like activity in C3H neonates, it had no effects in Tg8 neonates. MAOA deficiency in Tg8 pups increases both noradrenaline and 5-HT levels by \sim 50\% and 900\%, respectively (Cases et al. 1995; Lajard et al. 1999). It seems unlikely that this small noradrenaline change could be responsible for the maturational differences we observed, although this possibility cannot be totally rejected because noradrenaline effects persisted in Tg8 preparations. The substantial 5-HT excess in Tg8 pups, which has already been shown to be responsible for maturational differences in both the thalamocortical projections (Cases et al. 1996) and the central respiratory network (Bou et al. 1998a,b), is more likely to be involved. This perinatal 5-HT excess may have induced alterations in 1) the maturation of the locomotor network itself, 2) the maturation of the descending central projections, and/or 3) the expression of spinal 5-HT receptors. As of now, none of these three possibilities can be rejected. A role of 5-HT in the locomotor network development was already suggested (Nakajima et al. 1999). It seems unlikely that this small noradrenaline excess during their combined cultivation.

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