Electroacupuncture stimulation using different frequencies (10 and 100 Hz) changes the energy metabolism in induced hyperglycemic rats[1]

Eletroacupuntura usando diferentes freqüências (10 e 100 Hz) altera o metabolismo energético em ratos hiperglicêmicos induzidos

Lanese Medeiros Figueiredo[I], Agamenon Honório Silva[I], Alberico Ximenes do Prado Neto[II], Miguel Nasser Hissa[III], Paulo Roberto Leitão de Vasconcelos[IV], Sérgio Botelho Guimarães[V]

[I]Fellow Master degree, Department of Surgery, Postgraduate Program, UFC, Ceara, Brazil. Participated in the design of the study, performed the electro acupuncture procedures, literature search and acquisition and interpretation of data. The article is part of a master degree dissertation.
[II]Graduate student, UFC, Ceara, Brazil. Helped with technical procedures and acquisition of data.
[III]PhD, Associate Professor, Department of Clinical Medicine, UFC, Ceara, Brazil. Critical revision and interpretation of data.
[IV]PhD, Associate Professor, Coordinator, Postgraduate Program, Department of Surgery, UFC, Ceara, Brazil. Assistance in manuscript preparation, critical revision of discussion and statistical analysis.
[V]PhD, Associate Professor, Department of Surgery, Head, Experimental Surgery Laboratory (LABCEX), UFC, Ceara, Brazil. Tutor, conception, design, intellectual and scientific content of the study, manuscript writing, statistical analysis, critical revision.

ABSTRACT

PURPOSE: To investigate the effect of 10 and 100 Hz peripheral electro-estimulation (electroacupuncture, EAc) at *Zusanli* (ST-36) and *Zhongwan* (CV-12) acupoints on blood glucose and lactate levels and tissue (liver and kidney) concentrations of lactate in hyperglycemic induced anesthetized rats.

METHODS: Thirty-six rats were randomly assigned to 3 groups (n=12): G1: basal (anesthesia: ketamine (90mg kg^{-1} body weight)+ xylazine (10mg/kg^{-1} body weight, i.p.); G2: anesthesia+EA10Hz EAc and G3: anesthesia+EA100Hz EAc). EAc stimulation was delivered for 30 min at 10 mA at selected acupoints. Blood and tissue (kidney, liver) samples were collected at the end of the EAc application (n=6, T30) and 30 minutes later (n=6, T60) for biochemical analysis. G1 samples were collected at the same timepoints. ANOVA followed by Tukey's Multiple Comparison Test was used for statistical analyses.

RESULTS: Glycemia decreased significantly ($p<0.001$) in G2/G3 rats in all timepoints. Kidney and liver lactate concentrations decreased significantly ($p>0.001$) in G2/G3 rats at T-60 and at T30 timepoints in G2 compared with G1 rats. Lactacedemia decreased significantly at T30 timepoint in G2 compared with G1 rats. G1/G3 tissue lactate levels were not different.

CONCLUSIONS: Electroacupuncture (10 Hz) applied to St-36 and CV-12 acupoints decreases glycemia and lactacedemia and liver and kidney lactate concentrations. We hypothesize that the decrease in lactate levels may be related to greater energy production due to enhanced lactate to pyruvate conversion. Higher frequency (100 Hz) failed to promote the same effect.

Keywords: Medicine, Chinese Traditional. Electroacupuncture Glycemia. Rats.

RESUMO

OBJETIVO: Investigar o efeito da eletroacupuntura (10-100 Hz) aplicada nos acupontos *Zusanli* (ST-36) e *Zhongwan* (CV-12) sobre a glicemia, lactacedemia e concentrações de lactato no fígado/rim em ratos anestesiados.

MÉTODOS: Trinta e seis ratos foram distribuídos aleatoriamente em três grupos (n= 12): G1: basal (anestesia: cetamina (90mg kg^{-1})+xilazina (10mg/kg^{-1}, ip), G2: anestesia+10Hz EAc e G3: anestesia+100Hz EAc). EAc foi aplicada por 30 min (10 mA) em acupontos selecionados. Amostras de sangue e tecidos (rim, fígado) foram coletadas no final da aplicação da EAc (n=6, T30) e 30 minutos depois (n=6, T60) para análise bioquímica. Amostras de G1 foram coletadas nos mesmos tempos (T30 e T60). ANOVA seguido pelo teste de comparações múltiplas de Tukey foi utilizado para análises estatísticas.

RESULTADOS: A glicemia diminuiu significativamente ($p<0,001$) nos grupos G2/G3 em todos os pontos temporais. As concentrações de lactato nos rins e no fígado diminuiu significativamente ($p<0,001$) nos ratos G2/G3 ratos no T-60 e no T30 no G2, comparados com ratos G1. Lactacedemia diminuiu significativamente no T30 no G2 comparado com G1. Os níveis de lactato tecidual não foram diferentes comparando os grupos G1/G3.

CONCLUSÕES: Eletroacupuntura (10 Hz) aplicada aos acupontos ST-36 e CV-12 reduz a glicemia e lactacedemia bem como as concentrações de lactato no fígado e nos rins. Nossa hipótese é que a diminuição dos níveis de lactato possa estar relacionada à maior produção de energia devido ao aumento de conversão de lactato para piruvato. A utilização de uma freqüência mais alta (100 Hz) não produz o mesmo efeito.

Descritores: Medicina Tradicional Chinesa. Eletroacupuntura. Glicemia. Ratos.

Introduction

Electroacupuncture (EAc) is a form of treatment where small electrical currents are applied to needles previously inserted in the body and appears to have more consistently reproducible results in many specific clinical and research settings[1-3]. EAc at Zusanli (ST-36) or Zhongwan (CV-12) acupoints have been widely used to relieve symptoms of diabetes mellitus in Traditional Chinese Medicine. Eventual release of beta-endorphins from the adrenal gland to lower plasma glucose has been demonstrated by EAc at the Zhongwan acupoint in rats[4]. Plasma glucose lowering effect of 15 Hz EAc using bilateral Zusanli acupoints have been demonstrated in rats. Enhanced insulin sensitivity was also demonstrated by the same researchers[5].

EAc effectiveness is not only dependent on stimulation intensity but also dependent on its frequency. Due to different excitation threshold and refractory period of different types of afferent fibers, high intensity excitation (5.0 mA) with low frequency (5.0 Hz) EAc for excitation of the small (A-ß and C-group) fiber afferent can produce best analgesic effect, On the other hand, only excitation of the large (A- ß group) fiber afferent with EAc with low intensity (0.5 mA) and high frequency (50 Hz) EAc also can produce better analgesic effect[6]. All three types of opioid receptors (the mu, delta and kappa receptors) in the spinal cord of the rat play important roles in pain relieving when electrically stimulated[7,8]. Recent study has verified that in fed Sprague-Dawley rats, ketamine (100 mg/kg)/xylazine (10 mg/kg) produced acute hyperglycemia within 20 min. The hyperglycemic effect in fed rats was associated with decreased plasma levels of insulin, adrenocorticotropic hormone, and corticosterone and increased levels of glucagon and growth hormone[9].

The question raised here is if a single EAc application in ketamine/xylazine induced hyperglycemia in anesthetized rats can change blood and tissue metabolic parameters. Therefore we are aimed at assaying the glycemia and lactate contents in the blood, liver and kidney in healthy adult rats following a single acupuncture session, using the same excitatory intensity (10mA) and different frequencies (10 and 100 Hz).

Methods

Approval for experimental use of laboratory animals was obtained (Protocol #093/2007) from the Ethics Committee on Animal Research (CEPA) of the Federal University of Ceara, now Ethics Committee on the Use of Animals (CEUA), in view of the Federal Law No. 11794 of October 8, 2008, http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/ 2008/Lei/L11794.htm and Decree No. 6689 of July 15, 2009 that regulated the Law 11794, available at: http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2009/Decreto/ D6899.htm. The study was designed so as to minimize the number of animals required for the experiments.

Study outline

Figure 1 depicts all phases of the experiment.

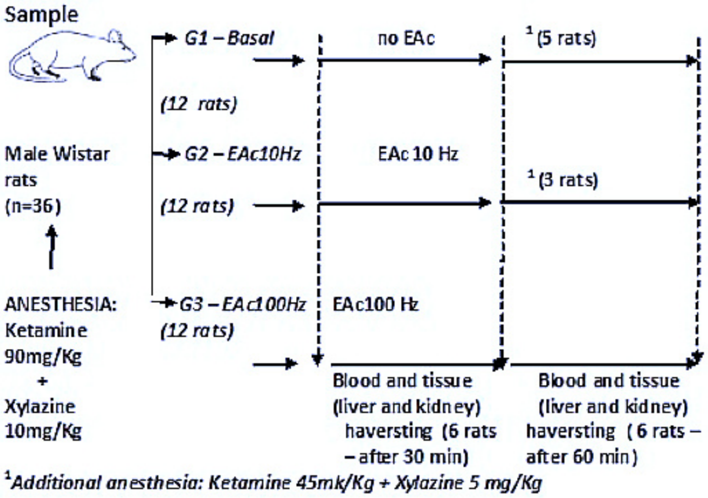

FIGURE 1 – Diagram outlining hyperglycemia induction with ketamine/xylazine, eletroacupuncture frequencies applied to G2/G3 rats and haversting timepoints.

Equipament and materials

EAc stimulation was delivered by an electrical stimulator EL-608 (NKL Electronic Products, Brusque, Santa Catarina, Brazil). A pulsed electrical stimulation (asymmetric balanced, rectangular shape, 10 and 100 Hz frequency, 10 mA intensity with automatic timing was used in the experiment. For puncture, sterile needles 0.25 mm diameter and 30 mm length (Hwato brand, Suzhou Medical appliance factory, China) were used.

Animal preparation

Male Wistar rats weighing 255–400g, provided by the Faculty of Medicine Small Animals Breeding Facility (Federal University of Ceara) were kept under controlled environmental conditions (24°C _relative humidity 40%–60%, 12-hour alternate light–dark cycles, food and water *ad libitum*). The equivalent of the human right ST-36 and CV-12 acupoints were chosen for needling and electrical stimulation. The acupoint nomenclature used follows WHO nomenclature[10]. ST-36 acupoint is located 5mm below the head of the fibula under the knee joint, and 2mm lateral to the anterior tubercle of the tibia. Puncture of ST-36 acupoint stimulates the lateral sural cutaneous nerve, the cutaneous branch of the saphenous nerve, and deeper, the deep peroneal nerve[11,12]. CV-12 acupoint is located in the anterior midline of the upper abdomen, 20 mm below the sternal synchondrosis. This region is innervated by the anterior cutaneous branch of the 8th intercostal nerve[12].

Experimental groups

All animals were anesthetized intraperitoneally with a fresh-prepared mixture of ketamine (90mg kg^{-1} body weight) and xylazine (10mg kg^{-1} body weight). Rats were randomly assigned to 3 equal groups (n = 12): G1 (anesthesia only); G2, treated with 10Hz EAc at ST26 and CV12; and G3, treated with 100Hz-EAc at ST36 and CV12.

Sterilized disposable stainless steel needles (0.25 mm × 30 mm) were inserted perpendicularly as deep as 2-3 mm at right ST36 and CV12 acupoints in G2 and G3 rats after routine skin disinfection with 75% ethanol. Electrodes were connected to both needles and to the electro-stimulator (NKL EL-608); pulsed square waves (10 mA), 10 Hz (G2) and 100 Hz (G3), were applied for 30 minutes. (Figure 2) Arterial blood (3.0 ml) from the abdominal aorta (Figure 3) and tissue samples (left liver lobe (Figure 4) and upper pole of right kidney (Figure 5) were collected at the end of the EAc application (T30) and 30 minutes later (T60).

Basal rats (G1) were anesthetized as described. After 30 minutes (T30) the first six animals underwent laparotomy for sample haversting, as described. Thirty minutes later the remaining six rats underwent the same procedures.

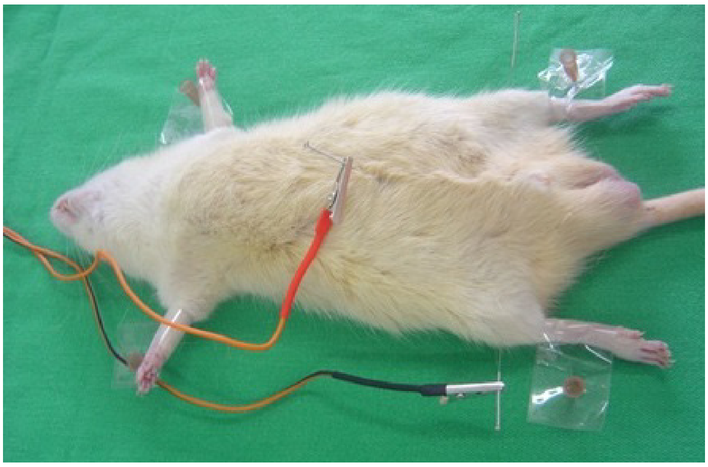

FIGURE 2 – Electroacupuncture (10 and 100 Hz, 10 Ma, 30 minutes) delivered to selected acupoints *Zusanli* (ST-36) and *Zhongwan* (CV-12) in anesthetized rat (G2 rat).

FIGURE 3 – Puncture of abdominal aorta for arterial blood collection at T30 (G1 rat).

FIGURE 4 – Liver left lobe haversting for biochemical analysis (T60, G1 rat).

FIGURE 5 – Upper pole of right kidney haversting for biochemical analysis (T60, G2 rat).

Data analysis

Graphpad Prism 5.0 (GraphPad Software, San Diego California USA, www.graphpad.com) was used for statistical analysis and graphics design. All results were expressed as mean±SD. All data were tested for distribution using the Kolmorogov-Smirnov test with Dallal-Wilkinson-Lilliefor P value). One-way ANOVA followed by *post hoc* analysis (Tukey) was performed to determine differences among groups. A probability value of $p<0.05$ was considered to indicate statistical significance.

Results

No animal died during the experiment. Normal Wistar rats were successfully induced to develop hyperglycemia using ketamine/xylazine anesthesia. Additional anesthesia (50% of original dose) was applied to eight rats 45 minutes after the onset of the experiment (group Control, five rats and group EAc, three rats). No additional anesthesia was required in 28 animals. Mild muscle twitching was observed during 10 Hz EAc. Hind paw contraction occurred in all rats subjected to 100 Hz EAc treatment.

Effects of 10 and 100 Hz EAc

Blood glucose concentrations (mg/dl) decreased significantly (p<0.001) in Group 2 (EAc10Hz) and Group 3 (EAc100Hz) 30 and 60 minutes from the beginning of the experiment, compared with Basal Group. Blood lactate concentrations decreased significantly in G2 and G3 rats, at the end of electrical stimulation (T-30) with 10Hz and 100Hz. Blood lactate concentrations were not different in G2/G3 rats compared with G1 at the end of the study (T60). (Figure 6).

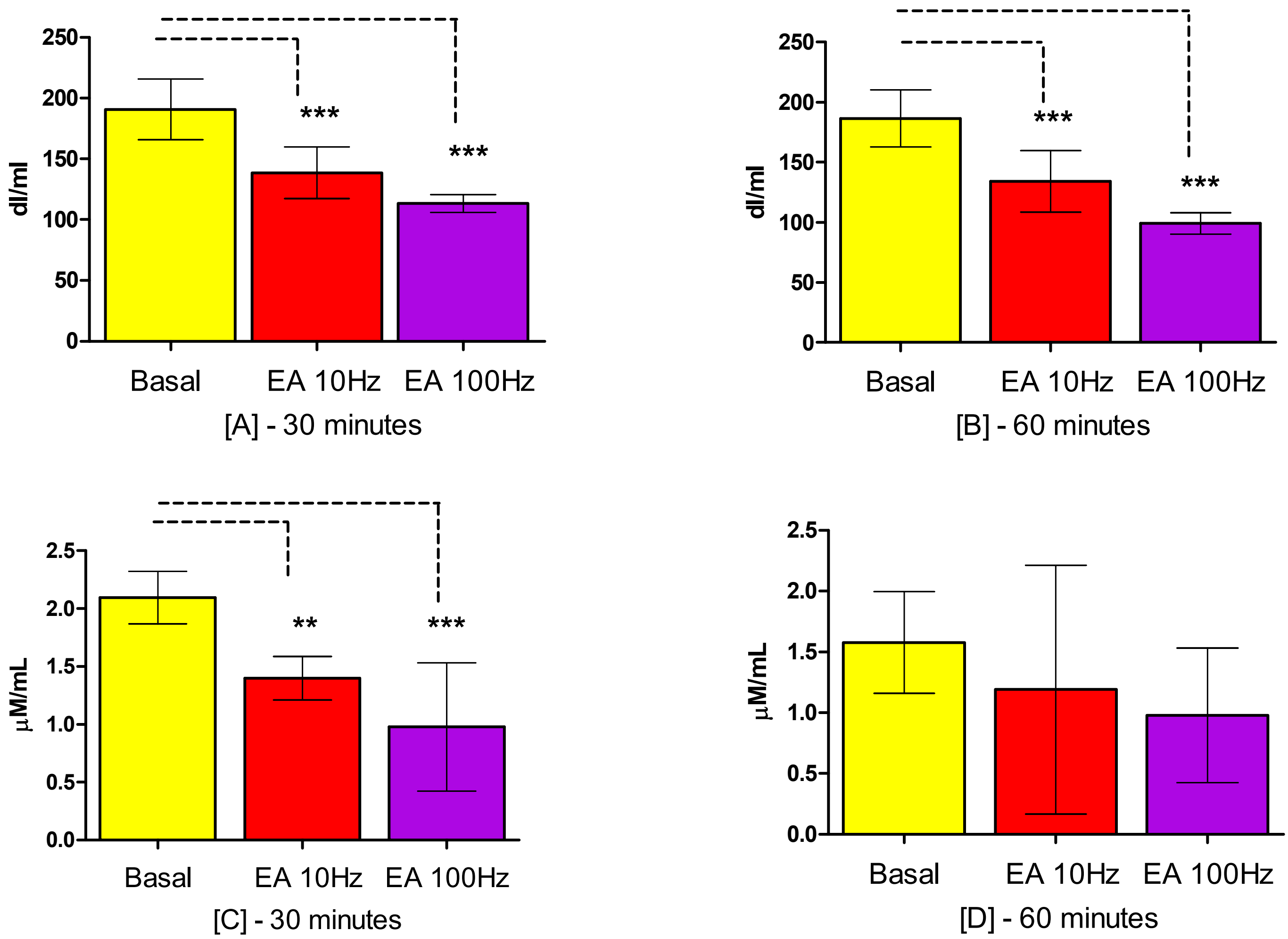

FIGURE 6 – Plasma glucose (**A**,**B**) and lactate (**C**,**D**) levels thirty/sixty minutes after the start of the experiment. Bars represent mean ± SD values for each group (Basal, EA10Hz and EA100Hz). Blood samples were obtained from the abdominal aorta of Basal and EAc-treated groups (10 and 100 Hz) (n=6, each group). Glucose and lactate levels expressed as dl/ml and µMol/ml. ANOVA/Tukey tests.

**p<0.01 compared with Basal; ***p<0.001 compared with Basal Group.

Kidney and liver lactate concentrations decreased significantly (p>0.001) 30 minutes after the end of electrical stimulation (T60) in rats treated with 10 and 100Hz. A similar decrease was observed at the end of the electrical stimulation (T30) in EAc10Hz group (Figure 7).

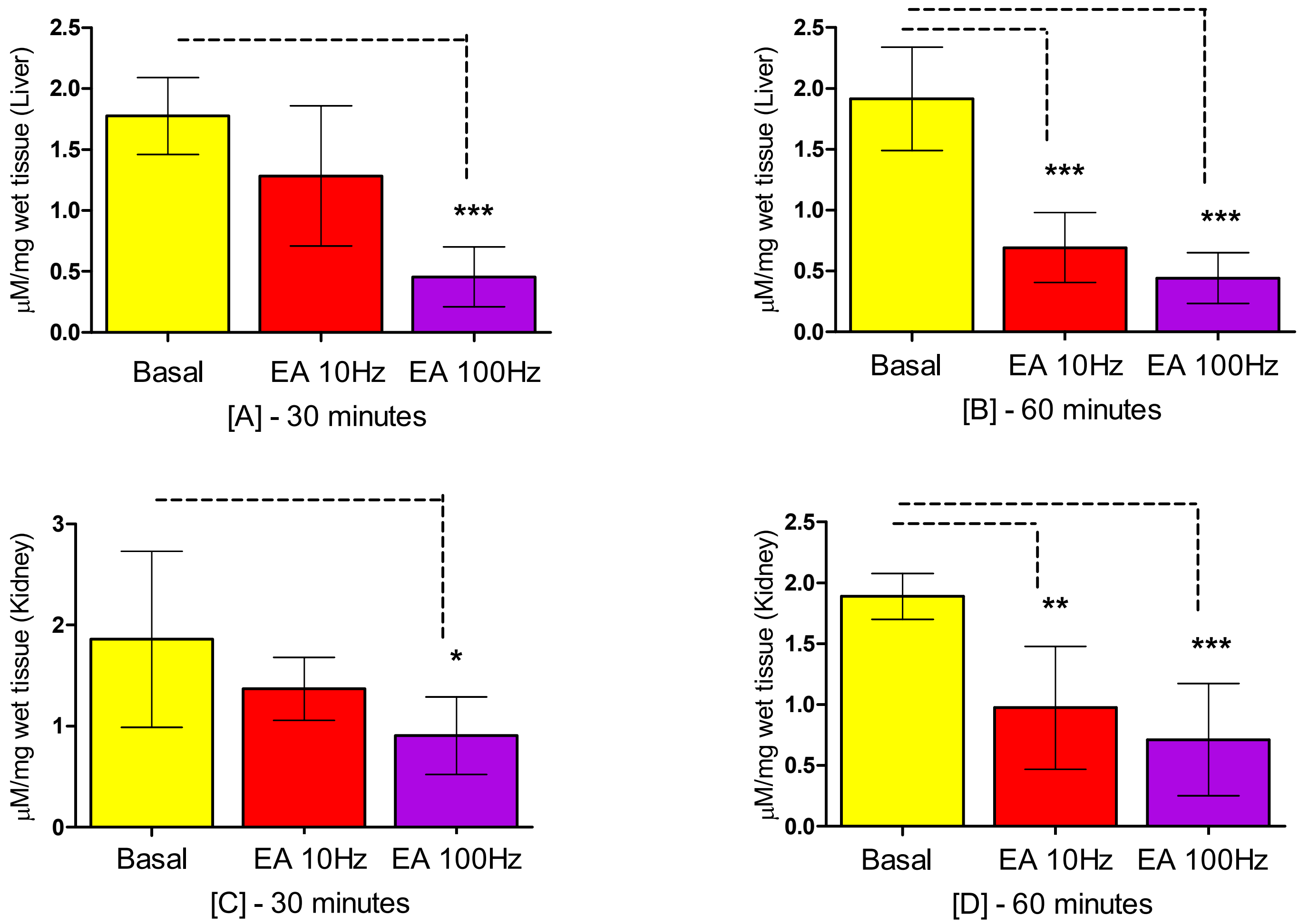

FIGURE 7 – Liver (**A,B**) and kidney (**C,D**) levels thirty/sixty minutes after the start of the experiment. Bars represent mean ± SD values for each group (Basal, EA10Hz and EA100Hz). Tissue samples were obtained from the left lobe of the liver and the upper pole of the right kidney of Basal and EAc-treated groups (10 and 100 Hz) (n=6, each group). Lactate levels expressed as µMol/g of wet tissue. ANOVA/Tukey tests. *p<0.05 compared with Basal; **p<0.01 compared with Basal; ***p<0.001 compared with Basal Group

Discussion

In the present study, the hypoglycemic potential of EAc was demonstrated in induced hyperglycemic rats. Hyperglicemia may be induced experimentally in normal rats with different cytotoxic glucose analogues. Alloxan is known for its selective pancreatic islet ß cell cytotoxicity and has been extensively used to induce a hyperglycemic state in animals[13]. Streptozotocin is a frequently used chemical to induce experimental diabetes in animals. The oxidative stress due to glucose overload may damage the pancreatic beta cells through oxidative stress and inflammation and may be a contributory factor in the pathogenesis of diabetes[14,15].

Recent study has verified that in fed Sprague-Dawley rats, ketamine (100 mg/kg)/xylazine (10 mg/kg) produced acute hyperglycemia within 20 min. The hyperglycemic effect in fed rats was associated with decreased plasma levels of insulin, adrenocorticotropic hormone, and corticosterone and increased levels of glucagon and growth hormone[16].

In order to evaluate the known hypoglycemic activity of EAC we used to different frequencies, 10 and 100 Hz. Previous studies from Chang *et al.*[5] have demonstrated that the use of 15 Hz EAc using bilateral Zusanli acupoints decreases plasma glucose levels. This effect was demonstrated here using lower (10 Hz) and higher (100Hz) frequencies in a single application. One can therefore infer that the power to reduce blood glucose levels is not directly related to the frequencies used. On the other hand, there was a reduction in blood lactate concentrations in rats submitted to the EAC of 10-100 Hz in the T-30 timepoint, suggesting that the resultant hypoglycemia could be related to a greater conversion of lactate for energy production. Since the effect was not persistent (no difference between the levels of lactate at timepoint T60) it can be assumed that the effect of a single acupuncture session, regardless of frequency used, persists during the acupuncture session, ending at the end of the application. However, whereas blood glucose levels remained low after 60 minutes of the beginning of acupuncture that explanation could not be consistent with the results obtained in this study.

EAc regulatory effects on glucose levels in hyperglycemic rats was demonstrated in this study. In Chang *et al*.[5] study, the rats had no ability to secrete insulin and the EAc hypoglycemic effects must have resulted from facilitating exogenous insulin activity or improving insulin sensitivity during insulin challenge test[5]. Some studies have demonstrated that insulin caused the release of the hepatic insulin-sensitizing substance (HISS) from the liver possibly through the activity of the hepatic parasympathetic nerves[17,18]. The involvement of the parasympathetic nerves is also supported by other researchers[19]. However the definite mechanism of action of EAC in lowering glucose levels still needs further studies for better understanding.

Conclusions

Electroacupuncture (10 Hz) applied to St-36 and CV-12 acupoints decreases glycemia and lactacedemia and liver and kidney lactate concentrations. The decrease in lactate levels may be related to greater energy production due to enhanced lactate to pyruvate conversion. Higher frequency (100 Hz) failed to promote the same effect.

References

1. Zhou L, Chey WY. Electric acupuncture stimulates non-parietal cell secretion of the stomach in dog. Life Sci. 1984;34(23):2233-8.
2. Li Y, Tougas G, Chiverton SG, Hunt RH. The effect of acupuncture on gastrointestinal function and disorders. Am J Gastroenterol. 1992;87(10):1372-81.
3. Lux G, Hagel J, Bäcker P, Bäcker G, Vogl R, Ruppin H, Domschke S, Domschke W. Acupuncture inhibits vagal gastric acid secretion stimulated by sham feeding in healthy subjects. Gut. 1994;35(8):1026-9.
4. Lin JG, Chang SL, Cheng JT. Release of beta-endorphin from adrenal gland to lower plasma glucose by the electroacupuncture at Zhongwan acupoint in rats. Neurosci Lett. 2002;326(1):17-20.
5. Chang SL, Lin KJ, Lin RT, Hung PH, Lin JG, Cheng JT. Enhanced insulin sensitivity using electroacupuncture on bilateral Zusanli acupoints (ST 36) in rats. Life Sci. 2006;79(10):967-71.
6. Wang XR, Xiao J, Sun DJ. Myocardial protective effects of electroacupuncture and hypothermia on porcine heart after ischemia/reperfusion. Acupunct Electrother Res. 2003;28(3-4):193-200.
7. Han JS, Chen XH, Sun SL, Xu XJ, Yuan Y, Yan SC, Hao JX, Terenius L. Effect of low- and high-frequency TENS on Met-enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF. Pain. 1991;47(3):295-8.
8. Chen XH, Han JS. Analgesia induced by electroacupuncture of different frequencies is mediated by different types of opioid receptors: another cross-tolerance study. Behav Brain Res. 1992;47(2):143-9.
9. Saha JK, Xia J, Engle SK, Chen YF, Glaesner W, Jakubowski JA. A model of controlled acute hyperglycemia in rats: Effects of insulin and glucagon-like peptide-1 analog. J Pharmacol Exp Ther. 2006;316(3):1159-64.
10. World Health Organization (WHO), 1993: Standard Acupuncture Nomenclature: a brief explanation of 361 classical acupuncture points and their multilingual comparative list. 2nd ed. WHO Regional Office for the Western Pacific.
11. Liu CZ, Yu JC, Zhang XZ, Fu WW, Wang T, Han JX. Acupuncture prevents cognitive deficits and oxidative stress in cerebral multi-infarction rats. Neurosci Lett. 2006;393(1):45-50.
12. Yin CS, Jeong HS, Park HJ, Baik Y, Yoon MH, Choi CB, Koh HG. A proposed transpositional acupoint system in a mouse and rat model. Res Vet Sci. 2008;84(2):159-65.
13. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26.
14. Murugan P, Pari L. Antioxidant effect of tetrahydrocurcumin in streptozotocin-nicotinamide induced diabetic rats. Life Sci. 2006;79(18):1720-8.
15. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7.
16. Saha JK, Xia J, Grondin JM, Engle SK, Jakubowski JA. Acute Hyperglycemia Induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. Exp Biol Med. 2005;230:777-84.
17. Guarino MP, Correia NC, Lautt WW, Macedo MP. Insulin sensitivity is mediated by the activation of the ACh/NO/cGMP pathway in rat liver. Am J Physiol Gastrointest Liver Physiol. 2004;287(3):G527-32.
18. Sadri P, Legare DJ, Takayama S, Lautt WW. Increased incidence of hepatic insulin-sensitizing substance (HISS)-dependent insulin resistance in female rats prenatally exposed to ethanol. Can J Physiol Pharmacol. 2005;83(4):383-7.
19. Shimoju-Kobayashi R, Maruyama H, Yoneda M, Kurosawa M. Responses of hepatic glucose output to electro-acupuncture stimulation of the hindlimb in anaesthetized rats. Auton Neurosci. 2004;115(1-2):7-14.

Correspondence:
Sergio Botelho Guimarães
Rua Professor Costa Mendes, 1608/3º andar, Bloco Didático
60430-140 Fortaleza – CE Brasil
Tel.: (55-85)3366-8083
Fax: (55-85)3366-8064
sergiobotelho@terra.com.br

Conflict of interest: none
Financial source: none

[1]Research performed at Experimental Surgical Research Laboratory (LABCEX), Department of Surgery, Postgraduate Program, Federal University of Ceara (UFC), Brazil.