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Frequency-dependent depression of V_{max} in K^+-depolarized guinea pig papillary muscle by tetrandrine

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ABSTRACT The effect of tetrandrine (Tet), a calcium antagonist, on the maximal upstroke velocity (V_{max}) of depolarization in K^+-depolarized guinea pig papillary muscles was studied by standard microelectrode method with computer. The results showed that: (1) the resting block of Tet on V_{max} was concentration dependent; (2) the drug (50 or 100 $\mu mol \cdot L^{-1}$) caused a marked frequency-dependent block of V_{max}, which accounted for 65 ± 8 % of total block at a concentration of 100 $\mu mol \cdot L^{-1}$ and the pacing frequency of 0.3 Hz; (3) the recovery kinetics of V_{max} could be characterized as a biexponential function, of which the second phase was prolonged by the drug; (4) compared with verapamil, nitrendipine, and diltiazem, the above-mentioned effects of Tet on V_{max} were similar to those of diltiazem. These results suggest that Tet can block calcium channel in both frequency-dependent and frequency-independent manner, mainly the former.

KEY WORDS tetrandrine; calcium channel blockers; verapamil; nitrendipine; diltiazem; electrophysiology

Selective calcium antagonists can be classified into 3 classes[1]: dihydropyridines (DHP, e g, nitrendipine), aralkylamines (e g, verapamil), and benzothiazepines (e g, diltiazem). Electrophysiologically, the depression of calcium channel caused by these 3 classes of drugs displays an obviously different character in frequency-dependent block (FDB)[2,3]. In addition, the amplitude of FDB of a given selective calcium channel antagonist is clinically in correspondence with its efficacy of anti-arrhythmia[4].

Tetrandrine (Tet), a bis-benzylisoquinoline alkaloid isolated from the Chinese medicinal herb *Stephania tetrandra* S Moore, has been proved to be a selective calcium antagonist[5] inactivating the benzothiazepine binding site of the calcium antagonist receptor complex[6], its frequency-dependent effect on reversing the positive inotropic action having been reported[7]. However, the FDB, characteristic for a given anti-arrhythmic, has not been identified. In the present study, the effects of Tet on FDB of V_{max} were studied and compared with those of the 3 classical calcium antagonists.

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MATERIALS AND METHODS

Guinea pigs of either sex weighing 350 ± *s* 50 g were used. The right ventricular papillary muscle was dissected out in normal Tyrode solution[8] at room temperature and quickly placed in a Plexiglas chamber perfused with high K^+-Tyrode solution containing KCl 22 $mmol \cdot L^{-1}$ and $BaCl_2$ 0.8 $mmol \cdot L^{-1}$, aerated with 95% O_2 + 5% CO_2 at 35 ± 0.2℃, pH 7.40 ± 0.05. Action potential (AP) and V_{max} signals were obtained by standard microelectrode technique. The signals were input through a microelectrode amplifier and a differentiator, both connected to a microcomputer(APPLE-Ⅱ) where the AP and V_{max} were analyzed and to an oscilloscope (SBR-Ⅰ) where the signals were scanned and photographed.

The experimental protocol was basically the same as that of Woods *et al*[8]. We chose low, moderate and high concentrations for Tet and the 3 classical calcium antagonists, which were 10, 50, and 100 $\mu mol \cdot L^{-1}$ for Tet; 0.1, 0.5, and 1.0 $\mu mol \cdot L^{-1}$ for verapamil (Ver) and diltiazem (Dil); 0.01, 0.05, and 0.1 $\mu mol \cdot L^{-1}$ for nitrendipine (Nit). At equivalent effective concentrations, the 4 drugs induced approximately similar depression on V_{max} at 0.3 Hz.

Resting block (RB) was measured by percent decrease in V_{max} at rested state ($V_{max(RS)}$) of drug exposure compared with that of control; FDB was calculated by comparing $V_{max(RS)}$ with the steady state values of V_{max} after a train of stimuli (also defined as use-dependent block), and a range of frequencies; and the recovery kinetics of V_{max} was analysed using paired stimuli separated by a variable interval (diastolic interval)[9]. The speed of recovery was represented as recovery time constant (τ_r) which was determined from the semilog plot of $1-V_{max(test)}/V_{max(RS)}$ as a function of diastolic interval. A single impalement was maintained throughout the experiment.

Tet was obtained from the Dept of Pharmacology, Tongji Medical University. Ver was the product of Shanghai 10th Pharmaceutical Factory. Nit was provided by Nanjing Pharmaceutical Factory. Dil was purchased from Reagent Shop, Shanghai.

Statistical significance was measured by *t* test.

RESULTS

After equilibration, the values of V_{max}, RP, APD, and APA at basal frequency of 0.1 Hz in drug-free state did not change in 6–8 h. All the solvents of drugs (Tris-HCl buffer and polyethylene glycol 400) showed no significant effect on the parameters mentioned above.

RB After a quiescent period in drug exposure, the V_{max} elicited by the first pulse in the train were of dissimilar amplitudes, indicating different degrees of RB. All the 4 drugs caused a concentration dependent depression on the V_{max} in rested state. The degree of depression by Tet (10, 50, and 100 μmol · L^{-1}) was more significant than the equivalent effective concentration of Ver (0.5 and 1.0 μmol L^{-1}, $P<0.05$) and less than that of Nit ($P<0.05$) but close to Dil.

FDB The depression of V_{max} by Tet, Ver, Nit, and Dil become gradually more marked with repeated depolarizations and approached a balanced state (quasi-steady state), the level of which varied in different frequencies. A progressive decline of V_{max} during subsequent depolarizations in a train of given frequencies, was deviated by Tet and the classical drugs (Fig 1).

Tab 1. Resting block of V_{max} by tetrandrine (Tet), verapamil (Ver), nitrendipine (Nit), and diltiazem (Dil). $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **the corresponding effective concentration of Tet.**

Drug	μmol · L^{-1}	Guinea pigs	Resting block of V_{max}
Tet	10	7	2.1 ± 2.3
Ver	0.1	5	0^{*}
Nit	0.01	5	$8.2 \pm 2.8^{***}$
Dil	0.1	4	$5 \pm 4^{*}$
Tet	50	6	11 ± 5
Ver	0.5	5	$2.4 \pm 2.3^{***}$
Nit	0.05	5	$29 \pm 3^{***}$
Dil	0.5	4	$14 \pm 6^{*}$
Tet	100	7	19 ± 6
Ver	1.0	4	$11.5 \pm 2.4^{**}$
Nit	0.1	6	$41 \pm 10^{***}$
Dil	1.0	5	$20 \pm 9^{*}$

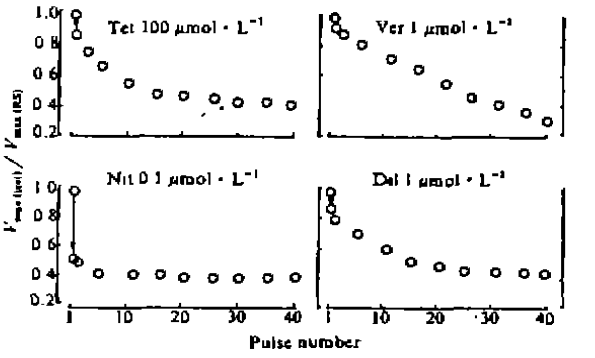

Fig 1. Depression of V_{max} by tetrandrine (Tet), verapamil (Ver), nitrendipine (Nit), and diltiazem (Dil) with repeated depolarizations at 0.3 Hz. All values are scaled relative to drug free $V_{max(RS)}$. Arrows indicate RB.

In total depression of V_{max} (about 60 %) at 0.3 Hz, the percentage of FDB induced by Tet(100 μmol · L^{-1}), Ver(1.0 μmol · L^{-1}),

Nit (0.1 μmol · L^{-1}), and Dil(1.0 μmol · L^{-1}) were 65 ± 8 (n=7), 83.5 ± 2.7 (n=4), 34 ± 6 (n=5), and 69 ± 12 (n=5), respectively (Fig 1), the effect of Tet was both diverged from that of Ver and Nit ($P<0.05$) but similar to that of Dil.

The percent depression of V_{max} by Tet became progressively more prominent with increasing frequency of stimulation over a range of 0.1–1 Hz, and so did Ver, Nit, and Dil. This depression was concentration-dependent for all 4 drugs.

Under normal conditions, there was a tendency that the higher the pacing frequency, the more the depression of V_{max} in drug-free state. So, it is better to get the net FDB of these drugs. The value of net FDB at different concentrations could be obtained by eliminating the influence of FDB in drug-free state and RB. A hypothetical regression line was fitted to illustrate the frequency-dependent changes expected, if V_{max} were altered from the rested-state value only in the same proportion as that seen in the drug-free state (Fig 2). The actual values diverged from the hypothetical regression line. The difference between the measured values and the expected ones represented the net FDB of drugs. A quantitative determination of net FDB was obtained by the linear regression of the different values stated above as a function of the lg pacing frequencies, the interception (expressed the magnitude of net FDB at 1 Hz) and the slope (representing the velocity that the depression increased with higher frequencies) indicating the different net FDB of 4 drugs in the chosen concentrations (Tab 2). Tet 10 μmol · L^{-1} showed little net FDB. However, the net effects of Tet 50 and 100 μmol · L^{-1} were similar to those of Dil (in both interception and slope), and diverged from those of Ver and Nit (in either the interception or the slope.)

Recovery kinetics The recovery of V_{max} following rested state depression was quantified by plotting lg $[1-V_{max(test)} / V_{max(RS)}]$ as a function of the diastolic interval and characterized as a biexponential function. Tet, Ver, Nit, and Dil promoted the appearance of 2 distinct phases in the course of recovery: a relatively rapid recovery phase (the time constant τ_1) just like that seen in the absence of drug (except Ver which slowed down τ_1 at 0.1, 0.5, and 1.0 μmol · L^{-1}) and a clear second phase with a slowing time constant (τ_2). The changes of τ_1 and τ_2 were shown in Tab 3. Tet and the 3 classical drugs prolonged τ_2 with a tendency of concentration-dependence. Tet (50, 100 μmol · L^{-1}) produced about the same changes in τ_1 and τ_2 as those of Dil (0.5, 1.0 μmol · L^{-1}) but significantly different from those of Ver and Nit.

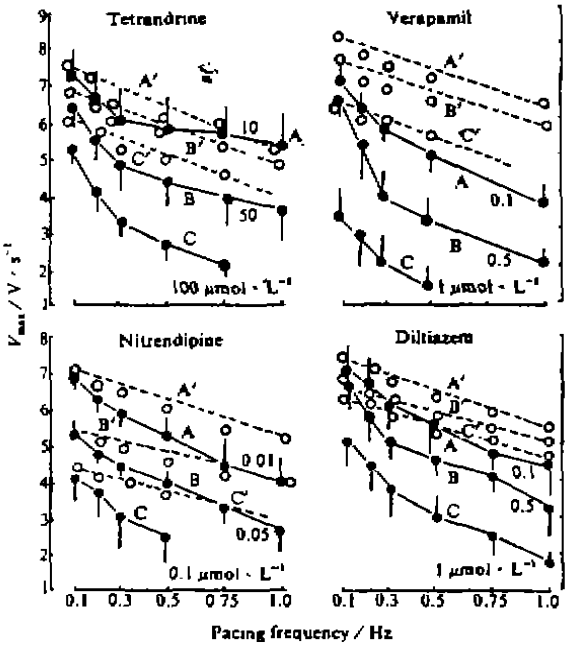

Fig 2. Effects of Tet, Ver, Nit, and Dil on V_{max} in rested state (RS) and as a function of frequency [$V_{max\ (measured)}$ (●)]. Dash line: expected FDB from RS in proportion to that of drug free state [$V_{max\ (expected)}$ (○)]. $\bar{x} \pm s$, n=4–7. The difference between the measured values and the expected ones represents the net FDB of drugs.

Tab 2. Linear regressions of the net depressions of tetrandrine (Tet), verapamil (Ver), nitrendipine (Nit), and diltiazem (Dil), where the abscissa being lg frequencies, the ordinate being net FDB, and the interception expressing net FDB at 1 Hz. $\bar{x} \pm s$, $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **Tet.**

Drug	μmol · L^{-1}	Guinea pigs	Interception	Slope	*r*
Tet	10	7	1.0 ± 0.7	0.6 ± 0.4	0.78 ± 0.09
Ver	0.1	5	2.49 ± 0.28***	1.4 ± 0.4***	0.93 ± 0.08
Nit	0.01	5	1.46 ± 0.12*	1.4 ± 0.3***	0.90 ± 0.08
Dil	0.1	4	1.4 ± 0.7*	1.2 ± 0.5**	0.90 ± 0.08
Tet	50	6	1.7 ± 0.3	1.1 ± 0.6	0.90 ± 0.08
Ver	0.5	5	3.87 ± 0.28***	2.7 ± 0.4***	0.92 ± 0.04
Nit	0.05	6	1.2 ± 0.3***	1.0 ± 0.4*	0.90 ± 0.05
Dil	0.5	4	1.8 ± 0.4*	1.5 ± 0.7*	0.91 ± 0.06
Tet	100	7	2.9 ± 0.6	2.0 ± 0.7	0.92 ± 0.05
Ver	1.0	4	4.31 ± 0.29***	1.3 ± 0.6*	0.89 ± 0.08
Nit	0.1	6	2.21 ± 0.19**	2.03 ± 0.26*	0.90 ± 0.03
Dil	1.0	5	3.0 ± 0.4*	1.8 ± 0.7*	0.97 ± 0.02

Tab 3. Effects of tetrandrine (Tet), verapamil (Ver), nitrendipine (Nit), and diltiazem (Dil) on biexponential recovery of V_{max} after rested state depression. $\bar{x} \pm s$, $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **drug free;** $^{+}P > 0.05$, $^{++}P < 0.05$, $^{+++}P < 0.01$ *vs* **moderate concentration;** $^{\S}P > 0.05$, $^{\S\S}P < 0.05$, $^{\S\S\S}P < 0.01$ *vs* **Tet.**

Drug / μmol · L^{-1}		Guinea pigs	Recovery time constant / s τ_1	τ_2
Tet	0	16	0.09 ± 0.05	4.0 ± 1.1
	10	6	0.10 ± 0.04*	5.2 ± 0.6^{**+}
	50	5	0.13 ± 0.06*	6.5 ± 1.4**
	100	5	0.14 ± 0.01**	9.4 ± 1.1^{***+++}
Ver	0	14	0.10 ± 0.03	4.0 ± 1.1
	0.1	4	0.14 ± 0.03**	8.8 ± 1.8$^{***+\S\S}$
	0.5	4	0.16 ± 0.03**§§	10.1 ± 2.0**§§
	1.0	6	0.174 ± 0.024***§§	12.2 ± 4.0$^{***+\S\S}$
Nit	0	8	0.098 ± 0.022	4.0 ± 0.4
	0.05	4	0.11 ± 0.03*§	5.8 ± 0.8***§
	0.1	4	0.121 ± 0.020*§§	7.3 ± 1.1$^{***+\S\S}$
Dil	0	9	0.099 ± 0.015	4.0 ± 0.9
	0.5	4	0.112 ± 0.023*§	5.4 ± 0.7**§
	1.0	5	0.14 ± 0.04**§	8.0 ± 1.4$^{***++\S}$

DISCUSSION

This study showed that Tet depressed V_{max} in both frequency-dependent and frequency-independent manner, the former being more important. The concentration-dependence of FDB and RB, the percentage of FDB and RB in total block, and the recovery kinetics of Tet were similar to those of Dil, which implied that the effects of Tet on electrophysiology may be closely approaching those of Dil.

According to the result that it produces more marked FDB than RB and its molecular structure of bis-tertiary amine, Tet might bind mainly to the calcium channel of inactive state. The mechanism that Tet slow the recovery of V_{max} might be due to its large molecular wt of 622.76, being difficult to egress from the binding site. But it was unknown that why the recovery kinetics shifts with a tendency of concentration-dependence that showed in our results.

Based on its physiological profile, Tet has been considered a verapamil-like calcium antagonist[5]. Though the subclassification of calcium antagonists by electrophysiologic or binding methods are not always the same, our results from electrophysiologic characteristics indicated that Tet may be a diltiazem-like drug, which is in line with the result of

receptor-binding experiments[6]. For anti-arrhythmic use, the classification of drugs by electrophysiologic methods is more important, our study suggested that, just like sodium channel blockers[10], studying the FDB may be an feasible means of subclassifying calcium antagonists. On the basis of its diltiazem-like FDB effect in the present study, we predicted that the clinical antiarrythmic effect of Tet, if any, would be less valuable than Ver.

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粉防己碱对豚鼠 K^+ 除极乳头状肌 V_{max} 的频率依赖性抑制

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提要 用标准微电极法研究粉防己碱(Tet)对离体豚鼠 K^+ 除极心肌 V_{max} 的影响. 结果提示: 静息抑制呈现浓度依赖性; 50 或 100 μmol · L^{-1} 频率依赖性抑制明显, 100 μmol · L^{-1}, 0.3 Hz 时占总抑制 65.1 ± 8.3%; 使第二相恢复延长; 上述作用均与地尔硫䓬相似. 本研究表明 Tet 可以频率依赖性和频率非依赖性两种方式抑制钙通道, 以前者为主.

关键词 粉防己碱; 钙通道阻滞剂; 维拉帕米; 尼群地平; 地尔硫䓬; 电生理学 心肌 豚鼠