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Effects of fenfluramine combined with electroacupuncture on monoamine release in periaqueductal gray of rat brain[1]

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KEY WORDS fenfluramine; biogenic monoamines; electroacupuncture; microdialysis; high pressure liquid chromatography; electrochemistry; periaqueductal gray

ABSTRACT

AIM: To study the changes of monoamines in ventrolatoral periaqueductal gray of rat brain before and after electroacupuncture (EA) analgesia (EAA) was enhanced by fenfluramine (Fen), a 5-hydroxytryptamine (5-HT) releaser. **METHODS**: Monoamines were collected by *in vivo* microdialysis and measured by HPLC connected with electrochemical detector. **RESULTS**: The level of norepinephrine (Nor) after EA was decreased ($P < 0.05$ *vs* NS group). The contents of 5-HT, 5-hydroxyindol acetic acid (5-HIAA), dopamine (DA), and homovanillic acid (HVA) in periaqueductal gray dialysate were increased ($P < 0.05$ *vs* NS group). When Fen was combined with EA, the level of 5-HT and 5-HIAA were further increased ($P < 0.05$ *vs* NS + EA group). There was no obvious change of Nor, DA, and HVA. **CONCLUSION**: Fen potentiating EAA may be related to further activation of serotoninergic system.

INTRODUCTION

Fenfluramine (Fen) enhancing electroacupuncture analgesia (EAA) and opioidergic system play an important role in its effect[1,2]. However, as a 5-hydroxytryptamine (5-HT) releaser, Fen potentiating EAA perhaps has a close relation with monoamine transmitters. In this study, we detected the contents of monoamines to get an understanding on the role of monoamines in Fen potentiating effect.

MATERIALS AND METHODS

Rats Male Sprague-Dawley rats ($n = 20$, 215 g ± 35 g, Grade Ⅱ, Certificate № 02-22-2) were bred by Experimental Animal Center, Shanghai Medical University.

Instruments Micro-infusion pump (CMA/100), microdialysis syringe 1.0 mL, microfraction collector (CMA/200), microdialysis probe (CMA/11, 3 mm long × 0.24 mm OD), HPLC (BAS200A system), LC-44 amperometric (the detector flow cell had a glassy carbon working and an Ag/AgCl reference electrodes), column (3-μm phase Ⅱ ODS column 100 mm × 3.2 mm, USA).

Reagents Fen of RA grade was from Shanghai Institute of Medical Engineering. Norepinephrine (Nor), dopamine (DA), homovanillic acid (HVA), 5-HT, and 5-hydroxyindol acetic acid (5-HIAA) of HPLC grade were purchased from Sigma.

Nociceptive test Nociceptive test was undertaken in quiet surrounding. The rat tail was inserted subcutaneously with needles which were connected with pain test apparatus (Model WQ-9E Pain Threshold Meter, Beijing). The least intensity of stimulating current that induced the tail flick was recorded as pain threshold. The pain threshold test was repeated thrice as the preadministration control, which ranged from 0.1 to 0.2 mA in normal rats.

Electroacupuncture (EA) EA was applied unilaterally at points (the needles were inserted 5 mm) of 'Zu-San-Li' (St 36, between muscle anterior tibialis and muscle extensor digitorum longus) and 'Kun-Lun'

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(UB60, between the tip of the external malleolus and tendon calcuneus) by EA apparatus (Model 6805-2, Shanghai) with the dense (60 Hz)-sparse (4 Hz) frequency of wave (< 1 mA), which provoked slight twitches of hindlimb.

Brain microdialysate Rats were anesthesized with sodium pentobarbital (3.5 mg · kg^{-1}, ip) and intracerebral guide with dummy was implanted in rat periaqueductal gray (PAG). The coordinates were A 0, R 0.6, H 6.0[13]. Two days after surgery, 20 rats were divided into 4 groups: 1) normal saline (NS) ip; 2) Fen 4.5 mg·kg^{-1} ip (Fen); 3) EA for 20 min (NS + EA); 4) Fen 4.5 mg·kg^{-1} ip for 10 min, then EA for 20 min (Fen + EA). The probe was implanted in intracerebral guide and perfused with artificial cerebrospinal fluid at 2 μL·min^{-1} using microinfusion pump. Dialysate before and after EA and Fen treatment for 30 min was collected for 15 min. At the end of the microdialysis, the rats were decapitated and the brain was sliced on freezing microtome for histological identification of the location of the implanted probe.

Monoamine analysis Monoamines in dialysate were measured by HPLC connected with chemical detector. Standard solution or sample 20 μL were injected into the column, separated with mobile phase consisted of edetic acid 0.4 mmol · L^{-1}, chloroacetic acid 0.15 mol · L^{-1}, C_{10}-7 (1s)-(+)-10 camphorsulfonic acid 7.5 mmol · L^{-1}, and 8 % methanol, detected using chemical detector at oxidation potentials 700 mV against the Ag/AgCl electrode and quantified using BAS ChromGraph programs. Data were analyzed by t-test.

RESULTS

Effect of Fen on EAA Changes of pain threshold in NS group were observed for 90 min. When unilateral EA was applied, the pain threshold was increased. There was no significant change in response to ip Fen 4.5 mg · kg^{-1}. After Fen + EA, the pain threshold was further increased and prolonged *vs* NS + EA group ($P < 0.01$), with the peak appearing at about 30 min following EA (Tab 1).

Monoamines in microdialysate In NS group, the contents of 5-HT, 5-HIAA, DA, HVA, and Nor in PAG dialysate were (22 ± 3), (27 ± 3), (1.30 ± 0.24), (2.16 ± 0.23), and (130 ± 6) μg · L^{-1}, respectively. After EA, the contents of 5-HT, DA, and their metabolites were increased, the increase values were (7.2 ± 2.5), (8 ± 3), (2.90 ± 0.14), and (2.67 ± 0.18) μg · L^{-1}, respectively, while the content of Nor in PAG dialysate was decreased about (25 ± 6) μg · L^{-1} (*vs* NS group, $P < 0.05$). In Fen group, the levels of 5-HT and 5-HIAA were also increased. However there was no obvious change in other monoamines.

When EA was combined with Fen, the contents of 5-HT and 5-HIAA in PAG dialysate were further increased about (13.4 ± 2.7) and (17.9 ± 2.3) μg · L^{-1}, respectively (*vs* NS + EA group, $P < 0.01$) and that of others unchanged significantly. (Tab 2)

DISCUSSION

Our present study showed that EA increased the level of 5-HT and 5-HIAA in PAG. The midbrain PAG has long been known to be a component of an endogenous pain-suppression system. Anatomical evidence has demonstrated that PAG contains much afferent input to and efferent output from raphe magnus nucleus and dorsal raphe nucleus where most serotoninergic neurons are located[4]. Thus, high

Tab 1. Increase of pain threshold (μA) of rats. $n = 5$. $\bar{x} \pm s$.
$^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* NS. $^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* NS + EA.

Time after treatment	NS group	Fen + NS group	NS + EA group	Fen + EA group
10 min	16 ± 11	40 ± 13^{a}	80 ± 29^{b}	86 ± 15^{bd}
20 min	18 ± 10	80 ± 11^{b}	120 ± 30^{c}	206 ± 27^{cf}
30 min	36 ± 9	70 ± 7^{b}	140 ± 11^{c}	226 ± 30^{cf}
50 min	28 ± 21	2 ± 8^{a}	43 ± 20^{a}	110 ± 15^{ce}
70 min	27 ± 19	12 ± 5^{a}	30 ± 11^{a}	71 ± 13^{ce}
90 min	26 ± 20	13 ± 9^{a}	20 ± 10^{a}	28 ± 11^{ad}

Tab 2. Changes ($\mu g \cdot L^{-1}$) of monoamine contents in microdialysate at 15 – 30 min after Fen + EA ($n = 5$. $\bar{x} \pm s$). $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* NS. $^dP > 0.05$, $^eP < 0.01$ *vs* NS + EA group.

	NS group	Fen + NS group	NS + EA group	Fen + EA group
5-HT	0.6 ± 1.2	3.4 ± 0.6^b	7.2 ± 2.5^c	13.4 ± 2.7^{ce}
5-HIAA	-0.9 ± 1.6	3.7 ± 0.6^b	8 ± 3^c	17.9 ± 2.3^{ce}
DA	-0.05 ± 0.19	0.31 ± 0.28^a	2.90 ± 0.14^b	1.98 ± 0.15^{bd}
HVA	0.13 ± 0.12	0.18 ± 0.22^a	2.67 ± 0.18^b	2.42 ± 0.18^{bd}
Nor	8 ± 4	6.5 ± 2.8^a	-25 ± 6^c	-23 ± 6^{ce}

content of 5-HT and 5-HIAA in PAG dialysate may be ascribed to the increase of activity of serotoninergic neurons by EA and facilitate EAA. Our previous results also showed that microinjecting p-CAP, a depletor of 5-HT, decreased greatly EAA[5].

When Fen was combined with EA, the content of 5-HT and 5-HIAA in PAG dialysate was increased moreover, while the contents of Nor, DA, and HVA were unchanged, in the meanwhile, EAA also continued to be enhanced. The possibility contributed to this effect was that the serotoninergic system was further activated via enhancing the release of 5-HT by Fen. The increase of 5-HT release may result in speeding up the metabolism, then increase its metabolite level. DA was also an important monoamine transmitter. Our study observed that EA also increased the content of DA in PAG dialysate, which received efferent output from dopamine neuron in A11. However dopaminergic system exerted a tonic inhibition on endogenous opioid peptide release[6], which played a key role in EAA and thus activating dopaminergic system could decrease EAA. Our lab work showed that dopamine receptor antagonist, droperidol, combined with EA improved EAA[7]. That is to say, EA could not only activate the facilitory factors such as serotoninergic system, but also activate the inhibitory factors such as dopaminergic system. It was perhaps one reason that EA had no perfect effect on analgesia.

As to Nor, another unfavorable factor for EAA, EA could decrease its level in PAG. Some evidence showed that there was a negative correlation between the changes of Nor and β-endorphin which was a key peptide in endogenous pain suppression system, indicating that β-endorphin may be related to the inhibition of Nor release during EAA[8].

The results in the present study suggested that Fen potentiating EAA may be related to activation of serotoninergic system via accelerating the release of the 5-HT.

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芬氟拉明合用电针对大鼠脑内中央灰质单胺类释放的影响[1]

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关键词 芬氟拉明；生物单胺类；电针；微透析；高压液相色谱法；电化学；水管周灰质

目的：研究 5-羟色胺释放剂芬氟拉明加强针刺镇痛前后大鼠脑内中央灰质（PAG）腹侧部单胺类递质的变化。**方法**：运用微透析及高效液相电化学检测方法。**结果**：电针后 Nor 的释放减少，而 5-HT，5-HIAA 和 DA，HVA 在 AG 部位含量升高（$P<0.05$，*vs* NS 组）。当芬氟拉明合用电针时，5-HT，5-HIAA 含量进一步升高，但 Nor，DA 及其代谢产物却无明显变化（$P<0.05$ *vs* NS + EA 组）。**结论**：电针能促进 DA 和 5-HT 释放但抑制 Nor 释放。芬氟拉明合用电针能进一步加强 5-HT 的释放。芬氟拉明加强针刺镇痛可能与进一步激活 5-羟色胺系统有关。

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