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Induction of delayed afterdepolarizations and triggered arrhythmias in isolated Purkinje fibers: comparison of resibufogenin and acetylstrophanthidin[1]

Jing-Tian XIE, Peter CUNNINGHAM, Stephen SHOROFSKY, Adriana MOSCUCCI, Chris CADMAN, Craig T JANUARY[2] (*Cardiac Electrophysiology Laboratories, Section of Cardiology, Department of Medicine, University of Chicago, Chicago IL 60637, USA*)

ABSTRACT The purpose of this study was to compare the electrotoxicological effects of resibufogenin (RBG) ($n = 14$) with acetylstrophanthidin (AS) ($n = 14$) to induce delayed afterdepolarization (DAD) and triggered activity (TA), and their alteration of the electrophysiological properties in sheep cardiac Purkinje fibers using the extracellular electrograms, signal averaging, and standard microelectrode techniques simultaneously. The results indicated: 1) Lower toxic dose of RBG (0.52 $\mu mol \cdot L^{-1}$) and AS (0.25 $\mu mol \cdot L^{-1}$) induced intracellular and extracellular DAD (DAD-I and DAD-E) at pacing cycle length of 990 and 690 ms. 2) Higher toxic dose of RBG (2.6 $\mu mol \cdot L^{-1}$) and AS (5.0 $\mu mol \cdot L^{-1}$) induced DAD and TA, nonsustained or sustained premature action potential and oscillatory potentials; 3) At the beginning period of superfusing the drugs, both RBG and AS caused changes of the electrophysiological characteristics. This study demonstrates that the electro-toxicological characteristics and electrophysiological properties of RBG are similar to that of AS and suggests that RBG belongs to the family of digitalis-like drugs.

KEY WORDS amphibian venoms; strophanthidins; arrhythmia; Purkinje fibers; action potentials; electrophysiology

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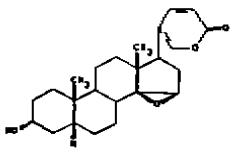
[2] Corresponding author: Craig T JANUARY, MD PhD.

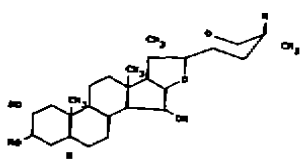
Triggered activity are used to describe impulse initiation in cardiac fibers that is dependent on a previous depolarization. Afterdepolarizations are oscillations in membrane potential that follow the upstroke of an action potential. Afterdepolarizations may occur early, ie, prior repolarization of the action potential (early afterdepolarization), or they may be delayed until after repolarization is complete (delayed afterdepolarization, DAD). When afterdepolarizations are large enough to reach the voltage threshold for activation of a regenerative inward current, they induce arrhythmias that are referred to as 'triggered'[1,2]. The prototypical experimental model used to induce DAD and triggered arrhythmias is to expose cardiac tissue to toxic concentrations of cardiac glycosides, such as acetylstrophanthidin (AS), ouabain, strophanthidin, digoxin, and resibufogenin (RBG)[3-5].

Resibufogenin is an extract from a traditional Chinese medicine, Venenum Bufonis, obtained from the skin gland venom of toads. RBG, one of important components, is similar to digitoxigenin in chemical structure. RBG is a positive inotropic agent, a respiratory stimulant, and a drug raising arterial blood pressure[5,6]. On the other hand, the toxicity of RBG and other cardiac glycosides that were obtained from Venenum Bufonis have been reported experimentally and clinically[7,8]. The toxic concentrations of RBG and AS inhibited the Na-K pump[9,10]. The purpose of this study was to compare the RBG with AS for

their toxicity or ability to induce DAD recorded intracellularly and to test whether extracellular electrograms could be used to record DAD-E and triggered activity in the isolated sheep cardiac Purkinje fibers.



Resibufogenin



Digitoxigenin

MATERIALS AND METHODS

Solutions Tyrode's solution containing NaCl 127, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 2.4, $MgCl_2$ 1.05, and dextrose 5.5 mmol · L^{-1}, was gassed with 95 % O_2 + 5 % CO_2 at 37 ± 1 C. RBG[5-8] [mono-hydroxy-14,15-epoxy-20,22-dienolide glycoside] was supplied by The First Chinese Medicine Factory of Tianjin, China. RBG was diluted with Tyrode's solution to 0.52 μmol · L^{-1} and 2.6 μmol · L^{-1} when used. Acetylstrophanthidin (AS, Sigma Chemical Co) was dissolved in distilled water and diluted with Tyrode's solution to 0.25 μmol · L^{-1} and 5.0 μmol · L^{-1} when used.

Preparation Sheep hearts were obtained at a local abattoir. The hearts were immediately placed in cooled Tyrode's solution. The single free-running Purkinje fibers of left ventricle (15.6 ± *s* 3.2 mm long and 1.1 ± 0.5 mm wide) were used.

Recording and stimulating The intracellular action potential was recorced with glass microelectrode (WPI, 1B150F-4 glass) filled with KCl 3 mol · L^{-1}, and having resistances of 10—20 ΩM and small tip potentials. Purkinje fibers were placed in a 2-ml tissue bath and superfused with normal Tyrode solution 1 ml · min^{-1}. The glass microelectrode was impaled mid-length of a Purkinje fiber as the voltage-recording electrode and was connected via an Ag/AgCl half-cell to the input of a high-impedance amplifier. To record the extracellular electrogram of a Purkinje fiber, two glass pipettes filled with KCl-3 mol · L^{-1} 2 % agar were placed in the bath at opposite ends of the fiber. Each piperte was connected via an Ag/AgCl half-cell to one input of high-impedance differential amplifier, thus permitting a bipolar recording. The amplifier outputs were displayed on an oscilloscope (Tektromx 5111). In most experiments data were digitized (band width > 1 kHz) and recorded on a laboratory computer using P-Clamp (version 5.5.1, Axon Instruments) for later measurement and analysis. Some of recordings also were recorded directly on a strip chart recorder (Gould). Data were signal averaged on-line to reduce random noise using the P-Clamp software to sample and average 25 sequential action potential and electrograms. The preparation was stimulated by bipolar extracellular silver electrodes placed close to one end of the preparation (World Precision Instrument). Stimulating current pulses usually were 2 ms in duration with amplitudes of 2 times the threshold.

Experimental protocol Each Purkinje fiber was equilibrated at basic cycle length of 990 ms stimulation for a minimum of 1 h prior to study. Because DAD can occur in freshly isolated fibers, our experiment required that each fiber first exhibited a normal resting potential, a normal action potential in the intracellular recordings, and a reasonable T-wave in the extracellular electrogram, and be free both spontaneous activity and afterdepolarizations. At the end of the equilibration period, control measurements were made of the action potential amplitude (APA_{MP}), resting potential (RP), maximum diastolic potential (MDP), action potential duration at 50% repolarization (APD_{50}), T-wave amplitude (T_{AMP}), and Q-T interval. After control measurements were obtained, the preparation was superfused with Tyrode's solution containing RBG (0.52 or 2.6 μmol · L^{-1}) or AS (0.25 or 5.0 μmol · L^{-1}) for 60 min, and then washout for 60 min. During superfusing the drug, DAD in the intracellular recordings and DAD-E in the extracellular recordings and/or triggered arrhythmias appeared. Transmembrane action potentials and electrograms were recorded every 30 min both the periods of superfusing and washing out. Besides preceding parameters of control, DAD and DAD-E amplitudes and their coupling intervals were also measured. Most of the data were obtained at the stimulation cycle lengths of 990 and 690 ms.

Thirty-one preparations were divided into 3 groups: Group RBG (0.52 μmol · L^{-1}, $n = 5$; 2.6

μmol · L^{-1}, n=9); Group AS (0.25 μmol · L^{-1}, n=9; 5.0 μmol · L^{-1}, n=5); Nontreatment Group (n=4), superfused with Tyrode's solution.

Data analysis Parameters of intracellular action potentials and extracellular electrograms were measured (Fig 1). Signals were analyzed by computer. All data are expressed as $\bar{x} \pm s$. Standard deviations within groups and between groups were analyzed by paired and nonpaired t tests, respectively.

RESULTS

DAD and DAD-E induced by lower toxic concentrations of RBG and AS Both lower toxic concentrations of RBG (0.52 μmol · L^{-1}) and AS (0.25 μmol · L^{-1}) induced DAD and DAD-E at the stimulation cycle lengths of 990 and 690 ms in all Purkinje fibers. For control condition (Fig 2, top panel), no DAD and DAD-E were present in the intra- and extracellular recordings. After exposure to RBG for 60 min (Fig 2, middle panel), DAD on transmembrane action potential recordings and DAD - E on electrograms

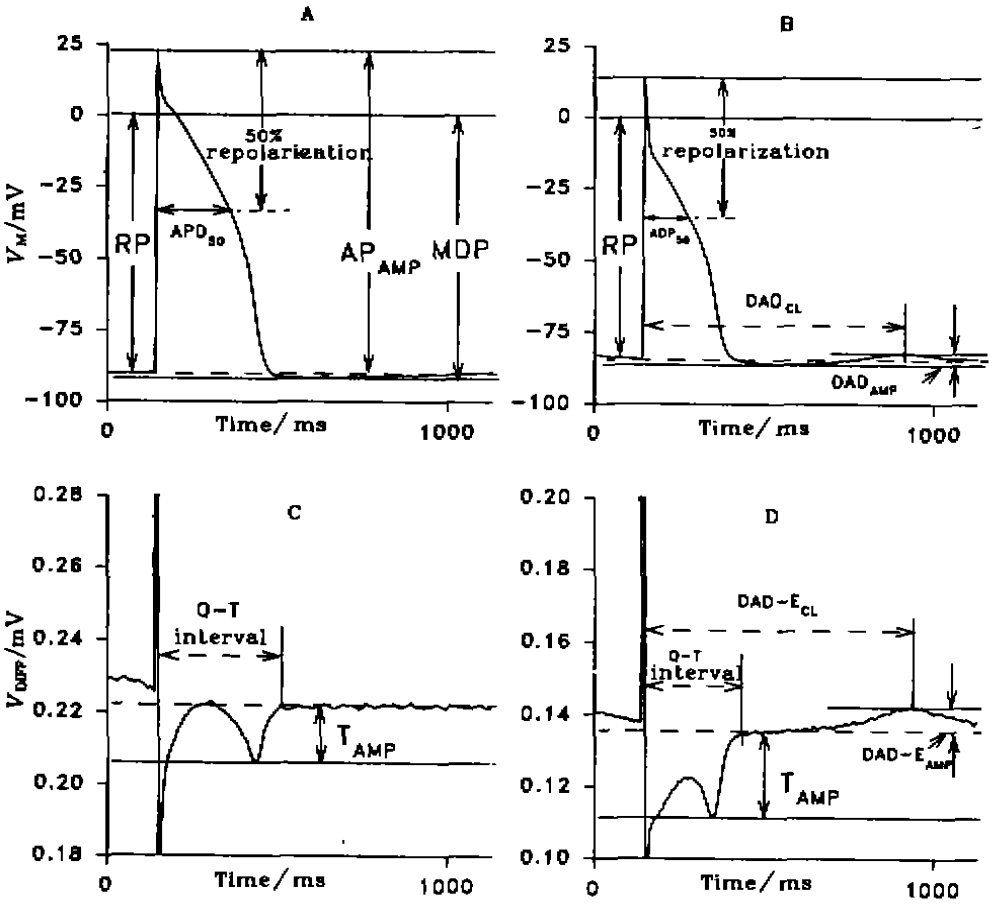


Fig 1. Transmembrane action potentials (A & B) and extracellular electrograms (C & D) before (A & C) and after (B & D) delayed afterdepolarizations induced by acetylstrophanthidin (AS, 0.25 μmol · L^{-1}). AP_{AMP}: action potential amplitude. RP: resting potential. APD_{50}: action potential duration at 50% repolarization. DAD_{AMP}: intracellular delayed afterdeopolarization amplitude. DAD_{CL}: coupling interval of intracellular delayed afterdepolarization. $DAD\text{-}E_{AMP}$: extracellular delayed afterdepolarization amplitude. $DAD\text{-}E_{CI}$: coupling interval of extracellular delayed afterdepolarization. T_{AMP}: T-wave amplitude.

developed obviously and simultaneously. The onset time of DAD and DAD-E were 49 ± 15 min at the pacing cycle lengths of 990 and 690 ms. For AS, DAD_{AMP} and DAD_{CL} depend on the stimulation cycle length. Both the intra- and extracellular recordings show that at the shorter pacing cycle length DAD_{AMP} and

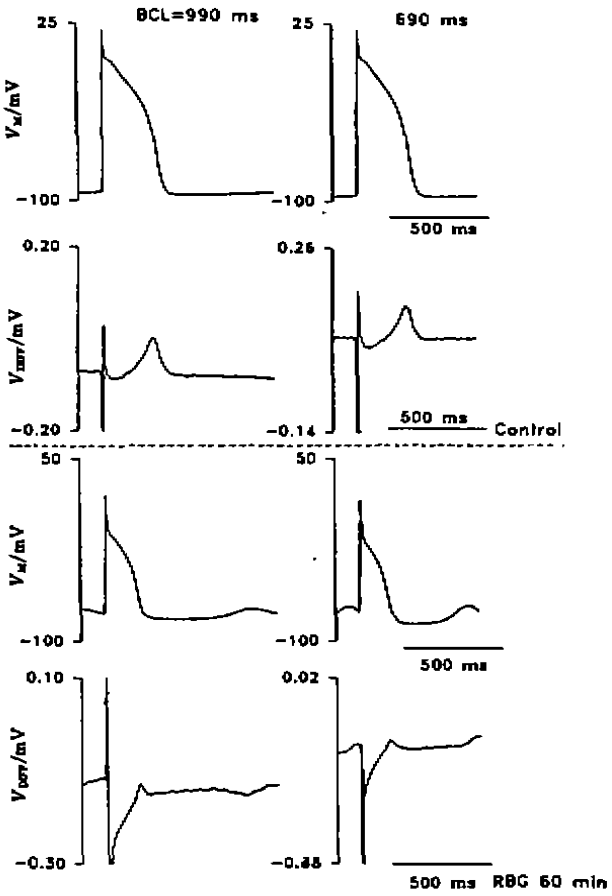


Fig 2. Effects of resibufogenin (RBG, 0.52 μmol · L^{-1}) on the transmembrane action potentials (upper tracings) and extracellular electrograms (lower tracings) in isolated sheep Purkinje fiber and induction of intracellular and extracellular delayed afterdepolarizations (DAD and DAD-E).

$DAD\text{-}E_{AMP}$ were greater and the coupling interval decreased. The intra- and extracellular recordings recovered to control condition after washing out of the drug for 60 min. Similar results were obtained using AS to induced DAD. In 2 of 5 preparations (40.0 %) 2 DADs were present at stimulation cycle length of 990 ms after RBG administration. Similarly, the AS group 5 of 9 preparations developed 2 DADs. Two DAD were not seen at cycle length of 690 ms both in RBG and AS Groups in these experiments.

Effects of lower toxic concentrations of RBG and AS on transmembrane action potential and electrograms AP_{AMP}, RP, MDP, and APD_{50} of in action potentials and T_{AMP}, and QT interval in electrogram did not change ($P > 0.05$) in nontreatment group during the period of observation, but changed after administration of RBG and AS.

After exposure to RBG, AP_{AMP}, absolute values of RP and MDP decreased progressively at the pacing cycle lengths of 990 and 690 ms. The AP_{AMP}, RP, and MDP at 990 ms were from 119 ± 7 to 99 ± 6 mV, from −93 ± 2 to −81 ± 4 mV ($P < 0.01$) and from −93 ± 2 to −84 ± 3 mV ($P < 0.01$), respectively. APD_{50} and Q-T interval at 990 ms were shortened (from 272 ± 44 ms to 129 ± 30 ms and from 443 ± 75 ms to 305 ± 67 ms, respectively. $P < 0.01$). All parameters recovered after washing out for 60 min ($P > 0.05$) similar changes in the electrophysiological characteristics and time couse occurred in Group AS (Fig 3).

DAD and triggered arrhythmias induced by higher toxic concentrations of RBG and AS Following exposure to RBG for 10 − 20 min, AP_{AMP}, RP, and MDP decreased, APD_{50}, and Q-T interval shortened, and DAD in action potentials and DAD-Es in electrograms developed markedly. DAD coupling interval (520 ms) was the same with DAD-E coupling interval (520 ms). When DAD_{AMP} were large enough to reach their threshold

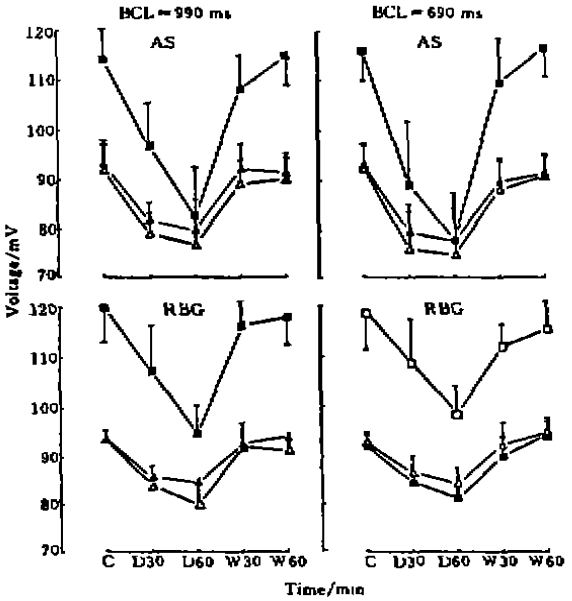


Fig 3. Effects of RBG (0.52 μmol · L^{-1}) and AS (0.25 μmol · L^{-1}) on action potential amplitude (AP_{AMP} ■), resting potential (RP ▲), and maximum diastolic potential (MDP △) at the pacing basic cycle lengths of 990 ms and 690 ms. Following exposure to RBG or AS, AP_{AMP}, RP, and MDP decreased progressively and minial peak values were at 60 min ($P<0.01$). After washing out for 60 min, the parameters recovered ($P>0.05$). BCL: basic cycle length; C: control condition; D30', D60': following drug administration for 30, 60 min.

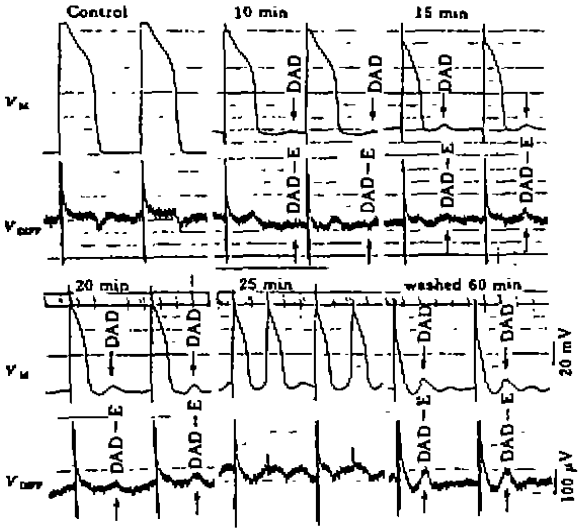


Fig 4. RBG (2.6 μmol · L^{-1}) induced DAD, DAD-E, and triggered activity in a Purkinje fiber at cycle length of 1000 ms. DAD (arrows) in action potentials (V_M) and DAD-E (arrows) in electrograms (V_{DIFF}) were induced simultaneously. The premature action potentials were induced from the peak of DAD. No recovery was seen in this experiment.

potential, triggered arrhythmias were induced. Premature action potentials in intracellular recordings and sustained ocsillations in extracellular recordings appeared. With washout for 60 min, the oscillatory potential (2 or 3 DAD) after action potential apperared and no recovery was seen (panel F). The toxic effects of RBG on induced triggered arrhythmias resembled those of AS (Fig 4).

DAD and complex arrhythmias were induced by higher toxic concentrations of both RBG and AS. DAD were seen in all preparations in Group RBG (9/9) and Group AS (5/5). There were various kinds of arrhythmias induced by RBG and AS at higher toxic concentrations, such as premature action poten-tials (9/9 in Group RBG and 5/5 in Group AS), spontaneous action potentials (4/9 in Group RBG and 2/5 in Group AS), and tachyarrhythmias (2/9 in Group RBG and 1/5 in Group AS).

DISCUSSION

These effects of RBG and AS were consistent with the previous reports[3,4,6,11,12].

This study demonstrates that there are similar electro-toxicological and electrophysiological actions between RBG and AS: 1) Both RBG and AS can induce DAD in the transmembrane action potential and DAD-E in the electrogram and triggered activity in isolated

cardiac Purkinje fiber at the cycle length of 990 and 690 ms. 2) The effects of RBG on all of parameters of transmembrane action potential and electrogram were similar to that of AS.

It is concluded that RBG belongs to the family of digitalislike cardiotonics.

REFERENCES

1 Cranefield PF, Aronson RS, editors. The role of triggered activity and other mechanisms. Mount Kisco (NY): Futura Publ Co, 1988.

2 Wit AL, Rosen MR. Afterdepolarization and triggered activity. In: Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE, editor. The heart cardiovascular system. New York: Raven Press, 1991: 2113—93.

3 Ferrier GR, Moe GK. Effect of calcium on acetylstrophanthidin-induced transient depolarizations in canine Purkinje tissue. Circ Res 1973; **32**: 508—15.

4 Karagueuzian HS, Katzung BG. Voltage clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. J Physiol (Lond) 1982; **327**: 255—71.

5 Xie JT, Belic N, McCullough J, Singer DH. The electrophysiological action of resibufogenin on mammalian cardiac fibers. Chin J Physiol Sci 1988; **4**: 145—54.

6 Lichtstein D, Kachalsky S, Deutsch J. Identification of a ouabain-like compound in toad skin and plasma as a bufodienolide derivative. Life Sci 1986; **38**: 1261—70.

7 Xie JT, Bufogenin. Drugs Future 1987; **12**: 837—9.

8 Kwan T, Paiusco AD, Kohl L. Digitalis toxicity caused by toad venom. Chest 1992; **102**: 949—50.

9 Wang LK, Lin QS. The inhibitory effect of steroid in chansu on the Na^+-K^+-APTase. Prog Biochem Biophys 1976; (4): 40—7.

10 Pamnani MB, Chen S, Bryant HJ, Schooley JF Jr, Ehades DC, Yuan CM, *et al*. Effects of three sodium-potassium adenosine triphosphatase inhibitors. Hypertension 1991; **18**: 316—24.

11 Rosen MR, Gelband H, Merker C, Hoffman BF. Mechanisms of digitalis toxicity. Effects of ouabain on phase four of canine Purkinje fiber transmembrane potentials. Circulation 1973; **47**: 681—9.

12 Moak JP, Rosen MR. Induction and termination of triggered activity by pacing in isolated canine Purkinje fibers. Circulation 1984; **69**: 149—62.

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脂布福吉宁和乙酰毒毛花甙诱发离体浦肯野氏纤维延时性后去极化及触发性心律失常的比较

解景田, Peter CUNNINGHAM, Stephen SHOROFSKY, Adriana MOSCUCCI, Chris CADMAN, Craig T JANUARY

(*Cardiac Electrophysiology Laboratories, Section of Cardiology, Department of Medicine, University of Chicago, Chicago, IL 60637, USA*)

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A **摘要** 用细胞外电图信号叠加技术微电极，于羊浦肯野纤维上，比较脂布福吉宁(RBG)与乙酰毒毛花甙元(AS)对诱发延时性后去极化(DAD)和触发性心律失常(TA)的作用. 结果：1) 低中毒剂量于刺激为 990 和 690 ms 时，诱发出胞内和胞外的 DAD；2)高中毒剂量诱发出DAD及TA，包含过早动作电位及触发性心动过速等；3) 均引起动作电位振幅、静息电位及最大舒张期电位的降低、50% APD 及 Q-T 间期的缩短. 故 RBG 属于洋地黄类药物.

关键词 两栖动物毒；毒毛花甙元；心律失常；浦肯野纤维；动作电位；电生理学