American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 14, No 8 (August), 2013: pp 808-817
Available online at www.jpain.org and www.sciencedirect.com

Histamine Produces Opposing Effects to Serotonin in the Knee Joint of Rats

Eduardo Souza-Silva, Daniel Teixeira de Oliveira, Carolina Eto, Taciane Stein, and Carlos Rogério Tonussi

Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, SC, Brazil.

Abstract: Formalin injected in the knee joint of rats produces concentration-dependent nociception, edema, and plasma leakage (PL). Herein, we investigated the effect of histamine H1 receptor (H1R) antagonists in this model. Articular nociception was inferred from the paw elevation time (PET; seconds) during 1-minute periods of stimulated walking, determined every 5 minutes, throughout a 60-minute experimental session. Edema was evaluated by the increase in articular diameter (AD; mm), and PL was measured by the amount of Evans blue dye in the synovial fluid (PL; μg/mL). Loratadine and cetirizine, given systemically, both increased the PET. None of the treatments changed the AD and PL. Loratadine given locally with formalin increased the PET but was without effect when given in the contralateral knee. Systemic loratadine was also without effect when formalin was coinjected with sodium cromoglycate. Histamine and the selective H1R agonist 2-pyridylethylamine decreased the PET and potentiated morphine spinal analgesia, but did not affect the AD and PL. Cetirizine prevented the antinociceptive effect of the H1R agonist. The *N*-methyl-D-aspartate/histamine-site agonist tele-methylhistamine coinjected with formalin only increased PET. Serotonin alone had no effect on the PET and increased the AD, and the highest dose increased the PL. When coinjected with formalin, serotonin only caused hypernociception, and the highest dose also increased AD. NAN 190, cyproheptadine, and ondansetron (respectively, 5-HT1, 5-HT2, and 5-HT3 receptor antagonists) decreased the PET without changing the AD or PL. Collectively, these results suggest that in rats, the H1R plays an antinociceptive role within the knee joint, while serotonin receptors play a pronociceptive role.

***Perspective:** The present study revealed an antinociceptive mechanism that has previously not been detected by traditional nociceptive tests. Our observations may help to improve the development of new pharmacological strategies for the treatment of clinically relevant pains that generally originate in deep structures.*

***Key words:** H1R antagonists, mast cells, articular pain, formalin test, cetirizine, loratadine.*

The nociceptive role of histamine has generally been studied in cutaneous tissue and it is accepted to be a hyperalgesic mediator in inflammation, but its role in deep tissues still remains poorly understood. Evidence that supports the cutaneous nociceptive role of histamine may have been, at least in some cases, overestimated. Thus, while locally applied first-generation histamine H1 receptor (H1R) antagonists can inhibit formalin-induced nociception,[25,32] this effect could well be due to a local anesthetic property of the antihistamine employed.[26] In other studies, the lack of selectivity of the antagonist used may be another problem.[7,24] Thus, in an acute inflammatory model, the peripheral H1R antagonist thiazinamium prevented the paw hyperalgesia induced by carrageenan, but this agent also possesses an antiserotoninergic property.[20] All of these studies were conducted in rodent models in which the possible contribution of serotonin derived from tissue mast cells cannot be omitted.

The local injection of histamine has also been used to support a role for endogenous histamine in the

Received October 2, 2012; Revised February 7, 2013; Accepted February 13, 2013.
This work received financial support from the following Brazilian government agencies: CNPq, CAPES, and FAPESC (pronex). E.S.S., D.T.O., and T.S. were recipients of graduate, and C.E. an undergraduate, fellowships from CNPq. C.R.T. was recipient of a research grant from CNPq.
The authors state that there is no conflict of interest.
Address reprint requests to Carlos Rogério Tonussi, Department of Pharmacology, Universidade Federal de Santa Catarina, 8804-0900 Florianopolis, SC, Brazil. E-mail: c.r.tonussi@ufsc.br
1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2013.02.006

formalin-induced nociception in skin[25] and articular tissue.[32] However, the concentrations of histamine used to produce nociceptive sensitization were far above that which is likely to exist per gram of rat skin.[10,31] Furthermore, the data available concerning the effect of histamine in articular afferents are scarce,[11] as are behavioral studies. In these studies, another source of ambiguity may be pruritus. Pruritus induced by histamine is not adequately discriminated from nociception by behavioral models, and therefore the summation of similar behaviors may be misinterpreted as sensitization.[28] In addition, the nociceptive behavior produced by formalin is only one component of a local reaction which also includes edema and plasma leakage (PL).[12] These vascular events can be influenced by the action of histamine in structures other than sensory fibers, such as blood vessels and immune cells, which may render their role even more complex. For example, the arthritis response induced by Freund's adjuvant was shown to be reduced by an H1R agonist[34] as well as by an antagonist.[3] In a previous report, we observed that the peripheral H1R antagonist loratadine, or low doses of the brain-permeant meclizine, produced a hypernociceptive effect in the new model of formalin-induced articular incapacitation,[17] suggesting that peripheral H1R activation could unexpectedly produce antinociception in the knee joint of rats.

In view of the above observations, the effect of antihistamines on deep nociception may not be easily predicted. Formalin presents an interesting tool to study the mechanisms underlying persistent pain in an early inflammatory condition with the involvement of amines released by mast cells,[25,19] and the model of knee incapacitation induced by formalin[17] may be an interesting tool to study the contribution of these amines to articular nociception. The present study was designed to assess the role of the H1R in the deep tissue nociception induced by formalin in the knee joint, in parallel with the edematous and articular PL effects, aiming to provide consistent information that may explain how antihistamines can produce hypernociception in this model.

Methods

Animals

The experiments were performed on male Wistar rats (250–300 g) housed in a temperature-controlled room (21 ± 2°C), under a 12/12-hour light/dark cycle, with free access to water and food. This study followed the ethical guidelines of the International Association for the Study of Pain[14] and was previously approved by the local ethics committee for animal use (CEUA-UFSC: 23080.042991/2008-16).

Substances and Vehicles

Cetirizine (MW 388.9), loratadine (MW 382.9), and sodium cromoglycate were obtained from Galena Química Farmacêutica LTDA (Campinas, SP, Brazil). Ondansetron and morphine were acquired from Laboratórios Cristália LTDA (Itapira, SP, Brazil). Histamine, serotonin (5-HT), and tele-methylhistamine were acquired from Sigma-Aldrich (St. Louis, MO), and 2-pyridylethylamine, NAN 190 and cyproheptadine from Tocris (Minneapolis, MN). Loratadine was dissolved in polyoxyethylenesorbitan monoleate (Tween 80), not exceeding 5% of the total volume in physiological saline. Cetirizine, sodium cromoglycate, ondansetron, NAN 190, cyproheptadine, histamine, serotonine, tele-methylhistamine, 2-pyridylethylamine, and morphine were dissolved in physiological saline. Systemic treatments (loratadine and cetirizine) were applied by intraperitoneal (i.p.) route, 60 minutes before formalin. Local treatments were applied intrathecally (morphine) 20 minutes before or coinjected (loratadine, histamine, 2-pyridylethylamine, sodium cromoglycate serotonin, NAN 190, cyproheptadine, and ondansetron) with intra-articular formalin. Formalin (formaldehyde 37%; Merck KGaA, Darmstadt, Germany) was dissolved in saline at 1.5%, considering the initial concentration as 100%. Control group treatments were carried out with the vehicle of the respective test drugs.

Knee Joint Incapacitation Induced by Formalin

The rat knee joint incapacitation test has been described in detail elsewhere.[33] Briefly, in this test, rats are placed on a revolving cylinder (30-cm diameter; 3 rpm) for 1-minute periods and a computer-assisted device measured the total time that a specific hind paw was not in contact with the cylinder surface (paw elevation time [PET]). Normally, control animals display a PET of approximately 10 seconds, whereas algogenic substances injected into the knee joint increased this value only in the affected limb.

In order to induce incapacitation, 50 μL of formalin solution, diluted in sterile saline, was injected into the right knee joints of the rats. The injection site was first shaved and treated with an iodine alcohol antiseptic solution. The animals were gently restrained in a supine position by hand, and the intra-articular injection was quickly performed with a 30-gauge needle. The PET was measured every 5 minutes after formalin injection.[17] In addition to the nocifensive behavior being scored automatically, the experimenter was blind to the treatment protocols.

Evaluation of Articular Edema

The animals were immobilized in the same way as for the intra-articular injection. Articular diameter (AD) was measured through the knee joint mediolateral axis applying the micrometer at 3 arbitrary levels along the knee joint proximodistal axis, and taking the highest value. The AD increase in millimeters was simply calculated subtracting the AD taken immediately before from that taken 1 hour after formalin injection.

Evaluation of the Synovial PL

Under isoflurane anesthesia, the animals received an intravenous injection of Evans blue (25 mg/kg;