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European Journal of Pharmacology 323 (1997) 283–286

ejp

Short communication

Histamine modulation of glutamate release from hippocampal synaptosomes

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Received 9 January 1997; revised 12 February 1997; accepted 18 February 1997

Abstract

Histamine is widely believed to act as a neuromodulator in the central nervous system. Histaminergic fibers arriving at the hippocampus could be involved in the modulation of glutamatergic neurotransmission. Therefore, we have investigated the effect of histamine on [^{3}H]glutamate release from hippocampal synaptosomes by using a superfusion system. Calcium-dependent [^{3}H]glutamate release was stimulated by KCl or 4-aminopyridine. When submaximal concentrations of the depolarizing agents were used (15 mM KCl or 50 μM 4-aminopyridine), histamine, acting via histamine H_1 and H_2 receptors, produced a concentration-dependent increase in the evoked release of glutamate. Maximal effect was obtained with 500 μM histamine. Histamine (up to 1 mM) did not modify basal release. These data suggest that histaminergic afferents may modulate activity-dependent glutamatergic presynaptic terminals in the hippocampus.

Keywords: Histamine; Glutamate; Synaptosome; [^{3}H]Glutamate, release; Hippocampus

1. Introduction

Much evidence exists that histamine could have a role as a neuromodulator in the mammalian central nervous system (Prell and Green, 1986; Pollard and Schwartz, 1987). Although the specific functions of histamine in the central nervous system remain unclear, it has been implicated in various neuroendocrine and vegetative processes (Prell and Green, 1986; Pollard and Schwartz, 1987). Histaminergic neurons are mainly located in the hypothalamus from where they send diffuse projections to most brain areas, including all parts of the hippocampal formation (Watanabe et al., 1984; Pollard and Schwartz, 1987; Panula et al., 1989).

What could be the role of the histaminergic fibers arriving at the hippocampus? One of the eventual functions could be the modulation of the excitatory synaptic transmission mediated by glutamate. This possibility has physiological relevance since glutamate is the main excitatory neurotransmitter in the hippocampus and is widely believed to play an important role in synaptic plasticity events involved in mammalian learning process like, for example, long-term potentiation (Bliss and Collingridge, 1993; Larkman and Jack, 1995). In this respect, it has been reported that histamine increases the excitatory post-synaptic potentials and facilitates the induction of long-term potentiation in area CA1 of the hippocampus (Segal, 1981; Brown et al., 1995).

How could histamine affect glutamatergic neurotransmission in the hippocampus? One possibility is by interacting with NMDA receptors. It has been shown that histamine is able to modulate the NMDA receptor activity (Bekkers, 1993; Vorobjev et al., 1993) probably through its binding to a polyamine modulatory site (Vorobjev et al., 1993). Another way in which histamine could modulate glutamate neurotransmission is by regulating the release of glutamate. This possibility is of particular interest since long-term potentiation has been associated with a presynaptic alteration of glutamate release (Dolphin et al., 1982; Lynch et al., 1994). Although histamine has been described to be able to modulate the release of neurotransmitters, mainly catecholamines and serotonin, in brain slices (Subramanian and Mulder, 1977; Young et al., 1988; Smits and Mulder, 1991), no clear data exist about its effect on glutamate release (Segal, 1981). Thus, in the present report, we have studied whether histamine has any effect on glutamate release in hippocampal synaptosomes.

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PII S0014-2999(97)00143-X

2. Material and methods

Crude synaptosomes from rat (Sprague-Dawley; 200–250 g) hippocampus were prepared essentially as described by Gray and Whittaker (1962). The final pellet was resuspended in a standard solution of the following composition (mM): NaCl, 125; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$, 1.2; $NaHCO_3$, 22; NaH_2PO_4, 1; glucose, 10 (gassed with 95% O_2 and 5% CO_2 at 37°C); pH 7.2–7.4. The synaptosomes were then labeled for 20 min with [^{3}H]glutamate (0.04 μM, 50 Ci/mmol) at 37°C. Aliquots of the synaptosomal suspension (0.20 mg protein) were gently layered onto 0.65 μm filters placed at the bottom of a set of parallel superfusion chambers (Raiteri et al., 1974). The synaptosomes were then superfused (0.62 ml/min) with standard medium for 20 min before starting the experiment. When Ca^{2+}-independent release was monitored, $CaCl_2$ was substituted by EGTA (1.2 mM) in the standard medium. Synaptosomes were depolarized either with KCl or with 4-aminopyridine for the indicated period of time. Generally, one 3-min sample and two 3-min samples were collected before and after the 6-min sample containing the glutamate released by the depolarization pulse. However, in some experiments (see Fig. 1A), fractions were collected each minute to have a better time resolution. The amount of [^{3}H]glutamate released into each fraction was expressed as a percentage of the total tritium present in the synaptosomes at the onset of the fraction collected. The evoked release was calculated by subtracting the basal values from the treated fractions. The effect of histamine on glutamate release was expressed as a percentage of KCl (15 mM)- or 4-aminopyridine (50 μM)-evoked release. Results are given as mean ± S.E.M. Statistical differences between the means were determined by the Scheffe's test. Similar experiments indicated that at least an 85% of the radioactivity released was recovered as glutamate (Feasey et al., 1986).

3. Results

After a 20-min perfusion, the basal release of [^{3}H]glutamate from rat hippocampal synaptosomes was constant for the duration of the experiment (following 15 min; Fig. 1A), and was not affected by the addition of histamine or by replacing $CaCl_2$ with EGTA (data not shown). When the synaptosomes were exposed to KCl (for 90 s) or 4-aminopyridine (3 min), a concentration-dependent increase in [^{3}H]glutamate release was observed. Maximal release was observed with 45 mM KCl and 1 mM 4-aminopyridine (60% and 73% increase over control respectively; data not shown). When $CaCl_2$ was substituted by EGTA in the superfusion medium, the KCl- or 4-aminopyridine-evoked release of [^{3}H]glutamate was significantly reduced, although the basal release was not affected (data not shown).

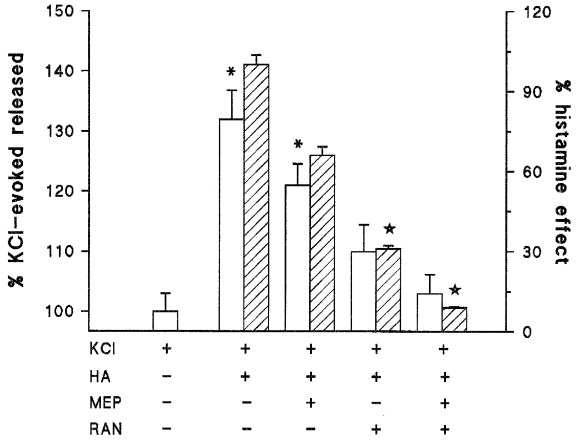

Fig. 1. Histamine increases depolarization-evoked release of [^{3}H]glutamate from hippocampal synaptosomes. Rat crude hippocampal synaptosomes were depolarized by 15 mM KCl (90 s pulse) or 4-aminopyridine (4-AP; 3 min pulse). Histamine (HA) was present during 9 min from the beginning of the depolarizing pulse. (A) Results represent [^{3}H]glutamate released in 1-min fractions. Horizontal bars represent the presence of KCl and HA in the superfusion medium. Data are the means ± S.E.M. of one typical experiment run in quadruplicate. Another independent experiment yielded similar results. (B) Results represent the effect of different doses of HA on KCl- (open bars) and 4-AP- (hatched bars) evoked release. The values are calculated as percent of KCl- or 4-AP-evoked release during the period in which HA was present in the superfusion medium and expressed as means ± S.E.M. of 3 (4-AP) or 4 (KCl) independent experiments performed in quadruplicate. * $P < 0.05$, ** $P < 0.01$ vs. depolarized-evoked release (Scheffe's test).

To assess the histamine modulation of the evoked release by KCl or 4-aminopyridine, we selected a submaximal concentration of the depolarizing agents (15 mM KCl or 50 μM 4-aminopyridine). Hippocampal synaptosomes were superfused with standard medium containing histamine (up to 500 μM) for 9 min and were stimulated with 15 mM KCl during the first 90 s. As shown in Fig. 1A, histamine did not increase the evoked release during the stimulation with KCl but produced a significant sustained increase in the evoked-released of [^{3}H]glutamate after removal of the depolarizing agent. The histamine effect was concentration-dependent (Fig. 1B), reaching a 48% increase over 15 mM KCl-evoked release with 500 μM histamine. Similar results were obtained when synaptosomes were stimulated with 4-aminopyridine for 3 min. The maximal effect (40% over 50 μM 4-aminopyridine) was also obtained with 500 μM histamine (Fig. 1B). It appears that the histamine effect on hippocampal glutamate release is independent of the depolarizing agent used to stimulate the synaptosomes.

The histamine-mediated increase in glutamate release was significantly reduced (33% and 69%) when the synaptosomes were superfused respectively in the presence of mepyramine (a histamine H_1 receptor antagonist) or ranitidine (a histamine H_2 receptor antagonist; see Fig. 2). A complete block of the histamine effect was observed

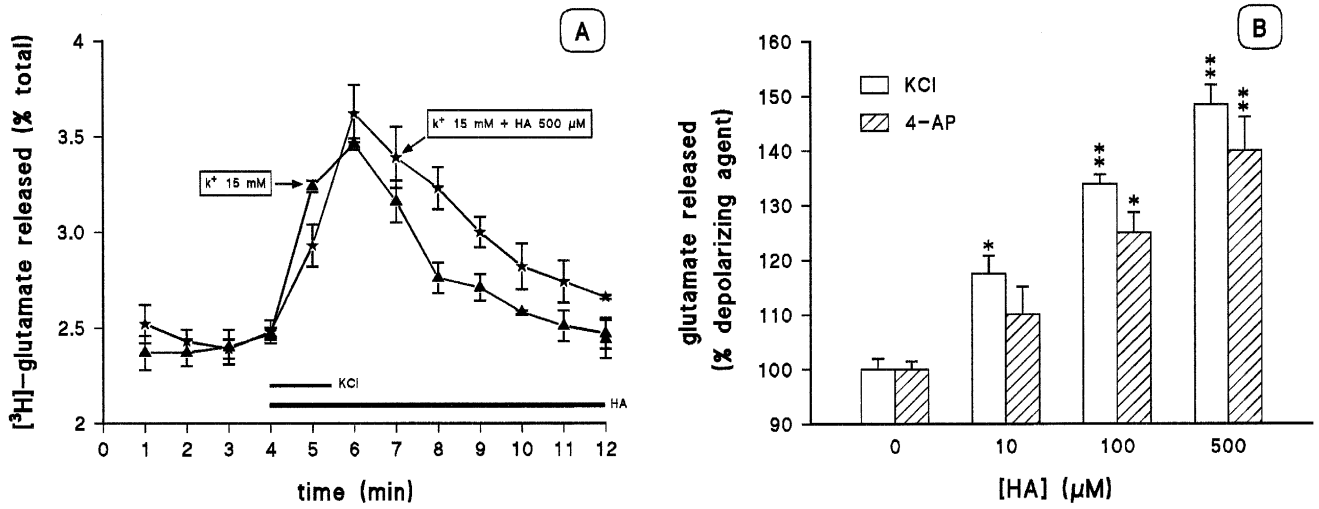

Fig. 2. Histamine effect on depolarization-evoked release of [^{3}H]glutamate is blocked by histamine H_1 and H_2 receptor antagonists. Rat crude synaptosomes were depolarized by 15 mM KCl (90 s pulse). Histamine (100 μM; HA) was present during 9 min from the beginning of the depolarizing pulse. When necessary, HA receptor antagonists were added to the superfusion media 10 min before the depolarization and were present during 9 min from the beginning of the depolarization pulse. Mepyramine (300 μM; MEP) and ranitidine (300 μM; RAN) were used to block HA H_1 and H_2 receptors respectively. Both antagonists did not modify basal release. The values are calculated as percent of KCl-evoked release (open bars) or percent of HA effect (hatched bars). Data are means ± S.E.M. of 3 independent experiments performed in quadruplicate. * $P < 0.05$ vs. KCl-evoked release; ★ $P < 0.01$ vs. HA effect (Scheffe's test).

when synaptosomes were superfused with both antagonists at the same time (Fig. 2).

4. Discussion

Glutamate is the main excitatory neurotransmitter in the hippocampus. Recently it has been described (Brown et al., 1995) that histamine modulates the excitatory transmission in the hippocampus and facilitates the induction of long-term potentiation in CA1.

Long-term potentiation is a long-lasting and use-dependent increase in glutamatergic synaptic strength implicated in learning (Bliss and Collingridge, 1993; Larkman and Jack, 1995). Several evidences indicate that long-term potentiation is associated with an activity-dependent increase in glutamate release (Dolphin et al., 1982; Bliss and Collingridge, 1993). Since our results show that histamine increases the release of glutamate evoked by depolarization but not the spontaneous release (basal release), only activated glutamatergic terminals would be modulated by the amine. It is then possible that the described effect of histamine on long-term potentiation (Brown et al., 1995) could be, at least in part, due to an increase in glutamate release.

What mechanisms are involved in the histamine modulation of glutamate release? The results, obtained with specific histamine receptor antagonists, have shown that histamine increases glutamate release by acting mainly on histamine H_2 receptors (ranitidine is able to block almost 70% of histamine effect). However, mepyramine, a histamine H_1 receptor antagonist, partially blocks the histamine effect, suggesting that histamine H_1 receptor also contributes to the observed increase in glutamate release.

We have previously reported that histamine increases Ca^{2+} uptake in synaptosomes by stimulation of histamine H_2 receptors (Rodriguez et al., 1987, 1988) and it is also well know that histamine H_1 receptor stimulation produces an IP_3-dependent mobilization of intracellular Ca^{2+} (Berridge, 1993; Claro et al., 1986). It is then possible that histamine could potentiate glutamate release by increasing the intracellular Ca^{2+} concentration in the presynaptic terminal.

In summary, our data suggest that stimulation of histamine H_1 and H_2 receptors by hippocampal histaminergic afferents may enhance the glutamatergic transmission by increasing the evoked glutamate release in active presynaptic terminals.

Acknowledgements

We thank Drs. J. Sabrìa and M.A. Lynch for helpful discussion. This work was supported by grants from the Fondo de Investigaciones Sanitarias. F.J.R. gratefully acknowledges the FPPI fellowship from the Ministerio de Educación y Ciencia.

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