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双乙酰香茶菜甲素抗离体大鼠工作心脏缺血再灌注损伤的作用

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关键词 双乙酰香茶菜甲素；心肌再灌注损伤；脂质过氧化

目的：观察双乙酰香茶菜甲素(DAA-A)对心肌缺血再灌注损伤的作用. 方法：离体大鼠心脏停灌 40 min，再灌 25 min，造成心肌缺血再灌注损伤模型. 结果：DAA-A 0.13，0.25，0.50 mmol·L^{-1}对再灌注所致心功能低下有心脏保护作用，降低室颤发生率及乳酸脱氢酶(LDH)，丙二醛(MDA)的生成量. DAA-A 0.25 mmol·L^{-1}改善心肌超微结构. 结论：DAA-A 抗心肌缺血再灌注损伤的作用与其抗脂质过氧化损伤有关.

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Effects of daurisoline on intracellular Ca^{2+} activity in myocardium[1]

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KEY WORDS microelectrodes; calcium; electrophysiology; myocardium; daurisoline

AIM: To explain the effect of daurisoline (DS) on delayed afterdepolarization (DAD). **METHODS**: Ca^{2+}-sensitive microelectrode technic was used to record intracellular Ca^{2+} activity (α^{i}_{Ca}) and triggered activity (TA) arising from DAD in myocardium. **RESULTS**: Strophantin G 3 μmol·L^{-1} yielded an increase in resting myocardial α^{i}_{Ca} by 0.19 ± 0.11 μmol·L^{-1} and transient elevations of α^{i}_{Ca} by 1.48 ± 0.55 and 4.96 ± 1.81 μmol·L^{-1}, respectively during the development of DAD and TA. By pretreatment with DS or verapamil, strophantin G-caused elevations of the α^{i}_{Ca} in resting and provoked myocardia were eliminated and TA disappeared. DS 50 μmol·L^{-1} reduced Na^{+}-free medium-induced elevation of dog Purkinje fibrous α^{i}_{Ca} and abolished caffeine-induced increase of dog myocardial α^{i}_{Ca}. **CONCLUSIONS**: DS inhibited DAD and TA by preventing an increase of α^{i}_{Ca} via transmembrane Ca^{2+} entry and Ca^{2+} release from the reticulum.

Although a substantial increase of cytoplasmic Ca^{2+} is a prerequisite for delayed afterdepolarizations (DAD) that has been implicated as a cellular mechanism for arrhythmias due to affecting normal impulse conduction and engendering triggered activity (TA)[1], little has been reported in literature on simultaneous measurements of intracellular Ca^{2+} activity (α^{i}_{Ca}) and DAD/TA in a same myocardium[2]. A neutral ligand ETH_{1001} Ca^{2+}-sensitive microelectrode (Ca-ISE), which developed to have a fine tip and show stable property, ample sensitivity and transient response to free Ca^{2+} in a submicromolar range[3,4], allowed to

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measure α^{i}_{Ca} (= coefficient × $[Ca^{2+}]_i$) during the development of DAD and TA in the myocardium. This experiment was performed to verify the direct relationship between the α^{i}_{Ca} and DAD/TA, and elucidate the mechanism responsible for effects of daurisoline (DS, RR, 7, 7′-demethyldauricine, $C_{37}H_{42}N_2O_6$) on DAD and TA, using Ca-ISE technic.

MATERIALS AND METHODS

DS[5] (white powder, mp 93 - 94 ℃, TLC showed only one spot, purity > 98.5 % by HPLC) was prepared by the Faculty of Pharmacy in Tongji University. Verapamil hydrochloride (Ver), strophantin G (Str) and Ca^{2+}-cocktail were purchased from Fluka.

Microelectrodes were pulled from aluminosilicate tubing to produce a requisite tip using PF-2 ventrial micropipette puller. Standard microelectrodes were filled with KCl 3 mol · L^{-1} and had a resistance of 15 - 30 MΩ. Similar electrodes, siliconized and baked, were back-introduced into the tip with the exchanger ETH_{1001} (Ca^{2+}-cocktail) and then filled with $CaCl_2$ 10 mmol · L^{-1} for constructing Ca-ISE. The Ca-ISE was calibrated before and after each experiment using a series of calibrating solutions[3].

Unbranched papillary muscles were isolated from right ventricles of guinea pigs (♀ ♂, n = 34, 300 ± 31 g). Canine hearts were also chosen because its cells are larger enough to tolerate and maintain impalements of both electrodes. Dogs (n = 8) weighing 18.3 ± 1.7 kg were stunned. Free-running Purkinje strand and myocardium were dissected from the ventricles.

The preparations were superfused with oxygenated Tyrode solution containing NaCl 145, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 11, and Tris 10 mmol · L^{-1} at 37 ± 0.3 ℃, and driven by rectangular pulses of 2 ms duration and twice threshold voltage from a stimulator (BMS-8303) through bipolar platinum electrodes.

The preparation was penetrated by a standard microelectrode and then impaled by the Ca-ISE. Both sites of the impalement were several cell legnths apart to make sure that there was no paraelectrode shunt. Both electrodes were connected via Ag/AgCl pellets to head stages of a 100 TΩ input-impedance microelectrode amplifier (SWF-I, made by Instrumental Factory of Chengdu) with individual and their differential outputs. The superfusate potential was difined as zero for both electrodes. The transmembrane resting potential (E_m) or action potential (AP) from a standard microelectrode and Ca-ISE potential (E_{Ca}) were fed to a storage oscilloscope (SJ-6) for photographical recording and monitored with a double beam oscilloscope (SBR-1). E_{Ca} and its subtracted difference from E_m (ΔE) were recorded on a bichannel recorder (LMS-2B). The level of myocardial α^{i}_{Ca} was obtained by comparing ΔE directly with the calibration curve.

Data were expressed as $\bar{x} \pm s$ and analyzed by t test.

RESULTS

Ca-ISE calibrating and testing of accuracy In the test of its response to Ca^{2+} in a series of calibrating solutions which mimic the cellular ionic background, the chosen Ca-ISE showed Nernstian response to free Ca^{2+} down to 0.1 μmol · L^{-1} and lower with more than 20 mV between 0.1 and 1 μmol · L^{-1}. The calibration curves of pre- and post-experiment were similar, both slopes were equal. The AP from the Ca-ISE resembled one from the standard microelectrode and the magnitudes of both AP plateaux were a good match (Fig 1).

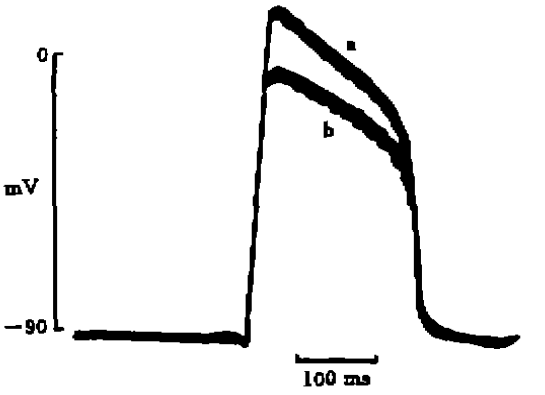

Fig 1. Superimposed tracings of transmembrane action potentials recorded with a standard microelectrode (a) and Ca^{2+}-sensitive microelectrode (b) during impalement of a guinea pig ventricular trabecula at a stimulation rate of 1 Hz.

Elevation of α^{i}_{Ca} during emergence of DAD/TA and effect of DS After 60-min incubation, measurements of DAD/TA and α^{i}_{Ca} started in guinea pig right ventricular trabecula. The resting myocardial α^{i}_{Ca} was 0.19 ± 0.06 μmol · L^{-1} (n = 16). At the end of 36 stimuli at cycle length (CL) of 250 ms, a small transient elevation in α^{i}_{Ca} was 0.28 ± 0.59 μmol · L^{-1} ($P > 0.05$, Fig 2A) and DAD or TA did not occur. Superfusion of trabecula with Str 3 μmol · L^{-1} for 15 min led α^{i}_{Ca} in resting myocardium to a steady increase of 0.19 ± 0.11 μmol · L^{-1}. After a drive train of 36 beats at a CL

of 250 ms, the α^i_{Ca} was further increased by 1.48 ± 0.55 μmol·L^{-1} in 7 preparations displaying DAD with the amplitude of 8.7 ± 1.2 mV, and 4.96 ± 1.81 μmol·L^{-1} in 9 preparations developing TA. This elevation of α^i_{Ca} was augmented by a train of stimuli at CL from 300 to 250 ms (Fig 2B).

When preparations were superfused with oxygenated Tyrode solution containing DS 50 μmol · L^{-1} for 20 min, Str was added to the superfusate (final concentration 3 μmol · L^{-1}). The resting level of α^i_{Ca} from 9 preparations was 0.23 ± 0.06 μmol·L^{-1}, which was similar to the control value ($P > 0.05$). At termination of 36 applied impulses at a CL of 250 ms, the transient elevation of α^i_{Ca} was 0.21 ± 0.63 μmol · L^{-1} ($P > 0.05$, Fig 2C) and TA disappeared (3/9 preparations showed DAD with the amplitude of 0.9 ± 0.2 mV and a transient elevation of 0.55 ± 0.29 μmol·L^{-1} in the α^i_{Ca}).

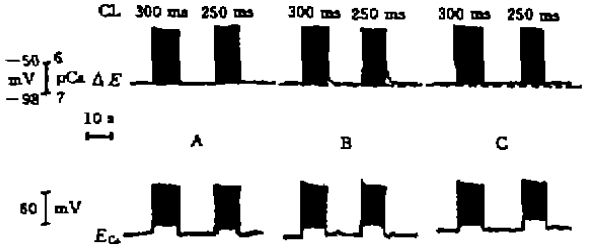

Fig 2. Effect of daurisoline (DS, 50 μmol · L^{-1}) on intracellular Ca^{2+} activity (α^i_{Ca}) after stimulation of a guinea pig ventricular trabecular exposed to strophantin G (3 μmol · L^{-1}) at drive cycle length (CL) of 300 and 250 ms for 36 impulses. Panel A: the control; panel B: the strophantin G-induced transient elevation of α^i_{Ca}; panel C: the block of strophantin G-induced elevation of α^i_{Ca} by pretreatment with DS. E_{Ca}: the signal from Ca^{2+}-sensitive microelectrode (Ca-ISE); ΔE: the potential difference from Ca-ISE and standard microelectrode. The dotted line indicates control level. pCa = − lg α^i_{Ca}.

In 7 preparations with continuous superfusion of Ver 11 μmol·L^{-1} for 15 min, the addition of Str 3 μmol · L^{-1} failed to elevate α^i_{Ca} in resting and provoked myocardium which remained 0.24 ± 0.10 and 0.38 ± 0.08 μmol·L^{-1}, respectively, and TA was not induced. In 4/7 preparations, DAD with an amplitude of 1.2 ± 0.4 mV developed and transient elevation in α^i_{Ca} was 0.11 ± 0.02 μmol · L^{-1}.

Effects of DS on Na^+-free medium-elicited elevation of α^i_{Ca} After dog ventricular Purkinje fibers were allowed to stabilize for 60 min, the α^i_{Ca} from 8 preparations was 0.36 ± 0.17 μmol · L^{-1}. When the preparation was switched to Na^+-free (choline substitute) Tyrode solution, the α^i_{Ca} gradually reached a peak increase of 68 ± 8 % within 10 min. After washout of the Na^+-free medium, the preparation was allowed to recover in normal perfusate. Subsequent superfusion with DS 50 μmol · L^{-1} for 25 min, the preparation was reexposed to Na^+-free medium in the presence of DS 50 μmol·L^{-1}. In this run, Na^+-removal gave a peak increase of 10 % ± 6 % in α^i_{Ca}, which indicated that DS decreased but did not eliminate the α^i_{Ca} elevation induced by Na^+-free.

Effect of DS on caffeine-caused increase in α^i_{Ca} After superfusion with Tyrode solution for 60 min, dog ventricular myocardium was measured to be 0.18 ± 0.04 μmol·L^{-1} in the α^i_{Ca}. Superfusion of caffeine 5 mmol·L^{-1} caused an elevation of 4.1 ± 0.4-fold in the α^i_{Ca} from 7 preparations. At 25 min after successive superfusion with DS 50 μmol · L^{-1}, the application of caffeine 5 mmol · L^{-1} elicited an increase of 4.4 % ± 4.3 % in the α^i_{Ca} ($P > 0.05$).

DISCUSSION

The Ca-ISE technic has been developed enough to monitor the α^i_{Ca} in cardiac tissue[3,4]. The present investigation found that this technic was well suited for simultaneous measurements of the α^i_{Ca} and DAD/TA in a same myocardium.

In this study, after α^i_{Ca} in resting myocardium was elevated by Str (3 μmol·L^{-1}), a single applied pulse did not elicit DAD or TA yet. Only when stimulation at a specific frequency lasted enough time, did Str induce DAD or TA, and the α^i_{Ca} further elevated transiently. It indicated that TA emerged when driven activities elicited a transient elevation of the α^i_{Ca} after the rise in a resting level. DS is a same kind of phenolic alkaloid as dauricine which is a novel type of anti-arrhythmic drug with electrophysiologic characteristics of class Ⅰ/Ⅲ. We found DS eliminated the resting and transient elevations of α^i_{Ca} induced by Str, and made TA disappear. Ca^{2+} channel blockers had been shown

to inhibit DAD induced by Str[6] but not α^{i}_{Ca} oscillations caused by Na^+-free medium[7]. The present experiment manifested that by pretreatment with Ca^{2+} channel blocker Ver, Str did not significantly induce elevations of α^{i}_{Ca} in resting and provoked myocardium, and TA disappeared. Those results supported the view that an elevation in α^{i}_{Ca} is essential for the development of DAD and TA, and suggested that inhibition of DS on DAD and TA was related to the prevention of an increase in the α^{i}_{Ca}.

The result showed that in the presence of Str which was found to activate T- and L-type Ca^{2+} current[8] and inhibit Na^+-K^+-transporting ATPase, driven activities potentiated the elevation of α^{i}_{Ca} and the emergence of TA, and demonstrated that the α^{i}_{Ca} elevation elicited by Na^+-free medium was significantly attenuated by pretreatment with DS. This implied that DS could reduce the accumulation of cytosolic Ca^{2+} as a consequence of Na^+-Ca^{2+} exchange.

Low concentration of caffeine, principally increasing the sarcoplasmic reticulum Ca^{2+} release[9], led to an elevation of α^{i}_{Ca}. This elevation in α^{i}_{Ca} could be eliminated by DS. It might be deduced that DS could forestall Ca^{2+} release from the sarcoplasmic reticulum.

In summary, this study provided an evidence that an increase in the α^{i}_{Ca} was a prerequisite to the generation of TA originating from DAD in myocardium. The inhibition of DS on DAD and TA might result from the amelioration of an increase in the myocardial α^{i}_{Ca} by prevention of the transmembrane Ca^{2+} gain and sarcoplasmic reticulum Ca^{2+} release.

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蝙蝠葛苏林碱对心肌细胞浆 Ca^{2+} 活度的影响

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关键词 微电极；钙；电生理学；心肌；蝙蝠葛苏林碱

A 目的：探讨蝙蝠葛苏林碱(DS)对迟后除极的作用. 方法：采用 Ca^{2+} 敏感性微电极技术. 结果：毒毛旋花甙 G (Str, 3 μmol·L^{-1})使豚鼠心室乳头肌细胞浆 Ca^{2+} 活度(α^{i}_{Ca})增加 0.19 ± 0 11 μmol·L^{-1}，在出现迟后除极和触发活动(TA)时又分别增加 1 48 ± 0.55 和 4.96 ± 1.81 μmol·L^{-1} 标本经 DS 作用后. Str 不再引起 α^{i}_{Ca} 增高和 TA DS 抑制无 Na^+ 和咖啡因引起的狗心肌 α^{i}_{Ca} 增加 结论：DS 可阻止 Ca^{2+} 跨膜入胞浆而防止 α^{i}_{Ca} 增高起抗 TA 作用

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