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Potassium concentration alters calibration sensitivities of dopamine but not serotonin

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Abstract

The use of carbon fibre microelectrodes and amperometric techniques for measurement of biological molecules has been widely studied. The use of calibrations to translate biological data is common practice between labs. Tris buffer is commonly used for conducting calibrations, where potassium ion concentrations are varied in buffers. However, little is known about how changes in this ion alter the calibration sensitivity of neurotransmitters. This work showcases that dopamine calibrations are highly dependent on the concentration of potassium ions, whilst serotonin is less affected. Our findings have implications on interpretation and comparison of measurements between different studies.

Electroactive substances have been monitored in biological settings over the past 50 years, with advancements in sensors and techniques. Carbon fibre microelectrodes (CFM) with diameters ranging from 5 - 10 μm are excellent minimally invasive tools for biological measurements. An important factor in these measurements is the use of buffers for sensor calibrations.[1] The main criteria of a buffer is to closely mimic the biological environment where measurements take place.[1]

Tris buffer contains most biological ions and maintains pH without a constant supply of CO_2. Its main component is the Tris molecule, a primary amine with three symmetric alcohol groups[2], mimicking the amine group present on most proteins in biological matrices. Due to its biocompatibility, stability, lack of reactivity and toxicity, this buffer is one of the most widely used in biochemical studies.[3] [4] [5]

In this paper, we aim to shed light on the effects of changing the composition of one of the monovalent ions, K^+ on the Tris calibration of catecholamine dopamine (DA) and indolamine serotonin (5-HT). As a principal ion in action potentials, changes in the concentration of K^+ in the extracellular space changes the membrane potential and in turn affects synaptic transmission. Larger changes in this cation are seen in different pathophysiologies such as epilepsy[6] or following spreading depolarizations[7]. Studies have previously explored the effect of altering divalent cation concentrations in buffers on the calibration of molecules such as DA and 5-HT. [8] [9] However as of yet, the effect of altering the concentration of monovalent ions, specifically K^+ are yet to be explored.

The importance of DA and 5-HT in reward, mood disorders, motivation etc. have caused them to be well-studied amperometrically using CFM. It is critical to understand if alterations in K^+ ions that can occur during biological measurements or those present in

different compositions of Tris buffers utilised would have an impact on the sensitivity factor of CFM to DA and 5-HT. Hence, a Tris buffer was utilised with differing concentrations of K^+ ranging 1-10 mM. The concentration range chosen covers the physiological range of K^+ present in the CNS and peripheral system and the fluctuations that can occur during biological measurement. [10] [11]

Figure 1A and 1B show the osmolality and conductivity of the Tris buffer solutions are not significantly altered as a result of increasing the K^+ concentration from 1-10 mM. This is expected as the changes in ionic concentration are negligent compared to the total ion concentration. Figure 1C shows no significant changes in the background capacitive current with increasing concentrations of K^+ in Tris, which hence means no change in the signal/noise ratio. This showcases that the interaction of K^+ with the surface occurs without any complex binding to the functional groups present on the CFM surface, as is known to happen with divalent cations such as Mg^{2+}, which forms complexes with carboxyl functional groups present on the CFM surface.[12]

As changes in K^+ concentrations are not causing any significant changes to the ionic composition or capacitive current, Tris buffer was utilised to calibrate CFM to DA and 5HT in the presence of these different concentrations to study their effects on the faradaic current. As seen in figure 2, the calibration of DA varies significantly with alterations in K^+ concentrations in Tris. Figure 2A shows representative traces of amperometric DA calibrations in different concentrations of K^+ in Tris. Current density calibration plots for the DA concentrations ranging 0.1 - 2 µM are shown in Figure 2B, with the resultant calibration sensitivity shown in Figure 2C. As previously seen for the divalent cations Ca^{2+} and Mg^{2+}, there is a clear dependency of DA sensitivity on CFM with

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alterations in the monovalent cation K^+.[8],[9] DA calibrations in the lowest and highest K^+ concentrations displayed the highest sensitivity in comparison to all other concentrations (3.25 mM, $p<0.05$; 5 mM, $p<0.001$; 7.5 mM, $p<0.05$, one-way anova with tukey *post hoc*, n=4), making the resultant sensitivity trend V-shaped. This trend could possibly be a result of multiple molecular and surface effects. Firstly there could be changes in the interactions occurring at the electrode surface that specifically perturbs positively charged DA from adsorbing onto the CFM surface.[12] Divalent cations present in the Tris buffer may displace increasing amounts of K^+ causing interactions with DA.[13] Secondly, Tris molecules which are well known to complex with divalent cations present in solution[4] could thus have a similar effect on K^+, again leading to localised ionic changes. Finally, another aspect that adds more complexity, is that Ca^{2+} and Mg^{2+} are kosmotropic, whilst K^+ ions are chaotropic. [14] Chaotropic ions are known to cause disorder in the hydrogen bonding between the water molecules in solution and as a result destabilizes proteins and biological molecules due to interference in intermolecular interactions and increasing entropy. [15] This effect is known to be exacerbated with increase in the chaotropic ion concentration and affects its interaction with the amine group on Tris and DA. [16] However, how this in turn affects the adsorption of DA onto the CFM, and the hydration layer present around the CFM, needs further investigation.

5-HT, on the other hand showed a similar trend to previous work performed with divalent cations. As can be seen from **figures 2D-F**, the current density calibrations, and sensitivity plot for 5-HT at different K^+ concentrations showed no significant alterations. This can be attributed to the hydrophobic nature of 5HT,[8] unlike DA, which allows it to

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be less affected by charged interactions occurring in solution and around the CFM surface.

The influence of this dopamine effect was also explored to understand if this effect occurred during voltammetric measurements, where **figure 3** shows that a similar trend was observed when cyclic voltammograms were obtained in Tris buffers of varying K^+ concentration. This suggest that the behaviour is not limited to just when a fixed voltage is applied but also when the voltage is ramped up over time.

To better mimic ionic changes that occur during biological measurements, it was important to determine if similar effects occurred during dynamic changes in K^+ concentration. To explore any changes in the capacitive current, injection of different concentrations of K^+ into a Tris solution with a pre-set amount of K^+ present (3.25 mM) was conducted. No significant changes in current (**figure 4A**) or current density (**figure 4B**) was observed.

A fixed concentration of DA or 5-HT were injected into a low concentration of K^+ in Tris (3.25 mM), followed by dynamic increases in the K^+ to determine if it would lead to any alterations in the DA or 5-HT signal. As can be seen in **figures 5A & C**, the CFM is able to detect the 1 µM injection of DA, and any further increase in the K^+ concentration does not significantly alter the DA signal. Similarly, **figures 5B & D** show no changes in the 5-HT signal with increasing concentrations of K^+**.** This showcases that the order in which the molecules interact with the CFM surface is crucial in terms of ion interaction. In this case, with DA being detected first at comparatively low K^+ concentrations, it was able to adsorb successfully and the time course for the K^+ ion effect is less dynamic to cause interference during biological measurements. This is contrary to what was observed in

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figure 2, where the DA calibration was performed in a Tris buffer with a set level of K^+ already present in solution and interacting with the CFM.

It is important to be critical of the dependency of biological molecules to alterations in cation concentrations that can occur physiologically during the measurement of these molecules, or due to pharmacological manipulation. This paper has showcased the important effects that buffer concentrations used for calibrations can have on sensitivities and thus interpretation of biological measurements, which can potentially lead to increased error in data interpretation and variations between studies. Work still needs to be completed to better understand the interaction of K^+ with the CFM and how it results in this calibration dependency in DA, as well as if this is a similar behaviour for different monovalent ions withs other catecholamines.

Experimental

Serotonin hydrochloride, Dopamine hydrochloride, Hexaammineruthenium (III) were purchased from Sigma-Aldrich (Gillingham, Dorset). Buffer solution was composed of 15 mM Tris, 140 mM NaCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 2 mM Na_2SO_4. 1.25 mM NaH_2PO_4 and different concentration of KCl (1 mM, 3.25 mM, 5 mM, 7.5 mM and 10 mM). All purchased from Sigma-Aldrich (Gillingham, Dorset)).

The CFM were constructed by aspirating a single F-180 Carbon fibre (10 μm, Goodfellow Cambridge, Huntingdon, England) into glass capillaries (internal diameter: 0.86 mm outer diameter: 1.5 mm, Harvard Apparatus Cambourne, UK). The capillaries filled with the CFM were positioned vertically into a pipette puller (Model PC-10, Narishige Group,

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London, UK) to form a carbon-glass seal under gravity. The CFM were cut to 70-120 μm in length. After cutting, the exact length was determined with a microscope and a moti connect camera. The carbon fibre was connected to a silver wire (diameter: 0.25 mm, Advent Research Materials Ltd. Eynsham, Witney, UK) which was soldered to a pin (Male Banana Plug, RS Components, Corby, UK). The silver wire was painted with conductive silver paint (RS Components, Corby, UK) and placed into the glass capillary. A heat shrink (RS Components, Corby, UK) was used to connect the glass capillary to the pin.

Cyclic Voltammogram and amperometry were performed using a CH instrument potentialstat/galvanostat CHI 760E (CH Instruments, Inc., Texas, USA). A three-electrode system was used for carrying out the electrochemical experiments. The system consisted of a Ag|AgCl (3 M KCl) reference electrode, a Pt wire counter electrode and the CFM as the working electrode. The electrochemical characteristics of the CFM were assessed using the common redox couple of 1 mM Hexaammineruthenium (III) chloride in 1 M KCl. at a scan rate of 0.01 V/s. The amperometry experiment was run in an electrochemical cell, while the solution was stirred at 0.65V.

Conflict of interest

The are no conflicts of interest.

Data Availability Statement

Electrochemical traces utilised to generate the data can be obtained from

http://researchdata.brighton.ac.uk/id/eprint/80

Figure legends

Figure 1. Exploring the ionic composition of the different Tris buffers. Measurement of **(A)** osmolality and **(B)** conductivity in different K^+ in Tris buffer. Data shown as mean ± standard deviation where n= 4. **(C)** Capacitive / background current for Tris buffer with different concentration of K^+ ions in Tris solution.

Figure 2. Effect of varying potassium concentration on calibration factors. **(A, D)** Representative traces of DA and 5HT calibration, respectively, in different K^+ compositions in Tris buffer. **(B, E)** DA and 5HT calibration plots, respectively, in Tris buffer with different K^+ compositions. **(C, F)** Sensitivity plot displaying changes in sensitivity of DA and 5HT calibration, respectively, with changes in K^+ composition in Tris buffer. Data shown as mean ± standard deviation where n= 4.

Figure 3. Representative cyclic voltammograms in different K^+ compositions in Tris buffer. Showing similar changes to those observed during amperometric measurements. Responses obtained in 100 µM DA, where scan rates were 50 mV s^{-1}.

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Figure 4. Effect of changing potassium concentration on electrode capacitance during amperometric measurements. **(A)** Representative amperometric traces of incremental injection of K^+ concentration in Tris solution with 3.25 mM K^+. **(B)** Current density plots following increases in K^+ concentration in Tris solution. Data shown as mean ± standard deviation where n= 4.

Figure 5. Effect of changing potassium concentration on dopamine and serotonin responses during amperometric measurements. **(A, B)** Representative amperometric traces of DA and 5HT injection in 3.25 mM K^+ respectively, followed by increments in K^+ concentration in Tris solution. **(C, D)** 1 µM DA and 5HT current density plots, respectively, following increases in K^+ concentration in Tris solution. Data shown as mean ± standard deviation where n= 4.

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References

[1]   C. J. Meunier, J. G. Roberts, G. S. McCarty, L. A. Sombers, *ACS Chemical Neuroscience* **2017**, *8*, 411-419.

[2]   J. Nagaj, K. Stokowa-Sołtys, E. Kurowska, T. Frączyk, M. Jeżowska-Bojczuk, W. Bal, *Inorganic Chemistry* **2013**, *52*, 13927-13933.

[3]   R. G. Bates, H. B. Hetzer, *The Journal of Physical Chemistry* **1961**, *65*, 667-671.

[4]   B. E. Fischer, U. K. Haring, R. Tribolet, H. Sigel, *Eur J Biochem* **1979**, *94*, 523-530.

[5]   S. P. Datta, A. K. Grzybowski, B. A. Weston, *Journal of the Chemical Society (Resumed)* **1963**, 792-796.

[6]   F. Fröhlich, M. Bazhenov, V. Iragui-Madoz, T. J. Sejnowski, *Neuroscientist* **2008**, *14*, 422-433.

[7]   M. L. Rogers, C. L. Leong, S. A. Gowers, I. C. Samper, S. L. Jewell, A. Khan, L. McCarthy, C. Pahl, C. M. Tolias, D. C. Walsh, A. J. Strong, M. G. Boutelle, *J Cereb Blood Flow Metab* **2017**, *37*, 1883-1895.

[8]   B. T. Chen, M. E. Rice, *Electroanalysis* **1999**, *11*, 344-348.

[9]   J. Kume-Kick, M. E. Rice, *Journal of Neuroscience Methods* **1998**, *84*, 55-62.

[10]   M. Zacchia, M. L. Abategiovanni, S. Stratigis, G. Capasso, *Kidney Dis (Basel)* **2016**, *2*, 72-79.

[11]   J. V. Raimondo, R. J. Burman, A. A. Katz, C. J. Akerman, *Front Cell Neurosci* **2015**, *9*, 419-419.

[12]   P. Takmakov, M. K. Zachek, R. B. Keithley, E. S. Bucher, G. S. McCarty, R. M. Wightman, *Analytical Chemistry* **2010**, *82*, 9892-9900.

[13]   J. A. Johnson, C. N. Hobbs, R. M. Wightman, *Anal Chem* **2017**, *89*, 6166-6174.

[14]   H. I. Okur, J. Hladílková, K. B. Rembert, Y. Cho, J. Heyda, J. Dzubiella, P. S. Cremer, P. Jungwirth, *The Journal of Physical Chemistry B* **2017**, *121*, 1997-2014.

[15]   A. Vemić, A. Malenović, M. Medenica, *Talanta* **2014**, *123*, 122-127.

[16]   M. W. Washabaugh, K. D. Collins, *J Biol Chem* **1986**, *261*, 12477-12485.