Increased Expression of Hypothalamic NADPH–Diaphorase Neurons in Mice with Iron Supplement

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Iron deficiency is known as the most important nutritional problem in the world. The loss of appetite is a common characteristic of iron deficiency. Iron-containing heme is required as a cofactor for nitric oxide synthase (NOS) which produces nitric oxide (NO). NOS in the central nervous system has been suggested to regulate food intake. Hence, we examined the expression of hypothalamic NOS at various levels of dietary iron. ICR mice (n = 30) were randomly divided into three groups based on the level of dietary iron and fed experimental diets for 4 weeks: the normal-iron diet group (7 mg/kg diet, n = 10), the low-iron diet group (21 mg/kg diet, n = 10) and the high-iron diet group (42 mg/kg diet, n = 10). Expression of NOS in the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) of hypothalamus was examined by histochemistry for nicotinamide adenine dinucleotide phosphate–diaphorase (NADPH–diaphorase). The high-iron diet mice showed significantly higher staining intensity of NADPH–diaphorase-positive neurons in the PVN and LHA than the normal- and low-iron diet mice.

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normal-iron diet group (21 g/kg, n = 10), and a high-iron diet group (42 g/kg, n = 10). After 4 weeks of experimental period, the mice were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and transcardially perfused with 50 mM phosphate-buffered saline (PBS). They were then fixed with a freshly prepared 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde. The brains were removed and postfixed in the same fixative overnight and then immersed in a 30% sucrose solution at 4°C. Serial 40 μm-thick coronal sections were made with freezing microtome (Leica, Nussloch, Germany). NADPH–diaphorase positive neurons were detected using NADPH–diaphorase histochemistry as described previously.\textsuperscript{12,13} In brief, free-floating sections were incubated at 37°C for 60 min in 0.1 M PB (pH 7.4) containing 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium and 0.1 mg/ml β-NADPH. Sections were washed three times with 0.1 M PB and mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, rinsed twice with distilled water and dried. Cover slips were mounted using Permount. Brain sections were analyzed using the atlas by Franklin and Paxions.\textsuperscript{14} The staining intensities of sections specifically for NADPH–diaphorase were assessed in a quantitative fashion according to the microdensitometrical method based on optical density from an image analyzer (Multiscan, Fullerton, CA).\textsuperscript{12} The results were analyzed statistically with a SPSS followed by Tukey’s test. The statistical significance level was considered at $P < 0.05$.

The staining intensities of NADPH–diaphorase-positive neurons in the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) of high-iron diet mice were higher than those of the normal-diet mice and the low-iron diet mice (Fig. 1). In the PVN regions, the number of small-sized stained cells and fibers was less in normal- and low-iron diet mice than in high-iron diet mice (Fig. 1A and 1B), even though those cells and fibers were more frequently observed in high-iron diet mice (Fig. 1C). Medium-sized stained neurons and fibers were scattered over the LHA of normal and low-iron diet mice (Fig. 1D and 1E), while higher staining intensities were observed from NADPH–diaphorase-positive neurons and fibers in the LHA of high-iron diet mice (Fig. 1F).

Table 1 shows quantitative differences in the optical densities of NADPH–diaphorase-positive neurons in the hypothalamic regions from each group. The optical densities of NADPH–diaphorase-positive neurons in the PVN and LHA of high-iron diet mice were significantly higher than in normal- and low-iron diet mice. Iron deficiency is a common nutritional problem due to increased iron requirements during adolescence in humans.\textsuperscript{11} Iron is essential as a metallic cofactor for

![Fig. 1. Distribution of NADPH–Diaphorase-Positive Neurons in Hypothalamic Regions.](image)
many enzymes and proteins containing either heme or non-heme iron. Goldblatt et al. suggested that iron deficiency affects NOS activity in the ileum. Iron deficiency also results in altered gallbladder and sphincter of Oddi motility, and cholesterol crystal formation due to decreased levels of neuronal nitric oxide synthase. This situation is related to the incidence of gallstones in premenopausal women. Iron deficiency transiently suppresses biliary neuronal nitric oxide synthase. This suppression results in a feeding center, in iron deficient mice. NO products generated from NOS will be studied in the future. In this manner, iron supplement in anemic conditions can suppress ileal nitric oxide synthase activity.

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References


