Electronic Supplementary Information

Counteranions in the Stimulation Solution Alter the Dynamics of Exocytosis Consistent with the Hofmeister Series

Xiulan He, Andrew G. Ewing*

Department of Chemistry and Molecular Biology, University of Gothenburg, 41296 Gothenburg, Sweden

Table of contents

S1. Experimental Section

1. Chemical Reagents. Chemicals and Solutions. Unless otherwise specified, all reagents were purchased from Sigma-Aldrich. All chemicals, of analytical grade, were obtained from Sigma-Aldrich and used as received. All solutions were prepared using 18 MΩ·cm water from Purelab Classic purification (ELGA, Sweden), adjusted to pH 7.4 and filtered prior for use. The isotonic saline solution included 150 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM glucose and 10 mM HEPES, pH 7.4. The K^+ stimulation solution contained 125 mM NaCl, 30 mM KCl (or KBr, KNO_3, $KClO_4$, KSCN), 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM glucose and 10 mM HEPES, pH 7.4. 10× Locke's buffer included 1540 mM NaCl, 56 mM KCl, 36 mM $NaHCO_3$, 56 mM glucose, 50 mM HEPES, 1% (v/v) penicillin, pH 7.4. This stock solution was diluted 10 times with distilled water (1× Locke's buffer) the day before the experiment.

2. Apparatus and Instruments.

2.1 Electrochemical measurements. Before single cell amperometry, the medium was removed and the cells were rinsed three times with the isotonic solution. The cells were kept 37 °C in the isotonic solution during the whole experimental process. Electrochemical recordings from single chromaffin cells were performed on an inverted microscope (IX81, Olympus), in a Faraday cage. The working potential was +700 mV versus Ag/AgCl reference electrode (Scanbur, Sweden) under the control of an Axopatch 200B potentiostat (Molecular Devices, Sunnyvale, CA). The output was filtered at 2.1 kHz and digitized at 5 kHz (Axoscope 10.4 software, Axon Instruments Inc., Sunnyvale, CA, USA). All the experiments were observed under an inverted microscope (IX81, Olympus) with 10x and 40x objectives. For single cell exocytosis, the micro-disk electrode was moved slowly by a Patch-Clamp Micromanipulator (PCS-5000, Burleigh Instruments, Inc., USA) to place it on the membrane of a chromaffin cell without causing any damage to the surface. Ten seconds after the start of recording, 30 mM K^+ stimulating solution in a glass micropipette was injected into the surrounding of the chromaffin cells with a single 30-s injection pulse.

2.2 Data Acquisition and Analysis. The amperometric traces were processed using an Igor Pro 6.22 routine originating from David Sulzer's group (Coumbia University). The filter for the current was 10 kHz (binomial sm., binomial switching median filter). The threshold for peak detection was three times the standard deviation of the noise. The traces were carefully inspected after peak detection and false positives were manually rejected. The number of molecules released by single cells was pooled, and the median of the data was calculated for each experimental condition. These parameters (Figure S1), the rise time (t_{rise}), defined as the time separating 25% of the maximum from 75% of the maximum on the ascending part of the spike; the half peak width ($t_{1/2}$), defined as the width of the exocytotic at half of its magnitude; the fall time (t_{fall}), defined as the time separating 75% of

the maximum from 25% of the maximum on the descending part of the spike. To compare between different conditions, the mean of median of molecules number calculated was used. The responding cells were also calculated from each experiment. Pairs of data sets were compared with Holm-Sidak method; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

3. Isolation of Adrenal Chromaffin Cells. Bovine adrenal glands were obtained from a local slaughterhouse, and the chromaffin cells were isolated as previously described.[1] Briefly, the vein was perfused with Locke's buffer to clear away blood cells. The medulla was isolated after collagenase (0.2%, Roche, Sweden) treatment, and cells were isolated using a series of homogenization and centrifugation steps. For single cell experiments, ~700 000 cells were seeded on collagen (IV) coated plastic dishes (D=60 mm, Corning Biocoat, VWR, Sweden) and maintained in a humidified incubator at 37 °C, 5% CO_2 for a maximum of 3 days prior to experiments.

The ionic composition of the cell medium included 1.05 mM $CaCl_2$, $5.2E^{-6}$ mM $CuSO_4$, 1.24 mM $Fe(NO_3)_3$, 0.0015 mM $FeSO_4$, 0.30 mM $MgCl_2$, 0.407 mM $MgSO_4$, 4.16 mM KCl, 14.29 mM $NaHCO_3$, 120.6 mM NaCl, 0.50 mM Na_2HPO_4, 0.45 mM NaH_2PO_4, 0.0015 mM $ZnSO_4$.

4. Fabrication of Disk Carbon Fiber Microelectrodes. The fabrication of disk microelectrodes was previously described.[2] Briefly, a 5 µm diameter carbon fiber was aspirated into a glass capillary (Sutter Instrument Co., Novato, CA). A micropipette puller (model PE-21, Narishige, Inc., Japan) was used to pull the glass capillary into two separate electrodes and epoxy (Epoxy Technology, Billerica, MA, U.S.A.) was used to seal the electrodes. The glued electrodes were then cured at 100°C overnight and subsequently beveled at 45° angle (EG-400, Narishige Inc., London, UK). Before the experiment, each electrode was tested with cyclic voltammetry (-0.2 to 0.8 V vs Ag/AgCl, 100 mV/s) in a solution of 100 µM dopamine in PBS (pH 7.4). Only electrodes showing good reaction kinetics and stable steady-state currents were used for experiments.

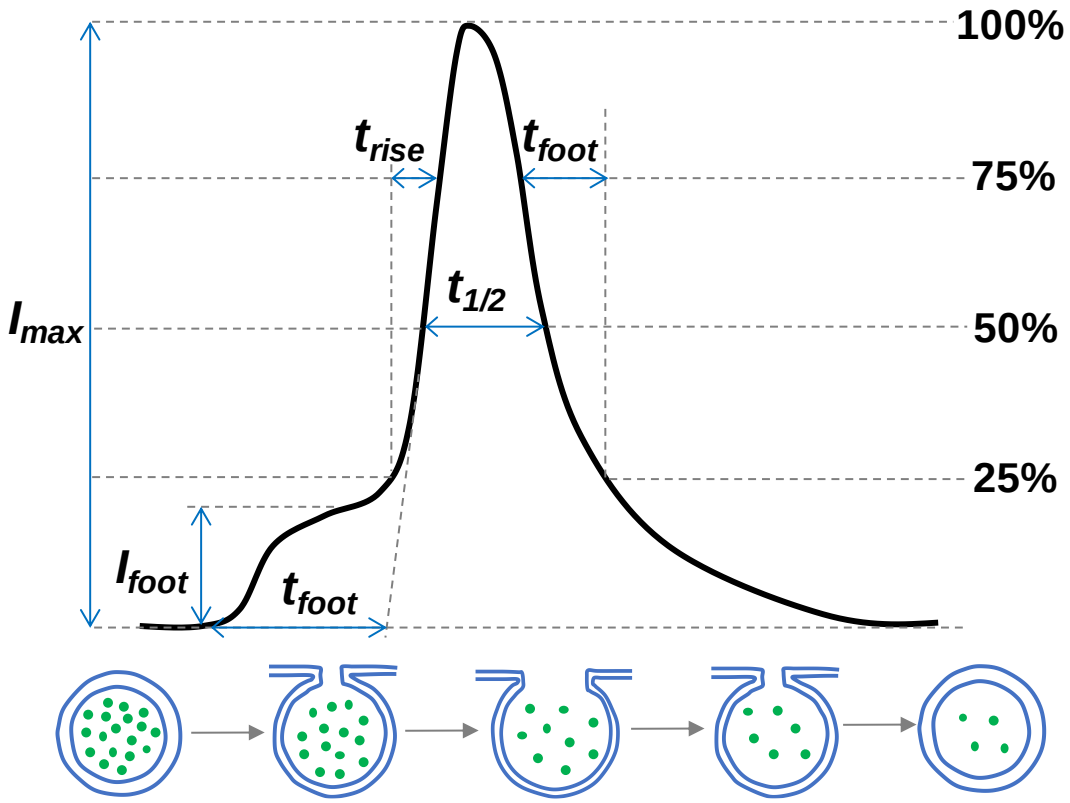

Figure S1. Illustration of the correlation of an amperometric peak and exocytotic release. Scheme of the different parameters used for the peak analysis for exocytosis. I_{max}=peak current, t_{rise}=rise time, $t_{1/2}$=half peak width, t_{fall}=fall time, I_{foot}=foot current, t_{foot}=foot duration.

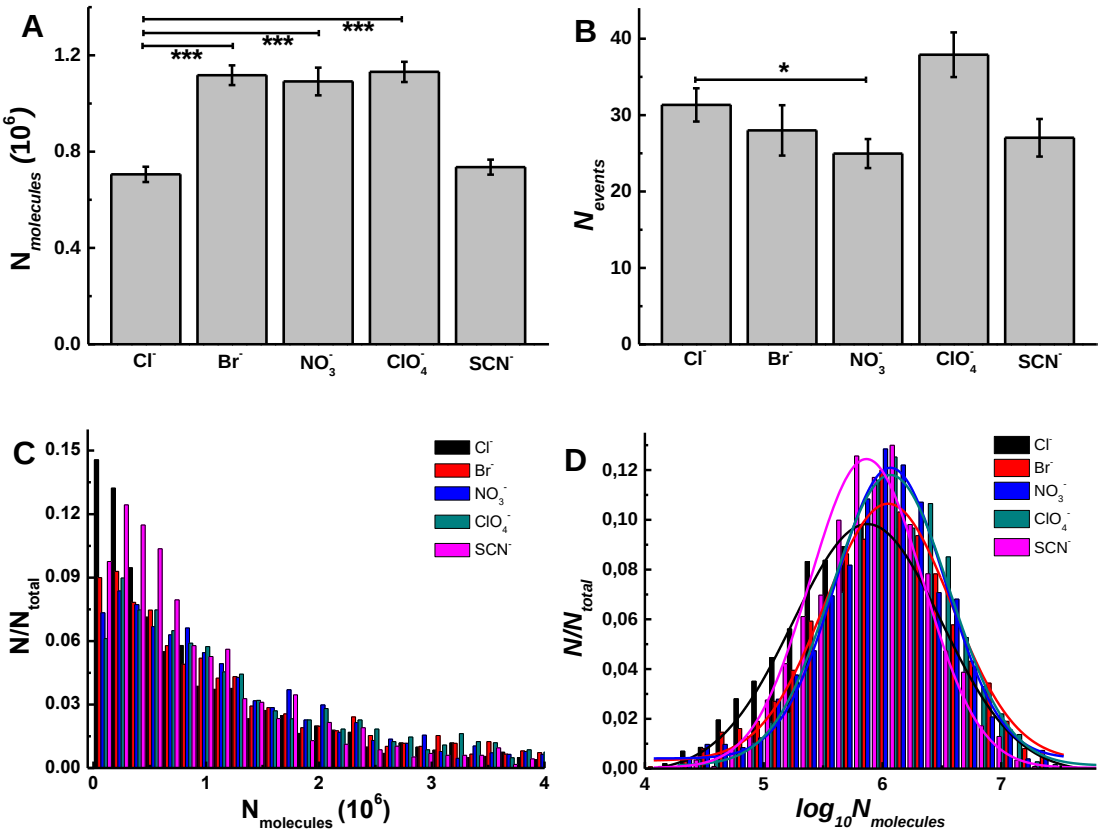

Figure S2. (A) Number of molecules ($N_{molecules}$) and (B) number of events (N_{events}) obtained from SCA with chromaffin cells (n=30) stimulated by 30 mM K^+ solution including different counter-anions (e.g., Cl^-, Br^-, NO_3^-, ClO_4^-, and SCN^-), Normalized frequency histograms describing the distributions of the $N_{molecules}$ (C) and $\log_{10}N_{molecules}$ (D) released from different conditions. The error bars are the standard error of the mean.

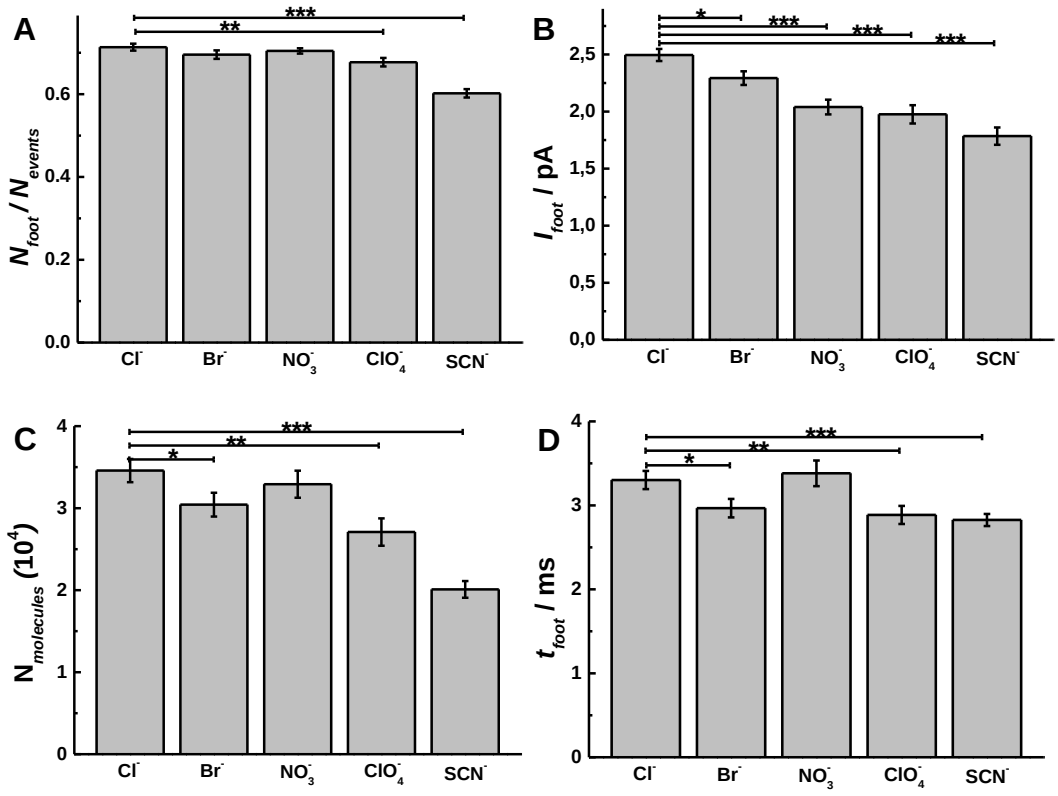

Figure S3. Foot parameters without cut off obtained from SCA with chromaffin cells (n=30) stimulated by 30 mM K^+ solution including different counter-anions (e.g., Cl^-, Br^-, NO_3^-, ClO_4^-, and SCN^-): N_{foot}/N_{events} (A), I_{foot} (B), $N_{molecules}$ in foot (C), and t_{foot} (D). Pairs of data sets were compared with T test; ***, p<0.001; **, p<0.01; *, p<0.05.

Table S1. P values (calculated by Holm-Sidak method) to compare the peak parameter $t_{1/2}$ obtained from SCA.

$t_{1/2}$	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.0156 *	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***
Br^-	0.0156 *	×	0.00629 **	0.00299 **	$<10^{-6}$ ***
NO_3^-	$<10^{-6}$ ***	0.00629 **	×	0.764	0.00231 **
ClO_4^-	$<10^{-6}$ ***	0.00299 **	0.764	×	0.00160 **
SCN^-	$<10^{-6}$ ***	$<10^{-6}$ ***	0.00231 **	0.00160 **	×

Table S2. P values (calculated by Holm-Sidak method) to compare the peak parameter t_{rise} obtained from SCA.

t_{rise}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.00218 **	0.000043 ***	0.000004 ***	$<10^{-6}$ ***
Br^-	0.00218 **	×	0.0288 *	0.00907 **	0.000031 ***
NO_3^-	0.000043 ***	0.0288 *	×	0.820	0.303
ClO_4^-	0.000004 ***	0.00907 **	0.820	×	0.409
SCN^-	$<10^{-6}$ ***	0.000031 ***	0.303	0.409	×

Table S3. P values (calculated by Holm-Sidak method) to compare the peak parameter t_{fall} obtained from SCA.

t_{fall}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.000364 ***	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***
Br^-	0.000364 ***	×	0.000005 ***	0.000168 ***	$<10^{-6}$ ***
NO_3^-	$<10^{-6}$ ***	0.000005 ***	×	0.838	0.00728 **
ClO_4^-	$<10^{-6}$ ***	0.000168 ***	0.838	×	0.0130 *
SCN^-	$<10^{-6}$ ***	$<10^{-6}$ ***	0.00728 **	0.0130 *	×

Table S4. P values (calculated by Holm-Sidak method) to compare the peak parameter I_{max} obtained from SCA.

I_{max}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	$<10^{-6}$ ***	0.00072 ***	0.000157 ***	0.000506 ***
Br^-	$<10^{-6}$ ***	×	0.00717 **	0.0593	$<10^{-6}$ ***
NO_3^-	0.00072 ***	0.00717 **	×	0.521	$<10^{-6}$ ***
ClO_4^-	0.000157 ***	0.0593	0.521	×	$<10^{-6}$ ***
SCN^-	0.000506 ***	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***	×

Table S5. P values (calculated by Holm-Sidak method) to compare number of molecules ($N_{molecules}$) obtained from SCA.

$N_{molecules}$	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***	0.503
Br^-	$<10^{-6}$ ***	×	0.714	0.814	$<10^{-6}$ ***
NO_3^-	$<10^{-6}$ ***	0.714	×	0.579	$<10^{-6}$ ***
ClO_4^-	$<10^{-6}$ ***	0.814	0.579	×	$<10^{-6}$ ***
SCN^-	0.503	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***	×

Table S6. P values (calculated by Holm-Sidak method) to compare the number of event (N_{events}) obtained from SCA.

N_{events}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.401	0.0315 *	0.077	0.195
Br^-	0.401	×	0.428	0.0284 *	0.815
NO_3^-	0.0315 *	0.428	×	0.000472 ***	0.509
ClO_4^-	0.077	0.0284 *	0.000472 ***	×	0.00614 **
SCN^-	0.195	0.815	0.509	0.00614 **	×

Table S7. P values (calculated by Holm-Sidak method) to compare the percent of foot in all events (N_{foot}/N_{events}) obtained from SCA.

N_{foot}/N_{events}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.913	0.00516 **	$<10^{-6}$ ***	$<10^{-6}$ ***
Br^-	0.913	×	0.00692 **	$<10^{-6}$ ***	$<10^{-6}$ ***
NO_3^-	0.00516 **	0.00692 **	×	0.000420 ***	$<10^{-6}$ ***
ClO_4^-	$<10^{-6}$ ***	$<10^{-6}$ ***	0.000420 ***	×	0.000066 ***
SCN^-	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***	0.000066 ***	×

Table S8. P values (calculated by Holm-Sidak method) to compare the peak parameter I_{foot} obtained from SCA.

I_{foot}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.377	0.000326 ***	0.000004 ***	$<10^{-6}$ ***
Br^-	0.377	×	0.0262 *	0.00137 **	0.000002 ***
NO_3^-	0.000326 ***	0.0262 *	×	0.212	$<10^{-6}$ ***
ClO_4^-	0.000004 ***	0.00137 **	0.212	×	0.000003 ***
SCN^-	$<10^{-6}$ ***	$<10^{-6}$ ***	$<10^{-6}$ ***	0.000003 ***	×

Table S9. P values (calculated by Holm-Sidak method) to compare the peak parameter t_{foot} obtained from SCA**.**

t_{foot}	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.557	0.166	0.000847 ***	0.696
Br^-	0.557	×	0.0707	0.00557 **	0.393
NO_3^-	0.166	0.0707	×	0.000126 ***	0.377
ClO_4^-	0.000847 ***	0.00557 **	0.000126 ***	×	0.00203 **
SCN^-	0.696	0.393	0.377	0.00203 **	×

Table S10 P values (calculated by Holm-Sidak method) to compare the number of molecules ($N_{molecules}$) in foot obtained from SCA.

$N_{molecules}$ in foot	Cl^-	Br^-	NO_3^-	ClO_4^-	SCN^-
Cl^-	×	0.00350 **	0.218	$<10^{-6}$ ***	$<10^{-6}$ ***
Br^-	0.00350 **	×	0.0518	0.0118 *	$<10^{-6}$ ***
NO_3^-	0.218	0.0518	×	0.000008 ***	0.000005 ***
ClO_4^-	$<10^{-6}$ ***	0.0118 *	0.000008 ***	×	0.923
SCN^-	$<10^{-6}$ ***	0.0113 *	0.000005 ***	0.923	×

References

(1) O'Connor, D. T.; Mahata, S. K.; Mahata, M.; Jiang, Q.; Hook, V. Y.; Taupenot, L. *Nat. Protoc.* **2007**, *2(5),* 1248-1253.

(2) Adams, K. L.; Engelbrektsson, J.; Voinova, M.; Zhang, B.; Eves, D. J.; Karlsson, R.; Heien, M. L.; Cans, A. S.; Ewing, A. G. *Anal. Chem.* **2010**, *82*, 1020-1026.