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ORIGINAL ARTICLE

Antinociceptive Effects of H_3 (R-Methylhistamine) and $GABA_B$ (Baclofen)-Receptor Ligands in an Orofacial Model of Pain in Rats

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Abstract The present study explored the antinociceptive effects of H_3 (R-alpha-methylhistamine) and $GABA_B$ (baclofen) receptor ligands in an orofacial model of pain in rats. Orofacial pain was induced by subcutaneous injection of formalin (50 µl, 5 %) in the upper lip region, and the number of jumps and time spent face rubbing was recorded for 40 min. Formalin produced a marked biphasic pain response; first phase, 0–10 min (jumps), and second phase, 15–40 min, (rubbing). Baclofen (50 µg) injected into the rat wiskerpad 5 min before formalin administration suppressed both phases of pain whereas R-alpha-methylhistamine (12.5 µg) abolished the first phase only. Brains were taken immediately after behavioral testing was completed. HPLC/ED analysis showed that 5-hydroxytryptamine (5-HT) turnover was increased in hippocampus, thalamus, and brain stem of all formalin groups, excepting the baclofen group in which the balance of 5-HT metabolism was restored to control values. These findings demonstrate that $GABA_B$ receptors represent peripheral targets for analgesia. Consequently, locally administered baclofen may be a useful approach in treating inflammatory trigeminal pain.

Keywords H_3 receptor · $GABA_B$ receptor · Orofacial pain · Formalin test · Rats

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Introduction

Pain is the primary reason for patients to visit a physician or dentist, and the diagnosis and management of pain in the face, mouth and jaws have been integral components of dental practice. However, for an adequate understanding of the neurological mechanisms underlying dental pain, with the objective of improving pain management, an animal model for dental pain assessment is frequently employed. Such models allow us to manipulate neural pathways for the study of those pathways and search for new treatment options (Raboisson and Dallel 2004; Rusina et al. 2010).

Among variety of different systems and associated receptors mediating nociception transmission and modulation, $GABA_B$ and histamine H_3 receptors have recently attracted most attention. $GABA_B$ is the principle inhibitory metabotropic receptor in the central nervous system of mammals. Activation of the $GABA_B$ receptor leads to the blockade of voltage-gated calcium channels, which results in inhibition of presynaptic mediator release as well as the inhibition of postsynaptic neuronal activity by indirect activation of K^+ conductance (Koyrakh et al. 2005; Mapelli et al. 2009). Baclofen, a specific agonist of the heterodimeric $GABA_B$ receptor, is effectively used in the treatment of rigidity and spasms of skeletal muscles.

Furthermore, baclofen also induces antinociception in several animal pain models (Franek et al. 2004; Potes et al. 2006). Its antinociceptive mechanism involves inhibition of glutamate release from Adelta and C primary afferent terminals in substantia gelatinosa and/or a decreased release of neurokinin in the spinal cord (Ataka et al. 2000; Lao and Marvizón 2005).

In the past 10–15 years, the neuronal histaminergic system in brain has become reasonably well characterized (Pollard et al. 1993; Brown et al. 2001). Cell bodies of histaminergic neurons are located exclusively in the tuberomammillary nuclei of the hypothalamus and give rise to widespread projections throughout the central nervous system, including subcortical nuclei and cerebral cortex. Four subtypes (H_1, H_2, H_3, H_4) of histamine receptors are currently recognized. The histamine H_3 subtype is the least defined, although histamine H_3 receptors are now known to be predominately located presynaptically, functioning as an autoreceptor that regulates the synthesis and release of histamine (Flik et al. 2011). Recently, histaminergic agonists and antagonists were shown to modulate antinociception induced by supraspinally administered mu-, epsilon-, delta-, and kappa-opioid receptor agonists (Suh et al. 1999). Histaminergic agonists and antagonists additionally modulate peripheral opioid-mediated antinociception (Fernández-Dueñas et al. 2010a) and may be involved in the regulation of nociception during cholestasis in rats (Hasanein 2010).

To the best of our knowledge, there are no literature data on locally applied histamine H_3 and $GABA_B$ receptor agonists on trigeminal mediated nociception. In an attempt to clarify this issue, we produced a model orofacial formalin test in rats and associated effects with monoamine levels in brain.

Materials and Methods

Animals and Treatment

Adult (8–10 weeks of age) male and female Wistar rats weighting 200–250 g were obtained from the University Animals Department (Katowice, Poland) and were housed in a well-ventilated room, at 22 ± 2 °C under a 12 h light:12 h dark cycle (lights on 7:00 a.m. to 7:00 p.m.), and with free access to food and water. All procedures were approved by the Local Bioethical Committee for Animal Care (permission no 62/2011 issued on 2011.09.14) and are in accord with principles and guideline described in the NIH booklet Care and Use of Laboratory Animals. Experiments were carried out in the morning and the animals were used only once.

Orofacial Formalin Test

Assessment of pain transmitted by trigeminal sensory pathway was evaluated by the orofacial formalin test in the rats (Park et al. 2011). Rats were divided into 4 groups (8 rats in each group) and placed in a clear plastic test chamber (30 × 30 × 30 cm) with three mirrored sides. Following a 30 min acclimation period, saline (50 µl) (2 groups), R- alpha-methylhistamine (12.5 µg/50 µl; 1 group), or baclofen (50 µg/50 µl; 1 group) was injected subcutaneously (30-gauge needle) into the right upper lip. Immediately afterward, rats were returned to the test chamber for 5 min, then injected again in the right upper lip with saline (50 µl) (1 control group) or 5 % formalin solution (remaining groups). Rats were then returned to the chambers, and nociceptive behavior was observed. Two parameter were evaluated, numbers of jumps (observed mainly in the first 10 min of testing) and time that animals spent rubbing and flicking (15–40 min). A reduction of formalin-induced behavior observed after administration of a given drug is interpreted as an antinociceptive response.

Assessment of biogenic amine and metabolite content

Immediately after behavioral testing rats were decapitated, and the frontal cortex, thalamus, and spinal cord were rapidly dissected and placed on dry ice, then weighed and stored at –70 °C, pending assay. Samples were homogenized for 15–20 s in ice-cold trichloracetic acid (0.1 M), containing 0.05 mM ascorbic acid. After centrifugation (5,000×g, 5 min), supernatants were filtered through 0.2 µm cellulose membranes (Titan MSF Microspin filters, Scientific Resources Inc., Eatontown GB) and supernatants was injected onto the HPLC/ED column. Levels of noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were assayed. The composition of the mobile phase was: 75 mm NaH_2PO_4, 1.7 mm 1-octanesulfonic acid, 5 µm EDTA (Avocado, Research Chemical Ltd., Morecambe, GB), 100 µl triethylamine (Sigma, St. Louis, USA), 9.5 % acetonitrile (J.T. Baker, Deventer, Holland), pH 3 adjusted with phosphoric acid (Fluka, Steinheim, Switzerland). The flow rate was maintained at 0.7 ml/min, at a temperature of 22 °C, and the oxidation potential was fixed at +700 mV, 10 nA/V sensitivity. Peaks were automatically integrated by universal chromatographic interface UCI-100 (Dionex Softron Gmbh, Germering, Germany). The instrumentation included an electrochemical detector (Gilson, Villiers-le-Bel, France) model 141 with flow cell, piston pump model 302 with head 5SC (Gilson, Villiers-le-Bel, France), manometric module model 802 (Gilson, Villiers-le-Bel, France), thermostat for STH 595 column (Dionex Softron Gmbh, Germering, Germany), precolumn Hypersil BDS C18, 10 × 4 mm, 3 µm (ThermoQuest, Waltham, GB)

and chromatographic column Hypersil BDS C18, 250 × 4.6 mm, 3 μm (ThermoQuest, Waltham, GB). The data were quantified using the area under the peaks and external standards, using Chromeleon software (Dionex, Germany) (Nowak et al. 2006; Korossy-Mruk et al. 2013).

Data Analysis

Group differences were assessed by an analysis of variance (ANOVA) and the post-ANOVA test of Newman–Keuls. A P value <0.05 was taken as the level of significant difference.

Results

Orofacial Formalin Test

Injection of formalin in the rat wiskerpad induces behavioral responses like jumping and rubbing. It consists of two distinct phases: a first "phasic" phase and a second "tonic" phase. In phase I called early or neurogenic (0–10 min) rats mainly jump; in phase II (late or inflammatory) (15–40 min) rats mainly rub their snout.

In the formalin group, we observed ~67.0 (±15.7) jumps during time testing, control animals (saline + saline) did not express this behavior at all. R-alpha-methylhistamine (12.5 μg) injected before formalin administration significantly diminished numbers of jumps (to 21.5 ± 8.76). Also, baclofen in a dose of 50 μg applied locally in the rat wiskerpad 5 min before formalin injection, reduced jumping behavior (to 11.0 ± 2.19) (Fig. 1).

Control animals (saline + saline) spent 14.0 ± 2.78 s on rubbing their snout. Rats in the formalin group rubbed their snout for 180.8 ± 35.5 s, similarly to rats pretreated with R-methylhistamine (176.6 ± 51.4 s) before formalin injection. In contrast, baclofen attenuated nociceptive behavior (34.8 ± 9.75 s) induced by formalin (Fig. 2).

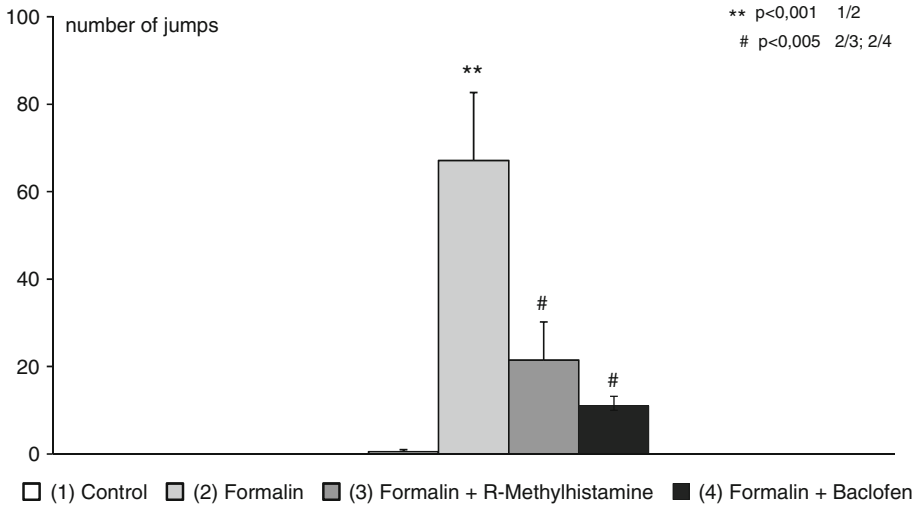


Fig. 1 Anti-nociception effects measured by numbers of jumps after locally applied R-methylhistamine (12.5 μg) and baclofen (50 μg), assessed in the orofacial formalin test in rats (x ± SEM; $n = 8$). *Open square* Control, *Light grey box* Formalin, *dark grey box* Formalin + R-alpha-methylhistamine, *black box* Formalin + baclofen

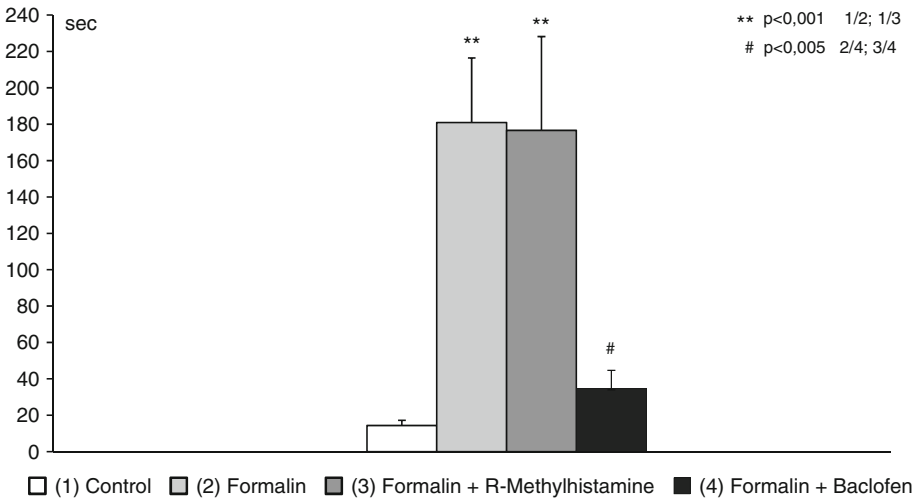


Fig. 2 Antinociception effects measured by time of rubbing, after locally applied R- alpha-methylhistamine (12.5 μg) and baclofen (50 μg), as assessed in the orofacial formalin test in rats (x ± SEM; $n = 8$). *Open square* Control, *Light grey box* Formalin, *dark grey box* Formalin + R-alpha-methylhistamine, *black box* Formalin + baclofen

Assessment of Biogenic Amine and Metabolite Content

Equally high levels of DA, DOPAC, HVA, 5-HT, and 5-HIAA in the prefrontal cortex were observed between all groups of rats (control, formalin, formalin + R-alpha-methylhistamine, and formalin + baclofen animals). A borderline significance ($p < 0{,}088$) was observed in NA content between control and formalin group (Fig. 3a–d). Also in other tested brain structures, equally high levels of NA, DA, DOPAC, HVA, and 5-HT were noted. Conversely, in the hippocampus, thalamus and brain stem of rats treated with formalin and formalin + R-alpha-methylhistamine, 5-HIAA content was increased, and with baclofen pretreatment 5-HIAA content was unchanged from control (Fig. 3b–d).

Discussion

The rat orofacial formalin test is a useful pre-clinical model of inflammatory trigeminal pain for evaluating antinociceptive activity of analgesics and their combinations. Injection of formalin in the rat wiskerpad induces jumping behavior and stereotyped response (rubbing), consisting of two distinct phases: a first "phasic" phase and a second "tonic" phase (Raboisson and Dallel 2004). In this work, we tested R-alpha-methylhistamine a histamine H_3 receptor agonist and baclofen a $GABA_B$ receptor agonist, each of which was given locally into the rat right upper lip. We have shown that baclofen reduced nociception both in the first (jumping) and in the second phase (rubbing), whereas R-alpha-methylhistamine induced antinociception in the

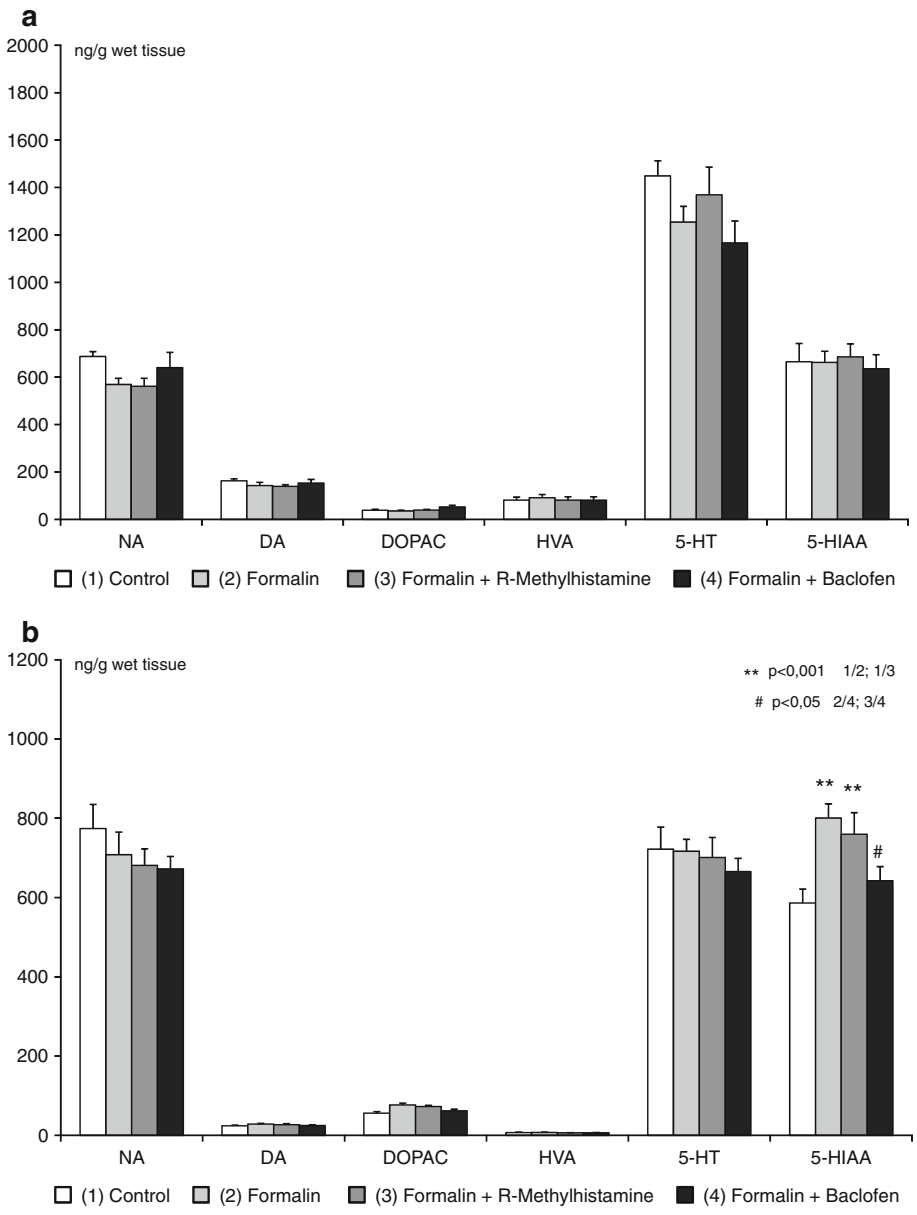
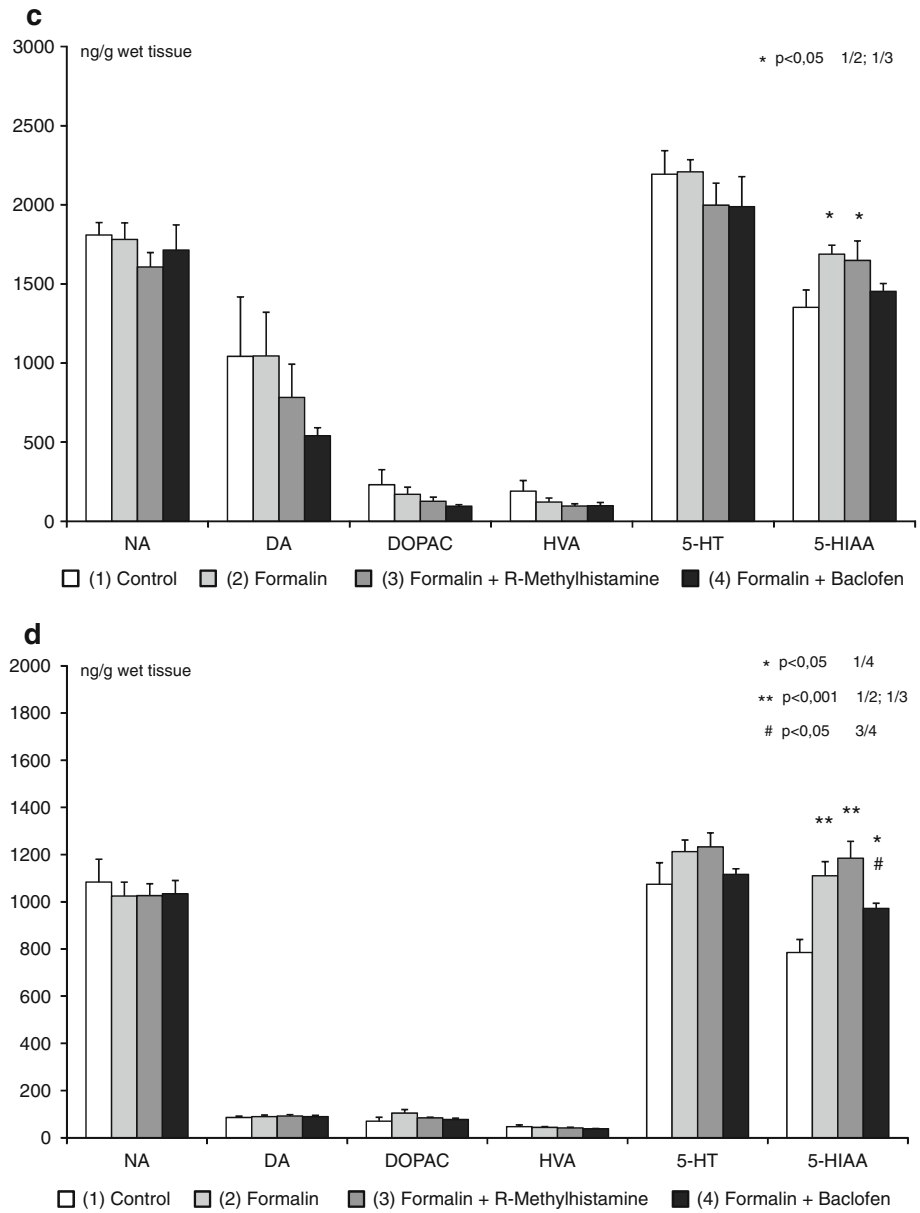


Fig. 3 Monoamine and metabolite levels in the prefrontal cortex (**a**), hippocampus (**b**), thalamus (**c**), and brain stem (**d**) after locally applied R-alpha-methylhistamine (12.5 μg) and baclofen (50 μg), as assessed in the orofacial formalin test in rats (x ± SEM; $n = 8$). Legend as in Figure 1

Fig. 3 continued



first phase only. Our results (with baclofen) are in line with Whitehead et al. (2012) who found that $GABA_B$ agonists like isovaline, baclofen, and GABA (a CNS-impermeant, unselective $GABA_B$ agonist) attenuated allodynia induced by prostaglandin E_2 injection into the mouse hindpaw and tested with von Frey filaments. They also demonstrated that immunohistochemical staining of cutaneous layers of the analgesic test site demonstrated co-localization of $GABA_{B1}$ and $GABA_{B2}$ receptor subunits on fine nerve endings and keratinocytes, which may account for antinociceptive effects. The only difference versus our study is that they administered agents systemically, not peripherally, so that the analgesic effect was also dependent on modification of nociceptive transmission in the trigeminal root ganglion neurons as well as in upper brain structures (Takeda et al. 2004). It is noteworthy that there are also some clinical observations on this topic. For example, Sanders et al. (2009) showed that intrathecal baclofen decreases acute and chronic postoperative pain after total knee arthroplasty. Patients from the baclofen group used less morphine than the control group.

We also found that R-alpha-methylhistamine-induced antinociception in the first phase of orofacial formalin test by decreasing number of jumps without an effect on the "inflammatory" test phase. Conversely, Fernández-Dueñas et al. (2010b) found that the systemic administration of fentanyl (0.005–0.1 mg/kg) plus a fixed dose of R-alpha-methylhistamine (0.5 mg/kg) induced a supra additive

effect on the inhibition of the thermal hyperalgesia and substance P accumulation in the hind-paw skin of inflamed mice. Others investigated the effects of systemically (s.c.) and intrathecally (i.t.) administered immepip (a histamine H_3 receptor agonist) in rats and mice. They showed that immepip produced robust antinociception in rats on a mechanical (tail pinch) test but did not alter nociceptive responses on a thermal (tail flick) test. In contrast, this treatment in mice did not change either mechanically or thermally-evoked nociceptive responses (Cannon et al. 2003). In a more recent rat study, systemic (s.c.) administration of the H_3 agonist immepip produced dose-dependent reductions (up to 70 %) in both phases of formalin-induced flinching. These effects were mimicked by i.t. immepip and blocked by systemic and i.t. thioperamide. Although s.c. immepip reduced formalin-induced flinching, this treatment had no effect on formalin-induced vocalizations in rats (Cannon et al. 2007). These results, in apparent contradiction to ours findings, could be the outcome of systemic versus local drug administration. One must be cognizant that histamine H_3 receptors are widely distributed within the brain, the spinal cord and on specific types of primary sensory neurons. Our "manipulation" was restricted solely to H_3 receptors located on certain Aβ fibers, keratinocytes, Merkel cells and peptidergic Aδ fibers terminating on deep dermal blood vessels in the skin (Hough and Rice 2011).

In our work, we also looked into brain monoamine metabolism. Immediately after the behavioral testing ended, brain specimens were taken for eventual HPLC analysis. We found that in the hippocampus, thalamus and brain stem of rats treated with formalin and formalin + R-alpha-methylhistamine, there was an increase in 5-HIAA levels, and baclofen pretreatment prevented a change in 5-HIAA levels. From these studies, we learn that persistent pain increased 5-HT metabolism in brain structures that are involved in pain perception. Based on the finding that baclofen relieved nociceptive effects and effectively maintained 5-HT levels, we hypothesize that 5-HT brain metabolism may be a simple biochemical indicator for the painful stimuli in rats. Our data are in accord with Burke et al. (2010) who found that 2 h after intra-plantar formalin administration in rats an increase in 5-HT and 5-HIAA concentration in the prefrontal cortex, hippocampus, thalamus; and 5-HIAA in amygdaloid cortex and cerebellum was observed. A similar "constellation" of marked effects upon 5-HIAA as opposed to 5-HT was found by Cox et al. (2011) who observed an increase in 5-HIAA, but no increase in 5-HT in the hypothalamus of rats exposed to a random pattern of mild stressors twice daily for 10 days (a model of chronic unpredictable stress). This resulted in an increased ratio of 5-HIAA/5-HT, suggesting increased turnover of 5-HT which is reflective of increased serotoninergic activity. An increase in 5-HT turnover in the hypothalamus was previously observed in response to chronic social stress (Blanchard et al. 1991).

The search for new peripherally restricted analgesics is desirable to avoid central nervous system side effects of opioids and overall toxicity of nonsteroidal anti-inflammatory drugs. Our work showed that $GABA_B$ receptors represent peripheral targets for analgesia; locally administered baclofen may be a useful approach in treating inflammatory trigeminal pain.

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