Temperature Control in Health and Disease

Edited by
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Minsk 1997

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ISBN 985-6178-05-3

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in Health and Disease

NITRIC OXIDE SYNTHESIS IN HYPOTHALAMIC NUCLEI OF EXPERIMENTAL ANIMALS AFTER APPLICATION OF FREUND'S COMPLETE ADJUVANT AND LOW AMBIENT TEMPERATURES

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In the last years, the involvement of nitric oxide (NO) in the central mechanisms of thermoregulation in heating [8] and experimental fever [1,3] has been demonstrated. At the same time it is still unclear how the production of this free radical molecule changes upon exposure of the organism to pyrogenic factors in a period preceding a body temperature rise and to cold. So, we studied changes in the content of NO-synthesizing cells in different hypothalamic nuclei after injection of Freund's complete adjuvant (FCA) in a period preceding a rise in body temperature and after prolonged exposure to low ambient temperatures.

Materials & Methods

Experiments were performed in Wistar male rats weighing 180-220 g. Group I experimental animals received FCA containing 0.1% killed and dried Mycobacterium butyricum into the cushions of hind paws as 0.1 ml per rat. Group II animals were exposed to 4-6°C for 48 h in laboratory premises. Intact animals, as those of group I, were kept at an ambient temperature 20-22°C. Control and experimental rats were anesthetized with intraperitoneal sodium thiopental in a dose 50 mg/kg. After opening of the thorax the right atrial wall was incised, and the cardiovascular system was perfused through the left ventricle with Ringer's solution containing 10 mM $NaNO_2$, 0.1 M phosphate buffer (pH 7.4), and 4% paraformadehyde in

0.1 M phosphate buffer (pH 7.4). After decapitation the brains were removed and the hypothalamus was isolated and fixed for 60 min in 4% paraformaldehyde on phosphate buffer (0.1 M, pH 7.4). Then it was washed off in cold six times for 30 min each using 0.1 M Tris-HCl (pH 8.0) and incubated in 10% and 25% sucrose on Tris-HCl (0.1 M, pH 8.0) for 1.5 and 12 h, respectively. After cryostat freezing of hypothalamic samples at -25°C sections 25 μm thick were prepared. The staining method for NADPH-diaphorase (NO-synthase) was used to identify NO-synthesizing cells [4,5]. NADPH-diaphorase-positive neurons were counted with a Leitz-TAS image analysis system (Germany) and expressed as per 1 mm^2 of the section area [2].

Results & Discussion

We found earlier that administration of FCA to rats increased deep body temperature 2.5-3 h after the injection [7] and that NO may be a factor of the antipyretic system in febrile states including adjuvant-induced hyperthermia [1,3,6]. The present study deals with NO synthesizing processes in neurons of the lateral preoptic (LPO), paraventricular (PAV) and periventricular (PEV) hypothalamic nuclei 2 h after application of FCA with a view to clarifying the involvement of this antipyretic system level in the central thermoregulatory mechanisms in a period preceding the body temperature elevation. It was shown that in this period the quantity of NO-synthesizing neurons (NADPH-diaphorase-positive) increased by 15.4% ($p<0.05$) in LPO, by 21.0% ($p<0.01$) in PAV, and did not significantly differ from control in PEV. The data suggest that the NO-synthase activity induction by FCA occurs in hypothalamic regions that have relation to the control of thermoregulatory processes and reflects activation of the NOergic link of the antipyretic system already in a period preceding a body temperature rise. This probably conforms to the known physiological principle that any excitation in the organism is combined with activation of inhibitory processes limiting the excessive response and making the quantitative and qualitative characteristics of the response correspond to the acting stimuli.

A study of the activity of NO synthesis in the hypothalamic nuclei after prolonged (48 h) exposure to cold did not reveal any marked changes in the indices as compared to control. This

may be due to the absence of response of NOergic system of the hypothalamus to low ambient temperatures in the range studied, as well as reflect compensatory processes in the CNS bringing about adaptation of the animal organism to cold.

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