Diamond Microelectrodes for *In Vitro* Amperometry

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We are developing innovative microcapillary techniques for separation and electrochemical techniques for detection of neurotransmitters released from nerves studied *in vitro* and *in vivo*. The major goals of the research are (i) to develop and apply diamond microelectrodes for monitoring neurotransmitter release from nerves studied *in vitro*, (ii) to identify specific differences in the neural control mechanisms of arteries and veins, and (iii) to identify changes that occur in neural control of arteries and veins in an animal model of hypertension. At the heart of this research is the diamond electrode, which is hard, lubricious, low in surface oxygen, and chemically stable (1). This new electrode has the potential to significantly impact *in vitro* and *in vivo* electrochemical measurements because two important problems often encountered with commonly used carbon fiber microelectrodes are minimized: the electrode fouling by adsorbed biomolecules and the pH-dependent background voltammetric response. This advanced carbon electrode is being used to study the mechanisms responsible for increased arterial tone and diminished venous capacitance in hypertension.

Sympathetic nerve activity is the most important determinant of venomotor tone, and there is increased sympathetically-mediated arterial and venous constriction in hypertensives. This may be due to increased sympathetic neural activity, increased efficiency of sympathetic neurotransmission, or both factors. Increased release of the sympathetic neurotransmitter, norepinephrine (NE), could account for increased sympathetic venoconstriction in hypertension. Although changes in sympathetic neuroeffector mechanisms associated with hypertension have been studied extensively in arteries, there have been few comparable studies in veins. A major obstacle to understanding the details of altered noradrenergic neurotransmission is the ability to make measurements of NE near the site of release and under physiological conditions. A novel aspect of this work is the study of sympathetic neural control of venous function and how this neural control may be altered in hypertension.

We have begun to employ diamond microelectrodes for the *in vitro* measurement of NE release in the mesenteric arteries and veins from normotensive and DOCA-salt hypertensive rats. The following summarize the important findings thus far: (i) diamond microelectrodes are much more resistant to fouling *in vitro* than are carbon fiber microelectrodes as less than a 7% response attenuation has been observed during 4 hours of continuous use, (ii) diamond microelectrodes exhibit a linear dose-response curve for NE from 0.01 to 10 μM, (iii) diamond microelectrodes exhibit lower background currents than carbon fiber microelectrodes and exhibit response variabilities of less than 6% when used *in vitro* to detect NE at an artery or vein surface, (iv) found that the frequency response curves for stimulation-induced contractions of veins are to the left of those from arteries indicating that veins are more sensitive to the effects of sympathetic nerve activity, (v) correlated the NE release with the % constriction of the veins and arteries, and (vii) found that nerve-mediated contractions of arteries and veins are blocked, at least in part by α1-adrenergic receptor antagonists indicating that NE is a vasoconstrictor transmitter.

The paper will discuss the properties of diamond microelectrode and their application in *in vitro* amperometry.

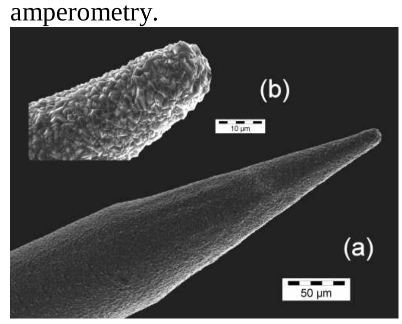

Figure 1. SEM images of boron-doped polycrystalline diamond deposited on a sharpened Pt wire.

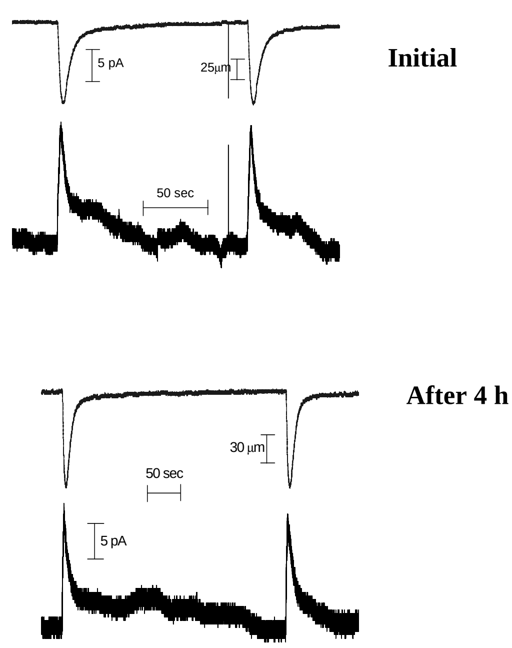

Figure 2. *In vitro* amperometric measurements of NE release from the mesenteric artery of a normotensive rat (bottom curve) and the corresponding constriction of the artery (top curve) after electrical stimulation. Detection was made with a diamond microelectrode at 0.8 V vs. Ag/AgCl in a flowing Krebs buffer, pH 7.4. The results show the corresponding artery constriction with NE release, the reproducibility of the current and constriction, and the diamond response stability over a 4 h period.

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References:

1. J. Cvacka, V. Quaiserova, J. Park, Y. Show, A. Muck Jr., and G. M. Swain, *Anal. Chem.*, 75, 2678 (2003).