Biochemical Pharmacology, Vol. 29, pp. 1429–1432.

0006-2952/80/0515-1429 $02.00/0

# SHORT COMMUNICATIONS

## Stimulatory effect of vanadate on cyclic AMP levels in cat papillary muscle

(*Received* 21 *October* 1979; *accepted* 27 *December* 1979)

Vanadate (vanadium in the +5 oxidation state; $VO_3^-$, $VO_4^{3-}$) increases the force of contraction of ventricular cardiac muscle [1–4]. This positive inotropic effect might be due to a vanadate-induced inhibition of $(Na^+ + K^+)$-ATPase which reportedly occurs in red blood cells [5, 6] and in membrane preparations from kidney [7, 8] and heart [2, 8, 9]. However, vanadate has also been shown to stimulate adenylate cyclase activity in particulate fractions from fat cells [10] and heart [4, 11]. But it is well known, especially from studies with fluoride (for review see ref. 12), that the ability of any agent to activate adenylate cyclase in broken cell preparations does not necessarily imply an increase in cyclic AMP levels in intact cells and hence a physiological significance. Therefore, we studied the effect of vanadate on cyclic AMP levels in intact cardiac muscle preparations and report here that vanadate actually produces an elevation of cyclic AMP levels, together with an increase in force of contraction in papillary muscles isolated from cats.

Cats (0.6–1.7 kg) were pre-treated with reserpine (Serpasil ampoules, CIBA, 5 mg per kg i.p., 15–18 hr before being killed) in order to avoid any influence from released catecholamines. The animals were anaesthetized with sodium pentobarbital (30 mg per kg, i.p.) and papillary muscles (diameter 1 mm or less) were dissected from the right ventricles. The preparations were attached to a platinum stimulating electrode and mounted individually in glass tissue chambers for recording isometric contractions as described previously [13]. The bathing solution (50 ml) containing (mM) NaCl, 136.9; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaH_2PO_4$, 0.42; $NaHCO_3$, 11.9; $Na_2EDTA$, 0.05; ascorbic acid, 0.28; glucose, 5.5 was equilibrated with 95% $O_2$ + 5% $CO_2$ and maintained at 35°; the pH was 7.4. The preparations were driven electrically at a frequency of 0.2 Hz (duration 5 msec, intensity about 10 per cent above threshold). Drugs used were anhydrous ammonium vanadate ($NH_4VO_3$; Merck, Darmstadt, F.R.G.) and (±)-isoprenaline HCl (Boehringer, Ingelheim, F.R.G.). The

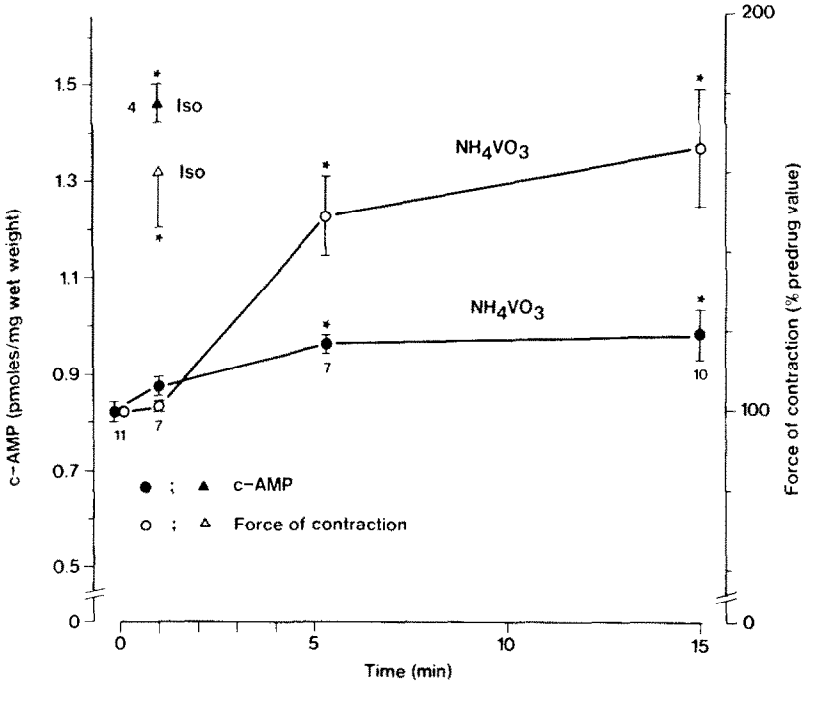


Fig. 1. Effect of 300 μM $NH_4VO_3$ and 0.3 μM (±)-isoprenaline (Iso) on force of contraction (open symbols) and cyclic AMP levels (filled symbols) in cat isolated papillary muscles driven electrically at a frequency of 0.2 Hz. Left ordinate: cyclic AMP in pmoles/mg wet wt. Right ordinate: force of contraction in % of the corresponding zero time values. The ordinates were scaled so that they coincided. Abscissa: time between drug addition and removal of the preparations from the organ bath. Significant differences from zero time values are marked with asterisk ($P \leq 0.05$; Student's *t*-test). One hundred per cent of force of contraction was 1.62 ± 0.14 g · mm$^{-2}$ (N = 39). Figures beside the symbols denote number of experiments for both cyclic AMP levels and force of contraction.

compounds were freshly dissolved in pre-warmed and pre-aerated bathing medium and injected into the tissue chamber in volumes of 1.5 ml or less at zero time. At the end of the experiment, the preparations were quickly frozen with metal tongs pre-cooled in liquid nitrogen. The cyclic AMP content of the preparations was determined by the method of Gilman [14] in the same manner as described previously [15]. The drugs tested did not interfere with the cyclic AMP assay. Cyclic AMP and force of contraction were always measured on the same preparations. Adenylate cyclase and phosphodiesterase activities of cat right ventricular preparations were determined by previously described methods [16–18] (for details see legend to Fig. 2). Protein was measured by the method of Lowry *et al.* [19].

The effects of 300 $\mu$M $NH_4VO_3$ (a submaximally effective positive inotropic concentration; see ref. 1) on cyclic AMP levels and force of contraction are illustrated in Fig. 1. Vanadate increased the force of contraction within 15 min by about 65 per cent. Cyclic AMP levels rose significantly within 5 min by about 20 per cent and remained elevated thereafter. Thus, the positive inotropic action of vanadate appeared to be greater than its cyclic AMP elevating effect. Isoprenaline was studied for comparison at an incubation time of 1 min. This time was chosen because the effects of isoprenaline on cyclic AMP levels and force of contraction

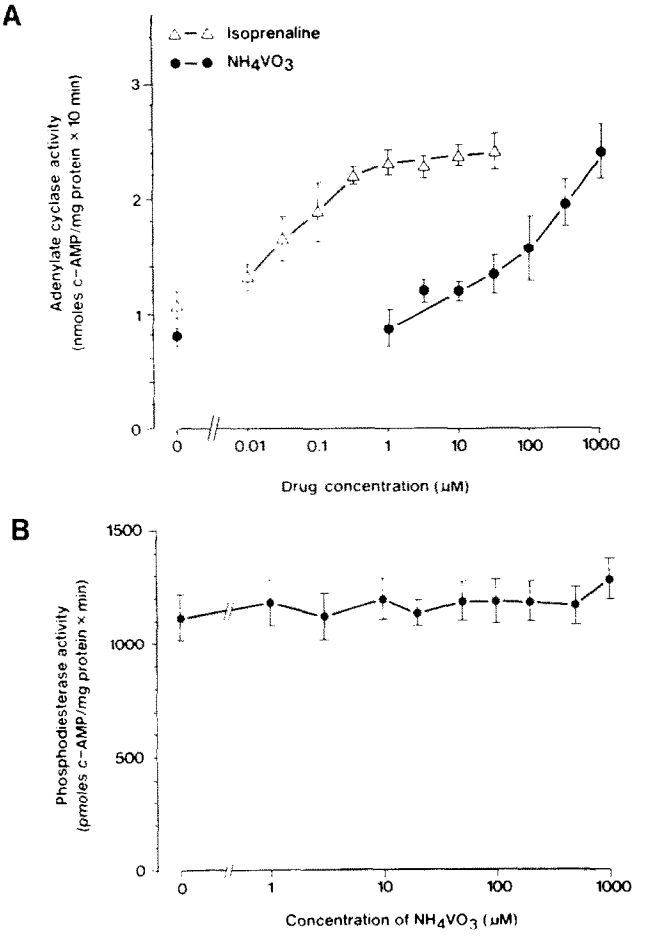


Fig. 2. Panel A. Concentration-dependent stimulation of adenylate cyclase activity by ammonium vanadate and isoprenaline. Particulate fractions were prepared from homogenates of cat right ventricles and adenylate cyclase activity was assayed as described previously [16], using the protein-binding assay of Gilman [14] to determine the cyclic AMP formed. The assay contained 40 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 0.3 mM ATP, 0.01 mM EGTA, 7 $\mu$M 5'-guanylylimidodiphosphate, 0.3 mM papaverine–HCl, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase, 1 mg/ml bovine serum albumin, 200 $\mu$M $KHCO_3$ and 20 $\mu$l enzyme suspension (approximately 40 $\mu$g protein) in a total volume of 100 $\mu$l. The reaction mixture was preincubated for 5 min at 37°. The reaction was started with the enzyme preparation, carried out for 10 min at 37° and was terminated by the addition of 500 $\mu$l ice-cold sodium acetate buffer, 200 $\mu$M, pH 4.0. Cyclic AMP was assayed after 60-fold dilution of the samples. Under these conditions, $NH_4VO_3$ and isoprenaline did not interfere with the assay procedure. Values are means ± S.E.M. of five experiments. Panel B. Failure of ammonium vanadate to affect cyclic AMP phosphodiesterase activity. Soluble phosphodiesterase (12,500 *g*) was prepared from homogenates of cat right ventricles according to Boudreau and Drummond [17]. Phosphodiesterase activity was determined by the method of Thompson and Appleman [18] but with chromatographical separation on QAE-Sephadex A-25 columns. Substrate concentration was 1 $\mu$M and protein concentration was 10.7–27.3 $\mu$g/ml. Values are means ± S.E.M. of five experiments.

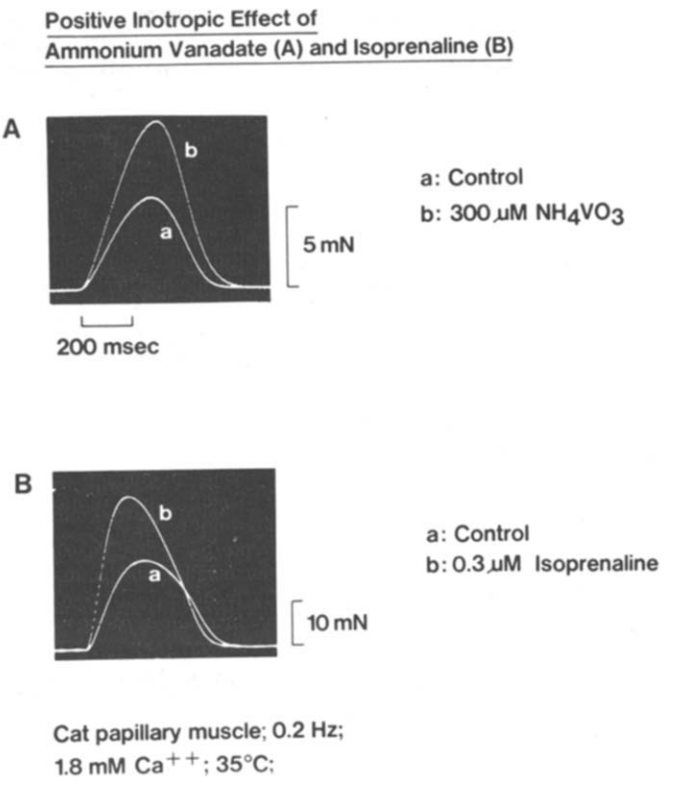


Fig. 3. Effect of 300 $\mu$M ammonium vanadate (panel A; incubation time 15 min) and 0.3 $\mu$M isoprenaline (panel B; incubation time 1 min) on isometric contractions (oscilloscope records) of cat isolated papillary muscles driven electrically at a frequency of 0.2 Hz. Curve a, control; curve b, after the addition of $NH_4VO_3$ or isoprenaline. The temperature was 35° and the extracellular $Ca^{2+}$ concentration was 1.8 mM.

are maximally developed after 1 min [20]. The isoprenaline concentration selected (0.3 $\mu$M) was such that isoprenaline produced a positive inotropic effect similar to that of vanadate. Figure 1 shows that isoprenaline, in contrast to vanadate, was nearly equally effective in increasing cyclic AMP and force of contraction.

The cyclic AMP elevating action of vanadate was entirely due to an effect on adenylate cyclase which was stimulated in a concentration-dependent manner (Fig. 2A). It should be noted that vanadate and isoprenaline, in the concentrations used for the above mentioned experiments (300 and 0.3 $\mu$M, respectively), stimulated adenylate cyclase to roughly the same extent. The vanadate-produced increase in adenylate cyclase activity was not affected by 1 $\mu$M (±)-propranolol (data not shown). Vanadate in concentrations up to 1 mM had no effect on cyclic AMP phosphodiesterase (Fig. 2B).

The results indicate that vanadate is capable of increasing cyclic AMP levels in intact cardiac muscle preparations. This effect is due to an activation of adenylate cyclase which in turn is not mediated by beta-adrenoceptor stimulation. These data suggest that the adenylate cyclase-stimulating effect of vanadate might actually be involved in the agent's positive inotropic action. However, in contrast to isoprenaline, the cyclic AMP-elevating action of vanadate seemed to be less pronounced than the positive inotropic effect. It is therefore probably safe to conclude that the increase in cyclic AMP may contribute to the positive inotropic effect of vanadate, but that other mechanisms appear to be also involved. It is also worth noting in this context that vanadate did not abbreviate the isometric contraction as characteristically did the beta-adrenoceptor agonist isoprenaline (for literature see ref. 21), but rather prolonged it, as is shown in Fig. 3.

*Acknowledgement*—This work was supported by grants Scho 15/8 and Er 65/2 from the Deutsche Forschungsgemeinschaft.

*Abteilung* III (*Biochemische Pharmakologie*),
*Institut für Pharmakologie und Toxikologie*,
*Medizinische Hochschule Hannover*,
3000 *Hannover* 61,
*F.R.G.*

INGELORE HACKBARTH
WILHELM SCHMITZ
HASSO SCHOLZ
EVA WETZEL

*Medizinische Klinik* I,
*Klinikum Großhadern*,
*Universität München*,
8000 *München* 80,
*F.R.G.*

ERLAND ERDMANN
WOLFGANG KRAWIETZ
GUNTER PHILIPP

## REFERENCES

1. I. Hackbarth, W. Schmitz, H. Scholz, E. Erdmann, W. Krawietz and G. Philipp, *Nature, Lond.* **275**, 67 (1978).
2. E. Erdmann, W. Krawietz, G. Philipp, I. Hackbarth, W. Schmitz and H. Scholz, *Nature, Lond.* **278**, 459 (1979).
3. U. Borchard, A. A. L. Fox, K. Greeff and P. Schlieper, *Nature, Lond.* **279**, 339 (1979).
4. G. Grupp, I. Grupp, C. L. Johnson, E. T. Wallick and

A. Schwartz, *Biochem. biophys. Res. Commun.* **88**, 440 (1979).
5. L. A. Beaugé and I. M. Glynn, *Nature, Lond.* **272**, 551 (1978).
6. L. C. Cantley, M. D. Resh and G. Guidotti, *Nature, Lond.* **272**, 552 (1978).
7. L. C. Cantley, L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, *J. biol. Chem.* **252**, 7421 (1977).
8. B. R. Nechay and J. P. Saunders, *J. envir. Path. Toxic.* **2**, 247 (1978).
9. E. E. Quist and L. E. Hokin, *Biochim. biophys. Acta* **511**, 202 (1978).
10. U. Schwabe, C. Puchstein, H. Hannemann and E. Söchtig, *Nature, Lond.* **277**, 143 (1979).
11. W. Krawietz, K. Werdan and E. Erdmann, *Biochem. Pharmac.* **28**, 2517 (1979).
12. G. A. Robison, R. W. Butcher and E. W. Sutherland, in *Cyclic AMP*, p. 203. Academic Press, New York (1971).
13. T. Meinertz, H. Nawrath and H. Scholz, *Naunyn-Schmiedeberg's Archs Pharmac.* **293**, 129 (1976).
14. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
15. C. Dönges, M. Heitmann, H. Jungbluth, T. Meinertz, B. Schmelzle and H. Scholz, *Naunyn-Schmiedeberg's Archs Pharmac.* **301**, 87 (1977).
16. E. Kruse and H. Scholz, *Experientia* **34**, 504 (1978).
17. R. J. Boudreau and G. T. Drummond, *Analyt. Biochem.* **63**, 388 (1975).
18. W. J. Thompson and M. M. Appleman, *J. biol. Chem.* **246**, 3145 (1971).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. J. G. Dobson, J. Ross and S. E. Mayer, *Circulation Res.* **39**, 388 (1976).
21. R. W. Tsien, *Adv. cyclic. Nucleotide Res.* **8**, 363 (1977).

Biochemical Pharmacology, Vol. 29, pp. 1432–1435. 0006-2952/80/0515-1432 $02.00/0


# On the mechanism of morphine action on rat striatal dopamine metabolism

(*Received* 17 *September* 1979; *accepted* 30 *November* 1979)

Morphine is known to decrease motor activity and to increase striatal dopamine metabolism in rats [1]. Several hypotheses have been invoked to explain these effects which are presumably mediated by activation of opiate receptors in the CNS since they are abolished by the morphine antagonist naloxone. Kuschinsky [1] and Celsen and Kuschinsky [2] have proposed that morphine causes a diversion of intraneuronal dopamine to nonfunctional metabolism and decreases the rate of dopamine release, actions consistent with both the akinesia and increased metabolism of dopamine. Others [3, 4] have proposed that the morphine-induced increase of dopamine metabolism may be due to indirect stimulation by morphine of dopaminergic impulse flow. Thus, turning behavior, induced by either an agonist of dopamine receptors, apomorphine, or an antagonist, haloperidol, in rats with unilateral striatal lesions, is inhibited by morphine [3]. Also, the morphine-induced increase of dopaminergic impulse flow has a slow onset of action compared with known direct acting dopamine agonists or antagonists [4]. Costa *et al.* [5] have proposed that a pre-synaptic enkephalinergic action on dopamine nerve terminals enhances release of dopamine per impulse. According to their proposal, apomorphine or haloperidol may either inhibit or activate a cholinergic to enkephalinergic loop feeding back on the dopamine nerve ending to either inhibit or activate tyrosine hydroxylase. The first two hypotheses are compatible in that decreased neurogenic release of dopamine would be expected to reflexly increase impulse flow in the nigral-striatal pathway. All three proposals are in harmony with the observed additive effects of morphine and chlorpromazine, a dopamine receptor blocker, to increase striatal dopamine metabolism [6].

The first two hypotheses mentioned above can be tested by using an impulse-dependent releaser of dopamine. Such a drug is the potent CNS stimulant amfonelic acid (AFA) [7]. The release of dopamine per impulse is enhanced by AFA such that, if haloperidol is co-administered in order to maintain impulse flow in the presence of AFA, a potentiated increase of dopamine metabolite concentrations results [8]. Inhibitors of dopamine impulse flow (i.e. apomorphine or γ-butyrolactone) prevent the synergism of these two drugs [8]. Shore *et al.* [7] proposed that AFA and other nonamphetamine stimulants enhance the transfer of dopamine from a large storage pool to a releasable pool so that greater amounts of transmitter are released per impulse. If morphine decreases the neurogenic release of dopamine and diverts dopamine to non-functional metabolism, then AFA, which alone causes a slight increase of dopamine metabolism [8], would be expected to either inhibit the morphine-induced increase of dopamine metabolism or show an additive effect, but not a marked synergism. However, if morphine is indirectly driving impulse flow [4], AFA would be expected to exhibit a supra-additive effect on dopamine metabolism when combined with morphine in a manner analogous to the interaction of AFA with haloperidol.

The hypothesis of Costa *et al.* [5] that the presumed dopamine autoreceptor is actually a pre-synaptic opiate receptor, activated by a cholinergic–enkephalinergic loop, can be tested by using the γ-butyrolactone (GBL) model for studying dopamine pre-synaptic receptors. Administration of GBL inhibits nigral dopamine neuronal impulse flow and increases dopamine levels and tyrosine hydroxylase activity in the striatum [9]. This effect of GBL may be due to either decreased extraneuronal dopamine available for binding to pre-synaptic autoreceptors [9] or activation of pre-synaptic enkephalinergic neurons subsequent to decreased dopaminergic activity [5]. The latter possibility can be tested by observation of the effects of pretreating with either morphine, which should enhance, or naloxone, which should inhibit the effect of GBL on dopamine levels.

Female Sprague–Dawley rats (Holtzman, Madison, WI), 200–250 g, were given various drugs s.c., except for GBL which was given i.p., and killed by chloroform asphyxiation at the times noted in the tables. The brains were rapidly removed, chilled in ice-cold saline, and the corpora striata dissected out and frozen over dry ice. Assays were performed the same day. Dihydroxyphenylacetic acid (DOPAC) was measured by organic extraction and fluo-