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Involvement of H_1 receptors in the central antinociceptive effect of histamine: pharmacological dissection by electrophysiological analysis

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Abstract. Intracerebroventricular (i.c.v.) administration of histamine (HA, 0.025–0.1 μM/rat) to arthritic rats induces a dose-related inhibition of the neuronal thalamic firing evoked by peripheral noxious stimuli. To characterize the type(s) of HA receptors involved in this depressing activity of the amine we used electrophysiological techniques to examine the effects of i.c.v. administration of H_1 and H_2 agonists and antagonists on the spontaneous and evoked nociceptive firing of the thalamic neurons in rats rendered arthritic by Freund's adjuvant.
The H_1 agonist 2-pyridylethylamine (0.4–1.0 μM/rat, i.c.v) displayed a dose-dependent antinociceptive effect very similar to that of HA, while the H_2 agonist dimaprit (0.05–0.2 μM/rat, i.c.v.) did not modify thalamic firing. Neither mepyramine (H_1 antagonist, 0.1 μM/rat, i.c.v.) nor zolantidine (H_2 antagonist, 0.01 μM/rat, i.c.v.) modified the evoked firing of rat thalamic neurons. When administered before HA (0.1 μM/rat, i.c.v.) mepyramine but not zolantidine was able to inhibit the antinociceptive effect of HA.
On the basis of the present electrophysiological results, we suggest that a specific interaction of histamine with H_1 receptors may be important for its antinociceptive effect on afferent peripheral inputs to the thalamus.
Key words. Histamine antinociception; histamine receptors; 2-pyridylethylamine; dimaprit; mepyramine; zolantidine.

A number of observations suggest that histamine (HA) can modulate nociceptive responses in the central nervous system, even though much about this function remains to be investigated[1]. Histaminergic neurons emanating from the posterior hypothalamus and innervating some brain areas involved in pain modulation have been detected by an immunohistochemical technique[2,3]. The thalamus is considered an important relay station in the pain pathway to the cortex so the presence of a high density of histaminergic fibers in this area[4] might be a clue to the involvement of HA in pain modulation. In agreement with this, we observed that intracerebroventricularly administered HA produced a dose-related inhibitory and long-lasting effect on the evoked firing activity recorded from thalamic neurons[5]. In addition, it has been reported that injection of histamine directly into the dorsal raphe nucleus of the periaqueductal grey zone[6], another region of the brain involved in pain modulation, produces analgesia in rats as shown in behavioural tests. By the hot plate[7,8], tail flick[7,9] and Randall-Selitto tests[10], histamine administered intracerebroventricularly elicited a clear antinociceptive effect. The role of histamine H_1-H_2 receptors in the analgesic activity of the amine has recently been reviewed[11,1] but because different methods and experimental conditions were used the prevalence of the effect for one or the other receptor is not clear. For example, in the hot plate test we observed that H_2 receptors mediate the antinociceptive effect of HA[12,8] while H_1 receptors seem to play a major role in the tail flick test[9].
The aim of the present study was to characterize, by an electrophysiological technique, the type(s) of HA receptors involved in the depressing activity of HA on neuronal thalamic firing evoked by a peripheral nociceptive stimulus. The possible involvement of H_1 and/or H_2 receptors in the inhibitory activity of the amine in such an experimental procedure was studied by evaluating the effect of central administration of specific histaminergic agonist and antagonist drugs on the spontaneous and evoked nociceptive firing of thalamic neurons in rats rendered arthritic by Freund's adjuvant, as a model of chronic pain[13].

Materials and methods

Preparation of the animals. Experiments were performed on male Sprague-Dawley rats weighing 200–270 g at the time of the electrophysiological recording. The rats lost weight during the development of arthritis. The arthritic syndrome was induced by intradermal injection of Freund's adjuvant, 0.1 ml of a suspension of killed *Mycobacterium butyricum* (5 mg/ml, DIFCO) in heavy mineral oil into the plantar surface of the left hindfoot[14]. The rats were kept in individual cages in an air-conditioned room with a 12 h light-dark cycle and were given

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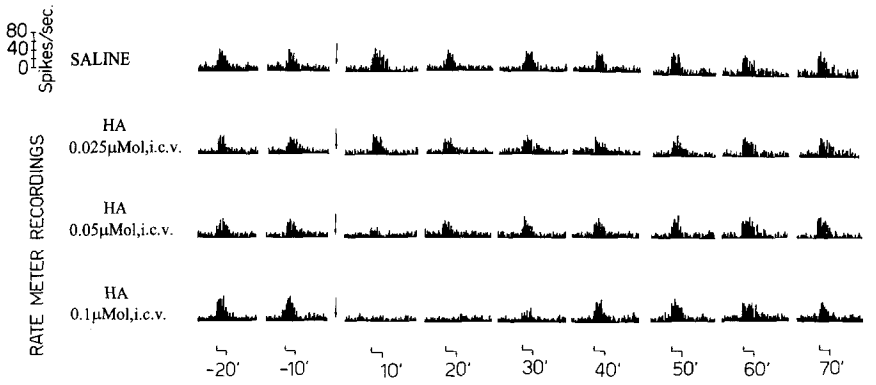

Figure 1. Examples of the spontaneous and evoked electrophysiological effects (ratemeter recordings) and time-course following administration of saline and increasing doses of histamine (HA). ↓ injection of drugs; ↑–↓ = start and stop of stimulus (10 sec); the numbers under each column are the times in minutes.

water and food ad libitum. To avoid the possibility of painful interactions between rats placed in close contact the animals were housed in individual cages. The ethical principles and guidelines for scientific experiments on animals proposed by the Swiss Academy of Medical Sciences published in Experientia (1992, 48: 1–3) and the guidelines on ethical standards for investigations of experimental pain in animals, published in a Guest Editorial in Pain (1983, 16: 109–110) were followed. In particular, the experiment duration was as short as possible and the number of rats used was kept to a minimum.

Electrophysiological investigations were done between the 14th and 18th day after the administration of the adjuvant, using rats with clearly visible evidence of arthritis (i.e. erythema and swelling to twice normal size of the paws and tibiotarsal joint) and related behaviour (vocalization, reduced motility). The animals were anesthetized with 1 g/kg (i.p.) of urethane, a dose smaller than usual because arthritic rats are more sensitive to anaesthesia than normal rats. After the animal was placed in a stereotaxic frame, a small hole (3 mm dia) was drilled in the skull and the dura mater was carefully removed to allow stereotaxic positioning. The cortical surface was covered with warm mineral oil and the animals were kept warm on a hot plate maintained at 37–38 °C. The colour and the vascularization of the extremities and their ability to return quickly to their previous state after application of pressure or movement of joints was checked and the experiment was terminated whenever an animal's condition deteriorated[15].

Single unit recording. Single-barrelled micropipettes were used to record extracellular single unit activity. The tips were broken back to a tip diameter of 1–2 μm and were filled by the glass filament technique with a 2 M NaCl solution, saturated with Pontamine sky blue,

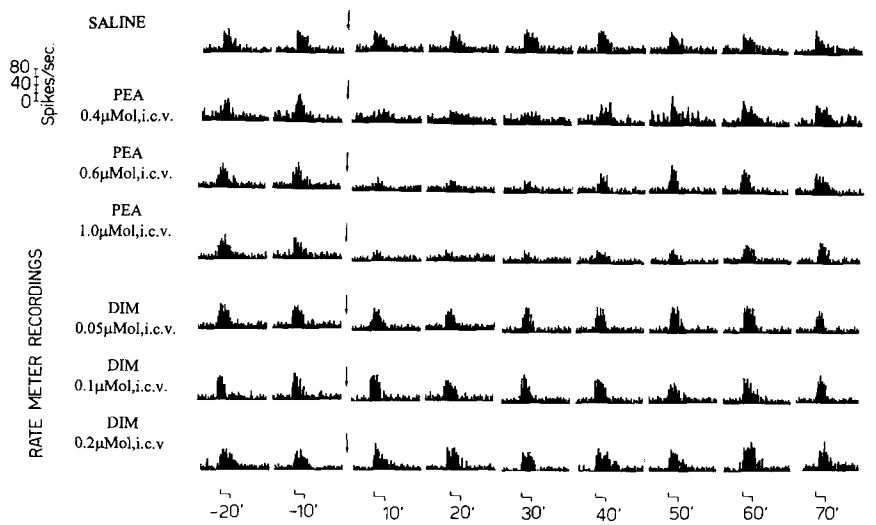

Figure 2. Examples of the electrophysiological effects of saline and increasing doses of 2-pyridylethylamine (PEA) and dimaprit (DIM). (Details as in fig. 1)

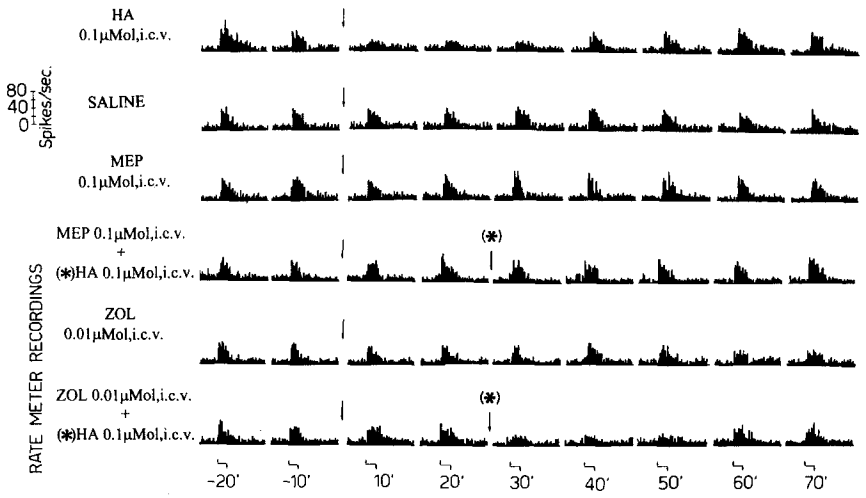

Figure 3. Examples of ratemeter recordings and electrophysiological behaviour before and after administration of histamine (HA), saline, mepyramine (MEP) and zolantidine (ZOL) alone, and HA (*) after pretreatment with MEP and ZOL. (Details as in fig. 1)

for subsequent localization. The resistance was 15–18 MΩ. The coordinates for correct electrode placement in the nucleus lateralis and ventrodorsomedialis of the thalamus were taken from the atlas of Paxinos and Watson[16]. The recording electrode was connected to a Digitimer amplifier and the neuronal activity was filtered, displayed on an oscilloscope and recorded on an FM tape recorder. A window discriminator was used to differentiate spikes from noise. The output pulses of constant amplitude and duration (TTL) were channelled into an integrator to obtain a continuous analogue ratemeter display of the firing.

The noxious test stimuli used were either extension or flexion of the ankle or mild lateral pressure on the heel. These stimuli did not induce any responses in non-arthritic rats. The stimuli were applied for 10 sec and were repeated every 10 min. Recordings were obtained from neurons that had low basal spontaneous firing rates and that are clearly excited by these stimuli. Neurons with spontaneous 'paroxysmal' firing rates can be found in arthritic rats[17]. These neurons were not studied because their irregular high frequency burst discharges interfered with the responses evoked. Care was taken to avoid damage to the skin and sensitization.

Drug application. Saline (for control rats) or other drugs were injected into the left lateral ventricle through a stereotaxically-positioned stainless steel cannula (outer diameter = 300 μm). Correct positioning of the cannula was confirmed by subsequent histological examination of the brain at the end of the experiment.

The following drugs were used: histamine dihydrochloride (Sigma, 0.025–0.05–0.1 μM/rat, i.c.v.); 2-pyridylethylamine dihydrochloride (Smith Kline & Beecham; H_1 agonist 0.4–0.6–1.0 μM/rat, i.c.v.); dimaprit dihydrochloride (Smith Kline & Beecham; H_2 agonist, 0.05–0.1–0.2 μM/rat, i.c.v.); mepyramine maleate (Sigma, H_1 antagonist, 0.1 μM/rat, i.c.v.); zolantidine dimaleate (Merck Sharp & Dohme Research Lab., USA, H_2 antagonist, 0.01 μM/rat, i.c.v.). We have chosen the doses of drugs on the basis of their relative affinity for histamine receptors[18].

All drugs were dissolved in saline except zolantidine, which was dissolved in a solution consisting of (in grams): NaCl, 7.46; KCl, 0.19; $MgCl_2$ dihydrate, 0.18 in 1 l of distilled water. All drugs were injected i.c.v. in a final volume of 5 μl. The pH of the solutions was between 5.2 and 7.3, which has been shown not to affect neuronal activity.

All drugs were injected alone, but in two experiments to investigate the interference of H_1 and H_2 antagonists on histamine response, mepyramine and zolantidine were injected 20 min before histamine.

A total of 82 neurons were studied, with only one neuron tested in each experiment. Electrophysiological recording was continued until the return to basal firing to obtain the time courses of the effects of the different doses of the drugs used.

The total number of spikes present in a 10-s-period, both spontaneous discharges and the evoked responses, were counted and expressed as percentage versus the mean values before drug administration for each neuron and each test.

Statistical analysis. Data are presented as the mean ± SEM. The area under the time-curve (AUC) of percentages of evoked firing was calculated by trapezoidal rule. Differences between groups were assessed by one-way analysis of variance (ANOVA). This analysis was followed by a Tukey test, with a probability of $p < 0.05$ considered statistically significant.

Results

A typical example of the nociceptive behaviour of thalamic neurons induced by ankle mobilization is shown in

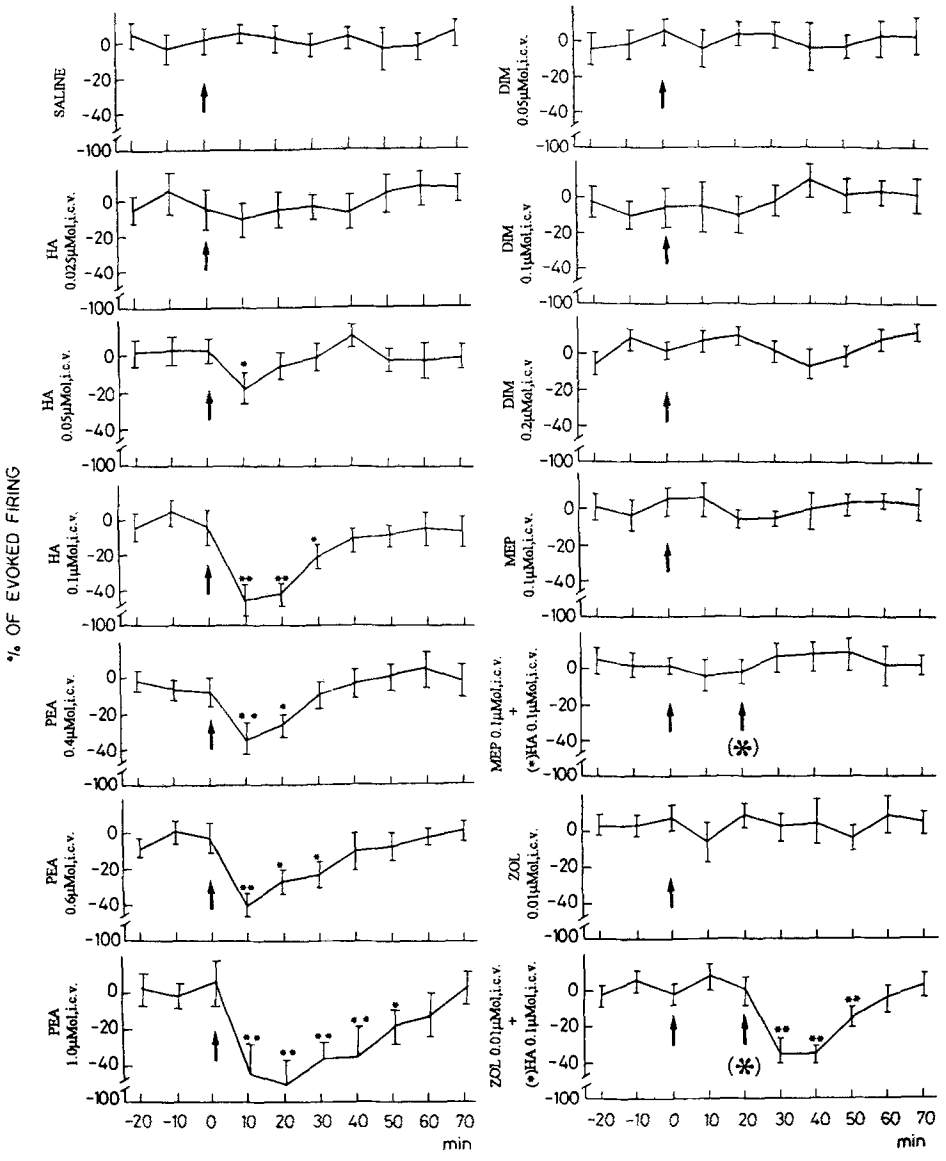

Figure 4. Overall view of the effects and time-courses of the drugs reported in figures 1, 2, 3. Data are expressed as percentage changes in firing rates in comparison to those before administration. Vertical arrows indicate the time of injection of drugs. *$p < 0.05$; **$p < 0.01$.

figure 1. Intracerebroventricular saline injection did not modify the nociceptive behaviour; in fact the ankle mobilization caused a very rapid excitatory effect with a clear increase in neuronal firing as recorded by the ratemeter, and this evoked response lasted for the period of stimulation. During this period there was no change in spontaneous activity. Figure 1 also shows the effects of increasing doses of HA, administered by the i.c.v. route, on the neuronal evoked firing. The degree and the time-course of the inhibitory effect of HA on the evoked nociceptive response was dose-related. As shown in figure 4, the highest dose of HA (0.1 μM/rat) caused a rapid and maximal (−46%) depressant effect on the neuronal firing after 10 min and the inhibitory response persisted up to 30 min. All these data are in agreement with those previously obtained[5].

The H_1 agonist, 2-pyridylethylamine (PEA) given i.c.v., induced a dose-dependent inhibition of the noxious neuronal firing very similar to that obtained with HA. However, the highest dose of PEA (1.0 μM/rat) caused a more pronounced and long-lasting antinociceptive effect compared with that observed with the highest dose of HA used. In contrast, the selective and potent H_2-agonist dimaprit (DIM), even if administered at a dose (0.2 μM/rat, i.c.v.) higher than HA, did not inhibit evoked firing behaviour (fig. 2).

The i.c.v. administration of mepyramine or zolantidine alone had no effect on normal response or response pattern of evoked firing. When these drugs were administered in combination with HA, only pretreatment (20 min before) with the H_1 antagonist mepyramine, but not with the H_2 receptor antagonist zolantidine, was

Table. Statistical analysis of area under the curves from figure 4.

Treatment (μM/rat, i.c.v.)		AUC (% of evoked firing × 70 min)
Saline		45.50 ± 26.37
HA	0.025	45.30 ± 28.70
	0.05	−51.33 ± 23.20
	0.1	−436.67 ± 34.12**
PEA	0.4	−200.00 ± 19.15*
	0.6	−307.67 ± 26.10*
	1	−710.00 ± 53.23**
DIM	0.05	34.83 ± 10.24
	0.1	16.50 ± 11.89
	0.2	64.00 ± 15.60
MEP	0.1	48.33 ± 27.50
MEP	0.1 + HA 0.1	32.00 ± 17.21•
ZOL	0.01	25.67 ± 11.74
ZOL	0.01 + HA 0.1	−367.00 + 16.05**

*$p < 0.05$; **$p < 0.01$ vs saline; •$p < 0.05$ vs HA 0.1 μM.

able to inhibit the antinociceptive effect of HA significantly (fig. 3).

For each dose and for each neuron investigated, the numbers of spikes collected for every time-interval were recorded, the mean was calculated, and the final values were plotted as percentage change in firing versus the firing values before administration of the drugs, to compare the collected findings. These data are shown in figure 4. Statistical evaluation of the AUC of the percentage of evoked firing calculated between 0 and 70 min is shown in the table.

Discussion

The present study confirms our previous data showing that HA is able to depress the thalamic neuronal firing activated by a peripheral noxious stimulus[5,8] and extends our knowledge of the type of HA receptors involved in such an effect. The similarity of the dose-related antinociceptive response of the H_1 agonist 2-pyridylethylamine to that obtained with HA, and the fact that the antinociceptive behaviour of HA in the thalamus is antagonized by pretreatment with the H_1 antagonist mepyramine, indicate that the H_1 receptors are involved in the inhibitory control of HA on evoked firing. The lack of effect of the selective H_2 agonist dimaprit on the nociceptive electrophysiological behaviour, even if administered at doses more potent on H_2 receptors than 2-pyridylethylamine on H_1 receptors, and the failure of the H_2 antagonist zolantidine to prevent the HA antinociceptive effect, strongly suggest that histamine H_2 receptors are not involved in the mediation of the HA response at the thalamic level. It is unlikely that the failure of zolantidine to remove the depressing activity of HA on thalamic neuronal firing could be due to the low dose used, since the same concentration was reported to be effective in inhibiting the naloxone-resistant foot shock-induced antinociception[19].

It is well documented that the somatosensory neurons of the ventrobasal thalamic complex in arthritic rats are activated by mechanical stimulation of the inflamed joints via activation of nociceptive afferents[20]. Such a stimulus provides the nociceptive afferent information to this region of the thalamus through spinothalamic inputs as well as less direct polysynaptic pathways[21,22]. Therefore, the depression of the activity of rat thalamic neurons by HA could be ascribed either to a direct action at this level or to influences on other CNS areas involved in the processing of pain. Since in the thalamus a high density of H_1 receptors has been observed[23] it is possible to suggest that a specific interaction of the amine with H_1 receptors may be important for the HA antinociceptive effect on afferent nociceptive peripheral inputs to the thalamus. This suggestion is supported by the observation that the inhibitory effect of HA on evoked neuronal firing appears soon after its administration, thus indicating that the drug did not have to diffuse far to exert its effect.

Based on behavioural tests, the pharmacological attempts to attribute the antinociceptive central activity of HA to H_1 or H_2 receptors indicate that both types of HA receptors may mediate HA antinociceptive central activity. The prevailing participation of H_1 or H_2 receptors in the processing of pain by HA could depend on where they are located in the CNS and on different neuronal pathways activated by different types of nociceptive stimuli[24].

It has been reported that H_2 receptors mediate antinociceptive responses to HA[6] and to morphine[25] in the periaqueductal grey matter (PAG), an area known to be a component of descending endogenous analgesic systems[26]. Considering that the PAG is innervated by histaminergic fibers originated from the hypothalamus[27], it is possible to suggest a modulatory role for histamine H_2 receptors in the descending inhibitory control of pain. On the other hand, the participation of H_1 receptors in the HA antinociceptive effect has been reported by Bhattacharya and Parmar[9] using the tail flick test, and furthermore we observed that both H_1 and H_2 receptors are involved in the inhibitory role of HA in the control of nociception in conditions of inflammatory pain[10]. Therefore it is likely that the interaction of histamine with H_1 receptors may be more relevant in attenuating spinally organized nociceptive responses.

Taken together these data indicate the existence of multiple antinociceptive mechanisms subserving HA antinociception, conceivably linked to the different type and/or localization of HA receptors.

In conclusion, the present study is an extension of our previous finding that HA is able to depress the thalamic neuronal firing activated by peripheral noxious stimuli and provides some knowledge of the type of HA receptors modulating this aspect of pain. Electrophysiologi-

cal investigations thus provide new insights into the modulatory role of HA in pain perception.

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