Journal of Neuroscience Methods, 28 (1989) 71–75
Elsevier

NSM 00923

The brain histamine system in vitro

H.L. Haas [1], R.W. Greene [2] and P.B. Reiner [3]

[1] *Institute of Physiology, Johannes Gutenberg-Universität, Mainz (F.R.G.),* [2] *Department of Psychiatry, Harvard University, Brockton, MA (U.S.A.) and* [3] *Kinsmen Laboratory, University of British Columbia, Vancouver, BC (Canada)*

(Received 3 October 1988)
(Accepted 10 October 1988)

Key words: Histamine-neuron; Hypothalamus; Hippocampus; Electrophysiology

The electrophysiological properties of identified tuberomammillary histamine neurones were investigated in explant and slice preparations. The effects of histamine were studied on target neurones, mainly in the hippocampal slice. The results describe an important modulatory role of this diffusely projecting system.

Introduction

The histaminergic system in the rat brain consists of about 2000 neurones located in the tuberomammillary (TM) nucleus. These large cells project to many regions of the central nervous system including hypothalamus, cerebral cortex, hippocampus, and even the spinal cord. Although most of these histaminergic projections had long been predicted mainly by the lesion studies of Garbarg et al. (1974; Haas and Wolf, 1977), the exact identity of the cells and their axons was defined relatively recently by immunohistochemistry (Panula et al., 1984; Steinbusch and Mulder, 1984; Watanabe et al., 1984). These TM neurones show specific immunoreactivity to histamine and to l-histidine decarboxylase, its synthetic enzyme. Subpopulations stain also for adenosine deaminase, GAD (Köhler et al., 1985; Pollard et al., 1985; Staines et al., 1987) and some peptides. Their anatomical disposition with the multifold arborising axons makes them a typical modulatory system (Prell and Green, 1986) like the other aminergic nuclei and projections (Crunelli et al., 1983; Grace and Bunney, 1983; Van der Maelen and Aghajanian, 1983; Williams et al., 1984; Reiner and McGeer, 1987) (Fig. 1).

We have studied the postsynaptic actions of histamine in hippocampal slices (Haas and Konnerth, 1983; Haas and Greene, 1986) and the properties of histochemically identified histaminergic TM neurones in a median eminence explant (Haas and Reiner, 1988).

Materials and Methods

Hippocampal slices of 500 μm thickness were completely submerged in a chamber modified from our original design (Haas et al., 1979; Greene and Haas, 1985) which had a volume of about 50 μl and was perfused with 0.3–0.5 ml/min of a standard medium at 30 °C or media specifically modified for an experiment. The median eminence explants were investigated in a similar chamber of slightly larger dimension. They were cut by hand with a razor blade and although up to 1 mm thick at the centre of the median eminence the tuberomammillary region with its superficially

Correspondence: H.L. Haas, Physiologisches Institut, Johannes Gutenberg-Universität, Saarstr. 21, D-6500 Mainz, F.R.G.

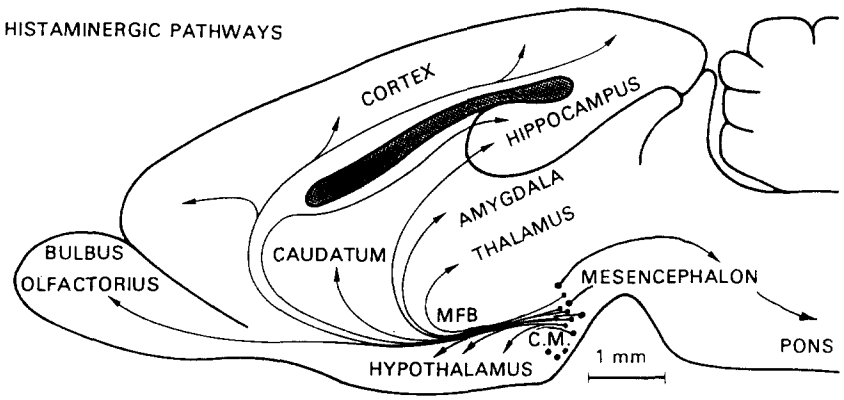

Fig. 1. Schematic diagram of histaminergic pathways in the rat brain as predicted by Garbarg et al. (1974) and confirmed by immunohistochemistry in recent years (see text). C.M., corpora mammillaria.

located histamine neurones (Reiner et al., 1987) was well-preserved for at least 12 h after preparation. The explant contained, at the rostral end, the supraoptic nucleus. Electrical stimulation in this region lead to antidromic invasion of several TM neurones.

Intracellular voltage recording and current injection were performed with a high input impedance bridge amplifier. The action of histamine in the hippocampus was also studied with single electrode voltage–clamp. Some of the investigated TM neurones were marked by intracellular ionophoresis of Lucifer yellow and, at the end of the electrophysiological experiment, the explant was fixed, coronal sections prepared and processed for histidine decarboxylase immunofluorescence with Texas red as the fluorochrome. Identification of labelled cells was by alternating inspection of the explant through appropriate selective filters for Lucifer yellow or Texas red. The TM neurones were also grown in organotypic cultures (Gähwiler, 1981; Reiner et al., 1988) explanted from newborn rats. Their morphological properties were very similar to those seen in vivo. Extensive ingrowth of histaminergic axons was observed in co-cultured hippocampus.

Results

Hypothalamus

Tuberomammillary histamine neurones in vitro displayed membrane potentials of about -50 ± 5 mV (all values are given as averages $\pm$ S.D.) and were spontaneously active at about 2 Hz. The action potentials had an overshoot of more than 20 mV, a half-amplitude duration of 1.8 ± 0.4 ms and were followed by an afterhyperpolarisation (a.h.p.) (Fig. 2). Input resistances, as determined by measuring the voltage responses to a weak hyperpolarising current injection (0.1–0.5 nA), were 176 ± 42 MΩ. Larger hyperpolarising current pulses revealed a progressive fall in resistance. This inward rectification was fully activated after several hundreds of microseconds and was not inactivating.

Furthermore, the TM neurones displayed a strong transient outward rectification. This was apparent by observation of the time courses of the onset and offset of the electrotonic potential in response to hyperpolarising current pulses. The charging profile followed a single exponential with a time constant of about 20 ms while the discharging profile exhibited at least two different exponentials, one of which was much slower (time constant ca. 500 ms). This transient outward rectification was greatest after hyperpolarising current pulses starting from near the resting potential and was absent when starting at -70 mV. This phenomenon has been attributed to the presence of an A current which is often sensitive to 4-aminopyridine (4-AP; Thompson, 1977). In our experiments however, high concentrations of 4-AP (2 mM) did not block this conductance.

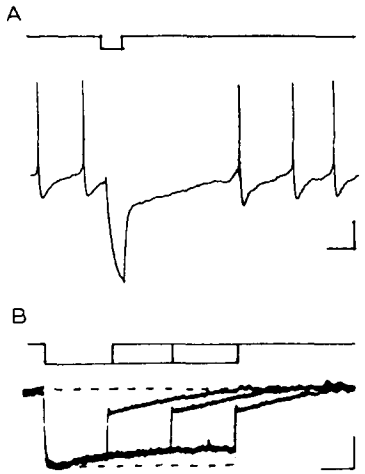

Fig. 2. Properties of tuberomammillary histamine (TM) neurons. A: spontaneously firing TM neuron, intracellularly recorded in a median eminence explant from rat. Action potentials with afterhyperpolarisations. Hyperpolarising current pulse injection (0.2 nA; upper trace) after second spike; note the slow return to resting potential. B: voltage responses (3 overlapping, lower trace) to −0.3 nA current pulses (upper trace) in a TM neuron, illustrating slow inward rectification and transient outward rectification. Inward rectification slowly develops during hyperpolarising current injection; transient outward rectification delays the return to resting potential (−52 mV, as in A). Calibration 20 mV, 500 ms.

The a.h.p. following the action potentials had an amplitude of about 14 ± 3 mV ($n = 12$) and a duration of about 400 ms (range 300–600 ms). Their reversal potential was in the region of −80 mV ($[K]_0$ 3.25 mM). The a.h.p.s were not reversed by intracellular chloride but were blocked by cesium loading through the recording electrode. With KCl filled electrodes spontaneous depolarising synaptic potentials could be observed in TM neurones, these were abolished by adding bicuculline (10 μM) or morphine (1 μM) but not TTX (1 μM) to the bathing fluid.

Hippocampus

The postsynaptic actions of histamine were studied in the hippocampal slice preparation (Haas and Konnerth, 1983; Haas and Greene, 1986).

Extra- and intracellularly recorded excitatory postsynaptic potentials (epsps) in the CA1 area were unaffected but the population spike was increased by histamine (1–10 μM). This effect was particularly prominent and often long lasting (more than 1 h) in slices from mice (Kostopoulos et al., 1988). In the dentate area histamine sometimes decreased extra- and intracellularly registered epsps, especially in the presence of the H_2-receptor blocker cimetidine (10 μM). In the presence of the H_1-receptor blocker mepyramine (10 μM) however, epsps were often enhanced. Population spikes following perforant path stimulation were increased by histamine (H_2-receptor).

Histamine (1–10 μM) caused a slight depolarisation of hippocampal CA1 and dentate granule cells. This effect was also seen in the presence of cadmium (200 μM) or TTX (0.3 μM) when synaptic inputs were completely removed. In cells loaded with EGTA from the recording electrode, however, when accommodation was blocked,

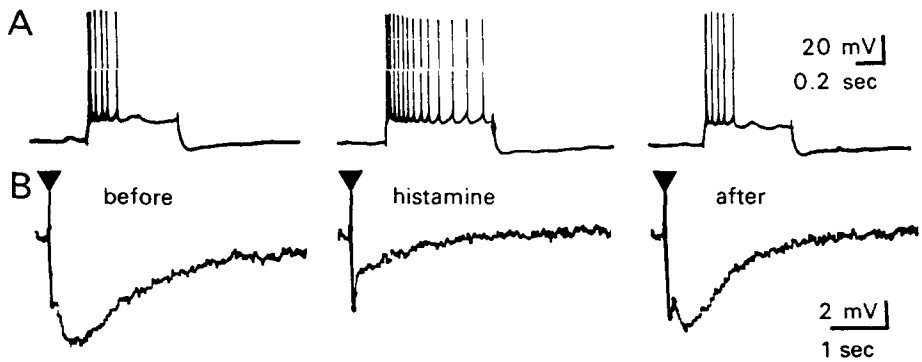

Fig. 3. Intracellular recordings from two CA1 hippocampal pyramidal neurones. A: positive current injection, accommodation of firing blocked by histamine (1 μM, middle record). B: afterhyperpolarisations following a calcium spike in a TTX-treated slice (clipped at triangle). The slow component is reversibly antagonised by 1 μM histamine. Averages of 4 sweeps.

histamine (up to 100 μM) had no effect. In both CA1 and dentate cells, histamine effectively blocked the long-lasting a.h.p. and the accommodation of firing (Haas and Konnerth, 1983; Haas, 1984; Haas and Greene, 1986), in the absence of significant changes in membrane resistance (Fig. 3). These effects of histamine are attributed to blockade of a Ca^{2+}-activated potassium conductance (gK_{Ca}). Histamine and impromidine (H_2-agonist) reduced amplitude and time course of the a.h.p. consistent with an interference with Ca^{2+}-sequestration. Ca^{2+}-spikes recorded in tetrodotoxin (TTX)-poisoned slices were never reduced but usually slightly enhanced and broadened by histamine. Voltage–clamp recordings with CsCl-filled electrodes using voltage jumps from -50 to around 0 mV never showed a decrease in inward but some decrease in outward current. These results indicate that the block of gK_{Ca} was not secondary to a decrease in gCa. Because the gK_{Ca} activation is proportional to the action potential dependent accumulation of internal Ca^{2+}, long duration excitatory inputs which elicit many action potentials are more affected than short duration inputs: a dynamic form of modulation.

Although interneurones could not be investigated directly, their spontaneous firing was reflected by depolarising inhibitory postsynaptic potentials (ipsps) recorded with KCl-filled electrodes. The number of these potentials always increased when histamine was added to the medium. It is, however, not certain that this is a direct effect of histamine on the interneurones.

Discussion

The median eminence explant offers an in vitro preparation which contains the histaminergic neurones in the mammillary bodies as well as some major targets of their axons. We have studied the properties of TM neurones in this preparation and the action of histamine on some target regions.

TM neurones have action potentials with sodium and calcium components and a delayed potassium conductance and are followed by a potassium-mediated a.h.p. which is relatively independent of calcium (Haas and Reiner, 1988). Their transient outward rectification (not blocked by 4-AP) may be an important factor for the firing rate of TM neurones as it is probably activated during the decay phase of the a.h.p.'s. It has been shown that TM neurones change their firing dramatically across behavioral states (Vanni-Mercier et al., 1984). This could be achieved through regulation of the a.h.p. by the transient outward rectification.

Although microionophoretic studies in vivo have usually revealed depressant and hyperpolarising actions of histamine in most brain regions (Haas, 1985), many cells in the hypothalamus were excited. These included supraoptic and paraventricular neurones which we have also investigated in vitro (Haas and Geller, 1982). This result is in keeping with the antidiuretic action of histamine locally applied to the supraoptic nucleus (see Haas and Wolf, 1977). A study of histaminergic activation of target neurones in the explant is underway. A detailed investigation of postsynaptic histamine actions is presented in the hippocampus which receives histaminergic afferents through a dorsal and a ventral route (see Schwartz et al., 1986). While H_1-receptor-mediated effects are subtle and inhibitory in the hippocampus (Haas et al., 1984), the H_2-receptor-mediated effect is, like the β-receptor-mediated effect of noradrenaline a striking block of accommodation and the long-lasting a.h.p. Cyclic AMP and forskolin mimick this effect, which is not secondary to a block of Ca^{2+} inflow but may well be mediated by an interference with Ca^{2+} sequestration, i.e. a reduction of free Ca^{2+} near the Ca^{2+}-sensitive K^+-channels.

Taken together these results establish the histaminergic system in the brain as a modulatory nucleus with diffuse projections to the whole central nervous system. Delineation of the multifacetted contributions of this system to brain functions remains an important challenge for the future.

References

Crunelli, V., Forda, S., Brooks, P.A., Wilson, K.C.P., Wise, J.C.M. and Kelly, J.S. (1983) Passive membrane properties

of neurones in the dorsal raphe and periaqueductal grey recorded in vitro, Neurosci. Lett., 40: 263–268.

Gähwiler, B.H. (1981) Organotypic monolayer cultures of nervous tissue, J. Neurosci. Methods, 4: 329–342.

Garbarg, M., Barbin, G., Feger, J. and Schwartz, J.C. (1974) Histaminergic pathway in rat brain evidenced by lesions of the medial forebrain bundle, Science, 186: 833–835.

Grace, A.A. and Bunney, B.S. (1983) Intracellular and extracellular physiology of nigral dopamine neurones, Neuroscience, 10: 301–315.

Greene, R.W. and Haas, H.L. (1985) Adenosine actions on CA1 pyramidal neurones in rat hippocampal slices, J. Physiol. (Lond.), 366: 119–127.

Haas, H.L. (1984) Histamine potentiates neuronal excitation by blocking a calcium dependent potassium conductance, Agents Actions, 14: 534–537.

Haas, H.L. (1985) Histamine may act through cyclic AMP on hippocampal neurones, Agents Actions, 16: 234–235.

Haas, H.L. and Wolf, P. (1977) Central actions of histamine, microelectrophoretic studies, Brain Res., 122: 269–279.

Haas, H.L., Schaerer, B. and Vosmansky, M. (1979) A simple perfusion chamber for the study of nervous tissue slices in vitro, J. Neurosci. Methods, 1: 323–325.

Haas, H.L. and Geller, H.M. (1982) Electrophysiology of histaminergic transmission in the brain. In B. Uvnäs and K. Tasaka (Eds.), Advances in the Biosciences, Vol. 33: Advances in Histamine Research, Pergamon, Oxford, pp. 81–91.

Haas, H.L. and Konnerth, A. (1983) Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells, Nature (Lond.), 302: 432–434.

Haas, H.L., Jefferys, J.G.R., Slater, N.T. and Carpenter, D.O. (1984) Modulation of low calcium induced field bursts in the hippocampus by monoamines and cholinomimetics, Pflügers Arch., 400: 28–33.

Haas, H.L. and Greene, R.W. (1986) Effects of histamine on hippocampal pyramidal cells of the rat in vitro, Exp. Brain Res., 62: 123–130.

Haas, H.L. and Reiner, P.B. (1988) Membrane properties of histaminergic tuberomammillary neurones of the rat hypothalamus in vitro, J. Physiol. (Lond.), 399: 633–646.

Köhler, C., Swanson, L.W., Haglund, L. and Wu, J.-Y. (1985) The cytoarchitecture, histochemistry and projections of the tuberomammillary nucleus in the rat, Neuroscience, 16: 85–110.

Kostopoulos, G., Psarropoulou, C. and Haas, H.L. (1988) Membrane properties, response to amines and to tetanic stimulation of hippocampal neurons in the genetically epileptic mutant mouse tottering, Exp. Brain Res., 72: 45–50.

Panula, P., Yang, H.-Y.T. and Costa, E. (1984) Histamine-containing neurons in the rat hypothalamus, Proc. Natl. Acad. Sci. U.S.A., 81: 2572–2576.

Pollard, H., Pachot, I. and Schwartz, J.-C. (1985) Monoclonal antibody against L-histidine decarboxylase for localization of histaminergic cells, Neurosci. Lett., 54: 53–58.

Prell, G.D. and Green, J.P. (1986) Histamine as a neuroregulator, Annu. Rev. Neurosci., 9: 209–254.

Reiner, P.B. and McGeer, E.G. (1987) Electrophysiological properties of cortically projecting histamine neurons of the rat hypothalamus, Neurosci. Lett., 73: 43–47.

Reiner, P.B., Semba, K., Watanabe, T. and Wada, H. (1987) En bloc immunohistochemistry reveals extensive distribution of l-histidine decarboxylase immunoreactive neurons on the ventral surface of the hypothalamus, Neurosci. Lett., 77: 137–142.

Reiner, P.B., Heimrich, B., Keller, F. and Haas, H.L. (1988) Organotypic culture of central histamine neurons, Brain Res., 442: 166–170.

Schwartz, J.-C., Garbarg, M. and Pollard, H. (1986) Histaminergic transmission in the brain. In F.E. Bloom, V. Mountcastle and S.R. Geiger (Eds.), Handbook of Physiology, American Physiological Society, Bethesda, pp. 257–316.

Staines, Wm.A., Yamamoto, T., Daddona, P.E. and Nagy, J.I. (1986) Neuronal colocalization of adenosine deaminase, monoamine oxidase, galanin and 5-hydroxytryptophan uptake in the tuberomammillary nucleus in the rat, Brain Res. Bull., 17: 351–365.

Steinbusch, H.W.M. and Mulder, A.H. (1984) Immunohistochemical localization of histamine in neurons and mast cells in the rat brain. In A. Björklund, T. Hökfelt and M.J. Kuhar (Eds.), Handbook of Chemical Neuroanatomy, Vol. 3: Classical Transmitters and Transmitter Receptors in the CNS, Part II, Elsevier, Amsterdam, pp. 126–140.

Thompson, S.H. (1977) Three pharmacologically distinct potassium channels in molluscan neurones, J. Physiol. (Lond.), 265: 465–488.

Van der Maelen, C.P. and Aghajanian, G.K. (1983) Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices, Brain Res., 289: 109–119.

Vanni-Mercier, G., Sakai, K. and Jouvet, M. (1984) Neurones spécifiques de l'éveil dans l'hypothalamus postérieur du chat, C. R. Acad. Sci., 298: 195–200.

Watanabe, T., Taguchi, Y., Shiosaka, S., Tanaka, J., Kubota, H., Terano, Y., Tohyama, M. and Wada, H. (1984) Distribution of the histaminergic neuron system in the central nervous system of rats: a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker, Brain Res., 295: 13–25.

Williams, J.T., North, R.A., Shefner, S.A., Nishi, S. and Egan, T.M. (1984) Membrane properties of rat locus coeruleus neurones, Neuroscience, 13: 137–156.