Spectroscopic Measurements of Physiological Elements in Microdialysis Samples from Rat Brain, Flowering Plum Fruit and Pea

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Abstract: The basal levels of magnesium and copper in rat brain and flowering plum fruit dialysates, and the background concentration of calcium in pea dialysates have been determined with sensitive spectroscopic techniques including atomic absorption spectrometry and spectrophotometry based on amino G acid chlorophosphonazo. It is found that the magnesium level in flowering plum fruit dialysates is much lower than that in rat brain dialysates, indicating a considerable composition difference present between a plant dialysate and an animal one.

Keywords: Microdialysis, spectroscopic methods, physiological element analysis, calcium, magnesium, copper.

Introduction

Microdialysis is an important bioanalytical sampling technique, which involves the implantation of a small probe of semipermeable membrane into the subject to be studied[1]. The method is minimally invasive and very suitable for studying parameters such as physiological elements[2-4], low molecular compounds and their metabolism[5-7] in living subjects. Up to now, however, there are much fewer reports on measuring such physiological elements as magnesium and copper in microdialysates from rat brain and especially from flowering plum fruit, and no reports on determining calcium in pea dialysates have been found in the literature, due to the limited volumes of microdialysates available and requiring highly sensitive analytical methods.

Physiological elements play a key role in a number of physiological processes of animals and plants. Determination of the background concentrations of the elements in extracellular dialysates should be conducive to further comprehension of their biological functions. In this paper, sensitive spectroscopic methods including atomic absorption spectrometry (AAS) and spectrophotometric method based on amino G acid chlorophosphonazo (AGCPA) have been examined for the determination of magnesium and copper in rat brain and flowering plum fruit dialysates, and calcium in pea dialysates. The results are discussed.

Experimental

Apparatus and Materials.

A Hitachi 180-70 polarized Zeeman atomic absorption spectrophotometer with an electrothermal atomizer was used to determine the concentrations of magnesium and copper, whose analytical wavelengths were set at 285.2 and 324.8 nm, respectively. A Model 721 spectrophotometer (Shanghai) was employed to quantify calcium levels in dialysates from pea. The working solutions of calcium, magnesium and copper were obtained by diluting their stock standard solutions (1 mg/mL) with water. AGCPA was a gift from Chemistry Department of Wuhan University, and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (Hepes) was purchased from Merck (Germany). 0.05% (w/v) of AGCPA solution and 0.02 mol/L of Hepes-NaOH buffer solution (pH 7.0) were employed for the spectrophotometric determination of calcium. All other chemicals used were of analytical grade, and the water was deionized and distilled. Male Wistar rats of 230 to 260 g, pea and flowering plum fruit were used.

Procedures.

Microdialysis probes (3 mm membrane; 0.22 mm diameter; 18,000 molecular weight cutoff) were inserted into the rat hippocampus, or the core region of pea or flowering plum fruit. The probes were continuously perfused at 1~1.5 μl/min with Ringer's solution (Na^+ 145 mmol/L, K^+ 4.0 mmol/L, Ca^{2+} 1.3 mmol/L, pH 7.4) for rat brain and pea, and with pure water for flowering plum fruit. Dialysates were collected every 30 min. The dialysate concentrations of magnesium and copper were determined directly by AAS using pyrolytic graphite coated tubes, and calcium levels quantified by spectrophotometry based on AGCPA [8]. Analytical data were expressed as mean ± SD (n = 3). The reported levels were not corrected for the probe recovery.

Results and Discussion

To keep the living subjects to be disturbed minimally, microdialysis is usually performed at a slow perfusion rate (1~5 μl/min) and only very small volumes of dialysates (10~30 μl) are collected. Therefore, highly sensitive methods are required for the determination of any species in dialysates. In our present studies, the detection limits by AAS are 0.6 and 10 ng/mL for magnesium and copper (S/N=3, 10 μl of injection volume), respectively, which are sensitive enough to achieve the direct determination. Additionally, the spectrophotometric method based on AGCPA offers a sufficient sensitivity to calcium, with a detection limit of 20 ng/mL[8]. The color reaction of AGCPA with calcium takes place rapidly in a neutral medium of pH 7.0 Hepes-NaOH buffer. The stable and greenish blue complex formed absorbs maximally at 670 nm with a molar absorptivity of 7.2×10^4 L $mol^{-1}cm^{-1}$. Using these methods, the dialysates from rat brain and flowering plum fruit were analyzed for magnesium and copper, and the dialysate from pea analyzed for calcium. The results are shown in **Table 1**. The recoveries of these methods are 94~102%. Owing to the absence of magnesium and copper in the used perfusate, the obtained values of magnesium and copper should represent their background concentrations in dialysates. Surprisingly, the magnesium level in dialysates from flowering plum fruit is much lower than that from rat brain. A possible explanation is that a plant dialysate differs significantly from an animal one in composition. Compared to 52 μg/mL (1.3 mmol/L) of calcium present in perfusates, a slight increase in the concentration of calcium in pea dialysates was observed after equilibration of dialysis exchanges, but such an increase had no statistically significant difference (Student's t-test, $P < 0.05$), suggesting that this calcium value reflects its basal level in microdialysates.

Table 1. Analytical results of physiological elements in microdialysates

Subject	Concentrations of elements in microdialysates (μg/mL)		
	Calcium	Copper	Magnesium
Rat brain	-	0.037 ± 0.009	16 ± 1
Flowering plum fruit	-	0.026 ± 0.005	0.33 ± 0.06
Pea	57 ± 6	-	-

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References

1. A. Mendelowitsch, H. Langemann, B. Alessandri, H. Landolt and O. Gratzl, "*Clinical Aspects of Microdialysis*", Springer, Wien, **1996.**
2. T. P. Obrenovitch and E. Zilkha, *J. Neurophysiol.,* **1995,** *73*, 2107-2114.
3. M. C. Linhares and P. T. Kissinger, *J. Pharm. Biomed. Anal.,* **1993**, *11*, 1121-1127.
4. C. E. Lunte, D. O. Scott and P. T. Kissinger, *Anal. Chem.,* **1991**, *63*, 773A-780A.
5. M. G. Boutelle and M. Fillenz, *Acta Neurochir.(Wien),* **1996**, *[Suppl] 67*, 13-20.
6. G. Damsma, D. P. Boisvert, L. A. Mudrick, D. Wenkstern and H. C. Fibiger, *J. Neurochem.,* **1990,** *54*, 801-808.
7. M. G. Boutelle, L. K. Fellows and C. Cook, *Anal. Chem.,* **1992,** *64*, 1790-1794.

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