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邻苯三酚与亚甲蓝舒张大鼠肠系膜动脉的不同机制<sup>1</sup>

R965.2

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**A** 摘要 在去内皮肠系膜血管, 邻苯三酚与亚甲蓝产生浓度依赖性舒张, SOD 可取消邻苯三酚对 TNS 所致缩血管效应的抑制作用, 但不影响亚甲蓝的效应. 邻苯三酚与亚甲蓝的舒血管效应不被过氧化氢酶、去铁胺、吲哚美辛和辣椒素所影响. 结果提示, 邻苯三酚的舒血管效应是其产生超氧阴离子所致, 而亚甲蓝的效应为直接作用于血管平滑肌.

**关键词** 肠系膜动脉; 邻苯三酚; 亚甲蓝; 亚硝基精氨酸甲酯; 超氧化物歧化酶; 吲哚美辛; 辣椒素; 过氧化氢酶; 去铁胺

Effects of toosendanin on electric and mechanical properties of guinea pig papillary muscles<sup>1</sup>

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**ABSTRACT** Effects of toosendanin (TS) on the action potentials and contractile force in guinea pig papillary muscles were examined using a standard microelectrode technique.

TS concentration-dependently increased the action potential duration at 90% repolarization (APD<sub>90</sub>) of the fast action potentials. In the presence of a I<sub>K1</sub> channel blocker BaCl<sub>2</sub>, the effects of TS on lengthening the APD<sub>90</sub> were completely abolished, thereby suggesting that TS inhibited the inward rectifier K<sup>+</sup> current I<sub>K1</sub>. The APD and contractile force of amino-

Received 1992-11-02 Accepted 1993-09-03  
<sup>1</sup> Project supported by the National Natural Science Foundation of China, No 38970831.  
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phylline-induced slow action potentials were potentiated by TS in a concentration-dependent manner. In the presence of  $\text{BaCl}_2$ , both effects of TS were completely abolished. The effect of TS on enhancing contractile force was abolished by the addition of  $\text{CdCl}_2$ , with the prolongation of APD preserved. Thus, TS selectively inhibited the inward rectifier  $\text{K}^+$  current  $I_{K1}$  with a positive inotropic effect, resulting from a delay in Ca channel inactivation which was secondary to delay in ventricular repolarization.

**KEY WORDS** toosendanin; papillary muscles; action potentials; myocardial contraction; ion channels

Toosendanin (TS) is a triterpenoid derivative ( $\text{C}_{30}\text{H}_{38}\text{O}_{11}$ ) extracted from the bark of *Melia toosendan* Seib et Zucc, which has been elucidated in structure<sup>1)</sup> and used as an ascaris vermifuge in China. TS influenced not only quantal but also non-quantal release of acetylcholine<sup>2)</sup>. As an effective anti-botulinic agent, TS showed an extracellular  $\text{Ca}^{2+}$  dependent initial facilitative action on miniature end-plate potentials (MEPP) discharge before the inhibition of MEPP<sup>3)</sup>. The effects of TS on the Ca channels may be attributed to an increase in calcium conductance<sup>4)</sup>. But there were no report available to show the effects of TS on the cardiac ion channels. Hence, using a standard microelectrode technique, we investigated the ionic mechanisms of the effect of TS in isolated guinea pig papillary muscles.

#### MATERIALS AND METHODS

**Reagents** The TS used were crystals from ethanol, >98% in purity, the same as described previously<sup>2)</sup>, and gifted from Prof LI Pei-Zhong, Academy of Military Medical Sciences; aminophylline (Shanghai XinYi Pharmaceutical Factory, lot 881006);  $\text{BaCl}_2$  (Tianjin 3rd Chemical Reagent Factory, lot 820912);

$\text{CdCl}_2$  (Beijing 57601 Chemical Engineering Factory, lot 850827).

**Protocol** Guinea pigs (either sex) weighing  $250 \pm 50$  g were stunned. Papillary muscle was taken from the right ventricle and was pinned in a 2-ml chamber. For recording fast action potentials (FAP), the Krebs solution ( $\text{NaCl}$  135,  $\text{KCl}$  5.0,  $\text{CaCl}_2$  2.0,  $\text{MgCl}_2$  1.0,  $\text{NaH}_2\text{PO}_4$  2.2,  $\text{NaHCO}_3$  11.9, and glucose  $5.5 \text{ mmol}\cdot\text{L}^{-1}$ ) was gassed with 95%  $\text{O}_2$  + 5%  $\text{CO}_2$  ( $34 \pm 0.5$  °C, pH  $7.4 \pm 0.5$ ). Bath solution was circulated at a rate of  $11 \text{ ml}\cdot\text{min}^{-1}$ . Force of contraction (FC) was measured with an isometric force transducer (Model HN-79, Shanghai Institute of Physiology, Chinese Academy of Sciences) which was connected to the tendinous end of the preparation. Initial length was adjusted to yield an optimal contraction amplitude upon stimulation. Transmembrane action potentials were recorded with conventional glass microelectrodes filled with  $\text{KCl}$   $3 \text{ mol}\cdot\text{L}^{-1}$  having a resistance of 15–25 M $\Omega$ . The maximal upstroke velocity ( $V_{\text{max}}$ ) was obtained by an electronic differentiator (Department of Physiology, Beijing University). Stimulation parameters were: stimulus duration 1 ms, frequency 1 Hz, voltage 20% above threshold. After recording the control FAP for 1 h, data were obtained cumulatively by increasing the concentration of TS every 20 min.

For slow action potentials (SAP), high  $\text{K}^+$  ( $22 \text{ mmol}\cdot\text{L}^{-1}$ ) Krebs solution was prepared by equimolar substitution of  $\text{KCl}$  for  $\text{NaCl}$ , containing aminophylline  $0.75 \text{ mmol}\cdot\text{L}^{-1}$  to elicit SAP after depolarization of the resting potential and inactivation of fast  $\text{Na}^+$  channels. Stimulation parameters were: stimulus duration 3 ms, frequency 0.5 Hz, voltage 20% above threshold. Experiments started after the recording of control SAP for 1 h.

Solutions of TS were prepared in either normal ( $\text{K}^+ 5 \text{ mmol}\cdot\text{L}^{-1}$ ) or high  $\text{K}^+$  ( $\text{K}^+ 22 \text{ mmol}\cdot\text{L}^{-1}$ ) Krebs solutions and were diluted to the final concentrations of 5, 10, 20  $\mu\text{g}\cdot\text{ml}^{-1}$  (pH 7.4). The electric and mechanical signals were displayed on the screen of an oscilloscope and photographed at regular intervals. The effects of TS on the FAP and SAP parameters ( $V_{\text{max}}$ , action potential amplitude APA, APD), and FC were observed. Data were analyzed for statistical significance by *t* test for paired values.

## RESULTS

With TS at concentrations of  $\leq 5 \mu\text{g} \cdot \text{ml}^{-1}$ , no effects on FAP parameters were seen. At higher concentrations, TS caused a concentration-dependent prolongation in the APD, with an increase at  $\text{APD}_{90}$  during the terminal phase of repolarization, while the  $V_{\text{max}}$  and APA were practically unaffected (Tab 1, Fig 1).

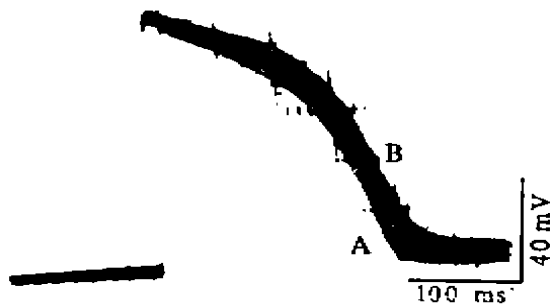


Fig 1. Effect of toosendanin on fast action potential (FAP) configuration in guinea pig papillary muscles. A: control; B: in the presence of toosendanin  $20 \mu\text{g} \cdot \text{ml}^{-1}$ , duration of FAP at 90 % repolarization was somewhat lengthened.

In another 6 papillary muscles, after recording the control FAP for 1 h, the addi-

tion of  $\text{BaCl}_2$   $50 \mu\text{mol} \cdot \text{L}^{-1}$  caused a depolarization of the resting potential from  $68 \pm 3 \text{ mV}$  to  $66 \pm 3 \text{ mV}$  ( $n=5$ ,  $P<0.01$ ), a reduction of the APA from  $103 \pm 3 \text{ mV}$  to  $100 \pm 4 \text{ mV}$  ( $n=6$ ,  $P<0.01$ ), and a prolongation of  $\text{APD}_{90}$  from  $185 \pm 43 \text{ ms}$  to  $220 \pm 29 \text{ ms}$  ( $n=6$ ,  $P<0.01$ ). Similar results have also been reported<sup>15,16</sup>, in which  $\text{BaCl}_2$  was used to block  $I_{\text{K1}}$ . In the presence of  $\text{BaCl}_2$ , the effects of TS on lengthening APD was completely abolished (Tab 1).

With TS  $\leq 5 \mu\text{g} \cdot \text{ml}^{-1}$ , no significant effects on the aminophylline-induced SAP parameters were seen. At higher concentrations, TS concentration-dependently prolonged the APD, without effecting the  $V_{\text{max}}$  and APA (Tab 2). TS ( $10$  and  $20 \mu\text{g} \cdot \text{ml}^{-1}$ ) potentiated the accompanying FC of the aminophylline-induced SAP concentration-dependently (Tab 2, Fig 2). TS  $20 \mu\text{g} \cdot \text{ml}^{-1}$  increased the FC to  $173 \pm 42 \%$  of the control ( $n=6$ ,  $P<0.01$ ).

In another 6 papillary muscles, after recording the aminophylline-induced SAP for 1 h, the addition of  $\text{BaCl}_2$   $70 \mu\text{mol} \cdot \text{L}^{-1}$  caused an

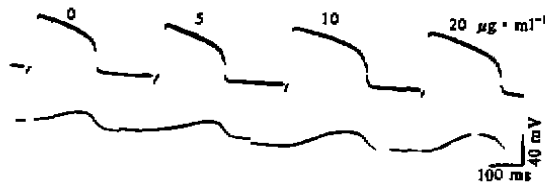
Tab 1. Effects of toosendanin on fast action potentials in guinea pig papillary muscles.  $n=6$ ,  $\bar{x} \pm s$ . \* $P>0.05$ , <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control. <sup>d</sup> $P>0.05$  vs control +  $\text{BaCl}_2$   $50 \mu\text{mol} \cdot \text{L}^{-1}$ .

Toosendanin/ $\mu\text{g} \cdot \text{ml}^{-1}$	$V_{\text{max}}/V \cdot \text{s}^{-1}$	APA/ mV	$\text{APD}_{30}/ms$	$\text{APD}_{50}/ms$	$\text{APD}_{90}/ms$
0	$195 \pm 36$	$99 \pm 6$	$102 \pm 21$	$135 \pm 25$	$163 \pm 27$
5	$185 \pm 29^a$	$101 \pm 3^a$	$106 \pm 19^a$	$138 \pm 25^a$	$165 \pm 27^a$
10	$180 \pm 39^a$	$102 \pm 4^a$	$108 \pm 18^a$	$143 \pm 25^a$	$171 \pm 28^a$
20	$181 \pm 54^a$	$101 \pm 3^a$	$112 \pm 19^b$	$150 \pm 22^c$	$180 \pm 24^c$
0	$167 \pm 32$	$103 \pm 3$	$109 \pm 31$	$154 \pm 40$	$185 \pm 43$
+ $\text{BaCl}_2$	$172 \pm 35^d$	$100 \pm 4^d$	$116 \pm 20^d$	$167 \pm 29^d$	$220 \pm 29^d$
5	$173 \pm 35^d$	$100 \pm 4^d$	$115 \pm 23^d$	$167 \pm 31^d$	$222 \pm 31^d$
10	$179 \pm 36^d$	$100 \pm 4^d$	$117 \pm 19^d$	$168 \pm 29^d$	$222 \pm 27^d$
20	$169 \pm 35^d$	$101 \pm 4^d$	$118 \pm 16^d$	$172 \pm 22^d$	$228 \pm 23^d$

$V_{\text{max}}$  = maximal upstroke velocity; APA = action potential amplitude;  $\text{APD}_{30}$ ,  $\text{APD}_{50}$ , and  $\text{APD}_{90}$  = action potential duration at 30 %, 50, and 90 %, repolarization.

**Tab 2.** Effects of toosendanin on slow action potentials induced by aminophylline in guinea pig papillary muscles.  $\bar{x} \pm s$ . <sup>a</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs control ( $n = 6$ ). <sup>d</sup> $P > 0.05$ , <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$  vs control + BaCl<sub>2</sub> 70  $\mu\text{mol} \cdot \text{L}^{-1}$  ( $n = 5$ ) or vs control + CdCl<sub>2</sub> 50  $\mu\text{mol} \cdot \text{L}^{-1}$  ( $n = 8$ ).

Toosendanin/ $\mu\text{g} \cdot \text{ml}^{-1}$	$V_{\text{max}} /$ $\text{V} \cdot \text{s}^{-1}$	APA/ mV	APD <sub>50</sub> / ms	APD <sub>90</sub> / ms	FC/ %
0	9.4 ± 4.0	68 ± 3	125 ± 27	145 ± 28	100
5	10.6 ± 6.8 <sup>a</sup>	68 ± 3 <sup>a</sup>	128 ± 34 <sup>a</sup>	149 ± 34 <sup>a</sup>	111 ± 30 <sup>a</sup>
10	12.3 ± 7.6 <sup>a</sup>	69 ± 4 <sup>a</sup>	135 ± 36 <sup>b</sup>	156 ± 38 <sup>b</sup>	147.8 ± 52.6 <sup>b</sup>
20	12.7 ± 6.3 <sup>a</sup>	69 ± 4 <sup>a</sup>	145 ± 31 <sup>b</sup>	167 ± 33 <sup>c</sup>	173 ± 42 <sup>c</sup>
<hr/>					
0	11.3 ± 4.7	68 ± 3	149 ± 33	180 ± 34	100
+ BaCl <sub>2</sub>	14 ± 4 <sup>a</sup>	70 ± 4 <sup>c</sup>	193 ± 41 <sup>c</sup>	236 ± 45 <sup>e</sup>	93 ± 28 <sup>c</sup>
5	13.5 ± 4.6 <sup>d</sup>	70 ± 4 <sup>d</sup>	192 ± 38 <sup>d</sup>	236 ± 43 <sup>d</sup>	99 ± 2 <sup>d</sup>
10	13.5 ± 4.6 <sup>d</sup>	70 ± 4 <sup>d</sup>	193 ± 39 <sup>d</sup>	236 ± 42 <sup>d</sup>	112 ± 23 <sup>d</sup>
20	15 ± 4 <sup>d</sup>	70 ± 4 <sup>d</sup>	191 ± 41 <sup>d</sup>	237 ± 43 <sup>d</sup>	126.6 ± 58.3 <sup>d</sup>
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0	12.9 ± 4.6	78 ± 8	122 ± 38	174 ± 56	100
+ CdCl <sub>2</sub>	10.9 ± 5.1 <sup>a</sup>	67 ± 7 <sup>c</sup>	113 ± 36 <sup>a</sup>	177.5 ± 72.0 <sup>a</sup>	22.4 ± 9.7 <sup>c</sup>
5	10.5 ± 4.9 <sup>d</sup>	66 ± 6 <sup>d</sup>	117 ± 28 <sup>d</sup>	179 ± 66 <sup>d</sup>	99 ± 12 <sup>d</sup>
10	10.7 ± 4.7 <sup>d</sup>	67 ± 5 <sup>d</sup>	123 ± 28 <sup>c</sup>	191.2 ± 66.4 <sup>f</sup>	98 ± 6 <sup>d</sup>
20	10.7 ± 4.4 <sup>d</sup>	67 ± 5 <sup>d</sup>	126 ± 27 <sup>c</sup>	201 ± 63 <sup>f</sup>	105 ± 20 <sup>c</sup>



**Fig 2.** Effects of toosendanin on aminophylline - induced slow action potentials (SAP, upper tracing) and force of contraction (FC, lower tracing) in guinea pig papillary muscles. Duration of SAP was lengthened and FC increased, in a concentration - dependent manner.

increase of the APA from 68 ± 3 mV to 70 ± 4 mV ( $n = 5$ ,  $P < 0.01$ ), a prolongation of APD<sub>50</sub> and APD<sub>90</sub> from 149 ± 33 and 180 ± 34 ms to 193 ± 41 and 236 ± 45 ms, respectively ( $n = 5$ ,  $P < 0.01$ ), without effecting the FC. The effects of TS (10 and 20  $\mu\text{g} \cdot \text{ml}^{-1}$ ) on lengthening APD and enhancing FC were completely abolished when BaCl<sub>2</sub> was used to block the  $I_{\text{K1}}$  (Tab 2).

In another 6 experiments, after recording the aminophylline-induced SAP for 1 h, the

addition of CdCl<sub>2</sub> 50  $\mu\text{mol} \cdot \text{L}^{-1}$  caused a reduction of APA from 78 ± 8 mV to 67 ± 7 mV ( $n = 8$ ,  $P < 0.01$ ), an attenuation of FC to 22.4 ± 9.7 % of the control ( $n = 8$ ,  $P < 0.01$ ), without effecting the  $V_{\text{max}}$  and APD. The effect of TS on enhancing FC was abolished when CdCl<sub>2</sub> was used to reduce the  $I_{\text{K1}}$ , whereas the concentration-dependent prolongation of the APD persisted with increasing concentrations of TS (Tab 2, Fig 3).

**DISCUSSION**

Our results indicated that the main effect of TS was to inhibit the inward rectifier K<sup>+</sup> current  $I_{\text{K1}}$ . Suppressing both the inward rectifier current and the delayed rectifier current by TS could lead to prolongation of the APD, but the primary currents blocked by TS were  $I_{\text{K1}}$ , since the current intensity of  $I_{\text{K}}$  was less than that of  $I_{\text{K1}}$  and APD at voltages of 0 mV or above did not increase substantially<sup>1,7,8</sup>. Thus, the  $I_{\text{K}}$  system appeared to play an insignificant role, at least in the case of the TS -

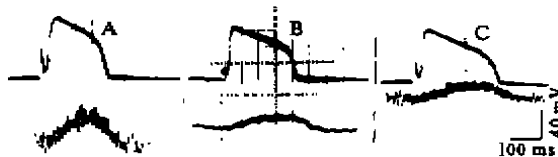


Fig 3.  $I_{K1}$ -blocking effect of  $CdCl_2$  on toosendanin-induced prolongation of slow action potential duration (upper tracing) and potentiation of force of contraction (FC, lower tracing) in guinea pig papillary muscles. A: control slow action potential (SAP) induced by aminophylline and the accompanying contraction; B: after the addition of  $CdCl_2$ ,  $50 \mu mol \cdot L^{-1}$ , FC was suppressed and without much effect on the duration of SAP; C: in the presence of  $Cd^{2+}$ , effect of toosendanin on enhancing FC was abolished, with the prolongation of APD preserved.

induced prolongation of APD in multicellular preparations.

The APD of aminophylline-induced SAP and FC were increased by TS. Following the application of  $BaCl_2$  to block  $I_{K1}$ , both the effects of TS on lengthening APD and enhancing FC could be completely abolished. Addition of  $CdCl_2$  abolished the effects of TS on enhancing FC, while the APD-prolonging effects of TS were preserved. Thus, the positive inotropic effects of TS on the accompanying FC of the SAP were not likely to be due to increased  $Ca^{2+}$  influx into the cells at depolarization phase of the SAP (no changes in  $V_{max}$  occurred), but resulting from slowing the calcium channel inactivation secondary to the delay in ventricular repolarization.

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川楝素对豚鼠乳头状肌电和机械特性的影响

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A 摘要 川楝素(TS)浓度依赖性地使快反应电位复极至90%的时程( $APD_{90}$ )延长。用  $BaCl_2$  阻断  $I_{K1}$ , 可取消 TS 延长  $APD_{90}$  的作用。TS 使慢反应电位的 APD 延长和收缩力(FC)增强。用  $BaCl_2$  后, 可取消 TS 的上述作用。  $CdCl_2$  取消 TS 增强 FC 的作用, 但延长 APD 的作用存在。提示, TS 抑制  $I_{K1}$ , 其正性肌力作用是继发于 APD 的延长及钙通道的失活减慢。

关键词 川楝素; 乳头状肌; 动作电位; 心肌收缩力; 离子通道

豚鼠

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