Effects of Dynorphin A(1-13) on DNA Synthesis and Free Radical Oxidation in Respiratory Organs of Newborn Albino Rats Pretreated with N^G-Nitro-L-Arginine Methyl Ester

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Dynorphin A(1-13) increased the number of DNA-synthesizing epitheliocytes in the bronchi and inhibited free radical oxidation in the lungs of newborn albino rats. Pretreatment with N^G-nitro-L-arginine methyl ester not only abolished, but also reversed the effect of this peptide.

Key Words: *DNA synthesis; nitric oxide; free radical oxidation*

Dynorphin A(1-13) is an endogenous opioid prodynorphin peptide displaying preferential selectivity for κ-receptors. Our previous studies showed that various opioid peptides, including dynorphin A(1-13), are involved in tonic regulation of DNA synthesis in epithelial and smooth muscle cells of the tracheal mucosa in newborn albino rats [3]. Published data suggest that morphogenetic activity of dynorphin A(1-13) is realized via the nitroxidergic mechanisms [5,10,12]. Here we studied the effects of dynorphin A(1-13) on DNA synthesis in epithelial and smooth muscle cells of the bronchi and free radical oxidation in the lungs of newborn albino rats pretreated with N^G-nitro-L-arginine methyl ester (L-NAME) inhibiting constitutive nitric oxide (NO) synthases.

MATERIALS AND METHODS

Experiments were performed on 114 newborn albino rats. The animals received intraperitoneal injections of the test substances from the 2nd to 6th day of life. Group 1 rats received dynorphin A(1-13) (H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys) in a dose of 0.82×10^{-7} mol/kg (Laboratory of Peptides, Russian Research-and-Production Center for Cardiology). Group 2 rats were injected with L-NAME (ICN Biomedicals Inc.) in a dose of 9.3×10^{-5} mol/kg. Group 3 rats received dynorphin A(1-13) 30 min after administration of L-NAME. Control animals were injected with isotonic NaCl. The rats were decapitated 24 h after the last treatment.

DNA synthesis was studied by autoradiography. The rats intraperitoneally received ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 1570 TBq/mol) 1 h before decapitation. Autoradiographs were prepared routinely. The number of S-phase epitheliocytes and smooth muscle cells of cartilaginous bronchi (index of labeled nuclei, ILN, %) was determined. The method of chemiluminescence (CL) was used to estimate the intensity of free radical oxidation in lung homogenates [1]. CL was recorded on a LS 50B luminescence spectrometer (Perkin Elmer). Signals were standardized using Finlab software. The intensity of spontaneous and Fe^{2+}-induced CL was evaluated as described elsewhere [2]. Total CL over 1 min of spontaneous CL (S_{SP}) correlated with the intensity of free radical processes. The maximum flash amplitude (h) of Fe^{2+}-induced CL reflected the content of lipid hydroperoxides. Total CL measured recorded over 4 min of the post-flash period (S_{IND}) reflected the rate of free radical formation. Kinetic parameters of H_2O_2-induced luminol-dependent CL [1,6,8] were analyzed by the maximum amplitude of the first flash (H_1) reflecting

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the intensity of radical generation in Fenton-like reactions, maximum amplitude of flash 2 (H_2) correlating with activity of antiradical systems, and interval (t) between H_1 and H_2 that depended on the state of the antioxidant systems. Parameters of CL were calculated per 1 mg lipids and expressed in relative units. Total lipid content was estimated by the phosphovanillin method using Lachema kits.

The results were analyzed by Student's t test.

RESULTS

Five injections of dynorphin A(1-3) increased the count of DNA-synthesizing epitheliocytes (1.5-fold increase in ILN), but not smooth muscle cells in the bronchi (Table 1). In the lungs we observed an increase in the buffer capacity of antioxidant protective systems, a decrease in the content of lipid hydroperoxides, inhibition of peroxide radical formation, and a shift of the redox potential toward the reduction component, as seen from changes in t, h, S_{IND}, and S_{SP}, respectively (Table 2).

The observed modulation of biogenesis of reactive oxygen species (ROS) with dynorphin A(1-13) was indirectly confirmed by published data [7,9].

Our previous studies showed that the blockade of NO synthesis with L-NAME induces various changes in the number of DNA-synthesizing nuclei, which depends on the type of cells [4]. This parameter decreases in epitheliocytes, but increases in smooth muscle cells. These changes are accompanied by the development of oxidative stress in the lungs of newborn albino rats (Tables 1 and 2).

Pretreatment with L-NAME not only abolished, but also reversed the effect of dynorphin A(1-13) on DNA synthesis in epitheliocytes. In these rats ILN decreased compared to animals receiving L-NAME. The peptide had no effect on the number of DNA-synthesizing smooth muscle cells during blockade of NO synthesis. ILN for smooth muscle cells did not differ from that observed after administration of L-NAME (Table 1).

Under conditions of NO synthesis blockade dynorphin A(1-13) produced an opposite effect on the redox potential in rat lungs. The changes in CL parameters indicated that combination treatment with L-NAME and dynorphin A(1-13) induces more severe disturbances in the free radical state compared to those observed after administration of the inhibitor alone (Table 2). The balance was shifted toward decompensated accumulation of ROS. Published data confirm the ability of dynorphin A(1-13) to aggravate oxidative stress [14,15]. Dynorphin A(1-13) decreases the content of reduced glutathione and induces overproduction of ROS (superoxide anion and hydroxyl radicals) in rat ischemic myocardium.

Under physiological conditions dynorphin acts as a vasodilator agent. Inversion of the effect of dynorphin was previously observed in newborn pigs with

TABLE 1. Effects of Dynorphin A(1-13) and L-NAME on DNA Synthesis in Epitheliocytes and Smooth Muscle Cells in the Bronchi of Newborn Albino Rats (ILN, %, $M\pm m$)

Cells	Control	Dynorphin A(1-13)	L-NAME	L-NAME and dynorphin A(1-13)
Epitheliocytes	1.86±0.12	2.79±0.13*	1.45±0.11*	1.07±0.10*+
Smooth muscle cells	0.526±0.034	0.526±0.034	0.854±0.071*	0.763±0.057*

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to the L-NAME group.

TABLE 2. Effects of Dynorphin A(1-13) and L-NAME on Chemiluminescence in Lung Homogenates from Newborn Albino Rats ($M\pm m$)

Parameter	Control	Dynorphin A(1-13)	L-NAME	L-NAME and dynorphin A(1-13)
S_{SP}, rel. units	6.27±0.20	4.83±0.13*	15.14±0.63*	17.50±0.25*+
Fe^{2+}-induced CL				
h, rel. units	0.086±0.002	0.059±0.004*	0.145±0.005*	0.192±0.006*+
S_{IND}, rel. units	44.48±1.75	27.35±1.14*	85.49±2.37*	93.23±1.99*+
H_2O_2-induced luminol-dependent CL				
H_1, rel. units	0.138±0.007	0.122±0.006	0.742±0.005*	0.853±0.008*+
t, min	0.84±0.04	1.18±0.04*	0.52±0.02*	0.40±0.03*+
H_2, rel. units	0.473±0.023	0.416±0.025	0.794±0.048*	0.945±0.021*+

brain trauma, and this inversion was abolished by preparations containing superoxide dismutase and catalase [13]. Administration of L-NAME is probably followed by oxidative structural modification of the peptide, which affects its biological properties. Our assumption is confirmed by published data that native dynorphin suppresses the respiratory burst in human polymorphonuclear leukocytes, while ROS-pretreated dynorphin activates this process [11].

Our experiments with L-NAME showed that the effects of 5-fold treatment with dynorphin A(1-13) on DNA synthesis in epitheliocytes of the bronchi and free radical oxidation in the lungs of newborn albino rats are realized via the nitroxidergic mechanisms.

REFERENCES

1. A. V. Arutyunyan, E. E. Dubinina, and N. N. Zybina, *Methods for Studying Free Radical Oxidation and Antioxidant System in the Body: Methodical Recommendations* [in Russian], St. Petersburg (2000).
2. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, *et al*., *Itogi Nauki Tekniki. Ser. Biofizika*, **29** (1991).
3. O. A. Lebed'ko and S. S. Timoshin, *Byull. Exp. Biol. Med.*, **205**, No. 2, 205-207 (2001).
4. O. A. Lebed'ko and S. S. Timoshin, *Ibid.*, **133**, No. 5, 501-503 (2002).
5. A. Capasso, L. Sorrentino, and A. Pinto, *Eur. J. Pharmacol*., **359**, Nos. 2-3, 127-131 (1998).
6. N. Gulyaeva, M. Onufriev, and M. Stepanichev, *Neuroreport*, **6**, No. 1, 94-96 (1994).
7. S. Hu, P. K. Peterson, and C. C. Chao, *Biochem. Pharmacol*., **56**, No. 3, 285-288 (1998).
8. A. I. Kuzmenko, *WMJ*, **71**, No. 4, 63-66 (1999).
9. B. Liu, L. Qin, S. N. Yang, *et al*., *J. Pharmacol. Exp. Ther*., **298**, No. 3, 1133-1141 (2001).
10. N. Nozaki-Taguchi and T. Yamamoto, *Anesth. Analg*., **87**, No. 2, 388-393 (1998).
11. A. Slaoui-Hasnaoui, M. C. Guerin, C. Le Doucen, *et al*., *Biochem. Pharmacol.*, **43**, No. 3, 503-506 (1992).
12. S. N. Thorat, P. L. Reddy, and H. N. Bhargava, *Brain Res*., **621**, No. 1, 171-174 (1993).
13. M. C. Thorogood and W. M. Armstead, *Anesthesiology*, **84**, No. 3, 614-625 (1996).
14. C. S. Yang, P. J. Tsai, W. Y. Chen, and J. S. Kuo, *Redox. Rep*., **3**, Nos. 5-6, 295-301 (1997).
15. C. S. Yang, P. J. Tsai, S. T. Chou, *et al*., *Free Radic. Biol. Med*., **18**, No. 3, 593-598 (1995).