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家兔血小板对离体豚鼠作功心脏的增强效应及组胺的参与

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摘要 用洗涤家兔血小板悬液和其上清灌注离体豚鼠作功心脏，心脏作功浓度依赖性增强。西咪替丁(Cim) 1 μmol·L^{-1}显著抑制增强相。经HPLC测出血小板悬液每10^9血小板含组胺2.6±0.7 μg。电镜显示受血小板攻击的心脏肥大细胞处于活动期。结果表明，心功能加强与血小板及心脏肥大细胞释放的组胺有关。

关键词 组胺；西咪替丁；血小板；心脏；血液动力学；肥大细胞；电镜

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Chlorpromazine attenuated electroacupuncture analgesia in conscious rabbits[1]

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ABSTRACT By measuring the defense behavior in response to the noxious stimulation induced by potassium iontophoresis on ear-lobe skin of concious rabbit, chlorpromazine (CPZ) (0.5 mg·kg^{-1}, iv) induced hyperalgesia, whereas it significantly attenuated electroacupuncture analgesia (EAA) efficacy. Monoamines and their metabolites in cerebrospinal fluid (CSF) were measured by high pressure liquid chromatography with electrochemical detector (HPLC-ECD) while the attenuation effect of CPZ on EAA was observed. CPZ markedly enhanced 3,4-dihydroxyphenylacetic acid (DOPAC) ($P<0.05$) and homovanillic acid (HVA) ($P<0.01$) contents in CSF both in the presence and absence of electroacupuncture. CPZ attenuated EAA with elevations of either DOPAC or HVA concentration in CSF. There was a positive cor-

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relation between the increase of DOPAC or HVA content in CSF and the attenuation effect of CPZ on EAA ($P<0.05$). These results suggested that the activation of dopamine system was unfavorable for EAA.

KEY WORDS electroacupuncture; analgesia; chlorpromazine; dopamine receptors; 3, 4-dihydroxyphenylacetic acid; homovanillic acid; cerebrospinal fluid; biogenic monoamines; high pressure liquid chromatography

Monoamines[1-3], endogenous opioid peptides (EOP)[1,4,5], and acetylcholine played important roles in electroacupuncture analgesia (EAA), which altered the levels of various neurotransmitters in the central nervous system (CNS). In previous experiments, involvement of dopaminergic mechanisms in EAA has been demonstrated[2,3], and dopamine (DA) receptor antagonists were consequently categorized as EAA synergists[3,6]. Chlorpromazine (CPZ), a neuroleptic mainly due to its anti-dopaminergic action, weakened EAA in rabbits[7]. CPZ possesses a broad spectrum of pharmacological actions, especially in modulating the actions of monoamine transmitters in CNS, ie, anti-DA, anti-serotonin (5-HT), anti-norepinepherine (NE), etc[8]. To probe into the intrinsic mechanism underlying the attenuation effect of CPZ on EAA, we observed the effect of CPZ on EAA, and simultaneously determined the content changes of monoamines and their metabolites in cerebrospinal fluid (CSF) of rabbits after CPZ and/or electroacupuncture (EA).

MATERIALS AND METHODS

CPZ was purchased from Shanghai Tianfeng Pharmaceutical Factory. NE, 3-metboxy-4-hydroxyphenylglycol (MHPG), DA, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic (5-HIAA) were the products from Sigma Co.

New Zealand rabbits (bred by Shanghai Medical University) of either sex, weighing $2.1 \pm s$ 0.2 kg, were used.

Rabbit skin pain model The potassium iontophoresis method was used to measure the pain threshold in conscious rabbits. K^+ were brought into the ear-lobe skin by continuously increasing direct current as noxious stimulus, and withdrawal reactions of forelimbs and/or head were served as a criterion of pain response, while the current value (mA) was recorded as pain threshold. Before administration of drug and/or EA, the pain threshold of each rabbit was measured 3 times at 5-min intervals, and the $\bar{x}$ was used as the baseline. Rabbits with pain thresholds $>$ 1 mA were discarded. Pain thresholds were tested at 10—20-min intervals during 100 min following administration.

Electroacupuncture EA was carried out at "Hegu" point (midpoint of medial edge of 2nd metacarpal of forepaw) and "Waiguan" point (dorsum of forelimb, 12 mm above wrist joint along the medium line between radius and ulna). Unilateral points were electrically needled for 30 min by EA apparatus (Model G-6805-2), at a frequency of 3 Hz with an intensity capable of inducing slight tremble of forelimb. Sham needling (SN) without electric stimulation was used as control.

Cannula implantation and CSF collection Rabbits were anesthetized with sodium pentobarbital (30 $mg \cdot kg^{-1}$, iv). A plastic cannula (OD=0.9 mm, ID =0.6 mm) was implanted into the 4th ventricle (coordinates P 13, L/R 0.5, H 0, according to Sawyer's Atlas). The cannula was then clogged up and secured by dental cement. Rabbits were individually caged until they were tested 3 d later. At the beginning and 30th min following administration, pain thresholds were recorded while CSF (30 μl) was collected within 30 s. The CSF were collected in tubes containing 10 μl HCl (0.1 $mol \cdot L^{-1}$), frozen with dry ice, and stored at −30℃ until assay.

High pressure liquid chromatography coupled to electrochemical detector (HPLC-ECD) Monoamines and their metabolites were detected by ion-pair reverse phase HPLC-ECD. The HPLC-ECD system (Waters) consisted of a M510 pump, a U6K injection system coupled to a M460 electrochemical detector and M740 data module. The separation was accomplished on a μ-Bondapak C_{18} column (300×3.9 mm ID) with a pre-

column (Guard Pak TMRCSS C_{18}). The eluent consisted of chloro-acetic acid-sodium hydroxide buffer 0.15 mol·L^{-1} containing EDTA 0.83 mmol·L^{-1}, *D*-camphor-β-sulfonic acid 9 mmol·L^{-1}, and 5% methanol (pH=4.2), at a flow rate of 1.5 ml·min^{-1}. The eluent was filtered through a 0.22 μm membrane and degassed before use. The working potential was +0.7 V. Samples of CSF were injected directly.

Protocol Rabbits were randomly divided into 4 groups: (1) NS+SN group; (2) CPZ+SN group; (3) EA+NS group; (4) EA+CPZ group. Results were expressed as $\bar{x}\pm s$, and statistical significance between groups was evaluated by *t* test.

RESULTS

Effect of CPZ on pain threshold and EAA Effect of CPZ on baseline pain threshold as well as EAA was examined on 32 rabbits. The rabbits elicited hypersensitivity to pain after CPZ (0.5 mg·kg^{-1}, iv) treatment. Compared with NS+SN control group, the pain threshold was significantly lowered. Given concurrently with EA, such a dose of CPZ weakened the EAA efficiency (Tab 1). The pain threshold change of EA+CPZ group was less than that of the sum of CPZ group and EA group, ie, CPZ markedly antagonized EAA. The attenuation action maintained for 80 min, the pain threshold remained little enhanced or even lower than the preadministration level during 100 min following administration.

Influence of CPZ and/or EA on monoamine levels in CSF NE, DA, MHPG, DOPAC, HVA, and 5-HIAA, but not 5-HT were detectable in CSF of rabbits, and the baseline monoamine contents of metabolites (50 — 300 ng·ml^{-1}) were higher than their prototypes (0 — 10 ng·ml^{-1}), owing to the rapid metabolic rate in the CNS. The NE and MHPG contents remained unchanged after CPZ and/or EA treatment. There was no significant change of DA content in all the 4 groups. But levels of DOPAC and HVA, DA metabolites, were significantly increased after CPZ injection. EA induced no changes of DOPAC or HVA contents. When EA was applied in combination with CPZ, DOPAC, and HVA levels were augmented. EA brought forth a marked elevation of 5-HIAA level. When CPZ was administrated together with EA, the 5-HIAA content was decreased, but no statistical significance was shown (Tab 2).

Correlation between DOPAC or HVA levels and EAA effect CPZ attenuated the EAA effect with elevation of either DOPAC or HVA level, showing a positive correlation (Fig 1). This suggested that increase of DA metabolites in CSF was unfavorable for EAA.

Tab 1. Effect of CPZ (0.5 mg·kg^{-1}, iv) on pain threshold (mA) and EAA in conscious rabbits. $n=8$, $\bar{x}\pm s$. **Statistical significance between changes of pain thresholds was examined by *t* test.** $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ **vs NS+SN;** $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ **vs NS+EA.**

Time/min	NS+SN	CPZ+SN	NS+EA	CPZ+EA
0	0.70±0.21	0.73±0.12^a	0.67±0.24^d	0.67±0.17^d
10	0.72±0.12	0.57±0.23^b	1.25±0.40^e	0.87±0.40^e
20	0.69±0.15	0.45±0.20^c	1.61±0.54^e	0.93±0.34^f
30	0.70±0.16	0.42±0.31^c	1.36±0.49^e	0.87±0.48^e
40	0.67±0.20	0.33±0.35^c	1.17±0.39^e	0.67±0.50^e
60	0.68±0.16	0.35±0.31^c	0.98±0.30^e	0.53±0.45^f
80	0.73±0.25	0.39±0.31^b	0.82±0.20^e	0.61±0.26^e
100	0.69±0.20	0.35±0.30^c	0.73±0.32^d	0.53±0.25^d

Tab 2. ***Content changes of monoamines and their metabolites in CSF of conscious rabbits after CPZ and/or EA administration.*** $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS+SN; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* NS+EA.

	Content increases of monoamines and their metabolites/ng·ml^{-1}			
(n)	NS+SN (8)	CPZ+SN (7)	NS+EA (8)	CPZ+EA (7)
NE	2.4±6.7	1.4±6.8^a	1.8±8.0^d	2.7±8.6^d
MHPG	1.3±8.8	0.8±9.8^a	5.8±11^e	6.5±11^d
DA	0.4±2.3	1.9±5.9^a	−0.6±2.3^d	−0.4±4.0^d
DOPAC	31±37	140±110^b	30±28^e	100±76^f
HVA	−30±78	180±180^c	−30±170^e	220±140^f
5-HIAA	−5.0±14	4.5±12^a	28±28^b	6.0±18^d

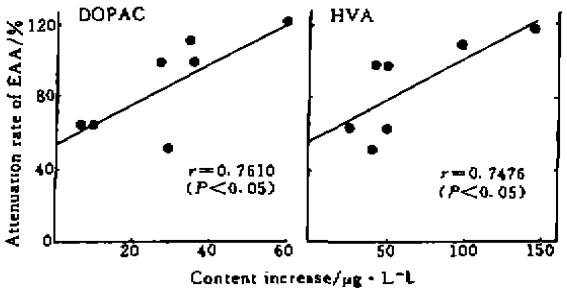

Fig 1. Correlation analysis between content increases of DOPAC and HVA in CSF of rabbits and attenuation rate of CPZ on EAA.

DISCUSSION

The hypothesis of the existence of two categories of DA receptors, D_1 and D_2, has been generally accepted[9]. It has been established that D_2, but not D_1 receptor, modulated the DA release and metabolism[10], whereas DA presynaptic autoreceptors were in good accordance with D_2 type[9]. The antagonists for D_2 receptor potentially stimulated DA release, especially in lower dosage[11]. CPZ, a potent DA receptor antagonist for both D_1 and D_2, had a preferential affinity for D_2 subtype. The present results showed a marked content elevation of DOPAC and HVA after CPZ (0.5 mg·kg^{-1}, iv) both in the presence and absence of EA. CPZ, used in low dosage in our study, blocked the presynaptic DA autoreceptors, activated the DA system, and thus provoked a content elevation of DA metabolites.

Studies concerning the influence of DA system on EAA has been intensively conducted. It was found that central dopaminergic activation was an unfavorable factor on EAA. Previous experiments indicated that DA receptor blockers consisted of a large family of EAA synergists, both from animal observations[3,6] and in clinical therapy[7]. In addition, it has been revealed that the anti-dopaminergic action on CNS contributed to the potential effects of these drugs on EAA[3,12]. Our data analysis also revealed a positive correlation between the elevation of DOPAC or HVA content in CSF and the attenuation rate of CPZ on EAA. These studies suggested that CPZ attenuated EAA, probably via blocking the central DA autoreceptors, and thus activating the DA system. Furthermore, EAA was a complicated process involving the integration of EOP, biogenic monoamines, and other transmitter systems in CNS. Opiates modulated the synthesis, release and metabolism of biogenic amines[13], and one-third of opioid receptors in striatum were present on the dopaminergic nerve terminals[14], which strongly suggested the bilateral regulatory actions of DA and EOP system. Whether the attenuation effect of CPZ on EAA was EOP-mediated still remained unclear in this study.

There have been sound evidences showing that increases in the activity of brain and spinal cord 5-HT neurons are associated with analgesia[15] as well as EAA[1]. CPZ is considered to be a moderate 5-HT receptor blocker[9]. Consistant with previous experiments, our results revealed an increase of 5-HIAA content in CSF after EA application. We also found that 5-HIAA was lowered when CPZ was used in combination with EA, which confirmed the attenuation action of CPZ on EAA.

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氯丙嗪在清醒家兔上使电针镇痛减效

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摘要 用钾离子透入法测定兔耳尖皮肤痛阈值，观察氯丙嗪(CPZ)对痛阈及电针镇痛(EAA)的影响. CPZ (0.5 mg·kg^{-1}, iv)不仅降低痛阈值且减弱 EAA. 高压液相-电化学检测法测定发现 NE, MHPG, DA 含量在给予 CPZ 和或电针后均无明显改变. 而在 CPZ＋伴针和＋电针组中, DOPAC 和 HVA 含量均明显升高, 且升高值与 EAA 减效率呈正相关, 提示激活 DA 系统不利于 EAA.

关键词 电针; 镇痛; 氯丙嗪; 多巴胺受体; 3,4-二羟基苯乙酸; 高香草酸; 脑脊液; 生物单胺类; 高压液相色谱法