Ann. Rev. Pharmacol. Toxicol. 1984. 24:501–24

MECHANISMS OF ACTION OF VANADIUM

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INTRODUCTION

Vanadium is widely distributed, the twenty-first most abundant element in the Earth's crust, with an average content of 135 ppm. In sea water, vanadium ranks thirty-fourth in abundance, with an average concentration of only 2 ppb. Because it evolved as an essential element for certain forms of life and also because of its wide industrial use, the biological actions of vanadium are of interest to scientists. Excellent accounts of the history and previous knowledge of vanadium are available. (1–7).

The chemistry of vanadium is complex because the metal can exist in oxidation states from −1 to +5 and forms polymers frequently (8). Recently Rubinson (9) reviewed the material concerning the form of biochemically active vanadium. The following generalizations appear justified. At low-normal concentrations in mammals and birds, any free vanadium will be in hydrated, monomeric form. In the body fluids at pH 4–8, the predominant species will be VO_3^- (+5 oxidation state), vanadate (metavanadate). VO_3^- may enter certain cells by an anion transport system and be reduced by glutathione to VO^{2+} (+4 oxidation state), vanadyl. By way of speculation, the oxidation-reduction reactions may be as follows: $H^+ + VO_3^- + 2GSH \rightleftarrows VO^{2+} + G_2S_2 + OH^- + e^- + H_2O$. Extensive binding to extra- and intracellular ligands may be expected. Since phosphate and Mg^{2+} are ubiquitous in biological processes, VO_3^- as the analogue of phosphate and VO^{2+}, which resembles the size of Mg^{2+} (respective ionic radii: 0.60 and 0.65 Å), potentially have many biochemical and cellular sites of action. For example, vanadium compounds inhibit ATP phosphohydrolases, ribonuclease, adenylate kinase, phosphofructokinase, squalene synthetase, glyceraldehyde-3-phosphate dehydrogenase (10),

0362-1642/84/415-0501$02.00

glucose-6-phosphatase (11), and phosphotyrosyl-protein phosphatase (12). The recent finding that VO_3^- is one of the most potent known inhibitors of $Na^+ + K^+$ ATPase (13–15), and a suggestion that vanadium may be a physiologic regulator of the Na^+ pump (16), have stimulated much research activity, and several reviews and editorials on the long-elusive role of vanadium in biology (9, 10, 17–25) have appeared.

This review is confined to selected aspects of vanadium interaction with transport ATPases and its expression on cellular, organ, and whole animal levels. For a biological perspective, updates on biochemistry, distribution, nutrition, and as yet unexplained observations on the effects of vanadium will be included. The symbol VO_3^- (for vanadate) will be used instead of the names of various salts of metavanadate (VO_3^-) or orthovanadate (VO_4^{3-}) employed in the works referenced.

DISTRIBUTION OF NATURALLY OCCURRING VANADIUM IN BIOLOGIC MATERIALS

The knowledge of vanadium content in biologic materials is far from complete. In addition to natural variability, inappropriate sensitivity of analytical methods, interference by other elements, nonavailability of standard reference materials in the past, and easy contamination of samples have contributed to a wide spread of vanadium values reported in the literature (26, 27). The most sensitive of commonly used methods for measuring vanadium are the techniques of neutron activation analysis (NAA) (26) and flameless atomic absorption spectroscopy (AAS) (28–9); detection limits are well under 1 ng of vanadium. The values given below are in ng per ml or g wet weight ($5.1 = 10^{-7}$ M).

Humans

Byrne & Kosta (26) and Cornelis et al (27, 30) scrutinized published information on vanadium concentration in the blood plasma or serum (ranging from 0.016–570) and whole blood (ranging from 0.5–1500) of healthy individuals and concluded that much of the disparity in these figures was methodological. The lowest ever reported vanadium serum concentration determined by NAA ranged from 0.016–0.139 in 37 women and from 0.024–0.939 in 37 men (30). No correlation was found between vanadium content and age or serum concentrations of cholesterol, triglycerides, or lipoproteins. The lowest mean vanadium concentration determined by NAA in whole blood was 0.5 (26). Values recently obtained by AAS were 3 for serum (29), 8 for plasma (31), and 6 for whole blood (32). According to only a few measurements, erythrocytes appear to have a vanadium content similar to that of plasma (30, 32). By a photometric method, <1 to 11 (<1 to 24 in chimney sweepers) was found in whole blood

(33). Byrne et al (26, 34–36) reported (and compared with published values) the following vanadium concentrations (by NAA) in tissues and body fluids: bone and teeth, <1–8; liver, 5–19; kidney, 3–7; heart, 1; skeletal muscle, <1–7; spleen and thyroid, 3–4; pancreas, 14; brain, fat, urine, <1; lung, 13–140; hair, 12–87; bile, <1–2; dry feces, 141–2210; dry milk and colostrum, <1–1. Other workers reported: liver, 3–13 (NAA) (37); kidney, 67–194 (AAS) (38); placenta, 3 (photometry) (39). The US Environmental Protection Agency (40) listed vanadium ranges of 4–140 for hair and 4–625 for nails. Mean vanadium content in neonates' hair was 50 (NAA) (41). Vanadium concentrations in hair appear to be sensitive to environmental exposure (42). US city dwellers showed increased lung concentrations in the fifth and particularly sixth decades of life (43).

A recent estimate of the total body pool of vanadium in the "reference man" was 100–200 μg (26), in contrast to Schroeder's earlier calculation of 22 mg (4). The daily dietary intake of vanadium was estimated as 10–60 μg (26, 34, 44, 45), with excretion mainly in the feces and urine (45). The mean urinary output per 24 hours was 10 μg (29). Intake via air and water may be significant (6, 26).

Other Mammals (26, 28, 32, 46, 47)

Researchers have shown the following values in other mammals: bone, 20–40 (pig, sheep); bone marrow, <1 (pig); liver, 2–10 (beef, pig, rat); kidney, 9–34 (rat, pig, dog, rabbit); heart, <1–9 (pig, rat, rabbit); skeletal muscle, <1–14 (beef, pig, rabbit, horse); brain, <1–3 (rat, cow); lung, 5–25 (rabbit, beef); fat, <1–2 (pork); butter, 1; milk, <1–3; plasma, 2–5 (rat); gelatin, 9–43; whole mouse, 66.

Chicken (26, 28, 48)

Researchers have shown the following values in chicken: dry bone, 370–760; (turkey bone, 86); liver, 38; kidney, 18 (107 on 3.5 μg vanadium/g diet); heart, 5–9; light muscle, 2–22; dark muscle, 12; egg white, <1–2; egg yolk, 2–21.

Aquatic Animals (26, 28, 47, 49, 50)

Researchers have shown the following values in aquatic animals: salt water fish, 3–28 (cod, mackerel, sardines, tuna); fish bone, 125–2000 (mackerel, tuna); fresh water trout, 0.4; lobster, 5–43; scallop, 22; dry blue crab, mussels, oysters, white shrimp, 455–1840. Vanadium content in shrimp and oysters was higher in specimens taken from industrialized areas compared to nonindustrialized sections around Galveston Island, Texas (49). An extensive list of vanadium concentrations in other biological systems has been published (1).

Plants (26, 28, 32, 47)

Researchers have shown the following values in plants: numerous fruits, vegetables, nuts, oils, <1–5; lettuce, radish, spinach, 21–52; dill, 140; dill seed, 431; parsley, 790; cereals, grains, beans, flour, bread, <1–93; dry wild mushrooms, 50–2000 (26 species); dry *Amanita muscaria*, 51,000 (as amavadin) (1); cocoa powder, 610; dry tea leaf, 150; dry black pepper, 204–987; wine, 4–32; beer, 8; tobacco, 1000–8000 (10 types); drinking water <1–2.

DISTRIBUTION OF EXPERIMENTALLY ADMINISTERED VANADIUM

The most complete picture of vanadium distribution emerges from autoradiographic studies of sagittal sections of whole mice prepared five minutes to seven days after an intravenous injection of $^{48}V_2O_5$. These studies included pregnant mice (51). Half-life of ^{48}V in blood was <1 hour. Bones and teeth had the highest persistent concentrations of vanadium, which reached a peak 1–2 days after the injection; ^{48}V accumulated to the greatest extent in zones of ossification. [In a similar study on 7–9 day-old rats, the greatest ^{48}V uptake was found in parts of the teeth and bones, where rapid mineralization was taking place (52)]. In soft tissues, ^{48}V rose rapidly after the injection and declined faster than in bone. The highest concentrations were found in the kidney cortex, the liver with a spotty distribution, and the lung parenchyma, with no radioactivity in the bronchi. Medium levels of radioactivity were found in the skin and salivary glands, with low concentrations in skeletal and heart muscles, cartilage, spleen, and brain. Intestinal and urinary bladder contents were high in vanadium. In the pregnant mice, high concentrations of vanadium were visible in the placenta, especially the visceral yolk sac epithelium, the fetal skeleton, and the mammary gland. Other investigators (53–61) found similar distribution in selected organs of mice or rats after giving various vanadium compounds by different routes. Some accumulation in testes was also noted. A similar organ and subcellular distribution of vanadium after intravenous injections in rats of labelled cationic and anionic compounds with different vanadium oxidation states suggests an in vivo conversion of dissimilar vanadium compounds to common vanadium species (62–63). In blood, less than 5% of radioactivity was associated with erthrocytes and over 95% was in plasma; some vanadium was bound to transferrin [liver and spleen ferritins also bind vanadium (64, 65)]. In fractions of liver and kidney homogenates, nuclei contained the highest quantity of vanadium, followed in descending order by mitochrondria, cytosol, lysomes, and microsomes.

VO^{2+} is used in electron microscopy; it stains cytoplasmic organelles, collagen fibrils, glycogen granules, secretion granules, and ribosomes (66).

An autoradiographic study on fish of ^{48}V uptake from water showed an

accumulation in skin, fins, intestines, liver, and bones. Vanadium content was low in the brain, eye, and muscles (47).

NUTRITIONAL IMPORTANCE

Vanadium is an essential nutrient for the chick and the rat (7, 67–71). Deficiencies have been induced in chicks and rats raised in vanadium-free isolator systems on diets containing <30 or <100 ng/g respectively. The requirement for growth probably lies between 50 and 500 ng/g. These figures appear high in view of the much lower vanadium content of most feeds (see the section on naturally occurring vanadium distribution above). However, in poultry farming too much vanadium is of concern because some rock phosphorus feed additives may contain high concentrations of vanadium; acceptable commercial chicken diets contain 1400–5400 ng V/g (72). Wild birds may obtain sufficient vanadium from grit and soil if their requirements are as high as those of chickens.

Vanadium-deficient chicks showed reduced vanadium concentrations in kidney, liver, and heart, reduced weight gain and feather growth, retarded skeletal development, increased plasma triglyceride concentrations and variably altered plasma concentrations of cholesterol. Signs of vanadium deficiency in the rat included impaired fertility, with a marked reduction in fourth-generation females and reduced pup survival. Deficiency in chicks and rats resulted in increased hematocrits. Evidence suggests that vanadium does not protect against experimental dental carries, as was once proposed (7, 35, 53).

Human diploid fibroblasts require 0.25 ng/ml of vanadium for optimal clonal growth. However, considerable background growth occurs in the absence of added VO_3^- (73). VO_3^- appears to act synergistically with epidermal growth factor in stimulating fibroblast DNA synthesis (74).

The mechanism of vanadium deficiency is not understood. No naturally occurring vanadium-deficiency disease has been described. No natural, functional mammalian or avian vanadium-containing metalloprotein is known.

$Na^+ + K^+$ ATPase

Vanadium has been known since 1965 to inhibit $Na^+ + K^+$ ATPase (75). However, it was shown only recently by three independent laboratories that certain vanadium compounds are among the most potent known inhibitors of this enzyme system (13–15). Cantley et al (13) and Quist & Hokin (15) tested VO_3^- because they identified it as an inhibitory contaminant present in certain ATP preparations (76–80). During a survey of metallic inhibitors of $Na^+ + K^+$ ATPase, Nechay & Saunders (14) worked with V_2O_5 dissolved in NaOH, which yields VO_3^- in solution, and discovered, together with Cantley et al (16),

the inhibitory properties of this ion. In view of the physicochemical properties of vanadium, its interaction with $Na^+ + K^+$ ATPase, its nutritional requirement, and its distribution in tissues, Cantley et al (16, 81) postulated that VO_3^- is a potential regulator of the Na^+ pump. Beaugé & Glynn (76) also considered a similar physiologic role for the inhibitor contained in samples of ATP before it was identified as vanadium because its effect could be altered by K^+.

VO_3^- binds to one high- and one low-affinity site of the enzyme molecule [under optimal conditions the dissociation constants are 4×10^{-9} M and 5×10^{-7} M respectively (16, 82)] and interferes with the activity by slowing $E_2 \rightarrow E_1$ conformational change (83, 84). The high-affinity VO_3^- site corresponds to the low-affinity ATP site and vice versa (16); ATP reduces VO_3^- binding (85, 86) and inhibition (14, 16). Mg^{2+} is required for the binding of VO_3^- to the enzyme (80, 85) and inhibition of the $E_2 \rightarrow E_1$ conformational change (84), and it facilitates inhibition of the enzyme's activity (14–16, 87, 88). Na^+ promotes $E_2 \rightarrow E_1$ conformational change, interferes with VO_3^- binding (85), and opposes inhibition of enzymatic activity (14, 87). Na^+ may also act by displacing K^+ (87). An effect of K^+ in facilitating inhibition (14, 16, 87) may be to displace Na^+ from sites at which it activates the enzyme (88). K^+ is not required for VO_3^- binding (85). Ca^{2+} or Mn^{2+} can substitute for Mg^{2+} in promoting VO_3^- binding, although Ca^{2+} is less effective and Mn^{2+} more effective than Mg^{2+} (85, 86, 89). Cations (Tl^+, Rb^+, Cs^+, NH^+, Li^+) that substitute for K^+ as activators of the enzyme also increase VO_3^- inhibition (87, 90, 91).

Overall, in a model in which VO_3^- can bind only to the E_2 conformational state, agents that favor the E_2 state, such as Mg^{2+}, K^+, ouabain, and dimethyl sulfoxide, increase VO_3^- binding, whereas those that favor the E_1 state, such as ATP, Na^+, and oligomycin, decrease VO_3^- binding (85, 92–95). VO_3^- is able to promote the binding of ouabain to $Na^+ + K^+$ ATPase in the absence of ATP (89, 92); based on this finding, a potentially clinically useful method was developed for measuring the number of ouabain binding sites in muscle biopsies (96). VO_3^- was reported to lower pH optimum for isolated renal $Na^+ + K^+$ ATPase activity from 7.8 to 7.0 (97).

Agents that interfere with VO_3^- inhibition include bovine serum albumin (14), which probably acts by chelation; anions such as citrate (14) and EDTA (14, 98), which may displace the anionic VO_3^- from the enzyme; reducing agents such as glutathione (90, 99), ascorbate (14, 90), NADH, methylene blue, imipramine, and chlorpromazine (100, 101), which convert the VO_3^- to less active VO^{2+}; and catecholamines, which may both bind and reduce VO_3^- (15, 98, 102, 103, 235).

Incorporation of $Na^+ + K^+$ ATPase in lipid bilayers confers high ion channel conductance when a cation gradient is present across the planar

membrane, and removal of the gradient results in low conductance. The high but not the low conductance state is inhibited by ouabain and VO_3^- (104).

In human erythrocytes (105, 106) and squid axons (107), VO_3^- is an effective inhibitor of the $Na^+ + K^+$ pump only when it interacts with the cytoplasmic surface of the $Na^+ + K^+$ ATPase, since VO_3^- binds to the phosphate site on the enzyme, located inside the cell. External K^+ (and Rb^+, Cs^+, NH^+, Li^+) controls VO_3^- inhibition of $Na^+ + K^+$ ATPase activity by an allosteric mechanism: low concentrations activate Na^+ efflux, high concentrations inhibit it (84, 105, 107).

$^{48}VO_3^-$ rapidly enters the erythrocyte, possibly by the same anion transport system as phosphate; there is also a slow equilibration process (56, 99, 106, 108). Inside the cell, VO_3^- is reduced nonenzymatically by glutathione (99), and probably by NADH, ascorbate, and catechols, to VO_3^- (56), a less active inhibitor of the $Na^+ + K^+$ ATPase activity (81, 90, 102, 109, 110). Similarly, entry of VO_3^- into rat adipocytes and its reduction to VO^{2+} has been reported (111). Also, after systemic administration of VO_3^- to rats, +4 is the predominant oxidation state of vanadium in homogenate of kidney and liver and subcellular liver fractions (112, 113). Binding of VO^{2+} to ATP (114, 115) and other phosphate and carboxyl ligands protects it from oxidation to VO_3^- (236), which would otherwise tend to occur at the intracellular pH. It is conceivable that the oxidation-reduction reactions could supply an appropriate concentration of free VO_3^- to modify the $Na^+ + K^+$ pump activity. However, the quantitative aspects and the physiologic control of free intracellular VO_3^- concentrations have yet to be established.

VO_3^- inhibits transepithelial Na^+ transport when applied at a high concentration (10^{-3} M) to the mucosal (but not to the serosal) surface of frog skin and toad bladder preparations (116, 117). Equally high VO_3^- concentration is required to inhibit $Na^+ + K^+$ ATPase activity in toad bladder homogenates. In keeping with the known ability of K^+ to promote the binding of VO_3^- and inhibition of enzyme activity, K^+ potentiates the VO_3^- effect on Na^+ transport in the toad bladder. VO_3^- exhibits delayed effects on both the Na^+ transport and the enzyme activity (117). The action of VO_3^- is not limited to $Na^+ + K^+$ ATPase, since it also blocks cyclic AMP-induced stimulation of Na^+ and water transport in amphibian epithelia (116, 118) and the H^+ pump in the turtle urinary bladder (119).

Of the 5b group of elements, niobate and tantalate are less effective $Na^+ + K^+$ ATPase (fish gills) inhibitors than VO_3^- (120).

$Ca^{2+} + Mg^{2+}$ ATPase

Studies have been done mainly on enzymes from sarcoplasmic reticulum of mammalian skeletal muscle and heart and from human erythrocytes. The red

cell Ca^{2+} + Mg^{2+} ATPase is several times more resistant to VO_3^- than Na^+ + K^+ ATPase (121). The Ca^{2+} + Mg^{2+} ATPase of sacroplasmic reticulum requires at least 10 times higher concentration of VO_3^- for 50% inhibition than that of erythrocytes (122–124). It is generally agreed that Ca^{2+} induces conformational transition of $E_2 \rightarrow E_1$ and stabilizes the E_1 state (125). VO_3^- stabilizes the E_2 state and inhibits the Ca^{2+}-induced conformational change. VO_3^- binds to the Ca^{2+}-free enzyme in a process that requires Mg^{2+} and is competitively antagonized by phosphate and ATP; the high concentration of ATP required indicates binding of VO_3^- to the low-affinity binding site. A similar number of sites for VO_3^- binding and phosphorylation suggests that the stabilization of the Ca^{2+}-free conformation is due to formation of a stable E-Mg-V complex at the site of phosphorylation (123, 126). The activators of the enzyme Mg^{2+}, K^+, Na^+ and calmodulin facilitate inhibition by VO_3^-, while ATP and Ca^{2+} at concentrations higher than 5×10^{-5} M protect (121, 124, 127, 128). Li^+ does not substitute for K^+ in this system (121).

In reconstituted erythrocyte ghosts, intracellular VO_3^- (5×10^{-5} M) inhibits active Ca^{2+} efflux; this inhibition is promoted by intracellular Mg^{2+} and K^+ and is antagonized by extracellular Ca^{2+} (129). The sensitivity of the Ca^{2+} pump to VO_3^- in vesicles made of purified red cell Ca^{2+} + Mg^{2+} ATPase is similar to that observed in whole erythrocytes ghosts (130). In the intact red cell, external VO_3^- (5×10^{-5} M) does not inhibit the Ca^{2+} pump (131). When exposed to 5×10^{-4} M VO_3^-, fresh erythrocytes become highly labelled with externally added Ca^{2+}, which suggests some penetration of VO_3^- into the cells as well as inhibition of the outwardly directed Ca^{2+} pumping ATPase (132). The VO_3^--induced accumulation of Ca^{2+} by red cells causes a massive efflux of K^+, suggesting either an activation of the Ca^{2+}-sensitive K^+ channel in the erythrocyte membrane (132) or that the intracellular VO^{2+} metabolite, similarly to Ca^{2+}, Mg^{2+}, and Pb^{2+}, can open the K^+ channel (133).

The newly characterized Ca^{2+} + Mg^{2+} ATPase of dog heart sarcolemma is about as sensitive to VO_3^- (Ki = 0.5 μM) as the Na^+ + K^+ ATPase. This high VO_3^- sensitivity has been used to distinguish the Ca^{2+} ATPase activity of sarcolemmal vesicles from that of the contaminating sarcoplasmic reticulum vesicles in heart microsomal fractions. The sarcolemmal enzyme could be responsible for ejecting Ca^{2+} during resting conditions when its intracellular concentration is very low (134). Also, in the intestinal smooth muscle, two Ca^{2+} transport activities resembling the sarcoplasmic reticulum and sarcolemmal Ca^{2+} pumps have been differentiated by sensitivity to VO_3^- (135).

VO_3^- inhibits uncoupled (probably Ca^{2+} ATPase-dependent) Ca^{2+} efflux from squid axon (136) and isolated rat neurohypophyses (137). The Na^+-Ca^{2+} exchange is not affected. ATP-dependent Ca^{2+} uptake by microsomal fractions of rat salivary glands is inhibited by VO_3^- (138). Isolated from rat livers,

Ca^{2+}-pumping ATPase of the endoplasmic reticulum (139), but not that of plasma membrane (140), is sensitive to VO_3^-.

$H^+ + K^+$ ATPase

VO_3^- inhibits microsomal gastric mucosa K^+ ATPase, which is an expression of a part of the gastric H^+ pump; proton transport by gastric microsomal vesicles and acid secretion by gastric glands are also reduced (128, 141, 142). VO_3^- also inhibits urinary acidification by the turtle bladder; the mechanism has not been determined (119). A bacterial membrane-bound proton-translocating ATPase was found to be sensitive to VO_3^- (143).

DYNEIN ATPases

Dynein is the collective name for either Ca^{2+}- or Mg^{2+}-requiring high molecular weight ATPases associated with microtubules. They function in the transduction of the chemical energy provided by ATP hydrolysis into mechanical work such as ciliary and flagellar motility and may have roles in chromosome movement, exoplasmic transport, and the intracellular movement of membrane-bound vesicles (144, 145). The sources of dyneins studied in detail have been the flagella and cilia of the *Tetrahymena* and the sea urchin.

VO_3^- at concentrations on the order of 10^{-6}–10^{-7} M reversibly inhibits both the isolated dynein ATPase activity and the motility of demembranated sea urchin or porcine sperm flagella and sea urchin embryo cilia (146, 147). Mg^{2+}-activated dynein is over 30 times more sensitive to VO_3^- than the Ca^{2+}-activated one (146, 148, 149). The inhibition of Mg^{2+}-stimulated enzyme activity is noncompetitive with ATP (146, 148, 150), as is the reduction of flagellar beat frequency by VO_3^- (146). The intact sea urchin spermatozoa are not inactivated by 10^{-2} M VO_3^-, and those of the pig require 10^{-3} M VO_3^- for complete paralysis (147). Other observations extend and amplify these findings (151–157).

Myosin and actomyosin ATPases are not inhibited by VO_3^- concentrations below 5×10^{-4} M (13, 146). Other studies, however, have demonstrated an irreversible, slow-onset inhibition of myosin and actomyosin ATPases by millimolar concentrations of VO_3^-; the mechanism is the formation of a stable myosin-ADP-vanadium complex (158–160). The difference in kinetics and sensitivity to VO_3^- offers an opportunity for distinguishing between the actions of dynein and myosin in different forms of cell motility (146, 161).

VO_3^- at $\sim 10^{-5}$ M has been shown to inhibit mitotic spindle in lysed cells (162) and translocation of pigment granules in permeabilized erythrophores (163) or when injected into the cell (164).

ADENYL CYCLASE

Cyclic AMP is formed by the catalytic action of adenyl cyclase and is inactivated by phosphodiesterase. VO_3^- ($>10^{-5}$ M), along with fluoride, catecholamines, vasopressin, prostaglandin E_1, parathyroid hormone, and glucagon, stimulates isolated adenyl cyclase activity from a variety of sources (165–170). The action of VO_3^- is not shared by V^{4+} and V^{3+} compounds (171) and is independent of hormones and inhibition of phosphodiesterase by theophylline (170, 172); it differs from that of fluoride (172–174). The postulated mechanism involves formation of an enzyme complex with VO_3^- via guanine nucleotide regulatory protein (174). Since VO_3^- does not attenuate the ability of hormone receptors to direct inhibition of adenyl cyclase, a routine inclusion of VO_3^- in studies directed at further elucidation of the mechanisms of receptor-mediated inhibition of the enzyme was suggested (172). One would expect VO_3^- to have cyclic AMP-mediated hormone-like effects in vivo. However, in toad bladder and skin, VO_3^- blocks cyclic AMP-induced (by vasopressin, theophylline, and exogeneous cyclic AMP) transepilial osmotic water flow by an unknown mechanism (116); it may also stimulate water flow in such systems (118).

Concentrations of VO_3^- in excess of 5 mM inhibit adenyl cyclase (172).

SMOOTH MUSCLE

In view of the dependence of vascular muscle tone on the electrogenic Na^+ pump, the effects of $Na^+ + K^+$ ATPase inhibitors on blood pressure are of great interest (175, 176). Indeed, an intravenous administration of VO_3^- acutely raises arterial blood pressure in rats (177, 178). Prolonged dietary administration of VO_3^- (100–200 ppm) to uninephrectomized rats produces hypertension that correlates positively with plasma vanadium concentrations ranging from 40–270 ng/ml (179). Note, however, that these vanadium levels are much higher than those encountered in human populations. In the dog or cat, intravenous VO_3^- infusions cause arterial hypertension, increased peripheral resistance, and a marked reduction of coronary, visceral, and renal blood flow, whereas the large arteries (femoral and carotid) escape constriction (180–183). There also may be centrally mediated effects of VO_3^- on blood pressure (184).

As expected of a $Na^+ + K^+$ ATPase inhibitor, VO_3^- (10^{-4} M) causes a contraction of isolated vascular smooth muscle preparations but does not inhibit the Na^+ pump (185–188). The VO_3^--induced contraction is blocked by a stilbene, an inhibitor of the anion transport system, suggesting an intracellular action of VO_3^- (185–188). Aortas of Dahl salt hypertension–sensitive rats react more vigorously to VO_3^- than do those from control rats (188).

It has been suggested that VO_3^- may act by inhibiting Ca^{2+} ATPase that controls intracellular Ca^{2+} concentrations (185). However, VO_3^- at a concentration as high as 10^{-4} M has been reported to have only minimal inhibitory effect on plasma membrane Ca^{2+} ATPase derived from rat mesenteric arteries and veins (189). VO_3^- also augments ^{3}H-norepinephrine release from the isolated pulmonary artery (190) and there is a pharmacological similarity between the contraction induced by VO_3^- and norepinephrine (187). Another observation is that actomyosin preparations of the carotid arteries of cattle contain VO_3^--sensitive phosphatase activity (191).

Intestinal smooth muscle may be more sensitive to VO_3^- than vascular muscle (185) and the VO_3^--induced contraction is inhibited by removal of external Ca^{2+} (192). Several vanadium compounds evoke contractions of the isolated rat vas deferens (193).

HEART

Since inhibition of $Na^+ + K^+$ ATPase has been implicated in the positive inotropic action of digitalis, there is much interest in the cardiac effects of VO_3^-. The subject has been clearly reviewed, and the reviewers concluded that VO_3^- has more action on the heart than does digitalis (17, 20). In intact dogs and cats, VO_3^- decreases the force of ventricular contraction, presumably due to a marked coronary constriction occurring at concentrations too low to have a direct effect on myocardium. In isolated cardiac muscle preparations, VO_3^- produces positive or negative inotropic effects, depending upon species, type of muscle, and experimental conditions; these actions do not appear to involve $Na^+ + K^+$ ATPase inhibition. VO_3^- stimulates adenyl cyclase and so can increase the concentration of cyclic AMP in cardiac muscle; this effect also seems to be unrelated to its inotropic actions. VO_3^- increases force of contraction of isolated rat atrial muscle by increasing the Mn^{2+}-sensitive superficial Ca^{2+} pool, which is related to the beat-to-beat control of force of contraction; on the other hand, VO_3^- lowers the force of contraction in guinea-pig atrial muscle by inhibiting slow Ca^{2+} channels (194). Compounds of vanadium in +4 and +3 oxidation states do not share with VO_3^- the positive inotropic effect on isolated cat papillary muscles and stimulation of adenyl cyclase (171).

High concentrations of VO_3^- ($>10^{-4}$M) may inhibit (like ouabain) or stimulate (like insulin) the uptake of K^+ by heart muscle cells from various species; both types of effects may be associated with the positive inotropic effect (195). It was shown previously for other tissues that VO_3^- mimics the stimulating effect of insulin on glucose oxidation (111) and transport, which appears to be associated with or mediated by a rise in cytoplasmic Ca^{2+} concentration (196). Another suggestion is that the stimulation by VO_3^- of rat heart protein kinase, which promotes the phosphorylation of the membranes of

the sarcoplasmic reticulum, may play a role in strengthening myocardial contraction by increasing sarcoplasmic reticulum stores of Ca^{2+} (197).

In chemically skinned right-ventricle hog preparations, inorganic phosphate and VO_3^- interfere with the chemomechanical energy transformation of myosin (198).

KIDNEY

The renal effects of vanadium have been reviewed previously (21, 23); they include a mixture of hemodynamic and parenchymal actions and, like cardiac effects, are characterized by unexplained and profound species differences. VO_3^- produces a large diuresis in the rat but not in the dog (181, 199, 200) and cat (182, 183); vasoconstriction, lowering of renal blood flow, and glomerular filtration rate (GFR) are prominent. In the rat, GFR may rise simultaneously with increased renal peripheral resistance, suggesting a postcapillary vasoconstrictor effect (201); in the dog and cat only a fall of GFR was seen.

Most authors have speculated that the diuresis induced by VO_3^- in the rat is due to inhibition of $Na^+ + K^+$ ATPase, since the ouabain-sensitive Na^+ pump accounts for up to 50% of renal Na^+Cl^- reabsorption, the urinary electrolyte excretion pattern is typical for ouabain, and renal $Na^+ + K^+$ ATPase is exquisitely sensitive to VO_3^-. Vanadium accumulates in the kidney, although this may be mainly in the form of VO^{2+}, with less activity with respect to $Na^+ + K^+$ ATPase. Rats on 15–day diets containing 5 and 25 ppm VO_3^- had renal vanadium concentrations in excess of 10^{-5} M but showed no changes in fractional Na^+ excretion or $Na^+ + K^+$ ATPase activity in kidney homogenates (46). On the other hand, chickens on diets containing subtoxic (25–50 ppm) and toxic (100 ppm) concentrations of VO_3^- for 15 months had reduced renal $Na^+ + K^+$ ATPase in proportion to renal concentrations of vanadium; the potency of vanadium for $Na^+ + K^+$ ATPase was similar in vivo and in vitro, showing I_{50} of 5×10^{-6} M. Unfortunately, diuretic studies were not performed in these birds (48). These enzyme observations are also of interest in determining the mechanism of vanadium-induced nephrotoxicity, since generalized proximal tubular transport defects (Fanconi syndrome) may be associated with interference with the active Na^+ pump (202).

VO_3^- reduces renal renin secretion in rat kidney slices (203) and in volume-expanded dogs (199). It produces a striking phosphaturia in acutely parathyroidectomized rats (204). $Ca^{2+} + Mg^{2+}$ ATPase of the rabbit kidney is resistant to VO_3^- (205).

Beside the fact that urine is the major excretory route for vanadium (45), nothing is known about the renal handling of vanadium compounds.

EYE AND EAR

Topical application of VO_3^- lowers intraocular pressure in the rabbit and monkey, presumably by inhibition of $Na^+ + K^+$ ATPase in the ciliary body and consequent reduction of intraocular fluid formation (18, 206). It proved only marginally effective in human ocular hypertension (207). VO_3^- ($>10^{-4}$ M) also inhibits active Na^+ and Cl^- transport in the isolated frog cornea (208). An ATPase from toad retinal rod outer segments that may have a dynein function involved in light-controlled structural changes (photoreceptor ?) is sensitive to VO_3^- (209).

Ototoxicity of locally applied VO_3^- was studied in guinea pigs. Both the endocochlear and microphonic potentials are inhibited by ouabain. Although VO_3^- inhibits $Na^+ + K^+$ ATPase of stria vascularis in vitro, it causes an increase in the cochlear potential followed by a gradual decrease and a depression of the microphonic potential when applied perilymphatically. These results were interpreted to suggest that VO_3^- acts by depolarizing the hair cells of the organ of Corti (210, 211).

BRAIN

Whole brain mirosomal $Na^+ + K^+$ ATPase is several times less sensitive to VO_3^- than is the kidney or heart enzyme in four mammalian species (14); it is not known whether this is related to the presence of two types of $Na^+ + K^+$ ATPase in the brain (212) or to other factors. Consistent with inhibition of $Na^+ + K^+$ ATPase, VO_3^- interferes with the uptake of ^{3}H-norepinephrine by rat cerebral cortex slices; the high concentrations ($>10^{-4}$ M) required suggest poor intracellular penetration of VO_3^-. Vanadium intoxication in rats causes changes in brain catecholamine levels (213). VO_3^- (10^{-3} M) also diminishes muscarinic binding sites in homogenates of rat corpus striatum (214).

The signs of vanadium toxicity in man include tremor and central nervous system depression (215). A group of Scottish investigators suggest that manic-depressive disorders may be associated with increased vanadium levels and a genetically defective Na^+ pump (examined in lymphocytes and erythrocytes) hypersensitive to inhibitors (216–218). An editorial on the subject appeared in *Lancet* (19). Plasma vanadium concentrations in manic-depressed subjects were about twice that of normal controls and declined after recovery (31). Encouraging results were obtained in the therapy of manic-depressive psychosis with a low vanadium diet and therapy with EDTA or reducing agents, ascorbic acid, and methylene blue (219). Antidepressants such as imipramine and indalprine may also reduce VO_3^- to VO^{2+}. Other investigators extended these observations to several ATPases in erythrocytes of patients with affective

disorders and found the best correlation between mood and Ca^{2+} ATPase activity (91).

MISCELLANEOUS

The common signs and symptoms of occupational industrial exposure to excess airborne vanadium are associated with the irritation of airways and conjunctiva and a green discoloration of the tongue. Recent measurements indicate that this may be accompanied by pronounced reversible reductions in forced vital capacity, forced expiratory volume, and forced mid-expiratory flow, together with increased urinary vanadium excretion (220). A few observations suggest that V_2O_5 particles may produce asthma, as judged by bronchiolar hyperreactivity to histamine (221). Alveolar macrophages isolated from rabbit lungs are inactivated only by very high concentrations ($>10^{-4}$ M) of V_2O_5 (222) and hence appear to offer a poor model for pulmonary toxicity testing of vanadium compounds. Green tongue may be due to deposition of hexaquo ion $[V(H_2O)_6]^{3+}$ (23).

In perfused rat livers, VO_3^- increases vascular resistance (at 3×10^{-5} M in the perfusate), decreases O_2 consumption, and reduces bile flow (at 6×10^{-5} M in the perfusate) (223). Some vanadium is excreted in the bile (224).

The effect of vanadium on drug biotransformation has been examined. Several hepatic microsomal mixed-function oxidase reactions are inhibited in vitro by VO_3^- with varying, generally low, effectiveness (225). Upon administration to mice, VO_3^-, and to a much lesser extent VO^{2+}, transiently inhibits oxidative demethylation of substrates of the cytochrome P-450-dependent monooxygenase system (226). Sulfite-induced lipid peroxidation is accelerated by VO_3^- (10^{-4} M) in several tissues (227).

Parathyroid hormone, prostaglandin E_2, 1,25-dihydroxycholecalciferol, and Na^+-stimulated Ca^{2+} release (resorption) from mouse bone is inhibited by ouabain and $\sim 10^{-5}$ M VO_3^- (228).

VO_3^- hyperpolarizes several types of cultured cells. This unique hyperpolarization is independent of Na^+, K^+, or the Na^+ pump, and requires $>10^{-5}$M VO_3^- for 50% effect (229).

The highest known accumulation of vanadium in any living system occurs in tunicate blood cells termed vanadocytes, reaching a concentration on the order of 1 M, or $\sim 10^8$ times higher than in sea water. The mechanism may involve entry of VO_3^- into acidic vacuoles where it is reduced by tunichrome and trapped as V^{4+}, and biologically unique V^{3+} states. The function of vanadocytes is unknown (10, 230, 231). Although crude petroleums contain vanadyl porphyrins of unknown origin, there is no convincing evidence that they have had, or currently have, functions as O_2 carriers or participate in photosynthetic reactions (232). Recently it was found that VO^{2+}, but not VO_3^-, enhances O_2

binding to bovine hemoglobin and myoglobin (233); the high vanadium concentration required ($>10^{-4}$ M) suggests that the effect is of no physiological significance.

Vanadocene dichloride, one of the numerous metallocene dihyalides, has an antiproliferative action in experimental mice leukemias (234).

CONCLUSIONS

Naturally occurring vanadium is among the lowest of trace elements in mammals (27, 30). Although nutritional essentiality has been established for the chicken and the rat, the physiologic role of vanadium remains unknown. It appears that vanadium exists in body fluids in +5 oxidation state as VO_3^- and intracellularly in +4 state as VO^{2+}. VO_3^- or VO^{2+} interferes with numerous enzymes involving phosphate at concentrations ranging from nM to mM. Of the enzymes studied, the most sensitive to VO_3^- is $Na^+ + K^+$ ATPase; this enzyme is more resistant to VO^{2+} than to VO_3^-. The proposal that vanadium may be a regulator of the Na^+ pump by means of a redox mechanism is yet to be proven. On the other hand, the differential sensitivity of various enzymes to vanadium has been exploited as an investigative tool. In general, it requires a large concentration ($>10^{-4}$ M) of VO_3^- outside the cell to influence even the sensitive intracellular enzymes. In the intact animal vasoconstriction is the most consistently observed pharmacologic-toxic effect of VO_3^-. There are obscure species differences in its renal, cardiac, and ocular actions: administration of VO_3^- may cause diuresis or antidiuresis, may have a positive or a negative inotropic effect on the heart, and may have variable effectiveness in reducing intraocular pressure. In spite of the recent advances, the understanding of vanadium effects remains incomplete because multiple mechanisms may be involved.

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