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298-301 (4)

放射配体结合分析法检测人肺动脉和胸主动脉β肾上腺素受体的亚型

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摘要 用β_1受体阻滞剂阿替洛尔和β_2受体激动剂沙丁胺醇与[^{125}I]pindolol对人肺动脉和主动脉膜上的β_1和β_2受体进行竞争性抑制反应，基于配基-高分子相互作用原理用LIGAND软件分析竞争结合数据. 结果表明肺动脉和胸主动脉上β_1和β_2受体是共存的，且以β_1受体占优势，其β_1：β_2的比率为2.9：1.0（肺动脉），2.1：1.0（胸主动脉）.

肾上腺素受体 测定

关键词 β肾上腺素能受体；肺动脉；胸主动脉；放射配体结合测定；阿替洛尔；沙丁胺醇；吲哚洛尔

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Jul; 14 (4): 301−305

Antifibrillatory effect of tetrahydroberberine

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ABSTRACT Electric stimulation and drug-induced ventricular fibrillation (VF), monopbasic action potentials (MAP), and triggered activity were studied before and after administration of tetrahydroberberine (THB) in rabbits, rats or guinea pigs. At doses of 5, 10, and 20 $mg \cdot kg^{-1}$, iv THB increased the ventricular fibrillation threshold, and the $BaCl_2$-induced VF was also prevented or terminated by THB in rabbits. Centrogenic VF induced by icv aconitine in rats was inhibited by pretreatment with THB in a dose-dependent manner, whereas VF induced by iv ouabain in guinea pig was inhibited to a lesser degree. For MAP, the duration at 90% repolarization ($MAPD_{90}$) was prolonged remarkably, whereas the $MAPD_{50}$, the MAP amplitude, and the maximal velocity of phase 0 were shortened or decreased slightly. The amplitudes of early afterdepolarization produced by cesium chloride (CsCl) were attenuated, while the cumulative threshold doses of CsCl for sustained ventricular tachycardia were elevated by THB.

These results indicated that THB had an potent antifibrillatory effect, which might be attributed to its

Received 1992-03-30 Accepted 1993-02-15

blockade of potassium, calcium, and sodium currents.

KEY WORDS tetrahydroberberine; ventricular fibrillation; action potentials; electrophysiology; barium; cesium; aconitine; ouabain

Tetrahydroberberine (THB) is an analog of tetrahydroprotoberberine. It possesses inhibitory effects on central nervous system. THB had a calcium antagonistic action similar to verapamil[1] and had protective effects on experimental myocardial infarction in rats[2]. THB also inhibited ventricular arrhythmia, especially ventricular fibrillation (VF). This work will focus on the effects of THB on VF.

MATERIALS AND METHODS

THB (Northeast Pharmaceutical Factory) was dissolved in H_2SO_4 0.1 mol·L^{-1}, then adjusted with NaOH 0.1 mol·L^{-1} to pH 4.3. The final concentration of THB was 1%.

Rabbits of either sex were anesthetized with iv a mixture of urethane (400 mg·kg^{-1}) and α-chlralose (17.5 mg·kg^{-1}. For recoding MAP and determination of ventricular fibrillation threshold (VFT), rabbits were immobilized with gallamine triethiodide under artificial respiration.

VFT was determined by electric stimulation[3]. The conscious rabbits restrained were iv barium chloride ($BaCl_2$, 5.5 mg·kg^{-1}) over 5—10 s to induce VF. THB (5, 10, and 20 mg·kg^{-1}, iv) served as pretreatment 2 min before iv $BaCl_2$ or as treatment 15 s after the onset of $BaCl_2$-induced VF.

Sprague-Dawley rats, ♂, weighing 238±*s* 20 g, were anesthetized with urethane (1.1 g·kg^{-1}, ip). On a stereotaxic apparatus, intracerebroventricular (icv) injection of aconitine (20 μg in 10 μl) was carried out after a period of stabilization[4]. Aconitine (E Merck) was dissolved in HCl 0.1 mol·L^{-1}, then diluted with artificial cerebrospinal fluid[5]. The final pH adjusted with NaOH 1 mol·L^{-1} was 6.5. Aconitine solution was refreshed daily.

Guinea pigs of either sex, weighing 510±*s* 34 g, were anesthetized with urethane (1.2 g·kg^{-1}, ip). The lead II of ECG was monitored. THB (5, 10, 20, 30 mg·kg^{-1}) or vehicle only were injected iv over 30 s as pretreatment. After 2 min, ouabain 200 μg·kg^{-1} was injected iv as a bolus to induce VF.

MAP was recorded with a silver contact electrode[1]. The amplitudes of MAP were adjusted to values between 20—30 mV by changing the position and pressure of electrode. To study the effects of THB on triggered activity, 24 rabbits were divided into 4 groups of iv pretreatments: (1) vehicle; (2) THB 5 mg·kg^{-1}; (3) THB 10 mg·kg^{-1}; (4) THB 20 mg·kg^{-1}. Cesium chloride (CsCl, 0.6 mmol·kg^{-1}) was injected iv as a bolus 2 min after the pretreatment. The MAP were recorded at 20, 30 s, 1, 3, 5, 10 min following the injection of CsCl, and the maximal amplitudes of EAD at each time were measured. Then the same dose of CsCl was similarly injected at 15 min intervals until sustained ventricular techycardia (VT) was induced[6]. To each rabbit, CsCl was injected for no more than 4 times, and the cumulative threshold dose was taken as 3.0 mmol·kg^{-1} if VT did not appear after injection of CsCl for 4 times.

Statistical tests used were *t* test and exact probabilities.

RESULTS

Electrically induced VF VFT was increased in a dose-dependent manner at 5—10 min after iv THB. At 25—30 min, the elevation of VFT was attenuated, but still statistically significant. Similar injection of vehicle only was ineffective (Tab 1).

Tab 1. Effects of iv tetrahydroberberine (THB) on ventricular fibrillation threshold in rabbits. $n=6-7$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* 0 min.

THB/ mg·kg^{-1}	Ventricular fibrillation threshold/V		
	0	5—10	25—30 min
Vehicle	8.3±2.1	8.1±2.2*	8.6±2.9*
5	9.1±1.7	10.4±1.5**	9.5±1.7*
10	8.2±1.3	11.4±1.7***	9.4±1.2**
20	8.6±1.5	14.2±2.1***	12.8±1.6***

$BaCl_2$-induced VF All of 10 rabbits in the control group developed VF after iv $BaCl_2$ (5.5 mg·kg^{-1}). After pretreatment with iv THB 5, 10 and 20 mg·kg^{-1}, the incidences of

VF after iv $BaCl_2$ were 5/10 ($P<0.05$), 2/10 ($P<0.01$), and 2/11 ($P<0.01$), respectively. THB quickly terminated the VF induced by $BaCl_2$, but did not completely suppress other ventricular arrhythmias.

Aconitine-induced VF In the control group, 9/10 rats developed VF after icv aconitine (20 μg in 10 μl). After pretreatment with ip THB 7.5, 15, 30, and 45 mg·kg^{-1}, the incidences of VF evoked by aconitine were 9/10 ($P>0.05$), 6/10 ($P>0.05$), 2/10 ($P<0.01$), and 1/10 ($P<0.01$), respectively. Thus, THB inhibited centrogenic VF in a dose-dependent manner.

Ouabain-induced VF All 10 guinea pigs developed VF about 5 min after iv ouabain (200 μg·kg^{-1}) in the control group. After pretreatment with iv THB 5, 10, 20, and 30 mg·kg^{-1}, the incidences of VF induced by ouabain were 8/10 ($P>0.05$), 5/10 ($P<0.05$), 4/10 ($P<0.05$), and 6/10 ($P>0.05$), respectively. Thus, THB inhibited the ouabain-induced VF only moderately.

MAP The duration of MAP at 90% repolarization ($MAPD_{90}$) was prolonged by THB, whereas $MAPP_{20}$ was shortened slightly or not prolonged in proportion to $MAPD_{90}$. The amplitude of MAP (MAPA) and maximal velocity of depolarization (V_{max}) was also decreased slightly about 10 min after iv THB. Verapamil (0.5 mg·kg^{-1}) shortened $MAPD_{20}$ and slowed the heart rate, but did not change other parameters (Tab 2).

Triggered activity induced by CsCl EAD developed quickly along with remarkable bradycardia after iv CsCl. The amplitude of EAD reached the maximum at about 30 s, then decreased progressively. In most cases, EAD were associated with ventricular ectopy. Pretreatment with THB depressed the amplitude of EAD and inhibited the ventricular arrhythmia (Tab 3). The cumulative dose of CsCl for sustained VT were also elevated from 1.4±0.3 in the control group to 2.3±0.5 and 2.5±0.5 mmol·L^{-1} in the groups pretreated with THB 10 and 20 mg·kg^{-1}, respectively ($P<0.05$), but pretreatment with iv THB 5 mg·kg^{-1} did not show significant effect ($P>0.05$).

DISCUSSION

THB increased electrically induced VFT significantly, and prevented or terminated the VF induced by iv $BaCl_2$ or icv aconitine, but inhibited the ouabain-induced VF to a lesser degree. These results indicated that THB had a potent antifibrillatory effect though it did not completely terminate other ventricular arrhythmias. This effect was similar to those of its analogs[7,8].

Similar to bretylium and amiodarone, THB prolonged the $MAPD_{90}$ *in vivo*, and the delay of repolarization occurred mainly at phase 3, which might reflect the suppression of potassium current. The drug that delayed repolarization might diminish the variation of effective refractory period (ERP), then exhibited antiarrhythmic action[9,10]. The $MAPD_{20}$, the amplitude of MAP and the maximal velocity of phase 0 were shortened or depressed slightly by THB, which indicated that it also suppressed the calcium and sodium currents. The electrophysiological changes caused by Ba^{2+} were mainly brought about by the increased sodium current of heart Purkinje fibers[11]. The inward sodium window current[12] and slow inward current carried by calcium[13] were essential to the occurrence of EAD and capable of triggering the arrhythmia. The results derived from $BaCl_2$ and CsCl further supported our deduction that THB could suppress calcium and sodium currents. Perhaps, the prolongation of action potential duration and suppression of these ion currents led to its antifibrillatory effect. Finally, it is

Tab 2. Effects of iv tetrahydroberberine (THB) and verapamil on monophasic action potentials (MAP) in rabbits. $n=7-9$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* 0 min. A=Vehicle; B=THB 5 mg·kg^{-1}; C=THB 10 mg·kg^{-1}; D=THB 20 mg·kg^{-1}; E=Verapamil 0.5 mg·kg^{-1}.

		0	1	5	10 min
MAPA/ mV	A	26.0±3.2	25.7±3.7*	25.4±3.6*	25.3±3.8*
	B	24.0±2.5	23.7±2.9*	22.9±3.0*	22.0±2.6**
	C	25.3±2.5	25.7±2.1*	24.5±2.6*	23.1±2.6***
	D	24.5±2.6	25.4±2.7*	23.8±2.4*	21.3±1.9***
	E	25.1±2.1	25.7±2.5*	26.0±2.5*	24.6±2.0*
V_{max}/ V·s^{-1}	A	2.34±0.60	2.30±0.62*	2.29±0.63*	2.28±0.65*
	B	2.46±0.36	2.47±0.42*	2.36±0.38*	2.30±0.37**
	C	2.44±0.41	2.51±0.40*	2.34±0.47*	2.22±0.45***
	D	2.70±0.42	2.77±0.35*	2.58±0.42**	2.35±0.38***
	E	2.45±0.48	2.55±0.43*	2.55±0.42*	2.41±0.47*
$MAPD_{90}$/ ms	A	127±6	128±7*	128±7*	127±7*
	B	128±6	133±7***	131±7*	127±7*
	C	129±9	138±9***	133±11**	132±9*
	D	130±8	141±8***	140±9***	136±7***
	E	127±9	124±9*	125±7*	125±7*
$MAPD_{50}$/ ms	A	64±6	64±7*	64±7*	64±8*
	B	64±4	63±5*	64±4*	64±4*
	C	62±5	59±5**	60±5*	62±4*
	D	61±5	58±5**	59±5**	61±5*
	E	60±5	49±6***	51±5***	54±4***
HR/ bpm	A	260±22	259±25*	261±21*	260±21*
	B	253±22	245±23***	251±23*	251±23*
	C	273±29	253±25***	259±28***	267±29***
	D	264±23	223±22***	235±23***	252±23***
	E	270±19	246±28***	255±26***	262±24**

Tab 3. Amplitude of early afterdepolarization (mV, induced by iv CsCl 0.6 mmol·kg^{-1}) after iv tetrahydroberberine 5--20 mg·kg^{-1} in rabbits. $n=6$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

THB/mg·kg^{-1}	0.5 min	1 min	3 min	5 min
Vehicle	6.5±2.0	4.8±1.5	2.9±1.9	1.1±1.1
5	5.3±1.2*	3.2±1.0*	1.7±0.9*	0.6±0.6*
10	4.1±1.6**	2.5±0.9**	1.0±0.9**	0.5±0.8*
20	3.8±1.9**	1.9±1.1***	0.8±1.0**	0.3±0.8*

necessary to point out that the changes of MAP *in vivo* derived from both the direct and indirect influences of THB on myocardium, and might differ from the results *in vitro*.

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四氢小檗碱的抗心室颤动作用

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摘要 四氢小檗碱(THB，5—20 mg·kg^{-1}) iv 显著提高兔室颤阈，预防或中止氯化钡所致室颤，ip 或 iv 抑制大鼠侧脑室注射乌头碱或豚鼠 iv 哇巴因所致室颤，兔心单相动作电位(MAP)时程可被 THB 延长，$MAPD_{20}$，MAPA 及 V_{max} 则被轻、中度缩短或降低. THB 还抑制氯化铯所致早后去极化及触发性心律失常. 提示 THB 有抗室颤作用，可能与其阻滞钾、钙、钠离子流有关.

关键词 四氢小檗碱；心室颤动；动作电位；电生理学；钡；铯；乌头碱；哇巴因

Instruction to Authors

Please read *Acta Pharmacol Sin* 1993 Jan; **14** (1) : 3—8.
Br Med J 1991 Feb 9; **302** (6772) : 388—41.
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