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HIPPOCAMPUS AND LEARNING

POSSIBLE ROLE OF HISTAMINE RECEPTORS

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Summary The effect of local administration of histamine and its receptor antagonists into the hippocampus on the learning process of an active avoidance response was studied. The task that the animals had to learn consisted in avoiding an electric shock on their feet after a conditioning ultrasonic 40 kHz tone was on. Latency time was defined as the time in sec rats took to avoid or escape the electric shock: % CAR was defined as the cummulative positive responses during learning session. All rats were implanted into the ventral hippocampus with guide cannulae. On the day of the experiment, rats were microinjected through the guide cannulae with 1 µl of saline solution containing 67.5 nmol of ranitidine or pyrilamine alone or in combination with 45 nmol of histamine. All groups were subjected to two sessions of learning. Results show that treatment with histamine was effective to block the adquisition of the response, since animals showed a learning curve significantly inferior to that of the controls. Ranitidine treatment was not able to block the histamine effect. Pyrilamine treatment, instead, was effective to block the inhibitory action of histamine on learning. Results suggest that histamine in hippocampus may be exerting a modulatory control on retrieval processes of memory.

Keywords: memory, hippocampus, histamine, avoidance-response

During the past 10 years histamine (HA) has been recognized as a widespread neuromodulator or neurotransmitter in the brain[1, 2, 3]. Immunohistological evidence has shown that histaminergic neurons appear to concentrate in the tubero magnocellular, caudal magnocellular and postmammillary caudal magnocellular nuclei of the posterior hypothalamic area of the rat brain[4, 5, 6, 7]. From these zones extensive projections of nerve fibers reach several structures of the telencephalon such as the olfactory bulb, basal ganglia, amygdaloid complex, hippocampus and septum[4]. Our laboratory is interested in studying the probable physiological role of HA in the hippocampal structure. Histamine fibers and histamine binding sites have been localized in this brain complex, suggesting that the imidazol amine could have some physiological function[4]. Previous work in rats have shown that HA locally applied into the ventral hippocampus was able to selectively inhibit some motor behaviours[8, 9, 10]. Furthermore, using a model of an active avoidance response learning, it was found that in the ventral hippocampus HA interfered with the retrieval of the response and this action seemed to involve H_1-histamine receptors[11, 12]. According to this evidence, it appears that besides its role in motor control the imidazolamine could participate in the complex mechanism of memory in the hippocampal formation. It was thought that if HA inhibits the mechanisms of memory recall, then the process of learning should also be affected, since retrieval is an important factor in the acquisition of the memory cues. The purpose of the present work was to investigate if HA locally applied into the hippocampal formation was able to interfere with the learning of an active avoidance response. A

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characterization of the histamine receptors was also intended.

Materials and methods

Animals

Male rats of the Holtzman-derived colony, weighing 250-300 g, 90 days old and maintained in thermoregulated (22-24°C) and light-controlled conditions (06.00-20.00 h) were used. Standard rat chow and water were available *ad libitum*.

Implantation procedures

Animals were anesthetized with ether and unilaterally implanted with guide steel cannulae (23-gauge, 15 mm length) into the caudal ventral hippocampus, as it was described elsewhere[13, 14, 15]. After implantation rats were caged individually and remained at rest for at least 72 h.

Drugs

Histamine dihydrochloride (Sigma Chemical Co., USA), Pyrilamine Maleate (Sigma Chemical Co., USA) and Ranitidine (R.B.I., USA) freshly prepared in saline solution were used.

Experimental schedule

The conditioned active avoidance response to an ultrasonic 40 kHz tone was used as experimental model of learning and memory. Animals were trained in a two-compartment wooden cage with a wall separating both compartments. Animals are allowed to pass from compartment 2 (the punishment box) to compartment 1 (the safe box) through a swinging door that can be locked in its place. Rats were conditioned to escape through by opening the door after the ultrasonic sinewave tone was on, as described in detail elsewhere[11, 12]. When animals avoided the electric shock passing through the door before the ultrasonic tone was off or escaped after the first electric shocks were given, a «positive response» was considered. If animals failed to escape through the door after 60 sec the ultrasonic tone was off, a «negative response» was considered. Electric shocks were given at a rate of 1 each 15 sec. Training sessions were composed of 8 trials with a maximum duration of (4.5) min each. Experiments were performed in three stages. *Stage 1. Implantation.* Animals were implanted with guide cannulae into the ventral hippocampus as described previously[13, 14, 15]. After that rats remained at rest for 72 h. *Stage 2. Adaptation.* Implanted animals were put in groups of 5 in compartment 2 with the communicating door unlocked, so rats could pass freely from one side to the other. No electric shocks and no ultrasonic tones were given this time. This period of adaptation to the cage lasted about 15 min. *Stage 3. Training.* There were two 8 trial training sessions. Twenty four h after the adaptation period, rats were subjected to the first training (Session 1). Ten minutes before rats were put in compartment 2, they were microinjected into the ventral hippocampus with 1 µl of saline solution containing 67,5 nmol of pyrilamine (PYR), ranitidine (RAN) or saline alone. Five min later, they were microinjected with 45 nmol of histamine (HA) in saline solution or saline alone. At time zero, rats were put in compartment 2 and training was begun. After 4 trials, rats were microinjected once again as explained before in order to cover possibility of fast inactivation of drugs in the hippocampal living tissue. The variables measures were: (i) «% CAR», the number of the accumulated avoiding responses divided by the total number of trials performed, and (ii) «escape latency time» (LT), the time in seconds the animals take to cross the door after the ultrasound is on. At the following day, rats were subjected to Session 2 with the same experimental schedule. Two experiments were performed.

Experiment 1: Effects of ranitidine, the H_2-histamine antagonist and HA locally applied into the ventral hippocampus on the adquisition of the ultrasonic conditioned avoiding response. Experimental groups were: SAL + SAL (n = 17), rats that received 1 µl of saline solution; SAL + HA (n = 15), rats that received saline and 45 nmol/µl of histamine; RAN+SAL (n = 15), rats that received 67.5 nmol/µl of ranitidine and saline; RAN+HA (n = 16), rats that received 67.5 nmol/µl of ranitidine and 45 nmol/µl of histamine.

Experiment 2: Effects of pyrilamine, the H_1-histamine antagonist and HA locally applied into the ventral hippocampus on the adquisition of the ultrasonic conditioned avoiding response. Experimental groups were: PYR+SAL (n = 15), rats that received 67.5 nmol/µl of pyrilamine and saline; PYR+HA (n = 16), rats that received 67.5 nmol/µl of pyrilamine and 45 nmol/µl of HA. Doses of antagonists were used according to previous results[11, 12]. In all these experiments a full activity of the histamine antagonists on its respective receptors at the equimolar doses was assumed. Once experiments were completed, all rats were sacrificed by ether excess and their brains dissected out for histological verification of sites of implants as described earlier[13, 14]

Statistics

Multiple comparisons between different experimental groups were performed using the Non Parametric Test of Dunn[16]. A p value less than 0.05 was considered significant. Data were presented as the median ± standard error.

Results

Experiment 1

The latency time to show the active avoiding response of implanted rats microinjected into the ventral hippocampus with HA and the H_2-histamine antagonist ranitidine in Session 2 is shown in Fig. 1 A. Control rats showed a latency time of 32 ± 4.03 sec in the first trial of Session 2. From there on, the animals reached a median of 3.5 sec in the remaining trials. Animals microinjected with 45 nmol of HA showed instead a latency time about 34 sec in the first 6 trials and reached a value of about 5 sec at the following final trials. Significant differences were found when trials 2-6 in this troup were compared with the respective trials in control group ($p < 0.01$). Groups of rats treated with RAN alone or RAN in combination with HA showed a latency time significantly different from SAL+HA group in trials 7 and 8.

Percentage of accumulated CAR in Session 2 in these same group of implanted rats is shown in Fig. 1B. Control animals showed an increasing learning curve during trials reaching a score of 75 ± 5.2% at the end of the trial 8. Rats treated with 45 nmol of HA showed a learning curve displaced to the right, reaching a score of 37 ± 6.5% at the end of the trial 8. When CAR responses of each trial of SAL+HA group were compared with the respective trials of control group (SAL+SAL), significant differences were found at trials 2-8 ($p < 0.01$). Rats treated with 67.5 nmol of RAN and SAL showed a flat curve of learning, reaching scores of about 13% at the two final trials. Significant differences were found when scores of trials 2-8 of this group were compared with the respective trials of the control group ($p < 0.01$). When RAN+SAL group was compared with SAL+HA group, significant differences were found in scores of trials 6 and 8 ($p < 0.05$). Finally, animals treated with 67.5 nmol of RAN and 45 nmol of HA showed no learning curve at all. Significant differences were found when scores of trials 2-8 in this group were compared with the control group ($p < 0.01$). Significant differences were also found in the scores of trials 4-8 of this group when compared with SAL+HA group ($p < 0.01$). No statistically differences were detected when RAN+HA and RAN+SAL groups were compared.

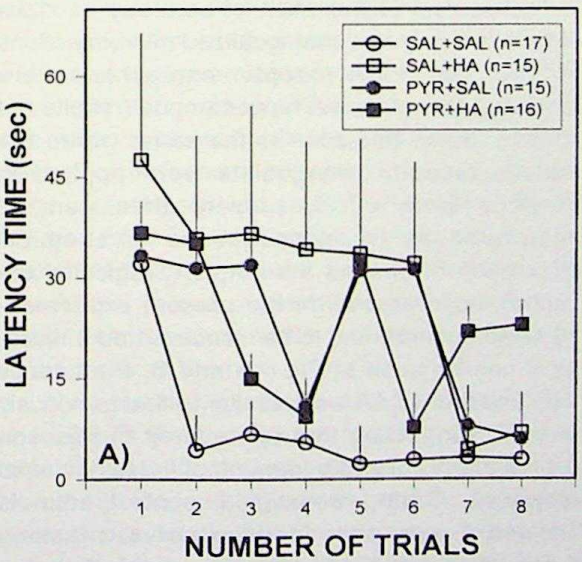

Fig. 1.— Latency time and % CAR curves during learning in implanted rats microinjected into the hippocampus with histamine and the H_2-histamine receptor antagonist ranitidine. A) Statistical comparisons against control (SAL+SAL group): SAL+HA group, trials 2 to 6, $p < 0.01$; RAN+SAL group, trials 2 to 8, $p < 0.01$; RAN+HA group, trials 2 to 8, $p < 0.01$. Statistical comparisons against SAL+HA group: RAN+SAL group, trials 7 & 8, $p < 0.01$; RAN+HA group, trials 7 & 8, $p < 0.01$.

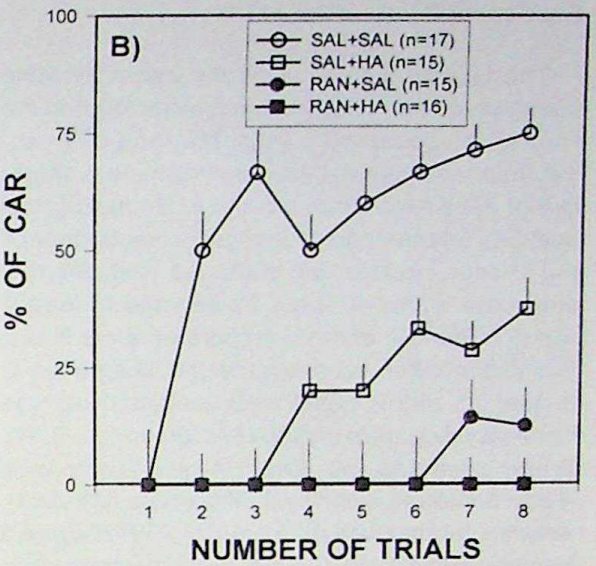

Fig. 1.-- B) Statistical comparisons against control: SAL+HA group, trials 2-8, $p < 0.01$; RAN+SAL group, trials 2-8, $p < 0.01$; RAN+HA group, trials 2-8, $p < 0.01$. Statistical comparisons against SAL+HA group: RAN+SAL group, trials 6 & 8, $p < 0.05$; RAN+HA group, trials 4-8, $p < 0.01$.

Experiment 2

The latency time to show the active avoiding response of implanted rats microinjected into the ventral hippocampus with HA and the H_1-histamine receptor antagonist pyrilamine is shown in Fig. 2A. Animals treated with 67.5 nmol of PYR and SAL presented a variable response through the 8 trial session. In trials 1-3 and 5-6 rats showed a score of about 31 sec and in trials 4 and 7-8 animals showed a score of about 8 sec. Significant differences were found when trials 2, 3, 5 and 6 of this group were compared with the corresponding trials of SAL+SAL group ($p < 0.01$). When compared with SAL+HA group, only trials 4 and 6 showed significant differences ($p < 0.01$). Animals treated with 67.5 nmol of PYR showed a learning curve similar to that of the controls. Only trial 2 in the PYR+HA was found significantly different from trial 2 of the control group ($p < 0.05$). When PYR+HA and SAL+HA groups were compared, statistically significant differences were found in trials 3 to 8 ($p < 0.05$).

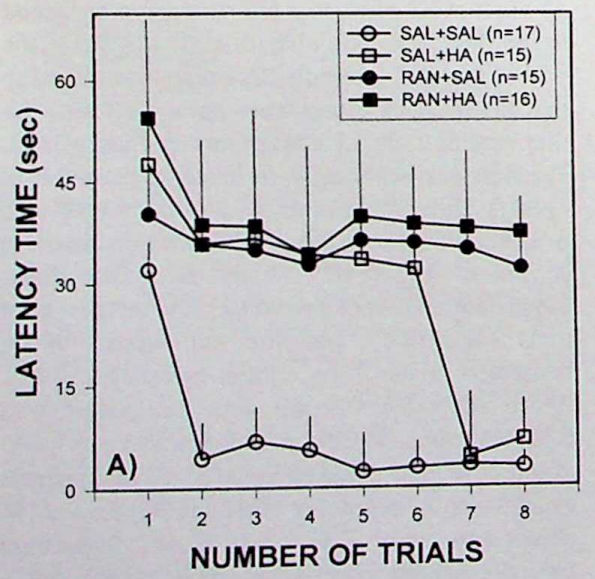

Fig. 2.— Latency time and % CAR curves during learning in implanted rats microinjected into the hippocampus with histamine and the H_1-histamine receptor antagonist pyrilamine.
A) Statistical comparisons against control: PYR+SAL group, trials 2, 3, 5 and 6, $p < 0.05$.

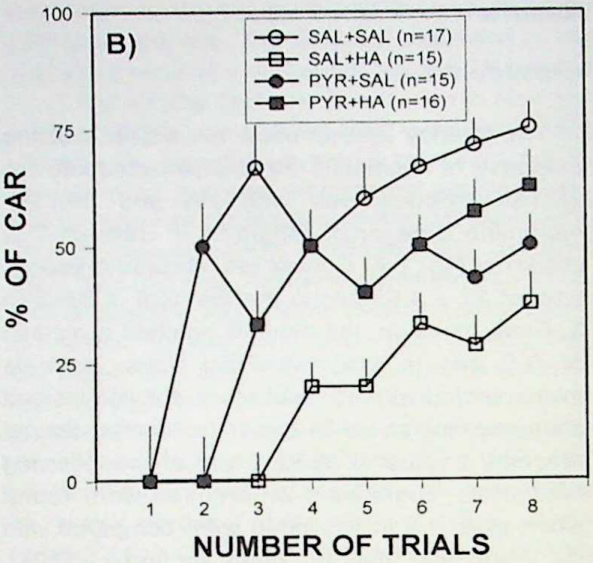

Fig. 2.-- B) Statistical comparisons against control: PYR+SAL group, trials 7 & 8, $p < 0.05$; PYR+HA group, trial 2, $p < 0.05$. Statistical comparisons against SAL+HA group: PYR+SAL group, trials 2-4, $p < 0.01$; PYR+HA group, trials 3, 4, 7 & 8, $p < 0.05$.

Discussion

Histological examination of coronary sections of rat brain revealed that localized microinjections of SAL, HA or HA receptor antagonists were restricted to the ventral hippocampus (results not shown). Since this zone is the same where HA and its receptor antagonists were applied in previous work[11, 12, 15], present data can be interpreted as the consequence of chemical stimulation of at least the CA_1-CA_4 region of the ventral hippocampus. In the present experimental setup, animals had to be microinjected 4 times. As it can be seen in Fig. 1A and B, the latency time and the %CAR were similar to that previously found[12], suggesting that procedures of injection did not affect adversely learning of the conditioned response. During Session 1 control animals showed a very poor learning curve, reaching criterium in trial 8 (results not shown). For that reason the study was focalized to Session 2 where consolidation of learning was made by control rats and the possible effects of HA and its antagonists should be more evident. In Experiment 1, HA treatment was effective in inhibiting the adquisition of the avoidance response (Fig. 1A). Interestingly, HA effect disappears after trial 6 even though a second reinforcement injection of

the imidazolamine was made before trail 5. Since this result can not be explained by an inactivation of HA in hippocampus, data suggest that some type of compensatory mechanisms is developed when reiterative conditioning stimuli are presented to the animal. Blocking of the H_2-histamine receptors by ranitidine did not abolish the inhibitory influence of HA (Fig. 1A). Although this evidence suggests that H_2-histamine receptors have no participation in memory processes in hippocampus, it can not be discarded some role to these receptors since animals treated with ranitidine alone did not show a normal latency time curve, and treatment of both the antagonist and HA also blocked completely the reaching of latency times less than 30 sec in the final trials of the sessions (Fig. 1A). Coherent findings were observed when % CAR were examined (Fig. 1B). Some evidence described by other authors seems to support the present results. It is known that HA hyperpolarizes the hippocampal CA_1 neurons *in vitro*[17] and a selective blocking of the late calcium-dependent current in hippocampal dentate granule cells by HA as a reduction of the field excitatory postsynaptic potential evoked by the perforant path stimulation has been also described[18].

In Experiment 2, blocking of H_1-histamine receptors by pyrilamine was able to counteract the inhibitory action of HA. These results give a further support about the importance of H_1-histamine receptors in learning processes. Memory mechanisms can be viewed as ocurring in two phases: (i) adquisition of information and (ii) recall or retrieval of that information. Latency time as it was measured in our experiments is estimating the recall mechanisms, while % CAR is an approximate index of learning or the acquisition process. In our laboratory previous data have shown that HA inhibits the recall phase of memory[11, 12]. Within this context, it was not surprising that HA interfered with the learning process and the hypothesis stated previously has been supported by the present results. Although there is agreement that HA may have a role in memory[11, 12, 21, 22, 23, 19, 20], the mechanisms and the specific effect of HA in these cognitive processes is uncertain. Some authors[19, 20, 21] have proposed that HA facilities memory consolidation. Meanwhile, others have found evidence supporting an inhibitory effect of HA on memory processes[22, 23] which is in agreement with our previous and present results[11, 12]. It is not clear the reason of discrepancy about this issue, but different experimental models, as routes of HA administration or doses of the imidazolamine used, can in part explain the conflicting results. Nevertheless, some other data are highly suggestive for an inhibitory action of HA on memory. For example, aging is usually associated with memory loss and normal aging in humans increase histamine levels and the number of mast cells in the brain[24, 25, 26, 27]. In senile dementia of the Alzheimer type, which is characterized by memory deficits, the cerebrospinal fluid and the brain contain higher HA levels than controls[28, 29, 30, 31]. Perhaps it is appropriate to speculate about the possible physiological role of HA in hippocampus in relation to memory mechanisms. As important to a living system as acquisition of information is *extinction* of that information[32]. It should be interesting to correlate hippocampal HA neuro-transmitter systems with at least part of the extin-ction processes in the brain. It is evident that ad-ditional research on this subject will be necessary in order to find out the exact role of HA on learning and memory mechanisms.

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Resumen

Hipocampo y aprendizaje. Posible papel de los receptores histaminérgicos

Se estudió en el presente trabajo el efecto de la administración en el hipocampo de histamina y los antagonistas de sus receptores sobre el aprendizaje de una respuesta de evitación activa condicionada en la rata. La tarea que los animales debían aprender consistió en evitar un golpe eléctrico en sus patas al escuchar un tono de ultrasonido de 40kHz. El tiempo que la rata tardaba en escapar a partir del momento del estímulo condicionante fue la latencia. El número de respuestas correctas acumuladas en el tiempo fue % de CAR. Todos los animales fueron implantados en el hipocampo ventral con cánulas de microinyección. En el día del experimento, los animales fueron inyectados con 1 µl de solución salina, o bien con 67,5 nmol de pirilamina o ranitidina, en combinación o no con 45 nmol de histamina. Todos los grupos fueron sometidos a 2 sesiones de 8 ensayos cada una del aprendizaje de la tarea. Los resultados mostraron que el tratamiento con

histamina bloqueó la adquisición de la tarea durante la mayor parte del ensayo provocando en los animales una curva de retención significativamente menor que la mostrada por el grupo control. La administración de ranitidina no contrarrestó el efecto de la imidazolamina pero el tratamiento con pirilamina fue efectivo en impedir los efectos de la histamina. Se concluye que la histamina podría estar participando en el hipocampo en la regulación de los mecanismos de evocación de la memoria.

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