ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Mar; 20 (3): 249 - 252
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Inhibitory actions of polysaccharide sulfate on action potentials and contraction of papillary muscles in guinea pigs

TANG Yun, TANG Shu-Ben[1], ZHANG Hai-Yan
(*Department of Physiology, Henan Medical University, Zhengzhou 450052, China*)

KEY WORDS polysaccharides; papillary muscles; action potentials; calcium channels; myocardial contraction; barium compounds; isoproterenol

ABSTRACT

AIM: To examine the effects of polysaccharide sulfate (PSS) on the action potentials and contractile force in guinea pig papillary muscles. **METHODS**: Using intracellular microelectrode to record fast (FAP) and slow (SAP) action potentials. **RESULTS**: PSS (≥ 50 mg·L^{-1}) caused concentration-dependent decreases in the contractile force and the action potential duration (APD) of FAP without affecting the resting potential (RP), action potential amplitude (APA), and maximal upstroke velocity (V_{max}). The V_{max}, APA, and APD of $BaCl_2$-induced SAP were concentration-dependently decreased by PSS (≥ 15 mg·L^{-1}) and the effects were antagonized by isoprenaline (1 μmol·L^{-1}). The APA and APD of isoprenaline-induced SAP were decreased by PSS (≥ 15 mg·L^{-1}) in a concentration-dependent manner and the effects were attenuated by elevation of extracellular Ca^{2+} concentration. **CONCLUSION**: PSS selectively inhibited the slow inward current.

INTRODUCTION

The polysaccharide sulfate (PSS) is a new type heparinoid, extracted from *Phylum phaeophyta*. After esterification and sulfonation, the PSS obtained was a semi-synthetic oceanic drug[1]. PSS improved microcirculation[2] and coronary blood supply[3], inhibited blood coagulation[4], and was effective in the treatment of ischemic heart disease[5]. Although PSS exhibited an inhibitory effect (auriculoventricular block) on the myocardium[6], electrophysiologic effects of PSS have not yet been examined. Therefore, we undertook the present studies to clarify the effects of PSS on cardiac ion channels.

MATERIALS AND METHODS

Drugs PSS (Qingdao 3rd Pharmaceutical Factory, lot № 941215); $BaCl_2$ (Tianjin 3rd Chemical Reagent Factory, lot № 820912); isoprenaline (Shanghai Tianfeng Pharmaceutical Factory, lot № 930101).

Guinea pigs Either sex, provided from the Experimental Animal Center of Henan Medical University (Certificate № 981002), $n = 30$, weighing 300 g ± s 50 g were stunned. Papillary muscle was taken from the right ventricle and was pinned in a 2-mL chamber of Tyrode's solution aerated with O_2 (pH 7.4 ± 0.5) at (36 ± 0.5) ℃ at a rate of 10 mL·min^{-1}. For fast action potentials (FAP), the Tyrode' solution contained: NaCl 140.0, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, NaH_2PO_4 0.3, HEPES 5.0, and glucose 5.0 mmol·L^{-1}. Force of contraction (FC) of papillary muscle was measured with an isometric force transducer connected to the tendinous end of the muscle. Initial length was adjusted to yield an optimal contraction amplitude upon stimulation. Transmembrane action potentials were recorded with a

[1] Correspondence to Prof TANG Shu-Ben. Phn 86-371-697-9165. Fax 86-371-696-0462. E-mail sbtang@hy.henmu.edu.cn
Received 1998-02-12 Accepted 1998-09-07

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Mar; 20 (3)
E-mail aps@server shcnc.ac.cn Phn/Fax 86-21-6474-2629

conventional glass microelectrode filled with KCl 3 mol·L^{-1}, having a resistence of 15 – 20 MΩ. The maximal upstroke velocity (V_{max}) was obtained by an electronic differentiator. Stimulation parameters were: stimulus duration 1 ms, frequency 1 Hz, voltage 50 % above threshold. After recording the control FAP for 1 h, experiments started. The electric and mechanical signals were displayed on the screen of an oscilloscope and photographed. The parameter of FAP were on-line analyzed by a microcomputer system TP-801B[7].

For slow action potentials (SAP), after impalement was stabilized for 30 min, high K^+ (25 mmol·L^{-1}) Tyrode solution was prepared by equimolar substitution of KCl for NaCl, containing isoprenaline 1 μmol·L^{-1} or $BaCl_2$ 0.2 mmol·L^{-1} to elicit SAP after depolarization of the resting potential (RP) and inactivation of fast Na^+ channels[8]. Stimulation parameters were: stimulus duration 3 ms, frequency 0.5 Hz, voltage 50 % above threshold. Experiments were started after the recording of control SAP for 1 h.

The preparations were perfused with the solutions of PSS 5, 15, 50, 150, 500 mg·L^{-1} (pH 7.4). The perfusion lasted 30 min at each concentration. The effects of PSS on the electric parameters of FAP and SAP were observed. Data were processed by computer using SAS software and analyzed by paired t test.

RESULTS

Effects on FAP PSS 50 – 500 mg·L^{-1}, caused concentration-dependent decreases in the FC, APD_{30}, APD_{50}, and APD_{90} of the FAP, but had no effect on the RP, V_{max}, and APA. PSS decreased FC from 100 % in the control to 62 % ± 17 % ($n = 7$, $P < 0.01$) after PSS 500 mg·L^{-1} and decreased APD_{30}, APD_{50}, and APD_{90} from (151 ± 16) ms, (186 ± 15) ms, and (234 ± 12) ms to (112 ± 23) ms, (138 ± 27) ms, and (186 ± 26) ms ($n = 7$, $P < 0.01$), respectively (Tab 1).

Effects on SAP induced by $BaCl_2$ PSS 50 – 500 mg·L^{-1} concentration-dependently suppressed the V_{max} of the SAP induced by $BaCl_2$. PSS decreased V_{max} from (9 ± 1) V·s^{-1} in the control to (6 ± 1) V·s^{-1} ($n = 6$, $P < 0.01$) after PSS 500 mg·L^{-1}. PSS 15 – 500 mg·L^{-1} concentration-dependently decreased the APA, APD_{30}, APD_{50}, and APD_{90} of the SAP induced by $BaCl_2$. PSS decreased APA from (69 ± 2) mV in the control to (58 ± 4) mV ($n = 6$, $P < 0.01$) after PSS 500 mg·L^{-1} and decreased APD_{30}, APD_{50}, and APD_{90} from (124 ± 8), (160 ± 9), and (187 ± 10) ms to (81 ± 16), (113 ± 18), and (135 ± 18) ms ($n = 6$, $P < 0.01$), respectively. All the effects of PSS on the SAP were attenuated after the addition of isoprenaline 1 μmol·L^{-1} to the solution (Tab 2).

Effects on SAP induced by isoprenaline

Tab 1. Effects of polysaccharide sulfate on fast action potentials in guinea pig papillary muscles. $n = 7$. $x \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	Control	Polysaccharide sulfate/mg·L^{-1} 15	50	150	500
V_{max}/V·s^{-1}	190 ± 25	178 ± 25^a	172 ± 14^a	179 ± 29^a	166 ± 15^a
APA/mV	115 ± 4	116 ± 4^a	115 ± 5^a	112 ± 5^a	109 ± 8^a
APD_{30}/ms	151 ± 16	141 ± 18^a	131 ± 14^b	123 ± 19^b	112 ± 23^c
APD_{50}/ms	186 ± 15	175 ± 22^a	164 ± 20^b	152 ± 28^b	138 ± 27^c
APD_{90}/ms	234 ± 12	226 ± 16^a	212 ± 22^b	201 ± 24^c	186 ± 26^c
FC/%	100	101 ± 8^a	84 ± 8^c	70 ± 12^c	62 ± 17^c

Tab 2. Effects of polysaccharide sulfate on slow action potentials induced by $BaCl_2$ (0.2 mmol·L^{-1}) or isoprenaline (Iso, 1 μmol·L^{-1}) in guinea pig papillary muscles.
$n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	Control	Polysaccharide sulfate/mg·L^{-1} 5	15	50	150	500	PSS 500 mg·L^{-1} + Iso 1 μmol·L^{-1}
+ $BaCl_2$							
V_{max}/V·s^{-1}	9 ± 1	8 ± 1^a	8 ± 1^a	7 ± 1^b	6 ± 2^c	6 ± 1^c	9 ± 1^a
APA/mV	69 ± 2	68 ± 3^a	66 ± 3^b	63 ± 5^b	61 ± 3^c	58 ± 4^c	68 ± 2^a
APD_{30}/ms	124 ± 8	123 ± 8^a	115 ± 4^b	109 ± 8^b	93 ± 12^c	81 ± 16^c	121 ± 9^a
APD_{50}/ms	160 ± 9	155 ± 7^a	146 ± 7^b	138 ± 4^c	120 ± 14^c	113 ± 18^c	158 ± 15^a
APD_{90}/ms	187 ± 10	183 ± 9^a	173 ± 9^b	166 ± 7^c	148 ± 12^c	135 ± 18^c	182 ± 12^a
+ Iso							
RP/mV	43 ± 4	40 ± 6^a	40 ± 4^a	38 ± 4^a	40 ± 3^a	40 ± 3^a	
APA/mV	73 ± 2	71 ± 4^a	68 ± 4^b	67 ± 3^c	64 ± 3^c	59 ± 4^c	
APD_{30}/ms	128 ± 15	121 ± 14^a	105 ± 20^b	99 ± 22^b	93 ± 22^c	84 ± 21^c	
APD_{50}/ms	165 ± 14	159 ± 15^a	145 ± 17^b	134 ± 18^c	123 ± 20^c	108 ± 24^c	
APD_{90}/ms	183 ± 15	173 ± 17^a	159 ± 19^b	152 ± 19^c	141 ± 21^c	130 ± 27^c	

PSS 15 – 500 mg·L^{-1} concentration-dependently decreased the APA, APD_{30}, APD_{50}, and APD_{90} of the SAP induced by isoprenaline, without affecting the RP. PSS decreased APA from (73 ± 2) mV in the control to (59 ± 4) mV ($n = 6$, $P < 0.01$) after PSS 500 mg·L^{-1} and decreased APD_{30}, APD_{50}, and APD_{90} from (128 ± 15), (165 ± 14), and (183 ± 15) ms to (84 ± 21), (108 ± 24), and (130 ± 27) ms ($n = 6$, $P < 0.01$), respectively (Tab 2).

The effects of PSS 150 mg·L^{-1} on the SAP induced by isoprenaline were seen at time intervals of 6, 12, and 24 min following the addition of the drug. The APA and APDs of the SAP decreased progressively in a time-dependent manner. After raising the extracellular Ca^{2+} content from 1.8 to 5 mmol·L^{-1}, the restitution of bioelectric parameters were noted at time intervals of 3, 5, and 10 min. All the parameters of the SAP were gradually recovered within 10 min (Tab 3).

DISCUSSION

PSS concentration-dependently decreased the APD of the FAP, but had no effect on the RP, V_{max}, and APA. The lack of effect on phase 0 characteristics strongly suggested that PSS did not exert an inhibitory effect on the fast inward sodium current. It is well known that the APD at plateau phase is determined by the

Tab 3. Progressive suppression by polysaccharide sulfate of slow action potentials induced by isoprenaline and restitution by raising the Ca^{2+} content from 1.8 to 5 mmol·L^{-1}.
$n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	Control	After addition of PSS 150 mg·L^{-1}/min 6	12	24	After elevation of $[Ca^{2+}]_o$ to 5 mmol·L^{-1}/min 3	5	10
APA/mV	75 ± 1	73 ± 3^a	70 ± 4^b	66 ± 3^c	71 ± 6^a	75 ± 6^a	79 ± 6^a
APD_{30}/ms	125 ± 10	123 ± 6^a	113 ± 6^b	100 ± 12^c	96 ± 13^c	110 ± 8^a	118 ± 11^a
APD_{50}/ms	158 ± 10	155 ± 11^a	144 ± 8^b	133 ± 13^c	127 ± 22^b	134 ± 22^a	143 ± 25^a
APD_{90}/ms	171 ± 8	166 ± 9^a	156 ± 10^b	144 ± 14^c	137 ± 20^c	147 ± 18^b	162 ± 19^a

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Mar; 20 (3)
E-mail aps@server shcnc.ac.cn Phn/Fax 86-21-6474-2629

balance between inward calcium current and outward potassium currents. The reduction of calcium current increased the net outward currents and thus caused a decrease of APD. Therefore, the reduction of APD of the FAP by PSS is thought to be induced by the suppression of calcium current. PSS produced a concentration-dependent decrease in peak contractile force in guinea pig papillary muscles, an effect which has been attributed to an inhibition of the transmembrane Ca^{2+} influx required for the conformational change of troponin I and normal excitation-contraction coupling[9].

The calcium current is the main inward current, contributing to the maintenance of the APD, determining the V_{max} and APA of the SAPs[9]. PSS decreased the V_{max} and APA, shortened APD of the SAP, indicating a preferential inhibitory action on the calcium current. All these effects could be restored by elevation of the extracellular Ca^{2+} concentration as well as administration of isoprenaline, which increased the open probability and the phosphorylation of the calcium channels[10]. Thus, PSS selectively inhibited the slow inward current.

REFERENCES

1 Zeng GJ, Han ZY. The clinical and laboratory observation of PSS in treating 120 cases of ischemic cerebrovascular diseases.
J Marine Drugs 1986; 5: 97 - 100.
2 Guo SS, Liu RJ.
Effect of PSS on microcirculation in the rabbit.
J Marine Drugs 1986; 5: 6 - 11.
3 Cao SQ, An WR, Cao DY. PSS in the treatment of chronic coronary insufficiency in 55 patients.
New Drugs Clin Remedies 1991; 10: 357 - 8.
4 Xu JM, Du Y. PSS in the treatment of hypercoagulable state in 76 patients.
New Drugs Clin Remedies 1991; 10: 350 - 1.
5 Li LM, Cong XZ. Polysaccharide sulfate in the treatment of coronary heart disease in 48 patients.
New Drugs Clin Remedies 1992; 11: 240 - 1.
6 Yuan JX. Auriculoventricular block induced by PSS in 2 cases.
Chin J Hosp Pharm 1993; 13: 471 - 2.
7 Yan GX, Tang SB, Cao XC. On-line analysis of intracellular cardiac action potentials with a microcomputer system.
Chin J Appl Physiol 1985; 1: 235 - 8.
8 Pappano AJ. Calcium-dependent action potentials produced by catecholamines in guinea pig atrial muscle fibers depolarized by potassium.
Circ Res 1970; 27: 379 - 90.
9 Noble D. The surprising heart: a review of recent progress in cardiac electrophysiology.
J Physiol (Lond) 1984; 353: 1 - 50.
10 Tsien RW, Bean BP, Hess P, Lansman JB, Nilius B, Nowycky MC. Mechanisms of calcium channel modulation by β-adrenergic agents and dihydropyridine calcium agonists.
J Mol Cell Cardiol 1986; 18: 691 - 710.

249-252

藻酸双酯钠对豚鼠乳头状肌动作电位和收缩的抑制作用

汤 蕴，汤树本，张海燕 （河南医科大学生理教研室，郑州市 450052，中国）

关键词 多糖；乳头状肌；动作电位；钙通道；心肌收缩；钡化合物；异丙肾上腺素

目的：观察藻酸双酯钠(PSS)对豚鼠乳头状肌动作电位和收缩力(FC)的影响。**方法**：利用细胞内微电极技术记录豚鼠乳头状肌的快反应动作电位(FAP)和慢反应动作电位(SAP)。**结果**：PSS (≥50 $mg \cdot L^{-1}$)浓度依赖性地使 FC 减弱和 FAP 的动作电位时程(APD)缩短，但对静息电位和动作电位幅度(APA)及 0 期最大除极速率(V_{max})均无显著影响。PSS (≥15 $mg \cdot L^{-1}$)浓度依赖性地使 $BaCl_2$ 诱发的 SAP 的 V_{max} 和 APA 降低、APD 缩短，在台氏液中加入异丙肾上腺素 1 $\mu mol \cdot L^{-1}$ 后，各项参数恢复正常。PSS (≥15 $mg \cdot L^{-1}$)浓度依赖性地使异丙肾上腺素诱发的 SAP 的 APA 降低、APD 缩短、提高细胞外 Ca^{2+} 浓度后各项参数均恢复正常。**结论**：PSS 选择性抑制慢内向电流。

（责任编辑 李 颖）