



Ionic mechanisms of histamine-induced responses in guinea pig intracardiac neurons

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Hardwick, Jean C., Amy F. Kotarski, and Melanie J. Powers. Ionic mechanisms of histamine-induced responses in guinea pig intracardiac neurons. *Am J Physiol Regul Integr Comp Physiol* 290: R241–R250, 2006. First published September 15, 2005; doi:10.1152/ajpregu.00498.2005.—Histamine, released from mast cells, can modulate the activity of intrinsic neurons in the guinea pig cardiac plexus. The present study examined the ionic mechanisms underlying the histamine-induced responses in these cells. Histamine evokes a small membrane depolarization and an increase in neuronal excitability. Using intracellular voltage recording from individual intracardiac neurons, we were able to demonstrate that removal of extracellular sodium reduced the membrane depolarization, whereas inhibition of K⁺ channels by 1 mM Ba²⁺, 2 mM Cs⁺, or 5 mM tetraethylammonium had no effect. The depolarization was also not inhibited by either 10 μM Gd³⁺ or a reduced Cl⁻ solution. The histamine-induced increase in excitability was unaffected by K⁺ channel inhibitors; however, it was reduced by either blockage of voltage-gated Ca²⁺ channels with 200 μM Cd²⁺ or replacement of extracellular Ca²⁺ with Mg²⁺. Conversely, alterations in intracellular calcium with thapsigargin or caffeine did not inhibit the histamine-induced effects. However, in cells treated with both thapsigargin and caffeine to deplete internal calcium stores, the histamine-induced increase in excitability was decreased. Treatment with the phospholipase C inhibitor U73122 also prevented both the depolarization and the increase in excitability. From these data, we conclude that histamine, via activation of H₁ receptors, activates phospholipase C, which results in 1) the opening of a nonspecific cation channel, such as a transient receptor potential channel 4 or 5; and 2) in combination with either the influx of Ca²⁺ through voltage-gated channels or the release of internal calcium stores leads to an increase in excitability.

tetraethylammonium; phospholipase C; mast cells; H₁ receptor

THE PARASYMPATHETIC CARDIAC PLEXUS integrates information from a variety of inputs, including sensory afferents, descending parasympathetic preganglionic fibers, sympathetic fibers, and interneurons (1, 22). These ganglia consist of a heterogeneous population of cells, including postganglionic neurons and numerous interneurons (1, 6, 20, 22). The convergence of inputs from such diverse sources allows these intracardiac neurons to rapidly respond to both central commands and local reflex circuits to regulate cardiac function.

In many mammalian species, there is also a high density of mast cells located within the heart (24). Previous studies in the guinea pig demonstrated that cardiac mast cells could be found in close proximity to, and even within, the intrinsic cardiac ganglia located within the wall of the atria. Stimulation of the cardiac mast cells, which results in the release of histamine, causes a depolarization of the intracardiac neurons and an increase in excitability via neuronal H₁ receptors (19). H₁

receptors have also been shown to mediate histaminergic modulation of neuronal activity in the canine intrinsic cardiac plexus (2). Thus histamine modulation of neuronal activity by H₁ receptors may be a common phenomenon in the mammalian heart.

The specific mechanisms underlying the histamine-induced responses in the guinea pig intracardiac neurons are unknown. In other neurons, histamine activation of H₁ receptors has been shown to produce a depolarization and an increase in firing frequency (4, 5) similar to that seen in the intracardiac neurons. The ionic mechanisms underlying histamine responses are diverse and include the inhibition of a background potassium conductance, stimulation of a chloride current, stimulation of a nonspecific cation current, and/or an increase in intracellular calcium levels (4). H₁ receptors are normally coupled to phospholipase C (PLC) activation and the subsequent increase in both diacylglycerol and inositol 1,4,5-trisphosphate (4). The present study investigated the potential cellular mechanisms underlying the histamine-induced changes in intracardiac neurons using a variety of pharmacological agents and ion substitutions. These experiments provide greater insight into the regulation of intracardiac neuron activity by inflammatory signals.

MATERIALS AND METHODS

General methods. Guinea pigs (male or female, Charles River), 300–500 g, were euthanized by CO₂ inhalation and exsanguination in accordance with procedures approved by the Ithaca College Institutional Animal Care and Use Committee. The heart was removed and placed into ice-cold Krebs-Ringer (in mM: 121 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, 8 glucose, aerated with 95% O₂-5% CO₂ for a pH of 7.4). The cardiac plexus, located in the epicardium of the atria, was dissected as previously described (8). Briefly, the region studied, which is located primarily in the wall of the left atria underlying the area of the coronary sinus, was exposed by opening the atria and removing the overlying muscle and connective tissue. The tissue was pinned to a Sylgard-lined 60-mm Petri dish and continuously superfused (6–8 ml/min) with 35–37°C Krebs-Ringer. Drugs were applied by inclusion in the circulating bath solution. In all cases, bath-applied solutions were allowed to equilibrate for a minimum of 10 min before testing. Histamine (10⁻⁴ M in Krebs solution) was applied by local pressure ejection (6–9 psi, Picospritzer, General Valve) through a small diameter (5–10 μm) glass electrode positioned 50–100 μm from the individual neuron. For multiple tests of histamine responses in the same cell, the cells were allowed to wash (via the circulating Krebs solution) for several minutes between histamine applications. This time frame allowed for multiple, repeatable responses, with no apparent desensitization.

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