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四氢原小檗碱同类物对大鼠中脑腹侧被盖区 D_2 多巴胺受体的作用

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关键词 四氢原小檗碱类；中脑腹侧被盖区；多巴胺 D_2 受体；阿扑吗啡；Sch-23390；氟哌啶醇

目的：阐明四氢原小檗碱同类物(THPB)对大鼠中脑腹侧被盖区(VTA)多巴胺(DA)受体的作用特性，并比较它们的作用强度. 方法：采用大鼠在体胞外单位放电记录. 结果：观察了 11 个 THPB 均可完全地翻转 DA 受体激动剂阿扑吗啡(20 $\mu g \cdot kg^{-1}$)所产生的放电抑制作用，为 D_2 受体拮抗剂的作用特性. THPB 对 D_2 受体的作用与 C_2 位上的 OH 基团有密切的关系. 它们的作用强度(ED_{50}，$\mu g \cdot kg^{-1}$)：THPB-143 (5.6) > SPD (8.5) > Iso (17.0) > THP (33) > THB (48) > THPB-18 (66) > THPB-1 (179) > THPB-19 (408) > THPB-126 (510) > THPB-104 (1019) > THPB-10 (4815). 结论：11 个 THPB 均为 VTA D_2 受体拮抗剂，以 C_2 位上有 OH 基团的 THPB-143 作用最强.

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Effects of clonidine on myocardial β-adrenergic receptor-adenyl cyclase-cAMP system after scalds in rats[1]

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KEY WORDS clonidine; burns; beta-adrenergic receptors; adenyl cyclase; phosphoric diester hydrolases; cyclic AMP; myocardium; yohimbine; prazosin

AIM: To study the role of clonidine (Clo) on the myocardial β-adrenergic receptor (β-AR)-adenyl cyclase (AC)-cAMP system after the scalds in rats. **METHODS**: A 30 % skin-full-thickness scald was produced by immersing rats in 95 ℃ water for 9 s. Clo 0.1 - 3.0 $mg \cdot kg^{-1}$ was injected ip to rats at 30 min before scalds, yohimbine (Yoh) 0.05 $mg \cdot kg^{-1}$ or prazosin (Pra) 0.03 $mg \cdot kg^{-1}$ to rats at 30 min before ip Clo. β-AR density and affinity, AC activity, phosphoric diester hydrolases (PDH) activity, and cAMP content were determined with

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radioreceptor assay, indirect method, enzyme-radiochemical assay, and radioimmunoassay, respectively. **RESULTS**: Clo inhibited the decrease of the myocardial β-AR density, the attenuation of AC activity, and the reduction of cAMP content at 12 h after the scalds. Yoh partially reversed the effects of Clo on the three parameters. But Pra did not. **CONCLUSION**: Clo reversed the changes of the myocardial β-AR-AC-cAMP system resulted from the scalds in rats.

Thermal injury to man and animals evokes myocardial dysfunction[1,2], not only in the acute resuscitation phase but also in the hypermetabolic phase of thermal injury[3,4]. Clonidine (Clo) alleviated the declines of myocardial function after the scalds[5]. The myocardial dysfunction after the scalds in rats was related to the depression of the β-adrenergic receptor (β-AR)-adenyl cyclase (AC)-cAMP system[6].

This study was to observe the effects of Clo on the myocardial β-AR-AC-cAMP system after the scalds in rats and to inquire into their possible mechanisms.

MATERIALS AND METHODS

Drugs and reagents Clo (powder, >98 % purity) was from Guilin Pharmaceutical Factory, Guangxi, lot No 911101. Propranolol, prazosin (Pra) and yohimbine (Yoh) were purchased from Sigma Co. All drugs were dissolved in saline. [^{3}H] Dihydroalprenolol (DHA, 1.7 TBq·mol^{-1}) was labelled by the Institute of Atomic Energy, Chinese Academy of Sciences. The kit of cAMP radioimmunoassay was produced by Department of Experimental Nuclear Medicine, Shanghai Second Medical University. The kit of phosphoric diester hydrolases (PDH)-radiochemical assay was from Department of Pharmacology, Institute of Basic Medical Sciences, Chinese Academy of Medical Science. Creatinine phosphate, creatinine phosphokinase, ATP, isoprenaline (Iso), NaF, forskolin were purchased from Sigma Co. Isobutylmethylxanthine was from the Boehringer Mannhein Co. QAE-Sephadex A-25 was from Pharmacia Co. All other reagents were AR.

Rats Wistar ♀ rats (n=81, weighing 202 ± s 18 g), obtained from the Laboratory Animal Center of our College, were divided into: A) the normal control, B) Clo control, C) scalded control, D) Clo, E) Pra + Clo, and F) Yoh + Clo groups (9 rats in each group).

Scalds Under ether anesthesia, a dorsal area corresponding to 30 % body surface area of rats in Groups C, D, E, F were depilated with Na_2S 1 mol·L^{-1}. After 24 h, this predefined area was immersed in 95 ℃ water for 9 s under anesthesia to produce skin-full-thickness scalds. The depth of scalds was confirmed histologically. The rats in Groups A, B were immersed in 25 ℃ water for 9 s. All rats were injected ip saline (2.5 mg·kg^{-1}) and kept in separate cages with water and food *ad lib*.

Medications Clo (0.1, 0.3, 1.0, and 3.0 mg·kg^{-1}) was injected ip to the rats in Group D at 30 min before scalds. Pra 0.03 mg·kg^{-1} or Yoh 0.05 mg·kg^{-1} was injected ip to the rats in Groups E, F at 30 min before ip Clo 1.0 mg·kg^{-1}, respectively. The rats in the 3 control groups were injected Clo or saline 1.0 mg·kg^{-1}, respectively. At 12 h after scalds, rats were anesthetized with ether and the hearts were stored in liquid nitrogen.

Methods Myocardial membranes were prepared from the hearts[7]. Radioligand binding assay of β-AR was conducted using [^{3}H] DHA[6]. AC activity was determined with indirect method[7] in the presence of stimulants, Iso 100 μmol·L^{-1}, NaF 20 μmol·L^{-1} and forskolin 10 μmol·L^{-1} or vehicle. Extraction and determination of cAMP were performed with radioimmunoassay[8]. PDH prepared from the frozen ventricular muscle was assayed with enzyme-radiochemical assay[9].

Statistic analysis Data were treated using the t test.

RESULTS

At 12 h after scalds, the myocardial β-AR-density was decreased, AC basal activity and cAMP content were reduced, but β-AR-affinity and PDH activity were around the normal control levels. Clo 0.1, 0.3, 1.0, and 3.0 mg·kg^{-1} enhanced β-AR-density by 1.03-, 1.26-, 1.47-, and 1.53-fold, intensified AC basal activity by 1.50-, 1.77-, 2.09-, and 2.18-fold, and increased cAMP content by 1.07-, 1.22-, 1.37-, and 1.45-fold, respectively. The effects of Clo on β-AR-density, AC basal activity and cAMP content were related to one another (r = 0.997 for β-AR-density and AC basal activity, r = 0.994 for AC basal activity and cAMP content, r = 0.999 for β-AR-density and cAMP content, P<0.01 for all). However, Clo showed no effects on β-AR-affinity and PDH activity in the scalded group and on all the parameters in the normal control group (Tab 1).

Iso, NaF, and forskolin stimulated *in vitro* the myocardial AC activity in the scalded rats. Clo 1.0 mg·kg^{-1} ip before scalds enhanced Isostimulated

Tab 1. Effects of Clo on myocardial β-AR-density (B_{max}) and -affinity (K_D), AC basal activity (AC-BA), PDH activity (PDH-A), and cAMP content (cAMP-C) at 12 h after scalds in rats. $n=9$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* normal control; $^eP<0.05$, $^fP<0.01$ *vs* scalded injury.

Groups /mg·kg^{-1}	β-AR-B_{max} /pmol·g^{-1}	β-AR-K_D /nmol·L^{-1}	AC-BA /pmol·g^{-1}·s^{-1}	PDH-A /pmol·g^{-1}·s^{-1}	cAMP-C /pmol·g^{-1}
Normal control	69±24	2.45±0.32	52±23	47±7	834±98
Clo control	67±18	2.20±0.26	46±16	46±8	847±103
Scalded injury	34±8^c	2.36±0.33	22±8^c	60±7	516±76^c
Clo 0.1	35±8^c	2.19±0.30	33±14^{be}	50±10	554±76^c
0.3	43±10^{be}	2.25±0.27	39±15^{be}	58±13	628±82^{be}
1.0	50±11^{be}	2.41±0.37	46±15^f	61±13	705±73^{bf}
3.0	52±12^{be}	2.38±0.34	48±16^f	61±15	747±85^{bf}

AC activity, but exerted no influence on NaF- and forskolin-stimulated AC activity (Tab 2).

Tab 2. Effects of Clo on Iso-, NaF-, and forskolin (For)-stimulated AC activity at 12 h after scalds in rats. $n=9$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* basal activity; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* Clo "0".

	Clo /mg·kg^{-1} 0	1.0
	AC activity/pmol·g^{-1}·s^{-1}	
Basal	22±8	46±15^f
Stimulated by		
Iso 100 μmol·L^{-1}	43±20^c	58±21^{be}
NaF 20 μmol·L^{-1}	47±21^c	50±23^d
For 10 μmol·L^{-1}	70±31^c	77±34^{bd}

Yoh 0.05 mg·kg^{-1} ip before Clo antagonized the effects of Clo on the myocardial β-AR-density, AC basal activity and cAMP content in scalded rats. However, Pra 0.03 mg·kg^{-1} ip exhibited no effect on the actions of Clo (Tab 3).

DISCUSSION

Clo intensified the myocardial AC activity and increased cAMP content in scalded rats, but showed little effect on PDH activity. These results indicate that the effect of Clo on the myocardial cAMP content in scalded rats was mainly related to its capacity to intensify AC activity.

Clo enhanced the myocardial β-AR density in scalded rats, and intensified the AC activity stimulated with Iso, an agonist to β-AR, but showed no effect on the activity stimulated with NaF, an excitant to G-protein or forskolin, an activator to AC catelytic subunit. It is inferred from these results that the intensification of the myocardial AC activity in scalded rats by Clo can be associated with its action on the β-AR density rather than on G-protein or AC catelytic subunit. However, the fact that the increasement of Iso-stimulated AC activity in Clo-treated group was lower than that in scalded control group contradicted the inference above. The intensification of AC activity by Clo may accelerate the generation of cAMP, which improved the cardiac function in scalded rats.

Yoh, a central α_2-AR antagonist, partially reversed the effects of Clo on myocardial β-AR density, AC activity, and the cAMP content in scalded rats, but Pra, a central α_1-AR antagonist

Tab 3. Effects of Pra (0.03 mg·kg^{-1}) and Yoh (0.05 mg·kg^{-1}) on the action of Clo (1.0 mg·kg^{-1}) on myocardial β-AR-B_{max}, AC basal activity (AC-BA), and cAMP content (cAMP-C) at 12 h after scalds in rats. $n=9$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* scalded control; $^eP<0.05$ *vs* Clo-treated.

	Scalded control	Clo	Pra + Clo	Yoh + Clo
β-AR-B_{max}/pmol·g^{-1}	34±8	50±11^b	52±14	37±10^e
AC-BA/pmol·g^{-1}·s^{-1}	22±8	46±15^c	45±15	33±12^e
cAMP-C/pmol·g^{-1}	516±76	705±73^c	639±84	540±78^e

showed no influences on these effects of Clo. It was suggested that the effects of Clo on the myocardial β-AR density partially involve its excitation on the central α_2-AR.

Clo showed no effects on the all parameters in the normal rats, it supported that the mechanism of Clo enhancing β-AR density might involve its suppression of the superexcitation of the peripherial sympathetic adrenomedullary system resulted from the severe superficial scalds[10] through exciting the central α_2-AR.

To sum up, Clo enhanced the myocardial β-AR density, intensified AC activity, and increased cAMP content in scalded rats. Considered together with our previous studies[5,6], the effect of Clo on the β-AR-AC-cAMP system might be one of important mechanisms of its alleviation of the declines of cardiac function after the severe superficial scalds.

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可乐定对大鼠烫伤后心脏 β-肾上腺素受体-腺苷酸环化酶-环腺苷一磷酸系统的作用[1]

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关键词 可乐定; 烧伤; β-肾上腺素受体; 腺苷酸环化酶; 磷酸二酯水解酶类; 环腺苷一磷酸; 心肌; 育亨宾; 哌唑嗪

目的: 研究可乐定(Clo)对大鼠烫伤后心脏 β-肾上腺素受体(β-AR)-腺苷酸环化酶(AC)-环腺苷一磷酸(cAMP)系统的作用. **方法**: 大鼠于 95 ℃水浴中烫 9 s, 造成背部 30 %皮肤全层烫伤. 用放射受体分析、间接方法、酶放射化学分析、放射免疫分析分别测定 β-AR 密度和亲和力, AC 和磷酸二酯酶(PDH)活性及 cAMP 生成量. **结果**: Clo (0.3、1.0 和 3.0 mg·kg^{-1})增加烫伤后 12 h 心肌 β-AR 密度、AC 活性及 cAMP 生成量($P<0.05$ 或 0.01); 但 Clo 对烫伤大鼠心肌 β-AR 亲和力、PDH 活性及正常大鼠上述各项指标均无影响($P>0.05$). 育亨宾 0.05 mg·kg^{-1}, 部分逆转 Clo 对 β-AR 密度、AC 活性和 cAMP 生成的作用; 哌唑嗪 0.03 mg·kg^{-1}, 对 Clo 的作用无明显影响. **结论**: Clo 抑制烫伤后大鼠心肌 β-AR-AC-cAMP 系统的变化.