Applicatin 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.

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 in-house de-ionized water using a NANOpure system (Barnstead/Thermolyne, Dubuque, IA, USA).

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.

<table>
<thead>
<tr>
<th>Rat ID Number</th>
<th>439</th>
<th>441</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark phase (7 p.m. - 7 a.m.)</td>
<td>0.21±0.04</td>
<td>0.17±0.05</td>
</tr>
<tr>
<td>Light phase (7 a.m. - 7 p.m.)</td>
<td>0.28±0.03</td>
<td>0.26±0.05</td>
</tr>
</tbody>
</table>

**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
1. www.empis.net
2. S.H. Khan and A. Shuaib, Methods, 23
3. L.J. Pellegrino, A.S. Pellegrino and A.J.
   Cushman, A stereotaxic atlas of the rat
4. M.G. Feenstra, M.H. Botterblom and S.
   Mastenbroek, Neurosci. 100 (2000) 741-
   8.
5. P.E. Paulson and T.E. Robinson, Behav

Empis, Raturn, ChromGraph and Honeycomb are
registered trademarks of Bioanalytical Systems,
Inc.

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.)