

Themed Issue: The Role of Microdialysis in Pharmacokinetics and Pharmacodynamics

Guest Editors - Markus Mueller and Ronald J. Sawchuk

## Microdialysis as a Tool in Local Pharmacodynamics

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### ABSTRACT

In many cases the clinical outcome of therapy needs to be determined by the drug concentration in the tissue compartment in which the pharmacological effect occurs rather than in the plasma. Microdialysis is an *in vivo* technique that allows direct measurement of unbound tissue concentrations and permits monitoring of the biochemical and physiological effects of drugs throughout the body. Microdialysis was first used in pharmacodynamic research to study neurotransmission, and this remains its most common application in the field. In this review, we give an overview of the principles, techniques, and applications of microdialysis in pharmacodynamic studies of local physiological events, including measurement of endogenous substances such as acetylcholine, catecholamines, serotonin, amino acids, peptides, glucose, lactate, glycerol, and hormones. Microdialysis coupled with systemic drug administration also permits the more intensive examination of the pharmacotherapeutic effect of drugs on extracellular levels of endogenous substances in peripheral compartments and blood. Selected examples of the physiological effects and mechanisms of action of drugs are also discussed, as are the advantages and limitations of this method. It is concluded that microdialysis is a reliable technique for the measurement of local events, which makes it an attractive tool for local pharmacodynamic research.

**KEYWORDS:** Microdialysis, pharmacodynamics (PD), neurotransmitter, glucose, hormone

### INTRODUCTION

Microdialysis is a catheter-based sampling technique that provides the opportunity to sample analytes from the interstitial fluid in tissues to measure the free pharmacologically active concentration of exogenous or endogenous com-

pounds at a site closer to the target site than plasma.<sup>1</sup> The method has been used extensively in animal and human studies for decades.<sup>1-5</sup> Pharmacodynamics (PD) is the study of the biochemical and physiological effects of drugs on the body. In a more quantitative sense, it describes the relationship between drug concentration and effect. The effects of pharmacological agents on biochemical processes can be inferred using endogenous compounds as biological markers; for example, lactate is a marker for glycolysis and glycerol is an indicator of lipolysis.<sup>2</sup> Physiological effects are often the parameter used to indicate a local effect of a drug; for example, blood flow is decreased in the presence of norepinephrine and norepinephrine but increased in response to urapidil.<sup>6</sup> Endogenous substances can also work as pharmacodynamic biomarkers to indicate the effects of drugs as well as further elucidate their mechanism of action in eliciting the physiological response.

Microdialysis requires sensitive analytical methods to measure low concentrations in small sample volumes. It is suitable for pharmacodynamic studies of complex interactions of drugs at their sites of action in intact living tissue, and it leaves endogenous metabolic and oxygenation pathways, as well as synaptic functions, largely intact.<sup>4,7</sup> *In vivo* microdialysis allows the direct assessment of endogenous substances and is used in PD studies alongside, or instead of, other sampling techniques, such as equilibrium dialysis,<sup>8</sup> ultrafiltration sampling,<sup>9-12</sup> saliva sampling,<sup>13-15</sup> and skin blister fluid sampling.<sup>14,16</sup> It has become an increasingly popular and powerful technique to determine extracellular concentrations of glucose, lactate, glycerol, hormones, and various neurotransmitters and/or their metabolites. This information is then used to assess the activities of various enzymes and the mechanisms of action of drugs.<sup>1-5,17-22</sup>

### MICRODIALYSIS

#### *Principle of Microdialysis*

Microdialysis is a sampling technique that is used to measure the concentration of the unbound fraction of endogenous and/or exogenous substances in the extracellular fluid of many tissues (eg, adipose tissue, brain, heart, lung, solid tumors).<sup>1,14,23</sup> It is applicable to both animal and human studies. The basic principle is to mimic the function of a

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capillary blood vessel by perfusing a thin dialysis probe with physiological fluid after it is inserted into the tissue of interest by means of a guide cannula. Continuous transfer of soluble molecules from the extracellular fluid into the probe occurs by means of a semipermeable membrane covering the tip of the probe. Samples are subsequently collected either (1) at intermittent time points for later analysis by standard chemical analytical techniques or (2) more recently, continuously for direct online analysis.<sup>1</sup>

### **Features of Microdialysis**

There are many advantages of microdialysis.<sup>1,14,24-27</sup> It is a straightforward technique that is not too difficult to establish on a routine basis. Microdialysis sampling does not change the net fluid balance of the surrounding sample matrix and provides clean samples in which analytes are separated from further enzymatic action. Because there is no net fluid loss, samples can be collected continuously for hours or days from a single freely moving animal. Most important, each animal can serve as its own control, so microdialysis requires fewer subjects than do some other methods, such as direct tissue assay. It can be used in humans in a relatively noninvasive manner. Moreover, microdialysis is easily coupled with other chemical analysis, such as high-performance liquid chromatography (HPLC),<sup>27,28</sup> mass spectrometry (MS),<sup>28,29</sup> capillary electrophoresis (CE),<sup>30,31</sup> and nuclear magnetic resonance (NMR).<sup>32,33</sup> These techniques are often combined (eg, HPLC-MS,<sup>28,34</sup> HPLC-NMR,<sup>33,35</sup> HPLC-NMR-MS,<sup>36-38</sup> CE-LIF (laser-induced fluorescence)<sup>30</sup>) to enhance the specificity, sensitivity, reliability, and efficiency of separation and detection.

As with any technique, there are limitations in the application of microdialysis.<sup>24-27</sup> Implantation of the probe almost certainly leads to tissue reactions that can interfere with the physiological system under investigation. To minimize this interference, optimal times after probe implantation must be determined specifically for the analyte of interest. For example, the optimal time to measure endogenous dopamine levels after probe insertion may differ from the optimal time to measure glutamate levels because of the continuing process of gliosis at the site of probe tissue damage. Another problem is that highly lipophilic drugs stick to tubing and probe components, complicating the relationship between dialysate and extracellular concentrations. Most important, microdialysis causes dilution of analyte levels in 2 ways. First, endogenous analyte levels may be decreased near the probe, leading to a tissue reaction and change in physiological status. Second, the diluting effect of the dialysis procedure leads to lower concentrations of analyte in samples compared with tissue, requiring both sensitive analytical methods and determination of *in vivo* recovery of the analyte to calculate true concentrations in the extracellular

fluid. This latter technique may impose temporal limitations (eg, the no-net-flux method may require hours of sampling) and may be confounded by physiological conditions that change over time.

## **MICRODIALYSIS AS A TOOL IN LOCAL PD**

### ***Analysis of Neurotransmitters Using Microdialysis***

There are more than 300 known neurotransmitters, including acetylcholine, norepinephrine, dopamine, serotonin (5-HT), amino acids, peptides, adenosine triphosphate, and gases (eg, nitric oxide). Neurotransmitters convey information within the brain and from the brain to all parts of the body. Measuring the changes in neurotransmitter extracellular levels in discrete brain areas can help identify the neuronal systems involved in specific behavioral responses or cognitive processes.<sup>39</sup> In 1974, Ungerstedt and Pycock first described the use of an osmotic membrane in the recovery of neurotransmitters.<sup>40</sup> However, it was not until the 1980s that it became possible to analyze brain chemistry quantitatively. Analysis of neurotransmitters and related substances in the dialysates from probes inserted into discrete brain areas has been extensively used to monitor extracellular levels of neurotransmitters, yielding a great deal of information about functional interactions of endogenous neurotransmitter systems.<sup>41</sup> Moreover, combined with either systemic or local delivery of drugs, microdialysis has been used to monitor drug-induced changes in neurotransmitters and other endogenous compounds, resulting in informative correlations between these levels and behavioral changes induced by psychotropic drugs, perhaps contributing to the elucidation of mechanisms of drug action. For example, interactions of biogenic amines with isozymes of monoamine oxidase, and interactions of biogenic amines and amino acids with each other, have been described using this technique.<sup>41,42</sup>

### ***Acetylcholine***

Acetylcholine (ACh) is the first neurotransmitter whose diffusion from the central nervous system was investigated and whose extracellular levels were correlated to changes in neuronal activity. ACh can be detected in the extracellular fluid even in the absence of cholinesterase inhibitors. However, to facilitate ACh quantification and reduce the duration of sample collection, a cholinesterase inhibitor is commonly added to the dialysis solution.<sup>39,43</sup> The dorsal striatum is a common target for early microdialysis studies because of its important role in locomotor and cognition function as well as its large size and easy accessibility to dialysis probes. Microdialysis has been used to demonstrate that dopamine directly inhibits striatal ACh release via D2 receptors in the striatum and indirectly stimulates ACh release via D1 receptors outside the striatum.<sup>27</sup> Since the

establishment of the microdialysis method in the striatum, pharmacological studies of ACh release in the terminal regions of the basal forebrain cholinergic system (BFCS)—the cortex, hippocampus, and amygdala—using microdialysis have become increasingly prevalent.<sup>27</sup>

In 1988, Maysinger et al detected striatal and cortical extracellular ACh and choline levels in samples collected using microdialysis coupled with a sensitive HPLC technique in normal and decorticated rats treated with saline or the monoganglioside GM1.<sup>44</sup> Picomole amounts of ACh could be measured when the acetylcholinesterase inhibitor (neostigmine) was included in the microdialysis perfusion medium. Maysinger et al found that GM1 treatment increased the ability of cortical cholinergic terminals to release ACh, allowing the brain to promote recovery following injuries to the central nervous system.<sup>44,45</sup> Watanabe and Shimizu found that the cholinergic antagonists atropine and scopolamine increased both striatal ACh release and motor activity, while the quaternary ammonium compounds atropine methylbromide and methscopolamine bromide increased striatal ACh release without motor excitation. These results demonstrated that anticholinergic drugs can cause an increase in striatal ACh release that does not always result in an increase in motor activity.<sup>46</sup> Since then, ACh release in the striatum has been shown to be stimulated by an orally active novel nicotinic agonist TC-1734,<sup>47</sup> an adenosine A2a receptor agonist,<sup>48</sup> N-methyl-D-aspartate (NMDA),<sup>49</sup> substance P,<sup>50,51</sup> estradiol, raloxifene,<sup>52</sup> the arousal-promoting peptide hypocretin,<sup>53</sup> and somatostatin<sup>54</sup>; and inhibited by gamma aminobutyric acid A receptor agonists,<sup>55</sup> adenosine A1 receptor agonist,<sup>56</sup> neuropeptide galanin,<sup>57</sup> and the general anesthetic isoflurane,<sup>58</sup> allowing for a much better understanding of the regulation of locomotor activity by the basal ganglia.

In many cases, drug delivery via reverse microdialysis (inclusion of the drug in the perfusing medium) either to the cholinergic terminal region being sampled or to other brain regions has elucidated the possible sites of action involved in regulating ACh release.<sup>27</sup> For example, administration of an NMDA antagonist to the cortex via reverse dialysis has no effect on cortical ACh release, whereas delivery of the drug to the cell body region of the BFCS neurons decreases cortical ACh release.<sup>43</sup> On the other hand, local administration of an NMDA antagonist to the lateral septum emulates the effects of intracerebroventricular injection to increase cortical ACh release.<sup>59</sup> Similarly, microdialysis using multiple probe implantation sites can be used to determine the anatomy of a given pharmacological effect.<sup>59-62</sup>

### Catecholamines

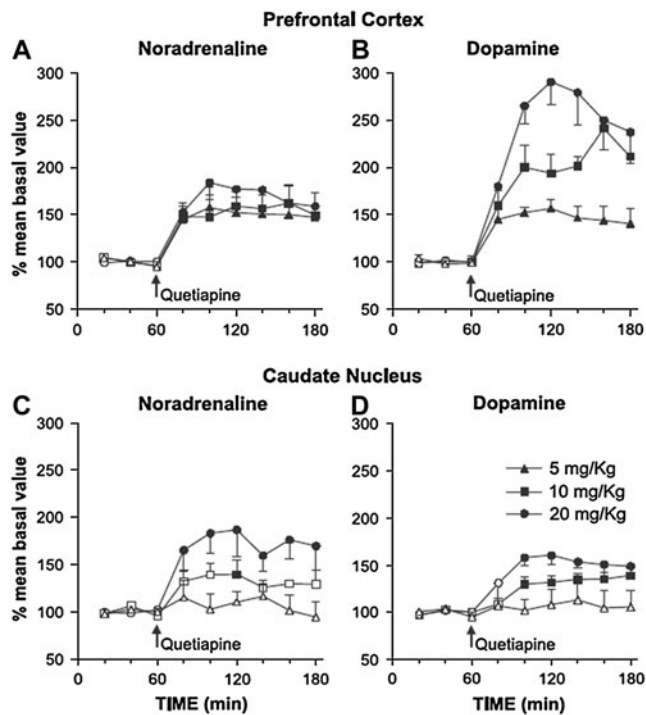
Microdialysis allows the in vivo measurement of catecholamines, such as epinephrine, norepinephrine (NE), and

dopamine (DA), aiding in the investigation of the mechanisms that underlie their local release and clearance as hormones and neurotransmitters. Zetterstrom et al<sup>63</sup> studied the effect of amphetamine on DA release and metabolism in 1983. Theirs was one of the first studies undertaken with the in vivo microdialysis technique. The microdialysis technique was used to compare the effect of amphetamine on the level of DA, NE, and their metabolites in the extracellular fluid of the striatum, nucleus accumbens (Nac), and lateral hypothalamus with alterations in behavior.<sup>64,65</sup> In 1992, Okuda et al used microdialysis to demonstrate increases in extracellular striatal DA levels and locomotor activity after deprenyl administration with L-dopa for the treatment of Parkinson's disease.<sup>66</sup> It was shown that deprenyl and *l*-methamphetamine (*l*-MeAmp) were both present in striatal dialysate at 10 and 60 minutes, indicating that both the deprenyl and the metabolically generated *l*-MeAmp contribute to the DA response.<sup>67</sup>

Recently, microdialysis has been used as a tool to measure extracellular DA and NE after atypical antipsychotic administration. Using in vivo microdialysis, Pira et al<sup>68</sup> found that quetiapine markedly increased extracellular levels of both NE and DA in the prefrontal cortex (Figure 1A and B) but had only a weak effect on DA levels in the caudate nucleus (Figure 1C). Quetiapine increased extracellular NE release in the caudate nucleus, as expected from its  $\alpha_2$ -adrenoceptor antagonist profile (Figure 1D). These findings might be related to the therapeutic action of quetiapine in schizophrenia and its low risk of inducing extrapyramidal side effects.

Stress-induced NE release in the medial amygdala, acting primarily through  $\alpha_1$  receptors, facilitates activation of the hypothalamic-pituitary-adrenal axis.<sup>69</sup> Kushikata et al<sup>70</sup> measured NE release by microdialysis in the preoptic area and the posterior hypothalamus before, during (30 minutes), and after (50 minutes) rats were anesthetized with isoflurane. They found that isoflurane increases NE release in these areas, suggesting that augmentation of NE release in the preoptic area is responsible for hypothermia during general anesthesia.

A large number of studies have focused on DA changes in the cell body and terminal regions of the mesolimbic and mesocortical pathways involved with drug reinforcement and addiction. Repeated use of cocaine produces behavioral sensitization that is associated with alterations in DA levels in the striatum,<sup>27,71,72</sup> the Nac,<sup>71-76</sup> and the medial prefrontal cortex.<sup>27,71</sup> Similarly, the different levels of morphine-induced locomotor hyperactivity found in 2 inbred mouse strains, DBA/2J and C57BL/6J, appear to be mediated by different sensitivity of both the dopaminergic and the serotonergic systems in the Nac and the dorsal striatum.<sup>72</sup> Ethanol reinforcement and subsequent consumption has been shown, primarily via the use of the microdialysis technique, to be regulated by the degree of neuronal firing in the mesolimbic



**Figure 1.** Effect of quetiapine (5, 10, and 20 mg/kg intraperitoneally) on extracellular noradrenaline and dopamine concentrations in the prefrontal cortex (A and B) and in the caudate nucleus (C and D), measured by microdialysis. Data represent the mean values  $\pm$  SEM of 4 to 6 rats. Statistical significance was calculated with a repeated-measures analysis of variance followed by a Dunnett test. Closed symbols indicate  $P < .05$  with respect to basal values.<sup>68</sup>

DA pathway. Initial exposure to low doses of ethanol increases mesolimbic DA transmission,<sup>73,74,76,77</sup> and repeated ethanol exposure further activates DA transmission.<sup>75</sup> This enhancement may increase alcohol cravings and consumption, since animals in which DA efflux in the Nac is disrupted exhibit reduced alcohol preference.<sup>75,78</sup> Ethanol-induced increases in DA also occur in the central nucleus of the amygdala, another region implicated in the formation of positive reinforcement associations,<sup>77</sup> whereas ethanol decreases DA efflux in striatal regions involved with motor function.<sup>79</sup> Finally, both naltrexone and acamprosate decrease ethanol self-administration behavior and ethanol-induced increases in DA in the Nac,<sup>75,80</sup> indicating an interaction of these agents with DA mediation of positive reinforcement by ethanol.

### Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is found in the brain, serum, and gastric mucosal membranes. Its role as a neurotransmitter in diverse functions includes learning, sleep, and control of mood. The structural similarity of 5-HT to several drugs known to cause mental aberrations, such as lysergic acid diethylamide, has prompted much

speculation as to the role of 5-HT in naturally occurring mental disorders such as schizophrenia and depression.

The microdialysis technique has been widely used to monitor the release of 5-HT.<sup>64,72,81-89</sup> In 1987, Hernandez et al<sup>90</sup> tested the effects of systemic and local amphetamine on 5-HT release in the Nac and ventral striatum in freely moving rats. Using microdialysis to measure cortical release of 5-HT in freely moving rats, Owen and Whitton suggested that combined treatment of clinically tolerated NMDA antagonists such as amantadine could reduce the delay in therapeutic onset of tricyclic antidepressants and selective 5-HT uptake inhibitors, as well as possibly enhance their efficacy.<sup>89</sup>

The extracellular concentration of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), is much higher than that of 5-HT. Therefore, the extracellular level of 5-HT, as monitored by the microdialysis technique, is closely related to serotonergic neuron activity, while the extracellular level of 5-HIAA has generally not been thought to be as directly related.<sup>91</sup> However, similar percentage increases for both 5-HT and 5-HIAA dialysate levels were found in the guinea pig brain after 5-HT<sub>1B</sub> autoreceptor blockade.<sup>92,93</sup> These findings challenged the general opinion and indicate the need to reevaluate the relationship between extracellular 5-HIAA concentrations and 5-HT release.<sup>91</sup>

### Amino Acids

Amino acids such as glutamate, aspartate, glycine, and gamma-aminobutyric acid (GABA) can serve as either excitatory or inhibitory neurotransmitters in the brain and spinal cord. Glutamate and aspartate are excitatory transmitters, and GABA and glycine are well-known inhibitory neurotransmitters. In 1986, microdialysis combined with HPLC was used to determine *in vivo* GABA release in the globus pallidus after a 6-hydroxydopamine lesion in the substantia nigra of the rat.<sup>94</sup> Since then, numerous studies have used microdialysis to indicate changes in basal or stimulated amino acid neurotransmitter levels during such diverse situations as severe insulin-induced hypoglycemia,<sup>95</sup> angiotensin antagonist-induced decreases in mean arterial pressure,<sup>96</sup> and subchronic administration of the DA D<sub>1</sub>/D<sub>2</sub> agonist apomorphine after lesions of the substantia nigra pars reticulata.<sup>97</sup> Microdialysis has also been used to investigate the actions of antidepressant<sup>87</sup> and antiepileptic<sup>98</sup> drugs in specific brain regions.

Amino acid neurotransmission is also altered by the addiction process. For example, acute ethanol treatment increases taurine levels in the Nac, while repeated ethanol exposure further increases ethanol-stimulated taurine release.<sup>99-102</sup> Behavioral evidence suggests that taurine alters the reinforcing properties of ethanol,<sup>103</sup> possibly via modulation of DA transmission. Along these lines, genetically selected

lines of rats differing in ethanol self-administration behavior exhibit alterations in both ethanol-induced taurine and DA efflux in Nac.<sup>100,104-107</sup> There is very little evidence that ethanol changes GABA, glycine, or glutamate levels in any brain region.<sup>99,101,102,104</sup> Instead, increases in glutamate are caused by ethanol withdrawal.<sup>107-111</sup> The use of microdialysis has not only elucidated these changes but also indicated a possible mechanism of action for the anti-alcohol craving drug acamprosate, since it has been shown to normalize glutamate levels after alcohol withdrawal.<sup>109,112,113</sup>

One important caveat in using microdialysis to study extracellular levels of amino acids in the brain and infer alterations in neuronal function is that many amino acids are also important intermediates in energy and nitrogen metabolism in the brain. Therefore, the question of whether interstitial glutamate, GABA, taurine, and glycine levels measured using microdialysis represent synaptic activity is quite controversial.<sup>2-5</sup> To clarify the origin of interstitial glutamate in the brain, Rooyackers has reviewed current studies on this issue.<sup>114</sup> Many findings have suggested that extracellular glutamate mainly represents the neurotransmitter functions of glutamate, but conflicting conclusions have also been drawn.<sup>2,5,114</sup> Current techniques for measurement of the release of these amino acids in vivo have been called into question<sup>115</sup> as detecting extrasynaptic glutamate and GABA from only nonneuronal origins. Although it is conceivable that amino acids from a nonneuronal origin may alter neuronal signaling,<sup>116</sup> it is imperative that a methodology be used with sufficient sensitivity to detect changes in amino acid content that reflect signaling and not only metabolic status. The use of a novel method to increase the sensitivity of detection of amino acids separated by CE has resulted in much higher temporal resolution for measuring extracellular levels of glutamate, GABA, taurine, and other amino acids.<sup>30,117</sup> This technique enables detection of transient increases in these substances shown to be derived almost exclusively from neurogenic release.<sup>118</sup> Thus, by using this methodology, changes in these amino acids associated with neuronal signaling can be distinguished from metabolic or osmotic events. Using stable isotope tracers in combination with microdialysis is expected to be useful for studying those issues in the human brain, helping to reveal more about the cause of the increased glutamate levels and about glutamate (in combination with glutamine) metabolism in the human brain in general.<sup>114</sup> For example, a simple question of whether increased interstitial glutamate levels are caused by increased glutamate release or by decreased clearance by glial cells could be addressed by this method.<sup>114</sup> An approach similar to this method has also been described to assess glucose kinetics in human muscle and adipose tissue, indicating that the fractional extraction of glucose tracers infused via microdialysis could be used as an index of glucose disposal in peripheral tissues or tissue beds.<sup>119</sup>

### Peptides

Over 100 peptides, such as substance P, enkephalins, endorphin, vasopressin, oxytocin, vasoactive intestinal peptide, somatostatin, and adrenocorticotrophic hormone, have been identified as neurotransmitters. Microdialysis has been used to study the extracellular concentrations of peptides in response to various drugs over the past 15 years. The peptide content of the samples in microdialysates can be directly analyzed without further preparation because the size limit for the peptide molecules passing through the membrane is small, and for this reason the chance of enzymatic degradation of the dialysis sample is decreased. Therefore, it is not necessary to add inhibitors for preventing peptide degradation, and possible pharmacological effects induced by such inhibitors can be avoided.<sup>120</sup>

In 1989, microdialysis combined with radioimmunoassay (RIA) was used to detect the release of neurokinin A-like immunoreactivity induced by amphetamine<sup>121</sup> or acute and subchronic haloperidol treatment.<sup>122</sup> Microdialysis was used to demonstrate the ability of typical (haloperidol) and atypical (risperidone) antipsychotic drugs to block amphetamine-stimulated neuropeptide Y levels in certain brain regions.<sup>123</sup> Landgraf et al used simultaneous microdialysis to monitor the release of vasopressin and oxytocin within the hypothalamic supraoptic and paraventricular nuclei and into the systemic circulation before and after central administration of interleukin-1 beta. The results indicated that the cytokine-induced central and peripheral release pattern appeared to be independent of the rise in body temperature.<sup>124</sup> In vivo microdialysis studies were also performed to examine the role of somatostatin receptors (sst<sub>1</sub>) in controlling somatostatin release.<sup>125</sup>

Beta-endorphin is an endogenous opioid that is released in the cell body and terminal regions of the mesolimbic pathway by cocaine, amphetamine, and ethanol.<sup>126-128</sup> Solinas et al<sup>129</sup> used microdialysis while rats performed a 2-lever choice discrimination procedure for delta-9-tetrahydrocannabinol (THC) to investigate whether THC-induced changes in endogenous levels of beta-endorphin regulate the discriminative effects of THC. They showed that THC produces large increases in extracellular levels of beta-endorphin in the cell body region (ventral tegmental area) and lesser increases in the terminals present in the shell of the Nac.

Cholecystokinin (CCK) is a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein, but it is also an abundant neurotransmitter in the brain. It is colocalized with DA, is known to modulate DA neurotransmission, and is involved in behavioral sensitization to psychostimulants. To better understand its role, CCK was measured by microdialysis in the Nac in response to cocaine in drug-naive and cocaine-sensitized rats. In cocaine-sensitized rats, cocaine-induced increases

in CCK levels were significantly higher than in drug-naive animals. These results provide evidence for an activation of the mesolimbic CCK system in response to repeated cocaine administration.<sup>130</sup>

Immunoassay, particularly RIA, is the approach most commonly used to analyze neuropeptides in dialysate.<sup>121-124</sup> However, because of the potential for cross-reactivity with peptides that contain similar epitopes, great care must be taken in applying RIA. Other techniques such as capillary liquid chromatography (LC) with electrochemical detection and capillary LC coupled with tandem MS have been used for determination of peptides *in vivo*.<sup>29,131,132</sup> Recently, Baseski et al<sup>29</sup> used *in vivo* microdialysis sampling coupled with capillary LC/electrospray ionization quadrupole ion trap MS to monitor [met]enkephalin and [leu]enkephalin in the striatum of anesthetized and freely moving rats. The method was successfully used to monitor these peptides under basal conditions and after stimulation by elevated K<sup>+</sup> concentration (Figure 2).<sup>29</sup> Microdialysis combined with other *in vivo* techniques has provided new insights into the secretory capacity of peptidergic neurons of the mammalian brain and has gained attention as a tool for direct monitoring of peptide neurotransmitters.

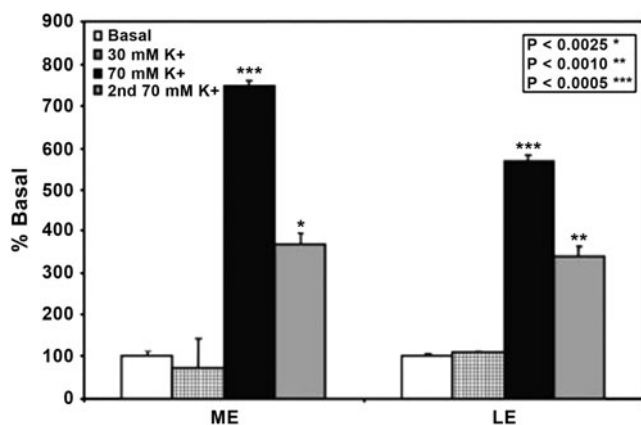
#### Other Neurotransmitters

Purines (adenosine triphosphate [ATP] and guanosine triphosphate [GTP], and their derivatives), a pyrimidine ring fused to an imidazole ring, are generally considered to be neurotransmitters in autonomic neurons. In 1991, Sijbesma et al examined the effects of intraperitoneal (IP) administration of 8-hydroxy-2-(di-n-propylamino) tetralin on the

efflux of cyclic adenosine monophosphate in the extracellular fluid of the dorsal hippocampus, using *in vivo* microdialysis.<sup>133</sup> Implanting microdialysis probes into the renal cortex and measuring adenosine and inosine levels in the dialysate exiting the kidney, the researchers documented that 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor, decreases renal cortical interstitial levels of adenosine and inosine.<sup>134</sup> Reverse dialysis of adenosine is capable of raising extracellular kynurenic acid in the rat striatum by interacting with postsynaptic neuronal A1 receptors.<sup>135</sup>

Anandamide and arachidonylethanolamine, also known as AEA, are naturally occurring endogenous cannabinoids that are thought to act as neurotransmitters. Giuffrida et al<sup>136</sup> found that anandamide release was increased 8-fold over baseline in the dorsal striatum of freely moving rats after local administration of the D2-like (D2, D3, D4) DA receptor agonist quinpirole, and this response was prevented by the D2-like receptor antagonist raclopride. These results suggest that functional interactions between endocannabinoid and dopaminergic systems may contribute to striatal signaling.

Nitric oxide (NO) and carbon monoxide (CO), both gases, can serve as signaling molecules between nerve cells. The NO level in a sample can be assayed by measuring the NO metabolites (NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>) using an automated NO detector-HPLC system.<sup>137,138</sup> In brief, a dialysate sample (20 μL) was collected into the sample loop of the automatic sample injector. NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were separated on a packed column, and NO<sub>3</sub><sup>-</sup> was reduced to NO<sub>2</sub><sup>-</sup> in a cadmium reduction column. NO<sub>2</sub><sup>-</sup> was mixed with Griess reagent in a reaction coil and measured using a UV detector at 540 nm.<sup>137,138</sup> The effect of erythropoietin on NO production in the rat hippocampus revealed that NO levels gradually increased after the injection of erythropoietin. The increase in NOx levels was blunted by nifedipine, a Ca<sup>2+</sup> channel blocker, but not by MK-801, an antagonist of NMDA receptors. These findings suggest that erythropoietin increased NO production in the rat hippocampus by activating voltage-gated Ca<sup>2+</sup> channels but not through NMDA receptors.<sup>137</sup> Hara et al examined the effect of CO poisoning on the NO system in the striatum of freely moving rats using *in vivo* brain microdialysis. The investigators found that the extracellular levels of the oxidative NO products decreased during exposure to CO at a condition that causes CO poisoning. The findings suggest that CO poisoning suppresses NO production in rat striatum *in vivo* though a mechanism that may be different from that of hypoxia.<sup>138</sup>



**Figure 2.** ME and LE levels resulting from perfusion of artificial cerebral spinal fluid through microdialysis probe with increasing concentrations of K<sup>+</sup> for 15 minutes into an anesthetized rat. Values are reported as a percentage of basal level. Basal levels were determined as the average of 2 to 5 assays collected prior to stimulation in each animal. Error bars represent ±1 SEM. ME indicates [met]enkephalin; LE, [leu]enkephalin.<sup>29</sup>

#### Analysis of Glucose, Lactate, Glycerol, and Local Blood Flow Using Microdialysis

Glucose is the primary fuel used by most cells in the body to generate the energy that is needed to carry out cellular

functions. In animals, glucose can be formed in the liver and skeletal muscle by the breakdown of glycogen stores or synthesized in the liver and kidneys from intermediates by a process known as gluconeogenesis. Lactate is a 3-carbon compound that is produced when insufficient oxygen is present in cells to break down pyruvate to acetyl CoA. Measures of glucose, lactate, and glycerol in peripheral and central tissues by microdialysis can be useful as biological markers for glycolysis and lipolysis.<sup>22,139-143</sup> Microdialysis allows studies on several tissues<sup>139</sup> or several regions of the same tissue at the same time<sup>22,142,143</sup> as well as allowing simultaneous measurement of glucose, lactate, and glycerol.<sup>142,143</sup>

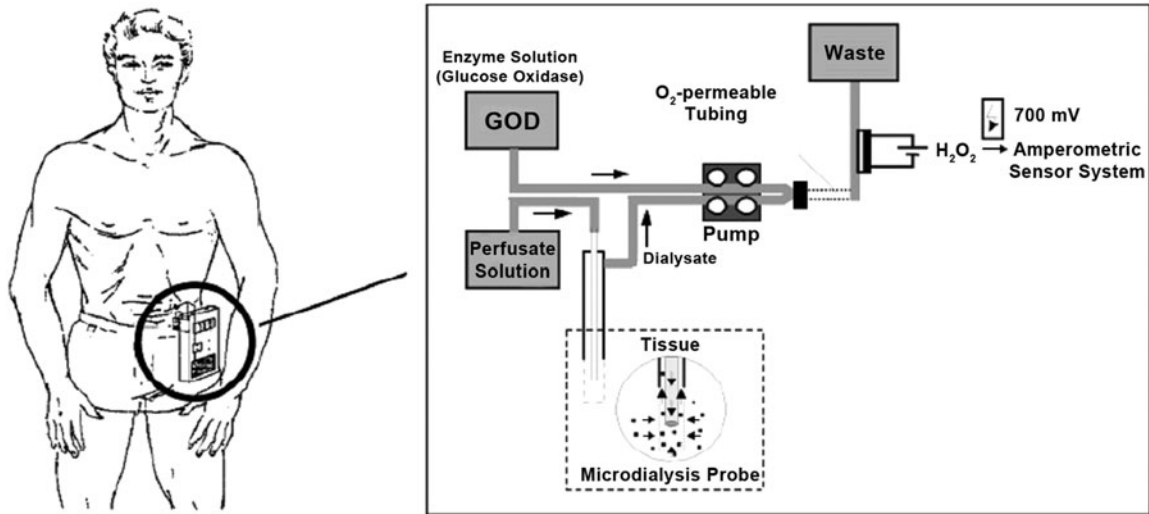
In vivo microdialysis has been used to monitor the effect of different substances on glucose uptake in different tissues, including skeletal muscle,<sup>139,140,143,144</sup> adipose tissue,<sup>22,139,144,145</sup> and the brain.<sup>141,142</sup> The technique was used to examine increases in glucose levels in the rat hippocampus during pentobarbital (45 mg/kg) or ketamine-xylazine (66 mg/kg, 7 mg/kg) anesthesia. The glucose levels of the extracellular fluid of the hippocampus were found to increase during anesthesia, supporting the view that extracellular fluid glucose levels in the hippocampus are dynamically coupled to local neural activity.<sup>141</sup> Microdialysis catheters were placed in the brain to measure interstitial concentrations of glucose, lactate, and glycerol at the same time after IV infusion of low-dose prostacyclin in an experimental model of vasogenic brain edema of piglets. Prostacyclin caused a decrease in glucose, an increase in lactate, an increase in the lactate/glucose ratio, and an increase in glycerol.<sup>142</sup> In another study, microdialysis was used to determine interstitial glucose concentrations in abdominal subcutaneous adipose tissue after the tissue was stimulated with the  $\alpha_1$ -agonist norfenefrine, the  $\alpha_{1,2}\beta$ -agonist NE, and the  $\alpha_1$ -antagonist urapidil in 24 severely obese human subjects. Both norfenefrine and NE caused a concentration-dependent decrease in interstitial glucose concentration. Preperfusion of adipose tissue with urapidil inhibited this decrease and enhanced extracellular glucose at high concentrations.<sup>22</sup>

Microdialysis has been successfully used in human subjects to monitor glucose levels in various peripheral tissues.<sup>17,22,23,143</sup> In Hamrin and Henriksson's study,<sup>143</sup> the local effect of the insulin-mimetic agent vanadate on glucose metabolism in human skeletal muscle was investigated using in vivo microdialysis. Reverse dialysis with vanadate dose dependently decreased interstitial glucose concentrations, most likely secondary to an increased cellular glucose uptake. The accompanying dose-dependent increase in lactate reflected the increased glucose uptake, which stimulates muscle glucose metabolism and lactate formation. The effect of in vivo insulin-stimulated glucose utilization in relation to glycolysis (lactate formation) in peripheral tis-

ues was investigated. The results indicated that vanadate mimics the effect of insulin in human skeletal muscle in vivo.

Microdialysis was also used to measure the blood flow and/or glucose levels in tissues such as in skeletal muscle and adipose tissue after drug treatment.<sup>22,144,146</sup> In Flechtner-Mors et al's studies,<sup>22</sup> 4 microdialysis probes were implanted in abdominal subcutaneous adipose tissue, and 3 of the probes were used to determine the effects on changes in interstitial glucose concentration and blood flow of the  $\alpha_1$ -agonist norfenefrine, the  $\alpha_{1,2}\beta$ -agonist NE, and the  $\alpha_1$ -antagonist urapidil, respectively, in a single concentration over time. The fourth microdialysis probe was implanted in all of the subjects to measure basal glucose concentration during the study period. Orciprenaline decreased adipose tissue glucose concentrations as well as increased nutritive blood flow. Local adipose blood flow decreased in the presence of norfenefrine and NE but increased in response to urapidil. The accelerated blood flow due to urapidil was counteracted by NE and norfenefrine. The observed changes in local adipose blood flow induced by adrenergic agents were not related to glucose uptake.<sup>22</sup> Djurhuus et al<sup>144</sup> assessed skeletal muscle blood flow using plethysmography applied to the thigh contralateral to the microdialysis probes. A cuff was inflated to 300 mmHg at the level of the patella to measure flow exclusively in the thigh. Djurhuus et al also assessed the impact of similar growth hormone (GH) and cortisol exposures on adipose tissue blood flow with the <sup>133</sup>Xe washout technique.<sup>144,146</sup> In brief, approximately 3 MBq of <sup>133</sup>Xe was injected into the subcutaneous adipose tissue of the abdomen ~5 cm sinistrolateral to the umbilicus. Disappearance of <sup>133</sup>Xe was continuously measured starting from 30 minutes after injection by use of an NaI detector.<sup>146</sup> The results indicated that coadministration of GH and cortisol does not influence adipose tissue blood flow.<sup>144</sup>

One of the most exciting clinical applications of microdialysis is its use in measuring glucose in diabetic patients. Recently, a portable glucose sensor was developed for diabetic patients (Figure 3) under insulin treatment or with an artificial pancreas, providing improved metabolic control that delays or prevents the development of the long-term complications of these treatments.<sup>20,147,148</sup> Reliable continuous glucose monitoring can provide more detailed information on postprandial glucose fluctuations than can single spot measurements, allowing feedback-controlled insulin delivery.<sup>1,17,18,20</sup> In Freckmann et al's studies, continuous glucose monitoring of type 1 diabetic patients administered 3 daily 60-g carbohydrate meals resulted in glucose concentrations ( $140 \pm 13$  mg/dL) close to the target value of 120 mg/dL as well as stable levels during the night (Figure 4).<sup>20</sup> Continuous microdialysis sampling does not withdraw body fluid, so there are no factors limiting the amount of samples that can be taken from patients. There is only minimal



**Figure 3.** Schematic diagram of the continuous glucose monitoring system. The combination of microdialysis and enzymatic amperometric glucose measurement implemented in this device marked a breakthrough in achieving reliable and precise continuous tissue glucose monitoring. GOD indicates glucose oxidase.<sup>20</sup>

perturbation of the system under study, making this technique more and more popular in preclinical and clinical studies.

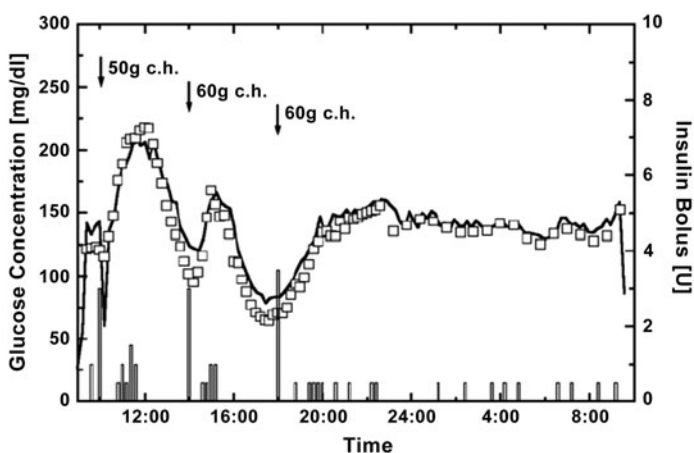
#### Analysis of Hormones Using Microdialysis

Hormone molecules are secreted directly into the bloodstream, other body fluids, or adjacent tissues and serve as a signal to the target cells. Melatonin helps regulate circadian rhythms, presumably because its secretion is regulated by the circadian pacemaker in the suprachiasmatic nucleus. Nakahara et al established a system that enables long-term monitoring of melatonin secretion by implanting a trans-

verse microdialysis probe in or near the pineal gland in a freely moving mouse. Two daily peaks of secretion were observed, with the first, small peak at dusk and the second, large peak at midnight. The large nighttime peak was suppressed by tetrodotoxin, an Na<sup>+</sup> channel blocker, and enhanced by both phenylephrine and isoproterenol, which are α- and β-adrenergic agonists respectively, whereas daytime melatonin levels were not affected by tetrodotoxin infusion.<sup>149</sup> Prolongation of NE-stimulated melatonin release was observed after reverse dialysis of the β-adrenergic and α-adrenergic receptor agonists into the pineal gland.<sup>150</sup>

Vascular endothelial growth factor (VEGF) is a peptide hormone and considered a key mediator of tumor angiogenesis, including neovascularization in human breast cancer cells. Microdialysis was used to sample the extracellular space where VEGF is biologically active.<sup>151,152</sup> Garvin and Dabrosin<sup>152</sup> concluded that tamoxifen decreased extracellular VEGF in vivo in solid MCF-7 tumors in nude mice and illustrated that microdialysis is a viable method that may be applied in human breast tissue to detect soluble VEGF in situ released by the tumor.

Cortisol is an important steroid hormone secreted by the cortex of the adrenal glands. In vivo microdialysis was used to measure the conversion of cortisone to cortisol in abdominal subcutaneous adipose tissue before and after carbenoxolone was used to inhibit metabolic conversion. Human obese subjects showed more rapid initial conversion of [<sup>3</sup>H]cortisone to [<sup>3</sup>H]cortisol than did lean subjects. Conversion in both lean and obese groups plateaued at a similar level after 3 hours. After the introduction of hyperinsulinemia by insulin infusion, there was a rapid, temporary fall in cortisol generation in lean subjects but no change in obese subjects. There was not significant conversion of



**Figure 4.** Continuous glucose monitoring with the comparative microdialysis technique in a type 1 diabetic patient with glucose-controlled subcutaneous insulin infusion over 24 hours. □ stands for venous blood glucose and – for tissue glucose (comparative microdialysis technique). Insulin doses are represented by vertical bars. c.h. indicates carbohydrates.<sup>20</sup>

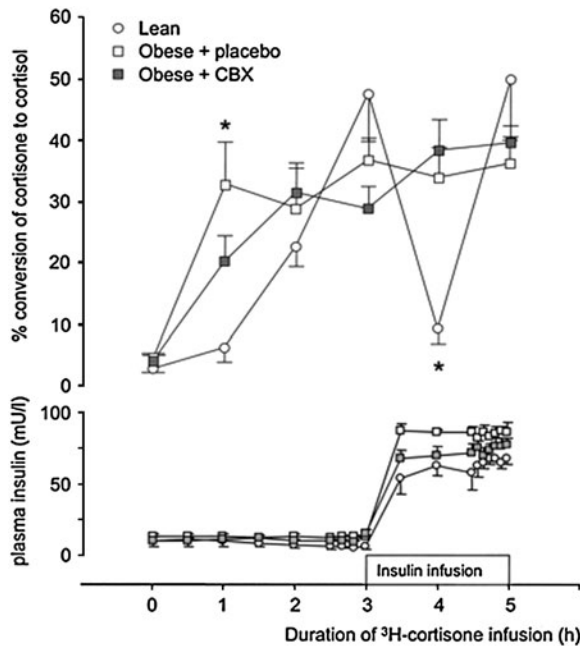
[<sup>3</sup>H]cortisone to [<sup>3</sup>H]cortisol after carbenoxolone, indicating that carbenoxolone did not reduce 11 $\beta$ -hydroxysteroid dehydrogenase type 1 activity in regenerating cortisol from cortisone within adipose tissue in obese men as compared with lean men (Figure 5).<sup>153</sup> Thus, microdialysis allows measurement of small local changes in human subjects that many other techniques cannot provide.<sup>1,14,153</sup>

Many times, the temporal pattern of release is an integral part of hormonal signaling, making the repeated measurement afforded by microdialysis indispensable.<sup>58,64,154,155</sup> To study pulsatile secretion of hypothalamic gonadotropin-releasing hormone (GnRH), the effects of arcuate nucleus-median eminence (AME) administration of desipramine (a specific NE transporter blocking drug) on GnRH release were examined. First, 0.2- to 10-mM doses of desipramine were delivered continuously for 1 hour via AME reverse dialysis into intact male rabbits. Desipramine stimulated a GnRH pulse, and the size of the GnRH pulse was proportional to the

dosage of desipramine. Simultaneous determination of the catecholamine content of microdialysis samples confirmed the specificity of desipramine for NE transport.<sup>154</sup>

## CONCLUSIONS AND PERSPECTIVES

Most drugs exert their pharmacologic effects on extravascular structures rather than in the bloodstream. Thus, the interstitial fluid of a target tissue may be regarded as the true anatomical “effect compartment.” Therefore, the local tissue levels of endogenous compounds can be a very important biological marker used to determine the drug concentration versus drug effect profile. Microdialysis allows direct assessment of endogenous substances in multiple sites and continuous sampling in the same individual and the same area with minimal tissue perturbation and high sensitivity in small sample volumes. Elegant separation methods are available. These features make microdialysis a very efficient diagnostic tool for local pharmacodynamic studies, both peripherally and centrally. The present examples are not meant to be exhaustive but to illustrate the application of microdialysis for measuring the effect of the drug on extracellular levels of endogenous substances in peripheral compartments. In addition, microdialysis has contributed greatly to the understanding of hormone signaling and the interactions between drugs and neurotransmitters in the nervous system. It can be expected that this technique will be further refined to afford even more brain region and cellular specificity as well as more detailed analysis of peptide, fatty acid, and gaseous signaling compounds. This information will also provide a gateway to the development of more selective and effective psychotropic drugs in the future.



**Figure 5.** In vivo microdialysis measurement of subcutaneous adipose 11HSD1 activity with and without hyperinsulinemia. Data are mean  $\pm$  SE for 6 subjects per group for the conversion of [<sup>3</sup>H]cortisone to [<sup>3</sup>H]cortisol (microdialysis; upper panel) and plasma insulin levels (lower panel). For microdialysis, comparison of lean and obese subjects was performed by repeated-measures ANOVA. The changes with time of perfusion ( $P < 0.001$ ) showed a highly significant interaction by group ( $P < .002$  lean vs obese). Post hoc testing by Student’s  $t$  tests showed significant differences in the first hour after cannula insertion ( $*P < .02$ ) and in the first hour after introducing hyperinsulinemia ( $*P < .002$ ). For microdialysis, the effect of CBX was determined by repeated-measures ANOVA of the difference between placebo and CBX; analysis at each time point revealed no effect of CBX ( $P = .64$ ). ANOVA indicates analysis of variance; CBX, carbenoxolone.<sup>153</sup>

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