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Role of muscarinic and nicotinic cholinergic receptors in an experimental model of epilepsy-induced analgesia

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Abstract

The blockade of GABA-mediated Cl^- influx with pentylenetetrazol (PTZ) was used in the present work to induce seizures in animals. The neurotransmission in the postictal period has been the focus of many studies, and there is evidence suggesting antinociceptive mechanisms following tonic–clonic seizures in both animals and men. The aim of this work was to study the involvement of acetylcholine in the antinociception induced by convulsions elicited by peripheral administration of PTZ (64 mg/kg). Analgesia was measured by the tail-flick test in eight albino Wistar rats per group. Convulsions were followed by significant increases in tail-flick latencies (TFLs) at least for 120 min of the postictal period. Peripheral administration of atropine (0.25, 1 and 4 mg/kg) caused a significant dose-dependent decrease in the TFL in seizing animals, as compared to controls. These data were corroborated by peripheral administration of mecamylamine, a nicotinic cholinergic receptor blocker, at the same doses (0.25, 1 and 4 mg/kg) used for the muscarinic cholinergic receptor antagonist. The recruitment of the muscarinic receptor was made 10 min postconvulsions and in subsequent periods of postictal analgesia, whereas the involvement of the nicotinic cholinergic receptor was implicated only after 30 min postseizures. The cholinergic antagonists caused a minimal reduction in body temperature, but did not impair baseline TFL, spontaneous exploration or motor coordination in the rotarod test at the maximal dose of 4 mg/kg. These results indicate that acetylcholine may be involved as a neurotransmitter in postictal analgesia.

Keywords: Postictal analgesia; Pentylenetetrazol; $GABA_A$ receptor; Acetylcholine; Muscarinic receptor; Nicotinic receptor; Epilepsy; Pain

1. Introduction

The neuroanatomical basis of analgesia and the neural substrates involved in the generation and propagation of epileptic activity in the central nervous system are the focus of many investigations in the field of neuroscience and experimental neurology (Frenk et al., 1978; Basbaum and Fields, 1984; Guieu et al., 1992; Garcia-Cairasco et al., 1996; Rosa et al., 1998; Dos Santos et al., 2000).

Recent findings have demonstrated antinociceptive processes in experimental models of pentylenetetrazol (PTZ)- or electroshock-induced seizures, in which opioid, serotonergic 5-HT_2 and cholinergic receptors may be involved (Coimbra et al., 2001a; Portugal-Santana et al., 2004). Other recent investigations have used invasive methods for studying the neural bases of experimental epilepsy (Peterson et al., 2000; Omori et al., 2001). In fact, some structures and neural networks of the brainstem, such as the periaqueductal gray matter, the dorsal raphe nucleus, the nucleus raphe magnus, the anterior pretectal nucleus, the nucleus reticularis gigantocellularis, pars alpha, have endogenous opioid-, monoamine- and acetylcholine-mediated mechanisms involved in the control of pain (Basbaum and Fields,

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1984; Rosa et al., 1998; Guimarães and Prado, 1999; Oliveira and Prado, 2001; Leite-Panissi et al., 2003), in the elaboration of defensive behavior (Monassi et al., 1997; Eichenberger et al., 2002, Osaki et al., 2003; Monassi and Menescal-de-Oliveira, 2004; Borelli et al., 2004) and epilepsy (Brodie and Proudfit, 1984; Ribak et al., 1997; Peterson et al., 2000; Azami et al., 2001; Omori et al., 2001).

Many of these nuclei are interconnected and send projections to the dorsal horn of the spinal cord controlling the synaptic connection between the first and the second neurons of the spinal–thalamic nociceptive pathways (Basbaum and Fields, 1984; Roychowdhury and Fields, 1996; Azami et al., 2001). In addition, the cholinergic system in the dorsal horn of the spinal cord is involved in antinociception, and a recent report showed that neuronal bodies of the dorsal horn express the muscarinic acetylcholine receptor (Stewart and Maxwell, 2003). In fact, muscarinic and nicotinic cholinergic receptor subtypes are involved in peripheral and central antinociception (Sahley and Berntson, 1979; Bartolini et al., 1992; Marubio et al., 1999; Wess et al., 2003). Neuronal nicotinic receptors appear to function at both pre- and postsynaptic membranes to modulate the release of acetylcholine and to mediate synaptic neurotransmission, respectively, in cell culture (Girod et al., 2003) and also in the spinal cord (Kommalage and Höglund, 2003).

A mapping of presynaptic nicotinic cholinergic receptor has been recently described (Girod et al., 2003), and cholinergic receptors are involved in many behavioral functions (Oliveira and Prado, 1994; Leite-Panissi et al., 2003), as well as in antinociceptive mechanisms (Metys et al., 1969; Guimarães and Prado, 1999; Li et al., 2002) and epilepsy (Berdiev et al., 2003). In addition, cholinergic neurons of the medial septum that project to the hippocampus, the cingulate cortex and the entorhinal cortex (Metys et al., 1969) receive inputs from a variety of brainstem and midbrain areas that participate in defensive behavior, arousal and neurovegetative reactions (De Lima and Rae, 1991), as well as in antinociceptive responses (Basbaum and Fields, 1984). These cholinergic pathways may represent a neural hodology eventually implicated in the generation and propagation of epilepsy (Peterson et al., 2000) and in the control of the perception of nociceptive stimuli during and after convulsive reactions in mammals (Coimbra et al., 2001a). In fact, PTZ-induced seizures in immature rats provokes endogenous acetylcholine-induced interictal-like discharges in adult hippocampal CA3 (Meilleur et al., 2003), and muscarinic analgesia may be mediated by M_2 and M_4 cholinergic receptors at both spinal and supraspinal structures (Duttaroy et al., 2002).

In this work, we demonstrate the effect of peripheral pretreatment with the specific muscarinic pharmacological antagonist atropine, and a nicotinic cholinergic receptor blocker, mecamylamine, on postictal analgesia induced by seizures elicited by a systemic blockade of Cl^- channels linked to GABAergic synapses in the central nervous system.

2. Materials and methods

2.1. Subjects

Male albino Wistar rats (n=6–8, per group), weighing 200–250 g, from the animal facility of the Campus of Ribeirão Preto of the University of São Paulo (USP) were used. They were housed in groups of four per cage, under a 12:12 dark/light cycle (lights on at 0700 h) at 23±1 °C, and given free access to food and water throughout the experiment. All experiments reported in this work were performed in compliance with the recommendation of the Brazilian Society for Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Nociceptive testing

All rats had their nociceptive thresholds compared using the tail-flick test. Each animal was placed in a restraining apparatus (Stoelting, Wood Dale, IL) with acrylic walls, and its tail was placed in a heating sensor (tail-flick Analgesia Instrument; Stoelting), whose calorimetric progressive elevation was automatically interrupted at the moment in which the animal removed its tail from the apparatus. The current raised the temperature of the coil (Ni/Cr alloy; 26.04 cm in length and 0.02 cm in diameter) at the rate of 9 °C/s (Prado and Roberts, 1985), starting at room temperature (approximately 20 °C). A small current intensity adjustment could be made, if necessary, at the beginning of the experiment, aiming to obtain three consecutive tail-flick latencies (TFLs) between 2.5 and 3.5 s. If the animal did not remove its tail from the heater within 6 s, the apparatus was turned off to prevent damage to the skin. Three baselines of control TFLs were taken at 5-min intervals. TFLs were also measured following seizures elicited by peripheral administrations of PTZ.

Behavioral tests were made by placing the rats in the interior of a circular arena, whose walls, made of transparent acrylic, measured 60 cm in diameter and 50 cm in height. This arena was located in an experimental compartment and illuminated by a fluorescent lamp (350 lx at the arena floor level). The evaluation of the effects of drug administration (PTZ, atropine, mecamylamine and saline) was made with the rats inside the arena.

3. Procedure for epileptic and antinociceptive reactions record

First, a baseline of the tail-flick test was recorded in every animal of each group. Independent groups received

either intraperitoneal (IP) administration of saline, PTZ (64 mg/kg), saline+saline or saline+PTZ. After the pretreatment, the animals were placed in the arena until the end of seizures (PTZ) or during 10 min (after saline), when their withdrawal reflexes (TFL) were determined. There were three different groups pretreated with saline+PTZ as independent control groups for random comparison with each pharmacological treatment (PTZ, atropine and mecamylamine groups).

Independent groups of animals received peripheral injections of atropine or mecamylamine followed by IP PTZ (64 mg/kg) after 10 min. The nociceptive responses (TFL) were measured immediately after seizures, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min after the convulsive reactions.

3.1. Rotarod test

The motor performance of the rodents was evaluated in the rotarod test for rats (Ugo Basile Rota-Rod, Stoelting). After receiving IP injections of physiological saline, saline+saline, PTZ (64 mg/kg), saline+PTZ, the animals were taken to the rotarod treadmill (62.5×50×45 cm), rotating at a gradually increasing speed from 11 to 18 rpm for 120 s and maintained for another 30-s period at 18 rpm, in which the time spent on cylinder was recorded in independent groups of animals after seizures (or 10 min after saline administration) and 10, 20, 30, 40, 60, 90, 120, 150 and 180 min after the IP administration of saline (NaCl; 0.9%), saline+saline, PTZ (64 mg/kg) or saline+PTZ (64 mg/kg). Other groups of animals were pretreated with IP administration of physiological saline+saline, mecamylanine (4 mg/kg)+saline or atropine (4 mg/kg)+saline, and they were submitted (after 15 min) to the same rotarod device described above. In these last groups, the time of falling, vertical and horizontal movements were recorded in only one measurement, taking into account that cholinergic mechanisms may influence visual attention, cognition and spatial working memory (Gold, 2003; Bentley et al., 2004; Elvander et al., 2004).

3.2. Core temperature record

The changes produced by the IP administration of cholinergic antagonists were measured by means of a digital pen-type thermometer (CE0044; Pro Check), with measurement accuracy of approximately 0.1 °C between 34 and 42 °C at ambient temperature of 18 to 28 °C. The clinical thermometer (tip with 0.6×0.3 cm) was carefully inserted in the rectal channel of the animals (n=6). Three baselines of control body temperature were taken at 5-min intervals. The animals then were pretreated with physiological saline+saline, atropine (4 mg/kg)+saline or mecamylamine (4 mg/kg)+saline. Fifteen minutes after the pharmacological treatment, the rectal temperature was recorded.

3.3. Drugs

Pentylenetetrazol (Sigma), atropine (Sigma) and mecamylamine (Sigma) were each dissolved in physiological saline (NaCl; 0.9%) shortly before use. Physiological saline also served as the vehicle control.

Drugs were administered in the following doses: atropine (0.25, 1 and 4 mg/kg), mecamylamine (0.25, 1 and 4 mg/kg) and PTZ (64 mg/kg).

3.4. Analysis of results

Data from experiments in which there were repeated measures of TFL were submitted to a repeated-measure MANOVA. In case of significant treatment×time interaction, one-way ANOVA followed by the Duncan test at each time interval were performed. Data from experiments performed to evaluate the effect of cholinergic antagonists on the motor performance in the rotarod test and core temperature were submitted to a one-way ANOVA and to paired Student's t-test, respectively. A level of $p<0.05$ was used to confirm statistically significant differences.

4. Results

PTZ induced severe tonic–clonic seizures in all animals. Convulsions were not preceded by wild running, and lasted from 1 up to 130 s, with an average of 89.7 s, considering the total number of crises (Table 1).

Control animals, submitted to the tail-flick test and placed also in the experimental situation for 10 min without receiving any type of drug afterwards, did not display significant changes in the nociceptive threshold at the time studied ($p>0.05$ in all cases).

There was a significant effect of treatment [$F(3,28)=902.16$; $p<0.001$], time [$F(9,20)=367.42$; $p<0.001$] and treatment×time interaction [$F(27,56)=119.66$; $p<0.001$]. One-way ANOVA showed a significant treatment effect from 0 to 120 min [$F(3,28)$ varying from 15.56 to 1234.16; $p<0.0001$; η^2 and estimate of explained variance ranging from 4.72 to 76.88 and from 0.63% to 1.01%, respectively]. Post hoc analyses showed that the saline pretreatment, used in another experimental group, also did not induce any significant change in the nociceptive threshold during the time observed, from 10 min after saline administration to 180 min after the drug, when compared to the control group (Fig. 1).

However, after the generalized tonic–clonic seizures induced by PTZ, we observed a strong analgesia, supported by increased TFLs recorded immediately after the end of seizures and from 10 to 120 min after seizures. The effect of the seizures on TFLs did not cause analgesia after 150 min post tonic–clonic convulsions, when compared to the control group (Fig. 1). There also was a significant treatment effect on TFLs after the saline

Table 1
Behavioral characterization, incidence and duration of tonic–clonic seizures evoked by intraperitoneal administration of pentylenetetrazol (64 mg/kg)

	Latency (s) of tonic–clonic seizures	Number and duration (s) of each tonic–clonic seizure				Total time (s) of seizures	Intensity of tonic–clonic seizures				Period of time (min) from the first to last seizure
		First	Second	Third	Fourth		First	Second	Third	Fourth	
Rat I	90	50	5	–	–	55	Intermediate	Weak	–	–	4
Rat II	60	30	50	40	–	120	Intermediate	Weak	Intermediate	–	10
Rat III	240	1	3	1	5	10	Weak	Weak	Weak	Weak	4
Rat IV	60	43	27	–	–	70	Weak	Weak	–	–	3
Rat V	60	21	25	25	–	77	Weak	Weak	Weak	–	4
Rat VI	86	130	16	–	–	146	Strong	Weak	–	–	5
Rat VII	120	21	45	84	–	150	Weak	Intermediate	Intermediate	–	7

Blank spaces in the table mean absence of seizure.

pretreatment followed 10 min later by peripheral administration of PTZ. In fact, the pretreatment with the vehicle of the pharmacological antagonists, followed by the ionophore blockade of Cl^- channel linked to GABA receptor, made as an additional control in this experiment, showed an expressive postictal analgesia recorded immediately after the end of seizures and from 10 to 120 min. These data are shown in Fig. 1.

Considering the experiments made with the aim of investigating the involvement of muscarinic cholinergic receptors in postictal analgesia, there was a significant effect of treatment [$F(3,27)=23.13$; $p<0.001$] and time [$F(9,19)=105.80$; $p<0.001$] and a significant treatment×time interaction [$F(27,53)=9.20$; $p<0.001$]. One-way ANOVA showed a significant treatment effect from 10 to 120 min [$F(3,27)$ varying from 4.34 to 24.83; $p<0.05$; η^2 and estimate of explained variance ranging from 1.2 to 29.44 and from 0.33% to 0.73%, respectively]. Post hoc analyses showed that the peripheral administration of atropine at the minor doses (0.25 or 1 mg/kg) used in the present work decreased TFLs 40 min (atropine: 0.25 mg/kg) and 30 min (atropine: 1 mg/kg) after seizures, as well as from 60 to 120 min postseizures. However, atropine pretreatment at the highest dose (4 mg/kg) was effective in antagonizing the postictal analgesia recorded from 10 to 120 min postseizures. Post hoc analyses also showed significant differences between the three doses of muscarinic antagonist from 10 to 60 min. These data followed a dose-dependent effect and are shown in Fig. 2.

These results were corroborated by peripheral administration of mecamylamine, but the blockade of nicotinic cholinergic receptors was effective in antagonizing the postictal analgesia recorded only in later stages, as compared to the effect of the blockade of muscarinic receptors. There was a significant effect of treatment [$F(3,28)=23.93$; $p<0.01$] and time [$F(9,20)=544.67$; $p<0.001$] and a significant treatment×time interaction [$F(27,56)=4.61$; $p<0.001$]. One-way ANOVA showed significant treatment effects from 10 to 120 min [$F(3,28)$ varying from 6.69 to 16.66; $p<0.01$; η^2 and estimate of

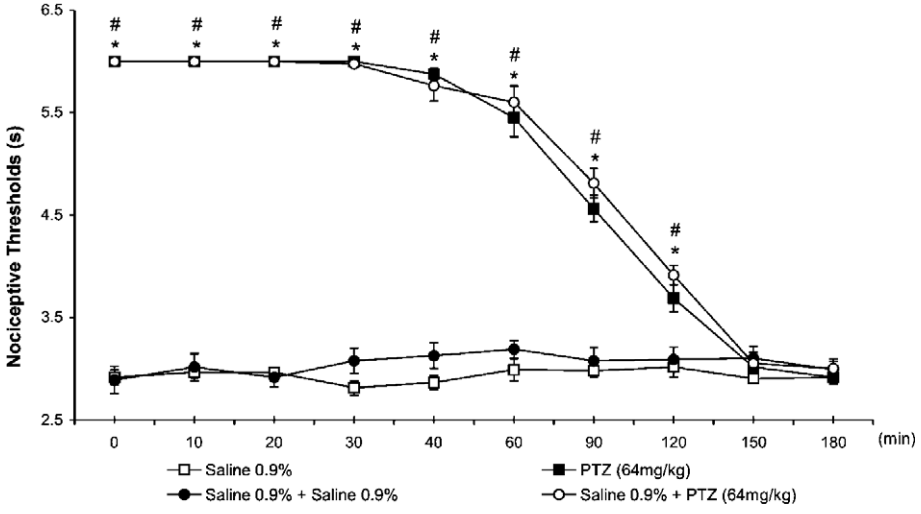

Fig. 1. Effects of administration of saline (□–□), pentylenetetrazol (PTZ; ■–■; 64 mg/kg, i.p.), saline (NaCl; 0.9%)+saline (●–●), and saline+PTZ (○–○) on nociceptive threshold. N=8 in all groups. Increases in nociceptive thresholds were presented in means of tail-flick latencies±S.E.M. *Statistically significant differences ($p<0.05$, according to Duncan test) as compared to physiological saline group; #Statistically significant differences as compared to saline+saline group.

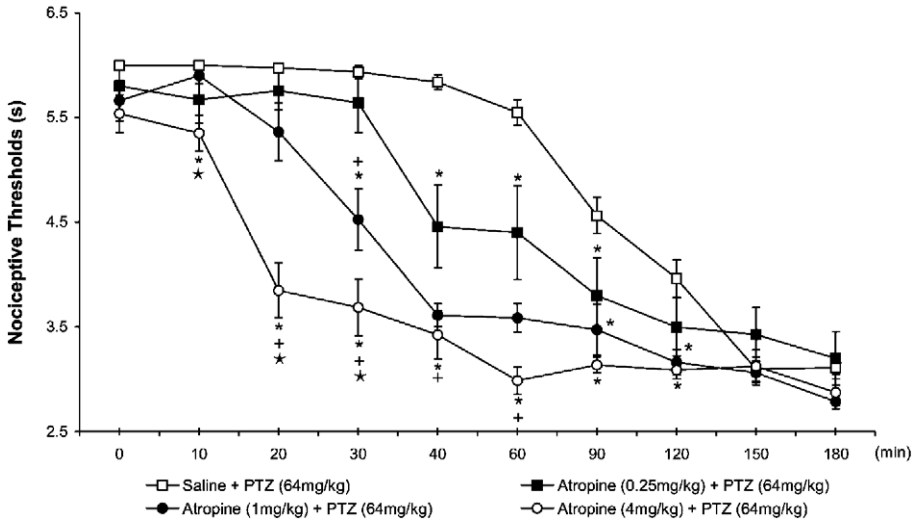

Fig. 2. Effect of administration of saline (NaCl; 0.9%) followed by pentylenetetrazol (PTZ; □–□; 64 mg/kg, i.p.), and effect of peripheral (i.p.) injections of atropine at doses of 0.25 mg/kg (■–■; N=7), 1 mg/kg (●–●; N=8) or 4 mg/kg (○–○; N=8), followed by PTZ (64 mg/kg, i.p.) on nociceptive threshold. *Statistically significant differences (p<0.05, according to Duncan test) as compared to saline+PTZ group; $^{+}$Statistically significant differences as compared to 0.25 mg/kg atropine+PTZ group; ★Statistically significant differences as compared to 1 mg/kg atropine plus PTZ group. Increases in nociceptive thresholds were presented in means of tail-flick latencies±S.E.M.

explained variance ranging from 2.34 to 11.31 and from 0.42% to 0.64%, respectively]. Post hoc analyses showed that there was a lack of effect of both the minor doses (0.25 or 1 mg/kg) and the highest dose (4 mg/kg) of mecamylamine in antagonizing the postictal analgesia recorded immediately after seizures or recorded at 10 or 20 min after convulsive reactions, in all cases, although the blockade of nicotinic cholinergic receptors (at both 1 and 4 mg/kg) was effective in antagonizing the postictal analgesia from 30 to 120 min postseizures. There were significant statistical differences between doses from 30 to 90 min of the postictal period. Particularly in the group treated with mecamylamine at 4 mg/kg, the convulsive reactions after the blockade of GABA-mediated Cl^{-} influx with PTZ were more expressive. These data are shown in Fig. 3.

These effects are not independent of the epileptic crisis induced by the blockade of the GABA-mediated Cl^{-} influx, because the peripheral administration of atropine or mecamylamine at the highest dose (4 mg/kg) did not cause any statistically significant effect on nociceptive thresholds [p>0.05 in all cases, as compared to the control; F(2,21) varying from 0.38 to 3.44; p>0.05 in all cases; η^2 and estimate of explained variance ranging from 0.14 to 0.93 and from 0.03% to 0.34%, respectively; Fig. 4].

The evaluation of the motor performance of animals submitted to the rotarod test showed no statistically

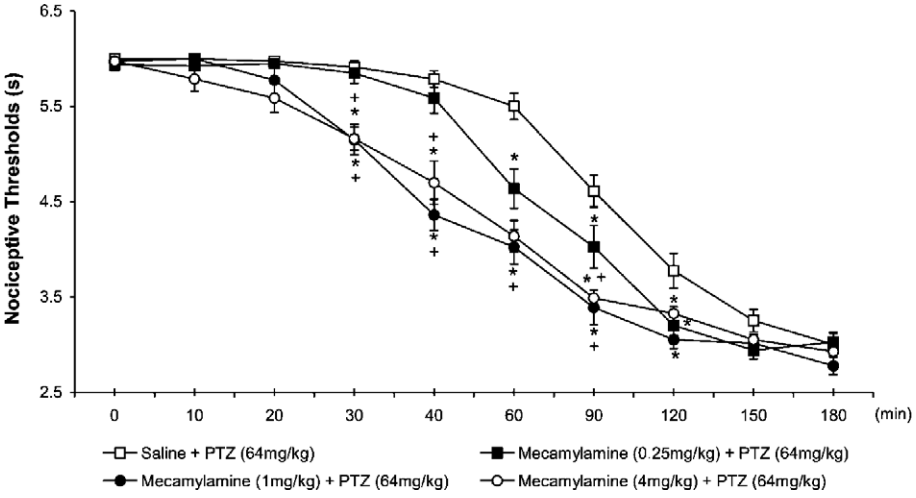

Fig. 3. Effects of administration of saline followed by pentylenetetrazol (PTZ; □–□; 64 mg/kg, i.p.), and effect of peripheral (i.p.) injections of mecamylamine at doses of 0.25 mg/kg (■–■; N=8), 1 mg/kg (●–●; N=8) or 4 mg/kg (○–○; N=8), followed by PTZ (64 mg/kg, i.p.) on nociceptive threshold. *Statistically significant differences (p<0.05, according to Duncan test) as compared to saline+PTZ group; $^{+}$Statistically significant differences as compared to 0.25 mg/kg mecamylamine+PTZ group. Increases in nociceptive thresholds were presented in means of tail-flick latencies±S.E.M.

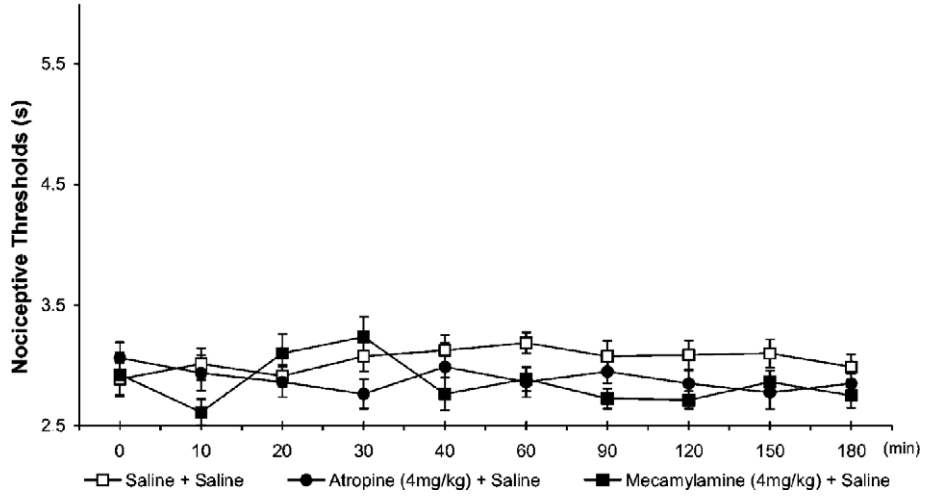

Fig. 4. Lack of effect of peripheral (i.p.) administration of saline (NaCl; 0.9%)+saline (□–□), atropine (4 mg/kg)+saline (●–●), and mecamylamine (4 mg/kg)+saline (■–■) on nociceptive thresholds of Wistar rats (N=8; p>0.05 in all cases, according to Duncan test, as compared to controls). Data were presented as means of tail-flick latencies±S.E.M.

significant difference among TFLs observed immediately after seizures and from 10 to 180 min of the postictal period [F(3,28) varying from 0.20 to 2.52; p>0.05 in all cases; η^2 and estimate of explained variance ranging from 8.84 to 75.59 and from 0.02% to 0.21%, respectively; Fig. 5].

Both atropine (at 4 mg/kg)+ physiological saline (Student's t-test: t=4.70; p<0.01) and mecamylamine (at 4 mg/kg)+saline (Student's t-test: t=2.58; p=0.049) groups, but not the saline+saline group (Student's t-test: t=2.13; p>0.05) showed a very small but significant decrease in core temperature. One-way ANOVA showed significant differences between delta values [F(2,15)=4.19; p<0.05; η^2=3.62; estimate of explained variance=0.36%; Table 2].

The pretreatment of animals with cholinergic antagonists at the highest dose (4 mg/kg) did not affect their motor performance in the rotarod test. In fact, atropine caused a significant increase in the time spent on the rotarod [F(2,15)=4.41; p<0.05; η^2=82194.78; estimate of explained variance=0.37%] and in horizontal [F(2,15)=4.73; p<0.05; η^2=94693.44; estimate of explained variance=0.39%], but not vertical motor behavior [F(2,15)=1.43; p>0.05; η^2=34.33; estimate of explained variance=0.16%]. The mecamylamine-treated group did not differ from controls. These data are shown in Table 2.

5. Discussion

In the current work, PTZ caused tonic–clonic seizures, followed by antinociception, and acetylcholine may be involved as a neurotransmitter in this antinociceptive process induced by GABA-mediated Cl^- influx. Interestingly, a recent report showed that pretreatment with bicuculline partially prevented nicotine-induced antinociception, suggesting that the $GABA_A$ receptor may contribute to the mechanisms involved in cholinergic-induced

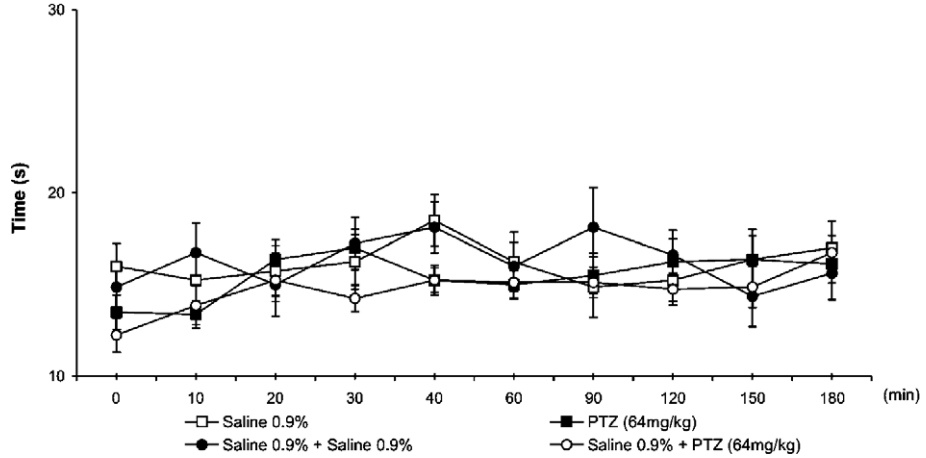

Fig. 5. Lack of effect of peripheral (i.p.) administration of saline (NaCl; 0.9%; □–□), saline+saline (●–●), pentylenetetrazol (PTZ; ■–■; 64 mg/kg. i.p.), and saline+PTZ (○–○) on motor performance of Wistar rats in the rotarod test (N=8; p>0.05 in all cases, according to Duncan test, as compared to the controls). Data were presented as means of time (seconds) spent on the cylinder±S.E.M.

Table 2
Effects of atropine and mecamylamine administered 15 min before testing on motor activity, rotarod performance and body temperature in *Rattus norvegicus*

	n	Vertical activity counts	Horizontal activity counts	Latency to fall (s)	Rectal temperature (°C)		Rectal temperature (Δ°C)
					Control	After drugs	
Saline+saline	6	4.67±2.18	57.5±40.64	64.83±42.75	36.61±0.14	37.17±0.17	0.55±0.26
Atropine (4 mg/kg)+saline	6	1.33±0.42	234.00±28.54*	229.68±36.34*	36.68±0.12	36.27±0.15#	−0.43±0.009*
Mecamylamine (4 mg/kg)+saline	6	2.50±1.02	163.33±50.27	160.33±38.88	36.90±0.22	35.22±0.59#	−0.37±0.37*

Data were presented as means±S.E.M. The control absolute values of the rectal temperature correspond to the average of three rectal temperatures recorded at 5-min intervals.

* Significantly different from saline ($p<0.05$), according to one-way ANOVA, followed by Duncan test.

\# Significantly different from control, according to Student's *t*-test for dependent samples.

analgesia (Mui et al., 1997), and the GABAergic system may modulate nicotinic receptor-mediated seizures (Dobelis et al., 2003).

Our results strongly suggest the involvement of the cholinergic system in seizure-induced analgesia. Acetylcholine seems to be released as a neurotransmitter in the synaptic connectivity of the neural network responsible for the elaboration of postictal analgesia in the initial steps of this phenomenon. Both muscarinic and nicotinic receptors are recruited in this postictal antinociceptive process. Although there is no evidence of a crucial involvement of the nicotinic receptor in the initial stage of postictal analgesia, this cholinergic receptor seems to be recruited in a later stage, after the first 30 min of postconvulsive antinociceptive reactions. However, the muscarinic receptor may be recruited shortly after the elicitation of tonic–clonic convulsive reactions.

The present work suggests the involvement of the muscarinic cholinergic receptor in this initial postictal period and is in accordance with previous findings obtained in our laboratory suggesting that, during postictal analgesia, acetylcholine and 5-HT_2/5-HT_{1C} monoaminergic mechanisms may be initially recruited after tonic–clonic convulsions, while endogenous opioid peptides, such as enkephalin and β-endorphin, would have a place in a later stage of postseizure antinociception, considering the first 30 min after tonic–clonic seizures (Coimbra et al., 2001a).

Curiously, after 30 min of postictal analgesia, mecamylamine at the intermediate dose (1 mg/kg) produced a stronger effect compared to the highest dose (4 mg/kg). This paradoxical effect may be related to the severity of convulsive reactions observed in the group of animals pretreated with the highest dose of this nicotinic cholinergic receptor antagonist.

Considerable evidence now points to muscarinic and nicotinic receptors as targets of cholinergic control of the perception of nociceptive stimuli in other kinds of antinociceptive reactions in both experimental animal and human models (Decker and Meyer, 1999; Irusta et al., 2001). There is also evidence that many of these mechanisms are centrally mediated in different species (Grau et al., 1991; Oliveira and Prado, 1994; Leite-Panissi et al., 2003).

The lack of effect of the pretreatment with muscarinic or nicotinic antagonists on TFLs recorded immediately after seizures cannot be related to a sudden motor deficit caused by either postictal depression or an eventual unexpected influence of cholinergic receptor blockade on motor ability, considering that previous treatment with saline, PTZ, atropine or mecamylamine+saline did not cause a motor deficit in the rotarod test. Interestingly, both cholinergic antagonists at the highest dose used in the present work increased the time spent on the rotarod. These data are corroborated by previous experiments investigating the possible effect of PTZ and cholinergic compounds on motor tasks (Decker et al., 1998; Coimbra et al., 2001a).

In addition, the lack of effect of pretreatment with mecamylamine and atropine (at all doses used in the present work) in postictal antinociception at the first period of seizure-induced analgesia does not seem to be related to postictal depression effects, as it has been demonstrated that acetylcholine may contribute to epileptogenesis in immature neocortex (Potier and Psarropoulou, 2001).

Although recent studies implicated the cholinergic regulation of body temperature (Takahashi et al., 2001a,b), the positive effect of the pharmacological antagonists used in the present work on postictal analgesia cannot be related to the small decrease in core body temperature, eventually affecting the responsiveness to the painful stimulus, considering that the pretreatment of the animals with the highest dose of each antagonist used in this work did not alter baseline TFLs compared to control.

In fact, previous findings suggest that decreases in skin temperature do not influence the peripheral effect of cholinergic antagonists, such as atropine and mecamylamine, on TFLs, considering that mecamylamine but not atropine antagonizes carbachol-induced antinociception from the dorsal periaqueductal gray matter, without significant changes in TFLs baseline values (Guimarães and Prado, 1999).

Nicotinic receptors do not only exist on neuronal cell bodies and dendrites but are also located on axon terminals and are involved in the modulation of multiple neurotransmitter releases (Wonnacott et al., 1989; Todorov et al., 1991; Wilkie et al., 1996; Vizi and Lendvai, 1999). Thus,

other chemical mediators may be involved in postictal analgesia in the different steps of this process.

We cannot discount the involvement of coreleased neurotransmitters in the organization of postictal analgesia. In fact, a recent report showed that presynaptic muscarinic cholinergic mechanisms and $GABA_B$ receptors may influence cholinergic analgesia (Li et al., 2002; Rashid and Ueda, 2002). In addition, spinal endogenous acetylcholine may mediate at least part of the analgesic effect of systemic morphine through both muscarinic and nicotinic receptors (Chen and Pan, 2001).

It is possible that different opioid systems, probably μ_1-opioid receptor-mediated, may be involved in the delayed stage of postictal analgesia, as suggested in previous experiments using PTZ- or electro-convulsive shock-induced analgesia (Urca et al., 1981; Coimbra et al., 2001b; Portugal-Santana et al., 2004).

The present results, suggesting that muscarinic and nicotinic cholinergic receptors are involved in the modulation of painful stimulus-induced reactions during epileptic-like attacks, are in agreement with previous reports showing that scopolamine blocks both brief shock-induced and conditioned hypoalgesia on the tail-flick test (Grau et al., 1991).

The involvement of the cholinergic system is also related to the generation and elaboration of epilepsy. In fact, a recent report suggests that the muscarinic M_1 receptor may have a critical role in the development of kindling epileptic activity (Eşkazan et al., 1999), and the involvement of this receptor on the initiation of cholinergically induced epileptic seizures in the brain was already considered by another study (Cruickshank et al., 1994).

However, we do not exclude the possibility of involvement of other neuronal circuitry on the elaboration of postictal analgesia and epilepsy. The varicose monoaminergic, glutamatergic and cholinergic axons are equipped with neuronal nicotinic acetylcholine receptors. These nonsynaptically localized receptors are of high affinity, and the receptor activation itself causes depolarization of the neuron, releasing chemical transmitter or may result in modulation of neurotransmitter release initiated by axonal firing (Wonnacott et al., 1989).

The modulation of presynaptic nicotinic receptors in synaptic and nonsynaptic neurochemical circuitry in the brain may provide a cholinergic mechanism recruiting other nuclei of the brainstem involved with monoaminergic descending control of the pain and activating complex neuronal assemblies that elaborate epileptic activity in the central nervous system and postictal analgesia (Coimbra et al., 1998a,b; Coimbra et al., 2001a,b; Shouse et al., 2001).

The nucleus raphe magnus may contribute at least partly in this complex neural circuitry (Abe et al., 2002; De Oliveira, 2003), and cholinergic excitatory innervations of the neurons of this brainstem structure may be important in the regulation of pain sensitivity (Brodie and Proudfit, 1984). In fact, a recent report showed immunohistochemical evidence suggesting the involvement of nonserotonergic brainstem neurons involved in picrotoxin-induced analgesia (Koyama et al., 2000).

The dorsal horn of the spinal cord is another crucial target for the action of cholinergic antagonists, considering strong evidence of the involvement of the spinal muscarinic M_4 receptor subtype in cholinergic mechanisms of analgesia (Mulugeta et al., 2003), as well as the property of the nicotinic acetylcholine receptor in stimulating spinal norepinephrine release (Li and Eisenach, 2002).

Despite the interesting report showing that nociceptive threshold seems to be spontaneously high in patients with temporal lobe epilepsy (Guieu et al., 1992), many patients with either occipital lobe epilepsy or temporal lobe epilepsy have related headache (Ito et al., 1999, 2000, 2004). In fact, headache and epilepsy are a common comorbidity in childhood, and occur mostly in children older than 10 years with idiopathic epilepsy (Yamane et al., 2004). The paradoxical findings reported above reinforce the importance of future research on the neurochemical bases of pain- and analgesia-induced mechanisms after tonic–clonic seizures.

In this respect, the study of the acetylcholine-mediated system in seizure-induced antinociception is relevant to understanding the neural hodology and the chemical mediation of the postictal phenomenon. The complete elucidation of the neurochemistry of the antinociceptive process evoked by convulsive crises may represent an important step for understanding the neural basis of the control of pain- and epilepsy-related mechanisms.

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References

Abe K, Ishida K, Kato M, Shigenaga T, Taguchi K, Miyatake T. Effect of morphine-induced antinociception is altered by AF64A-induced lesions on cholinergic neurons in rat nucleus raphe magnus. Acta Pharmacol Sin 2002;23;961–6.

Azami J, Green DL, Roberts MHT, Monhemius R. The behavioral importance of dynamic activated descending inhibition from the nucleus reticularis gigantocelularis pars alpha. Pain 2001;92;53–62.

Bartolini A, Ghelardini C, Fantetti L, Malcangio M, Malmberg-Aiello P, Giotti A. Role of muscarinic receptor subtypes in central antinociception. Br J Pharmacol 1992;105;77–82.

Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 1984;7;309–38.

Bentley P, Husain M, Dolan RJ. Effects of cholinergic enhancement on visual stimulation, spatial attention, and spatial working memory. Neuron 2004;41;969–82.

Berdiev RK, Chepurnov SA, Chepurnova NE, van Luijtelaar EL. Cholinergic mechanisms in the pathogenesis of genetically-caused absence epilepsy. Usp Fiziol Nauk 2003;34;20–30.

Borelli KG, Nobre MJ, Brandão ML, Coimbra NC. Effects of acute and chronic fluoxetine and diazepam on freezing behavior induced by electrical stimulation of dorsolateral and lateral columns of the periaqueductal gray matter. Pharmacol Biochem Behav 2004;77; 557–66.

Brodie MS, Proudfit HK. Hypoalgesia induced by the local injection of carbachol into the nucleus raphe magnus. Brain Res 1984;291;337–42.

Chen SR, Pan HL. Spinal endogenous acetylcholine contributes to the analgesic effect of systemic morphine in rats. Anesthesiology 2001; 95;525–30.

Coimbra NC, Castro-Souza C, Garcia-Cairasco N. Neuroanatomical and neurophysiological study of post-ictal antinociceptive processes in experimental models of epilepsy. Arq Neuro-Psiquiatr 1998a;56;91–2 [Abstracts].

Coimbra NC, Castro-Souza C, Segato EN. Neurotransmission in the post-ictal analgesia: A psychopharmacological study. Int J Psychophysiol 1998b;30;125.

Coimbra NC, Castro-Souza C, Segato EN, Nora JEP, Herrero CFPS, Tedeschi-Filho W, et al. Post-ictal analgesia: involvement of opioid, serotoninergic and cholinergic mechanisms. Brain Res 2001a;888; 314–20.

Coimbra NC, Freitas RL, Savoldi M, Castro-Souza C, Segato EN, Kishi R, et al. Opioid neurotransmission in the post-ictal analgesia: Involvement of μ_1-opioid receptor. Brain Res 2001b;903;216–21.

Cruickshank JW, Brudzynski SM, McLachlan RS. Involvement of M_1 muscarinic receptors in the initiation of cholinergically induced epileptic seizures in the rat brain. Brain Res 1994;643;125–9.

Decker MW, Meyer MD. Therapeutic potential of neuronal nicotinic acetylcholine receptor agonists as novel analgesics. Biochem Pharmacol 1999;58;917–23.

Decker MW, Bannon AW, Buckley MJ, Kim DJB, Holladay MW, Ryther KB, et al. Antinociceptive effects of the novel neuronal nicotinic acetylcholine receptor agonist, ABT-594, in mice. Eur J Pharmacol 1998;346;23–33.

De Lima TC, Rae GA. Effects of cold-restraint and swim stress on convulsions induced by penthylenetetrazol and electroshock: influence of naloxone pretreatment. Pharmacol Biochem Behav 1991;40; 297–300.

De Oliveira RC. Involvement of the nucleus raphe magnus/gigantocellularis complex and of 5-HT_2 receptor in the post-ictal analgesia MSc Thesis (in Portuguese), School of Philosophy, Sciences and Literature of Ribeirão Preto of the University of Sáo Paulo (USP), Ribeirão Preto (SP), Brazil, 2003;1;1-79.

Dobelis P, Hutton S, Lu Y, Collins AC. GABAergic systems modulate nicotinic receptor-mediated seizures in mice. J Pharmacol Exp Ther 2003;306;1159–66.

Dos Santos NF, Arida RM, Trindade-Filho EM, Priel MR, Cavalheiro EA. Epileptogenesis in immature rats following recurrent status epilepticus. Brain Res Rev 2000;32;269–76.

Duttaroy A, Gomeza J, Gan JW, Siddiqui N, Basile AS, Harman WD, et al. Evaluation of muscarinic agonist-induced analgesia in muscarinic acetylcholine receptor knockout mice. Mol Pharmacol 2002;62; 1084–93.

Eichenberger GCD, Ribeiro SJ, Osaki MY, Maruoka RY, Resende GCC, Castellan-Baldan L, et al. Neuroanatomical and psychopharmacological evidence for interaction between opioid and GABAergic neural pathways in the modulation of fear and defense elicited by electrical and chemical stimulation of the deep layers of the superior colliculus and dorsal periaqueductal gray matter. Neuropharmacology 2002;42;48–59.

Elvander E., Schott P.A., Sandin J., Bjelke B., Kehr J., Yoshitake T., et al. Intraseptal muscarinic ligands and galanin: influence on hippocampal acetylcholine and cognition. Neuroscience 2004;126;541–57.

Eşkazan E, Aker R, Onat F, Köseoğlu S, Gören MZ, Hasanoğlu A. Effect of pirenzepine, a muscarinic M_1 receptor antagonist, on amygdala kindling in rat. Epilepsy Res 1999;37;133–40.

Frenk H, MacCarty BC, Liebeskind JC. Different brain areas mediate the analgesic and epileptic properties of enkephalin. Science 1978;200; 335–7.

Garcia-Cairasco N, Wakamatsu H, Oliveira JAC, Gomes ELT, del Bel EA, Mello LEAM. Neuroethological and morphological (neo-Timm staining) correlates of limbic recruitment during the development of audiogenic kindling in seizure susceptible Wistar rats. Epilepsy Res 1996;26;177–92.

Girod R, Jareb M, Moss J, Role L. Mapping of presynaptic nicotinic acetylcholine receptors using fluorescence imaging of neuritic calcium. J Neurosci Methods 2003;122;109–22.

Gold PE. Acetylcholine modulation of neural systems involved in learning and memory. Neurobiol Learn Mem 2003;80;194–210.

Grau JW, Illich PA, Chen PS, Meagher MW. Role of cholinergic systems in pain modulation: I Impact of scopolamine on environmentally induced hypoalgesia and pain reactivity. Behav Neurosci 1991;105;62–81.

Guieu R, Mesdjian E, Roger J, Dano P, Pouget J, Serratice G. Nociceptive threshold in patients with epilepsy. Epilepsy Res 1992;12;57–61.

Guimarães AP, Prado WA. Pharmacological evidence for a periaqueductal gray-nucleus raphe magnus mediating the antinociception induced by microinjecting carbachol into dorsal periaqueductal gray of rats. Brain Res 1999;827;152–9.

Irusta AEC, Savoldi M, Kishi R, Resende GCC, Freitas RL, Carvalho AD, et al. Psychopharmacological evidences for the involvement of muscarinic and nicotinic cholinergic receptors on sweet substance-induced analgesia in *Rattus norvegicus*. Neurosci Lett 2001;305; 115–8.

Ito M, Nakamura F, Honma H, Takeda Y, Kobayashi R, Miyamoto T, et al. A comparison of post-ictal headache between patients with occipital lobe epilepsy and temporal lobe epilepsy. Seizure 1999;8;343–6.

Ito M, Nakamura F, Honma H, Takeda Y, Kobayashi R, Miyamoto T, et al. Clinical factors associated with post-ictal headache in patients with epilepsy. Acta Neurol Scand 2000;102;129–31.

Ito M, Adachi N, Nakamura F, Koyama T, Okamura T, Kato M, et al. Characteristics of postictal headache in patients with partial epilepsy. Cephalalgia 2004;24;23–8.

Kommalage M, Höglund AU. Nicotinic acetylcholinergic receptors regulate the intraspinal release of acetylcholine in male rats. Pharmacol Toxicol 2003;93;169–73.

Koyama N, Nishio T, Yokota T. Non-serotonergic midbrain neurons are involved in picrotoxin induced analgesia An immunohistochemical study in the rat. Neurosci Lett 2000;291;147–50.

Leite-Panissi CRA, Coimbra NC, Menescal-de-Oliveira L. The cholinergic stimulation of the central amygdala modifying the tonic immobility response and antinociception in guinea pigs depends on the ventrolateral periaqueductal gray. Brain Res Bull 2003;60;167–78.

Li X, Eisenach JC. Nicotinic acetylcholine receptor regulation of spinal norepinephrine release. Anesthesiology 2002;96;1450–6.

Li DP, Chen SR, Pan YZ, Levey AI, Pan HL. Role of presynaptic muscarinic and GABA(B) receptors in spinal glutamate release and cholinergic analgesia in rats. J Physiol 2002;543;807–18.

Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. Nature 1999;398;805–10.

Meilleur S, Aznavour N, Descarries L, Carmant L, Mamer OA, Psarropoulou C. Pentylenetetrazol-induced seizures in immature rats provoke long-term changes in adult hippocampal cholinergic excitability. Epilepsia 2003;44;507–17.

Metys J, Wagner N, Metysová J, Herz A. Studies on the central antinociceptive action of cholinomimetic agents. Int J Neuropharmacol 1969;8;413–25.

Monassi CR, Menescal-de-Oliveira L. Serotonin 5-HT2 and 5-HT1A receptors in the periaqueductal gray matter differentially modulate tonic immobility in guinea pig. Brain Res 2004;1009;169–80.

Monassi CR, Hoffmann A, Menescal-de-Oliveira L. Involvement of the cholinergic system and periaqueductal gray matter in the modulation of tonic immobility in the guinea pig. Physiol Behav 1997;62;53–9.

Mui WC, Zbuzek VK, Wu WH. $GABA_A$ antagonist and nicotine-induced antinociception. Pharmacol Lett 1997;61;221–5.

Mulugeta E, El-Bakri N, Karlsson E, Elhassan A, Adem A. Loss of muscarinic M_4 receptors in spinal cord of arthritic rats: implications for a role of M_4 receptors in pain response. Brain Res 2003;982;284–7.

Oliveira MA, Prado WA. Antinociception and behavioral manifestations induced by intracerebroventricular or intra-amygdaloid administration of cholinergic agonists in the rat. Pain 1994;57;383–91.

Oliveira MA, Prado WA. Role of PAG in the antinociception evoked from the medial or central amygdala in rats. Brain Res Bull 2001;54;55–63.

Omori N, Ishimoto T, Mutoh F, Chiba S. Kindling of the midbrain periaqueductal gray in rats. Brain Res 2001;903;162–7.

Osaki MY, Castellan-Baldan L, Calvo F, Carvalho AD, Felippotti TT, de Oliveira R, et al. Neuroanatomical and neuropharmacological study of opioid pathways in the mesencephalic tectum: effect of μ_1- and κ-opioid receptor blockade on escape behavior induced by electrical stimulation of the inferior colliculus. Brain 2003;179–92.

Peterson SL, Armstrong JJ, Walker MK. Focal microinjection of carbachol into the periaqueductal gray induces seizures in the forebrain of the rat. Epilepsy Res 2000;42;169–81.

Portugal-Santana P, Doretto MC, Tatsuo MAKF, Duarte IDG. Involvement of prolactin, vasopressin and opioids in post-ictal antinociception induced by electroshock in rats. Brain Res 2004;1003;1–8.

Potier S, Psarropoulou C. Endogenous acetylcholine facilitates epileptogenesis in immature rat neocortex. Neurosci Lett 2001;302;25–8.

Prado WA, Roberts MH. An assessment of the antinociceptive and aversive effects of stimulating identified sites in the rat brain. Brain Res 1985; 340;219–28.

Rashid MH, Ueda H. Neuropathy-specific analgesic action of intrathecal nicotinic agonists and its spinal GABa-mediated mechanism. Brain Res 2002;953;53–62.

Ribak CE, Manio AL, Navetta MS, Gall CM. In situ hybridization for c-*fos* mRNA reveals the involvement of the superior colliculus in the propagation of seizure activity in genetically epilepsy-prone rats. Epilepsy Res 1997;26;397–406.

Rosa MLNM, Oliveira MA, Valente RB, Coimbra NC, Prado WA. Pharmacological and neuroanatomical evidence for the involvement of the anterior pretectal nucleus in the antinociception induced by stimulation of the dorsal raphe nucleus in rats. Pain 1998;74;171–9.

Roychowdhury SM, Fields HL. Endogenous opioids acting at a medullarly μ-opioid receptor contribute to the behavioral antinociception produced by GABA antagonism in the midbrain periaqueductal gray. Neuroscience 1996;74;863–72.

Sahley TL, Berntson GG. Antinociceptive effects of central and systemic administrations of nicotine in the rat. Psychopharmacology 1979;65; 279–283.

Shouse MN, Staba RJ, Ko PY, Saquib SF, Farber PR. Monoamines and seizures: microdialysis findings in locus coeruleus and amygdala before and during amygdala kindling. Brain Res 2001;892;176–92.

Stewart W, Maxwell DJ. Distribution of and organization of dorsal horn neuronal cell bodies that possess the muscarinic m_2 acetylcholine receptor. Neuroscience 2003;119;121–35.

Takahashi A, Ishimaru H, Ikarashi Y, Kishi E, Maruyama Y. Opposite regulation of body temperature by cholinergic input to the paraventricular nucleus and supraoptic nucleus in rats. Brain Res 2001a;909;102–11.

Takahashi A, Ishimaru H, Ikarashi Y, Kishi E, Maruyama Y. Hypothalamic cholinergic regulation of body temperature and water intake in rats. Auton Neurosci 2001b;94;74–83.

Todorov L, Windisch K, Sershen H, Lajtha A, Papasova M, Vizi ES. Prejunctional nicotinic receptors involved in facilitation of stimulation-evoked noradrenaline release from the vas deferens of the guinea-pig. Br J Pharmacol 1991;102;186–90.

Urca G, Yitzhaky J, Frenk H. Different opioid systems may participate in post-electro-convulsive shock (ECS) analgesia and catalepsy. Brain Res 1981;219;385–96.

Vizi ES, Lendvai B. Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. Brain Res Rev 1999;30;219–35.

Wess J, Duttaroy A, Gomeza J, Zhang W, Yamada M, Felder CC, et al. Muscarinic receptor subtypes mediating central and peripheral antinociception studied with muscarinic receptor knockout mice: a review. Life Sci 2003;72;2047–54.

Wilkie GI, Hutson P, Sullivan JP, Wonnacott S. Pharmacological characterization of a nicotinic autoreceptor in rat hippocampal synaptosomes. Neurochem Res 1996;21;1141–8.

Wonnacott S, Irons J, Rapier C, Thorne B, Lunt GG. Presynaptic modulation of transmitter release by nicotinic receptors. Prog Brain Res 1989;79;157–63.

Yamane LE, Montenegro MA, Guerreiro MM. Comorbidity headache and epilepsy in childhood. Neuropediatrics 2004;35;99–102.