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Electrophysiologic effects of berberine on isolated sinoatrial and atrioventricular nodes of rabbit[1]

WANG Yu, LIU Lie–Ju [2], FANG Da–Chao (*Departments of Pharmacology and* [2]*Biomedical Engineering, Tongji Medical University, Hankou 430030, China*)

ABSTRACT Berberine (Ber) 0.1–30 μmol / L decreased the APA, V_{max}, maximal depolarization potentials (MDP) and rate of spontaneous impulse initiation in sinoatrial node (SAN) and atrioventricular node (AVN) cells with prolongation of APD_{50}, APD_{95} and ERP in a dose–dependent manner. The depression of SAN function was shown by increasing sinus cycle length (SCL), sinus recovery time (SRT) as well as corrected sinus recovery time (CSRT). In atria (crista terminalis, CT) higher concentrations of Ber were needed to prolong APD_{50}, APD_{95}, and ERP as compared with SAN and AVN. The effects of Ber on SAN were not antagonized or reversed by atropine (1 μmol / L), α_2–receptor agonist BHT–920 (10 μmol / L) or α_1–receptor agonsit phenylephrine (10 μmol / L); but those effects of Ber on SCL, APA and V_{max} could be reversed by norepinephrine (0.1 μmol / L). In calcium free Tyrode's solution, the inhibitory effects of Ber still revealed on SAN but on atria only prolongations of APD_{50} and APD_{95} were observed.

KEY WORDS anti–arrhythmia agents; berbines; berberine; sinoatrial node; atrioventricular node; microelectrodes; electrophysiology

Berberine (Ber) has been used clinically for many years as a bacteriostatic agent in China. Animal experiments revealed that Ber also possessed anti–arrhythmic effect[1]. It has been recently introduced in clinical practice for the management of supraventricular tachycardia[2,3].

Experimentally, Ber has been shown both positive inotropic and negative chronotropic activities in the superfused atrial preparations[4–6]. It prolonged the action potential duration (APD) and effective refractory period (EPR) in guinea pig papillary muscles[7]. But its effects on SAN and AVN have not been reported. The present work was undertaken to evaluate the effects of Ber on pacemaker cells in these nodular tissues.

MATERIALS AND METHODS

Rabbits were killed by a blow on the head. The heart was excised and immersed in oxygenated Tyrode's solution. Two kinds of preparations containing either SAN or AVN tissues were made[8]. The spontaneously beating preparation pinned on the base or a tisse bath with its endocardial surface upward was superfused with normal or Ca–free Thyrode's solution (pH 7.4 ± 0.5, 37 ± 0.5℃), bubbled with 95% O_2 + 5% CO_2. The tissue was superfused at a rate of 8 ml / min and equilibrated for 1 h before experiment. The normal Tyrode's solution contained (mmol / L): NaCl 136.9, KCl 5.4 , $MgCl_2$ 1.05, NaH_2PO_4 0.42, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, glucose 5.6. Two glass micorelectrodes filled with KCl 3 mol / L, resistance 20–40 MΩ, were conneted with a microelectrode amplifier (MEZ–7101). In the SAN preparation, one microelectrode was inserted into a dominant pacemaker cell (P cell), the other was in a crista terminalis (CT) cell. In the AVN preparation, one microelectrode was implanted into N cell, the other in CT cell[9]. Signals were amplified and displayed on oscilloscope. The action potential amplitude (APA), maximal rate of rise of phase 0 depolarization (V_{max}), action

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potential duration at 50% and 95% repolarization (APD_{50} , APD_{95}) spontaneous depolarization rate of phase 4 ($SDRP_4$) of SAN and AVN action potentials, sinus cycle length (SCL) and AVN cycle lenth (ACL) were sampled and measured with a multi-purposes electrophysiologic processing system automatically[10]. kPart of the results were read directly from oscilloscope photographs. Only those results obtained from a single cell thoughout the whole experimental period were subjected to analysis.

Measurement of the effective refractory periods (ERP) Measurement of ERP was conducted by the above mentioned programmer automatically. The stimulation impluse was delivered through a stimulus isolator. After 8 basic drive pulses (S_1) (300 ms duration, double threshold voltage), one premature stimulus (S_2) was interpolated. The duration and valtage of $S_2$was the same as S_1. The interval between trains was 6 s. The delay between S_1-S_2 was started from 40 ms and then lengthened progressively at an interval of 5 ms. The premature response was sampled. When the amplitude of the premature response reached 20 % of the basic response amplitude, the results were determined and printed as ERP. In each experiment this process was repeated twice and the mean value of the two readings was taken as the result[11].

Measurement of SRT and CSRT In the isolated rabbit SAN preparations, the measurement of CSRT was conducted by the Rame system. Rectangular pulses of 2-ms duration at 200% threshold voltage were used as stimuli. After a stimulation period of 30 s, the following parameters were sampled and printed: SCL, SRT, CSRT (CSRT = SRT - SCL)[11].

Statistic treatment The results were expressed as $\bar{x} \pm SD$ values, the significance of the difference between two means within the same experimental groups was analyzed by t test for paried data.

RESULTS

Effects of Ber on SAN and AVN action potentials Ber had significant dose-dependent suppressive effects on SAN as shown in Tab 1. The parallel increment of APD_{50} and APD_{95} indicated that the whole course of repolarization was prolonged. Among all indices, the change in V_{max}, APD_{95} and

Tab 1. Effects of berberine on sinoatrial and atrioventricular nodal action potentials in 7 rabbits. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Berberine (μmol / L)		APA (mV)	APD_{50} (ms)	APD_{95} (ms)	V_{max} (V / s)	$SDRP_4$ (mV / s)	SCL / ACL (ms)
Control	SAN	321 ± 20	74 ± 4	87 ± 6	168 ± 13	16 ± 4	57 ± 7
	AVN	430 ± 43	83 ± 4	84 ± 6	147 ± 16	25 ± 2	37 ± 6
0.01	SAN	326 ± 20*	73 ± 4*	88 ± 8*	171 ± 13*	14 ± 3*	55 ± 6*
	AVN	438 ± 43*	79 ± 2*	85 ± 6*	148 ± 17*	25 ± 2*	34 ± 5*
0.1	SAN	333 ± 19**	73 ± 4*	92 ± 6***	177 ± 11**	11 ± 2***	51 ± 6*
	AVN	452 ± 46**	78 ± 2*	89 ± 6*	155 ± 16***	20 ± 2***	33 ± 5***
1	SAN	349 ± 17***	72 ± 4*	96 ± 5***	186 ± 11***	8 ± 1.2**	46 ± 6***
	AVN	466 ± 46***	78 ± 2*	90 ± 2*	162 ± 17**	19 ± 3***	32 ± 5***
10	SAN	354 ± 17***	69 ± 4**	101 ± 6***	198 ± 15***	6 ± 0.8**	42 ± 5***
	AVN	498 ± 51***	76 ± 2*	97 ± 5***	177 ± 19***	15 ± 1.2***	29 ± 5***
30	SAN	390 ± 19**	65 ± 5***	105 ± 5***	203 ± 10***	4 ± 0.5**	37 ± 5***
	AVN	598 ± 63**	72 ± 4**	108 ± 7***	195 ± 17***	12 ± 0.6***	25 ± 5***

SAN: sinoatrial node, AVN: atrioventricular node; SCL: sinus cycle length; ACL : atrioventricular node length
$SDRP_4$: spontaneous depolarization rate of phase 4 of action potentials.

Tab 2. Electrophysiologic effects of berberine on isolated superfused rabbit sinoatrial node and crista terminals. $n=6$, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Berberine (μmol / L)		APA (mV)	APD_{50} (ms)	APD_{95} (ms)	V_{max} (V / s)	ERP (ms)	$SDRP_4$ (mV / s)	SCL (ms)	CSRT (ms)
Control	SAN	73 ± 9	102 ± 13	177 ± 17	14 ± 5	89 ± 4	24 ± 7	440 ± 82	53 ± 19
	CT	97 ± 9	65 ± 3	132 ± 24	172 ± 36	61 ± 7			
1	SAN	71 ± 11*	116 ± 21**	209 ± 23**	12 ± 4**	99 ± 5***	21 ± 7	505 ± 81**	127 ± 28**
	CT	96 ± 9*	71 ± 10*	160 ± 26**	170 ± 23*	73 ± 6***			
10	SAN	62 ± 12**	139 ± 33***	285 ± 32**	9 ± 2**	110 ± 7***	17 ± 8***	651 ± 115***	163 ± 45**
	CT	91 ± 8***	77 ± 9***	181 ± 27**	168 ± 32*	98 ± 6***			

ERP : effective refractory periods, CSRT : corrected sinus recovery time, CT : crista terminalis.

Tab 3. Electrophysiologic effects of berberine in calcium free Tyrode solution on the isolated superfused rabbit sinoatrial node and crista terminals. $n=6$, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* calcium free Tyrode solutoin; $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$.

		APA (mV)	APD_{50} (ms)	APD_{95} (ms)	V_{max} (V / s)	$SDRP_4$ (mV / s)	SCL (ms)	CSRT (ms)
Control	SAN	68 ± 16	110 ± 27	172 ± 19	11 ± 7	24 ± 8	455 ± 48	102 ± 43
	CT	103 ± 6	50 ± 11	107 ± 16	182 ± 28			
Calcium free	SAN	53 ± 11**	121 ± 27**	187 ± 28**	5.6 ± 2.9**	16 ± 4**	493 ± 34**	164 ± 35***
Tyrode solution	CT	72 ± 9***	47 ± 11*	95 ± 14*	188 ± 27*			
Berberine 0.1	SAN	51 ± 11^{+}	129 ± 25^{++}	195 ± 29^{++}	4.8 ± 2.4^{++}	16 ± 5^{++}	518 ± 37^{+++}	191 ± 58^{++}
(μmol / L)	CT	72 ± 12^{+}	46 ± 13^{+}	95 ± 12^{+}	184 ± 28^{+}			
1	SAN	50 ± 10^{+}	141 ± 20^{++}	212 ± 38^{+++}	4.1 ± 1.6^{+++}	15 ± 5^{++}	543 ± 43^{+++}	218 ± 63^{++}
	CT	64 ± 12^{++}	47 ± 13^{+}	96 ± 11^{+}	170 ± 30^{+}			
10	SAN	37 ± 6^{++}	154 ± 31^{++}	246 ± 11^{+++}	3.3 ± 1.4^{++}	13 ± 4^{++}	559 ± 47^{+++}	253 ± 79^{++}
	CT	59 ± 16^{++}	49 ± 11^{+}	96 ± 11^{+}	159 ± 28^{++}			
Tyrode solution	SAN	63 ± 15^{+}	124 ± 27^{+}	185 ± 27^{+}	6.6 ± 2.5^{+}	25 ± 7^{++}	461 ± 45^{++}	
washout	CT	90 ± 13^{++}	50 ± 12^{+}	101 ± 11^{+}	94 ± 36^{+++}			

$SDRP_4$ were the most pronounced.

Similar to SAN, AVN action potentials was also strikingly suppressed by Ber. Significant depression of V_{max}, APA, $SDRP_4$ and prolongation of ACL occurred in concentrations of 0.1 −30 μmol / L in a dose−dependent manner (Tab 1).

Effects of Ber on fast response and slow response cells The effects of Ber on the SAN and CT cells were compared on SAN−CT preparation (Tab 2). Ber again demonstrated its inhibitory effects in dose−dependent on all phases of CT action potentials but higher threshold concentrations (10 μmol / L) were needed and the decrease of V_{max} and APA was not significant.

Effects of Ber on ERP and CSRT In SAN−CT preparations, the ERP of SAN and CT cells were prolonged by 24% and 60% respectively with Ber 10 μmol / L. The CSRT prolonged from 53 ± 19 to 163 ± 45 ms by Ber 10 μmol / L ($P<0.01$) (Tab 2).

Superfusion with Ca−free solution The action potentials were recorded first on SAN−CT preparations in normal Tyrode's solution (Tab 3). After 20 min, Ca−free solution was used to substituted. The preparations again equilibrated for another 20 min, the tissue was superfused with solutions containing Ber (0.1 , 1 to 10 μmol / L). In both solutions, the APD_{50} ADP_{95}, V_{max}, $SDRP_4$, SCL, CSRT were suppressed by Ber 1

Tab 4. Effects of berberine on the isolated superfused rabbit sinoatrial node and crista terminalis exposed to atropine before and during addition of berberine. $n=5$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* atropine; $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$.

		APA (mV)	APD_{50} (ms)	APD_{95} (ms)	V_{max} (V/s)	$SDRP_4$ (mV/s)	CSRT (ms)	S–H (ms)	SCL (ms)
Control	SAN	62±13	104±6	176±11	10±1	28±1	92±42	20±2	440±42
	CT	99±2	48±9	99±14	178±12				
Atropine	SAN	62±12*	96±6***	165±13***	11±1*	28±1*	83±21*	20±1*	411±32***
1μmol/L	CT	98±4*	50±9**	97±14*	178±10**				
Berberine 1	SAN	61±12+	104±7++	177±14+++	9±1+++	26±1+++	86±46+++	15±2+++	436±36+++
(μmol/L)	CT	97±4+	51±10+	107±17++	176±13+				
10	SAN	58±15+++	112±5+++	189±15+++	7±1+++	23±2+++	89±47+++	12±1+++	460±41+++
	CT	97±3+	54±10+	120±17++	173±12+				
100	SAN	53±1+++	119±5+++	212±14+++	6±0.7+++	15±3+++	126±53+++	16±2+++	491±51+++
	CT	91±2++	66±14++	133±27++	169±14+				

S–H : interval of sinus nodel cell to His bundle cell.

μmol/L. But in Ca–free solution, the suppresive effects of Ber on the APD_{50} and APD_{95} in CT cells were almost completely obliterated.

Interaction of Ber and other drugs On the isolated SAN preparations, after superfusion with Ber 30 μmol/L, the addition on BHT–920 10 μmol/L or phenylephrine 10 μmol/L did not block the suppressive effects of Ber, but norepinephrine (NE) 0.1 μmol/L significantly blocked the effects of Ber on SCL, APA, and V_{max} (Fig 1).

In SAN–CT preparations superfused with Tyrode's solution containing atropine 1 μmol/L, Ber 1 μmol/L still showed inhibitory effects on all the action potential parameters except APA in both SAN and CT cells (Tab 4).

DISCUSSION

Similar to the results obtained from papillary muscle preparations[12], Ber has marked suppressive effects on the action potentials of SAN and CT. The only difference lies in that, in CT preparation the reduction of V_{max} was not significant in concentrations used in the present experiments. It is demonstrated that Ber also has marked inhibitory effect on the action potential of SAN and AVN,

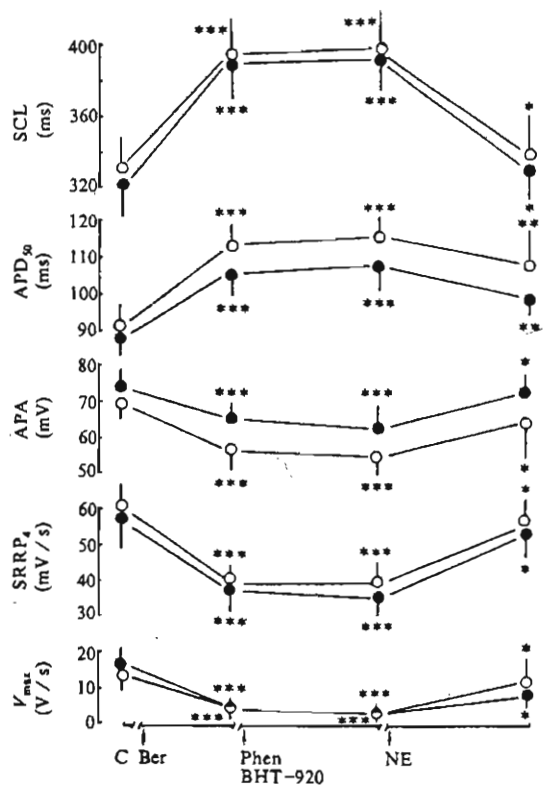

Fig 1. Interactions of berberine with phenylephrine (Phen) 10 μmol/L (O), BHT–920 10 μmol/L (●), and norepinephrine (NE) on action potentials in isolated superfused rabbit sinus node. $n=6$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control (C).

such as reduction of V_{max}, $SDRP_4$, APA,

prolongation of SCL, ACL, APD and ERP; among which the reduction of V_{max} and $SDRP_4$ were most obvious while APA was the least sensitive. The above results suggest that the suppressive effects of Ber might be related to its blocking action on Ca^{2+} and Na^+ influx and K^+ outward current in these nodul cells, comparable to the actions of a membrane stabilizer.

The effects of Ber on the SAN and AVN were similar, and both were concentration dependent. Under the same experimental condition, the slow response cells (SAN cells) were more sensitive to the inhibitory effects of Ber than fast response cells (CT cells). Both *in vitro* and *in vivo* experiments have demonstrated by many authors that Ber has negative chronotropic action on different animal preparitions[4-6]. It was noted that the prolongation of SCL was always accompanied by the decrease of $SDRP_4$, V_{max} and increase of MDP. While the atrial excitability unaltered[4,12]. So it seems that the negative chronotropic effect of Ber might be related to its actions upon $SDRP_4$, MDP as well as V_{max}.

It can be seen from Tab 4 that Ber still exerted its suppressive effects on SAN in the absence of calcium ions, suggesting that Ca^{2+} is not the sole ionic mechanism by which Ber cause negative chronotropic effect; although Ca-free solution significantly diminished the effect of Ber on APD_{50} and APD_{95} of CT[13,14]. Yc-170, a calcium modulator, has similar positive inotropic and negative chronotropic effects to Ber. As to its mechanism of action, it has been suggested that Yc-170 might facilitate Ca^{2+} inward current on hight membrane potential tissues and suppresse it on low membrane potentials[15]. Whether Ber behaves in a similar manner is worth of notice.

From the receptor point of view, since atropine couldn't block the effects of Ber showing its action is unrelated to M-receptor. In addition, BHT-920 and phenylephrine had also no effects on Ber action, while NE could almost completely antagonize them by virtue of its actions on β-receptor and calcium influx.

According to above results, it seemed reasonable to suggest that the anti-arrhythmic effects of Ber may be relevant to its actions on slow response cells of myocardium, which play an important role in the genesis and propagation of arrhythmias.

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小檗碱对离体兔窦房结和房室结电生理作用

王 玉、刘烈炬[2]、方达超 (同济医科大学药理教研室,[2] 生物工程室, 汉口 430030, 中国)

提要 小檗碱(Ber)0.1 到 30 μmol / L 能依剂量地降低兔窦房结(SAN)、房室结(AVN)细胞之 APA, V_{max}, 最大除极电位(MDP)及自博频率, 并延长 APD_{50}, APD_{95} 及 ERP. 同时抑制 SAN 功能, 即延长窦性周期(SCL)、窦房恢复时间(SRT)及校正窦房恢复时间(CSRT), 与 SAN 及 AVN 比较, Ber 延长心房(界嵴细胞)之 APD_{50}、APD_{95} 及 ERP 作用则需较高浓度、Ber 对 SAN 的作用不被阿托品(1 μmol / L)所拮抗; α_2-受体拮抗剂 BHT-920(10 μmol / L)或 α_1-受体激动剂苯肾上腺素(10 μmol / L)也不能翻转 Ber 的作用, 其抑制 SCL、APA 及 V_{max} 的作用可被去甲肾上腺素(10 μmol / L)所翻转. 在无钙液中, Ber 对 SAN 的抑制作用依然存在, 但在心房, 除 APD_{50} 及 APD_{95} 外, 对其它指标的影响则消失.

关键词 抗心律失常药; 小檗因类; 小檗碱; 窦房结; 房室结; 微电极; 电生理学

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绞股蓝总皂甙对实验性心肌梗塞的保护作用

熊维生、晏向东、沈 乃、丘芳龙[1]、陈 修 (湖南医科大学药理教研室, 长沙 410078, 中国)

Protective effects of gypenosides on experimental myocardial infarction

XIONG Wei-Sheng, YAN Xiang-Dong, SHEN Nai, QIU Fang-Long[1], CHEN Xiu (*Department of Pharmacology, Hu-nan Medical University, Changsha 410078, China*)

ABSTRACT In the model of myocardial infarction produced by occlusion of left anterior decending coronary artery (LAD) in rabbit, gypenosides (GP 100 but not 50 mg / kg, ip) reduced myocardical infarct size and decreased serum free fatty acid (FFA). In rat model of myocardial infarction, GP and the fractions of GP of non ginsenosides (FGNG) both in dose of 100 mg / kg, ip, protected significantly myocardial superoxide dismutase (SOD) activity and decreased the myocardial malondialdehyde (MDA). The results indicate that the protective effect of GP on myocardial infarction may be correlated with its prevention of myocardial lipid peroxidation, and attributed to the amelioration of FFA metabolic deterioration.

KEY WORDS gypenosides; ginseng; saponins; myocardial infarction; lipid peroxidation

提要 采用在体兔和大鼠冠状动脉结扎造成心肌梗塞动物模型.绞股蓝总皂甙(GP)能缩小兔心肌梗塞范围, 抑制心肌梗塞后 FFA 升高;并能降低大鼠梗塞心肌的 MDA 含量, 保护心肌 SOD, CPK 活性.结果表明 GP 对实验性心肌梗塞具有保护作用, 这种作用可能与它抗心肌脂质过氧化和改善心肌 FFA 代谢紊乱有关.

关键词 绞股蓝总皂甙; 人参; 皂甙类; 心肌梗塞; 脂质过氧化

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[1] Hu-nan Institute of Pharmaceutical Industry, Changsha 410014, China