Histamine in Cardiac Sympathetic Ganglia: A Novel Neurotransmitter?

Ryam M. Fryer1, Glenn A. Reinhart1, Timothy A. Esbenshade2

Department of Integrative Pharmacology1 and Neuroscience2, Abbott Laboratories, Abbott Park, IL 60064-6139

Historically, cardiac ganglia have been considered collections of cholinergic neurons that distributed most densely near the sinoatrial and atrioventricular nodes (1–3). However, it is now generally accepted that cardiac ganglia contain a heterogeneous population of neurons capable of synthesizing, and responding to, several different neurotransmitters and neuropeptides including adrenergic and purinergic agonists and antagonists (2). Recently, Li and colleagues (4) as well as others (2, 3) have proposed that histamine may in fact meet the definition of a neurotransmitter in the heart. However, whether histamine be classified as a true neurotransmitter in cardiac sympathetic ganglia is less clear.

Histamine produces a wide array of effects in the heart (6–8). In fact, the effects of histamine on cardiac function have been appreciated since the work of Dale and Leadon (9) in 1910 who showed that synthetic histamine, β-methylhistamine, modified cardiac rhythm in the mammalian heart. Subsequently, the effects of histamine and L-protein–coupled histamine receptor subtypes on cardiovascular function have been well characterized (reviewed in (8, 10)). Three of the four histamine receptor subtypes are present in the heart: H1 and H2 receptors are located postjunctionally whereas the H3 receptor is a prejunctional synaptic receptor (11, 12). A fourth histamine receptor, the H4 receptor, is widely expressed in hematopoietic cells (13–15) but to date has not been reported in the heart.

Histamine elicits multiple effects in the heart, including an increase in sinus rate, as demonstrated in transmembrane action potential recordings in sinoatrial (SA) nodal cells, increased ventricular automaticity (Box 1) through an H2-mediated enhancement of inward Ca2+ current (I\textsubscript{Ca}), and subsequent acceleration in phase 4 spontaneous depolarization (Box 2) (6–18). The subcellular mechanisms by which histamine increases the slope of spontaneously diabetic depolarization and thereby augments the firing rate of SA nodal cells includes stimulation of adenyl cyclase and increased adenosine 5′-monophosphate (cAMP) formation and the activation of protein kinase A (PKA) which produces Ca2+-channel phosphorylation and augmented Ca2+ influx. Also, histamine profoundly decreases atrioventricular (AV) conduction velocity—an effect that is mimicked by H2 agonists, antagonized by H2 receptor blockade (19), and has been observed in multiple species, including humans (8, 20). Although activation of H1 receptors stimulates phosphodiesterase turnover and increases intracellular cGMP in the myocardium, the precise mechanism of H1-mediated reductions in AV conduction has not been fully delineated (6). In spite of decreasing AV conduction velocity, histamine may actually increase AV nodal automaticity via H2 receptors through a mechanism analogous to that described for increases in sinus automaticity (8) that has been demonstrated in dogs following suppression of the sinus rhythm (21) and in isolated, blood-perfused, AV nodal preparations from dog (22).

Importantly, histamine increases the force of ventricular contraction. However, the receptor subtype, or subtypes, that mediate this effect are unclear and in fact, the coupling between histamine receptor subtypes and the transduction mechanisms responsible for positive inotropic effects varies considerably among species and also for different regions of the heart (reviewed in (8)). In the human, however, H1 receptors mediate the positive inotropic action of histamine in both atria and ventricles because increases in contractile activity are invariably blocked by cimetidine, but not pyrilamine (an H2 receptor antagonist) (23).

Finally, the release of cardiac histamine from both mast cell and non-mast cell sources during anaphylaxis or myocardial ischemia, or in response to drug treatment (e.g., anthracycline antibiotics, morphine, and d-tubocurarine) is known to provoke arrhythmogenic effects on the sinus node, atrial fibers, AV node, Purkinje fibers, and ventricular cells owing to changes in normal automaticity and conduction (reviewed in (24)). In fact, in experimental models of acute myocardial ischemia, concentrations of histamine in the coronary sinus increase concomitantly with the development of early ischemia.

Box 1: Definitions of Cardiovascular Terms

• **Automaticity and the Sinus Node:** Automaticity refers to the propensity for a cell to elicit an electrical impulse on its own. A single specialized location in the atria, the sinoatrial node, has a higher automaticity (i.e., a faster pacemaker) than the rest of the heart, and therefore is usually the one to start the electrical impulse resulting in a heartbeat. The sinus fibers connect directly with the atrial fibers, so that any action potential that begins in the SA node spreads immediately into the atria.

• **AV Conduction Velocity:** The speed with which the electrical impulse transmits from the atrial tissue and through the atrioventricular (AV) node to the Purkinje fibers and ventricular tissue of the heart.

• **Purkinje Fibers:** Conducting fibers within the ventricle responsible for the rapid transmission of the electrical impulse throughout the ventricle.

• **Inotropy:** The term applied to changes in the force of heart muscle contraction (e.g., a positive inotropic agent afflicts an increase in the force of ventricular contraction whereas a negative inotrope produces a reduction in the force of contraction).
induced ventricular arrhythmias and in proportion to their severity (25); thus, histamine receptors in the heart may represent novel therapeutic targets. Regardless of the well-characterized effects of histamine in the heart, whether histamine is truly a neurotransmitter in cardiac sympathetic nerve fibers necessitates the question, what defines a neurotransmitter?

Box 2: Phases of the Ventricular Action Potential

- **Phase 0**: Rapid depolarization of the cardiac cell first mediated by I_{Na} and later by activation of I_{K,1} resulting in rapid contraction of the ventricle.
- **Phase 1**: Activation of transient outward K+ current I_{To} that is present in subepicardial ventricular cells but to a small extent in the remaining ventricular myocardial cells.
- **Phase 2**: Outward phase of the action potential predominately mediated by L-type Ca^{2+} channels resulting in the sustained contraction of the heart.
- **Phase 3**: Repolarization of the cardiac cell, predominately mediated by K+ channels, resulting in ventricular relaxation.
- **Phase 4**: Maintenance of the resting membrane potential to allow for filling of the heart prior to the next contraction. Modified from (41).

Regardless of the well-characterized effects of histamine in the heart, whether histamine is truly a neurotransmitter in cardiac sympathetic nerve fibers necessitates the question, what defines a neurotransmitter? Schwartz (20) defines a neurotransmitter by four distinct criteria: 1) it is synthesized in the neuron, 2) the molecule is present in the presynaptic terminal and is released in amounts sufficient to exert a defined action on the postsynaptic neuron or effector organ, 3) when administered exogenously it mimics the action of the endogenously released transmitter, and 4) a specific mechanism exists for removing the molecule from the synaptic cleft. Several labs have taken even greater strides to more precisely define a neurotransmitter. This includes: 1) it is present in the presynaptic terminal and is released in amounts sufficient to exert a defined action on the postsynaptic neuron or effector organ, 2) when administered exogenously it mimics the action of the endogenously released transmitter, and 3) a specific mechanism exists for removing the molecule from the synaptic cleft. Several labs have taken even greater strides to more precisely define a neurotransmitter.

Do cardiac sympathetic neurons synthesize histamine and is histamine present in nerve terminals? Singh and colleagues (2) demonstrated that histamine-synthesizing enzymes are present in the neurons of adult human cardiac ganglia. They showed that 80% of neurons were immunopositive for histamine. Although Singh et al. (2) did not demonstrate the colocalization of histamine and NE to the same neurons in adult human cardiac ganglia, Li et al. (4) recently suggested that histamine and NE coexisted in 51% of cardiac sympathetic varicosities within guinea pig ventricles, although the evidence for the coexistence of the two substances in the neuronal soma is less convincing. Thus, cardiac sympathetic nerves appear to express the appropriate enzymes to synthesize and to store histamine intracellu larly prior to release.

Are histamine receptors present in the heart or on presynaptic or postsynaptic neurons? Three of the four of the known histamine receptors appear to be expressed in the heart or on sympathetic ganglia (11). In human tissue, evidence from both Northern and Western blot studies have indicated a variable distribution of H1 and H2 receptors and suggest that the relative distribution of the receptors may mediate the functional responses to histamine (20). H3 receptors also appear to be expressed on sympathetic ganglia in the heart although receptor mRNA or protein expression has not been conclusively demonstrated in cardiac synaptosomes or tissue. Despite the lack of physical evidence of H3 receptors in cardiac sympathetic ganglia, Imamura and colleagues (12) have functionally characterized these receptors on sympathetic ganglia. They suggested that H3 receptors present on sympathetic nerves in the human heart using H3-selective agents and demonstrated that the receptor is involved in the inhibition of K+-stimulated norepinephrine (NE) release and electrically-induced isoropic responses. Therefore, three histamine receptor subtypes have been physically or functionally characterized on either presynaptic or postsynaptic cells of the heart itself (11) and can respond to neuronal histamine release.

Is histamine release from presynaptic terminals Ca^{2+}-dependent? Li and colleagues (4) demonstrated that depolarization of cardiac sympathetic neurons (50 mM K+) stimulates modest (0.3–0.6 pM) but highly variable (± 0.4 pM) increases in endogenous histamine release, an effect that is not affected by Compound 48/80, a mast cell histamine-releasing agent, suggesting that cardiac histamine likely originates from a sympathetic neuronal source. Also, they demonstrated that the N-type Ca^{2+} blocker tetrodotoxin but not lacidipine, an inhibitor of L-type Ca^{2+} channels, attenuated histamine release,
suggesting that endogenous histamine release may be dependent on N-type Ca^{2+} channel activation. Although the modest and variable increases in histamine release following depolarization were statistically significant, as were the reductions in histamine release in the presence of ω-conotoxin, the relevance of these small changes in synaptic histamine concentrations under physiological conditions is less clear.

Are sufficient quantities of histamine released to exert electrophysiological effects on the heart or in pre- or post-synaptic neurons? Gross and colleagues (28) and Imamura et al. (29) have demonstrated that sympathetic stimulation of isolated guinea pig hearts produces a 1.5- to 3-fold increase in histamine overflow into the coronary perfusate. However, the concentrations of histamine under normal physiological conditions may be insufficient to activate sensitive H3 receptors because thioperamide, an H3 blocker, does not modify the resultant tachycardia or NE overflow (i.e., excess output of NE from neurons) following sympathetic activation, whereas thioperamide did increase NE overflow under conditions of ischemia and reperfusion. Thus, under physiological conditions, pre synaptic H1 receptors in the heart may be quiescent whereas endogenous histamine does likely play an important role in the regulation of NE release under pathological conditions including myocardial ischemia.

Does exogenous histamine mimic the action of the endogenously released histamine? Exogenous histamine (10 µL, 100 µM), when applied adjacent to spontaneously active canine right atrial neurons in vitro, increases neuronal activity and when administered into the local arterial blood supply of these neurons in vivo (100 µL, 100 µM) increases neuronal activity (from 8 ± 1 to 34 ± 4 impulses/min) and produces elevations in heart rate (from 119 ± 3 to 134 ± 4 beats/min) and right and left ventricular intramyocardial systolic...
pressures (from 106 ± 6 to 120 ± 7 mm Hg). Thus, exogenous histamine might activate a population of cardiac neurons relevant to cardiovascular function (30). These results are consistent with the effects of endogenous histamine in the heart as elucidated by selective pharmacological blockade or stimulation of specific histamine receptor subtypes (as detailed earlier). Also, both endogenous and exogenous histamine exert similar effects on NE release from cardiac sympathetic ganglia in experimental models (31–33) and in cardiac synaptosomes isolated from human atria (32). Endogenous NE release during ischemic stress has been linked to cardiac arhyth-

nonic effects (32, 33). Luo et al. (30) first demonstrated the ability of α1-agonists to modulate sympathetic neurotransmission using isolated guinea pig hearts whereby α1-selective agonists, a selective α1 receptor, inhibited the positive inotropic effects of electrical field stimulation. More recently, Li and colleagues (31) demonstrated that K+-evoked NE release was attenuated in cardiac sympathetic neurons preincubated with l-histidine, an effect reversed by thioperamide, further suggesting H3 involvement. Also, using α1-selective agents, Silver et al. (31) and Seyedi et al. (37) collectively demonstrated that H3-mediated reductions in NE exocytosis from cardiac sympathetic nerves results sequentially from H3 receptor G-protein coupling, inhibi-

tion of adenyl cyclase activity, and decreased cAMP formation, leading to a reduction in PKA activity and decreased Ca2+ influx through N-type Ca2+ channels. Thus, evidence suggests that under certain conditions both exogenous and endogenous histamine mod-

ulates cardiovascular function directly through H3 and H2 receptors and also modulates sympathetic NE release via stimulation of pre-

synaptic H3 receptors.

WJ Mokdad of histamine release present activity in pre-synaptic cells or effector organs? Li and colleagues (31) suggest that histamine reduces neuronal activity in the pre-synaptic region by receptor-mediated mechanisms (31) and also modulates serotonergic neuronal NE release via stimulation of pre-

synaptic H3 receptors.

Is histamine actively eliminated from the synaptic cleft? Two possi-

ble mechanisms could effectively limit histamine concentrations in the synaptic cleft: 1) histamine transport across the membrane of pre- or post-synaptic cells or 2) histamine degradation within the synapse. Histamine may be removed from the synapse by the high-capacity vesicular monoamine transporter 2 (VMAT2), and because the H3 receptor may potentially function as a postsynaptic autoinhibitory receptor that mediates the release of not only NE but also of hista-

mine itself (4), pre-synaptic H3 receptors may effectively reduce hista-

mine concentrations in the synaptic cleft. Whether the H3 receptor truly functions as a clearance receptor, however, to limit histamine concentrations in the synapse has not been conclusively demonstrat-

ed. Moreover, although the major histamine-metabolizing enzyme, histamine-N-methyltransferase, is present in the neuron (6), the presence of this enzyme or of histaminase has not been observed in the presynaptic cleft of sympathetic cardiac ganglia. Thus, although his-

tamine may be transported from the synaptic cleft by VMAT2, it is not clear whether H3 receptors effectively limit histamine concentra-

tions in the synaptic cleft or whether enzymes exist in the synapse of cardiac sympathetic ganglia to selectively degrade histamine. When the available evidence is assessed using a comprehensive definition of a neurotransmitter based on seven distinct criteria, histamine appears to fall short of classification as a neurotransmit-

ter in cardiac sympathetic ganglia. Clearly, histamine meets some of those criteria (figure 1). It appears to be synthesized and stored in the neurons and its receptors are present on both pre- and post-synaptic cells and in the heart itself (although conclusive physical evidence of pre-

synaptic H3 receptors in cardiac sympathetic ganglia has not been shown). Limited evidence also suggests that histamine release may be dependent upon N-type Ca2+ channel activation but whether sympa-

thetic ganglia in the heart actively release histamine to con-

centrations sufficient to exert effects under physiological conditions is less clear. On the other hand, endogenous histamine does appear to play an important role in the modulation of cardiac function dur-

ing pathological states (e.g., ischemia) and pharmacological studies have suggested that the effects of endogenous histamine on cardio-

vascular function and the regulation of NE release are similar to that of exogenous histamine. Selective modulation of histamine receptors clearly affects cardiovascular function in vivo and NE exocytosis in synaptosomes, thus it may be hypothesized that the blockade of histamine release would prevent this activity in postsynaptic cells, however, the actual blockade of postsynaptic activity in the presence of attenuated histamine release has not been conclusively demonstrat-

ed. Moreover with the exception of the possible functional absorption of histamine in the synapse by pre-synaptic H3 receptors or by VMAT2 transport, there is no known mechanism to selectively eliminate or degrade histamine from the synaptic cleft of cardiac sympathetic ganglia.

Regardless of the classification of histamine as a neurotrans-

mitter or simply as an important signaling molecule in the heart, histamine receptors do offer novel therapeutic targets in cardio-

vascular disease. The H1 and H2 receptor subtypes are expressed differen-

tially across types of cardiac tissue in a species-dependent manner (23), accounting for the pharmacological and functional dif-

ferences seen with histaminergic agonists and classical anti-allergi-

ical agents.
Viewpoint

H3 receptor and anti-ulcerogenic H2 receptor antagonists in these tissues. In humans, the predominant subtype in both normal arid and ventricular tissues is the H2 receptor (23), which when activated appears to produce a positive inotropic response. Histamine induces antiarrhythmic activity in diseased human heart tissue (15, 24), and in conditions of endotoxemia, expression of both H1 and H2 receptors are increased, leading to the augmented effect of histamine in cardiac tissue (38). Thus, it is conceivable that selective H3 receptor antagonists may be of benefit in such disease states. Interestingly, the H2 receptor antagonist lansoprazole has also been shown to lessen the severity of chronic heart failure, perhaps suggesting a new mode of treatment for this disease (39).

The H3 receptor has received considerable interest as a potential target for cardiovascular diseases in recent years because of its predominant role in the modulation of the release of cardiac neurotransmitters including norepinephrine. Myocardial ischemia is associated with reactivation of the sympathetic system, enhanced norepinephrine release, resultant dysrhythmias and metabolic demand, and aggravation of the initial ischemic event that can lead to additional heart damage and failure (10, 40). In fact, a greater incidence and longer duration of ventricular fibrillation, which was correlated with norepinephrine overflow, was seen upon repolarization in hearts from H3 knockout mice subjected to ischemia (38). Thus, activation of H3 receptors by histamine agonists with drug-like properties may carry a novel and attractive means for the treatment of myocardial ischemic arrhythmias.

In conclusion, the present literature does not definitively support the classification of histamine as a neurotransmitter in cardiac sympathetic ganglia. Promising therapeutic opportunities exist, however, for subtype-selective histamine receptor agents in several cardiac diseases ranging from the prevention of heart failure and ischemia-induced ventricular arrhythmias to the blockade of homeodynamic consequences that result from histamine overload during endotoxemia.

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Ryan M. Fryer, PhD is an Associate Research Investigator in Global Pharmaceutical Research and Development at Abbott Laboratories in the Department of Integrative Pharmacology. He also teaches at the University of Chicago. He can be reached at 788-5458.

Glen A. Reinhart, PhD is a Senior Group Leader in Global Pharmaceutical Research and Development at Abbott Laboratories in the Department of Integrative Pharmacology.

Timothy A. Eschenbacher, PhD is a Senior Group Leader in Global Pharmaceutical Research and Development at Abbott Laboratories in the Department of Neuroscience.