行政院國家科學委員會補助專題研究計畫
成果報告

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計畫編號：NSC 90-2320-B039-020
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計畫主持人：林昭庚

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行政院國家科學委員會專題研究計畫成果報告

不同頻率及俞穴電針探討內生性嗎啡調控小鼠血糖之機轉

Using different frequencies and acupoints of electroacupuncture to explore the mechanism which endogenous opioid peptides regulate plasma glucose in mice

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中文摘要

先前的研究發現電針麻醉的實驗動物，可經由內生性嗎啡依賴胰島素而調降血糖。本研究以相同實驗條件電針動物足三里及非穴區，並進一步探討其降血糖之機轉，期能比較不同俞穴電針調控血糖之機轉。

結果顯示 2Hz 足三里穴電針降糖作用優於非穴區，其調降血糖之作用除了內生性嗎啡外尚有血清素參與其中，腎上腺僅伴演一部份的角色。與先前中脘穴電針有大量的腦內啡來自腎上腺經由受體的活化而調降血糖有顯著的不同。同時也說明了針灸治療的遠部取穴及循經取穴確有實際義意。

關鍵詞：電針、足三里穴、血糖、腦內啡、胰島素、血清素、去腎上腺。

Abstract

An insulin-dependent hypoglycemic effect of electroacupuncture(EA) had been introduced in our previous studies, which were mediated by endogenous opioid(EOP) peptides. In the present study, we applied 2Hz EA to stimulate the animal model for 30min at bilateral Zusanli acupoints, and nonacupoint area as control group to realize the specific characteristic of acupoint in hypoglycemic effect. The determination of plasma β-endorphin and insulin by ELISA method was in order to investigate its mechanism. Furthermore, Adrenalectomized rats (ADXR) were applied to explore the role of adrenal gland, and using STZ-induced diabetic rats to examine the insulin-dependent character. In addition, Naloxone and μ-opioid receptor knockout mice (MOR-KOM) were applied to exame the mediation of EOP, and to explore relations with μ-opioid receptor. After injecting serotonin depleter (p-chlorophenylalanine, PCPA 300mg/kg, i.p. for 3 days) as a factor was added in for detecting serotonin involvement. As the data showed, 2Hz EA at Zusanli acupoints made a significant hypoglycemic effect that is better far from the rats receiving same stimulation at nonacupoint area. The levels of plasma insulin and β-endorphin were also elevated markedly. Electro-stimulating nonacupoint area of normal rats or Zusanli acupoint of ADXR did not have this phenomenon. This hypoglycemic effect was abolished in STZ induced-diabetic rats, but it did not block completely in ADXR . Furthermore, the sufficient dose of naloxone failed to abolish the hypoglycemic action completely, and there was still a significant decrease of plasma glucose by this EA in MOR-KOM. For this reason, we used PCPA as tool to explore the serotonin involvement besides EOP. Fortunately, The hypoglycemic effect can be totally blocked by sufficient dose of naloxone when pretreated PCPA however rats or mice, as the same pretreatment that can be totally blocked by knocking out μ-opioid receptor in mice.

From the obtained results, we suggest that stimulating Zusanli acupoints with 2Hz EA can induce a serotonin-involved hypoglycemic action, which is mediated by EOP through the secretion of insulin in acupoint specific way. Adrenal gland only play a role in part.

Keywords: Electroacupucture;Zusanli;Plasma Glucose ; β-endorphin;Insulin;Serotonin ; Adrenalectomy

Introduction

Main purpose of this study was to investigate the hypoglycemic mechanisms of 2Hz electroacupuncture(EA) at bilateral Zusanli acupoints(ST36). In the Meridian's viewpoint of Traditional Chinese Medical (TCM), Zusanli acupoint is belongs to The Stomach Channel of Foot –Yangming, and it is connected with abdominal organs especially digestive system. Previous study has known that the moxibustion at Zusanli can increase serum insulin levels [24]. Continually stimulating bilateral Shenshu and Zusanli points of Streptozotocin(STZ)-induced diabetic rats for five weeks can decrease plasma glucose levels, and also can elevate the lowered pain threshold [15]. A number of previous researches find that needle-stimulating Zusanli acupoint may produce physiology effects, such as analgesia, increasing immune function, and the inhibition of gastric acid secretion [8,22,16,10,23]. Analgesic mechanisms of acupuncture has closely relationship to the endogenous opioid peptide (EOP) which has been considered. In addition, EOP can enhance insulin secretion of β-cell in pancreas [11,5].

Therefore, acupuncture has an action to decrease plasma glucose levels that has been discussed extensively. Exercise also follows this way to lower down plasma glucose [6,1].

Our previous results indicate that EA applied to the Zhongwan acupoint of abdomen, can produce significant hypoglycemic effect, and this effect mediated by β-endorphin and increasing plasma insulin levels in NIDDM and normal wistar rats [3]. But, no similar hypoglycemic effect is noted in adrenalectomizd rats (ADXR) and μ-opioid receptor knockout mice (MOR-KOM). In addition, Naloxone and naloxonazine can block this hypoglycemic effect. Thus we suggest that the hypoglycemic effect of 2Hz EA at zhongwan acupoint mediated by the activation of μ_1 –opioid receptor through adrenal gland [12].

According to above paper review and our previous results, this study will observe the hypoglycemic effect when 2Hz EA applied to bilateral zusanli acupoints, and these results in rats and in STZ-induced diabetic rats compare with nonacupoint that located to lateral lower leg to verify acupoint specific character. Plasma β-endorphin and insulin by ELISA method were measured simultaneously. In addition, we studied the hypoglycemic mechanism of 2Hz at bilateral Zusanli acupoints, using naloxone pretreatment and MOR-KOM were to investigate the relationship between opioid receptor and 2Hz EA at Zusanli acupoint. A serotonin depleter p-chlorophenylalanine (PCPA) was also used to explore the involvement of serotonin in rats and mice, and using ADXR to observe the role of adrenal gland.

Results and Discussions

Hypoglycemic effect was found in rats, which was stimulated by 2Hz EA at Zusanli, and more significant than nonacupoint area. No significant hypoglycemic effect was found after 2Hz EA applied to bilateral Zusanli acupoints for 30min in STZ-induced diabetic rats. In ADXR, 2Hz EA at Zusanli still produce hypoglycemic effect (from 118±14 to 98±14 mg/dl, $p<0.01$). The hypoglycemic activity is -17±5(12)% that was only lower down than that of normal wistar. In the assay of plasma insulin and β-endorphin, They were remarkable elevation in 2Hz EA at Zusanli, Nonacupoint area did not has this phenomena(Fig 1). In ADXR, Changes were also no significant, however plasma β-endorphin (from 3.9±0.2 to 3.7±0.3 pmol/L , n=8) and plasma insulin (from 408±60 to 390±67 pmol/L, n=8) after 2Hz EA-stimulation.

In influence of naloxone and PCPA, the hypoglycemic effect of EA at Zusanli was not abolished completely by pretreated with naloxone or PCPA alone. Further, combination of them can block hypoglycemic effect by this EA (Table 2). In mice, significant hypoglycemic effect also found by 2Hz EA at Zusanli (from 187±11 to 125±19 mg/dl, n=8, $p<0.01$), and hypoglycemic activity was 33±11%. It has the same phenomena as rats in influence of naloxone and PCPA. They must combine together to block this hypoglycemic effect completely (Table 4). In another hand, MOR-KOM was pretreated PCPA that also can block this hypoglycemic effect totally. But 2Hz EA at Zusanli still has the ability of lowering down plasma glucose in MOR-KOM only (Table 3).

In STZ-induced diabetic rats whose insulin was absent, therefore no instant hypoglycemic effect produced after 2Hz EA at bilateral Zusanli acupoints for 30 min, whereas a significant elevation of plasma insulin was found in the normal wistar rats. According to this results, suggesting the decrease of plasma glucose levels results from EA promote insulin secretion. This mechanism was similar to EA of Zhongwan that we published previously, and that was acupoint specific character when compare with nonacupoint area [3,12].

Previous studies show that the hypoglycemic action of 2Hz EA at Zhongwan acupoint for 30 min did not occur in ADXR, and it was blocked by sufficient naloxone in normal wistar [12]. In this study, the hypoglycemic effect of 2Hz EA at bilateral Zusanli acupoints still produce in ADXR, and cannot be blocked completely by petreatment of naloxone in rats [3]. Therefore, we infer that the hypoglycemic effect of 2Hz EA at bilateral Zusanli acupoints possibly has another route participation, it is not from adrenal gland and Not via EOP. Further results find that the hypoglycemic action of Zusanli EA was blocked by PCPA plus naloxone, in the rats and mice (Table 2,4). This phenomenon indicated that serotonin participate in the hypoglycemic mechanism of 2Hz EA at Zusanli acupoints. In addition, PCPA pretreated can block the hypoglycemic effect of Zusanli EA completely in MOR-KOM; therefore we are sure that the hypoglycemic effect of 2Hz EA at Zusanli acupoint has the involvement of serotonin. In this study and previous one, the hypoglycemic effect of Zhongwan EA mediated via activating μ_1-opioid receptor, inferred that the hypoglycemic effect of EA is most connected with μ-opioid receptor. This mechanism is similar to that of analgesia by 2Hz EA stimulation [4].

According to physiological and anatomical viewpoints, Zusanli acupoint locates on front lower leg and Zhongwan locates on abdomen, Each of them belongs to different dermatome that have different physiological effects in modern or Traditional Medical theory. Zusanli acupoint is far from pancreas and adrenal gland, and its effect of EA in plasma β-endorphin and insulin is indirect, but it has more greater hypoglycemic effect than Zhongwan acupoint[12]. Therefore, we have sufficient reason to support that serotonin participated the hypoglycemic effect of Zusanli EA except EOP. In addition, 5-HT and specific 5-HT2A agonists can rapidly stimulate glucose uptake in skeletal muscle by a non-insulin dependent signaling pathway [7]. This result supports our data that insulin and β-endorphin does not increase significantly but still have partial hypoglycemic effect in ADXR after Zusanli EA.

According our results, we suggest that 2Hz EA

applied to bilateral Zusanli acupoints can induce a serotonin-involved hypoglycemic action, which is mediated by EOP through the secretion of insulin in acupoint specific way. Adrenal gland only plays a role in part.

Achievements of Self-evaluation

In the experience of this investigation, we obtain a new viewpoint, which EA seems to increase the sensitivity of insulin. Although it can not lower down the plasma glucose directly in STZ diabetic animal in short term, EA seems more sensitive than control in insulin challenge test. Higher frequency (15Hz) is more effective in hypoglycemic effect but more complicate in mechanism.

EA for plasma glucose regulation is a very interesting field. Different frequencies and different acupoints have different mechanisms. Not only diabetes control, but also the basic theory of TCM and Acupuncture will be proved.

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Table 1 Effect of 2Hz electroacupuncture on plasma glucose levels in normal and STZ-induced diabetic rats (mg/dl)

Group	Fasting	EA	Hypoglycemic Activity%
Zusanli(n=8)	132±4	94±4**	-30±3, A
Nonacupoint(n=8)	123±12	114±8**	-7±5, B
STZ-Zusanli(n=7)	555±132	527±132	-5±3, B

The values are given as mean ± SEM. In this and subsequent Tables: n= the number of the rat. Fasting= the plasma glucose levels were measured in rats with 12 h fasting state; EA= the plasma glucose levels were measured after electroacupuncure (EA) of 30 min; Hypoglycemic activity= the percentage in the decrease of plasma glucose levels; Zusanli= EA was applied to bilateral Zusanli acupoints of the normal rats; Nonacupoint= EA was applied to lateral aspect of right lower leg in the normal rats ; STZ-Zusanli= EA was applied to bilateral Zusanli acupoints of STZ-induced diabetic rats. ** $P < 0.01$ compared with the values of Fasting; Different characters tailing after hypoglycemic activity meams significant, A>B, P<0.05, by ANOVA.

Table 2 Effect of p-chlorophenylalanine and naloxone on plasma glucose levels in rats with 2Hz electroacupuncture treatment (mg/dl)

Groups	Fasting	EA	Hypoglycemic Activity %
PCPA(n=8)	114±5	93±4**	-18±3, A
Naloxone(n=7)	100±24	86±23*	-14±9, A
PCPA+Naloxone(n=10)	101±19	99±20	-2±5, B

The values are given as mean ± SEM. PCPA= rats with p-chlorophenylalanine (300 mg/kg, i.p.) treatment for 3 days prior to 2 Hz EA applied to bilaterlal Zusanli acupoints; Naloxone= rats with naloxone (1 mg/kg, i.v.) treatment 30 min prior to 2 Hz EA applied to bilateral Zusanli acupoints; PCPA+Naloxone= the methods were the same as PCPA and Naloxone group, but PCPA plus naloxone. * $P < 0.05$, ** $P < 0.01$ compared with the values of Fasting; Different characters tailing after hypoglycemic activity means statistic significantly, A>B, P<0.05, by ANOVA.

Table 3 Effect of p-chlorophenylalanine on plasma glucose levels in μ-opioid receptor knockout mice with 2Hz EA treatment (mg/dl)

Groups	Fasting	EA	Hypoglycemic Activity %
MOR-KOM(n=7)	172±22	134±28**	-21±14
PCPA+ MOR-KOM(n=11)	143±25	130±19	-7±19#

The values are given as mean ± SEM. PCPA+MOR-KOM= knockout mice with p-chlorophenylalanine (300 mg/kg, I.p.) treatment for 3 days prior to 2Hz EA applied to bilateral Zusanli acupoints. ** $P < 0.01$ compared with the values of Fasting; # $P < 0.05$ compared with the hypoglycemic activity from MOR-KOM group.

Table 4 Effect of p-chlorophenylalanine and naloxone on plasma glucose levels in mice with 2Hz EA treatment (mg/dl)

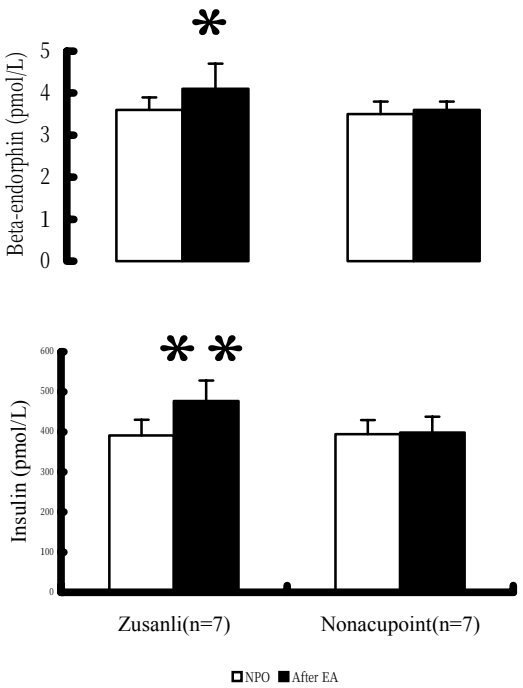
Groups	Fasting	EA	Hypoglycemic Activity %
PCPA(n=8)	144±25	112±32**	-22±14(8), A
Naloxone(n=7)	145±44	125±42*	-14±12(7), A
PCPA+Naloxone(n=10)	124±27	115±30	-8±13(7), B

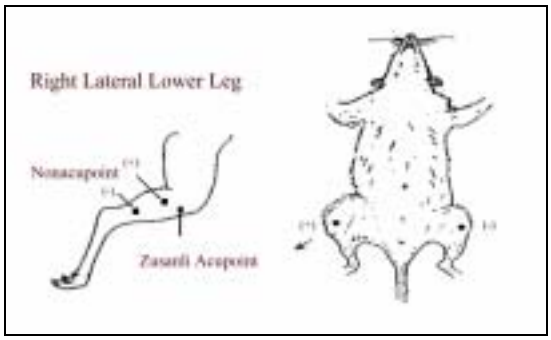
The values are given as mean ± SEM. PCPA= rats with p-chlorophenylalanine (300 mg/kg, i.p.) treatment for 3 days prior to 2 Hz EA applied to bilaterlal Zusanli acupoints; Naloxone= rats with naloxone (1 mg/kg, i.v.) treatment 30 min prior to 2 Hz EA applied to bilateral Zusanli acupoints; PCPA+Naloxone= the methods were the same as PCPA and Naloxone group, but PCPA plus naloxone. * $P < 0.05$, ** $P < 0.01$ compared with the values of Fasting; Different characters tailing after hypoglycemic activity means statistic significantly, A>B, P<0.05, by ANOVA.

Figure legends

Fig 1. Zusanli and Nonacupoint stimulated by 2Hz EA were observed the effect of plasma peptides (β-endorphin/Insulin). To illustrate that the hypoglycemic effect is mediated by EOP through insulin to lower down plasma glucose, and has acupoint specific manner. Values (mean ± SEM) were obtained from each group of 7 rats. * P< 0.05 and ** P<0.01 vs. data from NPO.

Fig 2. The location of acupoint and nonacupoint.
(+):positive charge connect to EA instrument;
(-):negative charge connect to EA instrument.


Beta-endorphin (pmol/L)
0
1
2
3
4
5
*
Insulin (pmol/L)
0
100
200
300
400
500
600
**
Zusanli(n=7)
Nonacupoint(n=7)
NPO
After EA


Right Lateral Lower Leg
Nonacupoint
Zusanli Acupoint