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Preproopiomelanocortin and preprodynorphin mRNA expressions in rat brain after electroacupuncture + droperidol[1]

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KEY WORDS endorphins; dynorphins; electroacupuncture; *in situ* hybridization; central nervous system; messenger RNA

AIM: To study the expression of preproopiomelanocortin (POMC) and preprodynorphin (PPD) mRNA following the combination of electroacupuncture (EA) with droperidol (Dro), a dopamine receptor antagonist. **METHODS:** The brains and spinal cords of Sprague-Dawley rats were sectioned after combination of EA with Dro, and the gene expression was investigated using nonradioactive *in situ* hybridization histochemistry (ISHH). **RESULTS:** Ten hours after EA, the POMC mRNA expression was enhanced; the expression was further enhanced when EA was combined with Dro. The expression of PPD mRNA showed regional difference in central nervous system (CNS): in spinal cord, EA enhanced the PPD mRNA expression and the combination of EA with Dro further promoted the expression; in the brain, the PPD mRNA expression after EA or combination of EA with Dro showed no obvious change in most regions (caudate-putamen, accumbens, arcuate nucleus of hypothalamus) or was decreased in supraoptic nucleus. **CONCLUSION:** Dro combined with EA promoted the expression of POMC mRNA in CNS and PPD mRNA in spinal cord, but reduced or had no effect on PPD mRNA expression in the brain.

Endogenous opioid peptides (EOP) including enkephalin, β-endorphin, and dynorphin play an important role in acupuncture analgesia[1]. Electroacupuncture (EA) induced expression of enkephalin precursor genes in central nervous system (CNS)[2,3]. Droperidol (Dro), a dopamine (DA) antagonist, potentiated EA analgesia and the preproenkephalin mRNA expression in CNS was further enhanced[4], and the release of β-endorphin in rat brain was accelerated[5]. Yet, the gene activity of endorphin and dynorphin following EA combined with Dro remains unclear. The present study was to observe the expression of preproopiomelanocortin (POMC) mRNA and preprodynorphin (PPD) mRNA following EA combined with Dro, so as to investigate the mechanism of potentiating effect of Dro on EA.

MATERIALS AND METHODS

Tissue preparation Sprague-Dawley rats (♂, $n = 14$, 170 - 230 g) were divided into 4 groups: A) normal saline (NS, 2 mL, ip, $n = 3$), B) NS + EA ($n = 3$), C) Dro (1.25 mg·kg^{-1}, ip, $n = 4$), and D) Dro + EA ($n = 4$). The treatment of rats conformed to the guidelines of International Association for Study of Pain[6]. EA was applied at right "Zu-San-Li" (St 36, between anterior tibial muscle and long digital extensor muscle) and "Kun-Lun" (UB60, between the tip of the external malleolus and calcaneal tendon) points with Model G6805 EA apparatus (Shanghai). The needles were inserted 5 mm. Dense (60 Hz)-sparse (5 Hz) frequency of wave was selected and the intensity of EA was adjusted to provoke a slight contraction of hindlimb. EA was applied at the 10th min after medication and kept on for 20 min. Ten hours after EA, the rats were anesthetized with sodium pentobarbital (40 mg·kg^{-1}, ip) and perfused with 4 % paraformaldehyde. Coronal brain sections of 50 μm were transferred to cryoprotectant solution[7].

***In situ* hybridization histochemistry (ISHH)** Digoxigenin (Dig)-labeled RNA probes were synthesized by *in vitro* transcription of POMC and PPD complementary DNA (cDNA) contained in plasmid vectors (POMC plasmid was a gift from Dr ZHU Xing-Zu, Shanghai Institute of Materia Medica, Chinese Academy of Sciences and PPD plasmid was a gift from Dr John HONG, National Institute of Environmental Health Sciences, Research Triangle Park NC, USA). The RNA probes were labeled using Dig RNA labelling kit (Boehringer Mannheim Biochemica, Germany). The PPE and PPD cDNA were linearized with SacI enzyme as template for the transcription.

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ISHH was done with a free floating method[8]. Briefly, the brain sections were hybridized with Dig-labeled POMC and PPD probes (0.5 mg·L^{-1}) in hybridization buffer at 37 ℃ for 16－24 h. The RNase (20 mg·kg^{-1}) was added to digest unhybridized probes, followed by incubating with antibody (anti-Dig alkaline phosphatase conjugate, 1∶500) The signals of POMC and PPD mRNA in neurons were detected with color reaction by Dig nucleic acid detection kit (Boehringer Mannheim, Germany).

Quantitation of signals was achieved by measuring the gray level of POMC or PPD mRNA in neurons using a computer-assisted image processing and analysis system (FG-100AT, Imaging Technology Inc, and TV-camera, RCA Inc, USA). Brain structures were identified[9]. Data were analyzed with t test.

RESULTS

POMC mRNA The POMC mRNA expressed mainly in arcuate nucleus of hypothalamus and periarcuate region as well as the spinal cord. Periaquductal gray (PAG) showed no obvious positive signals. In rats treated with NS, the expression of POMC mRNA was at low level.

Compared with NS-treated rats, the POMC mRNA expression showed no obvious change in Dro-treated rats, but an increase in EA-treated rats. When EA was combined with Dro, the POMC mRNA expression was further elevated (Tab 1, Fig 1, Plate 1).

PPD mRNA PPD mRNA positive neurons were more extensively distributed than POMC mRNA in cerebral cortex, caudate nucleus, accumbens, hypothalamic arcuate nucleus, PAG, supraoptic nucleus of hypothalamus, and spinal cord. Following EA or EA + Dro, only supraoptic nucleus of hypothalamus and spinal cord were found to have alterations in PPD mRNA expression: in supraoptic nucleus, EA caused decrease of PPD mRNA expression and EA + Dro led to a further decrease; in spinal cord, EA induced an increased expression of PPD mRNA and EA + Dro resulted in a further elevation. In PAG, the PPD mRNA expression showed a trend of decrease ($P>0.05$) after EA or EA + Dro. In cerebral cortex, caudate nucleus, accumbens and hypothalamic arcuate nucleus, the PPD mRNA expression following EA or EA + Dro showed no obvious changes (Tab 1, Fig 1).

Tab 1. Effect of combination of Dro with EA on POMC and PPD mRNA expression in rat brains (intensity of positive signals, indicated in gray level). $\bar{x} \pm s$. **[c]$P<0.01$ *vs* NS; [e]$P<0.05$, [f]$P<0.01$ *vs* NS + EA; [i]$P<0.01$ *vs* droperidol (Dro).**

Brain regions	NS group ($n=3$)	NS + EA group ($n=3$)	Dro group ($n=4$)	Dro + EA group ($n=4$)
POMC mRNA				
Arcuate N	56.7±3.8	66.8±4.3[e]	60.7±3.5	79.8±2.2[fi]
Layer I	23.3±4.6	31.4±2.8[e]	28.3±3.1	42.5±2.3[fi]
Layer II－IV	29.1±4.0	35.3±2.3	28.4±3.0	50.6±3.5[fi]
PPD mRNA				
Cerebral cortex	41.3±6.5	43.5±7.6	35.8±8.8	42.4±4.2
Caudate N	37.0±9.5	30.0±3.4	29.0±5.4	30.3±2.5
Accumbens	31.0±4.4	29.3±2.6	31.3±2.2	30.0±2.8
Arcuate N	32.0±4.7	33.7±3.1	36.0±2.7	39.0±6.0
Supraoptic N	54.8±5.6	44.0±2.2[b]	51.0±3.7	39.7±3.8[ei]
PAG	45.7±9.6	36.3±5.9	39.3±5.0	28.7±7.2
Layer I	53.6±4.0	63.3±2.8[e]	46.2±4.5	76.2±2.6[fi]
Layer II－IV	45.2±4.3	68.8±3.3[e]	44.6±5.3	80.8±5.1[fi]

DISCUSSION

POMC mRNA in brain encodes a kind of peptides known as endorphins, especially β-endorphin (β-END), which has potent analgesic effect. Previous work demonstrated that EA induced release of β-END in the brain[10]; our recent studies showed that when EA analgesia was potentiated by droperidol (Dro), an antagonist of dopamine, the EA-induced release of β-END was enhanced[5]. Present result that POMC mRNA expression was promoted by combination of Dro with EA further demonstrated that the activation of endorphin gene participated in Dro-potentiated EA analgesia. The increased expression of POMC mRNA may be compensative to the reduction of β-END caused by increased release, which is favourable to the effect of Dro on EA.

The PPD mRNA expression is more complex in CNS. In spinal cord, the enhanced PPD mRNA expression is consistent with the increased release of dynorphin after EA[11], which enhances EA analgesia. In the brain, the PPD mRNA expression after Dro + EA is different from that in spinal cord, indicating that the dynorphin gene expression in the brain may play a distinct role from spinal cord in EA analgesia. However, the PPD

mRNA expression alone cannot demonstrate clearly what role the dynorphin plays in the brain.

Although there were differences in POMC and PPD mRNA expression in CNS, the results in present study suggest that the genes of endorphins and dynorphins, together with enkephalins[4], express in cooperative manner in CNS, which underlies the central molecular basis of Dro-potentiated EA analgesia.

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氟哌利多与电针合用后大鼠脑内前阿黑皮原和前强啡肽原 mRNA 的表达

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关键词 内啡肽类；强啡肽类；电针；原位杂交；中枢神经系统；信使核糖核酸

目的：观察氟哌利多(Dro，多巴胺拮抗剂)与电针(EA)合用后前阿黑皮原(POMC)和前强啡肽原(PPD) mRNA 的表达. **方法**：大鼠经处理后取脑与脊髓切片，采用原位杂交技术观察. **结果**：EA 后 10 h，POMC *mRNA* 表达增强，Dro + EA 后，POMC mRNA 表达进一步增强. PPD mRNA 的表达在脊髓被 EA 增强，Dro + EA 进一步促进其表达；在脑内，EA 或 Dro + EA 后 PPD mRNA 的表达在尾壳核，伏核，下丘脑等脑区无明显变化，在视上核则减弱. **结论**：氟哌利多与电针合用促进了中枢 POMC mRNA 和脊髓 PPD mRNA 的表达，对脑内 PPD mRNA 的表达则有减弱作用(视上核)或无明显影响.