Original Article

Study of decavanadate on rat tracheal smooth muscle rings

Azzam Ali* B.V. Venkataraman*

Contractile responses of decavanadate were compared with a vanadate standard, metavanadate and a standard spasmogen, carbachol. Both the vanadate compounds were less active on the basis of maximal response and ED_{50}. Decavanadate was more potent and long acting compared to metavanadate. Vanadate induced contractions of the tracheal rings were not inhibited by pretreatment of tracheal preparations with atropine, verapamil, EDTA and prostaglandin inhibitor, indomethacin. Reduction in the concentration of calcium had no influence on the vandadate induced tracheal contractions. Vanadate induced contractions are feeble in calcium-free or sodium-free physiological salt solution, indicating that influx of extra cellular calcium, Na^+ K^+ ATPase inhibition and Na^+Ca^{++} exchange were not involved in the contractile process. Isoprenaline - β_2 - agonist, and nitroprusside - nitric oxide generator abolished the contractions induced by vanadates but they did not completely abolish the contractile responses of carbachol. Inorganic phosphate inhibited the contractile responses of both carbachol and vanadates.

Keywords: Metavanadate, carbachol, rat tracheal-rings.

Vanadium, a trace element in all living organisms, plays an important role in physiological and biochemical processes. Deficiency state of vanadium in human or animals is not reported [1,2]. Vanadium induces contraction of many visceral smooth muscles [3-5]. Pharmacological effects of vanadium on the cardiac smooth muscles are similar to digitalis, inhibitor of Na^+ - K^+ ATPase [6]. Isolated preparations of vascular and gastrointestinal smooth muscles contract by vanadate administration [4,7]. Vanadate is found to mimic the activities of noradrenaline in cardiac muscle and pulmonary artery. A structural configuration of decavanadate similar to phenylephrine and noradrenaline was identified to explain its α-adrenoceptor activity [8].

Toxicity of vanadium has become an area of great interest in view of increasing amounts of vanadium in atmosphere due to industrial process [9]. In man, acute inhalation of vanadium pentoxide resulted in respiratory disorders such as rhinitis, bronchitis, asthma, pneumonia etc. In certain cases prolonged exposure has produced bleeding from nasal mucosa due to irritation [10]. The pulmonary effects may be due to a direct action or antigen-antibody reaction [11]. Mechanisms such as inhibition of a variety of phosphates, Na^+, K^+ ATPase and Ca^{++} - ATPase have been proposed for the contractile response of air way smooth muscles with metavanadate and prevanadate.

* *Department of Pharmacology, St. John's Medical College, Bangalore - 560 034, India*
* *Associate Professor, Department of Pharmacology, St. John's Medical College, Bangalore - 560 034, India*

Decavanadate, a vanadium compound with cage-like structural of decamer, is not studied for its pharmacological properties in smooth muscles similar to other vanadates. In the present study, decavanadate has been studied on isolated rat tracheal smooth muscle for its pharmacological actions and compared with the established vanadium salt, metavanadate.

Materials and methods

Wistar rats (250-300 g) of either sex were killed by stunning and trachea was dissected out carefully trimming fat and connective tissues. It was cut into 5 mm wise transverse rings, similar to aortic ring preparations described earlier [12] preserving the inner epithelium. These rings were suspended by means of fine steel hooks with a resting tension of 2 g in 10 ml jacketed organ baths containing a physiological salt solution (PSS) maintained at 37 ± 0.5°C with a pH of 7.4. The composition of PSS was (mM): NaCl 119, KCl 4.7, $CaCl_2$ $2H_2O$ 2.5, $MgSO_4$ $7H_2O$ 0.5, NaH_2PO_4 1.0, $NaHCO_3$ 25, Glucose 11.0. A mixture of oxygen (95%) and carbon dioxide (5%) was bubbled through the PSS. The tissue was connected via a silk thread to a Grass FT-03 isometric force displacement transducer, which was coupled with the preamplifier of a Grass polygraph (Model 79) to record the tissue contractibility. After 1 hr period of equilibration, a submaximal dose of carbachol was applied to prime the tissue for 1-2 min and washed. Carbachol was used as reference against, since histamine was not found to be active on rat tracheal ring preparations. All the tissues were further allowed to equilibrate for 1½ hr before drug application. During the total equilibration period of 2½ hr, PSS was changed several times. Dose-response curves were constructed with cumulative doses of carbachol and decavanadate [13]. Dose-response curves of test and standard spasmogens were constructed before and in the presence of verapamil, EDTA and atropine at 100 Fg concentration. In an attempt to see whether vanadate was affecting calcium influx involvement excitation-contraction coupling, the responses of vanadate in PSS with 100%, 20%, 0% calcium were studied. To study whether decavandate contractions were mediated through prostaglandin production, tracheal preparations were incubated with indomethacin (30mm) for 30 min before addition of decavanadate doses. In some experiments, ascending doses of isoprenaline, sodium nitroprusside and inorganic phosphate were added at the plateau response of the spasmogens. The tissues were allowed to rest for 1 hr between two experiments [8]. Statistical analysis was conducted using student's 't' test with $P < 0.05$ regarded as significant.

Drugs

Decavanadate ($Na_6V_{10}O_{28}$ $18H_2O$) was prepared as described earlier [14]. Briefly excess of V_2O_5 was extracted with 0.3M NaOH for 12 hr with stirring and the orange-yellow solution (pH 7) containing predominantly the deca-form (as judged by NMR) was filtered and ethanol was layered over it. After 2-3 days in cold (0-5°C) yellow-orange crystals were separated. These were filtered and dried [8]. Indomethacin was dissolved in a 1:1 water/ethanol mixture and at the dilutions used the water/ethanol mixture had no effect on vanadate responses or intrinsic resting tone in the trachea. Phosphate solution was prepared from NaH_2PO_4 adjusted to pH 7.0 with NaOH. Other drugs were obtained from the following sources: atropine sulphate (Harson laboratories, Akota, Baroda), carbachol or cabamylchline chloride (Aldrich Chemical Co. Milwaukee, WI, USA), sodium nitroprusside (BDH Chemicals Ltd., Poole, England), isoprenanline sulphate (Burroughs Wellcome & Co. Bombay), indomethacin and verapamil hydrochloride (Sigma Chemical Co. St. Louis MO, USA). All the chemicals used in the study were analytical grade and all the solutions were freshly prepared. Tissue baths were protected from light when light sensitive chemicals such as isoprenaline, nitroprusside and verapamil were used.

Results

Decavanadate, metavanadate and carbachol produced dose-dependent contractile responses (Fig. 1). carbachol produced brisk and quick responses with a duration of 4 min for each contraction (Fig. 2). After completion of dose-response curve, the base line was obtained within 20 min by frequent washes. Decavanadate, in contrast, produced slow and sluggish response with a duration of 16 min at 10 µm dose and 30 min at 30 & 100 µm doses. Maximum responses of the vanadates returned to base line in 40 min after frequent washes. Maximum effects of decavandate and metavanadate were 84% and 82% that of carbachol. ED_{50} values (µm ± SE) of carbachol, decavanadate and metavanadate were 0.29 ± 0.04, 20.70 ± 1.83 and 86.30 ± 6.44 (Fig. 3). On the basis of ED_{50}, carbachol was 71 and 297 folds more potent than decavanadate and metavanadate.

Preincubation of the tissues with atropine abolished the contractile responses of carbachol while the contractile responses of decavanadate and metavanadate remained unaltered. This indicated that decavanadate did not act through specific receptors located on the tracheal smooth muscles. Responses of tissues either incubated with verapamil or calcium-chelating agent, EDTA were not significantly altered. The contractions were not also abolished in low calcium medium but they become feeble in 0% calcium free PSS. The contractile responses of decavanadate were not differed significantly from that of control by indomethacin treatment. Isoprenaline (100 µm), and nitroprusside (300 µm) relaxed completely decavanadate and metavanadate induced contractions respectively. Both the relaxants were partially (15-18%) effective with the contractions induced by carbachol (Fig. 4 & 5). However, inorganic phosphate (100 µm) had abolished the contractile responses of all the three spasmogen (Fig. 6).

Discussion

Decavanadate exerted prolonged effects on the tone of airway smooth muscle and was more potent compared to metavanadate. The action of vanadate was not mediated through mast cell degranulation or inhibition of prostaglandin synthesis [15]. Verapamil and EDTA did not block the responses of decavanadate. However, sluggish and diminished contractions to decavanadate was noticed in calcium free physiological medium. Though calcium is essential for contraction of trachea, the source of calcium available for vanadate response was not extracellular. Contractility may depend on the intracellular stores of skeletal and smooth muscles [15,16] and liver [17]. Presence of calcium in the exogenous medium was essential for carbachol induced-contractions. Responses of tracheal preparations, incubated with indomethacin, were not significantly altered from that of control and this confirmed that responses were not mediated through prostaglandins. It has been shown that inhibition of the Na^+- K^+ ATPase system by ouabain did not have a significant influence on the development of tone in tracheal smooth muscle [18].

The lack of effect of receptor blockade on vanadate induced tracheal contractions indicated that it probably acts at the post-receptor intracellular level. Chemical similarity of phosphate was suggested for the competitive inhibition of vanadate entry into the cell. Inhibition of vanadate uptake into tracheal smooth muscle cells by phosphate could then account for its specific inhibitory effect on vanadate - induced contractions in the trachea [19]. However, inorganic phosphate, but not isoprenaline and nitroprusside, inhibited the contractile responses of the trachea induced by carbachol, decavanadate and metavanadate.

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Fig. 1 Dose-response curves of Carbachol, Decavandate and metavandate on rat tracheal rings

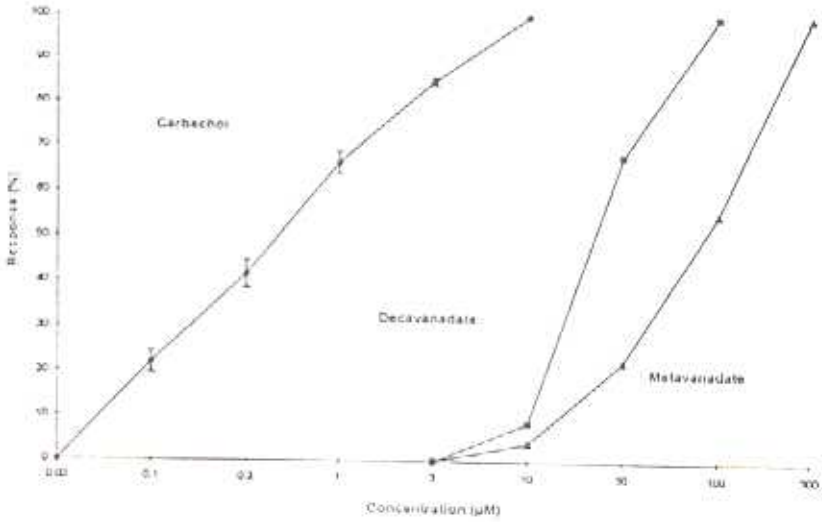


Fig. 2 Duration of Decavanadate compared to Carbachol and Metavanadate on rat tracheal rings

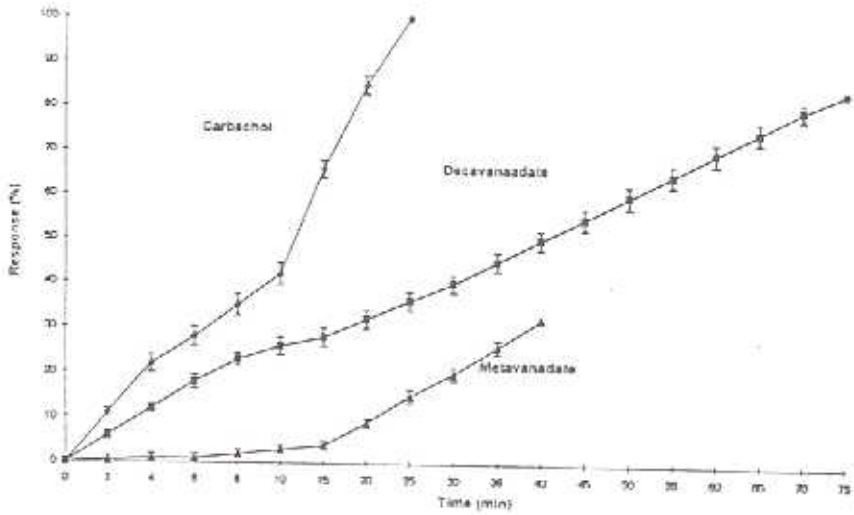


Fig. 3 Potency of Decavanadate compared to Carbachol and Metavanadate on rat tracheal rings

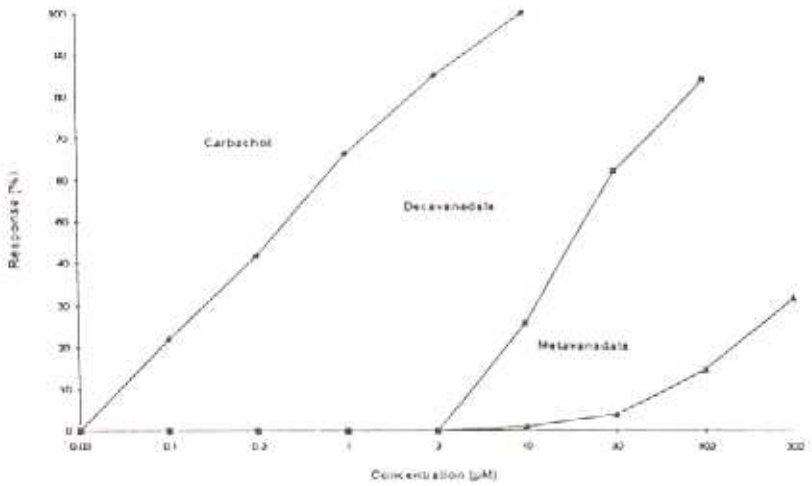


Fig. 4 Effect of isoprenaline on the contractile responses of Carbachol, Decavanadate and Metavanadate on rat tracheal rings

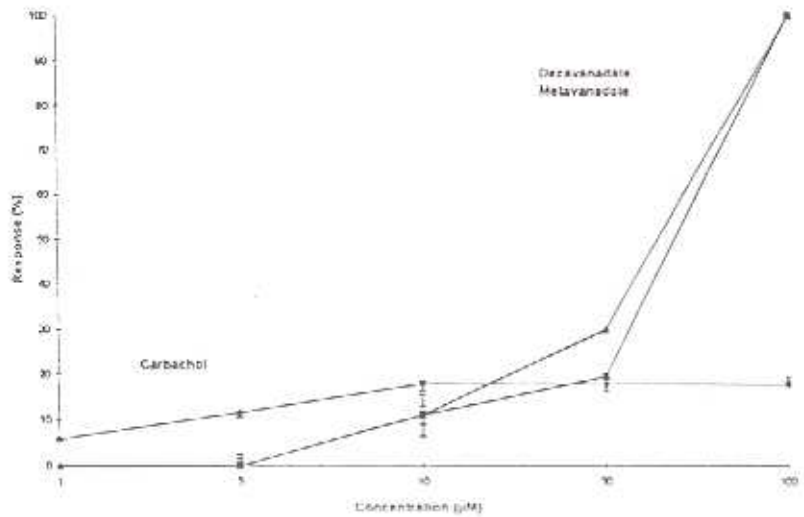


Fig. 5 Effect of nitroprusside on the contractile responses of carbachol Decavanadate & Metavanadate on rat tracheal rings

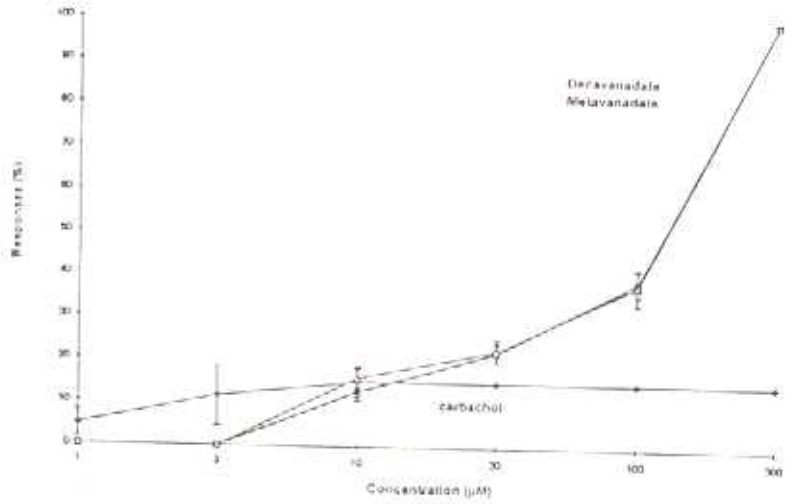
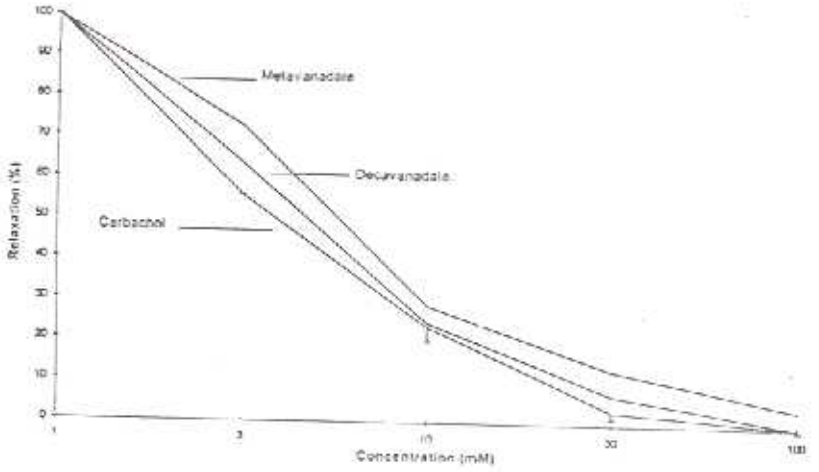


Fig. 6 Effect of Phosphate on the contractile response of Decavanadate and Metavanadate



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