Reactivity of Tracheal Smooth Muscles in Albino Rats with Experimental Diabetes Mellitus Treated with a New Complex Compound of Oxovanadium (IV) and Isonicotinic Acid Hydrazide

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We studied functional properties of tracheal smooth muscle cells in rats with diabetes mellitus. Reactivity of tracheal smooth muscles increased in rats with experimental alloxan-induced diabetes mellitus. A new complex compound of oxovanadium (IV) and isonicotinic acid hydrazide affected reactivity of tracheal smooth muscles in albino rats with experimental type I diabetes mellitus. This new organic vanadium-containing compound reduced contractility of tracheal smooth muscles in rats and potentiated relaxation of smooth muscle cells in the trachea in response to exogenous nitric oxide.

Key Words: *diabetes mellitus; albino rats; upper respiratory tract; vanadium; smooth muscle contractility*

Published data suggest that various endocrine disorders, including diabetes mellitus, affect functional activity of smooth muscle cells (SMC) in internal organs (*e.g.*, SMC of the upper respiratory tract) [3]. Reactivity of SMC in the trachea and bronchi under pathological conditions is an important medical problem. This is associated with growing incidence of allergic disorders, endocrine disturbances, and immune deficiency. It is necessary to evaluate the cause of pathological changes in the respiratory tract during endocrine disorders (*e.g.*, diabetes mellitus). Functional activity of SMC in the upper respiratory tract undergoes changes during diabetes mellitus, which is related to the impaired humoral regulation of smooth muscle reactivity [3].

Experimental insulin-dependent diabetes mellitus is associated with decreased release of endothelium-derived relaxing factor (nitric oxide) [8,9], which produces a permanent increase in basal tone and reactivity of SMC. Changes in acetylcholine secretion in nerve endings of parasympathetic fibers are an urgent problem [3]. It is probably associated with functional changes in M_2 muscarinic cholinergic receptors regulating acetylcholine release from parasympathetic endings in bronchial smooth muscles. The degree of changes in reactivity of SMC in the upper respiratory tract during diabetes mellitus and the role of nerve and humoral factors in this process should be determined. The methods for treatment of diabetes mellitus are proposed. The search continues for new potent preparations is in progress. Correction of functional activity in SMC with new antidiabetic preparations would complement main clinical approaches to the therapy of patients with diabetes mellitus and bronchoobstructive syndrome of different etiology.

Here we studied the effects of a new complex compound containing oxovanadium (IV) and isonicotinic acid hydrazide on reactivity of tracheal smooth

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muscles in rats with diabetes mellitus. The preparation was synthesized at the Laboratory of Coordinated Compounds (Kazan' State University). We evaluated changes in the reactivity of tracheal smooth muscles in rats with diabetes mellitus and efficiency of treatment with a new vanadium compound.

MATERIALS AND METHODS

The experiments were performed on 53 male and female outbred albino rats weighing 200-250 g. The animals were divided into 4 groups. Group 1 included rats with experimental diabetes mellitus treated with a new vanadium compound. Group 2 included control animals with experimental diabetes mellitus. Group 3 included intact rats receiving oxovanadium preparation. Group 4 included intact animals. Experimental diabetes mellitus was *in vivo* induced with alloxan injections. This method was developed by V. G. Baranov in 1983. The development and course of diabetes mellitus were monitored by glycemia (*o*-toluidine method), glucosuria, and 24-h volume of consumed fluid. The oral test for glucose tolerance developed by I. B. Penchev in 1962 was performed by the end of the experiments. The rats with diabetes mellitus received daily intraperitoneal injections of the vanadium compound in a single dose of $^1/_{30}$ LD_{50} for 28 days. Blood glucose level was measured after the course of treatment. This parameter characterized antidiabetic effect of the oxovanadium preparation. The animals were euthanized under ether anesthesia. Trachea preparation was placed in Krebs—Henseleit solution, incubated at 4-5°C for 10 h, and tested using a photomechanoelectrical transducer [2] under isometric conditions [1]. The curves were plotted using an H-3012 automatic recorder. The response of SMC was determined on a B7-35 voltmeter and expressed in mV. Trachea strips were placed in a bath of a single-channel tester with Krebs solution and incubated at 37°C for 1 h (the stretch was equal to 1 g). The ability of preparations to contract and relax was evaluated. Carbachol (CCh) in a concentration of 10^{-4} mol/liter served as the agonist of contraction. The exogenous donor of nitric oxide sodium nitroprusside in a concentration of 100 μmol/liter was used as a dilator. Moreover, open tracheal rings from experimental animals were tested with CCh in concentrations of 10^{-7}-10^{-4} mol/liter. The data were expressed in mV, and dose—response curves were constructed. The results were analyzed by Student's *t* test. The significance of differences was evaluated using Statgraf and Microsoft Excel software.

TABLE 1. Reactivity of Tracheal SMC in Guinea Pigs with Diabetes Mellitus Treated with a New Complex Preparation of Vanadium to CCh and Sodium Nitroprusside (Strength of Contractions, mV, *M*±*m*)

Group	CCh, 10^{-4} mol/liter	Sodium nitroprusside, 100 μmol/liter
Experiment	119±78	-103.5±52+
Control	77±31	13.5±7*
Intact	58±15	-54±16

Note. $p<0.05$: *compared to intact animals; +compared to control animals.

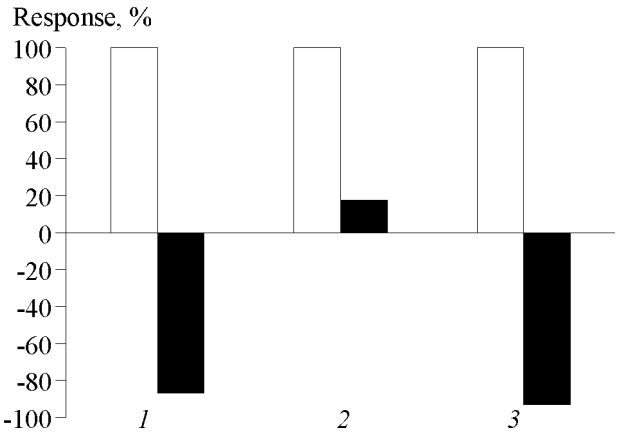

Fig. 1. Reactivity of tracheal SMC to CCh and sodium nitroprusside: experiment (*1*), control (*2*), and intact animals (*3*). Light bars: CCh-induced contraction. Dark bars: sodium nitroprusside-induced relaxation.

RESULTS

The test vanadium compound reduced the strength of contractions of tracheal smooth muscles induced *in vitro* by CCh in high concentrations. This compound potentiated sodium nitroprusside-induced relaxation of tracheal rings from rats with diabetes mellitus (Fig. 1). After the first treatment with CCh the strength of contractions of tracheal SMC from rats of groups 1, 2, and 3 was 119, 77.5, and 58 mV, respectively ($p>0.05$, Table 1). Further treatment with sodium nitroprusside caused relaxation of muscles in open tracheal rings from animals of groups 1 and 2 (-103.5 and 13.5 mV, respectively, $p<0.05$). Relaxation of SMC from group 3 animals reached -54 mV. Repeated treatment with CCh in a concentration of 10^{-4} mol/liter induced contraction of SMC from group 1 and 2 rats to 69.3 and 36.5 mV, respectively. Testing of tracheal preparations with CCh in various doses showed that the dose—response curves for group 2 animals were shifted toward lower concentrations of this substance. Therefore, the strength of SMC contraction in group 2 rats (mV) reached maximum at a lower concentration of CCh (compared to group 1 animals). These results suggest that the sensitivity of tracheal smooth muscles in group 2 rats was higher than in group

TABLE 2. Reactivity of Tracheal SMC in Rats to CCh in Various Dilutions (% of Maximum Value)

Group	Concentration of CCh, mol/liter						
	10^{-7}	5×10^{-7}	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}
Experiment	76.5±7.3	74.6±5.6	85.4±6.8	90.7±5.5	92.1±5.3	90.0±2.4^{+}	86.4±2.6^{+}
Control	73.9±9.4*	86.9±9.7*	88.4±6*	93.5±3.1	97.4±2.4	99.3±1.6	96.6±2.0
Intact	17.3±7.7	36.8±6.5	66.4±3.9	85.5±5.9	92.1±6.3	98.5±3.4	95.7±6.5

Note. $p<0.05$: *compared to intact animals; $^{+}$compared to control animals.

TABLE 3. Effect of Vanadium Preparation on Reactivity of Tracheal SMC in Intact Rats to CCh

Group	Concentration of CCh, mol/liter						
	10^{-7}	5×10^{-7}	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}
Intact	17.3±7.7	36.8±6.5	66.4±3.9*	85.5±5.9	92.1±6.3	98.5±3.4	95.7±6.5
Intact and vanadium preparation	-0.9±4.2	23.1±3.5	35.5±10.6	81.2±2.5	96.9±4.5	96.1±5.6	88.1±6.8

Note. $p<0.05$ compared to other group.

1 animals. In group 1 rats the strength of contractions induced by CCh in concentrations of 5×10^{-5} and 10^{-4} mol/liter was lower than in group 2 animals ($p<0.05$, Table 2). In group 3 rats reactivity of tracheal SMC to CCh in concentrations of 10^{-7}, 5×10^{-7}, and 10^{-6} mol/liter was lower than in group 4 animals ($p<0.05$, Table 3).

New organic vanadium compounds possess pronounced antidiabetic activity. The antihypertensive effect of organic preparations from vanadium is related to the influence of this microelement on Ca^{2+}-ATPase in the sarcolemma and intracellular structures of SMC [4,6]. It should be emphasized that inorganic vanadium compounds produce a spasmogenic effect on smooth muscles [7]. Attempts at using antidiabetic properties of inorganic vanadium salts in medical practice were unsuccessful, since they caused considerable side effects (body weight loss, impairment of renal functions, and disorders of the central nervous system) [10,11]. Our results show that in contrast to inorganic vanadium salts, a new complex compound of oxovanadium (IV) and isonicotinic acid hydrazide does not produce functional disturbances in smooth muscles of the respiratory tract by reducing reactivity of tracheal SMC to CCh in maximum concentration. By contrast, *in vivo* treatment with the vanadium preparation potentiates nitric oxide-induced relaxation after contraction produced by CCh in a maximum concentration. *In vivo* administration of the vanadium preparation decreases reactivity of tracheal SMC in intact rats. Our findings indicate that the effect of this preparation is not related to correction of diabetes mellitus.

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