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# Quantitative characterization of the time dependence of boron uptake by brain cells

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## Introduction

In boron neutron capture therapy (BNCT), relatively harmless thermal neutrons interact with a nonradioactive isotope of boron, thus creating high-energy α-particles able to selectively damage the brain cells in which the interaction had occurred. Dodecahydro-dodecaborate (BSH) and boronophenylalanine (BPA) are the most relevant candidates for BNCT of brain tumors [1—3]. There is therefore a strong interest in the study of the uptake of these two compounds by different species of brain cells, in order to elucidate the effects of BNCT.

In the present paper, we report a quantitative chemical analysis on primary cultures of granule and glial cells from postnatal rat cerebella, exposed to BSH or BPA after 8 days in vitro. We observed, by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES), that the concentration of boron in cells depends on the time of exposure to BSH or BPA. The differences in boron uptake between glial and granule cells will be also discussed.

## Material and Methods

Cells extracted from rat cerebella were allowed to grow for 7 or 8 days on Petri dishes pretreated with 5 μg $ml^{-1}$ of poly-L-lysine solution. Selective techniques [4] were used to obtain cultures with prevailing populations of different types of cerebellar cells, namely granule cell neurons or glial cells. At the end of the growth period, the cultured cells were washed and then exposed to BSH or BPA solutions in uptake buffer (500 μg $ml^{-1}$) [5]. Different exposure times (0—60 min) were used. After careful washing to remove the nonuptaken boron compound, the cultures were suspended in 1 N $HNO_3$ to solubilize boron ions.

ICP-AES performs chemical analysis of liquid samples. The liquid solution is

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heated to a cold-plasma temperature (8,000° C) by an electromagnetic field. At this temperature each element emits a specific near-ultraviolet light wavelength that can be easily identified. Measuring the emission intensity at each wavelength and comparing it to a standard reference makes it possible to determine the concentration of each element in the solution. The sensitivity reaches a few ppb. An example of the conceptual background of the ICP-AES technique can be found in [6,7].

## Results and Discussion

In Fig. 1, the results of the ICP-AES measurements on glial cells exposed to BSH (Fig. 1A) and to BPA (Fig. 1B) are reported. The results were normalized to the cell volume, i.e., 0.5 μl for $10^6$ granule cells and 1 μl for $10^6$ glial cells. Five cell culture separate samples were measured for each time point in Fig. 1A, two samples per point in Fig. 1B. The higher number of replicates in the case of BSH explains the smaller statistical errors of Fig. 1A as compared to Fig. 1B.

Control samples without cells were also investigated. They consisted of poly-L-lysine coated Petri dishes exposed to BSH or BPA for 60 min. In this case, we evaluated a "virtual" concentration in cells by normalizing the ICP-AES results of the control samples to the cell volumes. This procedure permitted us to compare directly the control samples (no cells) with the cell cultures in Figs. 1 and 2. We noticed the presence of a small amount of boron in the control samples without cells. The effect can be related to the existence of a weak adsorption phenomenon between boron compounds and poly-L-lysine on substrate.

The uptake of boron by glial cells exposed to BPA is higher (by about a factor of three) than the uptake obtained using BSH. Our results indicate an evident

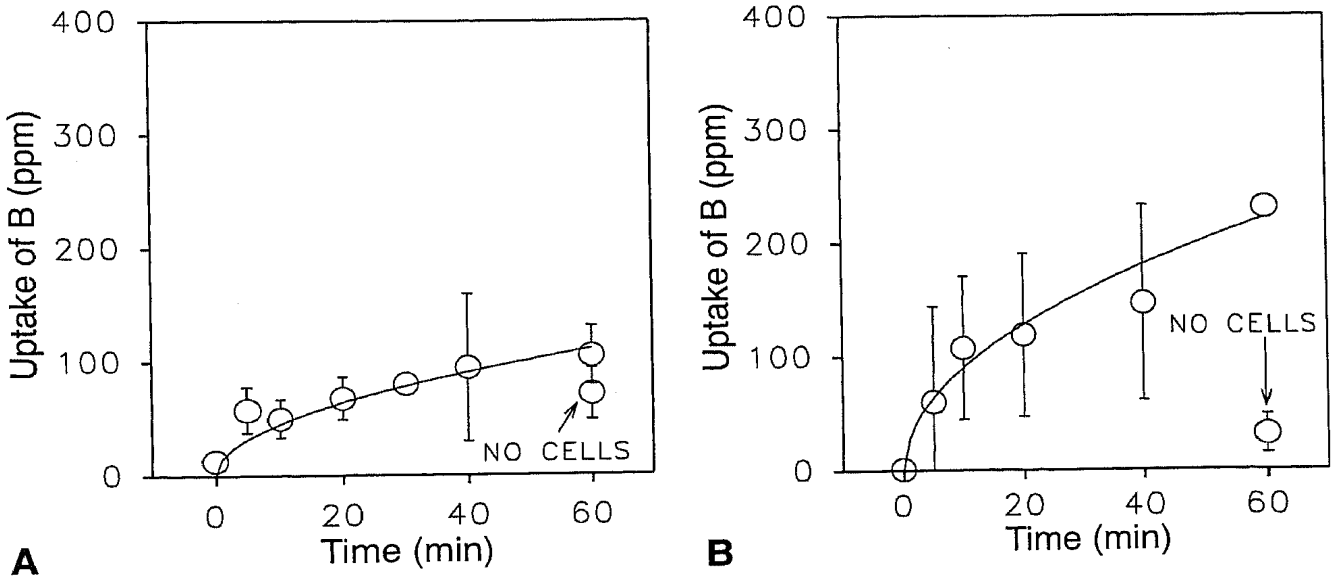


*Fig. 1.* Concentration of boron in glial cells exposed to BSH **(A)** and to BPA **(B)**. $7{\cdot}10^6$ cells were plated on 92-mm diameter Petri dishes and solubilized in 4 ml of nitric acid. Error bars are standard deviations. Solid lines indicate square root functional behavior.

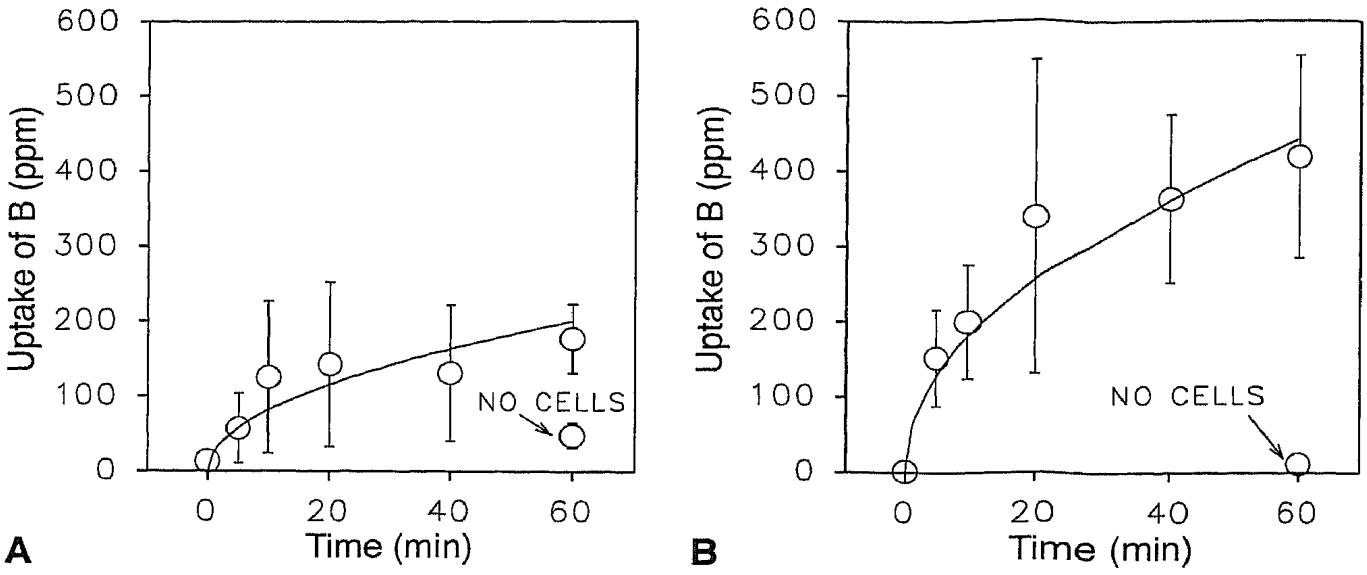


*Fig. 2.* Concentration of boron in granule cells exposed to BSH **(A)** and to BPA **(B)**. $7.5 \cdot 10^6$ cells were plated on 58-mm diameter Petri dishes and solubilized in 2 ml of nitric acid. Error bars and solid lines have the same meanings as in Fig. 1.

time dependence of the amount of boron in the glial cells: in fact, the quantity of boron detected in the cell's body by ICP-AES increases as the exposure time is increased. The time dependency of the boron uptake has been observed for both the investigated compounds. We remark that no saturation effect was observed in the investigated range of exposure times and the uptake of boron seems to follow a simple diffusion law, i.e., as the square root of time (continuous lines in Figs. 1 and 2).

The results of the ICP-AES experiments on granule cells exposed to BSH and BPA are reported in Fig. 2. In these cases the number of cell cultures investigated for each point was five for BSH and two for BPA. As in the case of glial cells (Fig. 1), the uptake of boron for BPA was higher with respect to that of BSH, showing a clear dependency on the exposure time for both the investigated compounds. Again, no saturation effect was observed in the investigated range. Note that BPA adheres to poly-L-lysine less than BSH (points indicated as "no cells" in Figs. 1 and 2).

## Conclusions

Our results indicate that: 1) glial cells take less boron up than granule cells; and 2) BPA enhances boron uptake with respect to BSH. These two results lead to the conclusion that BPA seems to be more promising for the success of BNCT (because of the higher uptake), and also that glial cells are less "attackable" by means of this therapy than granule cells.

Furthermore, we observe that with BSH or BPA, in glial or granule cells, boron concentration in cells increases with exposure time. We analyzed such a time dependence and found that in all cases it followed the physical behavior of a sim-

ple diffusion law. This suggests that much larger exposure times would be preferable to enhance the boron uptake in the investigated ranges.

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