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Effects of tetrandrine on action potentials and afterhyperpolarization potentials in toad dorsal root ganglia[1]

ZENG Wei-Zhong, LIU Tian-Pei
(*Department of Pharmacology, Nanjing Medical College, Nanjing 210029, China*)

ABSTRACT Intracellular recordings were obtained from the cytosome of type A primary afferents in the isolated toad dorsal root ganglia (DRG) preparations. Bath application of Ca^{2+} 8.0 mmol · L^{-1} led to prolong action potential duration of repolarization 100% (APD_{100}) by 23%, afterhyperpolarization potential duration of depolarization 50% ($AHPD_{50}$) by 46%, and increase afterhyperpolarization potential amplitude (AHPA) by 40%. Verapamil 3 μmol · L^{-1} shortened the APD_{100} by 7% and the $AHPD_{50}$ by 13%, and reduced the AHPA by 17%. The effects of Tet 3-100 μmol · L^{-1} consisted of APD and $AHPD_{50}$ shortening and AHPA reduction in concentration-dependent manner. Tet 100 μmol · L^{-1}, APD_{100} was shortened by 16%, $AHPD_{50}$ by 18%, and AHPA was reduced by 20%. The results suggested that the effects of Tet may be related to its Ca^{2+} channel blockade in DRG.

KEY WORDS tetrandrine; calcium channels; verapamil; action potentials; spinal ganglia

Tetrandrine (Tet) is an alkaloid isolated from medicinal plant *Stephania tetrandra* S Moore. The anti-arrhythmic effects[1] of Tet have been reported, and the electrophysiological mechanism was related to its Ca^{2+} channel blockade. However, the action of Tet on isolated neurons has so far been scarcely reported, and its mechanism for anti-ischemic effect on brain remains unclear. In this paper, through intracellular recording technique, the electrophysiological effects of Tet on action potentials (AP) and afterhyperpolarization potentials (AHP) in toad dorsal root ganglia (DRG) were investigated.

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MATERIALS AND METHODS

Toads (either sex) were decapitated, the vertebral column with attached spinal nerves was isolated and laminectomy was performed under chilled Ringer solution. DRG numbers 9 and 10, nomenclature of Gauppas described by Kudo[2] with attached dorsal root and spinal nerve were isolated and desheathed in a Sylgard lined dish. The preparation was pinned in a superfusion chamber (0.5 ml). The spinal nerve was mounted on wire electrodes and stimulated by SEN-3201 electronic stimulator with square-wave pulses (1 Hz, 0.2 ms, · 0.5-10 V). The distance between the cathode and the DRG was 15 mm.

The DRG was totally submerged in Ringer solution of the following composition (mmol · L^{-1}): NaCl 114, KCl 2, $CaCl_2$ 2, Tris 10, and glucose 5.6. The actions of Ca^{2+} was examined with Ringer solution containing $CaCl_2$ 1 mmol · L^{-1}. The Ringer solution was saturated with 100% O_2 (pH 7.4 ± 0.2) and the flow rate was 5.0 ml · min^{-1} at room temperature (25-28℃). The intracellular recording was done after the preparation had been equilibrated for 1-1.5 h.

Intracellular recordings were obtained using glass microelectrode filled with KCl 3 mol · L^{-1} (20-60 MΩ). Signals were fed through a MEZ-7101 microelectrode amplifier, displayed on a SBR-1 oscilloscope and recorded by a photographic recorder.

Verapamil (Sigma) was dissolved in Ringer solution. Tet was dissolved in HCl 0.1 mol · L^{-1} and diluted by Ringer solution adjusted to pH 6 with NaOH.

Statistical analysis employed was paired *t* test.

RESULTS

Stable intracellular recordings lasting up to 3 h were made from a total of 20 neurons

Tab 1. Effects of $CaCl_2$ on AP and AHP in toad DRG. $n=5$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **$CaCl_2$ 1 mol · L^{-1}.**

	$CaCl_2$ / mol · L^{-1}			
	1	2	4	8
APA / mV	76.6 ± 2.4	76.4 ± 2.3*	76.2 ± 2.5*	76.8 ± 2.8*
APD_{25} / ms	1.41 ± 0.16	1.62 ± 0.13**	1.86 ± 0.13***	2.13 ± 0.16***
APD_{50} / ms	2.26 ± 0.21	2.52 ± 0.20**	2.74 ± 0.21***	2.98 ± 0.23***
APD_{100} / ms	4.26 ± 0.17	4.58 ± 0.16**	4.92 ± 0.19***	5.22 ± 0.26***
AHPA / mV	6.72 ± 0.26	7.98 ± 0.11**	8.23 ± 0.27***	9.42 ± 0.53***
$AHPD_{50}$ / ms	6.78 ± 0.33	7.72 ± 0.19**	8.67 ± 0.15***	9.92 ± 0.65***

with conduction velocities of 5–20 m · s^{-1} (10.8 ± 3.8, $n=20$). According to the criteria of classification of Erlanger and Gasser[3], these cells were all type A cells.

Ca^{2+} on AP and AHP The Ringer solution for control contained Ca^{2+} 1 mmol · L^{-1}. The concentration–response relationship of Ca^{2+} was shown in Tab 1. APD was prolonged in the solution containing Ca^{2+} 2 mmol · L^{-1} with an increase of 15% for APD_{25}, 12% for APD_{50}, and 8% for APD_{100}. At this concentration, AHPA was increased by 18%, and $AHPD_{50}$ was prolonged by 14%. APD and AHPD were progressively prolonged and AHPA was progressively increased while the concentrations of Ca^{2+} were increased from 2 to 8 mmol · L^{-1}. But, APA was not affected significantly by Ca^{2+} at all these concentrations.

Verapamil on AP and AHP After 3 min of perfusion with Ringer solution containing verpamil 3 μmol · L^{-1}, the duration of AP, AHP, and the amplitude of AHP began to diminish. Eight minutes later, APD, AHPD, and AHPA were greatly reduced, attaining the maximal reduction after 15 min of perfusion: 12% for APD_{25}, 11% for APD_{50}, 7% for APD_{100}, 13% for $AHPD_{50}$, and 17% for AHPA (Tab 2). However, APA was not affected by verapamil 3 μmol · L^{-1}. The parameters of AP and AHP were completely recovered after 20 min of washing.

Tet on AP and AHP Various concentrations (1, 3, 10, 30, 100 μmol · L^{-1}) of Tet in an increasing order were applied to the preparation. Measurements were made after 10–15 min of exposure to each concentration, a time sufficient to obtain steady-state conditions. In toad DRG, Tet 1 μmol · L^{-1} did not affect the parameters of AP and AHP. Tet 3 μmol · L^{-1} caused a reduction of 6.4% for AHPA, a shortening of 4.6% for APD_{50}, 3.6% for APD_{100}, and 4% for $AHPD_{50}$. As the concentration of Tet was increased, APD_{25}, APD_{50}, APD_{100}, and $AHPD_{50}$ were progressively shortened and AHPA was reduced in a concentration-dependent manner (Tab 3). Tet 30 μmol · L^{-1} shortened APD_{100} by 12%, $AHPD_{50}$ by 13%, reduced AHPA by 16%. At 100 μmol · L^{-1}, APD was shortened with a reduction of 33% for APD_{25}, 20% for APD_{50}, and

Tab 2. Effects of verapamil 3 μmol · L^{-1} on AP and AHP in toad DRGs. $n=5$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

	Control	Verapamil
APA / mV	76.6 ± 2.4	76.8 ± 2.5*
APD_{25} / ms	1.46 ± 0.17	1.29 ± 0.13***
APD_{50} / ms	2.66 ± 0.24	2.36 ± 0.22***
APD_{100} / ms	4.34 ± 0.17	4.04 ± 0.19***
AHPA / mV	7.82 ± 0.32	6.48 ± 0.19***
$AHPD_{50}$ / ms	7.72 ± 0.81	6.69 ± 0.63***

Tab 3. Effects of tetrandrine on AP and AHP in toad DRGs. $n=5$. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

	Control	Tetrandrine / μmol · L^{-1} 1	3	10	30	100
APA / mV	76.6 ± 2.4	76.4 ± 1.7*	76.6 ± 2.1*	76.6 ± 2.3*	76.4 ± 2.1*	76.8 ± 2.2*
APD_{25} / ms	1.32 ± 0.13	1.31 ± 0.12*	1.26 ± 0.11*	1.14 ± 0.13**	0.96 ± 0.15**	0.88 ± 0.13**
APD_{50} / ms	2.14 ± 0.24	2.12 ± 0.22*	2.04 ± 0.21**	1.92 ± 0.16**	1.83 ± 0.21**	1.72 ± 0.23**
APD_{100} / ms	3.94 ± 0.24	3.92 ± 0.22*	3.80 ± 0.29**	3.68 ± 0.24**	3.48 ± 0.24**	3.32 ± 0.19**
AHPA / mV	7.81 ± 1.51	7.72 ± 1.30*	7.31 ± 1.41**	6.80 ± 1.40**	6.54 ± 1.50**	6.24 ± 1.14**
$AHPD_{50}$ / ms	7.74 ± 0.41	7.68 ± 0.37*	7.43 ± 0.35**	7.14 ± 0.31**	6.74 ± 0.31**	6.34 ± 0.29**

16% for APD_{100}, AHP was affected with a reduction of 20% for AHPA and 18% for $AHPD_{50}$. APA was not affected by Tet. After 30 min of washing, all the parameters of AP and AHP were completely recovered.

DISCUSSION

Neurons in DRG were classified into two types: Type A neurons with the condition velocity > 2.5 m · s^{-1}, and type C neurons with the condition velocity < 2.5 m · s^{-1}. Type A and Type C cells in the DRG were in the ratio of 2 : 1[3]. In this study, through stable recording most the DRG neurons were found to be type A neurons, because the size of type C was too small to make the recording stable when the resistance of the microelectrode ranged from 20 to 60 MΩ.

Three subtypes of voltage-sensitive Ca^{2+} channels have been identified in DRG neurons: T, N, and L type[4,5]. Ca^{2+} played a certain role in the AP, especially in the AHP caused by Ca^{2+}-activated K^{+} channel[6] which was opened at intracellular Ca^{2-} contraction > 0.05 μmol · L^{-1}. Verapamil mainly suppressed the L type at low concentrations[7]. Ca^{2+} channel blocker reduced AHP through reducing $[Ca^{2+}]_i$ by blocking Ca^{2+} influx[8]. The results of verapamil in this study seemed to be consistent with findings of others[9].

The Ca^{2+} channel blockade of Tet[10] could explain its actions on APD and AHP. The effect of Tet 30 μmol · L^{-1} on AHP was equivalent to that of verapamil 3 μmol · L^{-1}, but Tet had more remarkable effect on APD than verapamil did. It suggested that the mechanism for Tet on neurons might not be the same as verapamil. The subtype of Ca^{2+} channel affected by Tet might be different from that by verapamil, or Tet might also have some effects on Na^{+} or K^{+} channels. Ca^{2-} entered the ischemic neurons through voltage-dependent Ca^{2+} channels, which reduced the concentration gradient existing between the inside and the outside of the cell membrane[11]. Whittongham *et al*[12] reported AHPA was increased and AHPD was prolonged in ischemic neurons, AHP could be used to reflect the change of $[Ca^{2+}]_i$. Ca^{2+} channel blocker relieved neuronal damage during ischemia through reducing Ca^{2+} influx. The effects of Tet on AHP were related to its Ca^{2+} channel blockade which was one of its anti-ischemia mechanisms.

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粉防己碱对蟾蜍背根神经节细胞动作电位及后超极化电位的影响

曾维忠、刘天培 R965.1
(南京医学院药理教研室，南京 210029，中国)

提要 利用微电极方法记录蟾蜍背根神经节细胞内动作电位。结果表明：Ca^{2+}依浓度地延长 APD 及 AHPD，增大 AHPA。维拉帕米 3 μmol · L^{-1} 缩短 APD_{100} 7%及 $AHPD_{50}$ 13%，减少 AHPA 17%。粉防己碱依浓度地缩短 APD_{100} 及 $AHPD_{50}$，降低 AHPA。当其浓度达 100 μmol · L^{-1} 时，APD_{100}、$AHPD_{50}$、AHPA 分别减少 16%、18%及 20%。提示粉防己碱的作用可能与其阻滞钙通道有关。

关键词 粉防己碱；钙通道；维拉帕米；动作电位；脊神经节 史振蜂

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Effects of exogenous γ-aminobutyric acid on experimental arrhythmias

WANG Lai-Yi, MENG Juan-Ru, WU Tao
(*Beijing Heart Lung and Blood Vessel Medical Center, Beijing 100029, China*)
LI Rong-Zhi, HE Yun-Qing, ZHANG Qi-Bo
(*College of Pharmacy, Beijing Medical University, Beijing 100083, China*)

ABSTRACT The effects of exogenous γ-aminobutyric acid (GABA) 10 mg · kg^{-1} iv in preventing arrhythmias induced by drugs and ischemia were studied in mice, rats, and guinea pigs. It was found that the threshold dose of aconitine inducing arrhythmia in mice and the recovery rate to normal sinus rhythm increased significantly, ED_{50} of GABA was 5.4 – 5.8 mg · kg^{-1}. The duration of ventricular tachycardia (VT) induced by aconitine in rats was shortened ($P<0.01$). The incidence and the mortality of ventricular fibrillation (VF) in GABA group were decreased to 0/10 *vs* 6/10 and 5/10 in control, respectively ($P<0.05$). The doses of ouabain to induce ectopic beats (EB), VT, VF, and cardiac

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