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Electrophysiologic effects of total flavones of *Hippophae rhamnoides* L on guinea pig papillary muscles and cultured rat myocardial cells

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ABSTRACT The effects of total flavones of *Hippophae rhamnoides* L (TFH) were evaluated using conventional microelectrode technic. After administration of TFH 100—200 mg · L^{-1}, the action potential duration of 50% repolarization (APD_{50}) was shortened both in cultured rat myocardial cells and in guinea pig papillary muscles. The slope of phase 4 of depolarization (SP_4) in cultured rat myocardial cells was decreased and the contractile force (CF) in guinea pig papillary muscles was weakened. Arrhythmias evoked by strophantin G in guinea pig papillary muscles were suppressed by TFH 100 mg · L^{-1}. These findings suggested that the influence of TFH on myocardial cells may be resulted mainly from its inhibition of Ca^{2+} influx and its interference with intracellular Ca^{2+} reservoir.

KEY WORDS *Hippophae rhamnoides* L; flavones; papillary muscles; cultured cells; action potentials; ouabain; arrhythmia

The total flavones of *Hippophae rhamnoides* L (TFH) exerted an anti-arrhythmic action which was probably related to the inhibition of Ca^{2+} channel[1,2]. In order to determine its ionic and electric mechanisms, we studied the electrophysiologic effects of TFH on guinea pig papillary muscles and cultured rat myocardial cells.

MATERIALS AND METHODS

Forty guinea pigs weighing 250 ± *s* 20 g (both sexes) were killed by a heavy blow on the head. Papillary muscles were excised from the right ventricle and pinned to a recording chamber containing 50 ml of Tyrode solution (NaCl 136, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 1.8, and glucose 11 mmol · L^{-1}, pH 7.2—7.4) at 35 ℃ and gassed with oxygen 10 ml · min^{-1}. After an equilibration period of 1 h, the muscle was stimulated through a bipolar platinum electrode with pulses of 2-ms duration, delivered at 1.5 times threshold voltage at a frequency of 1 Hz.

Primary cultures of rat myocardial cells were isolated from 2-4-day-old Wistar rats by the method of Wenzel, Wheatley, and Byrd[3]. The hearts were excised under aseptic condition. After removal of the blood, the lower two-thirds of the ventricles were cut into small pieces. The fragments were washed twice in a flask of calcium-free and magnesium-free phosphate buffer solution. Then the fragments were digested twice in the presence of 0.06 % trypsin solution, 10 min each time at 37 ℃. The superabundant was discarded and Eagle's culture medium containing 20 % fetal calf serum was added. The cultures were incubated at 37 ℃ in culture flasks for 5 d before study.

Transmembrane action potentials were recorded using 3 mol · L^{-1} KCl-filled glass microelectrodes, tip diameter less than 0.5 μm, and displayed on the upper line of COS 5020 oscilloscope. The maximal rate of depolarization (V_{max}), obtained by electronic differentiator, and the contractile force (CF) were shown on the lower line of the oscilloscope in turn. The effective refractory period (ERP) was determined by measuring the shortest interval between an extra stimulus and a regular stimulus.

Data were measured by photograph. All results were expressed as $\bar{x} \pm s$. Statistical analysis was performed using *t* test.

RESULTS

Effects on electric activities of cultured

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rat myocardial cells and guinea pig papillary muscle The cultured cells with spontaneously firing action potentials (AP) were chosen. In the presence of TFH 100 mg · L^{-1}, the APD_{50} was shortened and the SP_4 was remarkably decreased. Upon further increasing the concentration of TFH to 200 mg · L^{-1}, a shortening of APD_{90} was seen (Tab 1).

On guinea pig papillary muscle, TFH 100 — 200 mg · L^{-1} shortened the APD_{50} and weakened the CF (Tab 1).

Effects on arrhythmias induced by strophantin G in guinea pig papillary muscle After an equilibration for 1 h, action potentials were elicited by the standard train stimulation with rectangular pulses of 0.5—1.0 ms duration and an amplitude of 1.5 threshold[4]. The oscillatory after-potentials were evoked by the addition of strophantin G 0.4 μmol · L^{-1}. TFH 100 mg · L^{-1} led to the disappearance of oscillatory after-potentials from a control amplitude of 7.1±0.8 mV within 15 min in the 8 preparations studied ($P<0.01$).

After the action potentials were elicited by stimulation, strophantin G 0.8 μmol · L^{-1} was added. Spontaneous electric activities were induced by gradually decreasing the stimulation frequency down to complete cessation. After 5 min, in the presence of TFH 100 mg · L^{-1}, the rate of spontaneous electric activity was decreased from 196±17 bmp to 67±9 bpm ($P<0.01$).

When both strophantin G 0.8 μmol · L^{-1} and TFH 100 mg · L^{-1} were added at the same time, oscillations did not appear in the tested preparations.

DISCUSSION

Our experiment demonstrated that TFH 200 mg · L^{-1} shortened the APD and lessened the SP_4 in cultured myocardium. This result indicated TFH blocked the transmembrane Ca^{2+} influx. However, in our preparation, the significant decrease expected in APA and V_{max} was not observed since Ca^{2+} was shown to be involved in the current underlying the upstroke of AP and the pacemaker activity in cultured myocardium[5]. Assumption is justified that TFH, at the concentration of 200 mg · L^{-1}, only partially inhibited the transmembrane Ca^{2+} influx and did not affect the

Tab 1. Effects of total flavones of *Hippophae rhamnoides* L (TFH) on electric activities of cultured rat myocardial cells and guinea pig papillary muscles. $n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **prior to TFH administration.**

Concentration/ mg·L^{-1}	AP/ mV	APD_{50}/ ms	APD_{90}/ ms	V_{max}/ $V\cdot s^{-1}$	SP_4/ $V\cdot s^{-1}$	ERP/ ms	CF/ %
Cultured cells							
0	72±8	44±9	77±16	15±6	0.44±0.15	—	—
50	69±6[a]	43±8[a]	76±17[a]	15±6[a]	0.42±0.18[a]	—	—
100	69±8[a]	34±7[b]	75±18[a]	14±6[a]	0.34±0.13[b]	—	—
200	67±8[a]	30±8[c]	65±15[b]	13±5[a]	0.30±0.15[b]	—	—
Papillary muscles							
0	110±4	180±10	210±11	125±15	—	215±12	97±6
50	109±5[a]	172±11[a]	208±11[a]	120±18[a]	—	218±9[a]	80±4[b]
100	106±5[a]	167±12[b]	207±13[a]	122±14[a]	—	212±13[a]	64±5[c]
200	105±8[a]	155±14[b]	201±15[a]	118±16[a]	—	209±14[a]	51±8[c]

AP=action potentials; APD_{50} and APD_{90}=action potential duration ar 50 % and 90 % repolarization; V_{max}=maximal rate of depolarization; SP_4=slope of phase 4; ERP=effective refractory period; CF=contractile force.

current determining upstroke of AP in cultured myocardium. In guinea pig papillary muscles, our findings also confirmed that TFH exerted a blockade effect on the transmembrane Ca^{2+} influx. Because TFH restricted the contractile force and, in the meanwhile, just abbreviated the plateau of AP without influencing the other parameters[6].

In addition, we found TFH could antagonize *arrhythmizs induced by strophantin G* which were proved to be related to the intracellular Ca^{2+} overload and a transient oscillatory movement of Ca^{2+} released from the sarcoplasm reticulum[7]. Therefore, it is likely that anti-arrhythmic effect of TFH was also resulted from the inhibition of transmembrane Ca^{2+} influx as well as the interference of the intracellular Ca^{2+} reservoir, sarcoplasm reticulum. In this paper, we cannot rule out the possibility that in cultured myocardium the absence of change in upstroke of AP was due to the deficiency which we did not make further study at higher concentrations of TFH.

REFERENCES

1 Liu FM, Li ZX, Shi S. Effects of total flavones of *Hippophae rhamnoides* L on cultured rat heart cells and on cAMP level and adenylate cyclase in myocardium. Acta Pharmacol Sin 1988; 9: 539—42.

2 Liu FM, Li ZX, Shi S. Antiarrhythmic effect of total flavones of *Hippophase rhamnoides* L in the isolated heart. Chin Pharmacol Bull 1989; 5: 44—7.

3 Wenzel DG, Wheatley JW, Byrd GD. Effects of nicotine on cultured rat heart cells. Toxicol Appl Pharmacol 1970; 17: 774—85.

4 Ferrier GR. Digitalis arrhythmias: role of oscillatory afterpotentials. Prog Cardiovasc Dis 1977; 19: 459—74.

5 Schanne OF, Ruiz-Ceretti E, Payet MD, Deslauriers Y. Influence of varied $[Ca^{2+}]$ and $[Na^{+}]$ on electrical activity of clusters of cultured cardiac cells from neonatal rats. J Mol Cell Cardiol 1979; 11: 477—84.

6 Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu Rev Pharmacol Toxicol 1977; 17: 149—66.

7 January CT, Fozzard HA. Delayed afterdepolarizations in heart muscle: mechanisms and relevance. Pharmacol Rev 1988; 40: 219—27.

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沙棘总黄酮对豚鼠心室乳头状肌和培养大鼠心肌细胞的电生理作用

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摘要 用传统微电技术研究了沙棘总黄酮(TFH)对心肌细胞的电生理作用. TFH 100—200 mg·L^{-1}使豚鼠心室乳头状肌 APD 缩短, 收缩力下降, 培养大鼠心肌细胞 APD 缩短及4相除极斜率降低. TFH 100 mg·L^{-1}可抑制毒毛花甙 G 诱发豚鼠乳头状肌心律失常. 提示, 上述作用主要与 TFH 抑制心肌细胞 Ca^{2+} 内流影响心肌细胞内 Ca^{2+} 储库有关.

关键词 沙棘; 黄酮类; 乳头状肌; 培养的细胞; 动作电位; 哇巴因; 心律失常