Effects of Atrial Natriuretic Peptide on DNA Synthesis and NADPH Diaphorase Activity in Epitheliocytes and Smooth Muscle Cells of the Respiratory Tract in Newborn Albino Rats

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Newborn rats received single intraperitoneal injections of atrial natriuretic peptide (1-28) in a dose of 3.2×10^{-8} mol/kg on day 6 of life. Autoradiography with ^{3}H-thymidine showed that the peptide inhibited DNA synthesis in smooth muscle cells of the respiratory tract. Pretreatment with nitric oxide synthase inhibitor N^{G}-nitro-L-arginine methyl ester attenuated, but did not abolish the effect of atrial natriuretic peptide (1-28). Histochemical assay for NADPH diaphorase showed that nitric oxide constitutively produced in the epithelium is involved in the growth-inhibitory effect of atrial natriuretic peptide (1-28) on proliferating smooth muscle cells.

Key Words: *atrial natriuretic peptide; DNA synthesis; nitric oxide; epithelium; smooth muscle cells; respiratory tract*

Our previous studies revealed an increase in the count of smooth muscle cells in large [6] and small airways [13] in patients with severe bronchial asthma. These changes are primarily realized via hyperplasia of smooth muscle cells (SMC) [3]. A variety of growth factors for respiratory SMC were identified. However, the mechanisms of their effects and inhibition of proliferation remain unclear. Secretion and synthesis of atrial natriuretic peptide (1-28) (ANP) and expression of guanylate cyclase-dependent (type A) and clearance receptors in the lungs were detected in the perinatal period [7]. ANP modulates basal and stimulated tone of the respiratory tract [10,11]. Nitric oxide constitutively synthesized in the respiratory tract possesses bronchoprotective activity and produces not only relaxing, but also antiproliferative effects on SMC [15]. We hypothesized that ANP also has antiproliferative properties. Here we studied the effects of single treatment with ANP on DNA synthesis and NADPH diaphorase activity in epitheliocytes and SMC of the trachea and cartilaginous and noncartilaginous bronchi in newborn albino rats during acute blockade of nitric oxide synthesis with the nitric oxide synthase (NOS) inhibitor N^{G}-nitro-L-arginine methyl ester (L-NAME).

MATERIALS AND METHODS

Experiments were performed on 98 newborn albino rats. Control and experimental groups were composed by the method of litter separation to reduce genetically determined differences between litters. The animals received single intraperitoneal injections of substances at 10.00-11.00 on day 6 of life. Group 1 and 2 rats were injected with 3.2×10^{-8} mol/kg ANP and 9.3×10^{-5} mol/kg L-NAME, respectively. Group 3 rats recei-

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ved ANP 30 min after administration of L-NAME. Control animals received isotonic NaCl.

DNA synthesis in epitheliocytes and smooth muscle cells of the tracheobronchial system was studied 24 h after treatment. The rats were intraperitoneally injected with ^{3}H-thymidine in a dose of 1 mCi/g (specific activity 1570 TBq/mol) 1 h before euthanasia. Autoradiographs were prepared routinely. The number of S-phase cells (index of labeled nuclei, ILN, %) and mean number of silver grains over the nucleus (labeling intensity, LI) were counted. Histochemical assay for NADPH diaphorase (specific NOS marker) was performed by the method described elsewhere [9]. This method is based on conversion of nitroblue tetrazolium into azure- or blue-colored diformazan under the effect of NADPH diaphorase (Fig. 1). In control series, the incubation medium contained no β-NADPH. Therefore, the slices were not stained and remained transparent. Light transmission was measured by the plaque method on a single-beam photometer equipped with an FMEL-1Uch attachment at 550 nm.

At least 50 cells in each preparation were examined. Light transmission in unstained tissues was taken as the baseline level. The results were analyzed by Student's *t* test.

RESULTS

ANP produced various effects on epitheliocytes and SMC of the respiratory tract in newborn rats. Single injection of the peptide did not affect epitheliocyte proliferation, but markedly inhibited SMC division in all portions of the tracheobronchial system: ILN and LI significantly decreased in the trachea (by 2.1 and 1.2 times compared to the control, respectively) and cartilaginous bronchi (by 2 and 1.4 times, respectively). Moreover, ILN in noncartilaginous bronchi decreased by 1.9 times (Table 1). The antimitotic effect of ANP is consistent with published data that the peptide in doses of 10^{-8}-10^{-6} M dose-dependently inhibits proliferation of SMC in human respiratory tract induced by thrombin and serum *in vitro* [5]

Studies of NADPH diaphorase activity showed that epitheliocytes, but not SMC, reacted to ANP (Table 1). The density of precipitated diformazan in epitheliocytes of the trachea and cartilaginous and noncartilaginous bronchi increased by 1.3, 1.3, and 1.2 times, respectively. However, in SMC this parameter did not differ from the control. The increase in NADPH diaphorase and NOS activities of the epithelium induced by ANP is consistent with published data that the peptide *in vitro* and *in vivo* activates these enzymes in the endothelium of large and small vessels [2]. It should be emphasized that NADPH diaphorase is a specific marker of tissue NOS (eNOS, nNOS, and iNOS). ANP activates eNOS via the interaction with clearance and guanylate cyclase-dependent (type A) receptors [8,12]. It can be assumed that the increase in total NOS activity of the epithelium is associated with eNOS activation.

L-NAME had no effect on proliferating epitheliocytes (similarly to ANP). However, the blockade of nitric oxide synthesis was followed by activation of SMC division. ILN significantly increased in the trachea and cartilaginous bronchi compared to the control. In noncartilaginous bronchi we observed the increase in ILN and LI (Table 1). Previous *in vitro* studies showed that nitric oxide donors S-nitroso-N-acetyl-penicillamine (10^{-4} M) and sodium nitroprusside (10^{-5}-10^{-3} M) dose-dependently inhibit SMC of the respiratory tract [5].

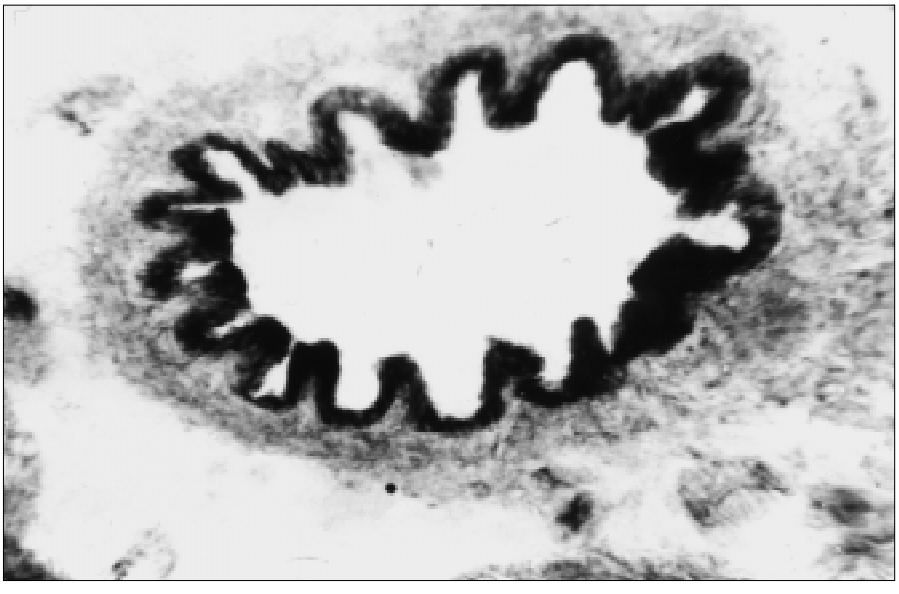

Fig. 1. Noncartilaginous bronchus from a 7-day-old control rat with NADPH diaphorase activity in epitheliocytes and smooth muscle cells. Hope-Vincent method (×75).

TABLE 1. Effects of ANP and L-NAME on DNA Synthesis and NADPH Diaphorase Activity in Epitheliocytes and Smooth Muscle Cells of the Tracheobronchial System in Newborn Albino Rats (*M*±*m*)

Series	Epitheliocytes			Smooth muscle cells		
	ILN, %	LI	NADPH diaphorase, optical density units	ILN, %	LI	NADPH diaphorase, optical density units
Control						
trachea	1.81±0.15	22.45±1.52	0.511±0.010	0.590±0.048	20.10±1.06	0.221±0.010
cartilaginous bronchi	1.77±0.10	20.15±1.21	0.449±0.015	0.533±0.055	18.67±1.10	0.187±0.009
noncartilaginous bronchi	1.70±0.08	20.55±1.05	0.392±0.012	0.523±0.044	19.43±0.94	0.148±0.008
ANP						
trachea	1.78±0.11	21.24±1.88	0.649±0.020*	0.277±0.012*	15.14±1.15*	0.221±0.008
cartilaginous bronchi	1.73±0.11	22.08±1.14	0.592±0.020*	0.260±0.010*	14.11±0.90*	0.182±0.013
noncartilaginous bronchi	1.74±0.09	19.31±1.22	0.489±0.017*	0.281±0.013*	20.19±1.14	0.166±0.015
L-NAME						
trachea	1.98±0.11	23.17±1.32	0.336±0.011*	0.851±0.065*	22.10±1.42	0.197±0.009
cartilaginous bronchi	1.83±0.08	20.21±1.38	0.311±0.012*	0.820±0.053*	20.33±0.96	0.186±0.011
noncartilaginous bronchi	1.76±0.09	21.72±1.30	0.190±0.010*	0.862±0.058*	24.34±1.07*	0.172±0.009
L-NAME+ANP						
trachea	1.88±0.11	23.07±1.23	0.483±0.016	0.562±0.043	19.88±1.11	0.207±0.009
cartilaginous bronchi	1.86±0.12	21.42±1.19	0.453±0.018	0.548±0.051	20.12±0.92	0.179±0.010
noncartilaginous bronchi	1.80±0.09	21.40±1.15	0.426±0.020	0.530±0.040	19.19±1.10	0.160±0.007

Note. *$p<0.05$ compared to the control.

L-NAME markedly decreased NADPH diaphorase activity in the epithelium of the trachea and cartilaginous and noncartilaginous bronchi (by 1.4, 1.4, and 1.2 times, respectively, Table 1). However, enzyme activity in SMC remained unchanged. Published data show that L-arginine analogues (NOS inhibitors) decrease NOS-associated catalytic activity of NADPH diaphorase [4]. L-NAME primarily blocks constitutive NOS [7]. During the neonatal period epitheliocytes of the respiratory tract express all isoforms of NOS, while SMC express only nNOS and iNOS [14]. Since L-NAME decreased NOS activity in epitheliocytes, but not in SMC, it can be assumed that this agent blocked eNOS. These data indicate that single injection of L-NAME decreases the amount of nitric oxide constitutively synthesized in the epithelium, which contributes to its growth-inhibitory effects on proliferating SMC.

Test parameters did not change in animals receiving both L-NAME and ANP (Table 1). Therefore, L-NAME attenuated, but did not abolish the effect of ANP. We hypothesized that the inhibition of constitutive nitric oxide synthesis in the epithelium induced by L-NAME was compensated by *de novo* expression and/or activation of eNOS after further treatment with ANP. On the other hand, the kinetics of SMC proliferation in the respiratory tract returned to the baseline level after the recovery of NOS isoenzyme activities in the epithelium.

Our results indicate that nitric oxide constitutively produced in epitheliocytes serves as an intercellular messenger and mediates the antimitotic effect of ANP on SMC.

During early ontogeny SMC of the tracheobronchial system are highly sensitive to mitogenic stimuli. The ability of ANP to suppress SMC proliferation holds much promise for the prevention and correction of changes in the respiratory tract that accompany obstructive bronchial diseases in the early postnatal development.

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