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*REVIEW ARTICLE*

# A PERSPECTIVE OF SMOOTH MUSCLE CONTRACTILITY THROUGH ACTIONS OF VANADIUM COMPOUNDS

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**Abstract :** Smooth muscle contraction has a characteristic step-response with successive additions of stimulating compounds, and instant reversal on withdrawing the stimulus, indicative of an equilibrium situation wherein continuous, rapid reactions are occurring.

Vanadium compounds, ortho- and meta-vanadates, decavanadate and peroxovanadate, were found to contract a variety of smooth muscles. Their actions were analyzed with respect to activation of receptors, increase in the intracellular calcium concentration, and increase in calmodulin-dependent myosin light chain phosphorylation leading to contraction. A new perspective of smooth muscle contractility has emerged from the studies with vanadium compounds suggesting control mechanisms involving phosphorylation for contraction and redox for relaxation.

**Key words :** muscle smooth muscle vanadium

**The growing importance of vanadium in biology**

Vanadium, a group V transition element (atomic wt. 50.94), is the 21st most abundant elment in earth-crust but occurs in traces in biosphere. Vanadium is now considered essential in trace quantities, has known therapeutic applications in pharmacological doses, and is toxic in excess. By restricting absorption the cells seem to have developed a mechanism to keep vanadium concentration low, and by sequestering the internalised vanadium compounds they avoid potential hazards. Absorption, accumulation, and excretion of vanadium compounds in animals appear to be under homeostatic control. Some characteristic deficiency symptoms are known in birds and plants, but none so far in animals. In support of its biological relevance, vanadium is now found naturally associated with two types of proteins, bromoperoxidases in marine organisms and an Azotobacter mutant nitrogenase, and it is essential in their reaction mechanisms (for early work, see reviews 1–5).

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The pharmacological value of metavanadate was recognized a century ago in France and it was acclaimed as "Panacee Universelle" for the treatment of extraordinarily diverse diseases : anaemia, malnutrition, tuberculosis, syphilis, and diabetes. A tonic (neogadine) containing small quantity of metavanadate based on original French formulation is available in the Indian market. Chronic treatment with vanadium compounds is beset with unacceptable toxic response such as anaemia, leucopaenia, renal and bone damage, diarrhoea, decreased sperm count, and paralysis. Consumption of excess metavanadate causes nausea, vomiting, giddiness, bradycardia and coronary insufficiency (6). Increased ingestion of vanadium oxide is now becoming common because of inhalation of dust and smoke arising out of burning of vanadium-rich fossil fuels in industries and heavy automobile traffic. This affects lungs causing oedema, bronchopneumonia and asthma (7).

Most food material used for human consumption contains vanadium in concentrations below 0.1 μg/g. Accessory food items such as black pepper, tea leaf, cocoa powder, tobacco, and mushrooms contain vanadium in μg/g concentrations (5). Dietary supplement of vanadate increases its tissue content which is stored in a non-toxic form. The importance of vanadium as a powerful inhibitor of the sodium pump was recognised after it was found to be the active inhibitory principle (8), present in the samples of crystalline ATP, derived from horse muscle, but not in yeast or synthetic samples.

**Major biological effects of vanadium**

The four following revelations on biological effects of vanadium during 1977–1980 raised the status of this element from that of a low adventitious contaminant to one of high biological relevance.

Cantley and co-workers (8) found in 1977 that vanadate occurring as a contaminant in Sigma crystalline ATP is a potent inhibitor of Na, K-ATPase (Ki=40 nM). Its action was reversed by the natural adrenergic receptor agonist, noradrenaline (9). This was the beginning of understanding of the potential of vanadate in enhancing effectiveness of a variety of phosphate esters, including phospho-proteins, by inhibiting their hydrolysis.

Ramasarma and co-workers (10) found in 1981 that oxidation of NADH by dioxygen was enhanced several fold in liver plasma membranes, microsomes, and the Golgi apparatus, and erythrocyte membranes by a polyvanadate preparation, and this oxygen-consuming activity was inhibited by noradrenaline and, paradoxically, by a low concentration of superoxide dismutase. This led to the discovery of peroxo-vanadate intermediates that act as powerful selective oxidants, and laid the foundation of redox basis of action of vanadium compounds (see review 11).

Dubyak and Kleinzeller (12), working with vanadate, and Shechter and Karlish (13) with vanadyl, showed in 1980 that these compounds had the insulin-mimetic action of enhancing glucose oxidation in rat adipocytes. These reports marked the

resurgence of interest in finding anti-diabetic vanadium compounds with low toxicity, and identification of peroxo-vanadates as possible active compounds that activate directly the cascade of enzymes that normally follows activation of insulin-receptor (11).

About the same time, vanadate was reported to mimic activity of noradrenaline in contracting pulmonary artery (14) and in increasing force of contraction of cardiac muscle with concomitant increase in cyclic AMP concentration (15).

**Vanadium and smooth muscle contractility**

Contractile effects of vanadate were found in many smooth muscle preparations. Some of these are listed in Table I. Most reports were based on the action of monomeric vanadates, ortho- or meta-forms, which form $VO_3^-$ and other anionic species in neutral buffer solutions. The concentration used varied from 0.01 to 10 mM. It is amazing that an ordinary inorganic salt is able to mimic a highly specialized receptor-mediated action obtained with endogenous organic molecules. A number of reviews on vanadium research are available. In this review we focus attention on smooth muscle contractility and the effects of vanadium compounds.

**Some key features of smooth muscle contractions**

The widely distributed smooth muscles perform a variety of functions in the body, as maintaining blood flow and blood presure, moving the contents in gastrointestinal tract, parturition, urination, defecation or sperm ejection (32). The core of these activites is the contraction of smooth muscle, and propagation of the wave of contraction across the tissue 2-10 μm in diameter and 20-600 μm in length. As in

TABLE I : Summary of reports on vanadium-dependent contraction of smooth muscles.

| *Species* | *Smooth muscle* | *Authors (year)* | *Reference No.* |
|---|---|---|---|
| Cannine | saphenous vein | Huot et al. (1979) | 16 |
| Guinea-pig | pulmonary artery | Ozaki & Urakwa (1980) | 14 |
| Rabbit | intestine | Hudgins & Bond (1981) | 17 |
| Guinea-pig | lung parenchyma | Nayler & Sparrow (1983) | 18 |
| Guinea-pig | trachea | Nayler & Sparrow (1983) | 18 |
| Guinea-pig | blood vessels | Shimada et al (1986) | 19 |
| Cat | cerebral artery | Sanchez-Ferrer et al (1988) | 20 |
| Cat | femoral artery | Sanchez-Ferrer et al (1988) | 20 |
| Guinea-pig | vas deferens | Sunano et al. (1988) | 21 |
| Guinea-pig | ureter | Sunano et al. (1988) | 21 |
| Rabbit | ileum | Candura et al. (1993) | 22 |
| Rat | aorta | St. Louis et al. (1995) | 23 |
| Rat | uterus | Ponce-Monter et al. (1995) | 24 |
| Rat | aorta | Venkataraman et al. (1997) | 25 |
| Rat | trachea | Azzam & Venkataraman (1997) | 26 |
| Rat | aorta | Rapp | 27 |
| Rat | vas deferens | Garcia et al. (1981) | 28 |
| Rat | blood vessels | Ozaki et al. (1982) | 29 |
| Rat | aorta | St. Louis & Sicotte (1992) | 30 |
| Rat | uterus | Gokita et al. (1994) | 31 |

striated muscle, contraction in muscle depends on thin (actin) and thick (myosin) filaments in sarcoplasmic reticulum. Smooth muscle, having only one-third of myosin and therefore lower ATPase activity than that in skeletal muscle, with a much greater actin : myosin ratio (3:1), produces equivalent forces and shortens to a very small faction of the length at rest. The thin filaments are large in number and are arranged randomly in relation to the thick ones.

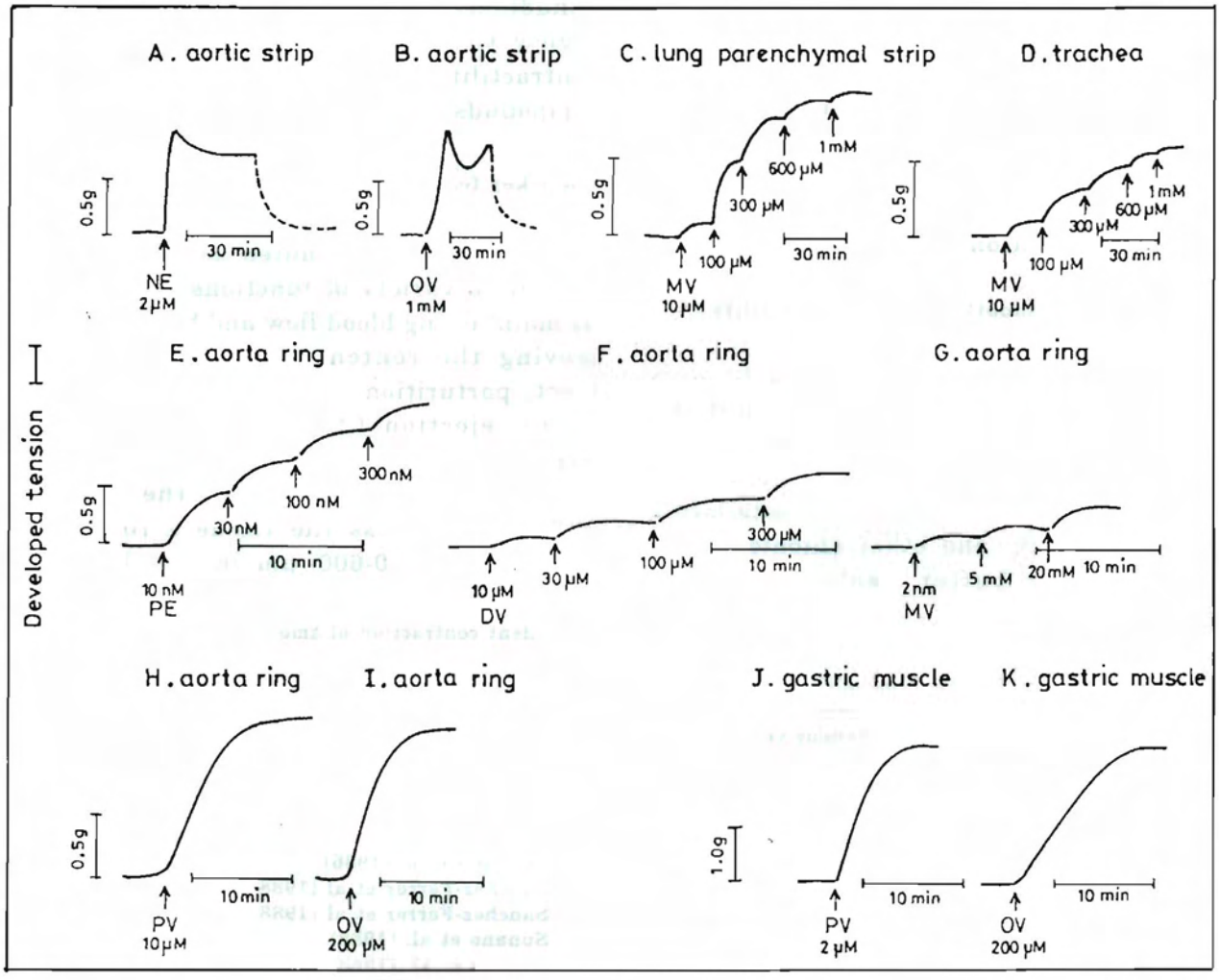


Fig. 1 : Some examples of vanadate induced smooth muscle contraction. Developed tensions with respect to time after treatment with different concentrations of vanadium compounds. (OV = orthovanadate, MV = metavanadate, DV = decavanadate, PV = peroxovanadate) and two reference compounds (NE = noradrenaline, PE = phenylephrine) are shown. The scales in each case are given. The samples are from guinea-pigs for A-D and from rats for E-K. Broken lines indicate the fall in developed tension on withdrawal of the effector. The data are collected from the following sources: A,B: Ozaki and Urakawa (14); C,D: Naylor and Sparrow (18); E-G: Venkataraman et al. (25); H-K: Laniyonu et al. (40).

Contraction is due to cross-bridge cycling which consumes ATP, and is initiated by phosphorylation of myosin light chain (20 kDa protein) by the kinase activated by increase in intracellular free calcium. Relaxation occurs by the reverse process when free calcium is sequestered or removed from the cell, and the myosin light chains are dephosphorylated.

In many smooth muscles excitation due to hormones and other initiator-agonists increases calcium permeability by opening voltage- or ligand-gated channels. In multiunit smooth muscles the signal is not coupled and elicits individual response, whereas in unitary smooth muscle the cells are electrically coupled and the cells respond as a unit in synchrony. Many muscles are supplied with inhibitory and excitatory nerves (33).

Smooth muscles develop force per unit comparable with that of striated muscles, but they consume lower amounts of ATP in view of low myosin ATPase activity. A characteristic of smooth muscles is that they sustain the developed tension, without further need of ATP and cross-bridge cycling. Indeed, a step response is obtained with increments of agonists or vanadate in serveral smooth muscle samples tested (Fig. 1). This indicates that the rapidly attained "phosphorylation status" is retained at each step, to be relaxed only on removing the initiating compound by washing the tissue.

The effects of vanadium on smooth muscle contractility have to be localised in one or more of the steps involved at the levels of the receptors, calcium concentration, phosphorylation status of protein and activity of ATPase. On the other hand the forms and actions of vanadium effecting these changes are to be considered. The available information is reviewed and evaluated in the following sections.

**Action of vanadate at the level of receptor and membranes**

In general muscarinic, histaminic ($H_1$) and adrenergic receptors are not involved in vanadate action as preincubation of the smooth muscle tissue with phentolamine, tolazolone, atropine and mepyramine do not abolish the contractile responses at concentrations that abolish actions of agonists such as noradrenaline (18, 25, 26, 34). As it is a weak effector, vanadate was used at high concentrations. In our laboratories, decavanadate ($V_{10}$) showed contraction of rat aortic rings at 0.3 mM and this effect was blocked by 0.3 mM concentration of phenoxybenzamine and prazosin and not at 10 nM sufficient to reverse the effect of 10 μM phenylephrine, an adrenergic agonist (25). These and other observations (35, 36) indicated that decavanadate possesses weak noradrenaline mimetic activity. It was found that decavanadate (35, 37), but not metavanadate even at high concentrations, decreased concentration of calcium and activity of pyruvate dehydrogenase in mitochondria in livers of rats which was similar to the effects obtained on treatment with noradrenaline (38). An integral part of the action of agonists of α-adrenergic receptor is an elevation of cytosolic calcium and increase in plasma membrane-located protein kinase C (PKC) activity. Both these responses were obtained on short-term perfusion of liver tissue with decavanadate before any

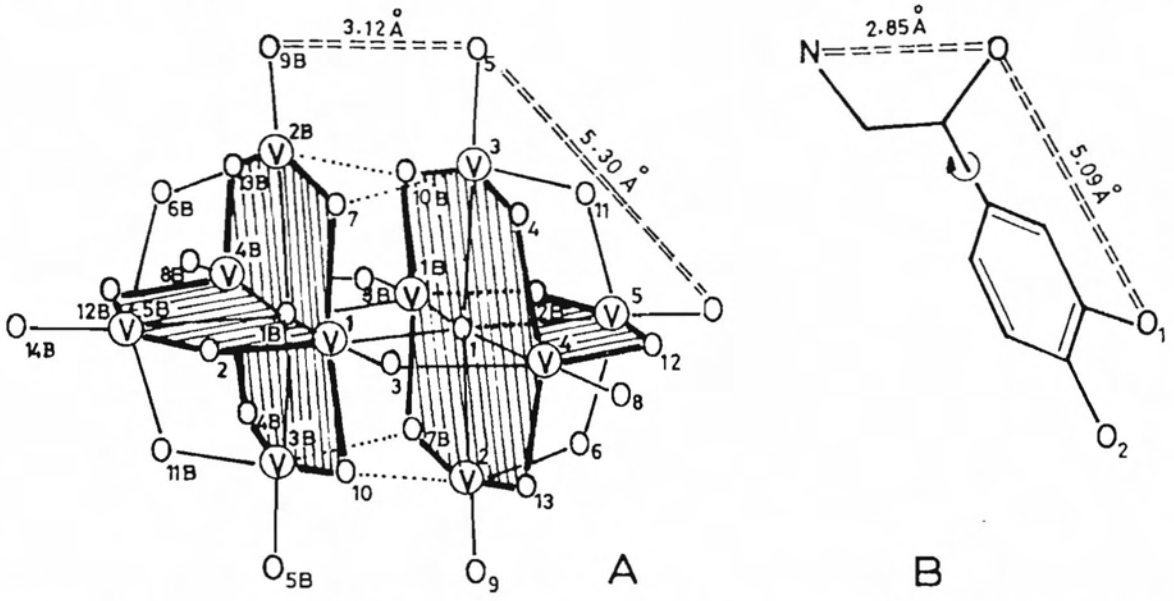


Fig. 2 : A common structural motif between decavanadate (A) and noradrenaline (B). Relative positions of three functional groups of amino-N, $O_3$ and $O_1$ in trans-β form of noradranaline show distances close to $O_{5B}$, $O_5$ and $O_{14}$ of decavanadate, which came into the motif only because of dimerization of two $V_5$ units identified by numbers and their B equivalents (V numbers as superscripts and O numbers as subscripts). Adapted from Venkataraman et al. (25).

detectable vanadium accumulation. This indicated membrane level of action and direct activation of α-adrenergic receptor by decavanadate.

The extraordinary feature of an inorganic oligomer, decavanadate, which possesses merely the structural elements of V-O bonds, to stimulate the specific action of a natural catecholamine is puzzling. Decavanadate ($V_{10}$), formed by dimerization of $V_5$ under acid conditions, possesses a structural feature of two pairs of unshared oxygen atoms at a distance of 3.12 Å, not found in its constituent units of $V_4$ or $V_5$. A structural motif of O-O-O using these oxygen atoms was recognised in $V_{10}$ which matches a motif of N-O-O formed with the essential amino- and hydroxy- groups of the side chain and m-hydroxyl group of the ring (25) (Fig. 2). This is found only in the trans-β form of noradrenaline that is identified with the α-adrenergic action (39). Decavanadate is likely to arise from metavanadate on storage or on acidification of alkaline orthovanadate solution because of characteristic easy polymerization of vanadate. Some of the reports on good contractile effects of metavanadate, including that on rat aortic rings by Srivastava and coworkers (23), may involve $V_{10}$ as the active form.

**Membrane depolarization and ion channels**

Depolarization of the resting negative potential (–40 to –70 mV) of smooth muscle membranes opens, "voltage-gated" calcium channels and trigger contraction of the tissue. On exposure to $K^+$ -depolarizing

solution, smooth muscles contract rapidly and revert slowly to initial resting tension. Addition of vanadate at this stage produced contraction (18) showing that its action is independent of depolarization of the membrane.

The variety of ion channels present in plasma membranes are elegantly used by smooth muscles for the diverse functions. These can be regulated by the direct action of effector molecules ("ligand-gated") in addition to membrane potential. Exposure of smooth muscle tissues to a sodium-deficient medium decreased contractile action of vanadate, indicative of the need for intracellular calcium for this effect (18, 34, 40). Blockers of calcium channel abolished tonic phase of contraction by preventing the availability of external source of calcium through voltage-gated channels (41, 42). Vanadate-dependent contraction in rat aorta and trachea was not affected by either lack of external source of calcium or pretreatment with calcium-chelator, EDTA (25, 26), and therefore calcium-channel blockers are of no use in the experiments with these tissues. In contrast, in rat uterine and ileal smooth muscles, vanadate effects were diminished (22, 24). This indicates variable dependence on external calcium among different smooth muscles.

#### Release of endogenous effectors

Vanadate may be releasing endogenous effector molecules that aid the contraction process. Some of these tested gave negative or ambiguous results. Endogenous prostaglandin production can be controlled by treating the tissue with indomethacin, an inhibitor of cycloxygenase. Pre-treatment of rat aorta with indomethacin before vanadate challenge had no effect on the contraction (18, 30). The response to vanadate was no different after two exposures of histamine-releasing mast-cell degranulation agent, compound 48/80, showing the mediators in this process are not involved in vanadate action (18).

Hydrogen peroxide ($H_2O_2$) is generated in small concentrations in all aerobic cells (43), including smooth muscles (44, 45). In pig coronary artery smooth muscle, $H_2O_2$ was found to hyperpolarize and relax $PGF_{2\alpha}$-induced contraction (46), and an unusually high concentration (4000 units/ml) of catalase was required to suppress this $H_2O_2$-effect. This is reminiscent of vanadate-$H_2O_2$ complex, diperoxovanadate, that is resistant to catalase action (47). Excess $H_2O_2$ may in fact be suppressing the contractile effect of endogenous trace amounts of vanadate, as free vanadate in addition to diperoxovanadate is required to generate some oxidant species.

#### Effects of ion-pump ATPase

Every effort to explain action of vanadate first deals with its powerful inhibition of Na,K-ATPase. Inhibition of this pump activity by ouabain and potassium-free solution produced contraction of guinea-pig aortic smooth muscle (14). With smooth muscle, all evaluations proved that the vanadate-dependent contraction phenomenon does not use the sodium pump. Indeed, vanadate produced contraction of vascular smooth muscle similar to that of noradrenaline even when Na,K-ATPase was inhibited with ouabain and in potassium-

free solution, and the ouabain-sensitive relaxation induced by restoring normal potassium remained unaffected by vanadate treatment (14). Such potassium-induced relaxation is indicative of involvement of Na,K-ATPase activity. Similar experiments with rat aorta smooth muscle also showed that vanadate did not influence the potassium-induced relaxation(27) and this even led them to wonder whether the enzyme activity in this tissue is sensitive to vanadate. On the other hand, they found that a known inhibitor of anion transport, 4,4'-diisothiocyano-2,2' -disulfonic acid stilbene, blocked vanadate-dependent contraction. Intracellular action of vanadate is thus indicated.

The contractile response of guiea-pig tracheal smooth muscle to vanadate remained unaffected in presence of ouabain sufficient to inhibit Na,K-ATPase (18). These workers demonstrated the need for anion pump activity for vanadate action by competing with phosphate in a dose-dependent fashion.

It is well established that tension development in contraction cycle correlates with intracellular calcium concentration. Vanadate-dependent contractions require calcium in the bathing solution for maintaining the developed tension. In calcium-free medium contractions diminished slowly. Also, blocking influx of extracellular calcium by verapamil had no effect on contraction produced by vanadate. These observations indicated that the intracellular store supplied calcium.

Vanadate has a distinct effect on mobilization of intracellular calcium in a variety of smooth muscles : guinea-pig taenia coli (48), toad stomach smooth muscle (49), guinea-pig airway smooth muscle (18), rat aortic smooth muscle (50), and canine tracheal smooth muscle (51). This appears to be a general cellular response as skeletal muscle (52) and liver (35) tissues also respond similarly. Mitochondrial calcium was found to be the source in the liver tissue for the released calcium which was also prevented from leaving the cell (35). This is obviously due to inhibition of Ca-ATPase, a general effect pervading all cells (53). It is interesting to note that direct measurement of inhibition of Ca-ATPase by vanadate in smooth muscle was not done and inferences were indirectly with studies on concentrations of efflux of calcium.

**Dependence on protein phosphorylation not universal**

As a natural consequence of calcium-involvement, protein phosphorylation is expected to be the mechanism of signal transduction process. Activation of PKC was implicated in the tonic contracton of smooth muscle maintained at the basal calcium concentration (54). Inhibitors of PKC, such as staurosporine and calphostin counteracted the contractile effect of vanadate (55, 56) over the entire time-course of testing.

Vanadate treatment enhanced protein tyrosine phosphorylation state in guinea-pig taenia coli during enhanced contraction both by inhibiting the phosphatase and activating the kinase activity (57). Genistein, a protein tyrosine kinase (PTK) inhibitor, reversed these effects, but it failed to do so with rat uterus (31).

Following increase in intracellular calcium, calmodulin-dependent phosphorylation of 20 kDa myosin light chain (MLC) by MLC-kinase occurs, and this is considered sufficient to initiate smooth muscle contraction (58). Indications are now available that contraction is possible in the absence of MLC phosphorylation. ATPase activity of a chemically modified gizzard myosin was activated by actin without phosphorylation (59). Polylysine was able to activate smooth muscle actin-myosin interaction in the absence of phosphorylation of the light chain (60). This is most unexpected since phosphorylation of a protein is considered to enhance its negative charges whereas polylysine will contribute many positive charges. Even more significiant than this is the observation that a slow cycling of cross-bridge was found under certain conditions (61) in swine carotid artery which was not dependent on calcium or myosin light chain phosphorylation. A similar situation was encountered in vanadate-induced contraction of guinea-pig taenia coli smooth muscle – the 20 kDa MLC phosphate band was inconspicuous (62). The foregoing evaluation of protein phosphorylation status does not support the well-accepted mechanisms for explaining vanadate-induced smooth muscle contraction.

The influence of various inhibitors and compounds on these vanadate actions on vascular and tracheal smooth muscles are summarized in Table II. These fit with their generally known effects and responses of the contraction process of skeletal muscle (33). Yet some differences are seen, prompting a careful look at the basis of vanadate actions. An overview of the chemical reaction potential of vanadate and its complexes given below is expected to aid in understanding vanadate actions.

**Forms of vanadium and their reactivity**

The beautiful colours of vanadium minerals prompted its naming after the Norse goddess of beauty. "Vanadis" by its Swedish discoverer. Vanadium compounds exist mainly in three valence states of 3+, 4+, and 5+. Of these trivalent - $V^{3+}$ is unstable at physiological pH and undergoes rapid oxidation and picks up a molecule of $O_2$ to form peroxo-$V^{5+}$. The tetravelent-$V^{4+}$ is stable in acid pH as the blue vanadyl cation, a powerful reducing agent. Vanadyl is not easily oxidized by $O_2$ and does not generate superoxide ($O_2^{\cdot-}$), on autooxidation (63), as mistakenly presumed with no experimental support (64). It can be oxidized by $H_2O_2$ and generates vandate-$V^{5+}$ and peroxo-complexes with the release of oxygen, as shown by the excellent studies of Brooks and Sicilio (65). Pentavalent vanadium ($V^{5+}$) compounds exist as monomers (ortho and meta-forms), and dimer ($V_2$) and tetramer ($V_4$) (66). The chemical reactivity of these is similar to that of the phosphate analogues. With P-O bond being shorter than V-O by about 0.1Å, competition between them occurs for transport and reactivity at active sites of enzymes. This explains the remarkable influence exerted by vanadate in phosphate enzymology. Vanadate has its own charateristic property of polymerizing into a cage-like structure of decamer ($V_{10}$) gaining a golden-yellow colour. This $V_{10}$ is easily formed in acid solutions by dimerization of two pentamers ($V_5$) (25).

TABLE II : Effect on vanadate-dependent vascular and tracheal smooth muscle contraction on treatment with some inhibitors and compounds.

| *Inhibitors and compounds* | *System and activity modified* | *Effect on smooth muscle contraction (reference no.)* | |
|---|---|---|---|
| | | *Vascular* | *Tracheal* |
| Indomethacin | cycloxygenase inhibitor (prostanoid synthesis) | no effect (30) | no effect (26) |
| Phenoxybenzamine (high concentration) | α-adrenergic antagonist | reversed (25) | |
| Prazosin (low concentration) | α-adrenergic antagonist | no effect (25) | no effect (18) |
| Prazosin (high concentration) | α-adrenergic antagonist | reversed (25) | |
| Atropine | muscarinic blocker | no effect (25) | no effect (26) |
| Mepyramine | histaminic blocker | no effect (25) | no effect (26) |
| Staurosporine | PKC inhibitor | reversed (40) | |
| Genistein | PTK inhibitor | reversed (40) | |
| EGTA | $Ca^{2+}$ chelator | no effect (25) | no effect (26) |
| Nifedipine | $Ca^{2+}$ Channel blocker | no effect (25) | no effect (26) |
| Verapamil | $Ca^{2+}$ Channel blocker | no effect (25) | no effect (26) |
| Ouabain | Na, K-ATPase inhibitor | no effect (27) | |
| 48/80 | Mast cell degranulation | | no effect (18) |
| Egg yolk (white) | Mast cell degranulation | | no effect (26) |
| Phosphate | Competitor for anion channel Vanadate uptake decreased | | reversed (26) |
| DIDS | Competitor for anion channel Vanadate uptake decreased | reversed (27) | |

PKC = Protein Kinase C; PTK = Protein Tyrosine Kinase; DIDS = 4,4'-diisothiocyano-2,2'-disulfonic acid stibene

Vanadium-$V^{4+}$ can be formed from $V^{5+}$ by cellular reductants such as ascorbate, glutathione and noradrenaline, and also by bromide at high concentration and at acid pH conditions (25). A microsomal NADH-dependent enzyme system reduces devavanadate-V, but not metavanadate (67). The reduced form, vanadyl, can enter into reactions with peroxo-compounds, as given below.

The crucial reactions of vanadate are the formation of addition complexes with $H_2O_2$ with mono-, di- and tri- peroxo-vanadates favoured as products in acid, neutral and alkaline pH, respectively (69). The product at physiological pH is diperoxovanadate. This is stable in aqueous solutions and is degraded by catalase at extremely slow rate compared to $H_2O_2$ (47). Thus, formation of diperoxovanadate is a simple way of preserving small quantities of cellular $H_2O_2$ in presence of abundant catalase and to make available the peroxide for use as substrate in peroxidase reactions and for selective oxidation of enzyme proteins far more efficiently than $H_2O_2$ e.g. glyceraldehyde-3-phosphate dehydrogenase (69) and phospholipase D (70).

A divanadate (VOOV) containing $H_2O_2$-derived μ-peroxo-bridge is formed by interaction of diperoxovanadate and vandyl [$OVOOV(O_2)$; $(O_2)$ represents bidentate peroxo-group] (71). This is shown to be more effective at physiological pH in oxidizing

bromide in bromoperoxidation, and in inactivating glucose oxidase (72). This complex with VOOV structure makes it reactive at this pH in contrast to the need for high acidic conditions for $H_2O_2$ (HOOH) to oxidize bromide.

The OVOOV($O_2$) is unstable and readily breaks up to OVO and an oxygen radical of peroxovanadate, °OV($O_2$), less powerful than the related °OH radical in reacting with quenching compounds (71). This radical species rapidly degrades and releases half-equivalent of oxygen (73), but it can also serve as an oxidant for NADH (71). The reaction squence of some chemical forms of vanadate is shown in Fig. 3.

**What is "pervanadate" ?**

Several laboratories have been working with a reagent containing an equimolar mixture of orthovanadate and $H_2O_2$ called pervanadate. The alkaline orthovanadate would be neutralized by protons released and depending on the rapidity of addition of $H_2O_2$ some decavanadate may be formed as indicated by the yellow colour. The bulk of vanadate will be converted to diperoxovanadate at neutral pH as is well known for this reaction (66). This has been reconfirmed by $^{51}$V-NMR spectra (69). Invariably, excess catalase was added and the mixture was further incubated for several minutes. This in fact is unnecessary and leads to ill-defined, partial loss of diperoxovanadate (47) reverting back to monomeric vanadate with release of oxygen. Such preparations used as "pervanadate" would contain a mixture of undegraded diperoxovanadate, monomeric vanadate

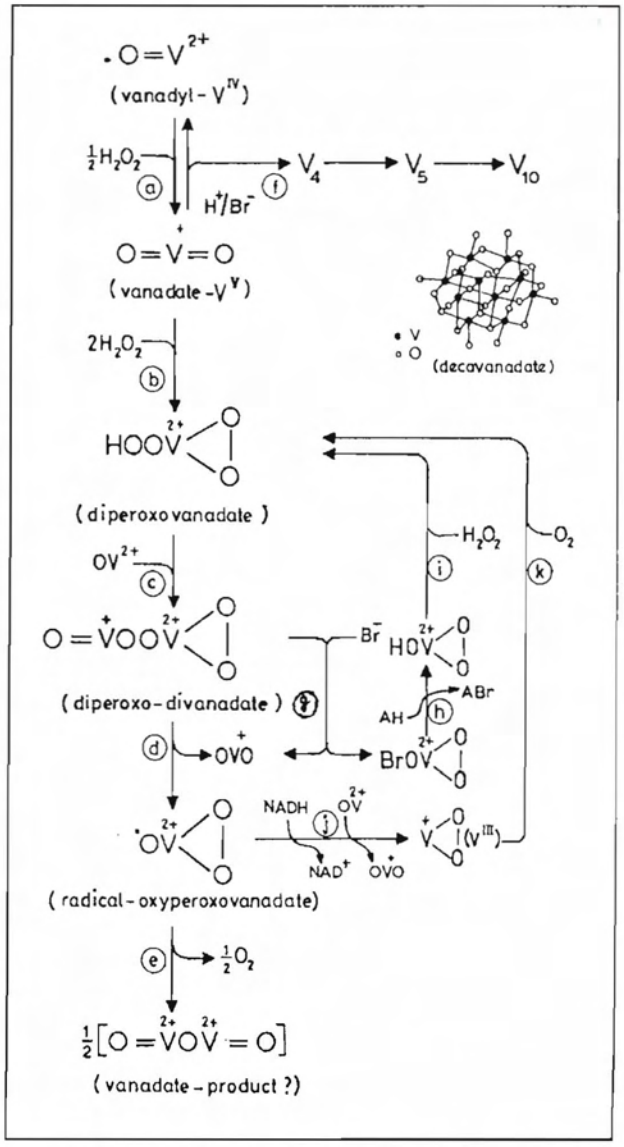


Fig. 3 : Some redox reactions of vanadium compounds: a. oxidation of vanadyl by $H_2O_2$; b. formation of diperoxovanadate from vanadate; c. addition complex of diperoxovanadate; d. generation of the radical species °$OVO_2$; e. dismutation of the radical releasing ½$O_2$; f. reduction of $V^{5+}$ to $V^{4+}$ and polymerization to decavanadate ($V_{10}$); g. oxidation of bromide; h. bromine transfer to acceptor AH; i. conversion of monoperoxo-to diperoxovanadate; j. oxidation of NADH by °$OVO_2$ radical and reduction of $V^V$ to $V^{III}$; k. oxidation of $V^{III}$ $O_2$ regenerating diperoxovanadate. All the intermediates provide powerful, selective oxidants. Adapted from References 11, 72 and 73.

and small amounts decavanadate. Notwithstanding this, pervanadate proved to be far more effective than vanadate and this is due to the peroxo-form and also due to possible availability of vanadyl by reduction of decavandate in this mixture.

**Peroxovanadates as biological oxidants**

It is becoming obvious that these reactions of peroxovanadates endow vanadium with selective biological oxidant status. More proteins and enzymes, it is anticipated, will undergo such redox reactions as part of their regulation. Examples of this are already available in the case of MAP kinase in the insulin-responsive cascade which is activated by vanadate (75) and pervanadate (76), and ERK-1 and ERK-2 by diperoxovanadate (70) and thus explains insulin mimetic action of vanadate.

In view of doubts being raised on the participation of phosphorylation reactions in vanadate-induced contraction of smooth muscle, it is necessary to look for alternative modes of action. Some vignettes are appearing in the *literature* and some approaches are discussed below which, on reinterpretation of the data, will fit with a redox-based mechanism.

**Decavanadate action in smooth muscle contraction – atternative explanation**

Decavanadate ($V_{10}$), an inorganic polymer of vanadate with a cage-structure (Fig. 2), produced contraction of rat aortic rings similar to phenylephrine, an agonist of α-adrenergic receptor. The effectiveness of phenylephrine, decavanadate and metavanadate respectively, was in nM, μM, and mM concentrations (25). To the list of actions of noradrenaline that polyvanadate can mimic was added the contraction of aortic rings. One paradox remained. A high concentraton of α-adrenergic receptor antagonist such as phenoxybenzamine was required to prevent this effect of decavanadate as well as the increase in hepatic plasma membrane-localized PKC activity (36). The phenylephrine-responsive receptor remained unaltered and needed only nmolar concentraction of the antagonist to inhibit. This prompted the question, "is it possible that devavanadate achieved its noradrenaline-like actions through a low affinity receptor, or an alternative intracellular mechanism ?" (36).

Inhibitors of PTK, genistein (57) and staurosporine (23) decreased vanadate-induced smooth muscle contraction. Such changes in protein phosphorylation status are also possible with monomeric vanadate if inhibition of phosphate hydrolysis is the main basis of action. Decavanadate invariably was found to be more effective in the noradrenaline-like actions (77). Because of easy polymerization, especially on acidification of solutions, decavanadate would definitely be present in concentrated vanadate solutions (25, 78).

Earlier we explained the weak α-adrenergic action of decavanadate by its possessing a structural motif of O..O..O.. that matches within 4-8% of distances of N..O..O.. motif of noradrenaline. If this enables decavanadate to interact with the receptor, more firmly binding antagonists could have counteracted its actions. A

standard antagonist like phenoxybenzamine did reverse many decavanadate actions (25, 35, 36), but at almost equimolar concentration. This raises the issue whether these molecules are interacting with protein(s) other than the adrenergic receptor, and thereby involve other reactions. One possibility is the NADH-$V_{10}$ reductase reaction specific for decavanadate. This will produce the reduced form, vanadyl (67), that is turn can lead to other oxidants mentioned above.

**Peroxovanadate also shows smooth muscle contraction**

The work of Hollenberg and co-workers (40) provided an example of peroxovanadate being more efficient than vanadate in contracting rat gastric longitudinal muscle strips and aortic rings. This effect was not due to release of a neurotransmitter or an endothelial cell-derived factor. It is not due to inhibition of Na,K-ATPase or Ca-ATPase. Extracellular calcium was in fact required for this contractility. "Pervanadate" is known to inhibit protein-tyrosine phosphatase more potently than vanadate (79). Importance of protein-tyrosin status is further enhanced by the findings that genistein and tyrphostin, PTK inhibitors, do inhibit peroxovanadate effects. Thus activation of PTK by peroxovanadate is also a possibility. Which proteins are involved in such increase in protein-tyrosine-phosphate and whether calcium is involved in the chain of these reactions are not known. Notwithstanding the gaps in information, it is obvious that alternative routes using tyrosine phosphorylation lead to crossbridge formation and contraction of smooth muscle.

**Protein-tyrosine phosphorylation and phospholipase D activity are enhanced by diperoxovanadate**

The work of Natarajan and co-wokers (70, 80) indicated direct effect of peroxo-compounds on the signal transducing systems in vascular endothelial cells of bovine pulmonary artery. Initially, $H_2O_2$ and fatty acid hydroperoxide were found to enhance the activity of phospholipase D activity (80). Later it was found that treatment of these cells with diperoxovanadate at 100-fold lower concentration caused an increase in tyrosine phosphorylation of several protein bands (20-200 kDa), determined by Western blot analysis with antiphosphotyrosine antibodies (70). This enhanced activity was sensitive to pretreatment with putative PTK inhibitors such as genistein, herbimycin and tyrphostin and by chelation of medium calcium but not with a PKC inhibitor, bisindolymaleimide. Most interesting of these findings is the attenuation of diperoxovanadate-induced enhancement of protein-tyrosine phosphorylation and phospholipase D stimulation by anti-oxidants such as N-acetyl cysteine and pyrrolidine dithiocarbamate. It became obvious that oxidant capacity of diperoxovanadate is used in these actions.

**Can muscular relaxation by nitric oxide occur through redox reaction?**

A very interesting piece of work was reported by Stamler and co-workers (81) on the involvement of nitric oxide in skeletal muscle action. Like reactive oxygen intermediates (82), nitric oxide was found to modulate contraction of rat skeletal

muscle. This tissue was found to have neuronal-type nitric oxide synthase. Contraction of these muscles was "augmented" by blockers of this enzyme, and by inhibition of nitric oxide-stimulated guanylyl cyclase activity, and was "depressed" by donors of nitric oxide and conditions wherein cyclic GMP levels increased. Inverse relationship between nitric oxide synthase activity and force development was indeed established (81). As expected of evaluation given in the above section, it is possible that a constant rate of formation of nitric oxide/cycle GMP may be maintaining a level of relaxation, counteracting the actions of contracting agents. Stamler's findings point out that nitric oxide may indeed have a secondary action of counteracting contraction dependent on oxidation of regulatory thiols in sarcoplasmic reticulum (83). It will be simple to explain this if nitric oxide were to react with diperoxovanadate or vandyl resulting in mutual deactivation. Such an implication of vanadate in contractile process amply justifies its presence in muscle tissue, rather than a contaminant, as in horse muscle that led to isolation of ATP-vanadate complex in some Sigma crystalline ATP samples!

**A projection of redox control of muscle contractility**

The characteristic of smooth muscle contraction is the step response with successive additions of stimulus in the medium, and instant reversal on withdrawing the stimulus. This indicates an equilibrium situation wherein continouous, rapid reactions are occurring. With the present understanding, the stimulus activates the α-adrenergic receptor, intracellular calcium concentration increases, calmodulin-dependent activation of MLC kinase occurs leading to actin-myosin interaction, cross-bridge formation and ATP hydrolysis (Fig. 4). Step increase may occur in the rate of each or any one of these on increasing stimulus concentration. After compensating for the concomitant loss at a constant rate this determines the balance. The aspect of intrinsic, coupled relaxation process has not been evaluated. This is indeed surprising in view of the high profile interest in the nitric oxide-mediated smooth muscle relaxation, the importance of which is recognised by the 1998-Nobel prize of Medicine or Physiology.

Vanadate mimics the effects of α-adrenergic agonists in contraction of smooth muscles but at high concentrations which are ordinarily toxic. This toxicity is attributed to its prime effect of inhibition of the essential sodium pump, which is certainly not invovled in the contraction process in all the smooth muscle systems tested. Recent experiments show the effect can be obtained with 100-fold less concentrations in the μmolar range of decavanadate (25) and diperoxovanadate (40) and they are less toxic. Several experimental evidences pointed out that alternative mechanism of action of these vanadium compounds must be operative subsequent to initial increase in calcium concentration in the cell. Indeed this thinking of the alternative had become possible only through the inputs from vanadate studies. Decavanadate has a similar structural motif as noradrenaline (25) and does activate the receptor and release calcium from mitochondria store

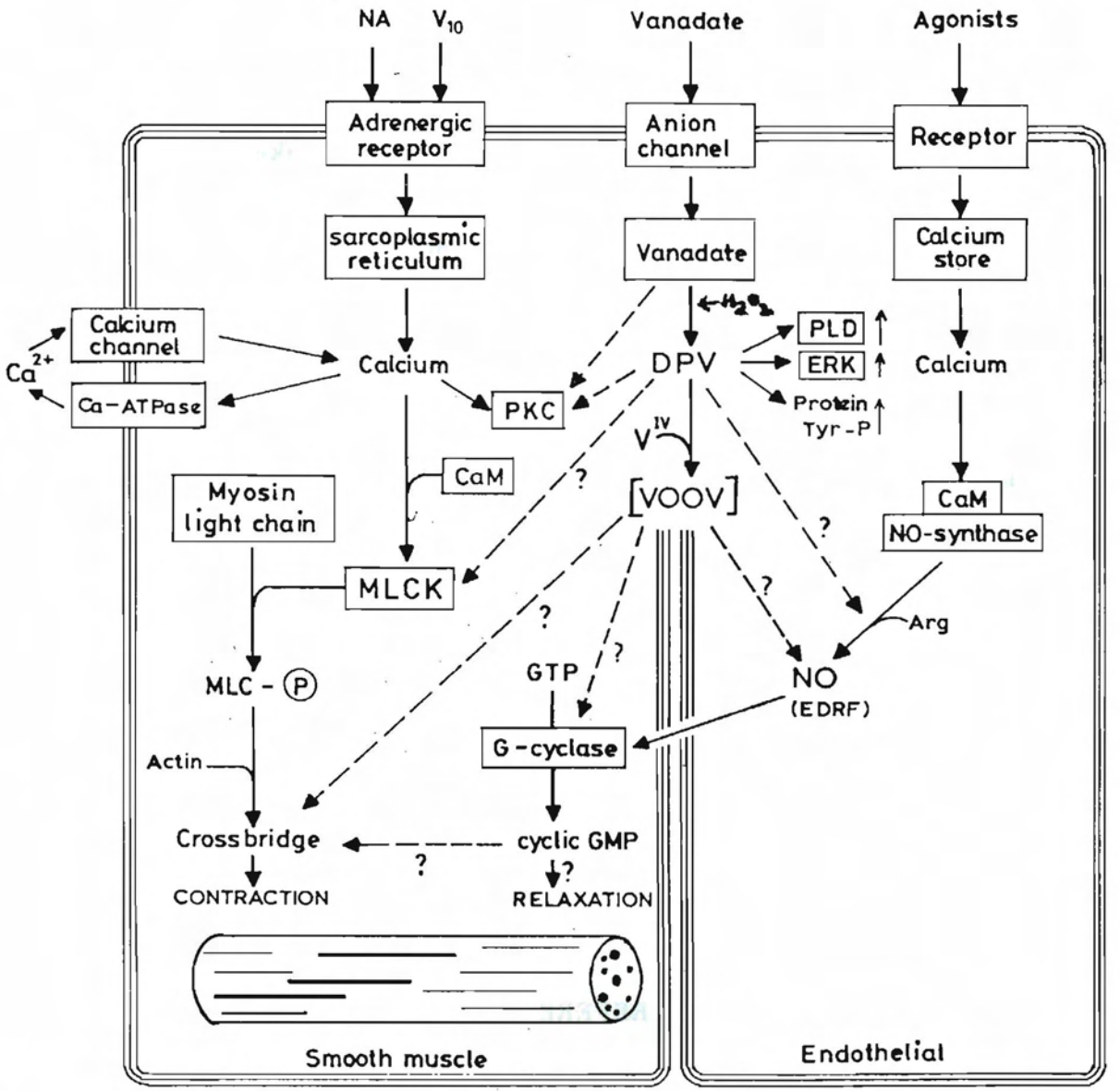


Fig. 4 : A schematic representation of events occurring in smooth muscle (left) and endothelial (right) cells of smooth muscle and the possible influences of vanadium compounds. Major steps of calcium-calmodulin (CaM)-dependent activation of myosin light chain kinase (MLCK) and its phosphorylation product interacting with actin in the contraction process are depicted. The formation, also CaM-dependent, and its effect of relaxation in intimal cell in producing cyclic GMP of the endothelial cell-derived relaxing factor (EDRF), nitric oxide (NO), are also shown. The outline of reactions of vanadate in producing oxidant species and their known effects on some enzymes as well potential targets (?) marked are given in the middle as they are expected to be common in both cell types.

The information is taken from many references discussed in the text. (NA: noradrenaline; $V_{10}$: decavanadate; DPV: diperoxovanadate; $V^{4+}$: vanadyl; [VOOV]: peroxo-bridged divanadate; PLD: phospholipase D; ERK: extracellular signal-regulated kinase).

(77). Peroxovanadate seems to achieve the same but depends on extracellular calcium as the source, both the subsequent steps seem to utilise protein-tyrosine phosphorylations, indicated by studies with specific inhibitors (40). These inhibitors also effect the diperoxovanadate stimulated protein-tyrosine phoshorylation and phospholipase D in endothelial cells, and it is not clear how these effects influence the muscle cells in their contraction process (80) (Fig. 4).

In this context it is interesting to note the findings that $H_2O_2$ produced a biphasic response in rabbit aorta consisting of an initial transient relaxation followed by contraction, and that the relaxation was prevented by removing the endothelium or by NO synthesis inhibitor (84).

The feature of redox based control of smooth muscle contractility had emerged from the studies with vanadium compounds from the papers cited above. Reduction of decavanadate by NADH to provide vanadyl appears a certain possibility, as microsomes from several tissues tested show this activity (67, unpublished experiments). Vanadyl could initiate the series of vanadate-dependent reactions and provide the oxidants as shown above. Similarly diperoxovanadate would join this chemical sequence, and make available vandyl by reduction, if the other oxidants are needed. Thus testing these compounds on the components and process of contraction, and of relaxation, of smooth muscle will yield valuable information. We suggest that an alternative way of controlling contraction is by affecting the relaxation process, and the redox basis for this is anticipated from the trend of research in this field. An elegant control mechanism will thus be unveiled: phos-dephos for contraction and redox for relaxation. We hope that this conjecture would stimulate work in this regard.

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