Subsecond detection of physiological adenosine concentrations using fast-scan cyclic voltammetry

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Abstract:
Adenosine modulates blood flow and neurotransmission and may be protective during pathological conditions such as ischemia and stroke. A real-time sensor of adenosine concentrations is needed to understand its physiological actions and extent of receptor activation. Microelectrodes are advantageous for in vivo measurements because they are small and can make fast measurements. The goal of this study was to characterize detection of physiological adenosine concentration changes at carbon-fiber microelectrodes with sub-second temporal resolution. The oxidation potential of adenosine is +1.3 V, so fast scan cyclic voltammetry (FSCV) was performed with an applied potential from -0.4 to 1.5 V and back at 400 V/s every 100 ms. Two oxidation peaks were detected for adenosine with T-650 carbon fibers. The second oxidation peak at 1.0 V occurs after the initial oxidation at 1.5 V and is due to a sequential oxidation step. Adsorption was maximized to obtain detection limits of 15 nM, lower than basal adenosine concentrations in the brain. The electrode was insensitive to the metabolite inosine and seven times more sensitive to adenosine than ATP. The enzymatic degradation of adenosine was monitored with FSCV. This microelectrode sensor will be valuable for biological monitoring of adenosine.
Adenosine is an important endogenous modulator, present in all cells, that plays a role in the regulation of physiological activity in various tissues and organs. Release of adenosine has been shown to protect heart cells from damage during an ischemic attack, where there is lack of oxygen delivery. In the central nervous system, adenosine can regulate cerebral blood flow and modulate neurotransmission. Because adenosine is a product of ATP degradation, its release from cells can also be a sign of a high metabolic rate or metabolic stress. Four adenosine receptors regulate a variety of adenosine functions with a range of affinities from low nM to tens of µM. Therefore, rapid measurements of adenosine in vivo are needed to understand of the amount of adenosine available to activate receptors and the time course of adenosine effects.

In vivo measurements are challenging because small quantities of analyte must be rapidly detected in a chemically-complex sample. Microdialysis sampling coupled to HPLC has been the most common method for monitoring chemical changes in vivo. However, the method suffers from low temporal resolution and tissue damage from implantation of the relatively large probes (250-500 µm). Tissue damage causes changes in cellular metabolism and reported basal levels of adenosine from microdialysis studies vary widely from 40 nM to 2 µM depending on the time elapsed from probe implantation. Therefore smaller probes that cause less damage would be better for adenosine measurements.

Electrochemical sensors either directly detect redox active molecules or an electroactive product formed after an enzymatic reaction. The direct electrochemistry of purines, such as adenine, was characterized by Dryhurst. Electrochemical oxidation of adenine proceeded in a series of 2 electron oxidations, with an oxidation potential of 1.3 V for the first oxidation at neutral pH. Similar mechanisms for oxidation of the nucleoside adenosine at carbon electrodes have been elucidated. These studies used traditional, large electrodes with slow scanning electrochemical techniques that would not be amenable to making fast measurements in tissue.
An enzyme-based electrochemical sensor has also been developed for adenosine that requires 3 enzymes to break down adenosine and produce hydrogen peroxide, which is detected by amperometry \textsuperscript{13,14}. This electrode is sensitive and a small, 25 µm microelectrode version has been fabricated \textsuperscript{15}, but it suffers from interferences of adenosine metabolites, has a slower temporal response (2 sec to 1 min), and is complicated to fabricate due to the necessity of 3 enzymes. An ideal sensor for adenosine would be sensitive, small, easy to fabricate and allow rapid detection of adenosine changes.

Carbon-fiber microelectrodes have been used extensively as \textit{in vivo} sensors because of their small size, good electron transfer properties, and ease of fabrication \textsuperscript{16}. Research has focused on rapid detection of dopamine neurotransmission using fast-scan cyclic voltammetry \textsuperscript{17}. Brajter-Toth and colleagues have studied the detection of adenosine at carbon-fiber microelectrodes \textsuperscript{18,19}. However, their peak oxidation potentials, between 0.9 and 1.2 V, were less than expected for adenosine oxidation and their limits of detection were only 2 to 5 µM, not sufficient for \textit{in vivo} use. Limits of detection under 40 nM will be necessary to monitor changes in adenosine from basal levels.

The objective of this study was to develop a fast scan cyclic voltammetry method for detection of physiological levels of adenosine at carbon-fiber microelectrodes. We found that depending on the type of carbon fiber used, two or three successive oxidation steps could be detected for adenosine. In addition, we show that the kinetics of adenosine detection are adsorption controlled and optimizing adsorption is the key to low nanomolar limits of detection. Finally, we demonstrate that the negative holding potential associated with fast-scan cyclic voltammetry allows higher sensitivities for adenosine than adenosine nucleotides such as adenosine monophosphate (AMP) and adenosine triphosphate (ATP). The sensor was used to monitor an enzymatic reaction degrading adenosine.
EXPERIMENTAL

Electrode construction. Carbon-fiber microelectrodes were fabricated by aspirating a single carbon fiber into a glass capillary (1.2 mm x 0.68 mm, A-M systems, Carlsburg, WA). Either 6 µm diameter T-650 fibers or 10 µm P-55 fibers were used (Cytec Engineering Materials, West Patterson, NJ). The capillary was pulled to form 2 electrodes on a vertical pipette puller (Narishige, Model PE-21, Japan). The extended fiber was trimmed with a scalpel at the glass/fiber interface under the microscope. The electrodes were epoxied to obtain a seal between the fiber and glass. The epoxy, Epon Resin 828 (Miller-Stephenson, Danbury, CT), was mixed with 14 % (w/w) m-phenylenediamine hardener (Fluka, Milwaukee, WI) and heated to approximately 80°C. Electrodes were dipped for 30 s in the epoxy and left overnight at room temperature. They were then cured in an oven at 100°C for 2 hours then at 150°C overnight. The electrode surface was polished on a beveling wheel (K.T.Brown Type; Sutter Instrument Co. Model BV-10, Novoto, CA) at an angle of 30° producing an elliptical electroactive surface. Electrical connection was made by back filling the capillary with a high ionic strength solution (4 M potassium acetate, 150 mM potassium chloride). The electrodes were soaked in 2-propanol for at least 10 min prior to use.

Chemicals. Adenosine, dopamine, ATP, AMP, guanine, and inosine were purchased from Sigma-Aldrich (Milwaukee, WI) and all other chemicals were purchased from Fisher scientific (Suwanee, GA) and used as received. Ten mM stock solutions were prepared in 0.1 M perchloric acid and diluted to the desired concentration in tris buffer on the day of the experiment. The tris buffer solution was (in mM) 15 Tris(Hydroxymethyl)aminomethane, 140 NaCl, 3.25 KCl, 1.25 CaCl₂, 1.25 NaH₂PO₄, 1.2 MgCl₂ and 2.0 Na₂SO₄ with the pH adjusted to 7.4. All aqueous solutions were made by using deionized water (Milli-Q Biocel, Millipore, Billerica, MA).
Instrumentation and electrochemistry. Fast scan cyclic voltammograms were collected using a GeneClamp 500B potentiostat (Molecular Devices, Union City, CA with a custom-modified headstage). The data acquisition software and hardware were the same as described by Heien et. al. Briefly, two computer interface boards, National Instruments PCI 6052 and PCI 6711 (Austin, TX), were used to apply the triangular waveform and collect the resultant current data through a breakout box. For detection of adenosine, the electrode was scanned from -0.4 to 1.5 V and back at 400 V/s every 100 ms. The reference electrode was a silver-silver chloride electrode.

Flow Injection analysis. The carbon-fiber electrode was positioned at the output of a flow injection apparatus consisting of a six-port, stainless steel HPLC loop injector mounted on a two-position air actuator (Valco, Houston, TX). The sample was loaded into a 500 µl sample loop before the experiment, then the air actuator would turn the valve to allow sample to flow by the electrode. The air actuator was controlled by a solenoid valve mounted to a digital voltage interface (DVI, Valco) which received digital signals from the breakout box. The buffer was pumped through the flow cell at 2 mL/min using a syringe pump (Low RFI Syringe Pump 22, Harvard Apparatus, Holliston, MA). Three second injections of the compounds were made to mimic fast concentration changes that occur in the brain.

All carbon-fiber electrodes were first tested by injecting dopamine, a compound with known electrochemistry. Current vs time traces were obtained from cyclic voltammetry by integrating the current in a 100 mV window centered around the oxidation peak for each cyclic voltammogram. Cyclic voltammograms were background-subtracted by averaging 10 background scans taken directly before the compound was injected into the flow cell.

Monitoring enzyme activity. Adenosine deaminase in 3.2 M ammonium sulfate (Sigma) had an activity of 1.5 units/µl. The stock enzyme was diluted 100 fold in Tris buffer and then 1 µl of the diluted enzyme was added to 5 ml of 10 µM adenosine in buffer. Aliquots were analyzed by flow injection analysis every minute.
Statistics. All values are given as the mean ± SEM. All statistics were performed in GraphPad Prism. Limits of detection were defined as a S/N ratio of 3.

RESULTS AND DISCUSSION

Adenosine cyclic voltammetry. With fast scan cyclic voltammetry, concentrations are sampled by repeating scans at regular intervals, and the current arises from analyte oxidation or reduction. For adenosine detection, the electrode was ramped from a resting potential of -0.4 to 1.5 V vs Ag/AgCl and back at 400 V/s every 100 ms as shown in Figure 1A. These fast scan rates produced a background charging current that was much larger than Faradaic currents. The background current of a T-650 disk electrode with (dashed) and without (solid) 5 µM adenosine is shown in Fig 1B. The background current is relatively stable over time and can be subtracted out, resulting in a background-subtracted cyclic voltammogram for 5 µM adenosine (Fig. 1C). Two oxidation peaks were observed, the main oxidation near the switching potential at 1.5 V (peak 1) and a secondary oxidation at approximately 1.0 V (peak 2). The oxidation appeared irreversible with no detectable reduction peaks. Detection of a low, physiological concentration, 200 nM, is shown in Fig 1D. The main oxidation peak at the switching potential is still easily observable.

Characterization of adenosine electrochemistry. Concentrations of adenosine were varied between 0.2 µM and 40 µM to determine the linear range of detection. The plot of peak current vs. concentration (Fig. 2A) shows linearity up to 20 µM with similar behavior for both oxidation peaks. The limits of detection for adenosine at carbon-fiber microelectrodes were estimated from the 200 nM cyclic voltammograms. Assuming a detection limit of three times the noise, the limit of detection was 14 ± 5 nM (n=8). This is 100 times lower than the previous report of adenosine detection at carbon-fiber microelectrodes and similar to studies performed with
diamond microelectrodes \textsuperscript{24} and enzyme electrodes \textsuperscript{15}. Basal adenosine concentrations are estimated to be 50 to 200 nM in the brain \textsuperscript{1}, so these limits of detection should be sufficient to measure changes from basal concentrations.

To test whether the detection of adenosine was adsorption or diffusion controlled, the scan rate was varied between 200 and 1200 V/s. The peak oxidation current for 5 µM adenosine was linear with scan rate, not the square root of scan rate, indicating the kinetics are adsorption controlled (Fig. 2B). As shown in Fig. 2A, the adsorption-controlled behavior extends until 20 µM. At higher concentrations, adsorption sites would be saturated and the behavior more diffusion controlled. This is consistent with the detection of other electroactive molecules at carbon-fiber microelectrodes, such as dopamine, which are also adsorption-controlled at low concentrations \textsuperscript{25}. Polished carbon surfaces have a significant amount of oxides \textsuperscript{26} and these sites are generally favorable for adsorption. Adsorption is likely due to molecular interactions of the adenine group with surface oxide groups. Adenosine has also been previously shown to adsorb well to conventional pyrolitic graphite electrodes \textsuperscript{27}.

Adsorption of adenosine to the electrode is due to the negative holding potential. Therefore, varying the holding potential should affect the amount of adsorption and the current measured. Indeed, the current increased as the holding potential was lowered from 0 to -0.4 V (Fig. 3A). However, lowering the holding potential to -0.6 did not increase the current for the first oxidation peak. An explanation for this is that the background current is less stable when lower holding potentials are used. Background subtraction errors are especially apparent at the switching potential where the change in current is steepest. Previous studies for dopamine detection have used -0.6 V holding potentials, but erroneous peaks due to background subtraction errors around the switching potential were present \textsuperscript{20}. Those switching potential errors do not affect the potentials where dopamine is detected but do interfere with adenosine observation.
The amount of adsorption is also controlled by the time spent at the holding potential. Using a holding potential of -0.4 V, the frequency of waveform application was varied (Fig. 3B). The higher frequencies resulted in lower currents because less time was available for adsorption at the holding potential. The 100 Hz frequency results in nearly continuous application of our waveform and the detection limits were substantially less than the normal 10 Hz application. The previous reports of cyclic voltammetry detection of adenosine at a carbon-fiber microelectrode achieved only 5 µM detection limits. Their method applied a cyclic voltammetry waveform from -1.0 to 1.5 V continuously, which would not allow time for adsorption. In contrast, applying the waveform only 5 or 10 times a second allows time for adsorption. Maximizing adsorption is the key to achieving low limits of detection.

Stability and selectivity. An ideal in vivo sensor responds identically to a concentration change every time. To test the stability, we repeatedly exposed the electrode to boluses of adenosine and measured the resultant currents (Fig. 4). The stable response indicates that the oxidation products were not fouling the electrode and the electrode is suitable for repeated measurements. The relative standard deviation for 10 injections was only 2 %, indicating good reproducibility.

Other compounds could act as interferents for adenosine detection in biological samples. For example, the adenosine nucleotides AMP and ATP are energetic molecules found in abundance in biological systems. ATP is the major energy unit of cells and in the brain, it can be released from vesicles as a neurotransmitter. Catabolism of ATP yields AMP and then adenosine. Fig. 5B and C show that the currents for 5 µM AMP and ATP are much smaller than for adenosine (Fig. 5A). The addition of phosphate groups gives these molecules a negative charge, which would cause repulsion at the negative holding potential of the electrode. ATP, with three phosphate groups, is more negatively charged and therefore is least likely to be adsorbed to the electrode. On average, the oxidation current for AMP was 36 ± 10% and for
ATP was 14 ± 5% of the current for adenosine. Therefore, the interference from ATP \textit{in vivo} is expected to be minimal. Dopamine is well characterized at carbon-fiber microelectrodes and the oxidation peak at 0.6 V indicates it would not interfere with adenosine detection (Fig. 5D). Similarly, guanine, another purine, had an oxidation potential of 1.1 V, which is 0.4 V lower than the oxidation potential for the main adenosine oxidation peak (Fig. 5E). Inosine, a product of adenosine metabolism, was not electroactive at these potentials and therefore would not be an interferent (Fig. 5F). The inability of carbon-fiber microelectrodes to detect inosine is an advantage over previously constructed enzyme sensors that were equally sensitive to adenosine and inosine \textsuperscript{15}.

**Mechanism of adenosine oxidation.** The adenine moiety undergoes oxidation and reduction in the adenosine molecule. Previous studies using larger electrodes and slower electrochemical methods have detected one peak for adenosine with a peak potential of 1.3 V vs Ag/AgCl \textsuperscript{12}. However, we observed two oxidation peaks for adenosine at T-650 electrodes, one at 1.5 V and one at 1.0 V. Oxidation potentials are often shifted in time (i.e. appear at higher potentials) in fast scan cyclic voltammetry because of the time necessary for electron transfer \textsuperscript{23}. Therefore, the peak at 1.5 V is likely to correspond to the literature peak for adenosine at 1.3 V.

To investigate the mechanism of formation of two peaks, the current versus time traces were examined for a flow injection analysis experiment (Fig. 6A). The current at each oxidation peak is plotted in the large graph and then normalized current in the inset. The current from the first oxidation peak at 1.5 V increased before the current from the second oxidation peak at 1.0 V. For example, when adenosine was first introduced to the electrode, the current from the first peak (solid triangles) was clearly elevated at 3.5 s while the current from the second oxidation peak was still at baseline. This indicates that the second oxidation peak is a secondary reaction and involves oxidation of a product made on the first scan. Indeed, the cyclic voltammograms confirm this because the first cyclic voltammogram after adenosine was introduced to the
electrode contained no secondary oxidation peak since the precursor oxidized species was not present. However, both oxidation peaks were present at 4 s, when the current was near its maximum.

The switching potential was also varied to demonstrate that the peak at 1.0 V is a secondary oxidation product (Fig. 6B). When the potential was extended to 1.5, two peaks were detected. But when the potential was swept to only 1.3 V, neither peak was observed, even though the potential was higher than 1.0 V where peak 2 appeared. This indicates that the second oxidation peak cannot be detected unless the primary oxidation at 1.5 V occurs.

Adenine is known to undergo a series of 2 electron oxidations\(^1\). Each product formed is easier to oxidize, so oxidation potentials should be less positive for each successive step. These data suggest the following scheme (scheme 1). At pH 7.4, the first 2 electron oxidation of adenosine results in the peak at the switching potential, 1.5 V. On subsequent scans, that oxidation product (II) can be oxidized again by another 2 electron oxidation, which results in the peak at 1.0 V. Both oxidations are adsorption controlled and the smaller current for the secondary reaction may indicate that the species is not as tightly adsorbed as adenosine. Some of II may also be oxidized on the scan back down when the potential is still sufficient for oxidation. Note that the current does not come back to 0 until after 1.0 on the back scan in Fig. 1C, indicating a species is still being oxidized.

Our fast scan data are different than slow-scan cyclic voltammograms that contain only one oxidation peak, present at 1.3 V, for a 6 e\(^-\) oxidation of adenosine. However, with slow-scan rates of 100 mV/s, the electrode would be at a potential sufficient to oxidize adenosine for several seconds. With fast scan cyclic voltammetry, the electrode is at a potential sufficient for the first step of oxidation for only 0.5 ms each scan. Insufficient time for the sequential reactions to occur on that scan would allow detection of a separate peak on the subsequent scan. Indeed, complete coulometry of adenosine at a pyrolitic graphite electrode took nearly 2 days to complete\(^1\).
A peak representing the oxidation of III to IV was not detected using T-650 disk electrodes. However, when using a different type of carbon fiber, P-55, a third peak was detected around 0.5 V (Fig. 6C). Two reduction peaks are also detected at 0.25 V and -0.05 V. The reduction peaks are believed to be due to the second and third redox reactions in the scheme, because no reduction peak is present in the very first cyclic voltammogram collected when adenosine is introduced into the flow cell. These results show that different types of carbon-fiber electrodes have different properties for electron transfer. Glassy carbon and pyrolytic graphite have also been shown to have different electron transfer properties for adenosine at traditional millimeter sized electrodes. The two carbon fibers we used are made of different precursor materials, T-650 fibers from polyacrylonitrile carbon and P-55 fibers from pitch. The geometry of the basal and edge planes may be different between the two fibers or the extent of surface oxide functionalization may differ. The adenosine oxidation products might be more stably adsorbed to the P-55 electrodes or the geometry of the carbon might facilitate electron transfer and detection of this third peak.

The last two oxidation steps for adenosine are very similar to the oxidation of guanine. Guanine (2-amino-6-hydroxypurine) has an oxy group at position 6 so oxidation gives a dioxyproduct, similar to III for adenosine. This product can then be reversibly oxidized to a diimine. For guanine, we also observe two peaks, at 0.6 V and 1.1 V (Fig. 5E), similar to peaks for the 2nd and 3rd oxidation steps for adenosine at a P-55 electrode.

**Monitoring an enzymatic reaction.** The utility of the carbon-fiber microelectrode to monitor a biological reaction is shown in Fig.7. Adenosine deaminase, an endogenous enzyme that metabolizes adenosine to inosine, was added to a solution of 10 µM adenosine. Every minute, an aliquot of the mixture was analyzed with flow injection analysis. Inosine is not electroactive (Fig. 5F) so the extent of reaction is observed by a decrease in the current for oxidation of adenosine. Adenosine was completely degraded within 4 min. This is in good agreement with
the stated enzyme activity that predicted the adenosine should be digested in 3.3 min. After the
experiment, the stock 10 µM adenosine solution was analyzed again and the current was 97± 5
% of the initial adenosine signal, indicating that the electrode was not fouled and could still
detect adenosine. This experiment shows that fast changes in adenosine produced in biological
environments should be able to be monitored and that adenosine metabolites do not interfere
with its detection.

Conclusions
A rapid, sensitive method has been developed for detection of
physiological concentrations of adenosine. The low nM detection limits are less than basal
adenosine levels in tissue and should enable use monitoring biological functions. In the brain,
adenosine is reported to increase after a stroke but exact concentration changes and the
potential effects for adenosine receptors are not known. An adenosine sensor would give
insight into the mechanism of adenosine release and the neuroprotective functions of
adenosine. Adenosine also has cardioprotective properties that may protect heart tissue during
ischemia, a lack of blood flow. The release of adenosine from myocardial cells could be
monitored under conditions of oxygen depletion to determine the actual levels of adenosine.
The adenosine sensor would also be useful for monitoring the adenosine concentrations
produced by drugs that potentiate adenosine release. The applicability of this sensor for
physiological experiments will allow real-time measurements of adenosine concentrations in
normal and disease states.

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FIGURE CAPTIONS

Figure 1. Example cyclic voltammetry of adenosine. A. The applied potential was scanned from -0.4 to 1.5 V and back at 400 V/s every 100 ms. B. This rapid scanning produced a large background charging current at the electrode (solid line). The current after 5 µM adenosine was added (dashed line) changed only slightly. C. An example background-subtracted cyclic voltammogram of 5 µM adenosine at physiological pH, 7.4, an average of ten cyclic voltammograms. Two oxidation peaks were detected: peak 1 at the switching potential, 1.5 V, and peak 2 around 1.0 V. D. An example cyclic voltammogram of a low, physiological concentration of adenosine, 200 nM. The main oxidation peak at the switching potential was still easily observable.

Figure 2. Characterization of adenosine electrochemistry. A. Effect of adenosine concentration on current. Peak oxidation currents are plotted for the first (triangles) and second (circles) oxidation peaks (at 1.5 and 1.0 V in the cyclic voltammogram, respectively). Error bars are the standard error of the mean (SEM) for 8 electrodes. The isotherms are linear up to 20 µM and the regression values for the linear portion were $r^2=0.98$ for the first peak and $r^2=0.95$ for the second peak. B. Scan rate dependence. Scan rate was varied from 200 to 1200 V/s and the resultant oxidative currents measured for detection of 5 µM adenosine. Current was linear with scan rate. For peak 1, $r^2$ was 0.99 while for peak 2, $r^2$ was 0.98. Error bars are the SEM ($n = 8$).

Figure 3. Evidence for adsorption. A. The holding potential was varied between 0 and -0.6 V and peak oxidative current for 5 µM adenosine is plotted for peak 1 (triangles) and peak 2 (circles) ($n=8$). B. The frequency of repeating the cyclic voltammetry waveform was varied from 5 to 100 Hz. The peak current ± SEM is plotted for oxidative peak 1 (triangles) and 2 (circles) ($n=8$).
Figure 4. Repeated exposure of a carbon-fiber microelectrode to 5 µM adenosine. Stable oxidation currents were observed for the main oxidation peak for 10 consecutive flow injection exposures of the electrode to adenosine. The relative standard deviation between peak heights was 2%.

Figure 5. Cyclic voltammograms of possible interferents. Cyclic voltammograms are plotted for A. 5 µM adenosine, B. 5 µM adenosine monophosphate, C. 5 µM adenosine triphosphate, D. 5 µM dopamine, E. 5 µM guanine and F. 5 µM inosine.

Figure 6. Mechanism of adenosine oxidation. A. Current versus time traces for the peak oxidative currents (triangles, peak 1; circles, peak 2) for 5 µM adenosine. In this flow injection analysis experiment, the three second exposure of the electrode to adenosine began at 3.5 s. The inset shows normalized current for each peak (maximum value is 100 for each curve). The left cyclic voltammogram, at the 3.5 s time point, demonstrates only the first oxidation peak was detected on the first scan adenosine was present. The right cyclic voltammogram was taken at 4.0 s when both peaks were nearly at the maximum. B. Cyclic voltammograms of 5 µM adenosine when the switching potential was 1.3 (dashed line) and 1.5 (solid line). Only the forward, oxidative scan is shown for clarity. No oxidation peaks are detected when the potential is only scanned to 1.3 V. C. Cyclic voltammogram of 5 µM adenosine at a P-55 disk electrode. Three oxidation peaks and two reduction peaks were detected.

Figure 7. Monitoring an enzymatic reaction. Adenosine deaminase (0.015 units) was added to 5 ml of 10 µM adenosine. Every minute, an aliquot was analyzed using flow injection analysis and the concentration of adenosine measured by monitoring the peak current at 1.5 V. The results are a plot of the normalized current before addition of enzyme. Adenosine was completely degraded within 4 minutes.
Scheme 1. Sequential oxidation steps for adenosine. R is a ribose unit.


Figure 1
Figure 2
Figure 3
Figure 4
A. Adenosine

D. Dopamine

B. AMP

E. Guanine

C. ATP

F. Inosine

Figure 5
Figure 6
Figure 7
Scheme 1