Electro-acupuncture reduces the need for additional anesthetics in experimental studies

Eletroacupuntura reduz a necessidade de doses adicionais de anestésicos em estudos experimentais

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ABSTRACT

Purpose: To evaluate the possible beneficial effects of electro-acupuncture in rats subjected to ketamine/xylazine (KX) intra-peritoneal (i.p.) anesthesia. Methods: Forty-eight male Wistar rats were distributed in four equal groups. All rats received i.p. injections of ketamine (90 mg/kg) + xylazine (10 mg/kg) anesthesia. Basal values group (control) rats (BV) received no additional treatment. The equivalent of the human right ST36 (Zusanli) and CV-12 (Zhongwan) acupoints were chosen for needling and electrical stimulation. AC rats were needled with sterilized disposable stainless steel needles at right ST36 and CV12 acupoints; needles were retained for 30 minutes. EAC10 rats, after needle insertion as described, had electrodes connected to both needles and to an electro stimulator model NKL EL-608; pulsed square waves, 10 Hz, 10 mA, was applied for 30 minutes. EAC100 rats were submitted to EA as described. However, a greater frequency (100 Hz) was used. Results: Thirty-seven rats remained under adequate anesthetic level during the experiment. However, maintenance anesthesia was required by 11 rats. Need for additional anesthesia decreased to 9.1% in EAC10 rats compared to BV (36.3%). Conclusion: Both the AC and the EAC10/100 prolong the anesthetic effect of the combination Ketamine-xylazine in rats, allowing longer duration of anesthesia with a lower dose of anesthetic, thereby reducing the occurrence of complications.

Key words: Ketamine. Xylazine. Anesthesia. Acupuncture. Eletroacupuncture. Rats.

RESUMO

Objetivo: Avaliar os possíveis efeitos benéficos da eletroacupuntura em ratos submetidos à anestesia intraperitoneal (i.p.) com ketamina / xilazina. Métodos: Quarenta e oito ratos Wistar foram randomizados em quatro grupos iguais. Todos os ratos receberam injeções i.p. de ketamina (90 mg / kg) + xilazina (10 mg / kg). Os ratos do grupo Valores Basais (controle - BV) não receberam nenhum tratamento adicional. Os acupontos equivalentes aos humanos E-36 (Zusanli) e VC-12 (Zhangwan) foram escolhidos para inserção de agulhas e estimulação elétrica. Os ratos do grupo AC foram estimulados com agulhas esterilizadas descartáveis, de aço inoxidável, nos acupontos E-36 direito e VC12. As agulhas foram mantidas por 30 minutos. Nos ratos do grupo EAC10, após agulhamento, como descrito, eletrodos foram conectados às agulhas e ao eletro-estimulador modelo NKL EL-608 e aplicadas ondas quadradas pulsantes, 10 Hz, 10 mA, por 30 minutos. Os ratos do grupo EAC100 foram submetidos à EA como descrito. No entanto, uma maior frequência (100 Hz) foi utilizada. Resultados: Trinta e sete ratos permaneceram no nível anestésico adequado durante o experimento. No entanto, a manutenção da anestesia foi se fez necessária em 11 animais. Nos ratos do grupo EAC100 a necessidade de anestesia complementar diminuiu para 9,1% em comparação com ratos do grupo BV (36,3%). Conclusão: Tanto a AC como a EAC10/100 prolongam o efeito anestésico da combinação ketamina-xilazina em ratos, permitindo maior duração da anestesia com menor dose de anestésico, reduzindo assim a ocorrência de complicações.

Introduction

Acupuncture (AC) is one of the main forms of treatment in traditional Chinese Medicine. It involves the use of sharp, thin needles that are inserted in the body at very specific points. Electroacupuncture (EAC) is a modification of this technique 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.

Acupuncture-induced analgesic effect has been used widely to alleviate pain. Increasing attention has been paid to exploring the physiological and biochemical mechanisms underlying acupuncture analgesia. The identification of brain regions associated with acupuncture analgesia in animal experiments has been confirmed in the human brain by the use of sophisticated examination techniques, such as functional magnetic resonance imaging.

Anesthesia is a prerequisite for surgical animal models. Ketamine is a commonly used short-acting anesthetic and analgesic agent that induces a trance-like anesthetic state known as dissociative anesthesia in both animals and humans. Xylazine is considered safe when used alone or in combination with other anesthetics such as ketamine in animal research. The combination of ketamine/xylazine is used by many researchers for small experimental animal anesthesia including mice and rats.

Methods

Male Wistar rats weighing 250-400 g provided by the Faculty of Medicine Small Animals Breeding Facility (Federal University of Ceará) were kept under controlled environmental conditions (24°C, relative humidity 40%-60%, 12-hour alternate light–dark cycles, food and water ad libitum). The animal protocols were approved by the Committee of Ethics in Animal Research of the Federal University of Ceará. The equivalent of the human right ST36 (Zusanli) and CV-12 (Zhongwan) acupoints were chosen for needling and electrical stimulation. The acupoint nomenclature used follows WHO nomenclature. ST36 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 ST36 acupoint stimulates the lateral sural cutaneous nerve, the cutaneous branch of the saphenous nerve, and deeper, the deep peroneal nerve. CV-12 acupoint is located in the anterior midline of the upper abdomen, 20 mm below the sternal synchondrosis of the rat. This region is innervated by the anterior cutaneous branch of the 8th. intercostal nerve.

Needles (sterilized stainless steel, 0.25 mm in diameter, 3 cm long) where purchased from Lautz, Brazil. EL-608 electro-stimulator was purchased from NKL Produtos Eletrônicos Ltda., Brusque, Santa Catarina, Brazil.

Experimental groups

All experiments were conducted from 9:00-11:00 h. All rats received a freshly-prepared mixture of ketamine (90 mg/kg) +xylazine (10 mg/kg) (KX) i.p. Rats were divided into 4 equal groups as follows:

- Group BV (Basal Values) - 12 rats
- Group AC (Acupuncture) - 12 rats
- Group EAC10 (Electro-acupuncture - EAC 10 Hz) - 12 rats
- Group EAC100 (Electro-acupuncture – EAC 100 Hz) - 12 rats

Group BV rats were anesthetized as described. No additional procedure was carried out.

Group AC rats were anesthetized as described. After routine disinfection with 75% ethanol 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 and retained for 30 minutes.

Group EAC10 – After needle insertion as described, electrodes were connected to both needles and to an electro stimulator model NKL EL-608; pulsed square waves, 10 Hz, 10 mA were applied for 30 minutes.

Group EAC100 - Rats were submitted to EAC as described. However, a greater frequency (100 Hz) was used.

Results

Duration of anesthetic effects

Most animals remained anesthetized throughout the entire duration of the study, typically 1 hr from the onset of the administration of anesthesia. A maintenance dose (50% of the regular dose) of KX (i.p.) was given to 11 rats (Tables 1 and 2) 37-59 min after induction.

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat’s serial number</th>
<th>Weight</th>
<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
<td>4</td>
<td>310</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>315</td>
<td>47</td>
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<tr>
<td></td>
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<td></td>
<td>11</td>
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<td>330</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>365</td>
<td>55</td>
</tr>
<tr>
<td>EAC10</td>
<td>28</td>
<td>295</td>
<td>43</td>
</tr>
<tr>
<td></td>
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<td>325</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>350</td>
<td>42</td>
</tr>
<tr>
<td>EAC100</td>
<td>48</td>
<td>340</td>
<td>59</td>
</tr>
</tbody>
</table>
TABLE 2 – Percentage (%) of rats requiring additional anesthesia before the end of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>% requiring additional anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
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<td>33.1</td>
</tr>
<tr>
<td>AC</td>
<td>12</td>
<td>27.3</td>
</tr>
<tr>
<td>EAC10</td>
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</tr>
<tr>
<td>EAC100</td>
<td>12</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Weight of animals

Rats weights were similar in all groups as no significant differences were statistically demonstrated (Figure 1).

FIGURE 1 - Weight of the rats (gram). Bars represent mean ±SEM of BV (yellow), AC (green), EAC10 (blue) and EAC100 (red) groups. Values are not significantly different from each other group by ANOVA test

Discussion

Ketamine, a dissociative anesthetic, is usually employed in combination with xylazine, which has analgesic, sedative and muscle relaxant effects. The use of KX anesthesia in experimental animals courses with some other side-effects. Recent study in fed Sprague-Dawley rats has demonstrated that ketamine (100 mg/kg) / xylazine (10 mg/kg) (KX) produces acute hyperglycemia (blood glucose 178.4 ± 8.0 mg/dl) within 20 min. The acute hyperglycemic effect of KX in fed rats was associated with decreased plasma levels of insulin, adrenocorticotropic hormone, and corticosterone and increased levels of glucagon and growth hormone. Higher doses (ketamine 100 mg/kg + xylazine 11 mg/kg), induced induced serum hemolysis and elevated glycogenolysis in the liver of rats. Alva et al. demonstrated that ketamine leads to increased plasmatic nitric oxide levels, induces metabolic acidosis, and causes oxidative damage, though without reaching hepatic toxicity.

Electrostimulation-induced analgesia effects are dependent on many parameters, such as frequency, intensity, pulse duration, stimulation location, stimulation duration, size of needles and depth of insertion. Some published studies have focused on the effects of manipulating stimulation frequency. Other studies have addressed the use of different levels of intensity. Huang et al. used a pain model of ‘tail flick latency’ in mice, and showed that incremental increases of stimulation intensity (0.5 - 2 mA) applied at both low (2 Hz) and high (100 Hz) frequencies for a 20-min period produced statistically significant and positive linear analgesic differences compared to a no-stimulation control group. Barlas et al. studied the effect of the stimulation using 2.9 to 9.9 mA applied to healthy volunteers and concluded that low-frequency electroacupuncture applied at a high, but sub-noxious, level of (subjective) intensity, had a significantly larger hypoalgesic effect than placebo stimulation.

Our study utilized a fixed current of 10mA and two high frequencies (10 and 100 Hz). Our aim was to verify if the use of either manual (AC) or electric (EAC) acupoint stimulation would lengthen the analgesic and anesthetic effects of the KX combination. Thirty-seven rats remained under adequate anesthetic level during the experiment. However, maintenance anesthesia was required by 11 rats. Need for additional anesthesia decreased to 9.1% in EAC100 rats compared to BV (36.3%) (Table 2). Study groups were quite homogenous. There was no significant difference in experimental rats weight compared with BV group and to each other.

Conclusion

The data collected support the hypothesis that both the AC and the EAC10/100 acupoint stimulation prolong the anesthetic effect of the combination Ketamine-xylazine in rats, allowing longer duration of anesthesia with a lower dose of anesthetic, thereby reducing the risk of complications.

References

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