

## Effects of nicotinamide on cardiac contraction force and slow inward current<sup>1</sup>

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**ABSTRACT** The effects of nicotinamide (Nic) on cardiac contraction force and on slow inward current ( $I_{si}$ ) in guinea pigs were studied with atrial strips and voltage clamp techniques. Verapamil (Ver),  $MnCl_2$ , nifedipine (Nif), and propranolol (Pro) depressed markedly the positive inotropic effect induced by Nic in a noncompetitive manner. The  $pD_2$  values of Ver,  $MnCl_2$ , Nif, and Pro were 6.19, 3.41, 5.00, and 6.43, respectively. The action of Nic was reduced by a low  $Ca^{2+}$  Tyrode solution and disappeared in a  $Ca^{2+}$ -free solution. Nic  $33 \text{ mmol} \cdot L^{-1}$  elevated the  $I_{si}$  from  $6.5 \pm 1.3 \mu A$  to  $10.3 \pm 2.2 \mu A$ . The results suggest that Nic promotes the  $Ca^{2+}$  influx and its site of action is different from that of both Pro and the calcium antagonists.

**KEY WORDS** nicotinamide; calcium; heart atrium; papillary muscle; calcium channel blockers; electrophysiology

Nicotinamide (Nic) shows a positive inotropic effect<sup>(1)</sup>, increases the slope of phase 4 of the action potential of the sinoatrial node cells and this action of Nic was antagonized completely by Ver but not by Pro<sup>(2)</sup>. This study aimed at elucidating the interrelation between Nic and calcium antagonists on  $I_{si}$ , to analyze the mechanism of action of Nic.

### MATERIALS AND METHODS

**Atrial strip experiment** Seventy-five guinea pigs of either sex weighing  $450 \pm 95 \text{ g}$  were killed by stunning. The atrial strip was prepared according to a previous report<sup>(3)</sup>.

**1 Cumulative concentration-responses to Nic**  
After having been equilibrated for 30 min, the bathing

fluid was changed by the same Tyrode solution<sup>(3)</sup>. After 30 min Nic was added to the bath in 1.5 times increment and the peak force of contraction response to each concentration was recorded as control. In the experimental group, the strip was equilibrated for 30 min in normal Tyrode solution which was then replaced by low- $Ca^{2+}$  Tyrode solution or by normal Tyrode solution containing Ver,  $MnCl_2$ , Nif, or Pro. After 30 min the responses were measured.

**2 Cumulative concentration-responses to  $CaCl_2$**   
After 60-min equilibration, the responses to  $CaCl_2$  were measured when the force of contraction began to decrease and then the preparation was washed 5 times with Tyrode solution. Ver,  $MnCl_2$ , or Nif was added to the bath. The responses to  $CaCl_2$  were measured again 30 min afterwards.

**Voltage clamp experiment** A fine papillary muscle (0.2-0.7 mm in diameter and 4-6 mm in length) dissected from the right ventricle of guinea pig was mounted on a single sucrose-gap chamber. Each compartment was perfused with Tyrode solution for 1 h. Then the compartments were perfused with 3 different solutions<sup>(4)</sup>. When the gap voltage reached the maximum (about  $-80 \text{ mV}$ ), the voltage clamp was set up. Details of chambers and the voltage clamp circuit were the same as that described by Sun *et al*<sup>(5)</sup>. All solutions were aerated with 100%  $O_2$ . The pH was adjusted to 7.4. Experiments were done at  $35 \pm 1^\circ C$ .

### RESULTS

#### Effect on positive inotropic action of Nic and $CaCl_2$

**1 Effect of extracellular  $Ca^{2+}$  on Nic action** Low- $Ca^{2+}$  ( $0.9 \text{ mmol} \cdot L^{-1}$ ) Tyrode solution produced an obvious reduction in the positive inotropic action of Nic. The action of Nic disappeared in the  $Ca^{2+}$ -free Tyrode solution (Fig 1).

#### 2 Effect of Ver, $MnCl_2$ , Nif, or Pro on

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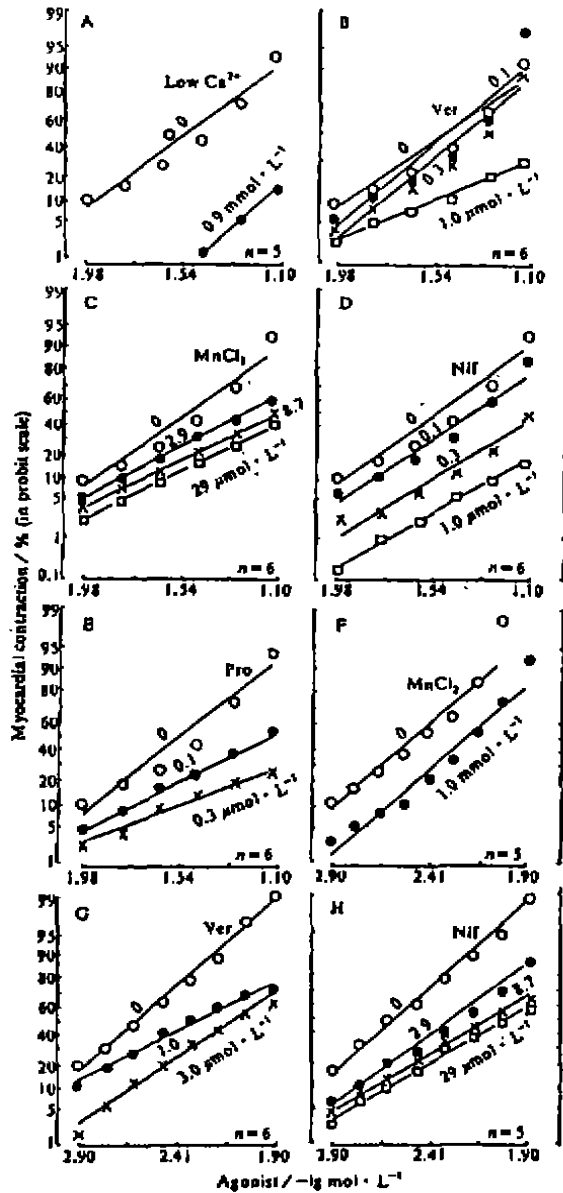


Fig 1. Effect of low- $\text{Ca}^{2+}$  Tyrode solution (Low  $\text{Ca}^{2+}$ ,  $\text{mmol} \cdot \text{L}^{-1}$ ), verapamil (Ver,  $\mu\text{mol} \cdot \text{L}^{-1}$ ),  $\text{MnCl}_2$  ( $\mu\text{mol} \cdot \text{L}^{-1}$ ,  $\text{mmol} \cdot \text{L}^{-1}$ ), nifedipine (Nif,  $\mu\text{mol} \cdot \text{L}^{-1}$ ), and propranolol (Pro,  $\mu\text{mol} \cdot \text{L}^{-1}$ ) on positive inotropic action induced by nicotine (A-E) and  $\text{CaCl}_2$  (F-H) in left atrial strip of guinea pig.

**Nic action** Lower Ver ( $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ ) made the positive inotropic action of Nic shifted to a

higher dose, and increased the maximal force of myocardial contraction induced by Nic. Higher Ver ( $0.3, 1.0 \mu\text{mol} \cdot \text{L}^{-1}$ ),  $\text{MnCl}_2$ , Nif, and Pro made the effect of Nic moving to higher doses, but depressed the maximal response. The  $\text{pD}_2^{(6)}$  values of Ver,  $\text{MnCl}_2$ , Nif, and Pro were 6.19, 3.14, 5.00, and 6.43, respectively (Fig 1).

**3 Effect of Ver, Nif, and  $\text{MnCl}_2$  on  $\text{CaCl}_2$  action**  $\text{MnCl}_2$  made the positive inotropic action of  $\text{CaCl}_2$  shifted to higher dose, and the maximal force of contraction induced by  $\text{CaCl}_2$  did not show any change. The  $\text{pA}_2$  value was 2.78. Ver and Nif made the effect of  $\text{CaCl}_2$  move to higher doses, but decreased the maximal response. The  $\text{pD}_2$  values of Ver and Nif were 5.48 and 4.61, respectively (Fig 1).

**Effect of Nic on  $I_{\text{Ca}}$**   $I_{\text{Ca}}$  was usually elicited by a  $-25 \text{ mV}$  rectangular depolarizing pulse (Command signal;  $E_c$ ) with duration of 550 ms ( $T_c$ ), starting from a slow holding potential ( $E_h$ ;  $-30 \text{ mV}$ ) at which the  $\text{Na}^+$  channel was sufficiently inactive<sup>(4)</sup>. The current was increased from  $6.1 \pm 1.5 \mu\text{A}$  to  $11.4 \pm 2.6 \mu\text{A}$  by  $\text{CaCl}_2$ . It indicated that the current was indeed  $I_{\text{Ca}}$ . After Nic was perfused for 15 min, the peak of  $I_{\text{Ca}}$  was elevated from  $6.8 \pm 1.3 \mu\text{A}$  to  $10.3 \pm 2.2 \mu\text{A}$  (Fig 2).

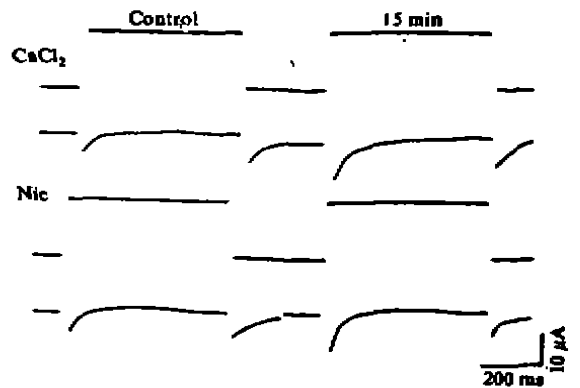


Fig 2. Effects of  $\text{CaCl}_2$  ( $5.4 \text{ mmol} \cdot \text{L}^{-1}$ ) and nicotine ( $33 \text{ mmol} \cdot \text{L}^{-1}$ ) on  $I_{\text{Ca}}$  of guinea pig papillary muscle. Upper tracing;  $V_m$ . Lower tracing;  $I_{\text{Ca}}$ ,  $E_h$ ;  $-30 \text{ mV}$ .  $E_c$ ;  $-25 \text{ mV}$ .  $T_c$ ; 550 ms.

DISCUSSION

We proved that Nic had indeed a positive inotropic effect on the isolated atrial muscles of guinea pigs. The range of myocardial reaction to Nic depended on the extracellular Ca<sup>2+</sup> concentration, and Nic increased the amplitude of I<sub>in</sub> markedly. It showed that Nic could promote the Ca<sup>2+</sup> influx.

In the present study MnCl<sub>2</sub> antagonized competitively the positive inotropic action of CaCl<sub>2</sub>. This result coincides with report<sup>(7)</sup>. But Ver, MnCl<sub>2</sub>, Nif, and Pro antagonized non-competitively the positive inotropic action of Nic. It shows that the site of action of Nic is different from that of both Pro and calcium antagonists.

It was reported<sup>(8)</sup> that calcium antagonist receptors had 2 affinity states, the high affinity state and the low affinity state. The former was related to excitement and the latter to inhibition. Calcium antagonist showed high affinity at lower dose. In this study the effect of Ver increasing the positive inotropic effect of Nic at lower dose might be related to this mechanism.

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514-516

烟酰胺对心肌收缩力和慢内向电流的影响<sup>①</sup>

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摘要 本文用心房条和电压钳技术研究了 Nic 对豚鼠心肌收缩力和 I<sub>in</sub> 的影响, Ver, MnCl<sub>2</sub>, Nif 和 Pro 以非竞争方式显著抑制 Nic 所致正性肌力效应. 其 pD<sub>2</sub> 值分别为 6.19, 3.41, 5.00 和 6.43. Nic 的作用被低钙台氏液减弱, 在无钙液中消失. Nic 33 mmol·L<sup>-1</sup> 使 I<sub>in</sub> 由 6.8 ± 1.3 μA 增至 10.3 ± 2.2 μA. 结果提示 Nic 确能促进钙离子内流, 其作用位点与 Pro 和钙拮抗剂均不相同.

关键词 烟酰胺; 钙; 心房; 乳头状肌; 钙通道阻滞剂; 电生理学

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