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Nafion-CNT Coated Carbon-Fiber Microelectrodes for Enhanced Detection of Adenosine

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Abstract

Adenosine is a neuromodulator that regulates neurotransmission. Adenosine can be monitored using fast-scan cyclic voltammetry at carbon-fiber microelectrodes and ATP is a possible interferent *in vivo* because the electroactive moiety, adenine, is the same for both molecules. In this study, we investigated carbon-fiber microelectrodes coated with Nafion and carbon nanotubes (CNTs) to enhance the sensitivity of adenosine and decrease interference by ATP. Electrodes coated in 0.05 mg/mL CNTs in Nafion had a 4.2 ± 0.2 fold increase in current for adenosine, twice as large as for Nafion alone. Nafion-CNT electrodes were 6 times more sensitive to adenosine than ATP. The Nafion-CNT coating did not slow the temporal response of the electrode. Comparing different purine bases shows that the presence of an amine group enhances sensitivity and that purines with carbonyl groups, such as guanine and hypoxanthine, do not have as great an enhancement after Nafion-CNT coating. The ribose group provides additional sensitivity enhancement for adenosine over adenine. The Nafion-CNT modified electrodes exhibited significantly more current for adenosine than ATP in brain slices. Therefore, Nafion-CNT modified electrodes are useful for sensitive, selective detection of adenosine in biological samples.

Introduction

Adenosine is an important nucleoside signaling molecule formed by ATP degradation. It acts as a neuromodulator to regulate cerebral blood flow, modulate neuronal excitability, and control energy delivery to the brain.^{1–3} Adenosine also protects against neuronal damage caused by oxidative stress and has been studied for possible protective effects in hypoglycemia, hypoxia, and ischemia.³ Traditionally, adenosine has been thought to act on a slow time scale and changes lasting minutes to hours have been measured *in vivo* with microdialysis sampling coupled to HPLC.^{4,5} However, faster adenosine changes have recently been detected *in vivo* and in brain slices using both fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes and amperometric enzyme sensors.^{6–8} FSCV can detect changes on the millisecond timescale which provides high temporal resolution for measuring transients *in vivo*. Detection of transient adenosine release is challenging because evoked adenosine changes are low, around 100–300 nM, and other purine species might interfere.⁷ In particular, ATP and adenosine have similar oxidation potentials and cyclic voltammograms (CVs) because the adenine moiety is the electroactive species in both molecules. Other purines such as guanine and hypoxanthine are similar in structure but have different electrochemistry.⁹ The development of an electrode with enhanced sensitivity and selectivity for adenosine will allow more reliable adenosine detection.

Carbon-fiber microelectrodes modified with Nafion have been used extensively to increase sensitivity to neurotransmitters such as dopamine and serotonin, but have not been used to

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study adenosine or ATP.^{10–14} Nafion is a permselective polymer that enhances sensitivity to cations and decreases sensitivity to anions.¹¹ At physiological pH, ATP is negatively charged while adenosine is neutral. Thus, coating carbon-fiber microelectrodes with Nafion is expected to increase the discrimination of adenosine from ATP because of the charge repulsion between Nafion and ATP. Different coating methods have been explored for depositing Nafion onto carbon-fiber microelectrodes and dip-coating and electropolymerization are the most common.^{10,15} Dip-coating has traditionally been used on disk electrodes, due to the limited surface area the Nafion polymer needs to adsorb,^{14,16} but cylinder electrodes can be dip coated using longer dip coating times.^{17,18}

Carbon nanotubes (CNTs) have also been used to modify carbon-fiber microelectrodes to enhance detection of neurotransmitters.^{19–22} CNTs offer unique chemical, electrical, and structural properties which can increase sensitivity and promote electron transfer kinetics.^{23–25} To purify and functionalize CNTs, treatments with strong oxidizing acids are used.²⁶ The resultant oxygen containing functional groups located on the sidewall defects and ends of CNTs change the overall hydrophobicity which affects the suspension of the nanotubes in various solvents.²⁷ CNTs are often dispersed in low concentrations of Nafion and deposited on electrodes to study the effects of CNTs on electrode sensitivity.^{19,28,29} Vertically aligned CNTs supported on thin Nafion-iron oxide layers have also been reported, although in this case the Nafion just aids in depositing iron oxide on the surface and is not deposited in a thick enough layer to exclude anions.^{30,31} Combining CNTs with higher concentrations of Nafion increases sensitivity for dopamine due to the combination of the preconcentration of the analyte in the Nafion and higher surface area of the CNTs.³² However, the combination of Nafion and CNTs has not been studied for purines such as adenosine.

In this study, we developed modified carbon-fiber microelectrodes with Nafion and CNTs to enhance the sensitivity for adenosine and decrease the interference by ATP. Using FSCV, we found that Nafion-CNT coatings increased the sensitivity for adenosine four-fold, which was twice as much as Nafion alone. Adenosine had higher sensitivity enhancements than adenine, showing an effect of the ribose unit, and enhanced sensitivity over guanosine or inosine, two nucleosides that contain carbonyl groups. Nafion-CNT electrodes had significantly higher ratios of current for adenosine than for ATP compared to bare electrodes after exogenous application of analyte in brain slices. Thus, the enhanced sensitivity for adenosine *in vitro* translates to improved performance in brain slices.

Methods

Solutions and chemicals

Purine, histamine, and dopamine standards were purchased from Sigma, dissolved in 0.1 M HClO₄ for 10 mM stock solutions and diluted daily in Tris buffer for testing. All compounds were tested at 5 μ M except dopamine, which was 1 μ M. The Tris buffer solution was 15 mM Tris(hydroxymethyl)aminomethane, 1.25 mM NaH₂PO₄, 2.0 mM Na₂SO₄, 3.25 mM KCl, 140 mM NaCl, 1.2 mM CaCl₂, and 1.2 mM MgCl₂ with the pH adjusted to 7.4 (all Fisher, Suwanee, GA). For in situ experiments, calibrations were performed in aCSF: 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH₂PO₄, 2.4 mM CaCl₂ dehydrate, 1.2 mM MgCl₂ hexahydrate, 25 mM NaHCO₃, 11 mM glucose, and 15 mM tris (hydroxymethyl) aminomethane, pH 7.4. All aqueous solutions were made with deionized water (Milli-Q Biocel, Millipore, Billerica, MA).

Preparation of microelectrodes

Carbon-fiber microelectrodes were fabricated from T-650 carbon-fibers (gift from Cytec Engineering Materials, West Patterson, NJ).²⁹ Detailed methods are in the supplemental methods. The Nafion coating procedure was adapted from Wiedemann *et al* and Hashemi *et al*.^{10,15} The electrode was dipped in a 5 wt % Nafion solution in methanol (Ion Power, New Castle, DE) for 5 minutes, air dried for 10 s, then baked in an oven at 70°C for 10 minutes. Electrodes were stored overnight at room temperature prior to use. To modify the electrode surface with Nafion and CNTs, the functionalized CNTs were suspended in the 5 wt % Nafion solution with a tissue sonication probe for 60 minutes. High pressure carbon monoxide conversion (HiPco) single-walled CNTs (Carbon Nanotechnologies, Houston, TX) were functionalized by a procedure adapted from Wei *et al*³³ that was described previously (see supplement for details).³² The coating procedure for the Nafion-CNT was the same as for the Nafion modified electrodes.

Scanning Electron Microscopy

Scanning electron microscopy images were taken on a JEOL JSM-6700F microscope (Tokyo, Japan) by using an accelerating voltage of 10 kV and a working distance of approximately 6 mm. Electrodes were sputter-coated before imaging with carbon (PECS, 682, Gatan Inc, Pleasanton, CA).

Brain slice experiments

All experiments were approved by the Animal Care and Use Committee of the University of Virginia. Male Sprague-Dawley rats (250–350 g) purchased from Charles River and brain slices were collected as previously described.⁷ The electrode position in the prefrontal cortex corresponded to approximately the following coordinates from bregma: AP: 4.20 mm, ML: 0.4 to 0.8 mm, and DV: –3.4 to –4.0mm.³⁴ A glass micropipette was pulled from a borosilicate glass capillary to an outer diameter of around 15 μ m and positioned in the tissue approximately 30 μ m away from the working electrode. The pipette was filled with 100 μ M adenosine and 60 pmol adenosine was pressure ejected (500–600 ms, 20–30 psi) using a Picospritzer III (Parker Hannifin Corp., Fairfield, NJ) 10 minutes after electrode implantation. A second injection was repeated 10 minutes later to ensure stability. Two pressure ejections of 60 pmol of ATP were performed 10 min apart, starting 10 minutes after the last adenosine application.

Statistics

All values are reported as the mean $\pm$ standard error of the mean (SEM). Paired t test were performed for comparing average bare electrode current to the average coated electrode current and for comparing electron transfer effects. All statistics were performed in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) and considered significant at the 95% confidence level ($p < 0.05$).

Results and Discussion

Surface Structure Characterization

The Nafion and Nafion-CNT modified electrodes were characterized using SEM to understand the surface coverage ($n=3$). Fig. 1 shows images which represent the overall characteristics observed for Nafion, 0.05 mg/mL CNT in Nafion, and 0.2 mg/mL CNT in Nafion modified electrodes. In Fig. 1A, the Nafion coating is not uniform but does cover part of the electrode sidewall. The lack of a uniform Nafion layer may be caused by the short dip time and using dip coating instead of electropolymerization.¹⁰ The spotty surface coverage from Nafion coating was consistent among all dip coated electrodes tested ($n=3$),

but the increases in current (*vide infra*) were relatively consistent, suggesting a similar layer was deposited each time. The Nafion-CNT modified electrodes (Fig. 1B and 1C) show CNT rod-like structures ranging from 30–50 nm in diameter covering the surface. Previously, polymers such as Nafion were shown to wrap CNTs and since individual single-walled CNTs are only 1–2 nm in diameter, the CNTs in Fig. 1B and C may be Nafion wrapped.^{25,35,36} Alternatively, the larger diameter may be a result of CNT bundle formation. Fig. 1B and Fig. 1C show differences in amount of CNT coverage on the electrode surface, demonstrating the 0.05 mg/mL CNT coated electrodes have fewer CNTs on the surface than the 0.2 mg/mL CNT coated electrodes. Eighty percent of electrodes coated with 0.05 mg/mL Nafion-CNT were usable electrodes, indicating that surface coverage was consistent with the dip coating method.

Nafion-CNT Carbon-Fiber Microelectrodes Increase Sensitivity to Adenosine

Fig. 2 shows representative CVs for 5 μ M adenosine (Fig. 2A) and ATP (Fig. 2B) on the same scale to illustrate the differences in sensitivity. Adenosine and ATP have similar electrochemistry as both contain an adenine group which is oxidized.⁹ When the electrode is scanned from –0.4 to 1.45 V and back, adenine can undergo a series of three, two-electron oxidations. The first oxidation potential of adenine is 1.3 V, however with FSCV the first oxidation peak of adenosine and ATP is observed around 1.40 V of the cathodic scan.^{9,37} The peak appears on the cathodic scan because of time required for electron transfer and the fast scan rate. When a switching potential of 1.5 V was used in previous experiments, the peak was observed at 1.5 V.³⁷ A slightly lower switching potential was chosen here because it produced more stable background currents and lessened the possibility of hydrolysis of water. The second oxidation peak for adenosine and ATP occurs at 1.0 V on the first anodic scan following the first oxidation, and the third oxidation peak is seldom seen at our cylindrical T-650 carbon-fiber microelectrodes. Quantitation for adenosine and ATP were performed at the first oxidation peak for all experiments.

The bare carbon-fiber microelectrodes had a 2.7-fold higher sensitivity for adenosine than ATP although the electrochemistry is similar (Fig. 2). At physiological pH, the phosphate groups on ATP are expected to be deprotonated, giving it a negative charge. The lower sensitivity for ATP is likely due to poor adsorption because of the negative charge or steric hindrance of the phosphate groups that prevent the adenine moiety from being properly aligned for oxidation.³⁸

Dip coating carbon-fiber microelectrodes in 0.05 mg/mL CNT in Nafion for 5 minutes increased the oxidation current for adenosine and ATP compared to bare carbon-fiber electrodes (Fig. 2). However, the increase for adenosine was larger than for ATP and the signal for adenosine was six times greater than the signal for ATP after Nafion-CNT coating. Nafion-CNT coating increased the sensitivity for adenosine and the ratio of current for adenosine compared to ATP. The electrochemical reactions observed for both adenosine and ATP on the Nafion-CNT coated electrode are primarily adsorption controlled (see Figure S1).

Optimization of CNT concentration

To test the effects of the amount of CNTs on the current detected, 0.05 mg/mL and 0.2 mg/mL CNTs suspended in Nafion were tested. In Fig. 3, the y-axis is a ratio of peak oxidation current of the coated electrode divided by the bare electrode. A ratio higher than 1.0 indicates an increase in current. As a control, electrodes were dip coated with only Nafion, which improved the current for adenosine 1.9 ± 0.3 fold but did not improve the signal for ATP. The data suggest that a thin Nafion layer is deposited onto the electrode, which causes a small increase for adenosine but not for the negatively-charged ATP. The oxidative current

increased more for adenosine than for ATP for both CNT concentrations tested (Fig. 3). For 0.05 mg/mL CNTs in Nafion, the coated electrode peak oxidation current was significantly greater than the bare electrode for adenosine (paired t-test $p < 0.005$) but not for ATP ($p=0.117$). The electrodes dipped in 0.2 mg/mL CNTs in Nafion did not have as large a signal improvement as for 0.05 mg/mL CNTs in Nafion and the increase was not significant for either adenosine or ATP (paired t-test, $p=0.284$ and $p=0.955$ respectively).

As a control, electrodes were also coated in 0.05 mg/mL CNTs in methanol, the solvent for Nafion, and the increases in sensitivity were minimal. These electrodes were noisy, possibly because of uneven coatings from poorly suspended CNT solutions. A greater increase in current for the lower CNT concentration in Nafion could be due to the stability of the Nafion-CNT solution, as the higher concentration of CNTs did not remain suspended for the entire length of coating and formed aggregates, which lead to noisier electrode.³⁹ On electrodes with thicker layers of CNTs, the electroactive sites on the CNTs may be harder to access. Thus, lower concentrations of CNTs were chosen for further analysis.

Selectivity, stability, and limit of detection (LOD) were evaluated for the optimized Nafion-CNT coated electrode. The signal for adenosine compared to ATP is three-fold higher for Nafion-CNT modified electrodes than bare electrodes, which allows improved confidence for *in vivo* use. The stability of the coated electrode was evaluated by observing the change in current over a three hour time period *in vitro*. The electrode sensitivity was 94 ± 2 % of original signal after three hours of continuous cycling ($n=3$). Biostability was also evaluated by observing the change in sensitivity after the Nafion-CNT electrodes were exposed to the brain slice for 1 hour. The coated electrodes maintained 72 ± 2 % of the original signal after slice exposure whereas bare electrodes had only 57 ± 7 % of their original signal ($n=4$ each). The LOD of the Nafion-CNT modified electrodes was 7 ± 2 nM for adenosine which was significantly different than the bare electrode LOD of 21 ± 3 nM ($p<0.01$ $n = 5$). The improvement in detection limit will allow more reliable detection of low nanomolar changes *in vivo*.

Time Response of Nafion-CNT Modified Electrodes

The response time of Nafion-coated and Nafion-CNT electrodes are compared to bare electrodes in Fig. 4. Normalizing to the same peak height (inset) allows a better comparison of the shape of the response. During flow injection analysis, a square plug of analyte flows by the electrode which ideally results in a square shaped, oxidation current versus time trace. However, the current versus time trace is not always ideal due to properties such as adsorption of the analyte to the surface and diffusion through the polymer to the electrode surface.⁴⁰ Nafion decreases temporal resolution because the analyte must diffuse across the layer⁴¹ and in Fig. 4, Nafion coating did slightly slow the response time to adenosine. Bare electrodes had an average 10% to 90% rise time of 1.2 ± 0.1 s which was significantly different than the Nafion only electrodes rise time of 1.9 ± 0.2 s ($p < 0.01$, $n = 6$). When the electrodes are coated with Nafion-CNTs, rise time does not change from the bare electrode (1.2 ± 0.2 s, $p=0.979$, $n = 24$). Therefore, the additional sensitivity of adding CNT to the Nafion did not come at the expense of slowing temporal resolution.

Investigation of other Electroactive Purines, Purine Derivatives, and Neurotransmitters

Table 1 reports average ratios of coated to bare peak oxidation currents to facilitate comparison of increases in sensitivity after Nafion-CNT coating for multiple species. Anions at physiological pH (ATP, AMP, uric acid) showed an attenuation in sensitivity because the Nafion and the negatively charged oxide groups on the CNTs cause repulsion of anions. However, a total repulsion is not observed, likely because the CNTs did increase the electroactive surface area and a complete layer of Nafion was not formed. The

improvements for ATP and AMP sensitivity are similar. Adenosine has the highest increase in sensitivity and the improvement was twice as big as that for dopamine. Adenosine signal improvement may be larger than dopamine because adenosine is a larger molecule and has more possible functional groups and nitrogen heteroatoms that could act as adsorption sites. Histamine and a basic pH change of +0.20 pH units were evaluated as other potential interferents. A two-fold increase was seen for histamine after Nafion-CNT coating whereas no increase was seen for the basic pH shift (Fig. S2, $n = 3$).

To study structural effects on sensitivity, purine bases and their corresponding nucleoside were compared: adenine and adenosine, guanine and guanosine, and hypoxanthine and inosine. Guanine is a purine base found in DNA like adenine and it contains a carbonyl group at the C6 position and an amine group at the C2 position, instead of at the C6 position as in adenine (see Fig. 5 for structures). Hypoxanthine is a xanthine derivative which has a carbonyl group at the C6 position like guanine; however, it does not contain an amine group. Inosine is the nucleoside of hypoxanthine and is also a downstream metabolite of adenosine.⁵ Fig. 5 shows an example CV for each analyte before and after Nafion-CNT coating and Figure S3 shows calibration curves for 50 nM to 10 μ M for all the analytes.

First, the amount of current observed was compared for each purine and corresponding nucleoside. In all cases, higher currents were observed at bare electrodes for each purine base compared to its nucleoside. Bare electrodes are about three to four-fold more sensitive to the purine base adenine than for adenosine (Fig. 5A, B and Fig. S3A, B), however guanine had only a slightly higher current than guanosine (Fig. 5C, D and Fig. S3C, D). The sensitivity for bare electrodes to hypoxanthine is lower than adenine or guanine, showing the absence of the amine group decreases sensitivity (note the difference in scale between Fig. 5A/C and E). The oxidation peak for inosine is not well defined, although the broad peaks on the cathodic scan increased with increasing inosine concentration, signifying they are due to inosine detection (Fig. S3F).

Second, the effect of different functional groups on purines was characterized. The initial sensitivity for adenine and guanine is similar at bare electrodes, although their oxidation peak potentials are different. The increase in current after Nafion-CNT coating is slightly higher for adenine than guanine (Table 1). After Nafion-CNT coating, lower improvements in sensitivity were observed for hypoxanthine than for guanine or adenine (Table 1). Comparison of changes in calibration slopes were also made, and the same trends were observed (see Figure S3 and Table S1). The data demonstrate that amine groups increase the oxidation current for purines, likely by increasing adsorption to the electrode, and that the presence of a carbonyl group decreases the sensitivity enhancements with Nafion-CNT coating.

Third, the effect of the ribose unit on sensitivity enhancement after Nafion-CNT was determined by comparing the purine bases and nucleosides. For guanosine and inosine, the enhancement in signal after Nafion-CNT was about the same as for their corresponding purine (Fig. 5 and Table 1). However, adenosine had two times the increase in sensitivity of adenine, when comparing current ratios and about a 1.5 fold increase in sensitivity over adenine when comparing changes in calibration slopes, indicating a positive effect of the ribose unit. The compounds with the carbonyl group showed no difference for improvement for purines and nucleosides. For adenosine, interactions between the ribose group and the CNTs, particularly repulsion by the oxide groups, might facilitate better orientation of the adenine group on adenosine and enhance oxidation current.

Adenosine vs ATP detection at Nafion-CNT Modified Electrodes in Brain Slices

The sensitivity of the Nafion-CNT coated electrodes for adenosine over ATP was demonstrated in rat brain slices. Adenosine and ATP were pressure ejected into a slice from a micropipette positioned 30 μm away from the working electrode. Both a bare and Nafion-CNT coated electrode were tested in each slice, allowing the ratio of adenosine to ATP current to be determined for a bare and a Nafion-CNT coated electrode in each slice. Figures 6 shows the responses of a bare electrode (6A) and a Nafion-CNT electrode (6B) to exogenously applied adenosine and ATP in the same slice. The Nafion-CNT coated electrodes have a 5.1 ± 0.5 fold greater signal for adenosine than ATP while bare electrodes have a 2.1 ± 0.4 fold bigger signal for adenosine (Figure 6C). The Nafion-CNT electrodes had significantly higher ratios of adenosine to ATP current than bare electrodes in brain slices ($n=4$, $p<0.005$) and the increase in adenosine to ATP current is about the same as that seen *in vitro*, where the electrode was six times more sensitive for adenosine. The Nafion-CNT coated electrodes will facilitate future studies by increasing the sensitivity for adenosine over ATP in the brain.

Conclusions

Combining Nafion and CNTs on the surface of carbon-fiber microelectrodes provided enhanced sensitivity for adenosine and reduced interference by ATP. A lower concentration of CNTs was optimal for enhanced sensitivity for adenosine. Temporal response was not affected by Nafion-CNT coating which makes these electrodes ideal for *in vivo* use because they maintain a fast response. Comparing pairs of different purine bases shows that the presence of an amine group enhances sensitivity and that purines with carbonyl groups do not have as great an enhancement after Nafion-CNT coating. The ribose group provides additional sensitivity enhancement for adenosine over adenine, but this trend is not observed for other purines and nucleosides. Nafion-CNT electrodes detect adenosine with greater sensitivity than ATP in brain tissue as compared to bare electrodes. Overall, Nafion-CNT electrodes provide more sensitive and selective detection of adenosine which should facilitate more reliable detection of adenosine in biological systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A. Nafion

B. 0.05 mg/mL CNT in Nafion

C. 0.2 mg/mL CNT in Nafion

Figure 1.

Scanning electron microscopy (SEM) images of Nafion and Nafion-CNT modified carbon-fiber microelectrodes at 100,000x resolution. Scale bar is 100 nm. A.) Nafion modified electrodes show a non-uniform layer of Nafion on the surface of the cylinder electrode B.) 0.05 mg/mL CNT in Nafion. Electrodes coated in this solution have a lower density of CNTs than electrodes coated with C.) 0.2 mg/mL CNT in Nafion. CNTs appear as 30–50 nm rod-like structures. The large diameter is attributed to either CNT bundle formation or the effect of Nafion wrapping the CNTs.

Figure 2.

Nafion-CNT coating improves sensitivity. The electrode was scanned from -0.4 V to 1.45 V and back at 400 V/s. Nafion-CNT electrodes were fabricated by dip coating in 0.05 mg/mL CNTs in Nafion for 5 minutes. A.) A cyclic voltammogram (CV) of 5 μ M adenosine shows the response to a bare electrode (solid) and the same electrode after Nafion-CNT modification (dashed). The oxidative current increased about four-fold with Nafion-CNTs. B.) A CV of 5 μ M ATP shows only a two-fold increase in sensitivity after Nafion-CNT coating. The response for ATP is about 6-fold less than adenosine after Nafion-CNT coating.

Figure 3.

Average peak oxidation current ratios for adenosine at microelectrodes modified with Nafion, 0.05 mg/mL CNT in Nafion, or 0.2 mg/mL CNT in Nafion. The y-axis is a ratio of the oxidative current after coating to the current before coating. Nafion-CNT modified electrodes with 0.05 mg/mL (striped) show enhanced sensitivity compared to electrodes coated with 0.2 mg/mL (grey) or Nafion only (white). The ATP current ratio remains about the same for each treatment with CNTs yet decreases slightly after Nafion only coating. (n = 6 for Nafion and Nafion+0.2 mg/mL CNT and n = 18 for Nafion+0.05 mg/mL CNT).

Figure 4.

Current versus time profiles of A.) Nafion and B.) Nafion-CNT modified carbon-fiber microelectrodes for 5 μ M adenosine. The insets are normalized currents to illustrate differences in shape. For flow injection analysis, buffer is initially flowed by the electrode for 4 s, then analyte flowed for 4 s followed by buffer again. A) The time response after Nafion modification is slightly slower due to slower diffusion. B) Nafion-CNT modified electrodes did not show slower response after modification.

Figure 5.

Cyclic voltammograms of each purine base and nucleoside. Insets show structures for each analyte. A.) 5 μM adenine (primary oxidation potential 1.4 V), B.) 5 μM adenosine (1.4 V), C.) 5 μM guanine (1.0 V), D.) 5 μM guanosine (1.2 V), E.) 5 μM hypoxanthine (1.3 V) and F.) 5 μM inosine (1.2 V). Higher currents were seen for all purine bases for bare electrodes. All analytes show an increase in current after Nafion-CNT coating (dotted line).

Figure 6.

Brain slice data comparing bare and coated electrodes sensitivity for adenosine and ATP. Adenosine and then ATP was pressure ejected into the medial prefrontal cortex 30 μm from the recording electrode. A) CV of adenosine (dashed) and ATP (solid) at a bare electrode show the current for adenosine is two times more than ATP. B) The Nafion-CNT coated electrode exhibits five times more current for adenosine than ATP in the same slice as the bare electrode. C) Average adenosine to ATP current ratio in a slice for both bare and coated electrodes ($n=4$, ** $p < 0.01$, unpaired t test).

Table 1

Average Current Improvement for Common Purines, Purine Derivatives, and Neurotransmitters

Nafion-CNT/Bare	
Adenosine (5 μ M)	4.2 $\pm$ 0.9
AMP (5 μ M)	1.7 $\pm$ 0.7
ATP (5 μ M)	1.6 $\pm$ 0.2
Uric Acid (5 μ M)	1.3 $\pm$ 0.1
Dopamine (1 μ M)	2.3 $\pm$ 0.3
Adenine (5 μ M)	2.0 $\pm$ 0.3
Guanosine (5 μ M)	1.8 $\pm$ 0.1
Guanine (5 μ M)	1.7 $\pm$ 0.1
Inosine (5 μ M)	1.5 $\pm$ 0.2
Hypoxanthine (5 μ M)	1.4 $\pm$ 0.1

Average data are the ratio of modified peak oxidation current to bare peak oxidation current for each analyte. Values given are $\pm$ SEM for n = 18 for all analytes except for guanine and inosine (n = 12).