Histamine stimulates ion transport by dog pancreatic duct epithelial cells through $H_1$ receptors

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Nguyen, Toan D., Charles N. Okolo, and Mark W. Moody. Histamine stimulates ion transport by dog pancreatic duct epithelial cells through $H_1$ receptors. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G76–G84, 1998.—Histamine affects pancreatic secretion, but its direct action on ion transport by pancreatic duct epithelial cells (PDEC) has not been defined. We now characterize the secretory effects of histamine on cultured, well-differentiated, and nontransformed dog PDEC. Histamine stimulated, in a concentration-dependent manner (1–100 μM), a cellular $^{125}$I⁻ efflux that was inhibited by 500 μM 5-nitro-2-(3-phenylpropyl)-amino-benzoic acid, 2.5 mM diphenylamine-2-carboxylate, and 500 μM DIDS and thus mediated through $Ca^{2+}$-activated Cl⁻ channels. Histamine-stimulated $^{22}Na^-$ efflux was 1) inhibited by 100 μM diphenhydramine, an $H_1$ receptor antagonist, 2) resistant to 1 mM cimetidine, an $H_2$ receptor antagonist, 3) not reproduced by 1 mM dimaprit, an $H_3$ agonist, and 4) inhibited by 50 μM 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid-AM, a $Ca^{2+}$ chelator, suggesting that it was mediated through $H_1$ receptors acting via increased cytosolic $Ca^{2+}$. Histamine also stimulated a $^{86}Rb^+$ efflux that was sensitive to 100 nM charybdotoxin and thus mediated through $Ca^{2+}$-activated $K^+$ channels. When PDEC monolayers were studied in Ussing chambers, a short-circuit current of 21.7 ± 3.1 μA/cm² was stimulated by 100 μM histamine. This effect was inhibited by diphenhydramine but not cimetidine, was not reproduced with dimaprit, and was observed only after serosal addition of histamine, suggesting that it was mediated by basolateral $H_1$ receptors on PDEC. In conclusion, histamine, acting through basolateral $H_1$ receptors, activates both $Ca^{2+}$-activated Cl⁻ and $K^+$ channels; in this manner, it may regulate PDEC secretion in normal or inflamed pancreas.

Since its discovery at the beginning of the century, the physiological and pathological role of histamine has been expanding. It is released from nerve endings to act as a neurotransmitter, from endocrine cells to act as a hormone, and from mast cells to act as a mediator of inflammation. These functions are mediated through the following three specific receptors: $H_1$ receptors acting through phospholipase C to increase cytosolic free $Ca^{2+}$ concentration ([Ca$^{2+}$]i); $H_2$ receptors activating the cAMP cascade, and presynaptic $H_3$ receptors inhibiting histamine synthesis and release (3, 9). In the digestive system, histamine stimulates secretion in certain cells [e.g., parietal cells and colonocytes (23)], whereas it inhibits secretion in others [e.g., duodenal cells and D cells (4, 22)]. The effect of histamine on pancreatic secretion has been examined using different experimental systems involving anesthetized animals, isolated pancreas, pancreatic lobules, or pancreatic segments. Depending on the model studied, histamine interacts with either $H_1$ receptors to stimulate pancreatic exocrine secretion, with $H_2$ receptors to stimulate or inhibit secretion, or with $H_3$ receptors to inhibit secretion (6, 8, 16–19). This varied response may reflect the different species studied; it also illustrates the complex components and determinants of pancreatic exocrine secretion.

Pancreatic exocrine function consists of the secretion of digestive enzymes, mediated by pancreatic acinar cells, and the secretion of fluid and electrolytes, mainly bicarbonate, mediated by pancreatic duct epithelial cells (PDEC). Most of the reports mentioned above characterized the effect of histamine on the final product of these two secretory processes, the pancreatic juice collected by cannulation. Although acinar and ductal function are generally reflected by the enzyme content and volume of pancreatic juice, pancreatic duct cannulation does not allow clear distinction between histamine effects on acinar cells and PDEC. The investigations using pancreatic lobules, on the other hand, focused mainly on the secretion of enzymes by acinar cells and did not address the effect of histamine on PDEC.

Of further note, because the pancreatic tissue studied in these reports contains many cell types, it is unclear whether the observed effects result from the direct interaction between histamine and the secretory cell or whether they are indirectly mediated through other cells. Indeed, histamine has been shown to interact with presynaptic $H_3$ receptors on intrinsic pancreatic nerves to inhibit pancreatic secretion (6).

Thus the direct effect of histamine on PDEC is yet undefined. Compared with acinar cells, studies of the secretory function of PDEC have been hampered by the lack of a practical model for these cells (11). Oda et al. (15) recently developed a method to culture well-differentiated dog PDEC without transformation; we recently demonstrated that these cells express functional cAMP- and $Ca^{2+}$-activated Cl⁻ channels (14) and $Ca^{2+}$-activated $K^+$ channels (14a). In this report, these cells were used to examine the direct effects of histamine on ion transport by PDEC. Our aims were to 1) determine the effects of histamine on ion transport pathways, such as $K^+$ and Cl⁻ channels, 2) characterize the subtype and localization of the responsible histamine receptors, and 3) define the signal-transduction pathway mediating this effect.
MATERIALS AND METHODS

Chemicals and reagents. Histamine, diphenhydramine, cimetidine, dimaprit, DIDS, charybdotoxin, and tissue culture medium and supplements were from Sigma (St. Louis, MO), and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid (BAPTA)-AM was from Calbiochem (San Diego, CA). Diphenylamine-2-carboxylate (DPC) was from Fluka (Ronkonkoma, NY), and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) was from Research Biochemicals (Natick, MA). Na$_{125}$I (16 mCi/mg iodide) was purchased from Americant. Ag-AgCl$_2$ electrodes, and the corresponding short-circuit current (Isc) was recorded continuously using a model MP100 analog-to-digital converter and the Acknowledge 2.0 software program (BioPak Systems, Goleta, CA). Instrument calibration was performed using membranes devoid of cells. In most cases, the magnitude of the Isc response was estimated using peak stimulated Isc increases, corrected for baseline activity; in some instances, the area under the curve was also calculated using the Acknowledge software.

Because the amplitude of the Isc response can vary with time (probably reflecting growth conditions and cell passage), comparative experiments used cell monolayers cultured under the same conditions and studied concurrently.

Statistics. All experiments were repeated at least three times, and results are expressed as means and SE. Comparisons were performed using unpaired two-tailed Student’s t-test, and the Stat View 512+ software (Abacus Concepts, Calabasas, CA) was used to determine the corresponding P values.

RESULTS

Iodide efflux studies. The effect of different concentrations of histamine on $^{125}$I efflux from preloaded PDEC was first evaluated. Marked increases in efflux were observed with concentrations of histamine $\approx$10 µM; these increases were rapid and transient, reaching a peak 30 s after the addition of histamine (Fig. 1A). The peak efflux rate coefficients were, respectively, 0.25 ± 0.02, 0.54 ± 0.05, 0.63 ± 0.04, and 0.69 ± 0.09/min for concentrations of histamine of 1 µM, 10 µM, 100 µM, and 1 mM (n = 6 experiments). The corresponding efflux rate coefficient in the absence of histamine was 0.23 ± 0.02/min. Thus histamine stimulated an increased $^{125}$I efflux in a concentration-dependent manner, with a maximal effect at $\approx$ 100 µM.

To determine that the $^{125}$I efflux was mediated through activated Cl$^-$ channels, the effects of previously established inhibitors of Cl$^-$ channels on these PDEC were evaluated. The $^{125}$I efflux stimulated by 100 µM histamine was completely abolished by 500 µM NPPB (Fig. 1B) and by 2.5 mM DPC (Fig. 1C). In addition, it was also markedly inhibited by 500 µM DIDS, from a control peak efflux rate coefficient of 0.82 ± 0.04/min to a peak rate of 0.30 ± 0.03/min in the presence of DIDS (mean peak stimulated efflux rate coefficient: 0.588 ± 0.049 vs. 0.205 ± 0.026/min, 65%
inhibition, P = 0.002 by unpaired 2-tailed t-test with 4 degrees of freedom; Fig. 1D). This inhibitory profile suggests that the $^{125}$I efflux is mediated through Ca$^{2+}$-activated Cl$^{-}$ channels (14).

The receptor subtype mediating histamine action was next defined using different histamine receptor agonists and antagonists. As shown in Fig. 2A, the $^{125}$I efflux stimulated by 100 µM histamine was abolished by 100 µM diphenhydramine, a specific H$_1$ antagonist. On the other hand, the histamine-stimulated $^{125}$I efflux was not affected by a high concentration (1 mM) of cimetidine, an H$_2$ antagonist (Fig. 2B). In addition,
the effect of histamine was not reproduced by a high concentration (1 mM) of the specific H2 agonist, dimaprit (Fig. 2C). In the aggregate, this profile suggests that the stimulatory effect of histamine was mediated by the H1, and not H2, histamine receptor subtype.

Because histamine effects mediated through H1 receptors are coupled to increased [Ca\(^{2+}\)], the signal-transduction pathway responsible for histamine-stimulated \(^{125}\)I-efflux was studied using BAPTA-AM, a membrane-permeant Ca\(^{2+}\) chelator. Once BAPTA-AM is loaded into PDEC, the ester bond is cleaved by cytosolic esterase, trapping the active chelator BAPTA intracellularly and depleting [Ca\(^{2+}\)]. When PDEC were pretreated with BAPTA-AM for 45 min, the increased...
125I efflux produced by histamine was abolished (Fig. 2D). This inhibition suggests that the effect of histamine is dependent on increased [Ca\(^{2+}\)].

86Rb\(^+\) efflux studies. Using 86Rb\(^+\) as a marker for K\(^+\), we demonstrated the presence on PDEC of Ca\(^{2+}\)-activated K\(^+\) channels (14a). These channels are likely to be activated by histamine, which stimulates an increased [Ca\(^{2+}\)]. The effects of histamine on the cellular efflux of 86Rb\(^+\) were therefore evaluated. Histamine stimulated an increased 86Rb\(^+\) efflux from PDEC, which reached a peak rate coefficient of 0.158 ± 0.003/min (peak stimulated efflux rate coefficient: 0.120/min after baseline correction, n = 3) 30 s after the addition of 100 µM histamine (Fig. 3A).

To verify that this efflux is mediated through activated K\(^+\) channels, the effect of charybdotoxin, an inhibitor of Ca\(^{2+}\)-activated K\(^+\) channels in these cells, was evaluated. As shown in Fig. 3B, 100 nM charybdotoxin inhibited the histamine-stimulated 86Rb\(^+\) efflux by 86%. Indeed, the mean peak stimulated efflux rate coefficient was 0.016 ± 0.001/min for charybdotoxin-treated cells vs. 0.111 ± 0.004/min for control untreated cells (P = 0.0001, 2-tailed unpaired t-test with 4 degrees of freedom).

Ussing chamber studies. An advantage associated with these PDEC is their ability to grow as monolayers of polarized cells with tight junctions, generating a transepithelial electrical resistance adequate for studies in Ussing chambers. Using this experimental system, we can localize the H\(_1\) receptors to either the apical or basolateral side of PDEC and characterize the effects produced by the sequential addition of antagonists and agonists.

Confluent PDEC monolayers were mounted in Ussing chambers; in this system, the serosal compartment contains the buffer in contact with the filter and basolateral membrane of the cell, whereas the luminal compartment contains the buffer in contact with the apical membrane. The addition of 100 µM histamine to both compartments produced a quick increase in the I\(_{sc}\) that reverted to normal over 5 min; occasionally, a small shoulder was also observed (Fig. 4A). Because the amplitude of the I\(_{sc}\) can vary with time (probably reflecting growth conditions and cell passage), the average peak I\(_{sc}\) increase (corrected for baseline I\(_{sc}\)) from 14 different experiments was calculated; it was 21.7 ± 3.1 µA/cm\(^2\).

The localization of the histamine receptors on PDEC is clarified in Fig. 4B, where 100 µM histamine was added to either the serosal or luminal compartment of the Ussing chamber. Histamine stimulated an I\(_{sc}\) increase only when added to the serosal compartment; but not when added to the luminal compartment. This finding suggests that expression of the histamine receptors is limited to the basolateral side of PDEC.

The receptor subtype was also verified using this system. As shown in Fig. 5A, addition of 100 µM diphenhydramine, the H\(_1\) antagonist, by itself did not affect the I\(_{sc}\). However, it fully inhibited the subsequent I\(_{sc}\) response to 100 µM histamine. In additional experiments, we observed that this inhibitory effect was reversible: histamine stimulated an I\(_{sc}\) increase from cells that were washed after treatment with diphenhydramine (data not shown).

The action of cimetidine, the H\(_2\) antagonist, was also evaluated. Cimetidine had no effect on the I\(_{sc}\) when added by itself; it also did not inhibit the subsequent I\(_{sc}\) increase stimulated by histamine (Fig. 5B). In some experiments, after the addition of cimetidine, the I\(_{sc}\) response was slightly sharper (higher amplitude and
not stimulate an $I_{\text{sc}}$ increase when added in isolation or when added after histamine. On the other hand, dimaprit appears to partially inhibit the subsequent response to histamine. Indeed, the peak $I_{\text{sc}}$ stimulated by 100 µM histamine was inhibited from a control value of $26.9 \pm 3.1 \mu A/cm^2$ to a value of $10.4 \pm 3.1 \mu A/cm^2$ after addition of 1 mM dimaprit ($n = 4$, $P = 0.009$ by 2-tailed unpaired $t$-test, 6 degrees of freedom).

To examine whether the inhibitory effect of dimaprit was mediated through H$_2$ receptors, the effect of 1 mM cimetidine on the ability of dimaprit to subsequently inhibit histamine was evaluated. When 1 mM cimetidine, 1 mM dimaprit, and 100 µM histamine were added in sequence, the final response to histamine was the same in the presence or absence of cimetidine (Fig. 5D). Because the inhibitory effect of dimaprit was not blocked by prior addition of the H$_2$ antagonist cimetidine, it probably was not mediated through an H$_2$ receptor.

**DISCUSSION**

Exocrine pancreatic secretion is composed of the secretion of pancreatic enzymes, mediated by acinar cells, and the secretion of fluid and electrolytes, mediated by PDEC. Histamine has been observed to affect this secretion. However, depending on the system and the species used, histamine may have different effects, mediated through different mechanisms. Injected arterially into anesthetized dogs, histamine stimulated pancreatic secretion as evidenced by an increased volume, bicarbonate concentration, and protein content of pancreatic juice obtained from main pancreatic duct cannulation. The agonist and antagonist profile of this effect suggested mediation through H$_2$ receptors (1, 5). When rabbits were studied instead of dogs, a more complex effect was produced by histamine: through H$_1$ receptors, histamine stimulated pancreatic secretion, but through H$_2$ receptors, it inhibited secretion (16, 17). When whole pancreata or pancreatic lobules from rabbits were studied, histamine stimulated an increased production in both the volume and enzymatic content of pancreatic juice obtained from main pancreatic duct cannulation. The agonist and antagonist profile of this effect suggested mediation through H$_2$ receptors (1, 5).

In these studies of relatively intact tissue, it is difficult to determine which cell type is directly affected by histamine and which receptor subtype mediates that specific action. The increased pancreatic secretion from cannulated ducts can reflect stimulation of both acinar and ductular components. In addition, regulatory interactions between different cell types can also occur; as discussed above, histamine may decrease pancreatic secretion indirectly through the inhibition of acetylcholine release (6). This report is, to our knowledge, the first study examining the direct action of histamine on PDEC.
Through studies of $^{125}$I and $^{86}$Rb effluxes, we demonstrated that histamine activated both Cl$^-$ and K$^+$ channels on dog PDEC. The uses of $^{125}$I and $^{86}$Rb effluxes to study Cl$^-$ and K$^+$ channels have been validated (21) and led to the successful characterization of Cl$^-$ and K$^+$ conductances in PDEC (Refs. 14 and 14a).

We previously demonstrated that dog PDEC express two distinct Cl$^-$ channels: a cAMP-activated channel corresponding to CFTR, inhibited by NPPB and DPC but resistant to DIDS, and a Ca$^{2+}$-activated Cl$^-$ channel inhibited by NPPB, DPC, and DIDS (14). Because the histamine-stimulated $^{125}$I efflux is inhibited by NPPB, DPC, and DIDS, it most likely is mediated through Ca$^{2+}$-activated Cl$^-$ channels.

The partial inhibition by DIDS merits further discussion. This partial inhibition was previously observed with the Ca$^{2+}$-activated Cl$^-$ channel in PDEC (14) and is consistent with a role for this channel in mediating histamine-stimulated $^{125}$I efflux; however, it does not exclude a partial role for the DIDS-resistant CFTR Cl$^-$ channel in this efflux. On the other hand, the abolition of this efflux by the Ca$^{2+}$-chelator BAPTA suggests dependence on increased [Ca$^{2+}$] and thus a principal

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**Fig. 5. Mediation of histamine effect in Ussing chambers by H$^1$ receptors.** Monolayers of confluent PDEC were mounted in Ussing chambers as described in MATERIALS AND METHODS, and I$_{sc}$ was measured (µA/filter of 0.95 cm$^2$). In A-D, both traces were derived from monolayers cultured and studied at the same time. A: after 5 min of baseline determination, histamine was added to a final concentration of 100 µM in the absence (tracing A) or presence (tracing B) of 100 µM diphenhydramine. Complete inhibition of histamine-stimulated I$_{sc}$ increase by diphenhydramine was verified in 4 experiments. B: after 5 min of baseline determination, histamine was added to a final concentration of 100 µM in the presence (tracing A) or absence (tracing B) of 1 mM cimetidine. Absence of significant inhibition was shown in 3 experiments (see text for details). C: after 5 min of baseline determination, 100 µM histamine (tracing A) or 1 mM dimaprit (tracing B) was added; after an additional 15 min, 100 µM histamine was added to cells previously exposed to dimaprit (tracing B) and vice versa (tracing A). The lack of a stimulatory effect of dimaprit and its inhibition of histamine was verified in 4 experiments (see text for details). D: effect of the sequential addition of 1 mM dimaprit followed by 100 µM histamine was assessed in the presence (tracing A) and absence (tracing B) of 1 mM cimetidine. The lack of an effect of cimetidine on the subsequent inhibition of histamine-stimulated I$_{sc}$ increase by dimaprit was verified in 3 experiments. A smaller scale is shown on the ordinate, corresponding to the smaller I$_{sc}$ observed in this particular series of experiment.
role for the Ca\(^{2+}\)–activated Cl\(^{-}\) channel. In control experiments, BAPTA has no effect on cAMP-activated \(^{125}\)I efflux mediated by CFTR and stimulated with forskolin (data not shown).

In PDEC, the function of Cl\(^{-}\) channels may be coupled to the Cl\(^{-}\)–bicarbonate exchanger; a potential effect of DIDS on this exchanger may indirectly affect \(^{125}\)I flow through Cl\(^{-}\) channels. However, such an indirect effect should affect both the CFTR and the Ca\(^{2+}\)–activated Cl\(^{-}\) channels; in our system, only the Ca\(^{2+}\)–activated, but not the cAMP-activated, \(^{125}\)I efflux is inhibited by DIDS (14). An indirect effect of DIDS is therefore unlikely, even though it cannot be excluded; either way, DIDS only inhibited the \(^{125}\)I efflux mediated through Ca\(^{2+}\)–activated Cl\(^{-}\) channels.

We also recently observed the presence, on PDEC, of Ca\(^{2+}\)–activated K\(^{+}\) channels sensitive to charybdotoxin (14a). The increased [Ca\(^{2+}\)] stimulated by histamine would be expected to activate these K\(^{+}\) channels. Indeed, histamine stimulated a \(^{86}\)Rb\(^{+}\) efflux from PDEC that was sensitive to the K\(^{+}\) channel inhibitor charybdotoxin. Activation of K\(^{+}\) channels is also consistent with the report that histamine evokes release of K\(^{+}\) from mouse and guinea pig pancreatic segments (18).

The secretory effect of histamine occurs at concentrations as low as 10 µM and appears to be mediated through specific receptors. Of the three histamine receptor subtypes described, H\(_1\) receptors are coupled to phospholipase C and increased [Ca\(^{2+}\)], H\(_2\) are coupled to adenylate cyclase and increased cAMP, and H\(_3\) receptors mediate neuroendocrine regulation. The secretory effects of histamine on PDEC, dependent on increased [Ca\(^{2+}\)], are most likely mediated through H\(_1\) receptors. H\(_1\) receptor involvement is further supported by the inhibitory effect of the specific H\(_1\) antagonist, diphenhydramine [no specific H\(_1\) agonist has been identified (9)]. The stimulatory role of H\(_2\) receptors was excluded by the ineffectiveness of dimaprit, an H\(_2\) agonist, and the resistance to cimetidine, an H\(_2\) antagonist. On the other hand, it remains possible that PDEC express H\(_3\) receptors that do not participate in these effects of histamine and therefore were silent when assessed for these functions. H\(_3\) receptors are only expressed in the nervous system and on endocrine cells (7, 9, 22); they would not mediate secretion in epithelial cells.

In using chambers, dimaprit inhibits the subsequent secretory response to histamine, raising the possibility that H\(_2\) receptors may mediate an inhibitory action. This action would be consistent with the in vivo studies in which H\(_2\) agonists and antagonists, respectively, inhibited and stimulated pancreatic flow and enzyme output in rabbits (17). However, the inhibitory action of dimaprit was resistant to cimetidine, suggesting that it was not mediated through H\(_2\) receptors. Considering the high concentration of dimaprit used relative to histamine (1 vs. 0.1 nM), it is possible that dimaprit may cross-react with the H\(_1\) receptor as an antagonist and inhibit histamine action in this manner.

Histamine acts mainly in a paracrine or neuroendocrine mode, and the secretory effects described in this report are only relevant if histamine is present in the pancreas. The presence and distribution of histamine have been determined fluorometrically and biologically in tissues of different species. The concentrations of histamine in human and canine pancreas are, respectively, 4.1 and 11.4 µg/g fresh weight. These concentrations are higher than the concentration of 1 µg/g found in fat, mesentery, or muscle; the highest concentration, 19 µg/g, is found in the body of the stomach (12). In addition to neurons and endocrine cells, mast cells are also a major source of histamine; these cells are present in guinea pig pancreas (20) and in both normal and inflamed human pancreas (T. D. Nguyen and M. P. Bronner, unpublished data). Histamine may also play a role in the inflammatory response of pancreatitis; elevated levels of blood and pancreatic histamine were observed in a rat model of pancreatic inflammation from chronic dietary magnesium deficiency (2). Histamine is therefore available for interaction with pancreatic duct cells.

In summary, we have shown that histamine interacts with basolaterally located H\(_1\) receptors on PDEC to increase [Ca\(^{2+}\)] and activate Cl\(^{-}\) and K\(^{+}\) channels. This effect may be relevant to the regulation of pancreatic ductal secretion and the pathological manifestations of pancreatitis.

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