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Activation of histaminergic H_3 receptors in the rat basolateral amygdala improves expression of fear memory and enhances acetylcholine release

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Abstract

The basolateral amygdala (BLA) is involved in learning that certain environmental cues predict threatening events. Several studies have shown that manipulation of neurotransmission within the BLA affects the expression of memory after fear conditioning. We previously demonstrated that blockade of histaminergic H_3 receptors decreased spontaneous release of acetylcholine (ACh) from the BLA of freely moving rats, and impaired retention of fear memory. In the present study, we examined the effect of activating H_3 receptors within the BLA on both ACh release and expression of fear memory. Using the microdialysis technique in freely moving rats, we found that the histaminergic H_3 agonists R-α-methylhistamine (RAMH) and immepip, directly administered into the BLA, augmented spontaneous release of ACh in a similar manner. Levels of ACh returned to baseline on perfusion with control medium. Rats receiving intra-BLA, bilateral injections of the H_3 agonists at doses similar to those enhancing ACh spontaneous release, immediately after contextual fear conditioning, showed stronger memory for the context–footshock association, as demonstrated by longer freezing assessed at retention testing performed 72 h later. Post-training, bilateral injections of 15 ng oxotremorine also had a similar effect on memory retention, supporting the involvement of the cholinergic system. Thus, our results further support a physiological role for synaptically released histamine, that in addition to affecting cholinergic transmission in the amygdala, modulates consolidation of fear memories

Introduction

There is increasing evidence that neuronal histamine can affect cognitive processes by modulating neuronal function throughout the brain. Both physiological and morphological characteristics of histaminergic neurons are coherent with such a role. Indeed, histaminergic cells project profusely to the whole brain and spinal cord (Watanabe *et al*., 1984; Panula *et al*., 1989), and fire spontaneously at variable rates (Reiner, 1987; Haas & Reiner, 1988) according to the circadian rhythm (Prast *et al*., 1988; Sakai *et al*., 1990), or the behavioural state (Weiler *et al*., 1998). In addition, recent evidence suggests that activation of histaminergic receptors subtypes can regulate neuronal mitogen-activated protein kinase (MAPK) phosphorylation (Blandina *et al*., 2001; Drutel *et al*., 2001), and modulate plastic changes (Brown *et al*., 1995; Selbach *et al*., 1997; Knoche *et al*., 1999; Ponomarenko *et al*., 2001), which are two early cellular mechanisms likely involved in memory consolidation (Schafe *et al*., 2001). Manipulation of the histaminergic central system affects animal behaviour during several learning paradigms (Meguro *et al*., 1995; Prast *et al*., 1996; Giovannini *et al*., 1999); however, the results are often contradictory, as both facilitatory and inhibitory effects of histamine on memory have been described (Passani *et al*., 2000). Possible confounding factors could be the systemic administration of histaminergic compounds, or extensive lesions of the histaminergic tuberomammillary nuclei, which are protocols that do not exclude effects on arousal, anxiety, perception or other homeostatic mechanisms in which histamine is involved (Sakai *et al*., 1990; Onodera *et al*., 1994; Philippu & Prast, 2001). As it is likely that the memory-modulating action of histamine affects several brain regions differently, the procognitive or amnesic effects of histamine can be evaluated with experimental protocols that interfere with the exact timing of histamine release in discrete brain regions, during the appropriate behavioural task. We recently demonstrated that injections of histaminergic H_3 receptor antagonists in the basolateral complex of the amygdala (BLA) immediately post-training, impaired memory consolidation of contextual fear conditioning (Passani *et al*., 2001). The lateral and basal nuclei of the amygdala are critical for learning information with highly emotional components (see Maren, 2001; for a review), and several studies showed that pharmacological manipulations affecting different hormones and neurotransmitters, such as glucocorticoids (Roozendaal & McGaugh, 1997), dopamine (Nader & LeDoux, 1999) and noradrenaline (Liang *et al*., 1986), within the amygdala modulate memory for aversive experiences (McGaugh & Izquierdo, 2000). One of the mechanisms implicated seems to rest on the modulation of the cholinergic function, as systemic or intra-BLA administration of muscarinic cholinergic compounds affects memory retention and/or the expression of fear responses in trained rats (Introini-Collison *et al*., 1996; Rudy, 1996; Vazdarjanova & McGaugh, 1999). Interestingly, when we perfused the BLA with H_3 receptor antagonists at

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concentrations similar to those that affected fear memory, the spontaneous release of ACh from the BLA of freely moving rats significantly decreased (Passani *et al*., 2001). According to our hypothesis, then, blockade of H_3 autoreceptors (Arrang *et al*., 1983b) upregulates spontaneous histamine release, which in turn impairs cholinergic neurotransmission and memory. The present experiments further test our assumption by investigating the effect of H_3 receptor agonists on cholinergic function in the BLA of freely moving animals and on contextual fear conditioning.

Materials and methods

Animal housing

Male Wistar rats (250–280 g) were housed in groups of three in a temperature-controlled room (20–24 °C), on a 12 h light : 12 h dark cycle and were allowed free access to food and water. All the experiments were carried out in strict compliance with the EEC recommendations for the care and use of laboratory animals (86/609/CEE), and were approved by the Animal Care Committee of the 'Dipartimento di Farmacologia Preclinica e Clinica' of the 'Universitá di Firenze'.

Surgery and microdialysis

Cannulae (Metalant, Sweden) were implanted as described in Passani *et al*. (2001). Briefly, rats were restrained in a stereotaxic frame (Stellar, Stoelting Co., Wood Dale, IL, USA) under chloral hydrate anaesthesia (400 mg/kg i.p.), and implanted with a guide cannula according to the following coordinates (Paxinos & Watson, 1998): AP = –2.8, L = –4.9, DV = 3.8 mm from bregma. The microdialysis experiments were performed 24 h after surgery during which rats, placed one per cage, recovered from surgery. On the day of the experiments, the stylet was removed from the guide cannula and a microdialysis probe (1.5 mm dialysing membrane molecular weight cut-off = 6000 Da; Metalant, Sweden) was lowered into the BLA, with the tip extending 4.5 mm beyond the tip of the guide cannula. Ringer's solution (in mM: NaCl, 147; $CaCl_2$, 1.2; KCl, 4.0; pH 7.0) was perfused at the rate of 1.35 μL/min using a microperfusion pump (CMA/100; Carnegie Medicine, Sweden).

Neostigmine bromide (0.1 μM), a cholinesterase inhibitor, was added to the medium perfusing the BLA to recover detectable ACh concentrations in the dialysate. Endogenous molecules, and exogenous compounds with molecular weight lower than 6000 Da, were allowed to cross the dialysis membrane according to their concentration gradient, and could be collected and administered, respectively. After 2 h equilibration period, fractions were collected at 20 min intervals. The BLA was perfused with control medium in the first three fractions to measure ACh spontaneous release and drugs were then added to the medium.

Histology

The placement of microdialysis membranes was verified post mortem. Rats were killed with an overdose of chloral hydrate and the brains were removed and stored in 10% formaline for 10 days. Sections (40 μm) were then cut on a cryostate, mounted on gelatin-coated slides and stained with cresyl violet for light microscopy. Data from rats in which the membranes were not correctly positioned were not included.

Assay of ACh

Acetylcholine was determined by HPLC-electrochemical detection as described previously (Giorgetti *et al*., 2000). Briefly, ACh was separated on a cation exchange column and hydrolysed by acetylcholinesterase to form acetate and choline in the postcolumn enzyme reactor. Choline was then oxidized by choline oxidase to produce betaine and hydrogen peroxide, which was detected by a platinum electrode with the potential set at 1.0 V. Peaks were identified by comparison of their retention times with those of the standards.

Quantification of ACh

The levels of ACh in the perfusates were calculated by comparison of sample peak heights with external standard peak height, and expressed as pmol/20 min. Calibration curves for ACh were constructed by plotting the heights of peaks against the concentrations. Regression lines were then calculated and determination of unknown samples was carried out by the method of inverse prediction. Spontaneous release of ACh was calculated for each experiment by averaging the mean of the three 20 min samples of perfusate collected before drug treatment. Release of ACh was expressed as a percentage of its spontaneous release value. The *in vitro* recovery of ACh from the dialysis membrane was about 60% at room temperature. Values reported here were not corrected for recovery.

Statistical analysis

All values are expressed as means ± SEM, and the number of rats used in each experiment is also indicated. The presence of significant treatment effects was first determined by a one-way analysis of variance (ANOVA) followed by Bonferroni's test. For all statistical tests, $P < 0.05$ was considered significant. For clarity purposes and for biological relevance, we reported in figures and figure legends only the significant differences vs. the last sample before drug treatment. Statistical analysis was performed using StatView (Abacus Concepts, Inc., Berkeley, CA, USA) and GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA).

Behavioural experiments

Experiments were performed on 7-day-old male albino Wistar rats (average body weight 290 g). The animals were housed individually in stainless steel cages in a room with a natural light–dark cycle (windows) and constant temperature of 20 ± 1 °C. The rats had free access to food and water throughout the experiments.

Apparatus

A basic Skinner box module (Modular Operant Cage, Coulbourn Instruments Inc. PA, USA) was used to induce contextual conditioned freezing as in previous experiments (Sacchetti *et al*., 1999). The top and the two opposite sides were aluminium panels and the other two sides were transparent plastic. The floor was made of stainless steel rods connected to a shock delivery apparatus (Grid Floor Shocker, model E13-08; Coulbourn Instruments Inc. PA, USA). The apparatus was connected to a stimulus programming device (Scatola di comando Arco 2340, Ugo Basile, Italy) to predetermine the number and duration of the electric shocks and the duration of the intervals between them. The apparatus was placed in an acoustically insulated room kept at a constant temperature of 20 ± 1 °C. Illumination inside the room was 60 lux.

Contextual fear conditioning procedure

The rat was gently taken manually from the home cage to the soundproof room. Once there, it was placed inside the conditioning apparatus. The rat was left undisturbed for 3 min After this time,

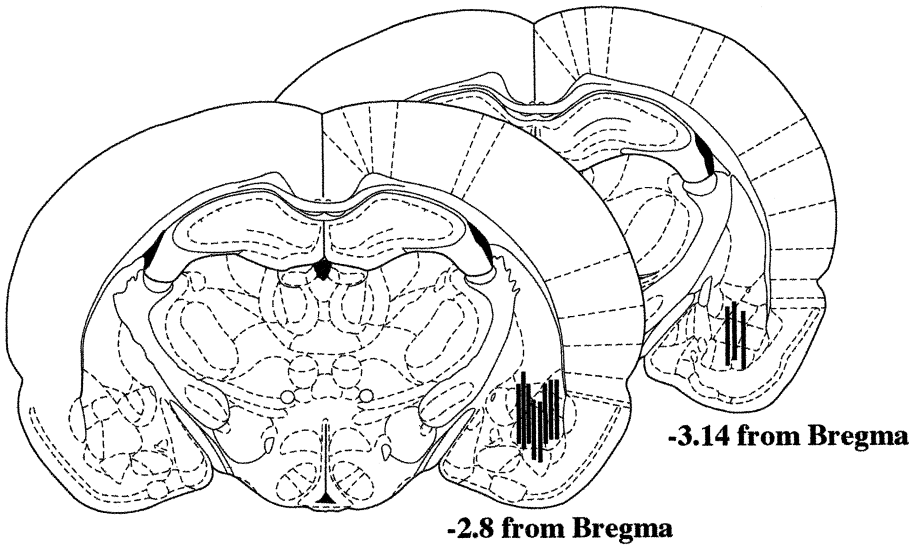

FIG. 1. Schematic drawing illustrating the placement of microdialysis probes in the BLA (n = 10). The black bars represent the dialysing portion of the probe. The position posterior to bregma were defined according to Paxinos & Watson (1998).

seven 1 s, 1 mA electric footshocks were administered at 30 s intervals. Two minutes after the end of the stimulation, the rats were removed, thus spending a total time of 8 min inside the conditioning apparatus.

Conditioned freezing measurements

One of the behavioural manifestations of conditional fear in rats is freezing, which consists of the complete absence of voluntary movements. Freezing duration was measured 72 h after conditioning by an experimenter unaware of the animal's treatment. To measure freezing, the animals were again placed inside the conditioning apparatus and left for 6 min, during which time they did not receive electrical stimulation. After that, they were returned to the home cage. The rat's behaviour was recorded by means of a closed circuit television system. Freezing time was measured with a stop-watch. All behavioural tests were performed between 10.00 h and 12.00 h.

Surgery and drug administration

Immediately after the training, rats were anaesthetized with ketamine (100 mg/kg i.p), and bilateral injections performed in the following 10 min. Drugs dissolved in physiological saline were injected into the BLA of rats restrained in a stereotaxic apparatus. The same coordinates were used as for the implantation of the microdialysis probes (according to Paxinos & Watson, 1998). The tip of the needle was placed 8.7 mm ventral to bregma. The injection needle (outside diameter, 0.3 mm) was connected with a short piece of polyethylene tubing to a Hamilton syringe that was fixed to an electrode holder. Solutions (0.5 μL per side) were injected over a 1–2 min period and the needle was left in place for another minute before withdrawing it. Control groups received bilateral injections of saline.

Histology

At the end of the experiments, rats were deeply anaesthetized and the brains were removed and stored in 10% formaline for 10 days. Sections were cut with a freezing microtome and Nissl-stained to verify injection sites.

Data analysis

For each session, data were expressed as seconds spent freezing within the 6 min of testing. One-way ANOVA and Neuman–Keuls *post hoc* tests were used. For all statistical tests, $P < 0.05$ was considered significant.

Chemicals

The substances used in this study included, neostigmine bromide dihydrochloride, R-α-methylhistamine dihydrochloride (RBI, Natick, MA, USA) and oxotremorine (Sigma Chemical Co. Ltd, UK). Immepip dihydrobromide was kindly provided by Drs R. Leurs and H. Timmerman (Vrije University, Amsterdam). All other reagents and solvents were of HPLC grade or the highest grade available (Sigma).

Results

Data analysis comprised only animals with correct placement of the dialysing membranes within the boundaries of the BLA (< 90%). Figure 1 shows the placement of the dialysing membranes in the BLA of experimental rats that received perfusion of the H_3 receptor agonists.

H_3 receptor agonists locally administered to the BLA increase spontaneous ACh release

Microdialysis membranes were introduced in the cannulae 24 h after surgery and perfused with Ringer's solution containing the cholinesterase inhibitor neostigmine (0.1 μM), which was necessary to recover detectable concentrations of ACh. After a 120 min equilibration period, the BLA spontaneously released ACh at stable rates (0.12 ± 0.01 pmol/20 min; n = 10, mean of all animals, irrespective of group assignment) and did not change significantly with time. The effect of selective H_3 receptor agonists were then tested on the spontaneous release of ACh. When 1 μM of the potent and selective histamine H3 receptor agonist, 4-(1H-imidazol-4-yl-

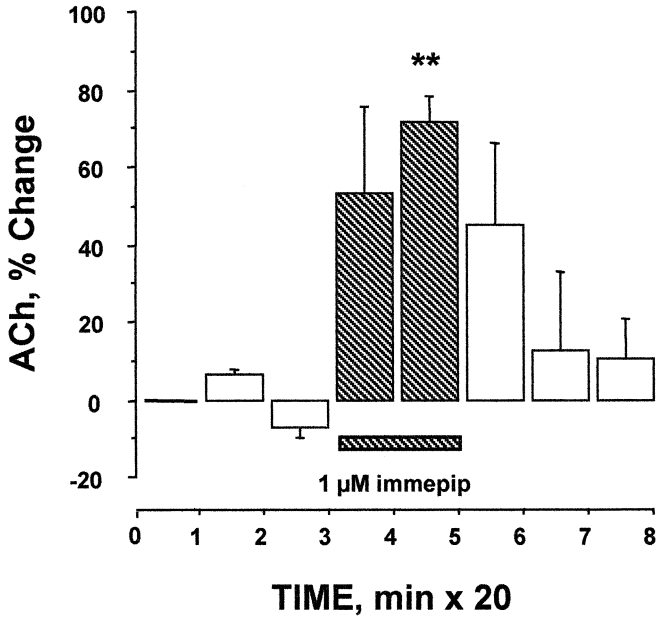

FIG. 2. Effect of 1 µM immepip on ACh spontaneous release from the BLA of freely moving rats. Acetylcholine levels were measured in fractions collected every 20 min, beginning 2 h after the onset of the perfusion. Spontaneous release was calculated for each experiment by averaging the mean of the three samples collected before the treatment. Shown are means ± SEM of five experiments. The mean of ACh spontaneous release value averaged 0.11 ± 0.01 pmol/20 min. The bar indicates the period of immepip application. $^{**}P < 0.01$ vs. last sample before drug treatment (ANOVA and Bonferroni's test).

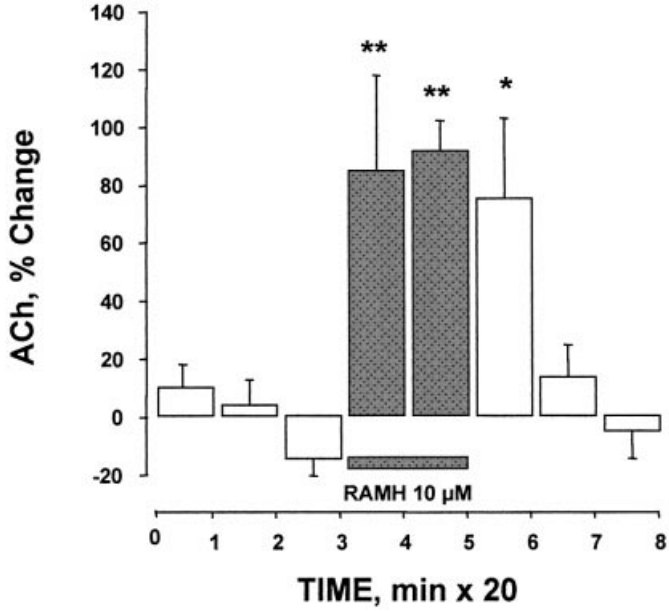

FIG. 3. Effect of 10 µM R-α-methylhistamine on ACh spontaneous release from the BLA of freely moving rats. Spontaneous release was calculated for each experiment by averaging the mean of the three samples collected before the treatment. The bar indicates the period of drug application. Data are means ± SEM of five experiments. Mean value of ACh spontaneous release averaged 0.12 ± 0.02 pmol/20 min $^{*}P < 0.05$ vs. last sample before drug treatment (ANOVA and Bonferroni's test).

methyl) piperidine (immepip; see Vollinga *et al*., 1994) was added to the BLA perfusing medium for 40 min, ACh spontaneous release increased by 72% ± 7% (Fig. 2). The maximum effect was achieved during the second 20 min period of perfusion with immepip. Baseline levels of ACh spontaneous release were quickly restored during subsequent perfusion with control medium. Spontaneous, basal release of ACh averaged 0.11 ± 0.01 pmol/20 min ($n = 5$). A similar effect was observed when the BLA was perfused for 40 min with another selective H_3 receptor agonist, R-α-methylhistamine (RAMH; Arrang *et al*., 1987). When 10 µM RAMH was added to the BLA-perfusing medium for 40 min, ACh spontaneous release increased significantly, with a maximal effect of 91% ± 11% (Fig. 3). The spontaneous, basal release of ACh averaged 0.12 ± 0.02 pmol/20 min ($n = 5$); levels returned to baseline on wash out with normal saline.

Post-training infusions of H_3 receptor and muscarinic receptor agonists in the BLA affect expression of contextual fear memory

The effect of RAMH and immepip on contextual fear conditioning was examined in rats that had received post-training, bilateral injections of these compounds in the BLA. Each drug was diluted in saline to permit injection of a constant volume (0.5 µL per amygdala). The position of the needle was examined post mortem, and when either one or both needles were outside the BLA, the animals were discarded (Fig. 4). The doses of RAMH and immepip injected were chosen according to the results of the microdialysis experiments. Therefore, 0.5 µL of a 10 µM solution of RAMH corresponded to a total amount of 626 pg RAMH, and 0.5 µL of a 1 µM solution of immepip corresponded to 83 pg immepip. Rats that received bilateral intra-BLA infusions of either RAMH or immepip showed a stronger response for the footshock-context association, as

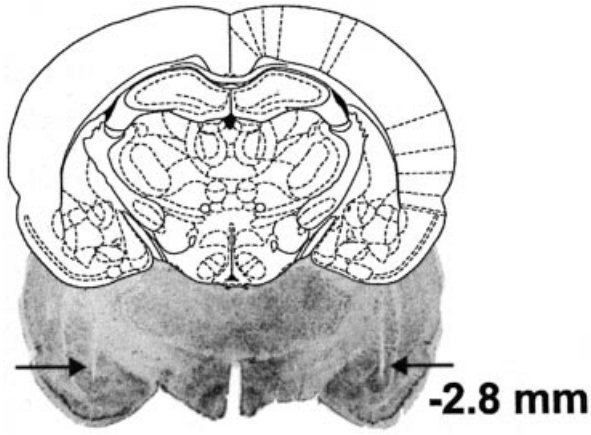

FIG. 4. Photomicrograph of an operated brain, illustrating the placement of injection needles in the BLA. Black arrows indicate the tip of the needles. The schematic drawing illustrates the position of the coronal section posterior to bregma according to Paxinos & Watson (1998).

assessed by measuring the time spent freezing in the 72 h retention test (Fig. 5). Analysis of variance on the freezing behaviour revealed a significant treatment effect ($F_{6,76} = 6.147$, $P < 0.0001$). Neuman–Keuls *post hoc* analysis showed that rats receiving either 626 pg RAMH ($n = 10$), or 82.6 pg immepip ($n = 13$) spent significantly more time freezing than saline-injected controls ($n = 14$; $P < 0.01$ and $P < 0.05$ vs. control). Similarly, rats that received intra-BLA injections of the muscarinic agonist oxotremorine (15 ng; $n = 14$) showed enhanced freezing (Fig. 5). There were no significant differences, however, between animals treated with 62.6 pg RAMH ($n = 12$), 8.3 pg immepip ($n = 10$), 1.5 ng oxotremorine ($n = 10$) and controls.

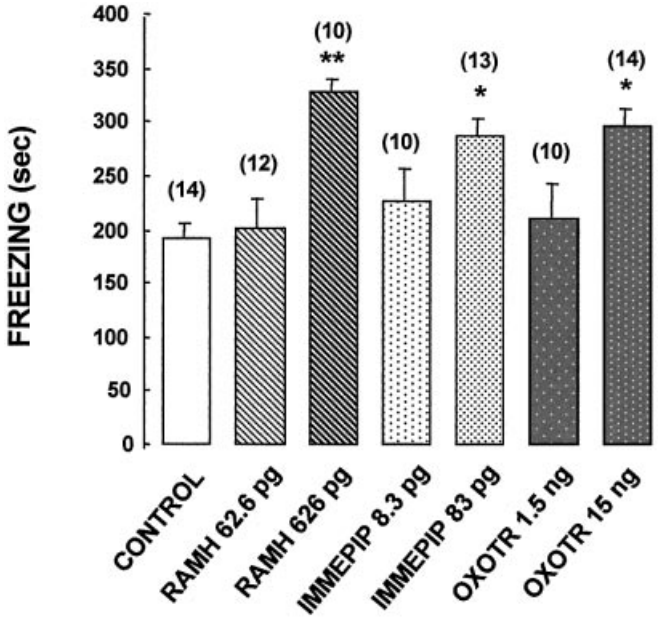

FIG. 5. Effects of post-training bilateral injection of H_3 receptor agonists and oxotremorine into the BLA on contextual fear conditioning. Immediately after training, rats were injected with drugs under general anaesthesia. Seventy-two hours after training, freezing was measured during the 6 min period of testing. Each bar represents the mean value ± SEM of (*n*) rats. $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. control (ANOVA and Neuman–Keuls's test).

Discussion

We have previously provided evidence that intra-BLA administration of histaminergic H_3 receptor antagonists impaired memory for contextual fear conditioning and decreased spontaneous ACh release (Passani *et al*., 2001). The findings of this paper show that post-training infusions of H_3 receptor agonists in the BLA increase the level of fear responses assessed at the retention test, and augment spontaneous ACh release from the BLA. Therefore, cholinergic tone within the amygdala can be modulated by histaminergic compounds in a bimodal fashion, and the expression of fear memories can be modified accordingly. Moreover, our data further support previous results demonstrating the requirement of muscarinic receptor activation in the BLA during consolidation of memory for aversive stimuli (McGaugh, 2000). Both histaminergic fibres from the tuberomammillary bodies (Köhler *et al*., 1985; Inagaki *et al*., 1990) and cholinergic terminals from the nucleus basalis magnocellularis (Mesulam *et al*., 1983) innervate the amygdala. According to receptor binding (Pollard *et al*., 1993) and *in situ* hybridization studies (Lovenberg *et al*., 1999), H_3 receptors are abundant in the BLA. Our results demonstrate a functional role for such receptors. Indeed, the spontaneous release of ACh from the BLA was increased by local perfusion with medium containing either 10 μM RAMH or 1 μM immepip, two selective and potent H_3 receptor agonists with pD2 values of 8.4 (Arrang *et al*., 1987) and 9.1 (Alves-Rodrigues *et al*., 2001), respectively, in rat brain cortical slices.

We previously demonstrated that H_3 receptor antagonists decreased spontaneous ACh release in the BLA, an effect fully antagonized by the simultaneous perfusion with cimetidine, an H_2 receptor antagonist (Passani *et al*., 2001). We proposed that the blockade of H_3 autoreceptors increased extracellular levels of endogenous histamine, which impacted on H_2 postsynaptic receptors; consequently, the activation of H_2 receptors decreased ACh release. In addition to preventing the inhibition elicited by H_3 antagonists, administration of cimetidine alone increased ACh spontaneous release, thus indicating the occurrence of a histaminergic, inhibitory tone (Passani *et al*., 2001). Our present data lend further support to this hypothesis, as local perfusion with selective H_3 agonists, which decrease spontaneous histamine release (Arrang *et al*., 1983a, b), augmented ACh spontaneous output from the BLA. Moreover, this study shows that local application of H_3 receptor agonists in the BLA, at concentrations similar to those that augment ACh release, improve retention in a contextual fear test. Increased availability of ACh in the synaptic cleft may account for this effect. Indeed, stimulation of muscarinic receptors increased the levels of freezing, as shown by post-training administration of oxotremorine. Although the injection sites were centred in the BLA, some spill-over to adjacent amygdaloid nuclei can not be excluded. However, the BLA receives the densest cholinergic innervation of all amygdaloid nuclei (Ben-Ari *et al*., 1977; Carlsen *et al*., 1985), therefore the observed effect was probably mediated by a local circuit within the BLA. Furthermore, the aversive effects on learning caused by lesions of cholinergic afferents from the nucleus basalis magnocellularis (NBM) are attenuated by infusions of cholinomimetic agents in the BLA (Power & McGaugh, 2002). Our observations confirm other reports demonstrating the involvement of the amygdala cholinergic system in Pavlovian fear-conditioning and aversive inhibitory avoidance (Anagnostaras *et al*., 1999; Passani *et al*., 2001; Power *et al*., 2002). They also suggest that the influences of the histaminergic system, similarly to those of other systems, such as the adrenergic, glucocorticoid, opioid and GABAergic, (McGaugh *et al*., 1996) on memories that involve the amygdala are ultimately mediated by the activation of muscarinic, cholinergic receptors within the amygdala itself. Post-training administration of these compounds excludes any influence of the treatment on acquisition and on other processes that indirectly affect learning, thus confirming our previous report that histaminergic drugs modulate memory consolidation processes (Passani *et al*., 2001). Histaminergic H_3 receptor activation modulates ACh release in other brain areas as well, apparently with modalities that differ according to the cytoarchitectonics of different regions. The cholinergic tone in the cortex is modulated by at least two mechanisms, one at the level of the cholinergic cortical terminals (Blandina *et al*., 1996), the other at the level of the cholinergic cell bodies in the NBM (Cecchi *et al*., 2001). Local H_3 receptor activation by histamine and H_3 receptor agonists inhibits ACh release from the cholinergic terminals of the neocortex, through a neuronal arrangement that involves GABAergic neurotransmission (Giorgetti *et al*., 2000), and impairs performance in both passive avoidance and object recognition tasks (Blandina *et al*., 1996). Conversely, in the NBM, where the cholinergic somata are located, augmented levels of histamine increased cortical ACh release via activation of H_1 receptors (Cecchi *et al*., 2001). The specific behavioural significance of the interactions between the NBM cholinergic and histaminergic systems, though, remains to be explored. In the hippocampus, ACh spontaneous release is facilitated by either histamine or H_3 receptor antagonists when applied to the cholinergic septum in freely moving animals, but not to the hippocampus itself (Bacciottini *et al*., 2002). Interestingly, post-training blockade of H_3 receptors in the septum improved retention in a T-maze avoidance task, whereas H_3 receptor agonists had the opposite effect (Flood *et al*., 1998). The scenario appears to be more complex in the ventral striatum, as ACh release appears to be regulated indirectly by the activation of H_3 autoreceptors and H_3 heteroceptors located on dopaminergic and GABAergic fibres (Prast *et al*., 1999).

The relevance of the neuronal histamine system in learning and memory is beginning to be evaluated. Several studies support a memory enhancing effect of histamine but there is also much evidence that histamine impairs cognitive functions (Passani *et al*., 2000). A partial explanation of these discrepancies could be the distinct action that the histaminergic system exerts on cholinergic neurotransmission in different brain structures (Bacciottini *et al*., 2001). Our data are in agreement with the impairing effect of histamine on the acquisition of an avoidance task, when administered locally to the basolateral amygdala (Alvarez & Ruarte, 2002). Similarly, histamine inhibits performance in an active avoidance task when injected directly in the ventral hippocampus (Alvarez & Banzan, 1995). In other brain regions, though, histamine exerts the opposite effect, as locally applied H_3 receptor agonists (that presumably decrease histamine availability) have memory-impairing effects on a T-maze avoidance task, as demonstrated in mice that received intraseptum injections of imetit (Flood *et al*., 1998).

In recent studies, several intracellular processes have been implicated in fear-induced memories, including protein synthesis, and activation of the MAPK pathway (Atkins, 1998; Schafe & LeDoux, 2000; Schafe *et al*., 2000; Weeber *et al*., 2000; Lin *et al*., 2002). Activation of H_3 receptors, as well, induces phosphorylation of ERK1/2 in rat hippocampal slices (Blandina *et al*., 2001) and in transfected COS-7 cells (Drutel *et al*., 2001). Therefore the memory enhancing effect of H_3 receptor agonists in the amygdala could rely on sequential or parallel mechanisms that include modulation of cholinergic neurotransmission and/or activation of the MAPK pathway.

Clearly, much remains to be done to elucidate the role of the histaminergic system in cognition. It is intriguing that the recently developed H_3 receptor-deficient mice display normal memory response in a passive avoidance task (Toyota *et al*., 2001). However, memory is a complex process that consists of related but dissociable events that involve distinct brain regions activated to different degrees and at different times in the elaboration of disparate learning situations. The amygdala constitutes one of the core elements of a circuit that regulates emotional responses (Davidson *et al*., 2000), and human neuroimaging studies have shown that the amygdala is active in response to emotionally salient cues (Whalen *et al*., 1998; Morris *et al*., 1999). Patients with bilateral damage of the amygdala have altered perception of fearful stimuli (Adolphs *et al*., 1994; Anderson & Phelps, 2001); in addition, an abnormal activation of the amygdala in disorders such as social phobia (Birbaumer *et al*., 1998) and in impulsive aggression has been postulated (Emery & Amaral, 2000).

Several investigations have supported the idea of a central histaminergic system implicated in arousal and in various homeostatic mechanisms. Histaminergic fibres innervate cholinergic neurons in the basal forebrain and pontine nuclei that provide cortical and thalamic inputs, respectively, and promote arousal. Indeed, *in vivo* injections of histamine into the pedunculopontine nuclei determines cortical desynchronization and promotes wakefulness (Lin *et al*., 1996). By contrast, activation of histaminergic fibres in the lateral geniculate appears to strengthen central transmission of afferent information, suggesting that sensory input enhancement could be one way in which the histaminergic system plays a role in arousal (Uhlrich *et al*., 2002). Recently it was demonstrated that the arousal effect of orexin, the neuropeptide associated with regulation of sleep and feeding, depends on the activation of histaminergic neurotransmission (Huang *et al*., 2001). In addition, there appears to be reciprocal contacts between histaminergic and orexin neurons, suggesting that the two systems may cooperate in the regulation of rapid eye movement sleep and feeding (Eriksson *et al*., 2001).

It has been proposed that brain histamine is a danger response signal, triggered by a variety of aversive stimuli such as stress, dehydration and hypoglycaemia (Brown *et al*., 2001). The present results, together with our previous data (Passani *et al*., 2001), are coherent with the hypothesis that the histaminergic system could provide a crucial mechanism to fine tuning amygdala activation for an adequate behavioural response.

Abbreviations

ACh, acetylcholine; BLA, basolateral amygdala; immepip, 4-(1H-imidazol-4-yl-methyl) piperidine; MAPK, mitogen-activated protein kinase; NBM, nucleus basalis magnocellularis; RAMH, R-α-methylhistamine.

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