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Research Report

Direct and indirect effects of neuropeptide Y and neurotrophin 3 on myelination in the neonatal brains

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ABSTRACT

Neuropeptide Y (NPY) is expressed in the developing central nervous system, however, its role in the brain development remains unclear. In this study, C57/B6 mice were intraperitoneally administered 1 nmol/capita/day of NPY, 10 nmol/capita/day of an NPY-receptor 1-specific antagonist (Y1R-A), or NPY and Y1R-A simultaneously (NPY+Y1R-A) from postnatal day (P) 7 to P14. Recombinant NPY reached the P14 cerebrum in 1 hour. These treatments didn't significantly affect body weight gain or P14 brain weight. The ratio of myelinated axons to total axons in the parietal cerebrum was significantly higher in the NPY group than in the control group. The expression of myelin basic protein (*MBP*)-mRNA in the cerebrum was significantly higher in the NPY group than in the control group and was significantly lower in the NPY+Y1R-A group than in the NPY group, while it was significantly higher in the NPY+Y1R-A group than in the control group. In cultured oligodendroglioma-derived B12 cells, NPY didn't influence the *MBP*-mRNA expression, while neurotrophin 3 (NT3) increased *MBP* mRNA via receptor-type tyrosine kinase type C (Trk C). NPY administration significantly increased *NT3*-mRNA expression in the P14 cerebrum as deduced by quantitative real-time PCR. The change in phosphorylated Trk C (P-Trk C) was proportional to that of the *NT3*-mRNA expression, and the proportion of P-Trk C was higher in the NPY group than in the control group. These results suggest that NPY, partially via Y1R, induces NT3 which, via Trk C phosphorylation, accelerates myelination by oligodendrocytes in the mouse brain during the neonatal period.

1. Introduction

Neuropeptide Y (NPY), a 36-amino-acid peptide (Jorgensen et al., 1990) has been shown to increase food intake in adults by stimulating the hypothalamus (Porter et al., 1993; Schwartz et al., 2000). On the other hand, *NPY* mRNA expression has been reported in the hippocampus of human embryos (Bai et al., 2005). NPY has also been reported to act on multipotent neuronal precursors in the olfactory epithelium (Hansel et al., 2001) and suggested to promote proliferation of neuronal precursors (Hansel et al., 2001; Udagawa et al., 2006). Further, transgenic mice lacking NPY receptors had fewer proliferating

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cells, migratory neuroblasts and less interneurons within the olfactory bulb compared to wild type mice (Stanic et al., 2008). These findings suggest that NPY plays some roles in the brain development. However, roles of NPY in development of the central nervous system (CNS) are not well understood.

NPY knockout adult mice have been reported to be more sensitive than wild-type mice to spontaneous and pharmacologically induced seizures (Erickson et al., 1996). In a later study, it was proved that NPY knockout mice had a reduced proliferation of glial cells, including oligodendrocytes, in the subcallosal zone (Laskowski et al., 2007). On the other hand, in the corpus callosum (CC) of rats, number of NPY-positive neurons and fibers increases temporarily with a peak at postnatal day (P) 7-10, although a role of this phenomenon is not clear (Ding and Elberger, 2000). Myelination in C57BL/6 mice starts in the parietal part of the cerebrum including the CC on P 9 (Okui et al., 2005), thus approximately corresponding to the period when NPY-positive neurons and fibers increase in number, while brain development is estimated to occur earlier in mice than in rats (Rivkin and Herrup, 2003). There have been a number of reports on the control mechanism of myelination in the CNS (for review see, Káradóttir and Attwell, 2007; Schmit and Chew, 2008) including the adenosine triphosphate (ATP) effect on oligodendrocytes to stimulate myeline formation (Stevens et al., 2002). However, to our knowledge, any relationship between NPY and myelination in the brain has not been documented. We thus hypothesized that NPY plays an unproven role in the myelination during the cerebral development.

The aim of this study was therefore to examine the effects of NPY on oligodendrocytes in the mouse brain during the second week after birth, during which normal myelination occurs there, by intraperitoneal administration of NPY and/or an Y1 receptor antagonist (Y1R-A), both of which can reach the brain during this period (see Results and Experimental Procedure), as well as in vitro studies using oligodendroglioma–derived B12 cells. We show NPY's effects on the promotion of myelination by oligodendrocytes in the brain during the neonatal period, i.e., the second week after birth.

2. Results

2.1. *Cerebral permeability of recombinant NPY*

We first assessed the ability of recombinant NPY to permeabilize the blood brain barrier (BBB) to the cerebral tissues by administering biotinized (B)-NPY (see 4.2) to P14 mice. We injected B-NPY and non-biotinzed (N)-NPY (1 nmol/capita) and collected the cerebra at 1 hour after administration. B-NPY was detected around the capillaries (yellow dots, Fig. 2a), but NPY and biotin were localized in the cerebral tissue separately (NPY: red dots; biotin: green dots; Fig. 2a). We detected some B-NPY around the outside of the endothelial cells of capillaries in the cerebrum, and found that N-NPY and biotin were localized separately in the cerebral tissue (Fig. 2a). It was suggested that B-NPY tended to be disturbed during its penetration into the cerebral tissue at the outside of capillaries, whereas the recombinant NPY crossed the BBB and reached the cerebral tissue in 1 hour. The level of NPY in the cerebra was significantly higher in the N-NPY group ($3.24 \pm 0.82 \times 10^{-4}$/1 g protein) than in the B-NPY group ($1.22 \pm 0.07 \times 10^{-4}$/1 g protein; $P<0.05$; Fig. 2b). It was suggested that biotinylation of NPY decreased the ability of NPY to permeate the BBB but we confirmed that NPY penetrated into the cerebral tissue. These results clearly showed that intraperitoneally injected NPY penetrated into the cerebral tissues.

BIBP3226 that was used as an Y1R-A in the present study was reported that its subcutaneous injection affected the combined Y1 and Y5 receptor populations of the hypothalamus in the P8 rat neonates (Stricker-Krongrad and Beck, 2004). In rats, inter-endothelial clefts in brain capillaries remain until P16 and those of the BBB are not fully developed until P24 (Schulze and Firth, 1992), while the BBB in mice is not fully developed until at least P14. NPY and the Y1R-A would therefore be similarly able to reach the CNS in the present study.

2.2. *Effects on weight gain and brain weight*

Weight gain in the control group C57BL/6 mice was 3.267 ± 0.448 g from P7 to P14, whereas in the NPY, Y1R-A and NPY + Y1R-A groups it was 3.331 ± 0.494 g, 3.461 ± 0.536 g and 3.173 ± 0.597 g respectively. The average P14 brain weights were 0.413 ± 0.011 g (control), 0.415 ± 0.012 g (NPY), 0.403 ± 0.010 g (Y1R-A) and 0.397 ± 0.017 g (NPY + Y1R-A). There were no significant differences in weight gain and brain weight among these groups. Thus, NPY and Y1R-A did not appear to affect gains of body and brain weights during this period.

2.3. *Effects of NPY and Y1R-A on MBP expression in the P14 cerebrum*

To examine the effects of NPY and Y1R-A on myeline basic protein (MBP) expression in the developing mouse brain, we first observed myelinated fibers by MBP immunohistochemistry in the coronary sections from the parietal region of the cerebrum of P14 mice in the NPY and control groups (Fig. 2c, d). It appeared that the myelinated areas differed in size between the NPY and control groups. We next checked the expression of *MBP* mRNA in the whole cerebrum. QRT-PCR indicated that cerebral *MBP* mRNA was significantly higher in the NPY group (ratio to 18S rRNA: 0.399 ± 0.056) than in the control (0.084 ± 0.043; $P<0.01$), Y1R-A (0.063 ± 0.026; $P<0.01$) and NPY + Y1R-A (0.216 ± 0.065; $P<0.05$) groups. *MBP* mRNA was significantly lower in the NPY + Y1R-A ($P<0.05$) than in the NPY group, while it was significantly higher in the NPY + Y1R-A group ($P<0.05$) than in the Y1R-A and control groups (Fig. 2e) (see 3).

2.4. *Effect of NPY on the myelin sheath/axon relationship*

TEM pictures of typical axon cross-sections in the control and NPY groups provided information on the myelin sheath radii in these groups (Fig. 3a, b). We counted cross sections of axons in the parietal part of the cerebra in the NPY and control groups. The axon density between the NPY group (250 ± 3.0 $X10^3/mm^2$) and the control group ($254 \pm 3.9 \times 10^3/mm^2$; Fig. 3c) was not significantly different. However, the ratio of myelinated axons to total axons was significantly higher in the NPY