Lymphocytic ppENKmRNA, MEK-IR, and Dyn-IR in Electroacupuncture

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We studied the effect of acupuncture analgesia on the expression of ppENKmRNA, MEK-IR, and Dyn-IR in circulating mouse lymphocytes. Electroacupuncture stimulated cell immunity. The release of *irDyn* during electrostimulation at 5 Hz frequency was less active than *irMEK* release.

Key Words: *ppENK; MEK-IR; Dyn-IR; lymphocytes; acupuncture*

Previous studies showed that the content of E-rosettes (particularly active ones), lymphoblast transformations, T4 subpopulation, circulating lymphocytes with methionin-enkephalin immunoreactivity, and their MEK-IR receptor increased in the acupuncture group [10-13]. Gene expression of ppENK in mouse lymphocytes and its relationship with MEK-IR and Dyn-IR were never studied before. Electroacupuncture with a frequency of 2 Hz promoted the release of MEK, while stimulation at 100 Hz was associated with dynorphine release in the cerebrospinal fluid [6]. We studied the effect of acupuncture on the expression of ppENK gene in mouse lymphocytes by *in situ* hybridization and RNA dot-blotting. MEK-IR and Dyn-IR in mouse lymphocytes were assayed using protein dot-blotting and immunohistochemical analysis.

MATERIALS AND METHODS

The study was carried out on BALB/c mice (n=20, 20-22 g). The animals were divided into 2 groups. Animals in the acupuncture groups were exposed to electroacupuncture (1.5 V, 5 Hz) in the bilateral E36 (Tzusanli) during 15 min. Controls received no treatment and were fixed for 15 min. Threshold pain sensitivity was determined before and after acupuncture or fixation by K^+ ionoelectrophoresis. Circulating lymphocytes were isolated from the peripheral blood by density gradient centrifugation. Lymphocyte suspension (1×10^5 cell/ml) was divided into 2 portions. One portion was blotted onto slides for evaluation of ppENK-mRNA by *in situ* hybridization [7] and of MEK-IR and Dyn-IR by immunohistochemical method. The other portion was blotted onto nitrocellulose membrane (NCM) for detecting ppENKmRNA by RNA dot blotting and detection of MEK-IR and Dyn-IR by protein dot-blotting (one dot per animal). BCIP/NBT was used as the substratum for violet color development, DAB as substratum for brown color development. Normal saline served as the negative control: it replaced the first antibodies and was subjected to proteolysis with ribonuclease. Protein dot blotting was carried out as described previously [13]. Dot blot signals were scanned by 528-nm waves on a Shimadu TLC scanner and analyzed statistically.

RESULTS

The threshold pain sensitivity alteration in mice exposed to acupuncture was 0.38±0.04, $p<0.01$, *vs.* virtually no alteration in the control group (0.02±0.03, $p<.0.05$).

NBT/BCIP stained the signals violet and DAB colored them yellow-brown. All ppENK, MEK-IR, and Dyn-IR signals were located in the lymphocyte cytoplasm and were stronger in the electroacupuncture group than in controls.

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Optical density of ppENK, MEK-IR, and Dyn-IR signals was scanned (Table 1).

Dot blot signals of ppENKmRNA, MEK-IR, and Dyn-IR were enhanced in the electroacupuncture group and positively correlated with the analgesic effect. The correlation (r) between optical density of dot blot signals and analgesic effect in the electroacupuncture group was 0.71 for ppENKmRNA ($p<0.01$), 0.79 for MEK-IR ($p<0.01$), and 0.71 for Dyn-IR ($p<0.01$). Moreover, there was a positive correlation between ppENK-mRNA (r=0/60, $p<0.05$) and Dyn-IR (r=0.67, $p<0.025$) alteration, but no correlation between ppENKmRNA and MEK-IR alteration ($p<0.05$).

Electrostimulation at 4 Hz and 100 Hz activates many nuclei in the brain stem, but some of them are selectively activated only at 4 Hz stimulation [8]. Pronounced expression of ppENKmRNA in the brain of rats can be caused by acupuncture at 2 Hz, while the increase of ppDmRNA is stimulated only by electroacupuncture at 100 Hz [4]. The level of irMEK in the cerebrospinal fluid of patients increased by 36.7% after transcutaneous stimulation (TENS) at 2 Hz, while irDyn increased by 49% at 100 Hz TENS [4].

Gene expression of ppENKmRNA in mouse lymphocytes can be superregulated by electroacupuncture at 5 Hz. Positive correlation between ppENKmRNA signals and analgesic effect is traced. The patterns of cerebrospinal fluid expression of ppEnk genes differed from lymphocytic patterns; ppENK expression was initiated after 4 h and reached the maximum level 48 h after electroacupuncture [1,3]. In this experiment the intensity of mouse lymphocyte irMEK did not correspond to the intensity of ppENKmRNA signals, despite the fact that ppENK is a MEK precursor. Lymphocytic MEK can be released from the cells and modify the nervous system and the pituitary [2]. Moreover, lymphocytic MEK-IR can develop methionine enkephalin in the lymphocyte cytoplasm and bind receptors for MEK to the lymphocyte periphery [9].

In our study lymphocytic ppENKmRNA and MEK-IR increased in the acupuncture group compared to the control, which proves the possibility of electroacupuncture stimulation of cellular immunity, particularly T-helpers involved in the neuroimmunological modulation through the neuroendocrinoimmune network.

TABLE 1. Alteration of ppENKmRNA, MEK-IR, and Dyn-IR Signals in Mouse Lymphocytes

Group	ppENKmRNA	MEK-IR	Dyn-IR
Control	1.88±0.25	1.35±0.21	2.32±0.37
Electro-acupuncture	2.35±0.41***	2.34±0.55**	3.02±0.52*

Note. *$p<0.05$, **$p<0.025$, ***$p<0.01$ compared to the control group.

Hence, it was found that Fos and Jun proteins are involved in ppD transcription easier than ppE gene expression of mRNA [5]. Preprodynorphine (ppD) is a dynorphine (Dyn) precursor. The results indicate that Dyn-IR in circulating lymphocytes was higher in the electroacupuncture group in comparison with the control, positively correlating with ppENKmRNA signals, and the number of animals with active lymphocytic Dyn-IR was higher than of those with active lymphocytic MEK-IR. The results indicate that irDyn is less actively released during electric stimulation at 5 Hz than irMEK.

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