BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Jul; 18 (4): 293-298

Original Research

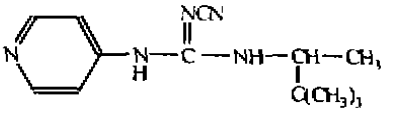
Differential effects of pinacidil, cromakalim, and NS 1619 on electrically evoked contractions in rat vas deferens[1]

HUANG Yu[2], LAU Chi-Wai (*Department of Physiology, Faculty of Medicine, Chinese University of Hong Kong, Shatin, NT, Hong Kong*)

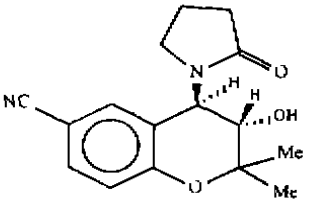
KEY WORDS pinacidil; cromakalim; NS 1619; glyburide; charybdotoxin; electric stimulation; potassium channels; muscle contraction; vas deferens

AIM: To compare the inhibitory action of electrically evoked contractions of rat epididymal vas deferens by pinacidil (Pin), cromakalim (Cro), and NS 1619. **METHODS:** Monophasic contractions were evoked by electric field stimulation in rat isolated epididymal half of vas deferens. **RESULTS:** Newly developed ATP-sensitive K^+ channel openers, Pin and Cro, concentration-dependently reduced the electrically evoked (0.3 Hz, 1 ms pulse duration, 60 V) contractions and glibenclamide but not charybdotoxin antagonized the inhibitory effects of both agents. Pin shifted the concentration-response curve for norepinephrine to the right with reducing the magnitude of the maximum contraction in a glibenclamide-sensitive fashion. The large-conductance Ca^{2+}-activated K^+ channel opener, NS 1619, inhibited the electrically evoked contractions in a concentration-dependent manner. Charybdotoxin (100 nmol·L^{-1}) partially reduced the effect of NS 1619 but glibenclamide (10 μmol·L^{-1}) showed no effect. None of these 3 agents affected the basal tension. **CONCLUSION:** Both ATP-sensitive and Ca^{2+}-activated K^+ channels presented in vas deferens smooth muscles involved in regulation of muscle contractility.

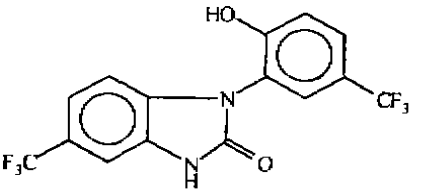
Pinacidil (Pin) and cromakalim (Cro), the potassium channel openers, belong to a new class of pharmacological agents which potently relax a variety of smooth muscles[1]. The effects of Pin and Cro are inhibited by the sulfonylurea antidiabetic drugs such as glibenclamide (Gli) and glyburide, selective blockers of ATP-sensitive K^+ (K_{ATP}) channels[2-5]. K_{ATP} channels on muscle membrane were activated by endogenous agents[6], which have distinct pharmacology from Ca^{2+}-activated K^+ (K_{Ca}) channels[7]. K_{ATP} channels are also present in nerves[8] which may be involved in regulation of neurotransmission in the periphery. Cro and levcromakalim inhibit neurogenic smooth muscle contraction *in vitro* in rabbit epididymal vas deferens[9], and the release of norepinephrine from nerve terminals in guinea-pig vas deferens[8] in a Gli-sensitive fashion. More recently, however, a novel group of the benzimidazolone derivatives, typified by NS 1619 and NS 004, also have smooth muscle



Pinacidil



Cromakalim



NS 1619

[1] Supported by Hong Kong RGC and UPCG Direct grants (CUHK 445/95M and A/C № 220404750).
[2] Phn: 852-2609-6882 Fax: 852-2603-5022.
Received 1996-10-18 Accepted 1997-04-30

relaxant actions[10]. These compounds relax smooth muscle by activating K_{Ca} channels in porcine coronary arterial cells[11] and rat cerebral artery smooth muscle[12]. Charybdotoxin (CTX), a selective large-conductance K_{Ca} channel blocker[13], inhibits the effect of NS 1619[12]. However, the differential effects of Pin and NS 1619 on neurogenic contractions of vas deferens have not be examined. The present study was to compare the inhibitory action of electrically evoked contractions of rat epididymal vas deferens by these 2 types of K^+ channel openers, Pin, Cro, and NS 1619.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 300 g were killed by cervical dislocation and a pair of vas deferens were dissected out. The epididymal portion was cut and cleared of surrounding connective tissues and visible blood vessels. Segments of the vasal duct about 2 cm in length were then mounted between 2 platinum electrodes placed 1 cm apart in a 10-mL organ baths containing Krebs-Henseleit solution of the following compositions (in mmol·L^{-1}): NaCl 119, KCl 4.2, $MgCl_2$ 1, $CaCl_2$ 2.5, KH_2PO_4 1.2, Na_2CO_3 25, Na_2-edetic acid 0.03, *d*-glucose 11.1, ascorbic acid 0.2. The bath solution was oxygenated with 95 % O_2 + 5 % CO_2 and maintained at 37 ℃ throughout the experiment. The tissue was positioned vertically with one end attached to a glass support and another end to the force transducer (Grass Instruments Co). Tissues were allowed to equilibrate under 1 g resting tension for 90 min while the bath solution was changed every 30 min.

The electric field stimulation was repetitively delivered to the vas deferens with use of Grass SD9 Stimulator via 2 *platinum electrodes (0.3 Hz, 1 ms pulse duration, 60 V).* The electrically evoked monophasic contractile responses were abolished by tetrodotoxin (3 μmol·L^{-1}) or by combination of prazosin 3 μmol·L^{-1} and α,β-methylene ATP 3 μmol·L^{-1} (30 min contact time), indicating the neurogenic origin of the contraction. Each of K^+ channel openers was added cumulatively to the bath to induce concentration-dependent inhibition of evoked contractions in the absence and presence of Gli or CTX at different concentrations. The mean amplitude of 5 consecutive twitches was measured at the start of application of next dose. In the 2nd set of experiments, preparations were contracted with norepinephrine (NE, 10 nmol · L^{-1} – 30 μmol · L^{-1}) to construct the first concentration-response curve. Once the maximum responses to NE had been reached, tissues were rinsed with Krebs' solution every 20 min until the tension fell to the basal level. Tissues were then equilibrated with different concentration of Pin for 10 min and another concentration-response curve to NE was repeated. In some experiments, Gli or CTX was added to the bath 10 mun prior to application of Pin.

Results were expressed as $\bar{x} \pm s$ of n experiments. The effects of K^+ channel openers on contractions evoked by field stimulation were presented as a percentage of the control value. Cumulative concentration-response relations were analyzed with a non-linear curve fitting by a logistic equation (Graft, Erithacuc Software Ltd) and IC_{50} values with 95 % confidence limits were calculated as the drug concentration causing a half-maximum inhibition. To study the effect of Pin on the NE-induced contractions, values of EC_{50} and maximal tension were compared in the absence and presence of the drug. *t*-Test was used to evaluate the significant difference between mean values.

The following chemicals and drugs were used: (−)-norepinephrine bitartrate, prazosin hydrochloride, Pin, Cro, tetrodotoxin, α,β-methylene ATP, Gli (Sigma, USA), NS 1619, charybdotoxin (Research Biochemicals, Natick MA, USA). All drugs were dissolved in Krebs' solution except for Gli, Cro, and NS 1619 in dimethyl sulfoxide. 0.2 % Me_2SO in organ baths did not affect muscle contractions induced by electric field stimulation.

RESULTS

Effects of Pin and Cro on electrically evoked contractions Monophasic contractions (6.29 ± 0.03 mN, $n = 45$) of the epididymal segment of *rat vas deferens were evoked by repetitive electric* field stimulation. Prazosin (3 μmol·L^{-1}) and α,β-methylene ATP (3 μmol · L^{-1}) decreased the amplitude of the electrically evoked contraction to 43 ± 22 % and 67 ± 20 % of control, respectively (n = 5 in each case), indicating that contractions were induced by NE and ATP probably co-released from the sympathetic nerve terminals in vas deferens upon stimulation.

Pin and Cro concentration-dependently reduced the amplitude of evoked contractions in the absence and presence of Gli. Gli shifted the concentration-response curve for the inhibitory effects of Pin and Cro to the right (Fig 1).

Fig 2A showed that Pin reduced the evoked contraction with IC_{50} values (95% confidence limits) of 0.5 ± 0.3 (0.1 − 2.4) μmol · L^{-1} in control (n = 8), 3.1 ± 0.8 (0.9 − 11.4) μmol·L^{-1} in Gli 1 μmol·L^{-1} (n = 5), 11.5 ± 1.3 (2.1 − 62.5) μmol·L^{-1} in Gli 3 μmol·L^{-1} (n = 5) and 31 ± 9 (1.4 − 65.4) μmol·L^{-1} in Gli 10 μmol·L^{-1}

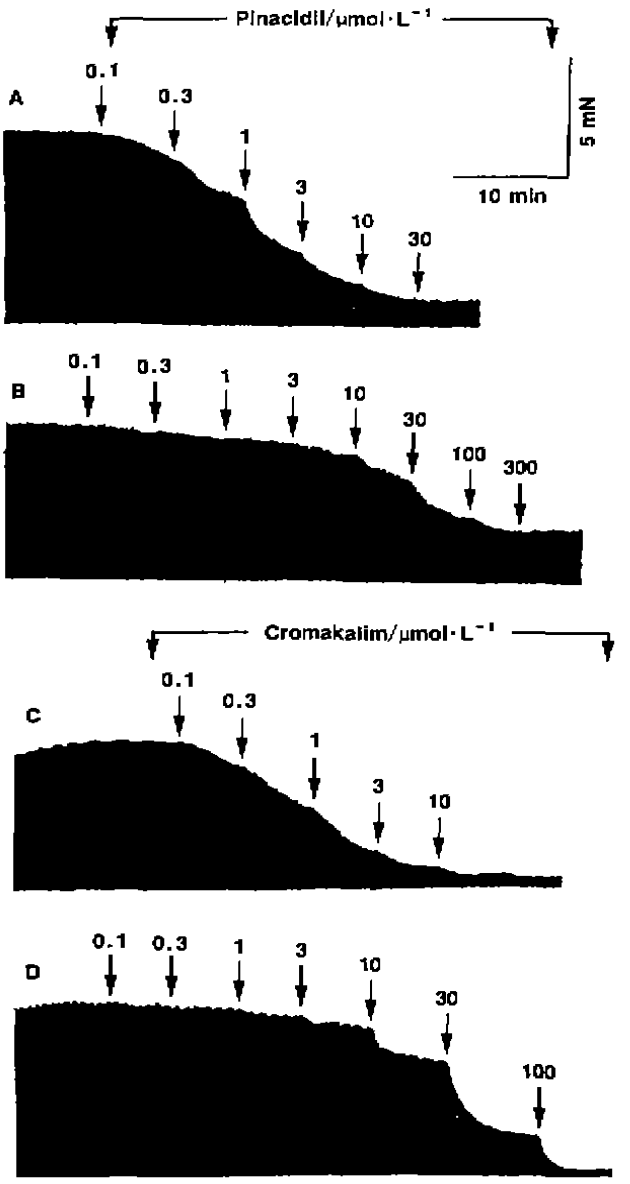


Fig 1. Inhibitory effects of Pin (A, B) and Cro (C, D) on monophasic contractions induced by electric field stimulation in rat epididymal vas deferens in the absence (A, C) and presence (B, D) of glibenclamide 10 μmol·L⁻¹ which was added 10 min before application of Pin and Cro. Calibration bars apply to all records.

($n=5$), respectively. Fig 2B shows that Cro inhibited the evoked contraction with respective IC_{50} values of 0.24 ± 0.12 (0.04 − 1.32) μmol·L⁻¹ in control ($n=6$), 1.7 ± 0.5 (0.5 − 6.0) μmol·L⁻¹ in Gli 1 μmol·L⁻¹ ($n=5$), 3.9 ± 0.8 (1.5 − 10.4) μmol·L⁻¹ in Gli 3 μmol·L⁻¹ ($n=5$) and 13.9 ± 1.7 (3.6 − 54.0) μmol·L⁻¹ in Gli 10 μmol·L⁻¹

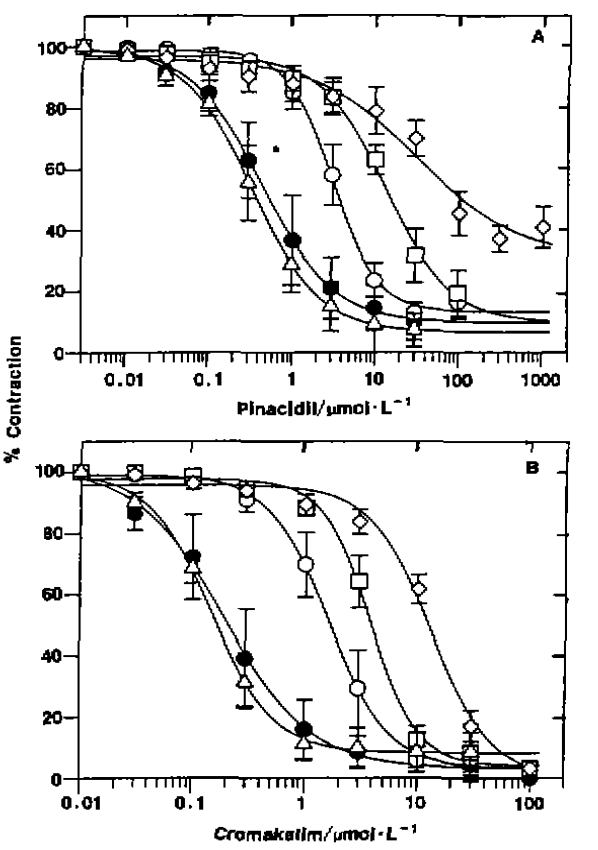


Fig 2. Effects of Pin and Cro on electrically evoked contractions. (A) Pin and (B) Cro: (●), $n=8$, in A control, $n=6$, in B control; (○), $n=5$, in Gli 1 μmol·L⁻¹; (□), $n=5$, in Gli 3 μmol·L⁻¹; (◇), $n=5$, in Gli 10 μmol·L⁻¹; (△), $n=4$, in CTX 100 nmol·L⁻¹. n = number of experiments, $\bar{x}\pm s$. Curves were drawn by fitting the data points to a logistic equation to give respective IC_{50} values.

($n=5$). On the other hand, the inhibitory effects of Pin and Cro were unaffected by CTX. The IC_{50} values for the Pin- and Cro-induced inhibition in the presence of CTX (100 nmol·L⁻¹) were 0.34 ± 0.10 μmol·L⁻¹ (0.05 − 2.48 μmol·L⁻¹, $n=4$) and 0.18 ± 0.04 μmol·L⁻¹ (0.03 − 0.98 μmol·L⁻¹, $n=4$), respectively (Fig 2). These values are not different from those obtained in the absence of CTX ($P>0.05$).

Effect of Pin on NE-indnced contractile responses NE evoked contractions of rat epididymal vas deferens consisting of intermittent spikes superimposed on a tonic component with an EC_{50} value of 1.01 ± 0.23 μmol·L⁻¹ ($n=15$) and

maximal rise in tension of 8.2 ± 2.1 mN (n = 15, measured at the mean height of intermittent spikes in the presence of NE 10 μmol·L^{-1}). Pin (up to 10 μmol·L^{-1}) did not affect the basal level of tension. Following construction of the first concentration-response curve for NE, the preparation was incubated with Pin at different concentrations for 10 min and another concentration-response curve for NE was repeated. The NE concentration-response curve was significantly shifted to the right in the presence of Pin (Fig 3).

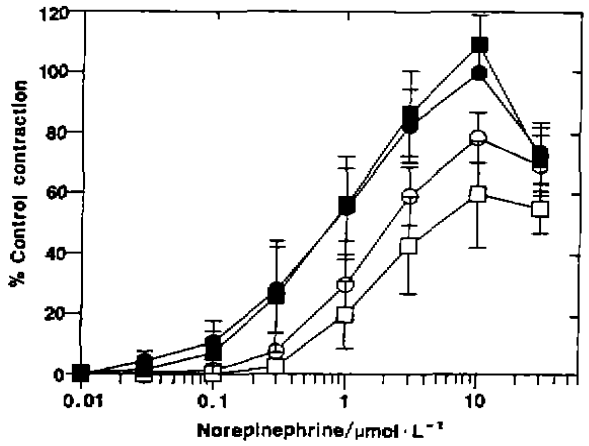


Fig 3. Inhibitory effect of Pin on the NE-induced contraction. (●), n = 15, in control; (○), n = 5, in Pin 1 μmol·L^{-1}; (□), n = 5, in Pin 10 μmol·L^{-1}; (■), n = 5, in Pin 10 μmol·L^{-1} plus glibenclamide 10 μmol·L^{-1}. n = number of experiments. $\bar{x} \pm s$.

EC_{50} values (95 % confidence limits) were 1.01 ± 0.23 (0.3 − 3.3) μmol·L^{-1} in control (n = 15), 1.5 ± 0.3 (0.4 − 6.4) μmol·L^{-1} in Pin 1 μmol·L^{-1} (n = 5), and 1.8 ± 0.3 (0.5 − 6.2) μmol·L^{-1} in Pin 10 μmol·L^{-1} (n = 5), respectively. Pin reduced the maximal contraction to NE by 22 % and 41 %, respectively, at concentrations of 1 and 10 μmol·L^{-1}. Gli 10 μmol·L^{-1} completely reversed the inhibitory effect of Pin 10 μmol·L^{-1} on the NE-induced contractions in rat vas deferens (EC_{50} = 1.16 ± 0.10 μmol·L^{-1} with 95 % confidence limits of 0.19 − 6.92 μmol·L^{-1}, n = 5, in Gli 10 μmol·L^{-1} + Pin 10 μmol·L^{-1}, P < 0.05 *vs* control).

Effect of NS 1619 on electrically evoked contractions Fig 4 showed that NS 1619 reduced the amplitude of electrically evoked contractions with an IC_{50} (95 % confidence limits) of 53 ± 14 μmol·L^{-1} (9.1 − 305.1 μmol·L^{-1}, n = 6). The concentration-inhibition curve to NS 1619 was shifted to the right in the presence of CTX (IC_{50} of 102 ± 24 μmol·L^{-1}, 18.2 − 466.9 μmol·L^{-1}, in CTX 100 nmol·L^{-1}, n = 5). CTX by itself increased the evoked contractions, therefore, the stimulation intensity was lowered to between 25 to 35 V in an attempt to match the initial levels of contractions in the absence of CTX. By contrast, Gli 10 μmol·L^{-1} (n = 4) failed to influence the inhibitory effect of NS 1619 (IC_{50} = 47 ± 14 μmol·L^{-1}, 6.9 − 315.2 μmol·L^{-1}, n = 4, P > 0.05 *vs* control). NS 1619 at 300 μmol·L^{-1} did not alter the basal tension of 1 g.

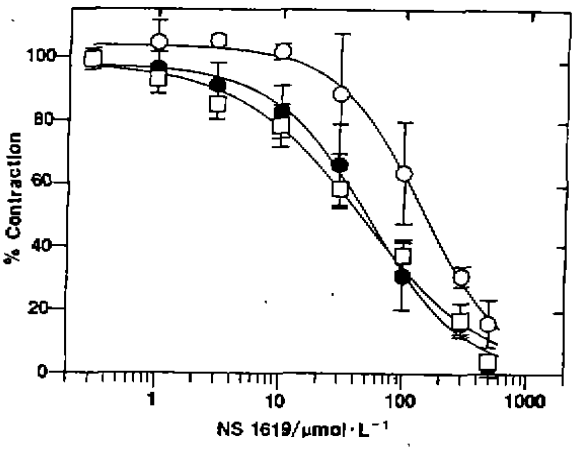


Fig 4. Inhibitory action of NS 1619 on electrically evoked contraction in rat vas deferens. (●), n = 6, in control; (○), n = 5, in charybdotoxin 100 nmol·L^{-1}; (□), n = 4, in glibenclamide 10 μmol·L^{-1}. n = number of experiments, $\bar{x} \pm s$.

DISCUSSION

Gli at concentrations up to 10 μmol·L^{-1} had no effect on the basal tension of rat vas deferens, but it significantly antagonized the inhibitory effects of both Pin and Cro which were previously shown to activate K_{ATP} channels in smooth muscle[1,4]. These results are in agreement with competitive antagonism by Gli of Cro inhibition of twitch contractions in rabbit vas deferens[9]. In contrast to about 56 % maximal inhibition by Cro 3 μmol·L^{-1} reported by Eltze in rabbit vas deferens[9], we found that Cro at the same concentration caused

approximately 90 % reduction of electrically evoked tension in rat vas deferens. Moreover, Gli (10 $\mu mol \cdot L^{-1}$) did not affect the maximal effect of Cro in our preparation, but it (3 – 10 $\mu mol \cdot L^{-1}$) enhanced the maximal inhibition by Cro in rabbit vas deferens[9]. This discrepancy may be attributed to the species difference or to different sensitivity to Cro. Interestingly, Gli at 10 $\mu mol \cdot L^{-1}$ reduced the maximal effect of Pin. It is possible that Pin may also act via a K^+ channel independent mechanism as suggested by Cai *et al*[14] in blood vessels.

Present results clearly showed that large-conductance K_{Ca} channels were not involved in the inhibitory effects of Pin and Cro despite the fact that these 2 agents were previously reported to increase the open state probability of the reconstituted aortic K_{Ca} channels[15]. CTX, a potent blocker of large-conductance K_{Ca} channels[13], did not affect the actions of Pin and Cro in rat vas deferens. Pin also reduced the NE-induced contractile response even at low concentrations (>0.03 $\mu mol \cdot L^{-1}$) which started to reduce electrically evoked contractions (data not shown), suggesting that Pin may primarily activate K_{ATP} channels on smooth muscle membrane. However, Cro 0.3 – 3 $\mu mol \cdot L^{-1}$ did not affect the NE-induced tension in Wistar Morini rat vas deferens[5]. This discrepancy may be caused by different strains of rats used between Grana *et al* and our group. Nevertheless, the possibility can not be discounted that presynaptic inhibition of neurotransmitter release by Pin may contribute in a small portion towards the observed inhibition of contraction in response of field stimulation. It was indicated that the Pin-induced vasorelaxation may also resulted from reduction of NE release from the sympathetic nerve[14].

In contrast to the Gli antagonism of the inhibitory effects of Pin and Cro, NS 1619, the newly developed activator of large-conductance K_{Ca} channels in many smooth muscles[10] concentration-dependently inhibited the electrically evoked contraction in rat vas deferens in a Gli-insensitive manner. CTX partially but significantly reduced the inhibitory effect of NS 1619. Similarly, iberiotoxin completely reversed membrane hyperpolarization induced by NS 1619 but only partially inhibited the NS 1619-induced vasorelaxation[12]. This indicates that NS 1619 may have additional sites of action in rat vas deferens as reported in vascular smooth muscle. Indeed, NS 1619 was recently found to inhibit L-type Ca^{2+} channels in arterial smooth muscle[15,17]. CTX alone significantly enhanced the amplitude of electrically evoked contractions, indicating that large-conductance K_{Ca} channels are active in sympathetic nerve terminals since this concentration of CTX did not significantly alter the NE-induced response in the same preparation (data not shown).

In summary, our results provide evidence of existence of K_{ATP} channels and large-conductance K_{Ca} channels in rat epididymal vas deferens. Gli selectively antagonized the inhibitory effects of Pin and Cro on electrically evoked contractions while CTX showed no effect. On the other hand, CTX but not Gli partially reduced the effect of NS 1619. It is suggested that both types of K^+ channels may play a role in regulation of contractility of rat vas deferens.

REFERENCES

1 Cook NS, Quast U. Potassium channel pharmacology. In: Cook NS, editor. Potassium channels. Chichester: Ellis Horwood Limited, 1990: 181.

2 Grover GJ, McCullough JR, Henry DE, Conder ML, Sleph PG. Anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker glyburide. J Pharmacol Exp Ther 1989; **251**: 98 – 104.

3 Eltze M. Glibenclamide is a competitive antagonist of cromakalim, pinacidil and RP 49356 in guinea-pig pulmonary artery. Eur J Pharmacol 1989; **165**: 231 – 9.

4 Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 1989; **245**: 177 – 80.

5 Grana E, Barbieri A, Zonta F. Inhibition by glibenclamide of the effects of cromakalim on responses of rat vas deferens to naphazoline. Eur J Pharmacol 1991; **202**: 93 – 6.

6 Nelson MT, Huang Y, Brayden JE, Hescheler J, Standen NB. Arterial dilations in response to calcitonin gene-related peptide involve activation of K^+ channels. Nature 1990; **344**: 770 – 3

7 Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am J Physiol 1990; **259**: C3 – 18.

8 Katsuragi T, Ogawa S, Furukawa T Contribution of intra- and

extracellular Ca^{2+} to noradrenaline exocytosis induced by ouabain and monensin from guinea-pig vas deferens.
Br J Pharmacol 1994; 113: 795 - 800.

9 Eltze M.
Competitive antagonism by glibenclamide of cromakalim inhibition of twitch contractions in rabbit vas deferens
Eur J Pharmacol 1989; 161: 103 - 6.

10 Olesen SP, Munch E, Moldt P, Drejer J. Selective activation of Ca^{2+}-dependent K^+ channels by novel benzimidazolone.
Eur J Pharmacol 1994; 251: 53 - 9

11 Hu SL, Kim HS, Fink CA.
Differential effects of the BK_{Ca} channel openers NS 004 and NS 1608 in porcine coronary arterial cells.
Eur J Pharmacol 1995; 294: 357 - 60.

12 Holland M, Langton PD, Standen NB, Boyle JP. Effects of the BK_{Ca} channel activator, NS 1619, on rat cerebral artery smooth muscle. Br J Pharmacol 1996; 117: 119 - 29.

13 *Miller C, Moczydlowski E, Latorre R, Phillips M.*
Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle.
Nature 1985; 313: 316 - 8

14 Cai BQ, Hao QZ, Greenberg SS, Debousblanc B, Gillott D, Goharderakhshan R, *et al*. Differential effects of pinacidil and cromakalim on vascular relaxation and sympathetic neurotransmission. Can J Physiol Pharmacol 1994; 72: 801 - 10.

15 Gelband CH, van Breemen C. Reconstituted aortic Ca^{2+}-activated K^+ channels: selectivity and pharmacological modulation by cromakalim (BRL 34915) and pinacidil.
Biophys J 1989; 55: 545a.

吡那地尔，cromakalim 和 NS 1619 对电刺激所致大鼠输精管收缩的作用[1]

黄 聿，刘志伟
（香港中文大学医学院生理系，新界沙田，香港）

关键词 吡那地尔；cromakalim；NS 1619；格列本脲；charybdotoxin；电刺激；钾通道；肌肉收缩；输精管

目的：本文研究新近研制的 ATP 敏感性钾通道开放剂，吡那地尔（Pin）和 cromakalim（Cro），以及钙离子激活性钾通道开放剂 NS 1619 对电场刺激所致大鼠输精管收缩的作用．方法：利用电场刺激（0.3 Hz，1 ms，60 V）反复性引致输精管单相性收缩．结果：Pin 和 Cro 浓度依赖性减低电刺激收缩．格列本脲（Gli）而非 charybdotoxin 拮抗上述两药的舒张作用．Pin 右移去甲肾上腺素的浓度－收缩曲线，同时降低最高收缩反应．Gli 抵消 Pin 的作用．Charybdotoxin 而非 Gli 减低 NS 1619 的平滑肌舒张作用．结论：ATP 敏感性和钙离子激活性钾通道参与调节输精管平滑肌的收缩性．