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Application of Empis in a Rat Brain Microdialysis Experiment

Empis® is a programmable infusion system, ideal for chronic infusions, in vivo *microdialysis, and dosing of animals in a Raturn® interactive cage. The application of Empis during brain microdialysis in freely-moving rats is demonstrated in this article. The dopamine concentration in the microdialysates collected from rat nucleus accumbens and rat activity were monitored for 48 hours before and after administration of the psychostimulant amphetamine.*

Empis (1) is a programmable infusion system recently developed at BASi (Bioanalytical Systems, Inc.). It is designed to deliver drugs into a targeted organ in an animal through an implanted catheter or probe, either in a continuous fashion or as a bolus at preset time intervals. The animal is housed in a Raturn interactive cage; therefore rat locomotor activity can also be monitored during the infusion experiment.

Since Empis has continuous fluid delivery capability, it can be used for prolonged *in vivo* microdialysis. A traditional microdialysis experiment is carried out through a gas-tight syringe pump delivery system (2). The fixed syringe volume can limit duration of the experiment during continuous perfusion. When multiple syringes are used, air bubbles may be introduced into the system, resulting in missing samples or bubble-trapped brain probes. In addition, more than one connector has to be used in order to connect the syringe pump with the rat brain probe, which makes the system prone to leakage. Using a large syringe is discouraged, since accuracy of the flow rate is compromised.

Empis, on the other hand, can refill itself continuously from a bag of saline or Ringer's solution without interrupting a closed delivery system. The long, thin Teflon tubing it employs allows direct connection from the syringe to the rat brain probe. Empis also incorporates an animal activity monitoring function, which provides additional information on pharmaco-dynamic effects of the test compound, especially those that target the central nervous system (CNS).

Experimental

Apparatus

The LC/EC system (all from BASi, West Lafayette, IN, USA) consisted of a chromatographic pump (PM-80), a UniJet™ C18 microbore column (150 x 1 mm, 5 mm), and a multi-channel amperometric detector (epsilon™) coupled to four glassy carbon working electrodes in a radial flow thin-layer cell. Potentials of +600, 650, 700, 750 mV vs. Ag/AgCl were applied to the working electrodes. The mobile phase contained MP-2 (BASi) and acetonitrile (100:40, v/v), delivered at 0.1 mL/min. All samples were injected via a 5.3 µL loop by a refrigerated autosampler (CMA, Sweden). Data were acquired and integrated using BASi ChromGraph® software.

Chemicals and Reagents

d,l-Amphetamine sulfate and dopamine HCl (USP grade) were purchased from Sigma (St. Louis, MO, USA). The Ringer's solution was purchased from B. Braun Medical Inc. (Irvine, CA, USA). Saline solution was obtained from Abbott (Chicago, IL, USA). Acetonitrile was of HPLC grade (Burdick and Jackson, Muskegon, MI, USA). Reagent grade water was prepared from in-house de-ionized water using a NANOpure system (Barnstead/Thermolyne, Dubuque, IA, USA).

The dopamine stock solution was prepared in 0.2 M acetic acid at a concentration of 1.0 mg/mL, and was kept at -20°C in the dark. A fresh calibration curve was constructed for each batch of samples by diluting the stock solution in Ringer's solution to yield a final concentration of 0.15-20 ng/mL. Quality control samples (0.4 and 1 ng/mL) were run every 8 to 10 unknown microdialysate samples. Deviations of less then 15% were observed in all the analytical batches.

Microdialysis Experiment

Sprague-Dawley rats (Harlan, USA) weighing 280-320 g were used. The animals were kept at constant room temperature (67±3°F) under a regular 12-hour light/dark schedule (lights on at 7:00 a.m.). Food and water were freely available. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication N. 85-23, 1985).

A guide cannula (BASi) was stereotaxically inserted into the nucleus accumbens area of an anesthetized rat at the following coordinates: AP -1.7 mm; ML -1.5 mm and DV -5.4 mm (3). The animal was allowed to recover from surgery for at least three days. On the day before the experiment, the rat was implanted with a jugular vein cannula (BASi) used to dose the animal.

At 4:00 p.m. on the first day of the experiment, a 2 mm brain microdialysis probe (BASi) was inserted in the guide cannula in place of the dummy stylet. The probe was perfused with Ringer's solution, delivered by Empis, at a constant rate of 1 µL/min. The brain-blood barrier was allowed to reconstitute for three hours before microdialysate was sampled to assess the basal concentration of dopamine.

At 12:00-12:30 p.m. on days two and

three, either vehicle or *d,l*-amphetamine at 2.5 mg/kg was administrated intravenously to the rat, respectively. The vehicle was saline solution containing 10% ethanol. Amphetamine (corrected for free base) was first dissolved in 70% ethanol to 17.5 mg/mL and then diluted to 2.5 mg/mL with saline. Samples of dialysate were automatically collected into a refrigerated fraction collector (HoneyComb™, BASi) every 30 minutes and then stored at -80°C until assayed by LC/EC. Animal activity data was recorded simultaneously as the brain microdialysates were collected.

At the end of each experiment, the animal was euthanized, the brain removed and location of the cannula verified histologically. Experiments where the cannula had been placed outside the nucleus accumbens were discarded.

Results

Empis was used to conduct rat brain microdialysis accurately and continuously for more than two days. The same device collected animal activity information. In the two rats studied, a similar basal concentration of dopamine in the nucleus accumbens was found, in both dark phase and light phase (***T1***). This is in agreement with the observations reported in literature

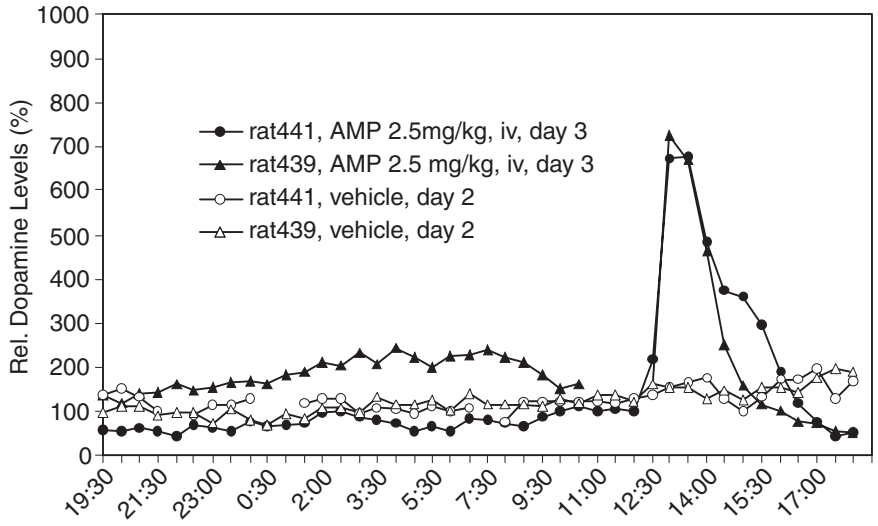
T1.

Average basal dopamine levels (ng/mL) in rat brain microdialysates collected from nucleus accumbens.

Rat ID Number	439	441
Dark phase (7 p.m. - 7 a.m.)	0.21+0.04	0.17+0.05
Light phase (7 a.m. -7 p.m.)	0.28+0.03	0.26+0.05

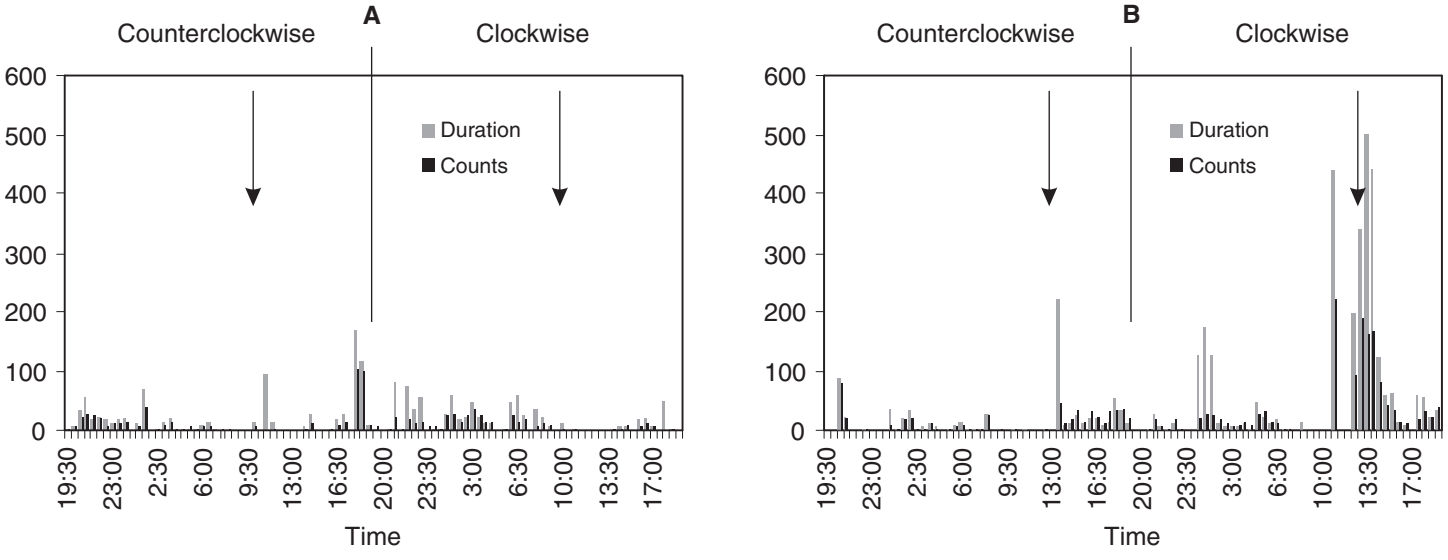
F1.

Relative dopamine levels in the nucleus accumbens in rat 439 and 441 during two 24-hour cycles.



F2.

Animal activity data (clockwise rotations and counterclockwise rotations) for rat 439 on day 2 (A) and day 3 (B), when vehicle and *d,l*-amphetamine at 2.5 mg/kg was administrated i.v., respectively. (Dosing time indicated with arrows.)



that dopamine concentration in rat nucleus accumbens did not change significantly across the light-dark cycle (4,5). Similar basal levels of locomotor activity was also found in the two rats, with more activity registered in the dark phase for both.

Upon intravenous administration of *d,l*-amphetamine, a dramatic increase of dopamine (up to 700% of basal) in the extracellular concentration was detected (***F1***), while more frequent and longer duration of preferential rat motor activity (clockwise rotation) was also observed (***F2*** and ***F3***). There was a positive correlation between the time of increased dopamine concentration and the duration of increased animal activity (about 4 hours). Of the two animals tested, one rat (439, ***F2B***) had much greater increased activity than the other (441, ***F3B***), even though both had similar level of increase in their extracellular dopamine concentration (***F1***). Therefore, the level of spontaneous motor activity *per se* may not be a primary function of the dopamine system in nucleus accumbens.

Conclusions

Empis is designed for chronic infusions, *in vivo* microdialysis, and programmed dosing of animals in a Raturn interactive cage. The current study demonstrated the reliability of Empis used in a prolonged rat brain microdialysis experiment. The same device can also collect information about animal activity.

References

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F3. Animal activity data (clockwise rotations and counterclockwise rotations) for rat 441 on day 1 (A) and day 2 (B), when vehicle and *d*,l-amphetamine at 2.5 mg/kg was administrated i.v., respectively. (Dosing time indicated with black arrows.)