Histaminergic regulation of 5-HT neurons in the dorsal raphe nucleus

K.M. Panetta[1], K.A. Wafford[2], J.J. Lambert[1] and D. Belelli[1]

[1]Division of Neuroscience, Medical Research Institute, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY
[2]Eli Lilly and Company Ltd, Erl Wood Manor, Windlesham, Surrey, GU20 6PH

Introduction

Our state of wakefulness results from the concerted action of neurotransmitter systems located in the brainstem (serotonin and noradrenaline), hypothalamus (histamine and orexins) and the basal forebrain (Saper *et al*., 2005). Serotonergic (5-HT) neurons of the dorsal raphe nucleus (DRN) fire tonically during waking, exhibit a reduced frequency of discharge during slow-wave sleep and cease firing during rapid eye movement sleep however little is know about how other arousal systems affect the firing of 5-HT neurons.

Labelling studies have revealed that the DRN is innervated by wake-active histaminergic neurons from the tuberomammilary nucleus (Lee *et al*., 2005) and binding studies have demonstrated the presence of histamine receptors in the DRN (H_1, H_2 and H_3 subtypes) (Barbara *et al*., 2002; Pillot *et al*., 2002). Here we have utilised extracellular single-unit recordings and whole-cell voltage-clamp recordings to better understand the role that histamine receptors play in regulating 5-HT neurons in the DRN.

Materials and Methods

Brain slice preparation:
Dorsal raphe slices were prepared from C57 BL6/J mice of either sex (P17-30). The brain was rapidly removed from the skull and placed in oxygenated ice-cold artificial cerebrospinal fluid (aCSF) solution containing the following (in mM): 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 glucose, 126 NaCl, 10 MgSO4. The tissue was maintained in ice-cold aCSF while coronal slices were cut using a Vibratome (Intracel; Royston, Hertfordshire, UK) . The slices were incubated at room temperature for 1 h in oxygenated aCSF before being used for recordings. All recordings were made from presumed 5-HT neurons located in the medial DRN as described previously (Calizo *et al*., 2011).

Extracellular single-unit recordings:
Slices were transferred to an interface recording chamber and perfused with a recording solution containing the following (in mM): 126 NaCl, 26 $NaCHO_3$, 2.95 KCl, NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose (flow rate: 1ml/min). Recordings were made at 35°C. Extracellular single-unit recordings were made from presumed serotonergic neurons firing spontaneously (1-3.5 Hz) using microelectrodes filled with NaCl (1-2MΩ)(Judge *et al*., 2004). Signals were recorded using an Axopatch 1D amplifier and fed to a PC via a 1401 computer interface analogue to digital convertor at a sampling rate of 100 Hz. Data were collected and analysed offline using Spike2 software (version 4, CED, UK).

Whole-cell patch-clamp recordings:
Slices were transferred to a submerged recording chamber and perfused with a recording solution, as above (flow rate: 6ml/min). Whole-cell voltage-clamp recordings were made from presumed 5-HT neurons at a holding potential of -60mV. Patchelectrodes were filled with an intracellular solution containing the following (in mM) K-gluconate 130, KCl 2, NaCl 2, EGTA 0.2, HEPES 10, ATP 2, GTP 0.5 , Phosphocreatine-tris 10 (4-7MΩ). Drugs were either bath applied (via the perfusion system) or focally applied (using a picrospritzer). All recordings were made using an Axopatch 200B amplifier and were analysed off-line using the Strathclyde Electrophysiological Software (WinEDR, University of Strathclyde).

Figure 1. Histamine increases the firing frequency of presumed 5-HT neurons in the DRN

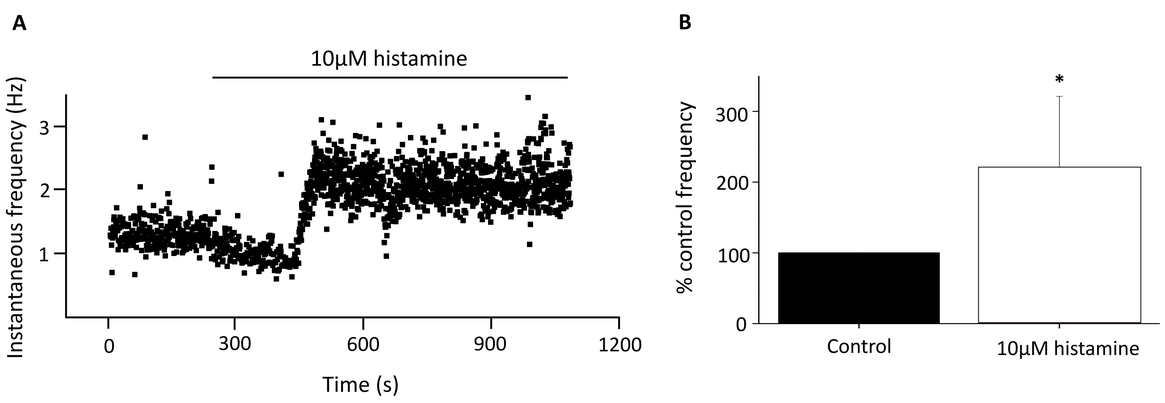


Figure 1. (A) A representative plot of an extracellular single-unit recording from a presumed 5-HT neuron illustrating the firing frequency before and after the bath application of histamine (10μM). Histamine causes an excitation that is characterised by a rapid increase in the firing frequency. (B) Bar graph showing that bath application of histamine on average causes a two-fold increase in firing frequency (n =7; $p < 0.05$).

Figure 2. Histamine causes an inward current in presumed 5-HT neurons in the DRN

Bath delivery	Current shift (n = 19)	36.1 4.1pA *
	ΔRMS (n = 19)	2.7 0.4pA *

Spritz delivery	Current shift (n = 49)	16.7 1.1pA *
	ΔRMS (n = 49)	1.8 0.1pA *

Figure 2. (A) A representative whole-cell voltage-clamp recording of the inward current before and after the application of histamine (10μM) from a presumed 5-HT neuron. Right: the corresponding all points histogram demonstrates the average current shift. Below: tabulated values of the average shift in the holding current and change in baseline noise (ΔRMS) following the application of histamine from 19 cells. (B) A representative recording of the inward current induced by the local delivery of histamine (300μM). Below: tabulated values of the average current shift and ΔRMS from 49 cells. $p < 0.05$.

Figure 3. The histamine-induced current is mediated via the H_1 receptor subtype

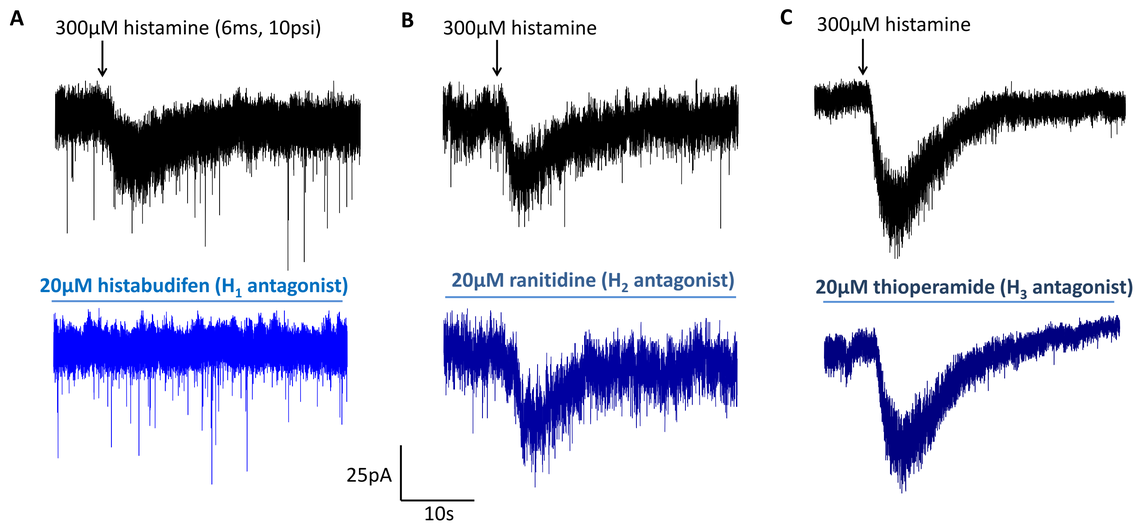
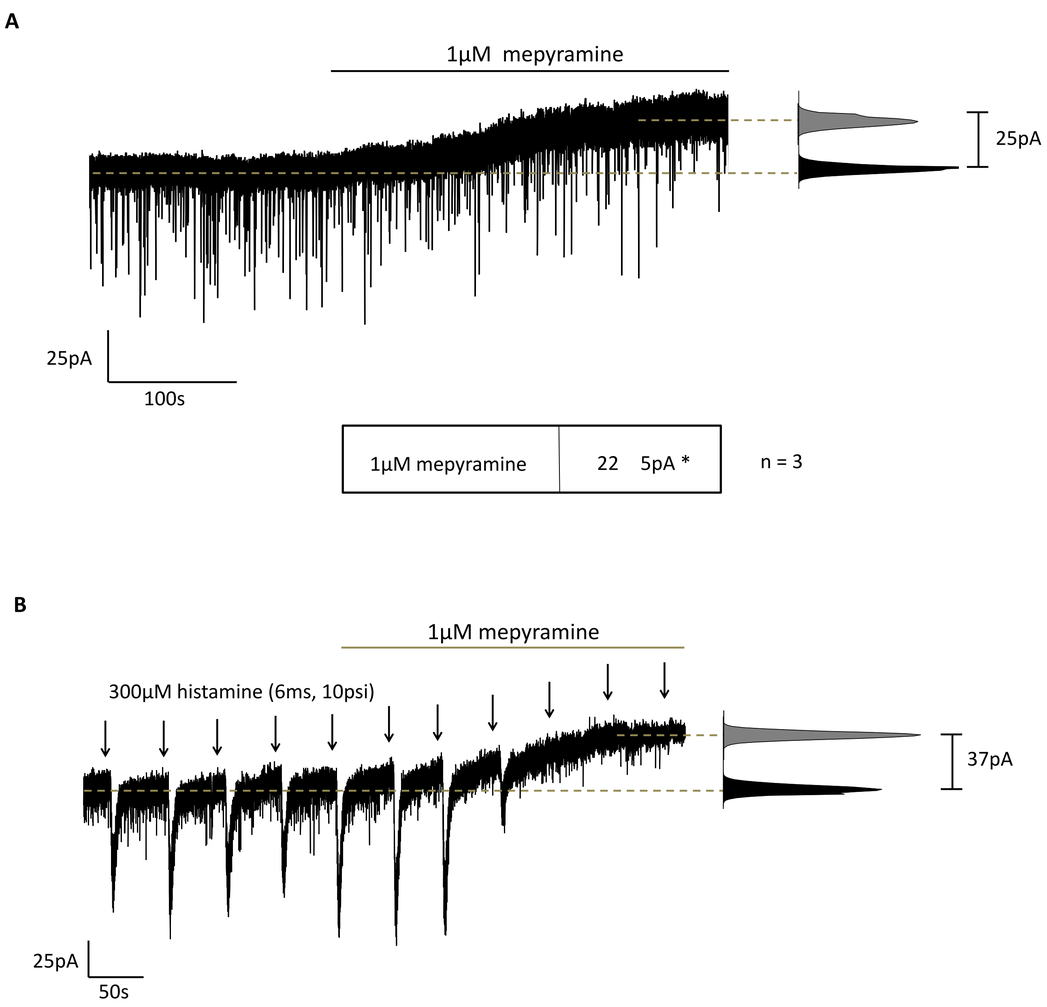


Figure 3. (A) Representative recordings from a presumed 5-HT neuron of an inward current induced by the local application of histamine in the absence (top trace) and presence (bottom trace) of (A) histabudifen (H_1R antagonist), (B) ranitidine (H_2R antagonist) and (C) thioperamide (H_3R antagonist). Note that histabudifen fully abolishes the histamine-induced current.

Figure 4. The application of H_1 inverse agonists reveals a tonic histamine conductance



	Current shift (pA)	ΔRMS (pA)	
Mepyramine (300nM)	32 ± 19	-0.8 ± 0.8	n = 3
Mepyramine (1μM)	24 ± 3 *	-0.7 ± 0.1 *	n = 7
Dimethindene (100nM)	23 ± 7 *	-0.6 ± 0.3	n = 6

Figure 4. (A) representative whole cell voltage-clamp recording from a presumed serotonergic neuron before and after the application of the H_1 inverse agonist, mepyramine (1μM). Right: note the outward shift in the holding current shown by the all points histogram suggesting a tonic histamine conductance is present. Below: table shows the average outward current shift in 3 cells. (B). A representative recording of inward current induced by the local application of histamine in the absence and presence of mepyamine (1μM). Note that in addition to blocking the histamine-induced current, mepyramine also causes an outward shift in the holding current. Right: all points histogram demonstrates the current shift. Below are the averaged values of the shift in current and ΔRMS in response to application of mepyramine and also the structurally distinct H_1 inverse agonist, dimethindene. $p < 0.05$.

Conclusions

(1) Histamine modulates presumed 5-HT neurons in the DRN; causing excitation in single-unit recordings and an inward current in whole-cell voltage-clamp recordings.

(2) The histamine effect is mediated via the H_1 receptor.

(3) The application of H_1 inverse agonists reveal the presence of a tonic histaminergic conductance.

(4) Experiments are in progress to explore the cellular conductances responsible for these observed effects.

References/Declarations

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