中国药理学报 *Acta Pharmacologica Sinica* 1990 Jan; 11 (1) : 36-39

# 偏钒酸钠对豚鼠乳头状肌动作电位的影响[1]

赵爱明[2]、刘泰棣 (北京大学生物系生理学教研室，北京 100871，中国)

**Effects of sodium vanadate on action potentials of guinea pig papillary muscles**

ZHAO Ai-Ming, LIU Tai-Feng (*Division of Physiology, Department of Biology, Peking University, Beijing 100871, China*)

**ABSTRACT** Sodium vanadate ($NaVO_3$, 800 μmol/L) diminished the plateau phase and the action potential duration of the action potentials in guinea pig ventricular fibers (VF). 200 μmol/L also reduced the maximal rate of depolarization of slow action potentials in the partial depolarized VF under high K superfusion and inhibited the delayed after depolarization induced by adrenalin. It is suggested that $NaVO_3$ may inhibit the slow channel current and does not reduce the sodium pump activity in VF significantly when it was used extracellularly.

**KEY WORDS** vanadates; papillary muscles; action potentials; membrane potentials

**提要** 偏钒酸钠($NaVO_3$) 800 μmol/L 使豚鼠心室乳头状肌快反应动作电位平台消失及动作电位时程缩短。200 μmol/L 抑制慢反应动作电位，使最大去极化速率显著降低。$VO_3^-$ 能抑制由肾上腺素 10 μmol/L 诱发的迟后去极化和异常节律活动。结果提示，细胞外施予 $NaVO_3$ 可能抑制心室肌慢通道电流，而对钠钾泵没有显著抑制作用。

**关键词** 钒酸盐类；乳头状肌；动作电位；膜电位

自从骨骼肌中发现有 Na-K-ATP 酶抑制物[1]并证实是钒酸盐 (Va)[2,3] 以来，关于 Va 对 Na-K-ATP 酶抑制作用的研究已有不少报道[4,5]。然而 Va 对心肌收缩的影响却存在着争论。在有些心肌标本上 Va 显示正性变力性作用。而在另些标本上则为负性变力性作用[6,7]。另一方面。近年的工作表明，钠钾泵抑制剂可引起心肌细胞产生迟后去极化 (DAD)及触发性活动(TA)[8]，但尚未见到有关 Va 能引起上述活动的报道。为了进一步阐明 Va 对心肌细胞的作用，本工作观察了 $VO_3^-$ 对豚鼠心室肌动作电位的作用。

## METHODS

豚鼠 42 只，♀♂不拘。击昏后速取心脏，在充以 95%$O_2$ + 5%$CO_2$ 的 Tyrode 液中分离出心室乳头状肌，将其固定在浴槽中并用 Tyrode 液灌流[9]。灌流液温度为 35±0.5℃，pH 7.4±0.5。经 1 h 灌流后开始实验。

动作电位的细胞内记录。采用常规玻璃微电极方法，与以前报道[9]相同。快反应电位(FAP)的驱动刺激频率是 1 Hz，刺激波宽为 1 ms，强度为 2 倍阈强度。进行慢反应电位(SAP)观察时，先用正常 Tyrode 液灌流 1 h，然后用 KCl 28 mmol/L 的 Tyrode 液(其他成分不变)灌流。待 FAP 消失后再经 30 min 用下列方式得到 SAP。

1 用含肾上腺素(Adr 10 μmol/L)或磷酸组织胺(HP 25 μmol/L)的高钾 Tyrode 液灌流，驱动频率为 0.6 Hz，刺激波宽为 1 ms，刺激强度为 2 倍阈强度。

2 驱动刺激频率为 0.6 Hz，波宽为 3-4 ms，刺激强度调到出现稳定的 SAP。灌流液仍为高钾 Tyrode 液。

$NaVO_3$ 50 mmol/L(北京房山陶瓷厂产，AR)，HP 5 mmol/L(北京药品生物制品检定所对照品)，Adr 5.4 mmol/L(北京制药厂产)，实验时用 Tyrode 液稀释到所需浓度。

结果均在同一细胞内获得，并采用配对 *t*

Recieved 1988 Sep 23 Accepted 1989 Jun 22
[1] Project supported by the National Science Foundation of China, № 3860135
[2] Now in *Department of Biology, Teachers College of Xiangtan, Xiangtan 411100, China*

检验进行统计处理。

## RESULTS

**$VO_3^-$ 对 FAP 的影响** 乳头状肌标本经低浓度 $NaVO_3$(50 及 200 μmol/L)处理后，动作电位幅度(APH)略有增高，动作电位时程(APD)略有缩短，但均无统计意义。经高浓度 $NaVO_3$(800 μmol/L)处理 20 min 后，平台消失，APD 显著缩短，但 APH 与动作电位零相上升最大速率($V_{max}$)无显著变化(Tab 1, Fig 1)。

**$VO_3^-$ 对 SAP 的影响** ($n=27$)在 10 个标本上用 Adr 诱发 SAP。所得动作电位的 APH 为 84±5 mV，$V_{max}$ 为 9±2 V/s。经 $NaVO_3$ 200 μmol/L 处理 3 min 后，SAP 消失，10 个标本无一例外(Fig 2)。

为排除 $VO_3^-$ 可能通过肾上腺素β受体的抑制而产生上述影响，用 8 个标本观察了由 HP 诱发的 SAP 以及 $VO_3^-$ 对它的作用。由 HP 所诱发的 SAP，其 APH 为 81±8 mV，$V_{max}$ 为 9±3 V/s。经 $NaVO_3$ 200 μmol/L 处理后，APH 为 73±17 mV，$V_{max}$ 为 6±3 V/s。前者虽有降低但无统计学意义，后者有显著差异，表明 $VO_3^-$ 对由 HP 诱发的 SAP 有抑制作用。

在 9 个标本上未用药物，而是直接用强刺激，在部分去极化标本上引起 SAP。其 APH 为 78±11 mV，$V_{max}$ 为 8±2 V/s。用 $NaVO_3$ 200 μmol/L 处理后，有 3 例标本 SAP 被取消，其余 6 例 APH 为 57±26 mV，$V_{max}$ 为 4±3 V/s 有显著差异。结果表明 $VO_3^-$ 对 SAP 有抑制作用。

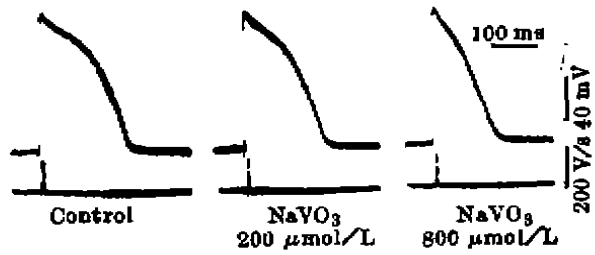



**Fig 1. Effects of $NaVO_3$ on the action potentials of guinea pig ventricular fibers. Upper tracing: action potentials; Lower tracing: $V_{max}$.**

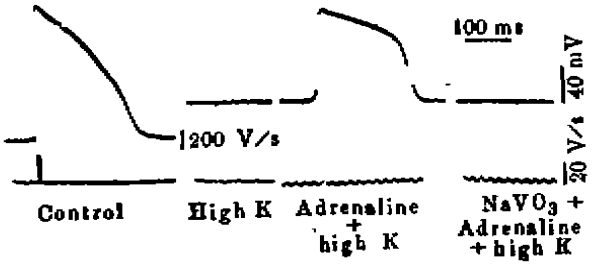



**Fig 2. Effects of $NaVO_3$ 200 μmol/L on the slow action potentials induced by high K (28 mmol/L) of guinea pig ventricular fibers.**

**$VO_3^-$ 对 DAD 的影响** 用 5 种浓度 $NaVO_3$ 处理(50, 200, 500, 800, 1000 μmol/L)以观察是否能诱发 DAD 的产生。结果是所有实验均未观察到 DAD 的产生，说明 $NaVO_3$ 在细胞外施予时，不能使细胞内钙离子浓度增高到产生 DAD 的程度，或者说不能引起显著的钠泵抑制。进一步用快速驱动(5, 10 及 20 Hz)也未观察到 DAD 发生，在实验中我们发现当 Adr 100 μmol/L 引起异常节律或产生 DAD 时，$NaVO_3$ 200 μmol/L 可以使之消除(Fig 3)，则进一步表明 $VO_3^-$ 不仅不能引起细胞内钙离子

**Tab 1. Effects of $NaVO_3$ on action potentials in guinea pig ventricular fibers. $\bar{x}\pm SD$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.**

| $NaVO_3$(μmol/L) | $n$ | APH(mV) | $V_{max}$(V/s) | $APD_{30}$(ms) | $APD_{50}$(ms) | $APD_{90}$(ms) |
|---|---|---|---|---|---|---|
| 0 | 9 | 123±9* | 142±8* | 117±21* | 148±20* | 189±16* |
| 50 | 6 | 127±6* | – | 103±20* | 130±20* | 172±19* |
| 200 | 5 | 126±5* | 145±9* | 102±36* | 128±34* | 168±34* |
| 500 | 6 | 124±6* | – | 78±23* | 97±33* | 146±34** |
| 800 | 8 | 124±8* | 145±9* | 72±29*** | 92±46* | 136±43*** |

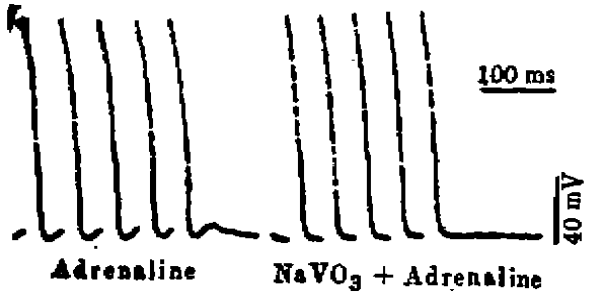



**Fig 3. The inhibitory action of $NaVO_3$ on the delayed afterdepolarization in ventricular fibers induced by adrenaline.**

浓度升高，反而有可能使之降低。

## DISCUSSION

本文表明，$VO_3^-$ 对乳头状肌慢反应电位有明显抑制作用，这至少可以解释 $VO_3^-$ 具有负性变力性作用[10]的部分机理。然而对其尚有正性变力性作用的问题，则须进一步的研究才能明确。关于文中所用 Adr 诱发 SAP 可由 $VO_3^-$ 所抑制的现象，在用收缩作为指标的工作用中也有相似的报道[11]并存在争议，有人认为该作用可能是 $VO_3^-$ 与儿茶酚胺发生化学反应的结果[12]但是 $VO_3^-$ 并不影响儿茶酚胺的生理学作用[13,11]，因而这一因素可以排除。总之，可以认为在实验条件下，既使存在着 $VO_3^-$ 与 Adr 的氧化还原反应可能性，但不影响 $VO_3^-$ 的作用。其次，$VO_3^-$ 既然能抑制由 Adr 诱发的 SAP 则 $VO_3^-$ 就有可能具有肾上腺素 β 受体阻断作用。关于这一点，本实验不能完全排除，但从 $VO_3^-$ 能抑制由 HP 及强刺激引起的 SAP 来看，即使它有 β-阻断作用，也不排斥其对慢通道的直接抑制作用。另有报道，$VO_3^-$ 能激动细胞腺苷酸环化酶[14]而能少量增加细胞内 cAMP 含量[15]，则似乎 $VO_3^-$ 并不具有 β-阻断作用或无强阻断作用。

本工作还表明较大量 $VO_3^-$ 从细胞外施予时，不能引起 DAD 这与 Brucker 等人[11]所得到的 $VO_3^-$ 不能加强 ouabain 的正性变力性作用相吻合。至于它能抑制 Adr 引起的 DAD 的机理则有待研究。但是 $VO_3^-$ 抑制慢通道使 $Ca^{2+}$ 内流减少可能起重要作用。

## REFERENCES


1 Charney AN, Silva P, Epstein FH. An in vitro inhibitor of Na-K-ATPase present in an adenosine triphosphate preparation. *J Appl Physiol* 1975; 39 : 156

2 Cantley LC, Josephson L, Warner R, Yanagisawa M, Lechene C, Guidotti G. Vanadate is a potent (Na, K)-ATPase inhibitor found in ATP derived from muscle. *J Biol Chem* 1977; 252 : 7421

3 Josephson L, Cantley LC. Isolation of potent (Na,K)-ATPase inhibitor from striated muscle. *Biochemistry* 1977; 16 : 4572

4 Erdmann E, Werdan K, Krawietz W, Schmitz W, Scholz H. Vanadate and its significance in biochemistry and pharmacology. *Biochem Pharmacol* 1984; 33 : 945

5 Meisch HU, Bielig HI. Chemistry and biochemistry of vanadate. *Basic Res Cardiol* 1980; 75 : 413

6 Hackbarth I, Schmitz W, Scholz H, Erdmann E, Krawietz W, Philipp G. Positive inotropism of vanadate in cat papillary muscle. *Nature* 1978; 275 : 67

7 Grupp G, Grupp I, Johnson CL, Wallick ET, Schwartz A. Effects of vanadate on cardiac contraction and adenylate cyclase. *Biochem Biophys Res Commun* 1979; 88 : 440

8 Wit AC, Cranefield PF, Gadsby DC. Triggered activity. In: Zipes DP, Bailey JC, Elharrar V, eds. *The slow inward current and cardiac arrythmias*. The Hague: Martinus Nijhoff Publ, 1980 : 437-54

9 Dong LW, Liu TF. Sodium *p*-chloromercuribenzoate induced delayed after depolarizations of guinea pig papillary muscle. *Acta Pharmacol Sin* 1988; 9 : 242

10 Borchard U, Fox AAL, Greeff K, Schlieper P. Negative and positive inotropic action of vanadate on atrial and ventricular myocardium. *Nature* 1979; 279 : 339

11 Brückner *R*, Schmitz W, Scholz H, *et al*. Negative inotropic effect of vanadate in ventricular myocardium in the presence of 3-isobutyl-1-methylxanthine or isoprenaline. *Naunyn Schmidebergs Arch Pharmacol* 1980; 315 : 147

12 Hudgins PM, Bond GH. Reversal of vanadate inhibition of Na,K-ATPase by catecholamines. *Res Commun Chem Pathol Pharmacol* 1979; 23 : 313
13 Krawietz W, Werdan K, Erdman E. Stimulatory effect of vanadate on the adenylate cyclase of cardiac tissue. *Biochem Pharmacol* 1979; 28 : 2517
14 Schwabe U, Puchstein C, Hannemann H, Söchtig E. Activation of adenylate cyclase by vanadate. *Nature* 1979; 277 : 143
15 Hackbarth I, Schmitz W, Scholz H, *et al*. Stimulatory effect of vanadate on cycle AMP levels in cat papillary muscle. *Biochem Pharmacol* 1980; 29 : 1429

中国药理学报 *Acta Pharmacologica Sinica* 1990 Jan, 11 (1) : 39-42

# Acute toxicity of dipfluzine and its effects on isolated vascular smooth muscle

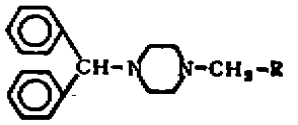
WANG Yong-Li, LI Yun-Shan, FU Shao-Xuan, JIN Sheng[1]

(*Department of Pharmacology, Hebei Medical College, Shijiazhuang 050017, China*)

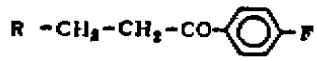
**ABSTRACT** Dipfluzine (Dip) is a new derivative of cinnarizine (Cin) first developed by Department of Chemistry, Beijing University. Dip showed a dose-dependently inhibitory effect on both KCl- and NE-induced contraction in the rabbit aortic rings. It was more effective in suppressing the contractile response evoked by KCl than that by NE. Dip also inhibited the KCl-induced contraction in porcine basilar, coronary and radial arteries. Their $pD_2'$ values were 5.7 ± 0.6, 5.4± 0.4 and 4.6 ± 0.5 respectively. The selectivity of Dip for vasodilation was proved by higher $pD_2'$ value of the basilar artery than that of the coronary and radial arteries, and this selectivity of Dip was more significant than that of Cin. The acute iv $LD_{50}$ of Dip and Cin in mice were 37 and 36 mg/kg, respectively.

**KEY WORDS** dipfluzine; cinnarizine; thoracic aorta; basilar artery; coronary vessels; calcium channel blockers; vascular smooth muscle

Dipfluzine (Dip), a new diphenylpiperazine compound, was first developed by Department of Chemistry, Beijing University, China, according to the characteristics of molecular formula of cinnarizine (Cin) and droperidol. Our previous studies[1] had shown that Dip possessed a dose-dependent depressive effect on blood pressure and femoral resistance, and a potent antagonistic effect on contraction induced by $CaCl_2$ in isolated central artery of rabbit ear. In order to examine the pharmacological characteristics of Dip, selectivities on various vascular smooth muscle preparation of Dip and Cin and their acute toxicity were compared in this paper.



Cinnarizine



Dipfluzine

## MATERIALS AND METHODS

Dip and Cin supplied by Department of Chemistry, Beijing University, were dissolved in tartaric acid solution 100 mmol/L separately as the stock solution, and further diluted with tartaric acid 10 mmol/L daily before use. The same concentrations of tartaric acid solution (solvent, pH 3.1)

Received 1989 Fab 2 Accepted 1989 Aug 29

[1] *Department of Chemistry, Beijing University, Beijing 100871, China*