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Preproenkephalin mRNA enhanced by combination of droperidol with electroacupuncture[1]

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AIM: To study the expression of preproenkephalin (PPE) mRNA following electroacupuncture (EA) combined with droperidol (Dro), an antagonist of dopamine receptors. **METHOD:** The brains of Sprague-Dawley rats were sectioned after combination of EA with Dro and nonradioactive *in situ* hybridization histochemistry (ISHH) technic was used. **RESULTS:** Ten hours after EA, the expression of PPE mRNA was enhanced; when EA was combined with Dro, the expression of PPE mRNA was further enhanced in many pain-modulation-related nuclei, such as caudate-putamen, accumbens, septal nucleus, diagonal band nucleus, amygdala, hypothalamus, periaqueductal gray (PAG), interpeduncular nucleus, substantia nigra, and the dorsal horn of spinal cord (layer I-II and III-IV). **CONCLUSION:** Dro promoted EA's action on the PPE mRNA expression, which underlie the mechanisms of Dro potentiation on acupuncture analgesia.

KEY WORDS enkephalins; droperidol; electroacupuncture; *in situ* hybridization; central nervous system; messenger RNA

Enkephalin is an endogenous opioid peptide with strong analgesic effect in central nervous system (CNS). It inhibits the activities of pain-related neurons[1]. Acupuncture promotes the synthesis and release of enkephalin in brain, which plays an important role in acupuncture analgesia (AA)[2,3]. Antagonists of dopamine receptors potentiated AA[4,5]. Preproenkephalin (PPE) mRNA increased in spinal cord and medulla following electroacupuncture (EA)[6], indicating EA can augment gene expression. This study was designed to investigate the PPE mRNA expression following EA combined with droperidol (Dro), a dopamine receptor antagonist, by using a nonradioactive *in situ* hybridization histochemistry (ISHH) technic, to explore the potentiating effect of Dro on EA.

MATERIALS AND METHODS

Tissue preparation Sprague-Dawley rats (♀, n = 16, 200±s 30 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[7]. EA was applied at right "Zu-San-Li" (St 36, between muscle anterior tibialis and muscle extensor digitorum longus) and "Kun-Lun" (UB60, between the tip of the external malleolus and tendo calcaneus) points with Model G6805 EA apparatus (Shanghai). The needles were inserted 0.5 cm. Dense-sparse frequency of wave was selected and the intensity of EA was adjusted to the extent which provoked 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[8].

ISHH Digoxigenin (Dig)-labeled RNA probes were synthesized by *in vitro* transcription of PPE com-

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plementary DNA (cDNA) contained in plasmid vectors (a gift from Dr J HONG, National Institute of Environmental Health Sciences, Research Triangle Park NC, USA). The RNA probes were labeled by using DIG RNA labelling kit (Boehringer Mannheim Biochemica, Germany). The PPE cDNA was linearized with SacI enzyme as template for the transcription.

The procedure of ISHH was done with a free-floating method[9]. Briefly, after prehybridization procedures, the brain sections were hybridized with Dig-labeled PPE cRNA probe (0.5 mg·L^{-1}) in hybridization buffer at 37 ℃ for 16-24 h. Then the RNase (20 mg·L^{-1}) was added to digest unhybridized probe, followed by incubating brain sections with antibody (anti-Dig alkaline phosphatase conjugate, 1∶500). The signals of PPE mRNA in neurons were detected by Dig nucleic acid detection kit (Boehringer Mannheim, Germany).

For identifying the specificity of hybridized signals, brain sections were treated with A) RNase to digest the PPE mRNA followed by incubation in probe-containing hybridization buffer, and B) incubation in hybridization buffer without probes.

Two indices were measured simultaneously to quantify the PPE mRNA changes in the neurons with computer-assisted image processing system (FG-100-AT, Imaging Technology Inc, and TV-Camera, RCA Inc, USA). The first was the gray level of cell image, indicating the quantity of expressed mRNA; the second was the area with PPE mRNA, indicating the number of positive neurons. Brain structures were identified by reference to the rat atlas[10]. Data were analyzed by t test between 2 samples.

RESULTS

In sections treated with RNase or incubated in hybridization buffer without probes, no signals were found.

In control rats, PPE mRNA positive neurons were found in caudate nucleus, accumbens, lateral septal nucleus, diagonal band nucleus, preoptic area, amygdala, nucleus of hypothalamus, nuclei of midbrain (such as interpeduncular nucleus, PAG, substantia nigra, and red nucleus). These results are consistent with previous study[11].

Compared with control animals, the PPE mRNA expression showed no obvious increase when Dro alone was used. EA caused an increase of PPE mRNA expression in caudate nucleus, accumbens, lateral septum, lateral preoptic area, ventrolateral and arcuate nuclei of hypothalamus, amygdala, PAG, interpeduncular nucleus, red nucleus, and spinal dorsal horn, but no obvious change in diagonal band, medial preoptic area and medial area of hypothalamus. When EA was combined with Dro, the PPE mRNA expression was further enhanced in all the nuclei mentioned above (Tab 1, Tab 2 and Fig 1, Plate 1). Little expression of PPE mRNA was noted in thalamus in 4 groups.

DISCUSSION

Why the combination of Dro with EA further enhanced the expression of PPE mRNA is a matter of interest. The increase in PPE mRNA could be due to either increased synthesis or decreased degradation, which is associated with an increased utilization of enkephalins. EA accelerated the biosynthesis and release of enkephalins[2,3], which resulted in the increase of PPE mRNA. On the other hand, dopamine inhibits the activity of enkephalinergic neurons. Dro, a dopamine receptor antagonist, can diminish to some extent the inhibition of dopamine on enkephalinergic system. Combination of EA and Dro may lead to the further increase of enkephalin release, which would necessitate increased synthesis of precursor. However, PPE mRNA can not demonstrate directly the release of enkephalin *in vivo*, which is the shortcoming of this study.

EA has not only an immediate effect, but also a long-term one (ie, when EA stopped, the effect still remains for a long time). The PPE mRNA expression does not seem to be

Tab 1. Effect of EA+droperidol on preproenkephalin mRNA in rat brains (intensity of positive signals, indicated in gray level, $\bar{x}\pm s$).

Brain regions	NS ($n=3$)	NS+EA ($n=3$)	Dro ($n=4$)	Dro+EA ($n=4$)
Telencephalon				
Caudate nucleus	36.2±2.1	66.7±3.8[b]	35.7±3.9	98.5±4.3[cfh]
Accumbens	26.0±4.5	41.2±3.2[c]	33.0±8.0	52.5±5.4[fh]
Septum	23.0±7.2	32.0±4.5[b]	26.0±3.6	34.0±3.5[bh]
Diagonal band	27.0±2.6	30.7±5.0	29.2±4.7	35.6±2.9[cfh]
Medial preoptic area	28.7±1.5	31.3±5.0	29.5±2.6	47.3±7.3[cfh]
Lateral preoptic area	26.7±4.7	40.7±3.1[b]	34.0±1.7	46.3±6.5[ceh]
Amygdala	31.0±1.2	47.0±3.6[c]	35.7±1.5	65.2±6.2[cfi]
Diencephalon				
Hypothalamus				
Medial nucleus	25.7±3.1	31.3±9.5	30.3±6.0	44.7±4.5[ceh]
Ventrolateral nucleus	31.3±2.6	46.0±3.0[b]	37.7±2.5	62.4±4.3[cfi]
Arcuate nucleus	27.5±3.4	43.5±1.3[c]	29.3±2.1	52.4±5.6[ceh]
Mesencephalon				
Interpedun nucleus	29.3±3.1	40.0±2.6[b]	33.0±3.5	69.3±2.4[cfi]
Substantia nigra	31.7±2.1	41.3±1.5[b]	32.3±3.2	45.7±2.1[cfh]
Periaqueductal gray	24.3±1.5	38.6±2.1[c]	26.3±3.2	46.7±4.9[cfi]
Red nucleus	32.3±2.3	46.5±4.8[c]	34.4±9.5	58.0±3.5[cfi]
Spinal cord				
Layer I-II	35.0±1.7	44.3±2.5[c]	33.3±1.5	47.0±2.3[ci]
Layer III-IV	26.0±2.3	34.3±2.1[c]	28.0±2.6	54.0±1.8[cfi]

[b] $P<0.05$, [c] $P<0.01$ *vs* NS; [e] $P<0.05$, [f] $P<0.01$ *vs* NS+EA; [h] $P<0.05$, [i] $P<0.01$ *vs* Dro.

Tab 2. Effect of EA+droperidol on preproenkephalin mRNA in rat brains (areas with PPE mRNA in neurons, μm^2/unit, $\bar{x}\pm s$).

Brain regions	NS ($n=3$)	NS+EA ($n=3$)	Dro ($n=4$)	Dro+EA ($n=4$)
Telencephalon				
Caudate nucleus	1585±200	5019±1485[b]	3320±1608[h]	8450±1789[cfi]
Accumbens	1960±136	4488±707[b]	1375±45[b]	5810±803[ceh]
Septum	1536±41	2704±35[c]	2013±55[b]	3406±99[cfh]
Diagonal band	787±32	1463±81[c]	1397±76[c]	1615±80[cfi]
Medial preoptic area	2547±286	3651±233[e]	2996±535[b]	4217±701[cf]
Lateral preoptic area	1847±579	3173±48	2712±274	3522±131[ci]
Amygdala	2012±129	6384±514[c]	2202±175	7202±660[ch]
Diencephalon				
Hypothalamus				
Medial nucleus	923±69	1274±75[c]	1257±88[c]	1419±86[ceh]
Ventrolateral nucleus	1106±40	3240±137[c]	1330±88	4596±136[cei]
Arcuate nucleus	1613±318	2652±302[c]	1406±119[b]	5161±676[ce]
Mesencephalon				
Interpedun nucleus	541±61	822±97[c]	419±69	1431±75[ceh]
Substantia nigra	1148±66	1913±35[c]	1246±72[b]	2689±214[cfi]
Periaqueductal gray	1114±85	1886±542	1510±71	2549±317[c]
Red nucleus	1366±143	1826±104[c]	1464±35	2247±101[ci]
Spinal cord				
Layer I-II	1479±286	2338±804[c]	1391±559	2374±245[ch]
Layer III-IV	3115±112	5272±339[c]	3543±270	8002±163[cfi]

[b] $P<0.05$, [c] $P<0.01$ *vs* NS; [e] $P<0.05$, [f] $P<0.01$ *vs* NS+EA; [h] $P<0.05$, [i] $P<0.01$ *vs* Dro.

the immediate effect of EA, but rather a long-term one. From this point of view, the results in present paper suggest that the antagonists of dopamine receptor also potentiate the long-term effect of EA, and the further enhancement of PPE mRNA may be one of the mechanisms of potentiating effect of droperidol on AA.

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201-204

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2

氟哌利多结合电针使大鼠脑内前脑啡肽原 mRNA 的表达进一步增加

R965.2

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目的：观察氟哌利多与电针(EA)合用后前脑啡肽原(PPE) mRNA 的表达。**方法**：氟哌利多与电针合用后取大鼠脑切片，以非放射性的原位杂交组织化学技术观察 PPE mRNA。**结果**：电针10 h 之后，PPE mRNA 的表达增强了；而 EA 与氟哌利多合用后，PPE mRNA 的表达在许多与痛觉调制有关的核团被进一步增强，如尾壳核，伏核，隔核，斜角带核，杏仁核，下丘脑，中脑导水管周围灰质，脚间核，黑质，脊髓背角。**结论**：氟哌利多促进电针对 PPE mRNA 表达的作用，与其加强针刺镇痛的机制有关。

关键词 脑啡肽类；氟哌利多；电针；原位杂交；中枢神经系统；信使核糖核酸