Supporting Information

Electrochemical Modulation of Carbon Monoxide-Mediated Cell Signaling

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Methods

Electrode preparation

Disc shaped electrodes with a diameter of 12 mm were punched from carbon paper (Toray, TGP-H-060, Fuel Cell Earth LLC) and inserted into a preheated tube furnace at 800 °C in static air for 10 min to generate OxCP. Catalyst ink was prepared by dispersing 5 mg of cobalt(II) phthalocyanine (CoPc, Strem Chemicals, Lot 24745700) in a mixture of 50 µL of 5 wt.% Nafion solution (Nafion 117 containing dispersion, Sigma Aldrich) and 5 mL N,N-dimethylformamide (DMF, Sigma Aldrich, 99.8%) with 15 min of sonication. The CoPc cathode was then prepared by dropcasting 20 µL of freshly sonicated ink onto the center of the OxCP. The droplet instantaneously spread throughout the entire carbon paper without penetrating onto the underlying aluminium foil. The electrode was then oven-dried in air at 80 °C for 10 min. This process was repeated two more times so that a total of 60 µL of the CoPc ink was deposited on each electrode. Fresh ink was prepared every time a new batch of electrodes was made, and electrodes were used within 48 h of preparation due to some observed decrease in activity upon using older inks or electrodes.

Electrochemical testing

Electrochemical measurements were conducted in a previously reported[1], customized three-compartment cell consisting of a counter electrode compartment, working electrode compartment, and gas compartment. The working electrode compartment was separated from the counter electrode compartment by an activated Nafion membrane (Nafion117, Fuel Cell Store). The counter and reference electrodes were platinum foil (99.9% metals basis, Alfa Aesar) and Ag/AgCl leak-free reference (LF-2, Innovative Instrument Inc.), respectively. The leak-free reference was calibrated every day before use against a saturated calomel electrode, (CHI150, CH Instruments) in a saturated potassium chloride solution (The potential at the SCE was assumed to be, according the manufacturer specification, +0.241 V vs SHE at 25 °C). CoPc/OxCP electrode was placed between the working electrode compartment and gas compartment.

Prior to experiments, 1.75 mL of CO2-saturated Tyrode’s solution (125 mM NaCl, 2 mM KCl, 2 mM MgCl2, 2 mM CaCl2, 25 mM HEPES and 51 mM D-glucose, pH adjusted to 7.4 with NaOH or HCl) was added into the working electrode compartment and counter electrode compartment, respectively. High-purity CO2 gas (Airgas, 99.999%, 10 sccm) was controlled by an
Alicat mass flow controller and introduced into the cell at atmospheric pressure; CO₂ gas entered the cell through the gas compartment, traversed the working electrode, and exited through the working electrode compartment and flowed directly to an on-line gas chromatograph (SRI Instruments, Inc., MG #5, Model 8610C). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and flame ionization detector (FID), a methanizer, and a Hayesep-D column. Notably, the high CO concentration in the outlet gas stream led to some imprecision in quantifying CO using the FID of the gas chromatograph, since the calibration curve used encompassed 10-1000 ppm whereas the exiting CO concentrations were at times up to 3000 ppm. At high CO concentrations, the FID may underestimate the true gas outlet concentration.

The electrochemical measurements were controlled with a VMP3 Multi-channel potentiostat. Resistance between the reference and working electrodes was measured with Potential Electrochemical Impedance Spectroscopy (PEIS) and automatically compensated by 85%. The remaining 15% was not manually compensated. For all experiments, the reaction was run for a total of 20 min, with gas products analyzed by an on-line gas chromatograph (SRI Instruments) every 5 min. The rates reported are calculated from average of the products detected at the 10, 15, and 20 min marks. Data collection began after 10 min to ensure that the sampled gas headspace composition reflected steady state conditions.

**HEK cell culture and transfection**

HEK 293FT cell was a gift from Feng Zhang (MIT), and the cell line was negative for mycoplasma contamination, as inspected by microscopic evaluations. HEK cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) with 10 % fetal bovine serum (FBS). Cells were seeded onto either glass coverslips (round, 5 mm or 12 mm in diameter, German Glass, Electron Microscopy) or well plates, which were pre-treated with Matrigel solution for at least 2 h. For transfection of α- and β-subunits of human sGC, 2 µL of Lipofectamine 2000 (Thermo Fisher Scientific) and 500 ng of each DNA (1000 ng in total) in 50 µL of Opti-MEM were added to each 24 well plate. *CMV-GUCY1A3-DDK* (Origene, NM000586) and *CMV-GUCY1B1-DDK* (Origene, NM000857) were utilized for transfection. After 48 h, cGMP measurements and immunohistochemical staining were performed.
Immunohistochemistry

Transfected cells seed on glass coverslips were fixed in 4% paraformaldehyde (PFA) in PBS for 20 min. The fixed cells were washed with PBS three times, and then the cells were permeabilized with 0.3 % (v/v) Triton X-100 in PBS for 10 min. After removing Triton X-100 solution, cells were blocked with 2.5 % goat serum in PBS overnight at 4°C. Cells were then incubated with primary antibody solution (200× dilution, rabbit-anti DDK (Origene, TA100023), 2.5% goat serum in PBS) for 3 h at room temperature on an orbital shaker. After three washes with PBS, cells were then incubated with secondary antibody solution (500 × dilution, goat anti-rabbit labelled with Alexa Fluor 488 (Invitrogen, A32731) in PBS) for 2 h at room temperature on an orbital shaker. Following three washes with PBS, the cells were transferred to the slide glasses, and then covered with mounting solution containing DAPI (Vectashield mounting medium with DAPI, H-1200, Vector Laboratories). Fluorescent imaging was performed with a laser scanning confocal microscope (Fluoview FV1000, Olympus) with 20× objective (oil, 0.85 NA).

Biocompatibility assay

According to the manufacture’s protocol, the biocompatibility of CoPc was evaluated with alamarBlue metabolic assay (Thermo Fisher Scientific) in 96 well plates. On day 0, HEK cells were incubated with alamarBlue solution (1:10 DMEM with 10% FBS medium) for 3 h in a cell culture incubator. After 3 h of incubation, fluorescence intensity of the solution was measured with a plate reader and used as baseline for cell viability (excitation: 545 nm, emission: 590 nm). Next, 0.1 mg/mL or 0.01 mg/mL of CoPc containing DMEM with 10% FBS solutions were added to HEK cells. The almarblue assay was conducted after 1 day of incubation with CoPc-containing solutions at 37 °C. To check cytotoxic responses in cells following acute exposure to CO₂-saturated Tyrode’s solution (pH 7.4), cells were incubated in CO₂-saturated solution for 5 min or 15 min. The solution was then replaced with fresh DMEM with 10% FBS solutions, and the almarblue assay was conducted after 1 day.

cGMP quantification

For cGMP quantification, HEK cells were washed with pre-warmed Tyrode’s solution
three times. Cells were then pre-incubated with Tyrode’s solution containing 200 µM cGMP phosphodiesterase inhibitor, 3-isobutylmethylxanthine (IBMX), for 20 min. CO was generated by applying –1.3 V versus SHE to the CoPc/OxCP electrodes (2.5 × 2.5 cm²) for 10 min in CO₂-saturated Tyrode’s solution containing 200 µM IBMX. Here, pH of CO₂-saturated Tyrode’s solution was pre-adjusted to 7.4 with NaOH or HCl. Control experiments were investigated by applying –1.3 V versus SHE to the electrodes for 10 min in 200 µM IBMX containing Tyrode’s solution without saturated CO₂ or incubating cells in CO₂-saturated Tyrode’s solution (pH 7.4) containing 200 µM IBMX for 10 min in the absence of an applied voltage. To investigate the effects of CO on NO-sGC-cGMP signalling pathway, cells were incubated with 5 µM NORM (DEA NONOate) and 500 µM CORM-2 (or electrochemically produced CO at -1.3 V versus SHE for 10 min) for 15 min. After stimulation, the solutions were carefully removed, followed by extraction of cells with a 0.1 M HCl solution. cGMP levels in the cell lysates were quantified with the cGMP enzyme-linked immunosorbent assay (ELISA) kit (Cayman chemical, no.581021).

**Hippocampal neuron culture and calcium imaging**

All the experimental procedures involving primary cultures were approved by the MIT Committee on Animal Care. Hippocampi were carefully extracted from neonatal rat pups (P1). After a dissociation process with Papain (Worthington Biochemical), hippocampal neurons were plated onto glass coverslips (round, 5 mm in diameter, German Glass, Electron Microscopy), which were pre-treated with Matrigel solution. After 3 days, glia inhibition was performed utilizing a FUDR (5-fluoro-2'-deoxyuridine, F0503 Sigma Aldrich) solution. Neurons were maintained in Neurobasal A-medium (Gibco) supplemented with B-27 (Gibco) and GlutaMax (Gibco). Calcium imaging experiments of neurons were conducted on day 10-14.

For calcium imaging, cultured hippocampal neurons were pre-incubated in the culture medium containing 1 µM Ca²⁺ indicator Fluo-4 (AM, cell permeable, Invitrogen) for 30 min. Neurons were then placed in 24 well plate, which contained 500 µL Tyrode’s solution. Fluorescence changes in Fluo-4 were monitored with an inverted fluorescence microscope (frame rate: 1 s). After 30 s of recording, CORM-2 and RuCl₃ stock solution was injected to each well plate. For experiments involving ion channel blockers or inhibitors of receptors, the neurons were incubated with blockers (or inhibitor) solutions (20 µM Iberiotoxin for BKCa, 12 µM Nitrendipine
for L-type Ca^{2+} channel, 400 µM Ivabradine for HCN, 10 µM 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) for sGC for 30 min prior to the calcium imaging. For calcium imaging with electrochemical CO-delivery system, CoPc/OxCP (1.0 × 2.0 cm²), Pt wire, and Ag/AgCl reference electrode were utilized as cathode, anode, and reference electrodes, respectively. CO₂-saturated Tyrode’s solution (pH adjusted to 7.4 with NaOH or HCl) or Tyrode’s solution without saturated CO₂ were used as electrolyte. Neurons cultured on the coverslips were positioned in the immediate proximity of the CoPc/OxCP electrode, and voltages were turned on after 30 s of recording. Fluorescent intensity of each neuron was analysed with ImageJ and F₀ value was calculated by averaging its fluorescent values during the initial 10 s of recording. Averaged ∆F/F₀ values were obtained utilizing 100 neurons randomly selected from at least three independently performed experiments.

Preform fabrication and fiber drawing

The macroscopic polymeric preforms were fabricated using standard machining techniques. Specifically, two rectangular grooves (2 mm × 2 mm) were milled into a 25 cm long polycarbonate (PC) slab (10 mm × 4 mm) to define the electrode tracks. Another rectangular groove (4 mm × 6 mm) was machined into a second PC slab (6 mm × 10 mm) to define a microfluidic channel. These two PC layers were consolidated in a vacuum oven at 185 °C for 1 h. The as-assembled preform was drawn into 100 m-long microstructured fibers in a custom built fiber drawing tower[2]. Particularly, the preform was heated in a three-zone furnace in which the top, middle and bottom zone were set at 140, 285 and 110 °C respectively. The preform was fed into the furnace at a rate of 1 mm/minute and drawn at a speed of 1600 mm/minute, which resulted in a draw-down ratio of 40.

Fiber device assembly

To establish electrical interfacing, a self-assembled CNT yarn (50 µm diameter, Dexmat) and a Pt-microwire (50 µm diameter, Goodfellow) were introduced into the microstructured grooves on the fiber surface under a microscope. The CNT served as a working electrode while Pt was the counter electrode in the final device. The as-assembled wires were connected to I/O electrical pins (Millmax) followed by addition of an insulation layer of UV-curable epoxy. For
interfacing with the microfluidic channel, the fiber was inserted into an ethylene-vinyl acetate tubing (0.5 mm diameter) and sealed off using epoxy. Finally, the I/O pins and microfluidic inlet tubing was embedded into a custom designed 3D-printed box and coated with epoxy to provide mechanical stability and ease of handling. CoPc was then deposited onto the exposed CNT microwire by dropcasting CoPc ink (1 mg/ml in ethanol). The electrode was then oven-dried in air at 60 °C for 15 min.

cGMP fluorescent imaging

For cGMP imaging, HEK cells were transfected with α-subunit of human sGC, β-subunits of human sGC, and Green cGull. 3 µL of Lipofectamine 2000 (Thermo Fisher Scientific) and 500 ng of each DNA (1500 ng in total) in 50 µL of Opti-MEM were added to each 24 well plate. CMV-GUCY1A3-DDK (Origene, NM000586), CMV-GUCY1B1-DDK (Origene, NM000857), Green cGull (Addgene, 86867) were utilized for transfection. Green cGull was a gift from Tetsuya Kitaguchi (Addgene plasmid # 86867). After 48 h, transfected cells were transferred to Tyrode’s solution, and Green cGull fluorescence in the cells was measured with an inverted fluorescence microscope (frame rate: 1 s). After 30 s of recording, 10 mM NORM in Tyrode’s solution was injected to confirm the functionality of expressed Green cGull. For cGMP imaging with electrocatalytic fiber, CoPc/CNT microwire and Pt microwire in the fiber were used as cathode and anode, respectively. Ag/AgCl reference electrode was placed in the electrolyte. Transfected cells cultured on the coverslips were transferred to each 6 well plate containing 6 ml of Tyrode’s solution, and the cells were then positioned below CoPc/CNT microwire. For CO generation, 1 ml of CO2-saturated Tyrode’s solution (pH 7.4) was delivered to each well plate before applying voltages to CoPc/CNT microwire. Voltages were turned on after 30 s of recording. ImageJ was used to analyse the fluorescent intensity of each cells. F0 value was calculated by averaging its fluorescent values during the initial 10 s of recording. Averaged ΔF/F0 values were calculated with 100 cells randomly selected from at least three independently performed experiments.

Statistical Analysis

In the case of multiple groups, statistical differences were determined using a one-way analysis of
variance (ANOVA) and Tukey’s multiple comparison test (***, p < 0.0001, **p < 0.01, *p < 0.05, ns: not significant). Two groups were compared by unpaired one-tailed Student’s t-test.

**Calculation methods for CO₂ degassing kinetics and diffusion profiles of CO**

**Details of equilibrium calculations:** Modeling of equilibrium and transport in CO₂ reduction reaction (CO₂RR) systems were modeled in Matlab and based on a previous report in the literature[1]. What is first needed is a description of the complex equilibria between CO₂, water, bicarbonate, and carbonate. We did this while also accounting for the effect of ionic strength on species activities. The following relevant constants were used:

<table>
<thead>
<tr>
<th>Physical context</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eq constant for CO₂(aq) + H₂O → H⁺ + HCO₃⁻</td>
<td>10⁻⁶.37 × 1000 mol/m³</td>
</tr>
<tr>
<td>Eq constant for HCO₃⁻ → H⁺ + CO₃²⁻</td>
<td>10⁻¹⁰.₂₅ × 1000 mol/m³</td>
</tr>
<tr>
<td>Eq constant for H₂O → H⁺ + OH⁻</td>
<td>10⁻¹⁴ × 1000² (mol/m³)²</td>
</tr>
<tr>
<td>Sechenov Coefficient for H⁺ (hₛ,𝐻⁺)</td>
<td>0</td>
</tr>
<tr>
<td>Sechenov Coefficient for OH⁻ (hₛ,𝑂𝐻⁻)</td>
<td>8.39 × 10⁻⁵ m³/mol</td>
</tr>
<tr>
<td>Sechenov Coefficient for HCO₃⁻ (hₛ,𝐻𝐶𝑂₃⁻)</td>
<td>9.67 × 10⁻⁵ m³/mol</td>
</tr>
<tr>
<td>Sechenov Coefficient for CO₃²⁻ (hₛ,𝐶𝑂₃²⁻)</td>
<td>14.23 × 10⁻⁵ m³/mol</td>
</tr>
<tr>
<td>Sechenov Coefficient for K⁺ (hₛ,𝐾⁺)</td>
<td>9.22 × 10⁻⁵ m³/mol</td>
</tr>
</tbody>
</table>

**Table S1.** Constants used for electrolyte equilibrium calculation

Activities of ionic species were accounted for using Davie’s equation:

\[ a_i = C_i \cdot 10^{-0.51z_i^2 \left( \frac{\sqrt{l}}{1+\sqrt{l}} \right)^{-0.3l}} \]

where \( a_i \) is activity of species \( i \), \( C_i \) is the concentration of species \( i \), \( z_i \) is the charge on species \( i \), and \( l \) is the total ionic strength of the solution, in mol/m³, given by:

\[ l = \frac{1}{2} \sum_i z_i^2 C_{i,0} \]

where \( C_{i,0} \) is the initial concentration of species \( i \).
Activity of dissolved CO\(_2\) was calculated using Henry’s law:

\[
a_{CO_2} = H_{CO_2} \cdot p_{CO_2}
\]

where \(p_{CO_2}\) is the partial pressure of CO\(_2\) in atm and \(H_{CO_2}\) is Henry’s constant, and calculated for CO\(_2\) as:

\[
H_{CO_2} = e^{\left(93.4517 \cdot \frac{100}{T} - 60.2409 + 23.3585 \log\left(\frac{T}{100}\right)\right)}
\]

where \(T\) is the temperature in Kelvin. Concentration of CO\(_2\) in the electrolyte (mol/m\(^3\)) was calculated as:

\[
C_{CO_2} = \frac{a_{CO_2}}{\gamma_{CO_2}}
\]

where \(\gamma_{CO_2}\) is the activity coefficient of CO\(_2\):

\[
\gamma_{CO_2} = e^{\sum c_i \cdot (h_{s,i} + h_G)}
\]

where \(h_{s,i}\) are the Sechenov coefficients for each ionic species (listed in Table S1 above), and \(h_G\) is the specific gas constant of CO\(_2\), (in m\(^3\)/mol) given by:

\[
h_G = -1.72 \cdot 10^{-5} - 3.38 \cdot 10^{-7} \cdot (T - 298.15)
\]

Concentrations of all species were solved for by enforcing that ratios of activities equaled equilibrium constants for the reactions listed in Table S1 above.

**2D Cartesian transport model:** We modeled the consumption of CO\(_2\) in an in vitro situation using a 2D reaction-diffusion model. We assumed a flat, planar electrode in a quiescent solution and assumed constant temperature and density throughout the entire solution. In this situation, the 2D, time-dependent governing equations take the form of:

\[
\frac{\partial C_i}{\partial t} = D_i \frac{\partial^2 C_i}{\partial x^2} + D_i \frac{\partial^2 C_i}{\partial y^2} + R_{gen,i}
\]

For carbonate-based buffers, we want to consider the following species \(i\) in solution:

1. CO\(_2^*\) (dissolved CO\(_2\)/H\(_2\)CO\(_3\))
2. HCO\(_3^-\)
3. CO\(_3^{2-}\)
4. H\(^+\)

For the chemistry, we assumed that CO\(_2\) hydration/dehydration (reaction 1) is slow, but all acid/base reactions are in equilibrium. Thus, the chemistry considered is the following:
Reaction 1: \( \text{CO}_2^* + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{HCO}_3^- \)  
\( \text{pK}_1 = 6.37 \text{ mol/L} \)  
\( k_{+1} = 3.71 \times 10^{-2} \text{ s}^{-1} \)

Reaction 2: \( \text{HCO}_3^- \rightarrow \text{H}^+ + \text{CO}_3^{2-} \)  
\( \text{pK}_2 = 10.25 \text{ mol/L} \)

Thus, we will need to solve the following system of equations (ignoring \( \text{CO}_3^{2-} \) since it is only part of an equilibrium reaction):

\[
\frac{\partial C_{\text{CO}_2}}{\partial t} = D_{\text{CO}_2} \frac{\partial^2 C_{\text{CO}_2}}{\partial x^2} + D_{\text{CO}_2} \frac{\partial^2 C_{\text{CO}_2}}{\partial y^2} + k_{-1} C_{\text{H}^+} C_{\text{HCO}_3^-} - k_{+1} C_{\text{CO}_2}
\]

\[
\frac{\partial C_{\text{HCO}_3^-}}{\partial t} = D_{\text{HCO}_3^-} \frac{\partial^2 C_{\text{HCO}_3^-}}{\partial x^2} + D_{\text{HCO}_3^-} \frac{\partial^2 C_{\text{HCO}_3^-}}{\partial y^2} - k_{-1} C_{\text{H}^+} C_{\text{HCO}_3^-} + k_{+1} C_{\text{CO}_2}
\]

\[
\frac{\partial C_{\text{H}^+}}{\partial t} = D_{\text{H}^+} \frac{\partial^2 C_{\text{H}^+}}{\partial x^2} + D_{\text{H}^+} \frac{\partial^2 C_{\text{H}^+}}{\partial y^2} - k_{-1} C_{\text{H}^+} C_{\text{HCO}_3^-} + k_{+1} C_{\text{CO}_2}
\]

Subject to the boundary conditions:

**Schematic S1.** A schematic for 2D Cartesian transport model

\[
C_i \left( t = 0, x, y \right) = C_{i,eq} \quad \text{(Initial equilibrium condition)}
\]

\[
\frac{\partial C_i}{\partial t} \bigg|_{t=0, y} = (k_{+1} a_i) C_i \bigg|_{t=0, y} \quad \text{(CO}_2 \text{ and CO degassing at surface of solution)}
\]

\[
D_i \frac{\partial C_i}{\partial y} \bigg|_{t, d < x < L, y = 0} = -\frac{v_i}{2F} \quad \text{(Species consumption or generation at electrode surface)}
\]

At all other boundaries, no flux.
Table S2. Parameters used for 2D Cartesian transport model. *not found in literature, and approximated to be similar to that of CO$_2$. Previous reports were used to get approximate $k_L a_i$ values for CO$_2$.$^{[3]}$.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>CO$_2$</th>
<th>HCO$_3^-$</th>
<th>H$^+$</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_i$</td>
<td>Diffusion constant [m$^2$/s]</td>
<td>$1.91 \times 10^{-9}$</td>
<td>$1.185 \times 10^{-9}$</td>
<td>$9.311 \times 10^{-9}$</td>
<td>$2 \times 10^{-9}$ *</td>
</tr>
<tr>
<td>$k_L a_i$</td>
<td>Degassing mass transfer coefficient [s$^{-1}$]</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>0.02 *</td>
</tr>
<tr>
<td>$v_i$</td>
<td>Reaction stoichiometry on a 2-electron basis</td>
<td>$-1$</td>
<td>0</td>
<td>$-2$</td>
<td>1</td>
</tr>
</tbody>
</table>

To solve numerically, we used the method of lines to turn the problem from a partial differential equation (PDE) to an ordinary differential equation (ODE). The problem was then discretized along the $x$ and $y$ directions to express the spatial differentials using algebraic expressions. Then, the grid of concentrations at discretized $x$ and $y$ values was evolved in time using a built-in Matlab ODE integrator (ODE15s).
Figure S1. The Faradaic efficiency (FE) for H$_2$ and CO (mean ± standard error of the mean (s.e.m.), n = 3) at negative voltages ≤ −1.5 V. Notably, the degradation of CoPc catalyst was also observed in this potential range over time.
**Figure S2.** Faradaic efficiency for active chlorine produced at the Pt anode in CO$_2$-saturated Tyrode’s solution (pH 7.4). Quantification of active chlorine was conducted using N,N-diethyl-p-phenylenediamine (DPD)-based active chlorine test kit (Hach, DR 300).
Figure S3. a, Intracellular cGMP levels (mean ± s.e.m.) in 10⁶ sGC⁺ HEK cells following the addition of NORM (500 μM, DEA NONOate) or CORM-2 (50 μM, 500 μM). The statistical significance of an increase in cGMP levels after NO delivery was confirmed by one-way ANOVA and Tukey’s multiple comparison test (n = 6, F₃,2₀ = 261.3, ** p = 3.3 × 10⁻¹⁶ < 0.0001). b, Intracellular cGMP levels (mean ± s.e.m.) in 10⁶ sGC⁺ HEK cells after the addition of CORM-2 (50 μM, 500 μM) or RuCl₃ (one-tailed Student’s t-test, n = 6, ** p = 0.003 < 0.01, **** p = 3.3 × 10⁻⁶ < 0.0001, and n.s. p = 0.46 > 0.05). Unlike electrochemically produced CO, CORM-2 failed to increase the cGMP levels in sGC⁺ cells, presumably due to its NO scavenging activity⁴.
Figure S4. a, Cell viability in the presence or absence of CoPc (mean ± s.e.m.). No statistically significant differences were observed between groups as identified by one-way ANOVA and Tukey’s multiple comparison test (n = 7, F2,18 = 2.72, p = 0.093). b, Cell viability following acute exposure to CO2-saturated Tyrode’s solution at pH 7.4 (mean ± s.e.m.). No statistically significant differences were observed between groups as identified by one-way ANOVA and Tukey’s multiple comparison test (n = 5, F2,12 = 0.62, p = 0.55).
Figure S5. a, A schematic illustrating an experimental setup for CO delivery to cells. Cells seeded on a coverslip were positioned at the bottom of the well plate, and the distance from the coverslip to the interface between the gas (air) and liquid (CO$_2$-saturated Tyrode’s solution) was approximately 6.3 mm. b, Calculated CO$_2$ concentration profiles as a function of distance from gas-liquid interface considering CO$_2$ degassing kinetics. Inset shows negligible changes in CO$_2$ concentration near the cells (6.3 mm from the gas-liquid interface) over 30 min. c, Diffusion profile of electrochemically generated CO at various current densities and electrolysis time.
Figure S6. Maximum of the normalized fluo-4 fluorescence changes in 100 neurons following the addition of 50 μM CORM-2 in the presence or absence of ion channel blockers (or inhibitors): 20 μM Iberiotoxin for BK_{Ca} channel; 12 μM Nitrendipine for L-type Ca^{2+} channel; 400 μM Ivabradine for HCN; 10 μM 1H-[1,2,4]oxadiazo[4,3-a]quinoxalin-1-one (ODQ) for sGC.
**Figure S7.** Individual fluo-4 fluorescence traces for 100 neurons pre-incubated with 12 μM nitrendipine, followed by exposure to electrochemically produced CO in CO$_2$-saturated Tyrode’s solution (pH 7.4). Voltage of –1.3 V was turned on at 30 s (dashed line).
Figure S8. Averaged Green cGull fluorescence traces of cells (n = 100 cells for each trace) following the addition of NORM (DEA NONOate, 10 mM, blue) or Tyrode’s solution (control, green) at 30 s (dashed lines). The solid lines and shaded areas indicate the mean and s.e.m., respectively.
Figure S9. **a,** Averaged fluo-4 fluorescence traces for dorsal root ganglion (DRG) neurons (n = 100 neurons for each trace) following the addition of 50 μM CORM-2 (blue) or 50 μM RuCl₃ (green) at 30 s (dashed lines). The solid lines and shaded areas represent the mean and s.e.m., respectively. **b,** Time-lapse images of Ca²⁺ responses in DRG neurons following the CORM-2 infusion (scale bar, 50 μm).
References


