Original Article

Role of GABA-A receptors in the dorsolateral pons on pain modulation in rats

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ABSTRACT

Background & Objective: The parabrachial (PB) region definitely is a collection of neurons which are in the dorsolateral pons. Considering strong nociceptive projection coming from the lamina I neurons of the spinal cord into the lateral parabrachial nucleus (LPBN) and the existence of GABA-A receptors in the LPBN in the present study we investigated the effects of the GABA-A receptor agonist (muscimol) and GABA-A receptor antagonist (bicuculline) into the PB nucleus on formalin-induced nociceptive behavior. Materials & Methods: Rats were anesthetized with sodium pentobarbital (55 mg/kg) and then special cannulas were inserted stereotaxically into the parabrachial nucleus. Different groups of seven rats were injected with formalin into the rear paw 5 min after administration of either GABA-A receptor agonist, muscimol (62.5, 125,250 ng/0.5µl) or GABA-A receptor antagonist, bicuculline (50, 100 and 200 ng/0.5µl) into the LPBN. The scores of nociceptive behaviors calculated immediately after formalin injection and continued for 60 min. Results: Intra- LPBN microinjection of muscimol (250 ng) caused a decrease in formalin-induced nociceptive behaviors in phases 2 compared to vehicle-treated rats ($P = 0.002$) but failed to show any significant change in interphase or phase 2 from AUC calculated value in the vehicle group. Microinjection of muscimol (62.5, 125 ng) had no effect on AUC in any phase of the formalin test compared with the vehicle-injected rats. Administration of 50, 100 and 200 ng of bicuculline into the LPBN resulted in similar pain behaviors compared to the vehicle group in three phase with similar AUC. Conclusion: The present study indicates that indicating GABA A receptors in the PB nucleus may be involved in the modulation of nociception in tonic pain (phase 2).

Keywords: Parabrachial, GABAA Receptors, Tonic pain, Muscimol, Bicuculline

Introduction

lateral parabrachial nucleus (LPB) is a nociceptive relay between the spinal laminas and intralaminar thalamus that mainly project to prefrontal cortex[1]. laminas I of the spinal cord receive nociceptive information through Aδ and C fibers from periphery. Many of lamina I neurons that project to LPB neurons are nociceptive sensitive, and a considerable portion of all of them respond just to noxious stimuli [1–3]. In cat much larger number of lamina I cells project to PBN than to the PAG and thalamus [4]. Projections originating in nociception-specific neurons in lamina I of the dorsal horn to the lateral parabrachial nucleus and then to the central amygdala play critical role in the nociception-emotion link and its tightening in chronic pain [5].

A dense group of immunoreactive varicosities for GABA was recognized in LPB nucleus, indicating that the neurons of this region is under a significant GABAergic inhibition [6]. In addition, LPB nucleus is targets of significant GABAergic afferent projections. GABAergic neurons are plentiful within numerous places through the neuraxis of the rat brain, consists of those brain regions that send projections to the PB nucleus [7, 8]. Activation of GABA receptors reduces excitatory transmission between axons of the pontine parabrachial nucleus and neurons of the central amygdala [9].

Considering strong nociceptive projection coming from the lamina I neurons of the spinal cord and trigeminal nucleus into the LPBN, and the existence of GABAA receptors in the LPBN in the present study we investigated the effects of the GABAA receptor agonist muscimol and GABAA receptor antagonist bicuculline into the PB nucleus during formalin test that

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produced a two phase response ، the direct stimulation of nociceptors followed by production of inflammatory mediators.

Materials and Methods

Subjects and surgery

Animals were handled in accordance with the criteria outlined in the *Guide for Care and Use of Laboratory Animals* [10]. Adult male Wistar rats (bodyweight 250–320 g) kept to a 12/12 h light/dark cycle with food and water availablead libitum. Rats were anaesthetized with sodium pentobarbital (55 mg/kg, i.p.) and two stainless-steel guide cannulas (23 gauge) were lowered stereotaxically until their tips were 2 mm above the LBN of rats. The cannulas were secured on the skull with dental cement. All animals were allowed 1 week to recover and clear from the anaesthetics.

At the end of each experiment, 0.25 µl of methylene blue was injected into each side of LPN. The brain was removed and placed in formalin and, and just a few days later, the brain was sectioned coronally and viewed under a microscope to localize the injection site.

Behavioural nociceptive testing

A transparent acrylic test box (30 × 30 × 30 cm) with a transparent glass floor was used to observe the paws of the animals without obstruction. The rat was placed in the test box for at least 30 min before microinjection of different doses of muscimol ،bicuculline or vehicle followed 5 min later by subcutaneous injection of 50 µl of 2% formalin into the plantar surface of the hind paw. Pain behaviors were scored as follows:

0, the injected paw was not favored

1: the injected paw had little or no weight placed on it with no toe splaying

2: the injected paw was elevated and not in contact with any surface

3: the injected paw was licked or bitten.

Recording of the nociceptive behaviors was started immediately after formalin injection (time 0) and continued for 60 min.

The scores of nociceptive behaviors for each 5-minute interval were calculated as the weighted average of the number of seconds engaged in each behavior. The behavioral responses of each rat were evaluated separately for the first phase (1–10 min), inter-phase (10–15 min) and the second phase (15–60 min).

$$\text{Nociceptive score} = ((t0\times0) + (t1\times1) + (t2\times2) + (t3\times3))/((t0 + t1 + t2 + t3))$$

Experimental Groups

Muscimol group

The first phase of this study was to test the bilateral injection of different doses of muscimol (62.5, 125,250 ng/0.5µl) or vehicle into the lateral parabrachial area followed 5 min later by subcutaneous injection of 50 µl of 2% formalin into the plantar surface of the right hind paw.

Bicuculline group

In the second phase of this study we test the bilateral injection of different doses of bicuculline (50, 100 and 200 ng/0.5µl) into the lateral parabrachial area followed 5 min later by subcutaneous injection of 50 µl of 2% formalin into the plantar surface of the right hind paw.

All drugs were injected in a volume of 0.5µl. Bicuculline and muscimol were purchased from Tocris Bioscience (Ellisville, MO).

Statistical Analysis

The obtained results are expressed as mean ± SEM (standard error of mean). In order to assess the nociceptive responses, area under the curves (AUCs) was calculated as raw pain scores × time by linear trapezoidal method. The calculated AUC in all groups were analysed using one-way ANOVA with Tukey's test for post hoc comparisons. P values less than 0.05 were considered to be statistically significant.

Results

Effect of Muscimol on Formalin-induced Pain Behaviors

Intra- LPBN microinjection of muscimol (250 ng) caused a decrease in calculated areas under curve (AUC) values of pain scores in phases 2 (mean AUC ± SEM: 51.05± 4.29) compared to vehicle-treated rats (mean AUC ± SEM: 82.00± 4.76) (P = 0.002) and bicuculline (50 ng/0.5µl saline) when given 5 min before muscimol abolished this antinociceptive effect (Fig. 1,2). This dose of muscimol failed to show any significant change in pain scores during interphase or phase I of the formalin test. Microinjection of muscimol (62.5, 125 ng) had no effect on pain scores in any phase of the formalin test compared with the vehicle-injected rats (Fig. 1,2).

Effect of Bicuculline on Formalin-induced Pain Behaviors

In the second set of experiments, we examined the dose response effects of different doses of bicuculline (50, 100,200 ng/0.5µl saline), microinjected into the LPBN, on formalin-induced nociceptive behaviors. Intra LPBN administration of 50, 100 and 200 ng of bicuculline resulted in similar pain behaviors compared to the vehicle group (Fig. 3, 4).

Discussion

In the present study, we found that muscimol reduced the behavioral hyperalgesic response induced by formalin injection. Furthermore, its inhibitory effect was significant in

the second phase of formalin test. The analgesia observed was blocked by bicuculline, suggesting activation of classical bicuculline-sensitive GABA receptors are responsible for muscimol induced antinociception.

Physiological and pharmacological researchs have shown that GABA performs a fundamental function in the control of different actions of the parabrachial nucleus. Saleh and Cechetto showed that GABAA receptors in the PB regulate the parabrachil thalamic pathway such that inhibition of GABAA receptors in the PB increase natural activity of thalamic neurons [11]. Data by Higgs and Cooper demonstrated that, benzodiazepines manipulations of GABAA receptors in the PB highly effect on ingestive behaviour [12]. Additionally, GABA agonists injection into the lateral parabrachial nucleus increases the sodium intake in the rats [13] and muscimol decreases parabrachial neurons activity induced by footshock stimulus [8].

The present study indicates that GABA A receptors in the PB nucleus may involved in induction of antinociception in tonic pain (phase 2 of formalin test). The first phase of the formalin test begins immediately after formalin injection and lasts approximately for 5-10 min. The first phase has been attributed to TRPA1-mediated excitation of nociceptors [14]. This phase is a model of acute pain [15]. We showed that muscimol decreased pain-related behaviors in this phase but this effect was not statistically significant. Similarity blockade of GABA A receptors by bicuculline had no effect in this phase. Rats subjected to the formalin test do not usually display any pain behaviors between the two phases, as it was also observed in the present study. Actually, this interphase is a result of hyperpolarization and transient inactivation by formaldehyde of the surviving neurons [14]. The second phase starts 15–20 min after formalin injection and has been called the inflammatory pain phase, also denominated as tonic or chronic pain phase. [14]. Inflammatory mediators like histamine, serotonin, prostaglandins and bradykinin seem to be involved in late phase of formalin test [15].

In this study, muscimol had antinociceptive action in the second phase of the formalin test. Additionally, blockade of GABAA receptors by bicuculline (at a dose which does not affect the formalin-induced basal hyperalgesic response) prior to muscimol injection was able to abolish the antinociceptive effect elicited by muscimol injection. Most likely, the reduction of all pain-related behaviors in the second phase was a consequence of a direct effect of muscimol on GABAA receptors during this period, and hence GABAA receptors from the LPBN probably also have a role on tonic pain modulation.

The lack of effect of bicuculline on formalin induced nociceptive behaviors suggest that there is little tonic activation of LPBN GABAA receptors after injection of formalin.

In general, both clinical and preclinical experiments indicate that agents known to increase GABAergic transmission display antinociceptive activity [16–18]. However, the response to such agents varies as a function of route, dose, and site of administration, and the nature, strength, and duration of the pain stimulus [19].

Our finding is in part in agreement with previous reports in that GABA, muscimol or baclofen given i.t. produced antinociception in the tail flick, hot-plate and formalin tests. In addition, muscimol or baclofen given i.c.v. produce an antinociception in the formalin test [19]. On the other hand, our previous study indicate thal muscimol microinjection into the LPBN produced hyperalgesia in tail flick test [20]. Furthermore, microinjection of baclofen in the ventromedial medulla of rats produced antinociception at low doses, whereas hyperalgesia at high doses [21]. Additionaly, peripheral GABAA receptors and the endogenous GABA play an important role in persistent inflammatory hypersensitivity[22]. The exact reasons for the differential pharmacological profiles of GABAergic receptor agonists in perceiving nociception in different modes of analgesic tests are currently unclear. Although the exact reasons for these differential actions of muscimol on antinociception in the different analgesic tests are currently unknown, different results may be due to the modulation of nociceptive or analgesic pathway at various levels of the neuraxis and/or different modulatory involvement of GABAergic receptors in nociceptive or antinociceptive pathway mediated by the activation of different nociceptors activated by phasic thermal and tonic chemical stimuli, respectively. The data leave little doubt that, under certain circumstances, stimulation of neuroanatomically discreet GABA receptor sites could be of benefit in the management of pain.

Conclusion

Our results indicate that GABA A receptors in the LPBN involved in tonic pain modulation.

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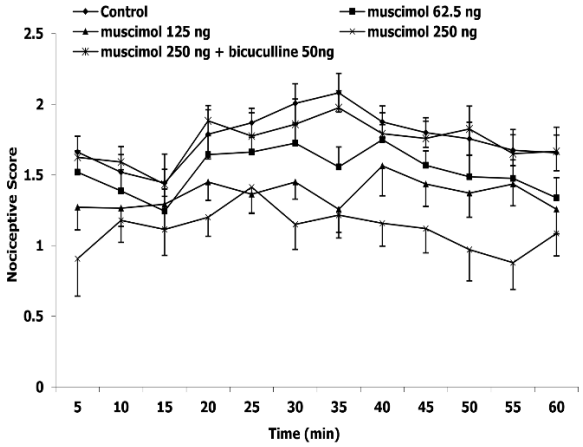

Figure 1: Time course for formalin induced nociceptive behavior score (±S. E.M.) following intra-LPBN infusion of saline (0.5µl), muscimol (62.5, 125,250 ng/0.5µl saline) and muscimol (250 ng/0.5 µl) + bicucullin (50 ng/0.5 µl) measured every 5 min for 60 min.

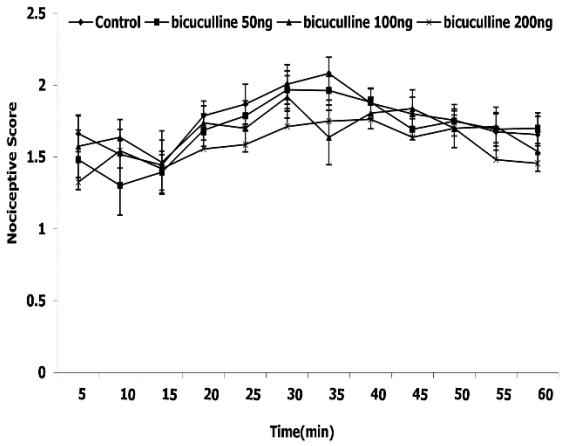

Figure 3: Time course for formalin induced nociceptive behavior score (±S. E.M.) following intra-LPBN infusion of saline (0.5µl) and bicuculline (50, 100,200 ng/0.5µl saline) measured every 5 min for 60 min.

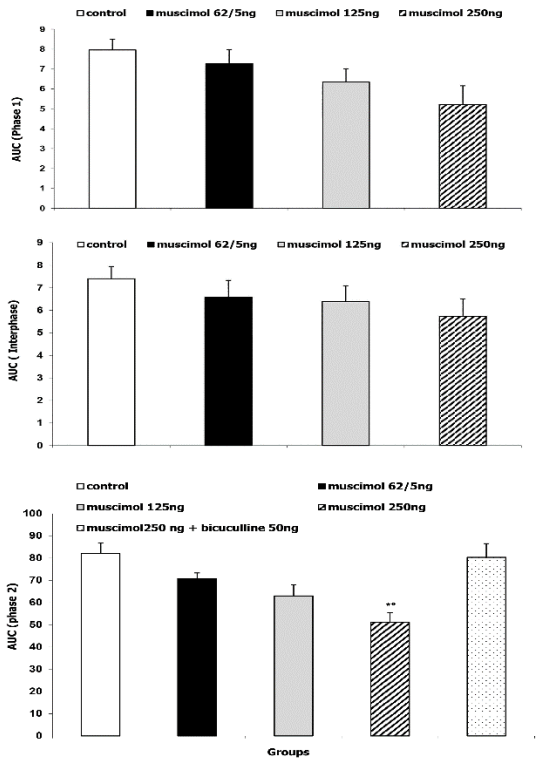

Figure 2: Area under the curve (AUC ± S. E.M) in phase 1 (0–10 min), inter-phase (10-15 min) and phase 2 (15– 60 min) for formalin induced nociceptive behavior score following intra-LPBN infusion of saline (0.5µl), muscimol (62.5, 125,250 ng/0.5µl saline) and muscimol (250 ng/0.5 µl) + bicucullin (50 ng/0.5 µl).

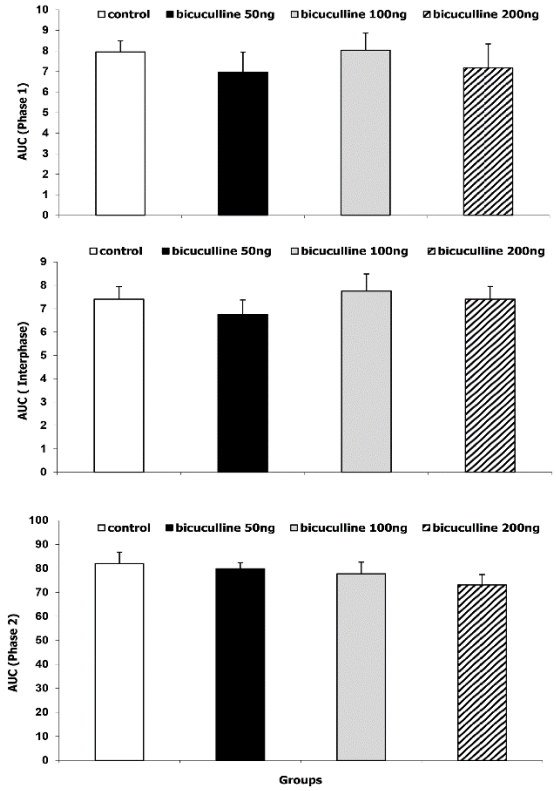

Figure 4: Area under the curve (AUC ± S. E.M) in phase 1 (0–10 min), inter-phase (10-15 min) and phase 2 (15– 60 min) for formalin induced nociceptive behavior score following intra-LPBN infusion of saline (0.5µl) and bicuculline (50, 100,200 ng/0.5µl saline)