Local sympathetic function in human skeletal muscle and adipose tissue assessed by microdialysis

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**Abstract**  
**Background** In response to stressors and pathophysiologic conditions, sympathetic neuronal outflows can change heterogeneously among body organs and tissues. This study examined the validity of microdialysis and measurements of microdialysate concentrations of catechols, to assess local sympathetic function in skeletal muscle and adipose tissue in humans. **Methods** Based on preliminary experiments, a microdialysate perfusion rate of 3 µl/min and collection duration of 30 minutes were chosen. To assess responses to a stimulus that increases sympathetic outflow to skeletal muscle, microdialysate norepinephrine and dihydroxyphenylglycol concentrations in quadriceps muscle, abdominal subcutaneous adipose tissue, and plasma were measured during orthostasis in 8 healthy normal volunteers. To assess responses to decreased post-ganglionic sympathetic nerve traffic, norepinephrine and dihydroxyphenylglycol concentrations were measured during i.v. infusion of trimethaphan in 5 volunteers. **Results** All subjects had detectable norepinephrine and dihydroxyphenylglycol concentrations in microdialysate from both skeletal muscle and adipose tissue, and plasma were measured during orthostasis in 8 healthy normal volunteers. To assess responses to decreased post-ganglionic sympathetic nerve traffic, norepinephrine and dihydroxyphenylglycol concentrations in microdialysate from both skeletal muscle and adipose tissue. Orthostasis significantly increased microdialysate norepinephrine in skeletal muscle (0.38 ± (SEM) 0.07 nmol/L supine to 1.48 ± 0.24 nmol/L standing, p < 0.01) and in adipose tissue (0.31 ± 0.02 nmol/L supine to 0.68 ± 0.11 nmol/L standing, p < 0.01). Orthostasis also increased microdialysate dihydroxyphenylglycol in both tissues (1.76 ± 0.30 nmol/L to 3.08 ± 0.43 nmol/L, p < 0.01; 1.37 ± 0.15 nmol/L supine to 1.99 ± 0.34 nmol/L standing, p < 0.01). Trimethaphan decreased norepinephrine concentrations in skeletal muscle microdialysate by 50%, adipose tissue by 70%, and antecubital venous plasma 50%, with non-significant decreases in dihydroxyphenylglycol concentrations at each site. **Conclusions** Microdialysate concentrations of norepinephrine and dihydroxyphenylglycol can be detected reliably and respond appropriately during manipulations that increase or decrease the sympathetically mediated release and turnover of norepinephrine. This approach may provide a means to assess sympathetic neuronal function in skeletal muscle and adipose tissue in humans with known or suspected dysautonomias.

**Key words** Norepinephrine · Sympathetic · Microdialysis · Skeletal Muscle · Adipose · Orthostasis · Trimethaphan
Introduction

According to the classical view propounded by Cannon [1], the “sympathoadrenal system” becomes activated homogeneously during emergencies threatening homeostasis, with no important role of the sympathetic nervous system under resting conditions [2]. Accumulating evidence over the past two decades, however, indicates that sympathetic neuronal activation occurs in a tissue- or organ-specific manner, including during non-emergency activities of daily life [3–8].

Measurement of concentrations of the sympathetic neurotransmitter, norepinephrine, in interstitial fluid sampled using microdialysis provides a potential means to assess sympathetic activity within specific tissues. Microdialysis offers several advantages, including relative technical simplicity and the ability to carry out neuropharmacologic assessments by administering drugs via the perfusate.

Clinical research reports have described microdialysate norepinephrine concentrations in the setting of local administration of tyramine [9], desipramine [10], isoproterenol [11], or the nitric oxide synthase inhibitor N(G)-monomethyl-L-arginine [12]; systemic administration of epinephrine [13] or insulin [14]; and physiological manipulations such as cold exposure [15] and exercise [12]. Low microdialysate concentrations of norepinephrine in human tissues (typically only a few pg/fraction under resting conditions) strain the limits of detection of available assays. Studies to date have not examined whether manipulations that inhibit the sympathetically mediated release of norepinephrine decrease microdialysate norepinephrine concentrations, leaving open the possibility of a “floor effect,” below which norepinephrine would not be distinguishable from background noise. A floor effect would be especially relevant to assessments of local sympathetic function by clinical microdialysis in patients with known or suspected autonomic failure. Whether the relatively mild stressor of daily life, orthostasis, affects microdialysate norepinephrine concentrations has also not been reported.

In this study we used a sensitive assay method for catechols [16], to test whether clinical microdialysis can validly detect changes in local sympathetic function in skeletal muscle and adipose tissue. We measured microdialysate concentrations not only of norepinephrine but also of its main intraneuronal metabolite, dihydroxyphenylglycol. Simultaneous measurements of norepinephrine and dihydroxyphenylglycol provide information about specific aspects of sympathetic function, such as norepinephrine release, reuptake, turnover, and metabolism, better than either measurement alone [17, 18].

To assess effects of a manipulation that increases exocytotic release of norepinephrine, we used orthostasis, a classical stimulus that decreases afferent inhibitory traffic to the brain from baroreceptors in the heart and major blood vessels and reflexively increases sympathetic outflow to skeletal muscle. To assess effects of a manipulation that decreases exocytotic release of norepinephrine, we used i.v. infusion of trimethaphan, which inhibits ganglionic neurotransmission by blocking ganglionic nicotinic cholinergic receptors and virtually abolishes bursts of sympathetic nerve traffic to skeletal muscle [19].

Methods

Subjects

The subjects were healthy normal volunteers. Each gave written informed consent to participate in the protocol, which was approved by the Intramural Research Board of the NINDS. All had unremarkable medical histories and physical examinations, were nonobese nonsmokers, and had not taken any medications for at least 5 plasma half-lives of that medication before the study. Eight volunteers (3 men, 5 women, mean age 38 ± 5 (SEM) years) participated in the study of orthostasis, and 5 (4 men, 1 woman, 46 ± 2 years) in the study of ganglionic blockade.

Microdialysis procedure

Microdialysate samples were obtained from quadriceps muscle and abdominal subcutaneous adipose tissue, as described previously [20–22]. A CMA/60 microdialysis probe (CMA, Stockholm, Sweden) with membrane length 3 cm and molecular weight cut-off 20 kD was inserted percutaneously after local anesthesia with intradermal 1% lidocaine. Each probe contained an inner cannula, to deliver perfusate into the tissue, and an outer cannula, to remove dialysate after equilibration with the surrounding interstitial fluid. Ringer’s lactate or normal saline solution was perfused through the probe, using a Medfusion 2010 syringe infusion pump (Medex Inc., Duluth, GA) and high pressure tubing (NAMIC, Glen Falls, NY).

Samples were obtained in 30-minute fractions into microvials placed on ice during the collection. In the study of orthostasis, each vial contained 20 µl of an acidic preservative (80% vol/vol of 0.1 M acetic acid, 20% vol/vol of 0.1 M phosphoric acid); in the study of ganglionic blockade, no preservative was used. Immediately upon completion of each 30-minute collection, each microvial was placed on dry ice to freeze the microdialysate. The samples were stored at −70 °C until assayed, usually within one month.

Antecubital venous blood was sampled from an indwelling i.v. catheter, with normal saline infused at a slow rate to keep the vein open between blood samples.

Calibration studies

An in vitro experiment was performed with a CMA/60 probe in a beaker that contained normal saline at room temperature. To the beaker were added 0.90 nmol/L of norepinephrine and 3.5 nmol/L of dihydroxyphenylglycol. Relative recovery was calculated from the concentration in the probe divided by that in the bathing solution [23]. Absolute recovery, in units of fmol/min, was calculated from the concentration of analyte recovered, in fmole/µL, multiplied by the perfusion rate, in µl/min.

In vivo calibration experiments were performed over approximately 8 hours in 5 normal volunteers. Each subject was tested during supine rest, with microdialysis probes in the abdominal adipose tissue and quadriceps skeletal muscle. The microdialysis perfusion