Simultaneous Determination of Caffeine from Blood, Brain and Muscle Using Microdialysis in an Awake Rat and the Effect of Caffeine on Rat Activity

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The ability to deliver a formulation and to monitor a drug in an awake and freely moving rat is demonstrated using microdialysis probes in the jugular vein, brain striatum and pectoral muscle. Caffeine, which is an important cytochrome P_{450} isozyme marker substrate, was employed as the model drug. Methods for successful determination of caffeine levels simultaneously through three microdialysis probes are described in detail. Delivery of the intraperitoneal dose without disturbing the rat and the ability to monitor the animal's activity during the experiment are added enhancements for performing measurements in the awake animal.

Introduction

The purpose of this study was to demonstrate the ability to deliver a formulation and to monitor a drug in an awake, freely moving animal using simultaneous multiple-site microdialysis sampling. In addition, the movement of the rat was recorded before and after the dose so that the effect of the drug on rat activity can be correlated. In this study, caffeine was chosen as the drug for administration. Monitoring caffeine and its metabolites is widely performed in the study of induction of liver enzymes in drug interaction studies. Specifically, caffeine is used as the marker substrate for the cytochrome P_{450} isozyme designated 1A2, which metabolizes, among other things, tricyclic benzodiazepines such as clomipramine, clozapine and imipramine.

Microdialysis sampling allows continuous, long-term sampling from the extracellular fluid of tissues with good temporal resolution and no net change in fluid volume in the animal. The protein-free samples can be analyzed directly on-line. The small size of the probes results in minimal perturbation to surrounding tissue, thus facilitating simultaneous sampling from several sites. In microdialysis sampling, an animal serves as its own control, thus reducing the number of animals needed for a given study, an advantage further enhanced by multiple-site sampling. Simultaneous multiple-site sampling facilitates continuous and long-term monitoring of parent compound and metabolite profiles from peripheral tissues and the bloodstream of the same animal.

The BAS Raturn® accommodates numerous fluid lines and prevents their tangling, making it ideal for simultaneous multiple-site sampling. Movement by the animal triggers an optical sensor, activating the motorized turntable and signaling the software to record direction and duration of movement. The operator can record event markers for future reference.

The methyl xanthines (i.e., theobromine, theophylline and caffeine), are well-studied compounds, with molecular weights of about 200 g/mol. Caffeine is a widely used—and perhaps abused—psychoactive drug. It has been shown to affect every body system that is controlled by the central nervous system (1). Caffeine has very low binding affinity to plasma proteins (2). It is metabolized in the liver by the CYP 1A enzymes system and is one of the compounds used for assessment of liver function (3). Because caffeine and its metabolites are well-characterized compounds and because caffeine could be expected to influence animal activity, we chose caffeine for this simultaneous multiple-site microdialysis sampling / activity monitoring study.

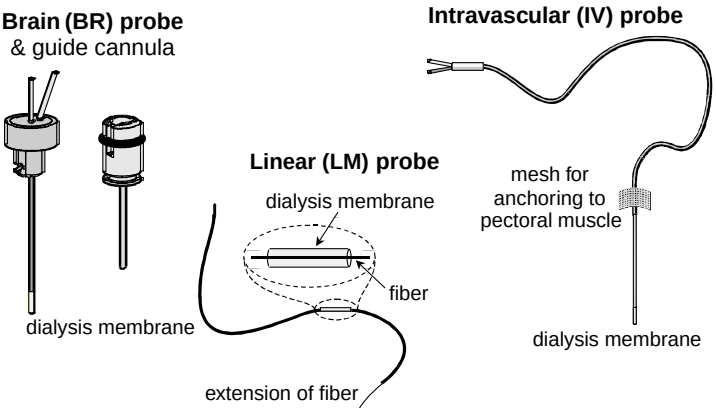
Experimental Procedure

Materials

Caffeine, CA (anhydrous); theobromine, TB (3,7-dimethylxanthine); theophylline, TP (1,3-dimethylxanthine, anhydrous); paraxanthine, PX (1,7-dimethylxanthine); and trimethyluric acid, TMU (1,3,7-trimethyluric acid) were purchased from Sigma Chemical Co. (St. Louis, MO). Tetrahydrofuran (HPLC grade), methanol (HPLC grade), ammonium hydroxide, phosphoric acid, sodium chloride, potassium chloride, and calcium chloride were obtained from Fisher Scientific (St. Louis, MO). All chemicals were re-

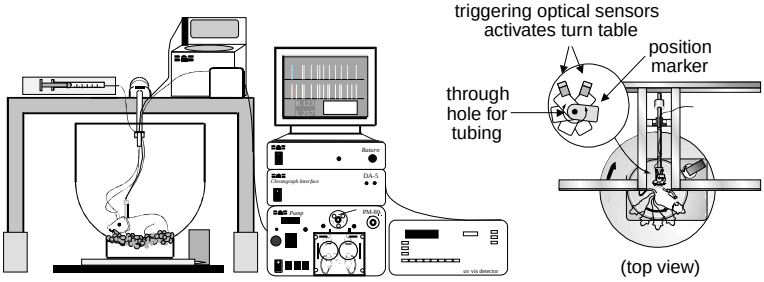
F1

Diagrams of BAS probe geometries optimized for implantation in various tissues.



F2

BAS Raturn® animal system with activity monitoring. Samples from two probes were coupled on-line to an LC system using a Pollen-8 injection valve. Dialysate from the third probe went to the HoneyComb fraction collector for later analysis.



agent grade or better and used as received.

Sterile lactated Ringer's solution (McGaw, Inc., Irvine, CA) was used to perfuse the brain probe and to prepare solutions perfused through the brain probe. The intravascular and linear probes were perfused with Ringer's solution (155 mM NaCl, 5.5 mM KCl, and 2.3 mM $CaCl_2$) prepared in-house. All solutions were prepared using NANOpure deionized water (Barnstead, Boston, MA).

All microdialysis probes, connecting tubing (FEP), syringe pumps and other microdialysis accessories were obtained from Bioanalytical Systems, Inc. (BAS) (West Lafayette, IN). The liquid chromatographic system, columns, detector, ChromGraph software, HoneyComb fraction collector, Raturn® animal containment system and activity monitoring software were also from BAS.

Methods

Surgical procedures

A locking intracerebral cannula was implanted in the striatium of an anesthetized rat according to the instructions provided. Following several days of recovery, the rat was again anesthetized. An intravascular probe (IV-5) and a linear probe (LM-10) were implanted in the jugular vein and pectoral muscle, respectively. The stylet of the intracerebral cannula was replaced with a brain probe (BR-4). The probe designs are shown in ***F1***. A dosing cannula was inserted into the abdominal cavity. All probe conduits and the cannula were externalized at the back of the neck. The animal was tethered in the Raturn® and the necessary fluid connections were made using 1 m or less FEP tubing. At least a 24 hour recovery period was allowed prior to administration of the dose.

In vivo calibration

The in vivo extraction efficiencies (EE) for caffeine and four metabolites were determined by a delivery experiment prior to administration of the dose. Extraction efficiencies by delivery (EE_{del}) were calculated according to the equation:

$$EE_{del} = \left(\frac{C_{init} - C_{dial}}{C_{init}}\right) \text{x } 100\% = \left[1 - \frac{C_{dial}}{C_{init}}\right] \text{x } 100\%$$

where C_{init} is the initial concentration of the analyte in the perfusate, and C_{dial} is the analyte concentration in the dialysate as it exits the probe. EE_{del} are reported as the average of consistent deliveries for a period greater than or equal to 1 hour ($n \geq 4$ samples) with the uncertainty expressed as the standard deviation of the averaged values. Each probe was perfused with 200 µM each of CA, TB, TP, PX, and TMU. Perfusion flow rate for the BR and IV probes was 2.5 µL/min and samples were collected directly to loops of the Pollen-8 valve. BR and IV samples were alternately injected for LC analysis at 6 minute intervals, making the interval for each probe 12 minutes. Perfusion rate for the LM probe was 4 µL/min and the samples were collected at 12 minute intervals to the refrigerated HoneyComb fraction collector and analyzed later using manual injections. The in vivo EE_{del} determinations were repeated after the administered dose had completely cleared.

Dose administration

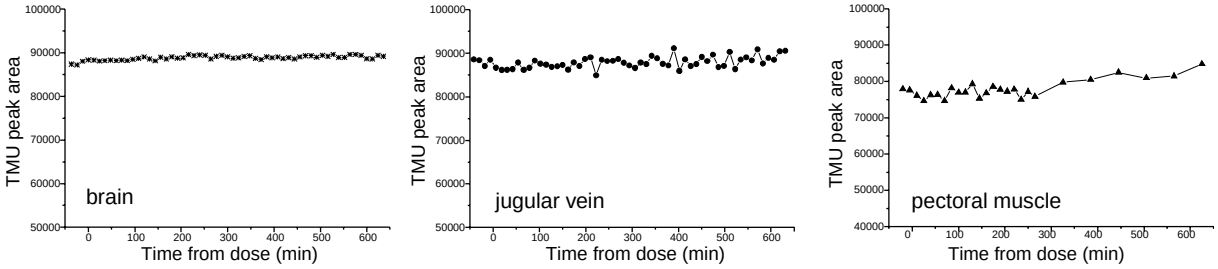
Caffeine (22.1 mg/kg dissolved in 1 mL of Ringer's solution) was administered intraperitoneally via an implanted cannula. The dose was followed by 1.5 mL Ringer's solution to completely flush the cannula. Microdialysis sampling was performed via the three probes just prior to and for about 11 hours following the dose.

Activity monitoring

The animal was maintained in a Raturn® animal containment system and Raturn® software was used to record direction and duration of turning by the animal throughout the experiments. The Raturn® feedthrough port accommodates numerous conduits to and from probes as well as dosing cannulae. The probe outlet conduits can be connected directly on-line to the LC injection valve or to a fraction collector. ***F2*** illustrates the on-line microdialysis-LC system with the activity monitoring animal containment system.

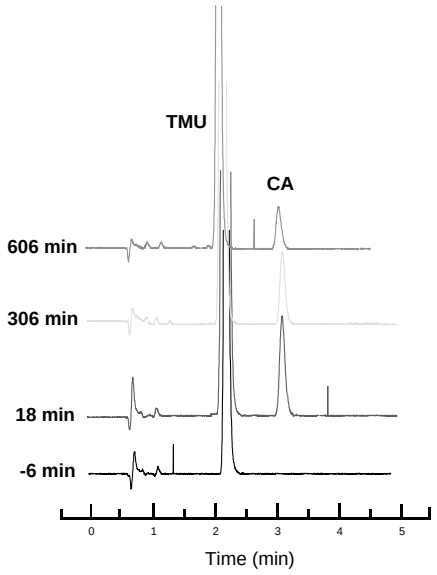
F3

In vivo delivery of TMU via microdialysis probes during monitoring of dosing experiment.



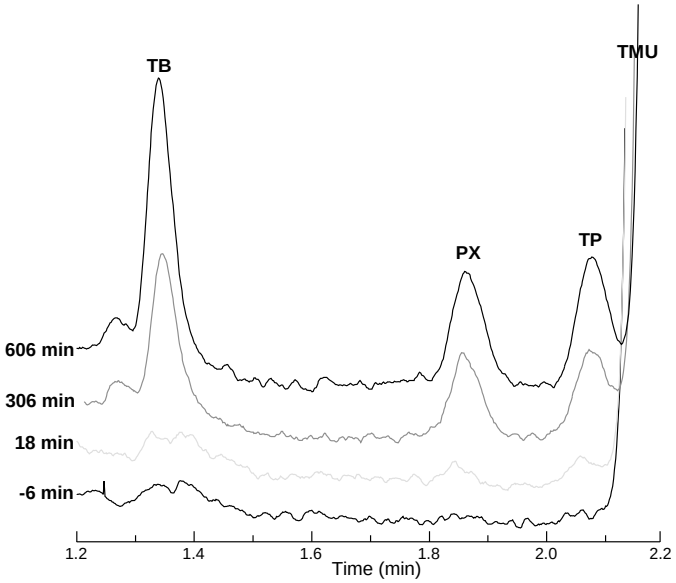
F4

Typical chromatograms from jugular probe dialysates at time points just prior to and following i.p. administration of a 22.1 mg/kg dose.



F5

*Detail of metabolite regions of jugular vein dialysates from same time points as **F4**.*



T1

In vivo probe calibration for caffeine and trimethyluric acid. Extraction efficiency by delivery (%).

	Pre-dose		Post-dose		Perfusate flow (as % of pump setting)	
Probe	*CA*	*TMU*	*CA*	*TMU*	*Pre-dose*	*Post-dose*
Jugular (IV-5) @2.5 µL/min	11.28 ± 1.88	6.92 ± 1.63	10.98 ± 1.68	6.60 ± 1.46	87.75 ± 7.60	83.75 ± 4.64
Brain (BR-4) @2.5 µL/min	9.26 ± 1.13	2.32 ± 0.57	4.91 ± 1.31	2.22 ± 1.11	88.91 ± 1.64	79.71 ± 4.97
Muscle (LM-10) @4 µL/min	20.17 ± 2.97	11.46 ± 2.33	12.08 ± 1.09	6.37 ± 1.08	56.68 ± 6.29	77.85 ± 6.24

Analytical method

Dialysate samples and standards were analyzed by reverse-phase LC with UV detection. The column was a 3.2 mm x 100 mm BioPhase, ODS III column with 3 µm particles preceded by a 3.2 mm x 15 mm guard column containing 7 µm ODS packing. The mobile phase consisted of 20 mL THF, 20 mL MeOH, and 940 mL of 50 mM ammonium phosphate buffer at pH 2.5 for a total volume of 980 mL. The column and mobile phase were maintained at 35 °C. The mobile phase flow rate was 1 mL/min.

A BAS 116 UV detector operated at 273 nm was used. Detector responses at two ranges, 0.1 and 0.001 AUFS, were simultaneously collected by ChromGraph software. The software was also used to integrate the chromatographic data.

A Pollen-8 on-line 10 port valve was configured to alternately collect dialysate samples from the brain and vascular probes to matched 3.2 µL loops. The injection interval was 6 minutes, resulting in a temporal resolution for each probe of 12 minutes. Samples from the linear probe (muscle) were collected at 12 min intervals by a HoneyComb refrigerated fraction collector and analyzed later.

Results and Discussion

In vivo extraction efficiency (EE)

In vivo EEs for CA and TMU, determined pre- and post-dose, are shown in ***T1***. The pre-dose experiment was conducted 24 hours just prior to dose administration, whereas the post-dose EEs were performed about 48 hours after the dose. Diffusion rate of the analyte through the surrounding tissue is one pa-

T2

Jugular probe in vivo extraction efficiency by delivery (%) for caffeine and four metabolites.

Analyte	Pre-dose	Post-dose
TB	9.91 ± 1.74	9.56 ± 1.46
PX	9.66 ± 1.75	9.63 ± 1.61
TP	9.98 ± 1.78	9.74 ± 1.58
TMU	6.92 ± 1.63	6.60 ± 1.46
CA	11.28 ± 1.88	10.98 ± 1.68

T3

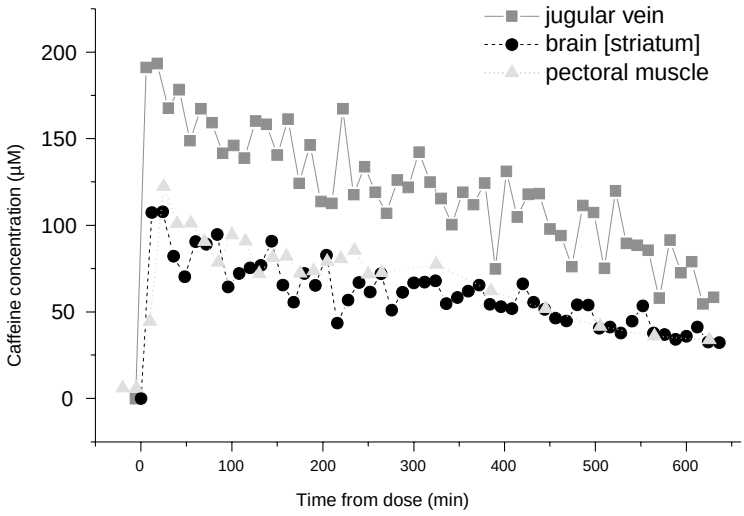
Caffeine pharmacokinetic parameters estimated from vascular microdialysis data.

Area Under the Curve (AUC) mg •min/mL	Plasma Clearance (CL) L/kg/min	Volume of Distribution (V_d) L/kg	Half-Life ($t_{1/2}$) hr
8.0	0.0015	0.71	5.5

F6

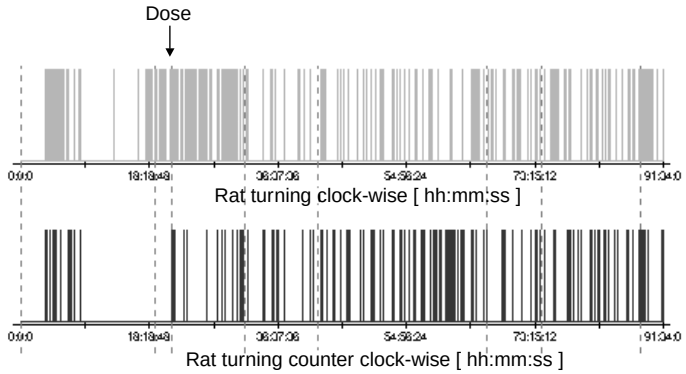
Concentration - time profiles of caffeine in blood, brain and muscle sampled by microdialysis in an awake rat.

(Concentrations were corrected using the in vivo EE_{del}.)



F7

Raturn® activity monitoring software output collected during the 5 day multiple microdialysis probe experiment.



rameter that influences the in vivo EE of the probe. If diffusion through the tissue is slower than diffusion through the dialysis membrane, then diffusion through the tissue becomes the limiting factor with respect to EE. Thus, the EEs measured at different times may reflect changes in the surrounding tissue as a response to the in-dwelling probe. Other researchers have reported similar results (4,5). Since the jugular vein presents a hydrodynamic situation, diffusion through the probe membrane is the limiting factor. That the in vivo EE for the IV probe did not change with time indicates that systemic circulation was not blocked by the presence of the probe. The pre- and post-dose in vivo EE for the vascular probe was consistent for CA and the four metabolites as shown in ***T2***.

Although it is a metabolite of caffeine, TMU was not detected in the microdialysates during preliminary studies. Therefore, it was included in the perfusate during the dosing experiment to monitor the stability of probe behavior. The delivery of TMU was consistent for each probe throughout the dosing experiment (see ***F3***). This indicates that the extraction efficiency of the probes did not change during the dosing study.

Profiles of caffeine and metabolites in dialysates

Representative chromatograms of jugular dialysates show the appearance and decrease of the caffeine peak (***F4***). ***F5*** details the metabolite region of the same chromatograms. The appearance and increase in TB, PX, and TP can be seen. The metabolites did not appear at quantitatable levels in the muscle or brain dialysates.

The concentration-time profiles for caffeine in blood, brain and muscle are shown in ***F6***. The concentrations were corrected using the in vivo EE_{del} of CA for each probe. Thus, the values represent an estimate of the tissue concentrations. The concentration-time profile and estimated tissue levels from this study are in reasonable agreement with the findings of others (6). The pharmacokinetic parameters shown in ***T3*** were estimated from the caffeine levels in blood following a 22.1 mg/kg i.p. dose.

Activity monitoring

F7 shows the activity data for the 96-hour multiple-site sampling experiment. The time of administration of the dose is indicated and a period of increased activity follows the dosing.

In a separate experiment, a rat with an i.p. dosing cannula was

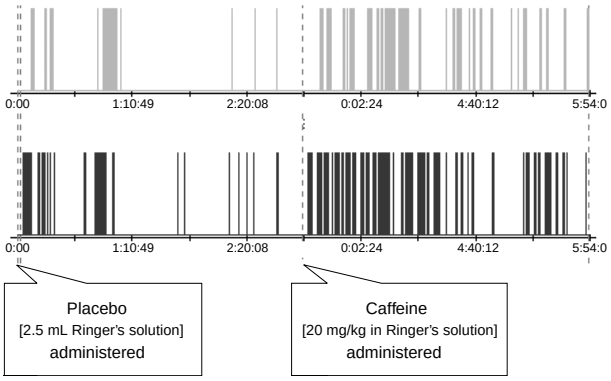
T4

Activity data following i.p. administrations of placebo and caffeine doses.

Activity for 3 hours after:	Placebo (Ringer's solution)	20 mg/kg caffeine dose
Number of counts, left	56	161
Number of counts, right	130	323
Max. duration left (s)	3	7
Max. duration right (s)	5	7
Mean duration left (s)	1.25	1.40
Mean duration right (s)	1.62	1.87
Total duration left (s)	70	225
Total duration right (s)	210	605
Standard deviation left	0.48	0.71
Standard deviation right	0.81	1.18

F8

Activity monitoring during administration of placebo and caffeine doses.



monitored for 3 hours following administration of a Ringer's solution placebo. A 20 mg/kg dose of caffeine in Ringer's (total volume equal to the placebo volume) was then administered and the activity monitored for an additional 3 hours. The activity data are shown in ***F8*** and summarized in ***T4***. The caffeine dose increased the number of movements by the rat about 2.5 times compared to the placebo. During the placebo period, the maximum duration for a single movement was 5 sec compared to 7 sec during the period following the dose. The directional ratio of turning appeared unchanged by the dose.

Conclusions

A method for the successful determination of caffeine levels simultaneously through three microdialysis probes in an awake animal has been demonstrated. Use of an indwelling i.p. cannula allowed administration of the dose without restraining or disturbing the rat. The ability to monitor activity during the experiment is an added enhancement to performing measurements in an awake animal.

References

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