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Mini Review

Fast-scan Cyclic Voltammetry for the Characterization of Rapid Adenosine Release

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ABSTRACT

Adenosine is a signaling molecule and downstream product of ATP that acts as a neuromodulator. Adenosine regulates physiological processes, such as neurotransmission and blood flow, on a time scale of minutes to hours. Recent developments in electrochemical techniques, including fast-scan cyclic voltammetry (FSCV), have allowed direct detection of adenosine with sub-second temporal resolution. FSCV studies have revealed a novel mode of rapid signaling that lasts only a few seconds. This rapid release of adenosine can be evoked by electrical or mechanical stimulations or it can be observed spontaneously without stimulation. Adenosine signaling on this time scale is activity dependent; however, the mode of release is not fully understood. Rapid adenosine release modulates oxygen levels and evoked dopamine release, indicating that adenosine may have a rapid modulatory role. In this review, we outline how FSCV can be used to detect adenosine release, compare FSCV with other techniques used to measure adenosine, and present an overview of adenosine signaling that has been characterized using FSCV. These studies point to a rapid mode of adenosine modulation, whose mechanism and function will continue to be characterized in the future.

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1. Introduction

Adenosine signaling was first discovered in 1929 and it regulates numerous physiological processes at the cellular level. Adenosine

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modulates sleep [1], breathing [2], heart rate [3], blood flow [4], and neurotransmission [5]. Adenosine is considered a neuromodulator, since it acts over extended periods of time and over a diffuse area [6]. Adenosine is also neuroprotective and released during events such as stroke and ischemia [7]. A breakdown product of ATP, adenosine can build up due to energy consumption and metabolic processes [6]. The effects of adenosine are complicated because it can be both excitatory and inhibitory. Pharmacological studies have demonstrated that A_1 receptors primarily mediate inhibitory effects while A_{2a} receptors facilitate excitatory effects of adenosine [8]. For example, the A_1 adenosine receptor modulated preconditioning against anoxia [9] and A_{2a} receptors modulated rapid eye movements and breathing [10]. Both A_1 and A_{2a} receptors regulated glutamate-induced depression of excitatory post synaptic potentials (EPSPs) [11]. Pharmacological studies can reveal which receptors adenosine acts at but do not give any information about the levels of adenosine that are available for signaling.

Techniques that directly measure adenosine release can be used to understand the amount of adenosine in the extracellular space. Early studies used radiometric labeling of adenosine coupled with HPLC analysis to examine the breakdown of ATP to adenosine [12]. Microdialysis coupled to HPLC was also used to measure adenosine increases [13]. These methods measured adenosine on the minute time scale but recently, electrochemical techniques have been developed that allow direct measurements on the second and even sub-second time scale. Fast, discrete release of adenosine has been characterized, which shows that adenosine exhibits characteristics of a neurotransmitter, as it is tightly regulated and cleared on a fast time scale. However, while adenosine has recently been reported in vesicles [14], there is currently no direct evidence that adenosine is released through exocytosis [15]. Enzyme biosensors specific for adenosine [16] have a response time of 2 s and were used to show that increases in adenosine occur within 2 min following ischemic events [17]. Fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes directly measures adenosine on a sub-second time scale [18,19], with a sampling rate of 10 times per second. FSCV has been used to study stimulated release *in vivo* [20,21] and in brain slices [22–24]. These studies revealed that adenosine can be released and cleared in only a few seconds. However, the function of rapid adenosine release is still being elucidated. FSCV is the fastest method currently available for measuring adenosine changes and combined with pharmacology and electrophysiology, it has the capability of revealing how adenosine signals on a rapid time scale. In this review, we examine the fundamental principles of adenosine detection by FSCV, compare it to other measurement techniques, and highlight the biological applications and possible future studies that rapid measurements with FSCV may enable.

2. Adenosine Detection With Fast-scan Cyclic Voltammetry (FSCV)

FSCV is an electrochemical technique that was developed to measure changes in electroactive neurotransmitters, especially dopamine [25]. To measure adenosine with FSCV, a triangular potential is applied scanning from −0.40 V to 1.45 or 1.50 V and back versus a Ag/AgCl reference electrode at 400 V/s (Fig. 1A). The scan takes less than 10 ms and scans are repeated at 100 ms, which is the temporal resolution of the technique. The working electrode is a carbon-fiber microelectrode with a 7 μm diameter, which allows measurements in discrete brain regions. The fast scan rates cause a large background charging current (Fig. 1B) due to double layer charging at the electrode. The background current is stable over time and can be subtracted out from the signal [26, 27]. The result of subtracting the background current of the dashed line (buffer only) and the red line (buffer and adenosine) in Fig. 1B is a characteristic cyclic voltammogram for 1 μM adenosine (Fig. 1C).

Adenosine is an electroactive molecule that can undergo a series of three, two-electron oxidations (Scheme 1) [28]. The initial oxidation of adenosine from product I to product II in Scheme 1 is observed at 1.4 V with FSCV. A secondary oxidation, from product II to product III, is detected at 1.0 V. The first two oxidation steps are irreversible and reduction peaks are not observed. The third oxidation in the scheme is seldom observed with FSCV at our carbon-fiber microelectrodes. Thus, the characteristic cyclic voltammogram (CV) for adenosine has two oxidation peaks, with the largest peak being near the switching potential at 1.4 V (Fig. 1C) [19].

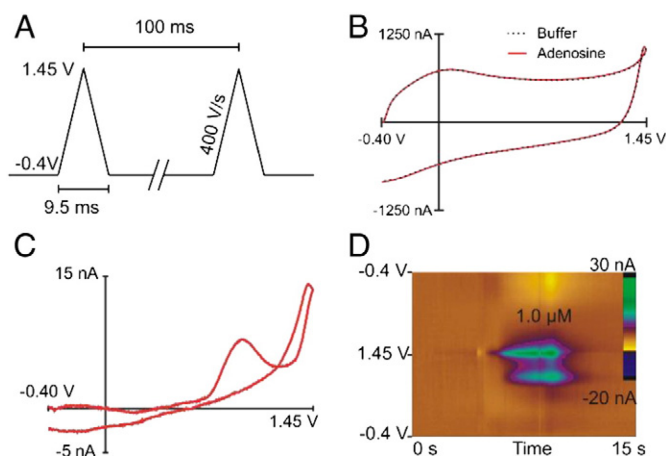

Fig. 1. *Detection of adenosine with FSCV.* A) Applied potential waveform. The electrode is held at −0.40 V, ramped up to a switching potential of 1.45 V and back at 400 V/s. The scan is repeated every 100 ms. B) The cyclic voltammogram (CV) is large due to the background charging current in the buffer (black dotted line) and the addition of 1.0 μM adenosine (red line). C) Subtracting out the background yields a background-subtracted CV of adenosine oxidation. The primary oxidation is observed at 1.4 V and the secondary oxidation at 1.0 V. The primary peak current is proportional to the concentration of adenosine detected at the electrode. D) False color plot of multiple background subtracted CVs. The *x*-axis is time, the *y*-axis is applied potential, and the color is current. This plot depicts an *in vitro* calibration experiment where the buffer is flowed by the electrode for 5 s, then 1.0 μM adenosine is flowed by for 5 s and finally buffer is flowed again. The large green oval in the center of the plot is the primary oxidation peak and the smaller green oval below is the secondary oxidation peak.
Data adapted from Nguyen et al. [29].

Since many CVs are collected over time, it is useful to visualize multiple voltammograms simultaneously in false color plots (Fig. 1D). A vertical slice through the color plot at 7.5 s gives a CV of adenosine (Fig. 1C). The primary peak appears about a half second before the secondary peak on the color plot [29]. A horizontal slice through the color plot at 1.4 V shows how the oxidation current of adenosine changes against potential. With an appropriate calibration value, that current can be converted to concentration.

Traditionally, FSCV has been used to detect catecholamines such as dopamine [30] and norepinephrine [31] but it can also detect serotonin [32], histamine [33], and hydrogen peroxide [34]. Dopamine, serotonin, and norepinephrine have oxidation peaks around 0.6 V and reductions peaks between 0.2 V and −0.2 V [35,36] and the peaks do not interfere with adenosine detection. Hydrogen peroxide has a similar oxidation peak as adenosine at 1.2 V, but has no secondary peak, which distinguishes it from adenosine [34]. Histamine also has a similar oxidation potential as adenosine, however, the secondary peak potential is lower than that of adenosine [33].

The same electroactive adenine moiety is present in ATP and adenosine, so the electrochemical signatures of adenosine and ATP are similar. However, interferences can be minimized and adenosine distinguished from ATP with FSCV. Regular carbon-fiber microelectrodes were more sensitive for adenosine than for ATP when the applied waveform has a negative holding potential of −0.4 V [19]. Electrodes coated with Nafion and carbon nanotubes were six-fold more sensitive for adenosine than for ATP [37]. Recently, our laboratory developed a sawhorse waveform that helped distinguish adenosine and ATP [38]. The altered waveform was more sensitive for adenosine and gave distinct signals for adenosine over ATP; however there was still some overlap between the two molecules. To positively identify adenosine release *in vivo*, pharmacological

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