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# Simultaneous Detection of pH Changes and Histamine Release from Oxyntic Glands in Isolated Stomach

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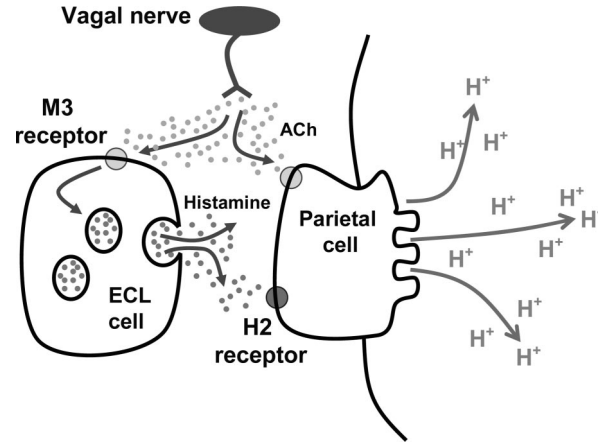
**Real-time simultaneous detection of changes in pH and levels of histamine over the oxyntic glands of guinea pig stomach have been investigated. An iridium oxide pH microelectrode was used in a potentiometric mode to record the pH decrease associated with acid secretion when the sensor approached the isolated tissue. A boron-doped diamond (BDD) microelectrode was used in an amperometric mode to detect histamine when the electrode was placed over the tissue. Both sensors provided stable and reproducible responses that were qualitatively consistent with the signaling mechanism for acid secretion at the stomach. Simultaneous measurements in the presence of pharmacological treatments produced significant variations in the signals obtained by both sensors. As the H2 receptor antagonist cimetidine was perfused to the tissue, histamine levels increased that produced an increase in the signal of the BDD electrode whereas the pH sensor recorded a decrease in acid secretion as expected. Addition of acetylcholine (ACh) stimulated additional acid secretion detected with the pH microelectrode whereas the BDD sensor recorded the histamine levels decreasing significantly. This result shows that the primary influence of ACh is directly on the parietal cell receptors rather then the ECL cell receptors of the oxyntic glands. These results highlight the power of this simultaneous detection technique in the monitoring and diagnosis of physiological significant signaling mechanisms and pathways.**

The role of transmission on gastric acid release from the oxyntic (parietal) glands within the stomach has been well studied. The majority of these investigations focus on the enterochromaffin-like (ECL) cell and parietal cell axis,[1] where the role of histamine and gastrin on acid secretion has been investigated. Luminal acid (HCl) is secreted by the oxyntic cells, which are located mainly in the middle third of the oxyntic gland.[1,2] The ECL cells, which are located only on the base of the oxyntic glands, are considered to be the primary source of histamine, containing at least 80% of mucosal and submucosal histamine with <20% being stored in mast cells.[3] Histamine released from the ECL cell is believed to reach the parietal cells via capillary transport[4] or by interstitial diffusion[5] sequentially triggering secretion of luminal acid.

**Scheme 1**




However, luminal acid can also be released by other pathways, of which one is a neuronal route utilizing acetylcholine (ACh) stimulation. Cholinergic neurons can directly or indirectly stimulate the muscarinic (M3) receptors of the oxyntic cells or ECL cell to increase acid secretion,[1−3] as shown in Scheme 1. There have been many claims on how this neuronal stimulation from the vagal nerves influences acid secretion.[3] Multiple sensing methods can provide the means of understanding the role of a secondary molecule.

To date, histamine levels in the stomach have been monitored with microdialysis and off-line separation-based methods that provide good selectivity but are limited in temporal resolution.[5,6] Histamine has been measured previously using boron-doped diamond (BDD) microelectrodes and produced stable responses with limited fouling for several hours.[7−9] We have shown that

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the BDD microelectrode has excellent stability, sensitivity, and reproducibility for the detection of neurotransmitters in complex biological matrixes.[9−11] Stable responses for the detection of serotonin and melatonin from the mucosa of isolated ileum and colon have also been reported.[12] These compounds, like histamine, are known to foul carbon-based electrodes and reduce the life span of the electrode,[7,9] preventing accurate long-term recordings. In this paper, we utilize BDD microelectrodes for the detection of histamine released from the ECL cell.

Acid secretion from the oxyntic glands of the stomach was monitored with a pH sensor. The typical glass membrane pH sensor, although used extensively for biomedical and physiological measurements, is mechanically fragile with the silicate glass membrane being susceptible to biofouling, which causes inaccurate pH measurements.[13] A more promising pH-sensing device based on a metal−metal oxide proton-sensitive film has been well documented.[14−17] In particular, iridium oxide electrodes have fast response times and show great potentiometric stability over a wide pH range. Anodic iridium oxide films have been used in biological systems to monitor extracellular myocardial acidosis during acute ischemia[18,19] and implanted in porcine intervertebral discs.[13]

In this paper, we describe the fabrication and application of two sensors that allow the rapid real-time monitoring of pH and histamine levels on guinea pig isolated stomach. The simultaneous measurement provides a means to understand the influence of the neuronal stimulation of acetylcholine on acid secretion through pharmacological treatments of the isolated tissue.

## EXPERIMENTAL SECTION

**Materials and Reagents.** All experiments were carried out in oxygenated (95% $O_2$ and 5% $CO_2$) Dulbecco modified Eagle's media (DMEM) high-glucose buffer solution, pH 7.4 (PAA Laboratories). Histamine, acetelycholine, and cimetidine were purchased from Sigma Aldrich, and 1 $\mu$M solutions were prepared fresh each day. Gold wire (99.99%, A-M Systems) with a diameter of 75 $\mu$m was used to fabricate the needle-type pH electrode. Iridium tetrachloride hydrate (99.9%), hydrogen peroxide solution (30% w/w), oxalic acid dihydrate, and anhydrous potassium carbonate used to make the $IrO_2$ deposition solution were all purchased from Sigma-Aldrich and used as received. Britton-Robinson (BR) buffer (0.1 M boric acid, 0.1 M acetic acid, 0.1 M othophosphoric acid) was used to test the pH sensor.

**pH Sensor Fabrication and Characterization.** A needle-type Au microelectrode was fabricated in-house and then modified with an iridium oxide film. Initially, a 75-$\mu$m gold wire insulated in Teflon (overall diameter 140 $\mu$m, A-M Systems Inc.) was threaded through a 27-gauge hypodermic needle. A copper or silver wire was attached to the gold wire with silver epoxy resin to form an electrical contact. Epoxy resin (Robnor Resins, CY1301 and HY1300) was used to fill the internal volume of the needle and left for 2 days to cure according to the manufacturer's instructions. The lower end of the needle was cut perpendicularly using a diamond saw (Buehler) to expose the 75-$\mu$m Au disk microelectrode. Successive polishing with aqueous slurries of 1-, 0.3-, and 0.05-$\mu$m alumina in deionized water with rinsing and sonication at each polishing stage was necessary to ensure a flat electrode surface. Cyclic voltammetry (CV) in 0.5 M $H_2SO_4$ was used to electrochemically clean the gold electrode surface prior to deposition. Using the same technique, the transport-limited currents were recorded in 1−10 mM $Ru(NH_3)_6^{3+}$ in supporting electrolyte to assess the surface of the gold electrodes. When the recorded limiting current had reached the theoretical value for a 75-$\mu$m-diameter microelectrode in a known concentration of analyte, the electrode was considered ready for use.

Anodic electrodeposition of the iridium oxide film onto gold microelectrodes was performed using a deposition solution described elsewhere by Yamanaka.[20,21] Briefly, 0.15 g of iridium tetrachloride, 1 mL of 30% w/w $H_2O_2$, and 0.5 g of oxalic acid dihydrate were added gradually in a 100 mL of water at 0.5-h intervals and left to dissolve in a stirred solution. Anhydrous potassium carbonate was then added gradually to the solution until the pH reached ~10.5 forming a pale yellow solution. The solution was then covered and left at room temperature for 48 h to stabilize until a color change to pale blue was achieved. This blue solution was stored in the refrigerator and was used for a few months to successfully produce $IrO_2$ films.

The anodic electrodeposition of the $IrO_2$ films on gold microelectrodes was achieved amperometrically using a constant-potential method. A potential between 0.6 and 0.7 V versus double junction reference electrode (DJRE) was applied for several minutes to produce a thin, uniform, and defect-free film. The DJRE with a calomel inner junction reference system was used with a sodium sulfate (0.1 M) outer junction solution to prevent chloride ion permeation.[13] The deposition reaction responsible for the iridium oxide formation on the electrode is given below (eq 1), where the oxalate ligand is oxidized to form $CO_2$ leading to the deposition of a hydrated form of iridium oxide.

$$[Ir(COO)_2(OH)_4]^{2-}_{aq} + 2OH^- \Rightarrow [IrO_2(OH)_2 \cdot 2H_2O]_s + 2CO_2 + 2e^- \quad (1)$$

Assuming 100% faradic efficiency of electrodeposition, the total amount of iridium oxide was calculated to be 0.006 $\mu$g when the deposition potential, $E_d$, of 0.65 V was applied for 2 min (current density 0.83 mA cm$^{-2}$). The coated microelectrodes were washed and placed in deionized water for at least 2 days prior to use to


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hydrate the film and stabilize the potential reading. The pH sensors were stored in deionized water between uses. The pH sensor was initially calibrated in BR buffer by measuring the open-circuit potential between the pH electrode and a standard reference electrode (SCE or Ag|AgCl). Upon acid/base additions (HCl/NaOH), the potential of the electrode changes according to the negative logarithm of the proton ($H^+$) concentration. Similarly, the pH sensor was calibrated in DMEM buffer prior to in vitro measurements. After in vitro use the pH sensor was placed in 0.5 M $H_2SO_4$ and scanned from 0 to 1 V versus SCE at 100 mV $s^{-1}$ to observe whether any changes in the current response were observed following biological measurements.

**BDD Microelectrode Fabrication and Characterization.** BDD thin film was deposited on a 40-$\mu$m-diameter Pt wire (99.99%, Aldrich Chemical) by microwave-assisted chemical vapor deposition (1.5 kW, 2.54 GHz, ASTeX, Woburn, MA), as detailed previously.[7,9,12,23,24] The diamond-coated Pt wire was affixed to a longer copper wire using conductive Ag epoxy, and the entire assembly was insulated with polypropylene from a pipet tip. The insulation was applied by inserting the microelectrode into a pipet tip with ~500 $\mu$m exposed from the end and carefully heating the tapered end using the coil of a micropipet puller. This softened the polypropylene and caused it to conformally coat the rough, polycrystalline diamond surface.[7,9,23,24] The resulting microelectrode was cylindrical with a diameter of ~40 $\mu$m. The length of the exposed electrode was 100−200 $\mu$m. The electrode can be reproducibly insulated with a thin and continuous polymer film using this method, but precise control of the exposed electrode length is difficult to achieve.

Differential pulse voltammograms (DPV) of 50 $\mu$M histamine were carried out to identify the potential where histamine was oxidized. Measurements were obtained in the potential range from 0 to 1.4 V versus Ag|AgCl, using a pulse height of 50 mV and a pulse step of 50 s. Amperometric measurements were carried out using flow injection analysis (FIA) to calibrate for histamine. An in-house flow cell was produced, using silicone elastomer, as described previously.[25] Measurements were carried out using a stainless steel counter electrode and a commercial "no leak" Ag|AgCl (3 M KCl, model EE009, Cypress Systems Inc.) reference electrode. The flow cell was connected to a HPLC pump (HP1050, Aligent), where the flow rate was set at 2 mL $min^{-1}$. For calibration measurements, 10-$\mu$L injections of 1 − 40 $\mu$M concentrations of histamine were analyzed. Electrochemical measurements were carried out using amperometric detection at +1.2 V versus Ag|AgCl. All measurements were carried out in DMEM buffer pH 7.4.

**Biological Preparation.** All animal experiments were carried out in compliance with the relevant laws and institution guidelines. Male guinea pigs weighing 300−400 g were euthanized using $CO_2$ gas. The stomach was isolated and placed in oxygenated (95% $O_2$ and 5% $CO_2$) Krebs' buffer solution, pH 7.4 (composition in mM: 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 $NaH_2PO_4$, 25 $NaHCO_3$, and 11 glucose) prior to sample preparation. A segment of stomach tissue was then cut along the mesenteric border, lightly stretched, and pinned flat in the recording bath using stainless steel pins (50-$\mu$m diameter). The mucosal surface was face-up at the bottom of the chamber. This tissue was constantly perfused with warm (37 °C) oxygenated (95% $O_2$ and 5% $CO_2$) DMEM buffer. The tissue was exposed to the flowing solution for ~30 min prior to measurements.

**Biological Experiments.** For the continuous amperometric recording of histamine overflow in vitro, a three-electrode system was also used. A Pt wire counter and a commercial "no leak" Ag|AgCl (3 M KCl, model EE009, Cypress Systems Inc.) reference electrode were also mounted in the bath with the BDD microelectrode to complete the electrochemical circuit. For the potentiometric recording of pH, a two-electrode system was used, where a Ag|AgCl reference was placed into the flow bath. Both the amperometric and potentiometric modes were controlled using a Biostat potentiostat (ESA Biosciences). The Teflon recording bath was about 6.5 cm wide, 6.5 cm long, and 0.4 cm deep and was lined with a silicone elastomer (Sylgard 184, Dow Corning). The bath was mounted on the stage of an inverted microscope (model 3030, Accu-Scope) and superfused continuously with warm (37 °C) DMEM buffer solution at a flow rate of 2 mL $min^{-1}$. The solution temperature was controlled with an immersion heating circulator (model 1130A, VWR Scientific) and the solution flow was controlled with a peristaltic pump (Masterflex, Cole Parmer).

The BDD microelectrode was mounted on one micromanipulator (model 25033, Fine Scientific Tools) and the pH sensor affixed to another for reproducible placement near the mucosa. The tissue sample was positioned in the center of the flow bath. For in vitro measurements, the electrodes were placed within the bulk of the perfusing media (at least 1 cm from the tissue) to establish a stable baseline response on both electrodes. During recordings, the BDD and pH electrodes were carefully moved to 0.5 mm over the tissue for 40 s and then removed back into the bulk of the media. This measurement was made over four or five regions of each tissue section to gain an understanding of variation within a tissue section. The differences in the current ($\Delta i_H$) and pH ($\Delta$pH) between the perfusing media and immediate vicinity of the tissue were recorded and assessed.

Measurements were then conducted on the same tissue sample to assess the influence of the H2 receptor antagonist cimetidine (1 $\mu$M) and sequentially 1 $\mu$M acetylcholine in DMEM buffer. The pH electrode response to 1 $\mu$M histamine was evaluated to assess interference. The tissue was sequentially perfused continuously with the following treatments. Recordings were commenced after the tissue was perfused with the specific treatment for 30 min.

**Data Analysis.** The mean changes in current and pH from baseline to over tissue measurements were measured and reported as $\Delta i_H$ and $\Delta$pH, respectively. A schematic of the responses is shown in Figure 1, where the gray box indicates the area where measurements over the tissue were obtained. The results with various treatments were compared using Student's *t*-test.

## RESULTS AND DISCUSSION

**Measurement of Histamine on the BDD Microelectrode.** DPV of histamine was recorded on the BDD microelectrodes to

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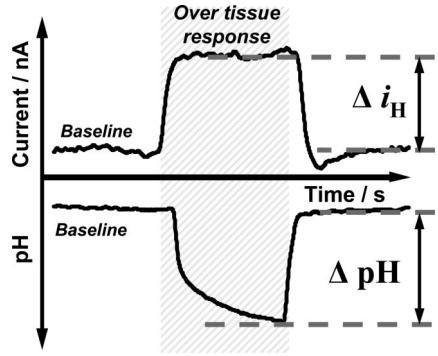


**Figure 1.** Schematic showing example of the traces and data analyzed. The current and pH traces are shown, where the gray-shaded box indicated the region where measurements over the tissue are carried out and the measured parameters, $\Delta i_H$ and $\Delta$pH, are indicated.

obtain the oxidation potential of histamine. Figure 2A shows the response of 50 $\mu$M histamine and the background response of DMEM media on the BDD microelectrode. Histamine is oxidized on the BDD microelectrode at 1.2 V versus Ag|AgCl.

Figure 2C shows a FIA calibration for histamine on the BDD electrode, where the detection potential was set at 1.2 V (vs Ag|AgCl). Stable responses are observed from 1 to 40 $\mu$M, and there is good reproducibility (less than 5% error at lower concentrations) between the three replicates carried out for each concentration was observed. The calibration graph from these data is shown in Figure 2D, where the limit of detection is 2.76 $\mu$M and the sensitivity is 0.25 nA $\mu M^{-1}$ on the BDD electrode. The stability and reproducibly of the response are similar to that observed by Sarada et al., where measurements of histamine and serotonin on BDD thin-film electrodes showed good reproducibility and dynamic range with no fouling or deactivation of the electrode for several hours.[26] The BDD microelectrodes have previously been shown to provide stable and reproducible measurements of serotonin from isolated ileum tissue with minimal signal attenuation.[9,11,12] These reasons make the BDD microelectrode ideal for histamine measurements from the isolated stomach preparation.

**$IrO_2$ Electrode Response to Solution pH.** Iridium oxide electrodes are known to exhibit a super-Nernstian response of $\sim -80$ mV per pH unit depending on preparation compared with glass pH electrode, where the typical Nernstian behavior of $-59$ mV/pH unit is achieved. The complex equilibrium interfacial reaction that accounts for the super-Nernstian behavior is shown in eq 2:[16]

$$2[IrO_2(OH)_{2-x}(2+x)H_2O]^{(2-x)-} + (3-2x)H^+ + 2e^- \Leftrightarrow [Ir_2O_3(OH)_3 \cdot 3H_2O] + 3H_2O \quad (2)$$

where $0.12 < x < 0.25$ to produce pH-sensitive films with slopes from $-73$ to $-81$ mV/pH unit. Equation 2 accounts for the variability between sensors that are due to the variation in hydration levels of iridium oxide films.

Figure 3A shows cyclic voltammograms recorded in BR buffer before and after the in vitro experiments. There are only modest changes in the appearance of the CV, indicating that the electrode reaction is essentially unchanged. Initial pH calibrations were made in BR buffer by additions of acid or base to a stirred solution. Typical potential−time responses are shown in Figure 3B. Response times (5−95%) were <2 s for these additions and the electrode potential was stable.

Calibration plots, shown in Figure 3C, of the electrode potential versus the pH of the solution were obtained at frequent intervals over a period of 50 days to assess the effective lifetime of the sensor. There was no significant change ($p < 0.001$) in the slope

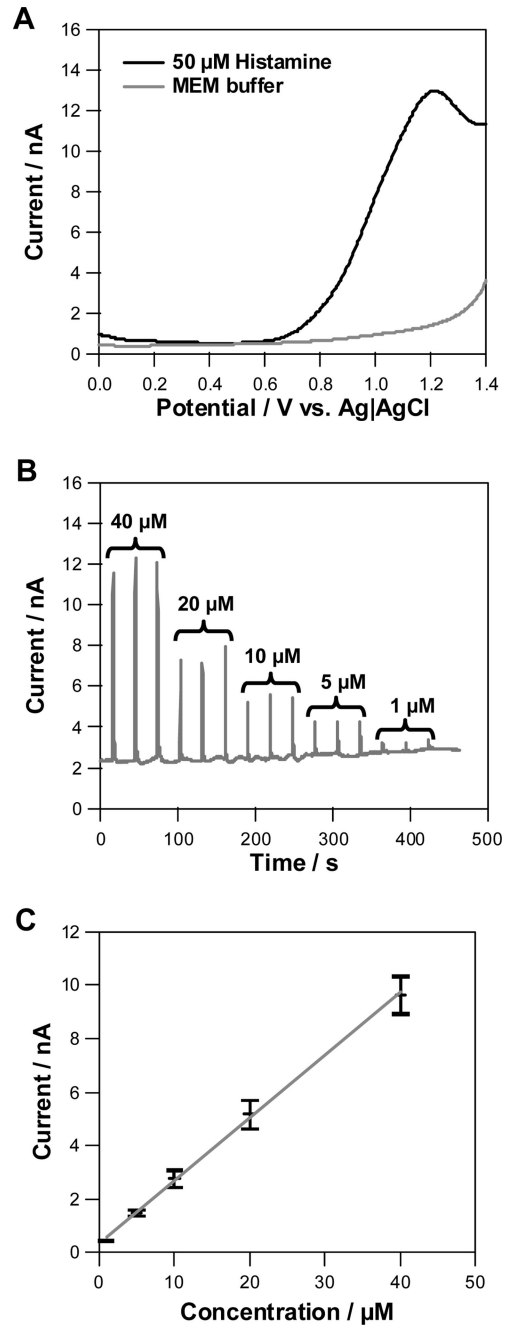


**Figure 2.** Assessment of the BDD electrode for detection of histamine. (A) shows a differential pulse voltammogram of histamine in DMEM buffer. (B) shows a FIA trace obtained with various concentrations of histamine, where three repeated measurements were obtained and the calibration curve obtained from these data are shown in (C). All measurements were conducted using a 40 $\mu$M boron-doped diamond microelectrode.

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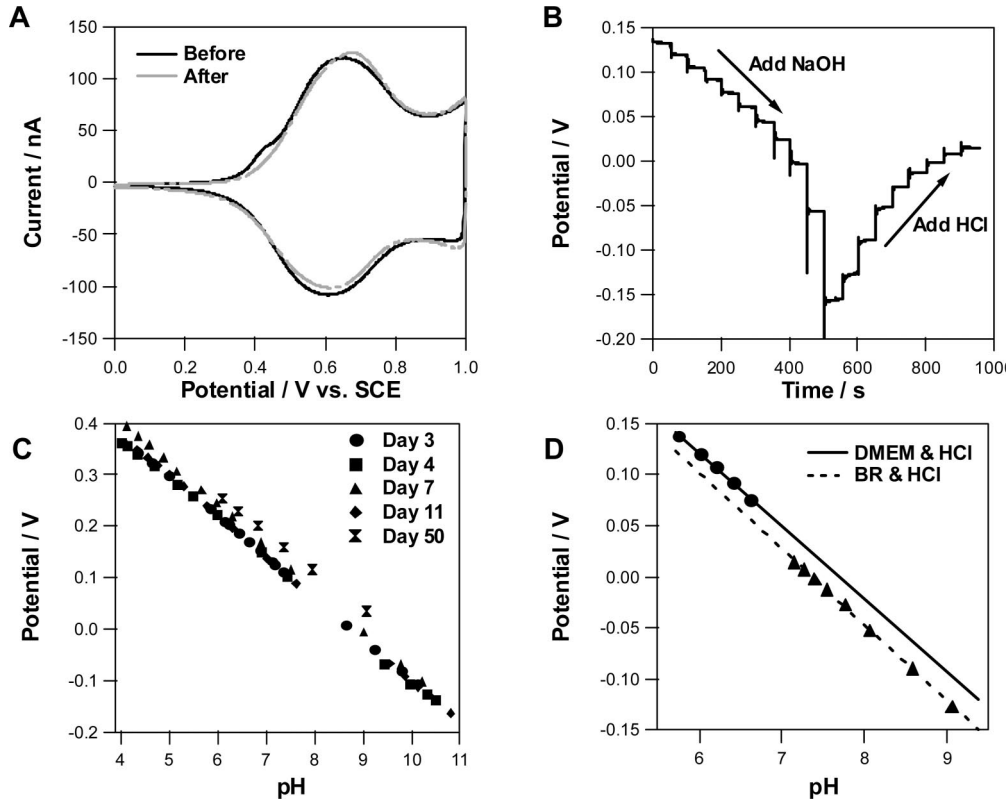


**Figure 3.** Characterization of the pH sensor. (A) Cyclic voltammogram of the anodic electrodeposited iridium oxide film on a 75-$\mu$m Au electrode in 0.5 M $H_2SO_4$ vs SCE at 100 mV $s^{-1}$ ($E_d$ = 0.65V, for 2 min) before and after tissue experiment. (B) Typical open-circuit potential response (vs SCE) of a 75-$\mu$m $IrO_2$ pH sensor produced by consecutive additions of NaOH and HCl in BR buffer. (C) Typical calibration plot (potential vs pH) of an $IrO_2$ electrode in BR buffer for wide pH range from 4 to 11 over a period of 50 days. (D) Calibration plot in the physiological pH range 6−9 of a 75-$\mu$m $IrO_2$ pH sensor in BR and DMEM media when aliquots of HCl were added.

of the calibration curve over 50 days with repeated use and storage in deionized water. The extrapolated pH = 0 voltage intercept gives $E_{IrO_x,H^+}$ of the electrode reaction. This also remained unaffected over 50 days indicating that the potential-controlling electrode reaction was unchanged.

Tissue experiments were conducted using DMEM media; thus, the iridium oxide pH electrode was assessed using this buffer. A calibration plot using the same 75-$\mu$m pH sensor is shown in Figure 3D comparing the straight line fits between a calibration in BR and DMEM buffer when HCl is added. The value of the intercept (pH 0) produced in both media is 0.54 V and indicates that there are no interfering ions in the DMEM buffer that affect the electrode reaction. The slope obtained in the two media varies slightly from 74.4 ± 0.0008 mV/pH for the BR buffer to 71.2 ± 0.002 mV/pH for the DMEM media when HCl was added, which suggests a small decrease of the electrode sensitivity to pH shifts in the DMEM buffer. Furthermore, the effect of dissolved oxygen was investigated and no interfering effect was observed.

Figure 4 shows a potential versus time plot recorded (vs SCE) in oxygenated DMEM buffer (pH 7.4) when histamine, cimetidine, and acetylcholine were added in concentrations ranging from 1 to 5 $\mu$M. During the additions, some potential drift (~3 mV/min; maximum error of ±0.04 pH unit/min) is recorded possibly due to noncontinuous buffer oxygenation. Although the added quantities exceeded physiological concentrations, none of these additions caused any significant change in the electrode potential.

**In Vitro Characterization.** For conducting in vitro measurements over the tissue, both sensors were initially placed far away

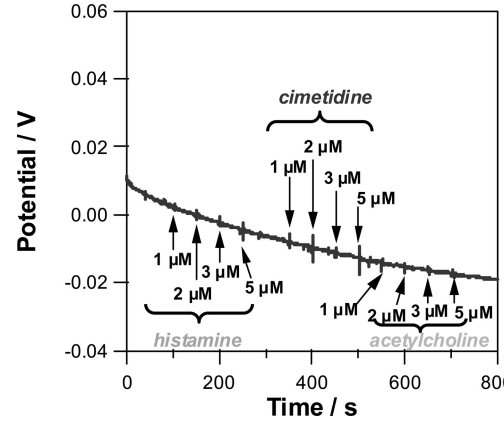


**Figure 4.** Potential versus time plot using an $IrO_2$ pH sensor in DMEM buffer while adding histamine, cimetidine, and acetylcholine to achieve solution concentrations 1, 2, 3, and 5 $\mu$M each.

from the tissue surface in the bulk of the media to obtain background measurements where responses from the tissue would have negligible effect. At a fixed period of time, both electrodes were brought to a position ~0.5 mm over the tissue using a micromanuipulator, where measurements were carried out, which are indicated by the gray boxes in Figure 5. Following this time, both electrodes were then moved away again into the bulk of the media to establish the baseline. This protocol was repeated at least five times over each tissue section. The change in current ($\Delta i_H$) on the BDD electrode and the change in pH (ΔpH) on moving the electrodes from the background to the

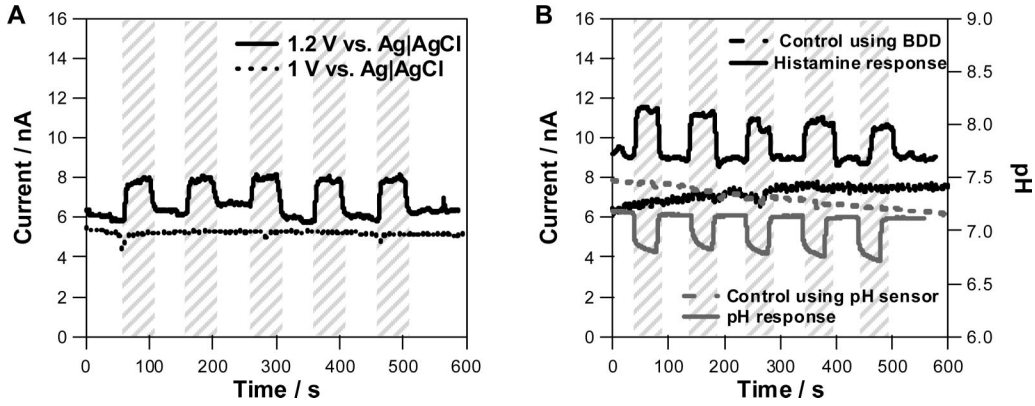


**Figure 5.** Experimental controls conducted for biological in vitro measurements. (A) shows repeated response over the tissue at a potential of 1.2 V, which is sufficient to oxidize histamine and at 1 V, which shows if any of the signal is due to catecholamines and serotonin. (B) shows the response obtained from the BDD and $IrO_2$ pH sensor with and without the tissue, showing that the responses obtained are from the isolated stomach tissue. The shaded gray box indicates the duration when the sensor is located within 1 mm over the tissue.

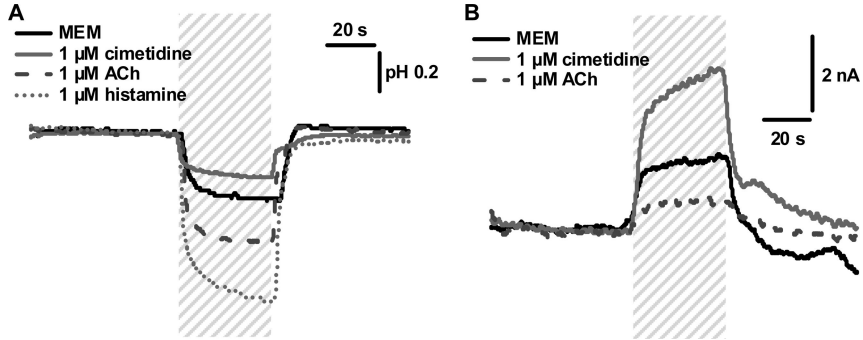


**Figure 6.** Experimental trace showing the influence of various pharmacological treatments. The response obtained from the iridium oxide pH sensor is shown in (A), and in (B), the response from the BDD microelectrode is shown. The shaded gray box indicates the duration when the sensor is located within 1 mm over the tissue.

tissue surface were assessed. Repeated measurements were conducted from isolated stomach tissue, where the BDD electrode over tissue response had a $\Delta i_H$ of 1.94 ± 0.41 nA ($n$ = 31). For the pH electrode, we observed ΔpH of −0.33 ± 0.12 ($n$ = 35). Responses obtained on both electrodes were unaffected by exposure to the tissue.

During calibration experiments, a potential of 1.2 V versus Ag|AgCl was required for the oxidation of histamine on the BDD electrode; however, during amperometric measurements at this potential, other electroactive biological molecules could also be detected such as serotonin and dopamine. As amperometric detection offers little selectivity, measurements were carried out over the tissue at a potential sufficient for the oxidation of histamine and also at 1 V, which would be able to show the response of any other interferences from the tissue. Figure 5A, shows that when the potential is set at 1 V versus Ag|AgCl and the sensor is placed over the tissue, no response is observed, indicating that the signal measured is solely due to histamine. Other neurotransmitters are known to be present within the stomach, such as serotonin from mast cells[27,28] and nitric oxide from neurons within the myenteric plexus.[29,30] These transmitters if present are below the detectable limit (≥0.41 $\mu$M for 5-HT and ≥46 nM for NO on the BDD electrode[12,30]), and studies on single isolated mast cells have shown that very small quantities of serotonin and histamine are present within these cells.[27,28,31] The histamine detected on the BDD microelectrode is most likely to be solely from the ECL cell.

A typical experimental trace from the use of the two sensors is shown in Figure 5B, where $\Delta i_H$ and ΔpH were observed during measurements in the presence of the tissue. The measurements of histamine oxidation on the BDD electrode had no influence on the measurements carried out on the pH electrode. A control experiment was also carried out (Figure 5B), where measurements were carried out using the same experimental protocol without the presence of the tissue. In this case, the electrodes were brought close together to a fixed distance over the Sylgard polymer base within the flow cell. Such measurements showed no artifacts or deviations within the current or pH trace on the two sensors.

**In Vitro Measurements from Isolated Stomach Tissue.** Figure 6 shows the responses of pH and histamine when the tissue is perfused with cimetidine (H2 receptor antagonist), acetylcholine, and histamine. One response is shown during measurements over the tissue, when the flow bath is perfused with different pharmacological treatments. The pH response on the iridium

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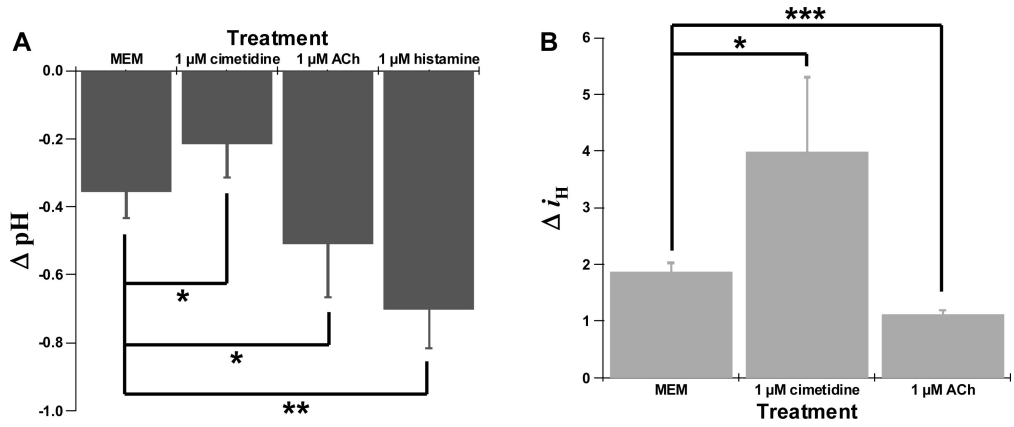


**Figure 7.** Influence of pharmacological agents on the biological signals, where (A) shows the response obtained from the pH sensor and (B) shows the current amplitude obtained from the BDD microelectrode. Data presented as mean ± st dev, $n = 5$, *$p < 0.01$, **$p < 0.01$, and ***$p < 0.001$.

oxide electrode is shown in Figure 6A, and the histamine response on the BDD microelectrode is shown in Figure 6B. The electrodes are initially held in the bulk of the perfusate to obtain a background response and then brought over the tissue. In the absence of any pharmacological intervention, we observed a ΔpH of −0.35 ± 0.07. The small change in pH is due to the sensor location some distance away from the site of release. Also, acid secreted from the parietial cells is buffered by bicarbonate located in the mucosal layer and from the buffer within the flow bath. Measurement in DMEM buffer gave a $\Delta i_H$ of 1.87 ± 0.16 nA, which corresponds to ∼7.5 ± 0.7 $\mu$M from the calibration curve shown in Figure 2D.

When cimetidine, a H2 receptor antagonist, is added to the physiological buffer, there is a significant decrease in ΔpH when compared to the control, which is coupled with a 2-fold increase in $\Delta i_H$. On the pH sensor, a ΔpH of −0.21 ± 0.1 ($n = 5$, $p < 0.05$) was observed and $\Delta i_H$ was 3.98 ± 1.32 nA (∼15.2 + 5.4 $\mu$M; $n = 4$, $p < 0.05$). The decrease in ΔpH with respect to the control is as expected; however, the increase in the level of $\Delta i_H$ is surprising as no significant change in the histamine levels was expected in comparison to the control response, as others have observed.[32,33] However, certain H2 antagonists, including cimetidine, may have the potential to alter the secretory activity of histamine decarboxylase.[34] Huszti et al. showed that 10 mg/kg cimetidine enhanced serum levels of newly formed histamine in the rat stomach by 57%.[35] The result obtained provided no complete understanding on how histamine levels are influenced by the use of H2 antagonists.

When histamine was added to the flow bath, ΔpH increased by 2-fold (ΔpH −0.70 ± 0.11, $n = 5$, $p < 0.01$) when compared to the control. The histamine-induced pH change was the greatest observed of all the pharmacological treatments investigated, which suggests that histamine plays the key role in influencing gastric acid secretion, consistent with other investigations.[5,36]

Acetylcholine is also known to increase gastric acid secretion via a neuronal mechanism. When acetylcholine was added, the ΔpH was −0.51 ± 0.16 ($n = 5$, $p < 0.01$). This decrease in pH is greater than that observed during the measurements in the absence of pharmacological treatments, but significantly smaller than that observed in the presence of histamine. The $\Delta i_H$ decreased to 1.12 ± 0.08 nA (∼4.5 ± 0.3 $\mu$M) on the BDD microelectrode ($n = 4$, $p < 0.001$), which indicates a lower histamine concentration during this treatment.

The summary data from the traces shown in Figure 6 are in Figure 7. The responses provide important information about the influence of histamine and acetylcholine on acid secretion. Acid secretion is well-known to be regulated primarily from histamine released from the ECL cell; however, there are two other chemical mechanisms that can influence acid secretion. Neuronal innervation of the gastric acid release mechanism is via the vagal nerve and from the enteric nervous system, where the neurotransmitter acetylcholine is released to influence acid secretion. The results shown in Figure 7 indicate that the acetylcholine influences muscarinic receptors on the parietal cell via a direct mechanism, rather than through the ECL cell, as previously postulated.[1,6] The use of the multiple sensing during this study has been the ideal means of obtaining such mechanistic information and could be the only means of understanding how acetylcholine influences acid secretion. The future application of such dual sensing methods will allow for greater understanding of secondary molecules in complex biological systems or where there are multiple biological targets.

## CONCLUSION

We have used two novel sensors to illustrate physiologically significant aspects of the signaling mechanism that influences acid secretion: BDD microelectrodes were used to detect histamine released from ECL cell, and a miniature iridium oxide pH sensor was used to study pH changes during acid secretion. Both electrodes demonstrated extremely stable responses for a long duration and excellent sensitivity. The pH sensor suffered no interference from high concentrations of all pharmacological treatments utilized within this study.

For biological measurements, stable and reproducible measurements were obtained when the sensors were placed over the tissue surface. No interference from serotonin or other catecholamines on the BDD microelectrode was observed during measurements. No cross talk was observed between the two sensors

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during measurements and no responses were observed without the presence of the tissue.

Simultaneous measurements were conducted during pharmacological treatments, where cimetidine showed the expected trend of increased histamine overflow and decreased the acid secretion, while histamine elevated the pH change, which indicated that the simultaneous measurements conducted were physiological relevant. The addition of acetylcholine caused a greater change in the pH level in comparison to the control; however, histamine overflow decreased, indicating that acetylcholine influences acid secretion via a direct mechanism through receptors on the parietal cell. The application of multiple sensors in this paper provided new understanding of the mechanism of acid secretion and demonstrated their utility in elucidating complex biological signaling pathways.

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