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5. HISTAMINE AND THE CENTRAL NERVOUS SYSTEM

Analysis of histamine actions by intra- and extracellular recording in hippocampal slices of the rat[1]

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Abstract

Extra- and intracellular recordings from CA1 pyramidal and dentate granule cells in hippocampal slices of the rat revealed a depressant effect of histamine which was usually accompanied by a hyperpolarization but no changes in conductance of the cell membrane. Synaptic potentials were unaffected. It is concluded that histamine has a post-synaptic action on ionic conductance in the dendrites, that allows a modulation of quick information transfer but no rapid synaptic transmission.

Introduction

Histamine (HA) probably functions as a neurotransmitter or neuromodulator in the mammalian brain. Evidence for such a role comes from biochemical, pharmacological and electrophysiological studies [1–4]. Although a number of electrophysiological studies describes depressant and excitatory actions of HA on the firing frequency of individual neurones in several regions of the brain [4] little is known on the mechanism of the action of HA on the membrane level. HA has been reported to produce a hyperpolarization [5] and a conductance decrease [6] in motoneurones of the cat. In aplysia neurones which are innervated by a HA-containing cell, hyperpolarization due to an increase in potassium conductance and depolarization due to an increase in sodium conductance were shown by CARPENTER and GAUBATZ [7] and WEINREICH [8]. Both these actions were mediated by H_1- or H_2-receptors. The involvement of H_2-receptors in depressant effects of histamine has also been demonstrated in mammalian brain [4, 9]. With recent advances in brain slice techniques, intracellular recording from neurones which are likely to receive HA-releasing afferents has become possible. The hippocampus retains physiological properties in vitro [10] and was a first obvious choice for an investigation of HA effects as biochemical [11] and electrophysiological [4, 11] studies suggest histaminergic inputs into this region.

Materials and methods

Transverse hippocampal slices were prepared as described previously [12] and kept in a perfusion chamber [14] at 33 °C. Micropipettes for intracellular recording were filled with 3 *M* potassium chloride or potassium acetate and had resistances of 50–200 MΩ. Pipettes with lower resistance (less than 10 MΩ) were used for recording extracellularly single units or field potentials and for electrical stimulation. Substances were applied under microscopical inspection locally by microionophoresis (0.2 *M* solutions of histamine dihydrochloride, pH 3.5, HA; serotonine creatinine sulphate, pH 3.5, 5-HT; noradrenaline hydrochloride, pH 4, NA; D,L-homocysteic acid, pH 8, DLH) or pressure ejection (10^{-4} *M* neutral solutions) from multibarrelled micropipettes. Microdrop application (10^{-4} *M*, 1 nl) or diffusion from a single pipette orifice on the slice surface and addition of drugs to the perfusion fluid were also employed.

Results

Field potentials were recorded from the CA1 and the dentate area after stimulation of the stratum radiatum and the perforant path fibres respectively of 23 slices. Population spikes registered in the soma regions were reduced when HA was added to the perfusion fluid (10^{-5} *M*) or when it was applied locally by microdrops (1 nl, 10^{-4} *M*). Epsp's recorded extracellularly from the dendritic layers or intracellularly from the somata were usually unaffected. Extracellular recordings

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were made from 5 dentate granules and 5 CA1 pyramidal neurones. HA was applied locally as a neutral solution (10^{-4} *M*) by pressure or microdrops (*ca.* 1 nl). Two of the dentate and one of the pyramidal cells were excited, these excitations were not blocked by adding mepyramine (up to 10^{-5} *M*) to the perfusion fluid. The spontaneous and DLH-evoked firing of 6 cells were reduced. Examples of drop and pressure application on 2 dentate granule cells are shown in Figure 1. On 3 further cells HA was added to the perfusion fluid. Although HA was ineffective in changing the firing rate (10^{-6}–10^{-4} *M*) it reduced the peak firing rate reached during regularly spaced ionophoretic DLH applications. Such an experiment is illustrated in Figure 2: the insert shows a ratemeter recording of one DLH application (5 nA for 10 sec). The peak firing reaches 11 spikes per second in this example. Each of the points in the upper trace represents one such peak firing rate. This DLH-evoked firing is reduced by HA (10^{-5} *M*) and the effect is reversed by adding the H_2-antagonist metiamide (10^{-5} *M*) to the perfusion fluid.

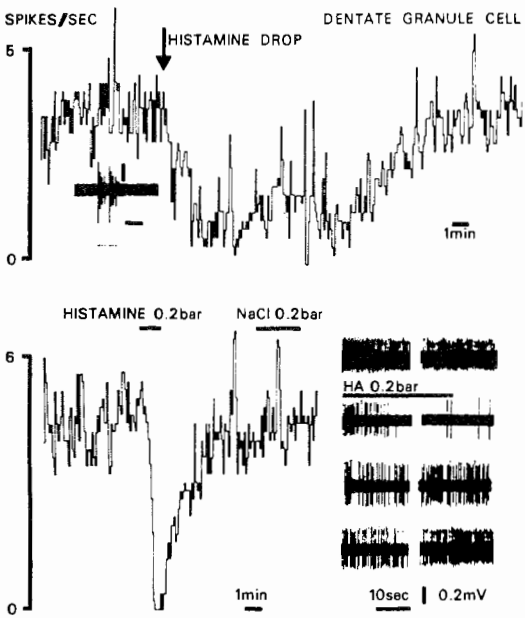

Figure 1
Ratemeter recordings from two dentate granule cells. Upper record: histamine was applied as a microdrop (1 nl, 10^{-4} *M*) to the slice surface, 200 μm from the cell soma in the dendritic layer. Insert shows superimposed oscilloscope traces (calibration 0.2 mV, 50 msec). Lower record: pressure application through micropipette within the slice in the dendritic region. On the right, oscilloscope traces showing the original action potentials.

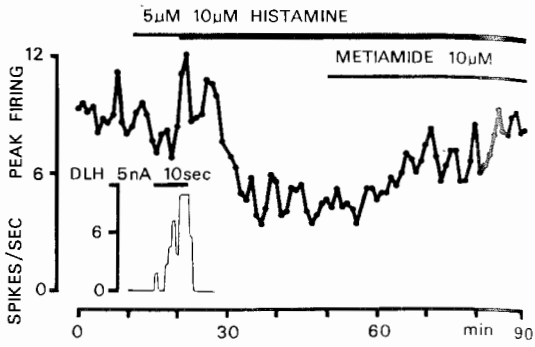

Figure 2
Peak firing rates of a CA1 pyramidal neurone in response to ionophoretic application of D,L-homocysteic acid (DLH 5 nA for 10 sec) in regularly spaced intervals to the apical dendrites. One response (ratemeter recording) is shown as an insert. Each point in the upper trace represents the peak of one such response. Histamine and metiamide are added to the perfusion fluid.

Intracellular recordings were obtained from 31 CA1 pyramidal and 6 dentate granule cells. Resting potentials were between 50 and 85 mV with an average of 67.0 ± 9.4 (S.D.). Perfusion with HA (10^{-6}–10^{-4} *M*) hyperpolarized 7 out of 12 cells tested (2 dentate, 5 CA1) by 2–5 mV. Spontaneous and DL-homocysteic acid evoked firing as well as excitability, tested by depolarizing current injection, was reduced. Local administrations of HA by ionophoresis or pressure ejection hyperpolarized 13 cells by 3–12 mV and was ineffective on 9 cells. Other cells showed variable and biphasic effects. It was possible in most cells to repeat the application several times on different locations (soma and dendrites) and although contrary actions were sometimes found on different locations no clear pattern related to the application site was found so far. Three cells displayed a change from hyper- to depolarization after repeated applications. Neurones with lower resting potentials between 50 and 70 mV showed hyperpolarizing responses more often, whereas neurones with resting potentials above 70 mV tended to be depolarized or unaffected. Hyperpolarizations were accompanied by a reduction in spontaneous firing and excitability (Fig. 3B); depolarizations were usually accompanied by an increased excitability (Fig. 3C). The membrane conductance, monitored in most experiments by intracellular injection of hyperpolarizing pulses, was not consistently changed. Increasing the magnesium concentration in the perfusion fluid to 10 m*M* blocked synaptic

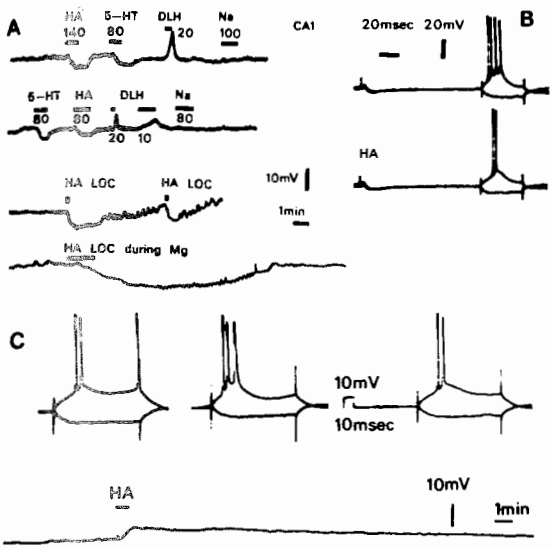

Figure 3
Intracellular recordings from CA1 pyramidal cells. A: membrane potential recordings from 4 different cells. Histamine (HA), serotonine (5-HT), D,L-homocysteic acid (DLH) and sodium (Na) were applied by ionophoresis (ejecting current given in nA) or by local diffusion from a micropipette (HA LOC, 10^{-4} *M*). Time of application is indicated by bars. Lowest trace during 10 m*M* magnesium in the perfusion fluid. B: epsp–ipsp sequence after stimulating stratum radiatum (100 μA) and response to intracellular current injection ($\pm$ 0.5 nA) before and during local histamine administration. Hyperpolarization 5 mV. C: Depolarizing action of histamine, administered locally close to the soma of a neurone which had shown a hyperpolarizing response previously. Oscilloscope traces are taken before, during and after the HA action. Intracellular current injection $\pm$ 0.5 nA, resting potential 76 mV.

transmission but did not prevent the histamine actions. Synaptic potentials (usually epsp–ipsp sequences after stimulation of the afferent fibres) were unchanged (Fig. 3B). D, L-Homocysteic acid (DLH) was applied from the same multibarrelled micropipette in order to assure the location within the dendritic tree of the recorded neurone. DLH always caused a rapid depolarization and usually a conductance increase. Noradrenaline and serotonine were also applied on several occasions. Both amines hyperpolarized the cells more effectively than histamine.

Discussion

Extracellular field potential and unit recordings from hippocampal pyramidal or dentate granule cells have revealed a depressant effect on excitability measured as the size of antidromic population spikes, and on spontaneous or DLH-evoked firing. This effect is reversed by metiamide and seems to be related to H_2-receptors. As pressure application of neutral isotonic solutions was employed these effects are not pH- or osmotic artifacts. They are in keeping with previous results from in vivo recordings. A hyperpolarization but sometimes also depolarization or complex effects are found when HA is administered during intracellular recording. Although a clear pattern of the mode and location of HA action has not yet emerged from these results they suggest the following tentative conclusions:

1. HA has a post-synaptic action, as epsps and ipsps were unaffected, DLH-evoked firing was reduced and synaptic isolation (by 10 m*M* Mg^{2+}) did not prevent its actions.
2. HA does probably affect an ionic conductance in the dendrites as its action seemed to be voltage dependent but no conductance changes could be detected by soma recording.
3. Compared with amino acids like GABA or glutamate the actions of HA are slow in onset and usually last much longer. Such action allows modulation of the quick information transfer but no rapid communication.

The occurrence of depolarizing effects seems to be in contrast to the almost exclusively depressant action in this structure of HA administered by classical ionophoresis to hippocampal neurones in vivo. However, this discrepancy may be explained by the lack of anaesthesia and, possibly, other differences between the in vivo and in vitro situation. Furthermore in some cells depolarized by HA the spontaneous firing was actually reduced.

There is no reason to believe that mammalian neurones should be less complicated than snail neurones. The complex actions described here may well be similar to those reported for aplysia neurones [7, 8] and further work should be aimed at the biophysical and pharmacological dissection of histamine effects as it has become available in brain slice preparations.

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