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Thiopental alters long term nociceptive response of young rats

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ABSTRACT

The objective of this study was to evaluate the long term nociceptive response determined by use of two general anesthetics, one intravenous and the other inhalatory, in young animals. In the first experiment, the animals of 21 days of age were divided into control (saline) and thiopental (35 mg/kg, i.p.) groups. In the second experiment, rats of the same age were divided in two groups – halothane (2%) and control. In experiment 1, there was difference between groups – reduction of tail-flick latency in the group thiopental (P< 0.05). In experiment 2, there were no differences between groups or interaction between time *versus* group ($F_{(1,19)}$=0.11 for groups, P>0.05; $F_{(1,19)}$=0.032 for the interaction, P>0.05). The results obtained in this study showed that halothane did not alter the nociceptive response in young animals. However, the thiopental induced hyperalgesic response in rats.

Key words: Rats. Sodium thiopental. Halothane. Nociception. Tail flick latency.

INTRODUCTION

Anesthesia in the neonate, infants and children is inevitable in some cases, and recently it has been associated with long-term detrimental side effects (Ririe et al., 2003; Fitzgerald & Beggs, 2001). Previous studies showed that exposure to drugs in early life can induce long-lasting implications for the developing nervous system, such as permanent alterations in pharmacological responses and cell signaling (Stanwood & Levitt, 2004). One of the most discussed topics in neurobehavioral sciences and pediatric anesthesia is the concern of the anesthetics ability to cause widespread cellular death in the developing animal brain (Jevtovic-Todorovic & Olney, 2008; Loepke et al., 2008), which has led some to seriously question the safety of pediatric anesthesia (Olney et al., 2004), while others have cautioned against the direct applicability of animal data to pediatric anesthesia practice (Loepke et al., 2008; Anand, 2007). Recently, data from two epidemiological human studies have suggested an association between anesthesia and surgery in young children and subsequent behavioral abnormalities and learning disabilities (Di Maggio et al., 2009; Wilder et al., 2009).

In addition, the neonatal nervous system is structurally and functionally immature, and significant changes in nociceptive pathways occur before and after birth (Beland & Fitzgerald, 2001). Newborns develop the descending inhibitory pathways later than the excitatory input (Berde & Sethna, 2002). Additionally, neuronal and behavioral responses to noxious stimuli and non-harmful are not under the same control as is observed in an adult organism, and the reason is the immaturity of the circuitry. Early in development, the pup rats features a super-expression of AMPA and kainate receptors, which decreases when it reaches adulthood (Wang & Gondré-Lewis, 2013). In the spinal cord, these receptors appear to be more sensitive in young rat than in the adult (Monyer et al., 1994). In previous study, your group observed a decrease in the nociceptive responses in the first phase of formalin test after fentanyl administration in rats at postnatal day 14

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(P14) (Medeiros et al., 2012). Also, in another study, we demonstrated that neonatal rats are sensitive to low doses of opioids (Rozisky et al., 2008, 2011). Moreover, it has been reported that patients undergoing surgery early in life have more postoperative pain when they are aged between 7 and 13 years, compared with children who underwent surgery in the same age group, but were not operated when they were younger (Caumo et al., 2000).

Taken it all in account, it is relevant to analyze the effect of anesthetics in the early life when pain pathways are immature. Thus, the objective of this study was to evaluate the effect of administration of general anesthetics, one intravenous and the other inhalatory, in the nociceptive response of young rats.

MATERIALS AND METHODS

Animals

Male and female Wistar rats (n=56), with 21-days-old at the beginning of the experiment, were used. It was chosen the age of 21 days (P21: postnatal day 21), because the neurodevelopmental stage of rats in this period is similar to that observed in child of one year old (Fitzgerald & Anand, 1993) when the physiological state is immature (Pattinson & Fitzgerald, 2004).

The animals were housed in groups of 5 in home cages made of polypropylene (49x34x16 cm), with the floor covered with sawdust. They were maintained on a standard 12-h dark/light cycle (lights on 7:00 a.m.) at room temperature (22 ± 2°C), with free access to food and water.

The Institutional Research Committee (protocol number 03-448) approved all animal procedures that were planed to minimize pain and discomfort.

Nociceptive response

Nociception was assessed with the tail-flick apparatus (D'amour & Smith, 1941). Twenty-four hours before the experiment, the animals were exposed to the apparatus to familiarize them with the procedure, since the novelty can itself induce anti-nociception (Netto et al., 1987). Rats were wrapped in a towel and placed on the apparatus; the light source positioned below the tail was focused on a point 2.3 cm rostral to the tip of the tail. Deflection of the tail activated a photocell and automatically terminated the trial. In the day of the experiment, the animal was submitted to the same procedure, before and after to be exposed to the proposed anesthetic drugs. Light intensity was adjusted so as to obtain a baseline Tail Flick latency (TFL) of 3 to 4 seconds (s). A cut-off time of 10 s was used to prevent tissue damage.

Drugs and chemicals

Sodium thiopental (powder for injection, 1 gram diluted in solution NaCl 0.9%, 35 mg/kg, via i.p.) and halothane (250 ml flask for inhalation) were purchased from Cristália (São Paulo-SP). The doses were used according previous studies (Muthuraman & Singh, 2011, Bankar et al., 2013)

Experimental design

In the first experiment, the male rats were divided into two groups that received saline solution (NaCl 0.9% - CONT, n = 19) or sodium thiopental (THIO, n = 18). TFL measures were taken once time before anesthesia (BASAL), and once a week for four weeks (TFL1 to TFL4).

In the second experiment, the male and female rats were also divided into two groups. The anesthetized group (HALO, n = 10) was exposed to 2% halothane (Cristália®), delivered via a nose cone, during 2 minutes, before the tail flick latency (TFL) measures. Control group (CONT, n = 9) was only exposed to the environment and submitted to the TFL measures.

The animals were put on a warm mattress to avoid hypothermia, a concern in animals submitted to inhalatory anesthesia. TFL measures were taken once time before anesthesia (BASAL), and once a week for four weeks (TFL1 to TFL4).

Male and females were used, since in the baseline we did not find any differences in tail flick latency between them.

Statistical analysis

Data were expressed as mean + SEM. For evaluation of the basal measurements, Student t test was used. For the comparison of the tail-flick latencies in the different periods of time, repeated measures ANOVA were performed followed by multiple comparisons test (Bonferroni), when indicated. Differences were considered statistically significant if $P<0.05$.

RESULTS

In both experiments (Figures 1 and 2), there were no differences between groups at the baseline measures: control (5.15 ± 0.44 s) *versus* thiopental (4.49 ± 0.43 s); and control (5.28 ± 0.34 s) *versus* halothane (4.51 ± 0.43) (Student t test for all, $P>0.05$).

In the thiopental experiment (Figure 1), there was difference between groups ($F_{(1,37)}=23.22$; $P<0.05$; repeated measures ANOVA, followed by Bonferroni test). It was observed difference between the times, and interaction time *versus* group ($F_{(1,37)}=120.17$ for time, $P<0.05$; $F_{(1,37)}=5.86$ for interaction, $P<0.05$; repeated measures ANOVA, followed by Bonferroni test). In the control group, there were also differences among the periods of time ($P<0.05$; repeated measures ANOVA, followed by Bonferroni test).

In the halothane experiment, it was observed difference in the tail flick latencies among the time points – baseline, TFL3, and TFL4, in both groups ($F_{(1,19)}=48.72$; $P<0.05$; repeated measures ANOVA, followed by Bonferroni test) (Figure 2). However, there were no differences between the two groups evaluated ($F_{(1,19)}=0.000$, $P>0.05$) or interaction between groups *versus* time ($F_{(1,19)}=1.07$, $P>0.05$) (repeated measures ANOVA, followed by Bonferroni test).

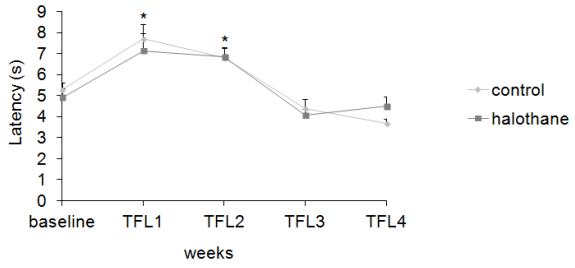

Figure 1. The weekly time effect of the thiopental administration on nociception response, evaluated by Tail-Flick Test.

TFL measures were taken before anesthesia (BASAL) and once a week for seven weeks (TFL1 to TFL7). Latency in seconds (s) after the thiopental administration i.p. (35mg/kg, n=18) and control (n=19). Data were expressed as mean + SEM.

Significant difference of TFL in times marked from baseline and TFL 4 in tiopenthal group (repeated measures ANOVA, followed by Bonferroni test, P< 0.05).

* Difference between the two groups (repeated measures ANOVA, followed by Bonferroni test, P< 0.05).

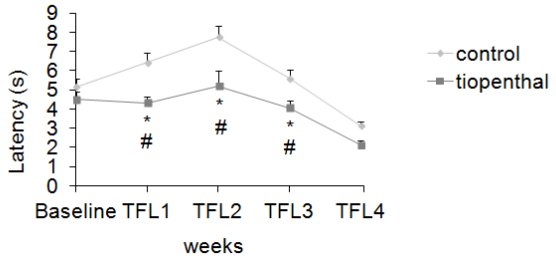

Figure 2. The effect of the inhaled anesthetic halothane on nociception response, evaluated by Tail-Flick Test.

Tail-Flick latency (TFL), expressed in seconds (s), in the halothane (2% by inhalation, n=10) and control (n=9) groups. Data were expressed as mean + SEM.

* Significant different from baseline, TFL3, and TFL4 measures in both groups (repeated measures ANOVA, followed by Bonferroni test, P< 0.05).

Male and females were used in halothane experiment, since in the baseline we did not find any differences in tail flick latency between them ($F_{(1,19)}$= 2.65; P>0.05, Student t test). And the same effects were observed when it was analyzed separately female and male animals. In female analysis, it was not observed difference for group [F(1,11)=2.87, P>0.05] or for interaction between groups *versus* time [F(1,11)=2.13, P>0.05]. However there was significant difference in times TFL3 and TFL4 [F(1,11)=6.06; P< 0.05; repeated measures ANOVA, followed by Bonferroni test]. In male analysis, it was not observed difference for group [F(1,8)=0.005, P>0.05] or for interaction between groups *versus* time [F(1,8)=1.00, P>0.05]. And again there was difference in times TFL3 and TFL4 [F(1,8)=35.69; P< 0.05; repeated measures ANOVA, followed by Bonferroni test] (Data not shown).

DISCUSSION

This study showed that young rats (P21) anesthetized with sodium thiopental presents a nociceptive response that lasted 3 weeks. Sodium thiopental is a barbiturate that acts on central nervous system (CNS), suppressing synaptic responses (Mihic et al., 2011). Facilitation is decreased and the inhibition is increased (Mihic et al., 2011). The increased inhibition occurs primarily in the synapses mediated by GABA neurotransmission, acting specifically on GABA-A receptors. Activation of these receptors leads to the increase in the Cl- conductance and a reduction of Ca2+ currents (Patel et al., 2011). Barbiturates, as thiopental, are GABAergic agonists, leading to decreased brain activity and metabolism. Its adverse effects include respiratory depression and hypothermia (Patel et al., 2011). Hyperalgesia associated with the use of sodium thiopental in rats has been described previously for the intravenous administration of lower doses (Tatsuo et al., 1997; Archer et al., 1994). But there is no report in the literature of persistent hyperalgesia for weeks, as noted in our study.

The cause of the reduction of the tail-flick latency, observed with the administration of thiopental, is not clear. One possibility is that hyperalgesia is, at least in part, a result of stress caused by barbiturate-induced hypothermia. Exposure to cold can change the nociceptive threshold, raising it or decreasing it (Imbe et al., 2006). It is known that exposure to stressors can alter the increased nociceptive threshold, producing analgesia induced by stress (stress-induced analgesia) (Menendez et al., 1993; Amir & Amit, 1978), or it can decrease the nociceptive threshold, producing hyperalgesia (Torres et al., 2003). The occurrence of one or another effect depends on the type of stressor and the duration of treatment (Torres et al., 2002; López et al., 1999). In our study, the animals exposed once to sodium thiopental took two hours to the recuperation. One hypothesis is that the hypothermia eventually produced by the anesthesia could have been sufficient to produce hyperalgesia, even using heated mattress during the recovery period.

Another explanation for the observed hyperalgesia may be related to the action of thiopental sodium on central neurotransmitter systems, mainly the GABAergic system. In a study of the effect of GABAergic activation upon the pain threshold, it was observed that non-hypnotic doses of phenobarbital, pentobarbital and thiopental induced hyperalgesia, verified by testing withdrawal latency of the tail. The effect was reversed by previous administration of a non-convulsant dose of the GABA antagonist, picrotoxin, highlighting the role of the GABA-A receptor in hyperalgesia (Tatsuo et al., 1997). However, long-term effects related to the barbiturate require additional experiments.

The increase in tail flick latencies over time, observed in animals of the control groups of both experiments, may be associated to the functional maturation of inhibitory modulation of descending pathways, which are not

functional until P21 in the rat (Fitzgerald, 2005), and the maturation of the local circuit inhibitory interneurons in the dorsal horn. The descending inhibitory pathways, which come from the brainstem, only establish synapses in the dorsal horn a few weeks after birth (Morton, 1998). It was shown that electrical stimulation of the PAG only produces analgesia in rats from P21 (Van Praag & Frenk, 1991). PAG neurons project to the raphe nuclei act directly inhibiting neurons or projection of exciting inhibitory interneurons (Millan, 2002). The P27 excitatory postsynaptic currents in miniature are five times larger than those of inhibitory interneurons (Dahlhaus et al., 2005). Another explanation may be related to the fact that, while the animals grow, their tails get more keratin and become thicker. Thus, while keeping the same light intensity, the tail flick occurs more rapidly in younger children. Thus, the tail flick is slower in older, when the tail is thicker.

For another side, halothane did not alter the nociceptive response, since control and anesthetized groups did not differ at any measures. However, the results showed changes in nociceptive threshold during development, in these two groups, which was different from the observations reported in literature (Falcon et al., 1996). Previous reports showed increased nociceptive response earlier in the animal development, around postnatal day 15 (P15), and decreased response in P21. These results are attributed to progressive functional maturation of spinal synaptic transmission of nociceptive information (Falcon et al., 1996). And, in the literature, data suggest that halothane could act potentiating GABA-mediated inhibition (Nicoll et al., 1975; Scholfield, 1980). Here we report an increased nociceptive response after P21. Factors that are contributing to our results could be investigated, but one possibility is the maturation level of descending modulation (Fitzgerald, 2005), which is not functional until P21.

In our study we utilized females and males. Although, we do not find any se differences in tail-flick measures between the genders. There is increasingly greater recognition of sex differences in response to noxious stimuli and to analgesic drugs, which can be observed both in humans and in laboratory animals (Berkley, 1997; Mogil & Chanda, 2005). These differences are likely caused by many factors, including genes, gonadal hormones, and environmental and sociocultural factors (Berkley, 1997).

The results obtained in this study suggest that halothane did not alter the nociceptive response in early life. However, the thiopental use induced hyperalgesic response. The mechanisms that explained how this kind of response occurs should be investigated in further studies.

In addition, our findings highlight the importance of extending the investigation of the effect of drug administration in early life into adulthood life. And, this study may be used as a basis for future research that aims to understand the mechanisms of action and side effects of drugs used in infant rats.

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Conflict of Interest

There is no financial relationship between any of the authors or any commercial interest in the outcome of this study.

RESUMO

Tiopental altera a resposta nociceptiva de ratos jovens em longo prazo

O objetivo desse estudo foi avaliar a resposta nociceptiva a longo prazo relacionada ao uso de dois anestésicos gerais – um intravenoso e outro inalatório, em animais jovens. No primeiro experimento, os animais de 21 dias de idade foram divididos nos grupos controle (solução salina) e tiopental sódico (35 mg/kg, i.p.). No segundo experimento, animais de mesma idade foram divididos em dois grupos – halotano (2%) e controle. No Experimento 1, houve redução da latência de retirada da cauda no grupo tiopental (P<0,05). No Experimento 2, não houve diferença entre os grupos ou interação entre grupo x tempo ($F_{(1,19)}$=0,11 para grupos, P>0,05; $F_{(1,19)}$=0,032 para a interação, P>0,05). Os resultados obtidos nesse estudo demonstraram que o halotano não altera a resposta nociceptiva em animais jovens. Entretanto, o tiopental induziu resposta hiperalgésica nestes ratos.

Palavras-chave: Ratos. Tiopental sódico. Halotano. Nocicepção. Latência de retirada da cauda.

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