Environmental Boron Exposure and Activity of δ-Aminolevulinic Acid Dehydratase (ALA-D) in a Newborn Population

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Following boron intake, multiple effects have been observed in animal experiments. However, human data is lacking, and no data is available on the ability of boron to accumulate in fetal tissues. Positive responses in animal species suggest that developmental toxicity may be an area of concern in humans, following exposure to boron. Two hypotheses have seemed to account for the multiple effects described in scientific findings. One hypothesis is that boron is a negative regulator that influences a number of metabolic pathways by competitively inhibiting some key enzyme reactions. The other hypothesis is that boron has a role in ionic membrane transport regulations. To better understand boron potential toxicity, the present study examined the relationship between boron exposure and some key enzymes, well-known for their affinity for mineral elements, such as δ-aminolevulinic acid dehydratase (ALA-D), and two fundamental enzymes having a role in ionic membrane transport regulations (Ca-pump and Na⁺ K⁺-ATPase). We investigated the potential effects of an environmental boron exposure on the activity of these enzymes in an urban population of 197 “normal” newborns. Environmental boron exposure was assessed in placental tissue. Because of the well-known inhibiting effect of lead on these enzymes, cord blood and placental lead were also analyzed. After adjustment for potential confounders, including lead, placental boron levels were negatively significantly correlated to ALA-D activity while Ca-pump and Na⁺ K⁺-ATPase activities did not seem to be affected by the level of boron exposure. Given boron’s ability, as a Lewis acid, to complex with hydroxyl groups, we suggest that such a mechanism would explain the inhibiting effect of boron on ALA-D.

Key Words: boron; human placenta; δ-aminolevulinic acid dehydratase; Na⁺ K⁺-ATPase; Ca-pump; epidemiology.

In humans, boron exposure occurs primarily through oral intake of food and drinking water. Elemental boron is inert in the presence of water, boron compounds rapidly transform to borates, the main naturally occurring form of boron (Smith and Ascherl, 1999).

Numerous experimental studies have shown that boric acid and borax are absorbed from gastrointestinal and respiratory tracts, as indicated by increased levels of boron in blood and tissues (Bai and Hunt, 1996; Hunt, 1989; Shuler et al., 1990).

To our knowledge, no epidemiological studies are available regarding the effects of boron on the developing human fetus. Experimentally, fetal toxicity was observed in rats, mice, and rabbits (Heindel et al., 1992; Price et al., 1996). Average fetal body mass index was significantly reduced in a dose-related manner in all exposed groups compared to controls. In vivo experiments also found various amine carboxyboranes to exert a diverse set of effects including lowering serum cholesterol, and blocking calcium resorption (Hall et al., 1994).

Although human data is lacking and no data is available on the ability of boron to cross the placenta or accumulate in foetal tissues, positive responses in animal species suggest that developmental toxicity may be an area of concern in humans following exposure to boron.

Boron could be a negative regulator that influences a number of metabolic pathways by competitively inhibiting some key enzyme reactions: Elevation of δ-aminolevulinic acid (ALA) level, due to inhibition of δ-aminolevulinic acid dehydratase (ALA-D) enzyme, is a major neurotoxic mechanism. Elevation of ALA results in overproduction of active oxygen species (Bechara et al., 1993; Hermes-Lima et al., 1991), inhibition of Na⁺ K⁺-ATPase enzyme (Russell, 1983), action potential deficiency and intramitochondrial calcium liberation (Hermes-Lima et al., 1991). ALA elevation also leads to perturbation of GABAergic system (Minnema and Michaelson, 1986) and changes in the adrenergic system (Cutler et al., 1985). Moreover, ALA-D plays an important and well-known role in hematopoiesis (Suzen et al., 2003).

The calmodulin-regulated Ca-pump [Ca²⁺ Mg²⁺-ATPase; Mg²⁺-dependent, Ca²⁺-activated ATPase] is responsible for extrusion of calcium ions (Ca²⁺) from the cell and for maintenance of the very low intracellular Ca²⁺ concentration (Carafoli, 1987).
The Na\(^+\)K\(^+\)-ATPase is a transport protein responsible for
maintaining ionic gradients across the plasma membranes of
animal cells. Hydrolysis of ATP is coupled with the exchange
of Na\(^+\) and K\(^+\) ions, and is specifically inhibited by ouabain. This
plasma membrane enzyme, ubiquitous in animal cells, is
involved in generating the plasma membrane electrical potential.

To better understand boron potential inhibition of enzyme
reactions, the present study examined the relationship between
placental boron levels and whole blood ALA-D, erythrocyte
membranes Ca-pump and Na\(^+\)K\(^+\)-ATPase activities among
197 newborns environmentally exposed to boron through
ternal environmental exposure. Because of the well-known
inhibiting effect of lead on ALA-D (Campagna et al., 1999),
cord blood and placental lead were also analyzed.

MATERIALS AND METHODS

Subjects. One hundred ninety-seven mother-newborn pairs were recruited
in the Robert Debré Maternity Hospital. This large pediatric hospital predomi-
nantly serves the suburbs and Northeastern parts of Paris, France. All mothers
gave informed consent. Information regarding obstetrical history was used from
medical records for exclusion criteria: stillbirths, multiple births, congenital
malformations, pregnancies under regular drug treatment, and births with caes-
sarean sections or at a gestational age lower than 37 weeks. A standardized
questionnaire was administered to the mothers by the same observer on the
third day after delivery. It detailed the daily maternal average number of cigare-
ettes smoked, daily intake of tap water, coffee, and tea by cup, and consumption of
wine, beer, cider, and liquors per week. Mothers were classified as “smokers” if
they smoked at least one cigarette a day, and as “alcohol drinkers” if they drank at
least one alcoholic beverage a week on a regular basis during the entire course of
pregnancy. Mothers were considered to be “coffee or tea drinkers” if they drank
at least one cup of tea or coffee daily throughout the pregnancy.

Sampling and Biochemical Analyses

Determination of boron placental contents. Placental samples were col-
clected immediately after birth and placed in polystyrene containers. Four pla-
cental specimens of approximately 10 g were cut from the dorsal (fetal) side then
were frozen and stored at \(-80^\circ\)C. Concentrations of boron were determined in
placental samples by atomic emission spectrophotometry (Spectrametrics Spectra-
traspin V). The wavelength used was 249.8 nm. Results were corrected for
reagent blank and expressed as microgram of boron per gram of wet weight
placenta (\(\mu g/g\)).

Determination of cord blood lead levels. Cord blood were sampled at
delivery in a heparinized Vacutainer\textsuperscript{®} tube and kept at \(+4^\circ\)C until analysis.
Lead concentrations were measured by flameless atomic absorption spectro-
photometry using a Zeeman effect corrector (Perkin Elmer 4100 ZL) and results
were expressed in \(\mu g/dL\). The accuracy and reliability of blood lead determination
were checked by national evaluation of quality control.

Determination of ALA-D activity in cord blood. ALA-D activity was
measured less than 24 h after blood sampling using the European Standardized
Method (Berlin and Schaller, 1974). Spectrophotometric determination was
conducted at 555 nm and an extinction coefficient of porphobilinogen of
0.0621 \(\mu M^{-1} cm^{-1}\) was used. Results were corrected for reagent blank and
were calculated as the mean of two measurements, each of them done in triplicate.
Activity was expressed in \(\mu M min^{-1} l^{-1}\).

Determination of Na\(^+\)K\(^+\)-ATPase and Ca-pump activities. Red blood
cell membrane suspensions (ghost) were obtained according to a method mod-
ified from Hanahan and Ekholm (1974). Na\(^+\)K\(^-\)-ATPase and Ca-pump activities
were measured using a modification of the method of Beutler et al. (1983).
Colorimetric assays were performed according to the Hurst method
(Hurst, 1964). Analyses were done in triplicates. The activity was expressed in
nM of inorganic phosphate produced per mg of protein per hour
(\(nM mg^{-1} h^{-1}\)).

Statistical analyses. Statistical analyses were performed using SAS version
8.02 (SAS Institute Inc., Cary, NC). Because of skewed distribution, placental
boron (\(B_{PL}\)) and lead (\(Pb_{PL}\)) contents, cord blood lead levels (\(Pb_{C}\)), ALA-D
activity (ALA-D\(_C\)), membrane Na\(^+\)K\(^+\)-ATPase and Ca-pump activities were
transformed to their decimal logarithms. The potential confounders considered
for the relationship between enzymes activities and \(B_{PL}\), \(Pb_{PL}\), \(Pb_{C}\) were mother
and father’s ages at delivery, alcohol, tea, coffee and smoking habits during
pregnancy, gestational age, placental weight, and child’s sex. To isolate an
optimal subset of potential confounders, a forward stepwise linear regression
analysis was performed with the confounders included as independent variables
to explain mother and cord ALA-D activities. Independent variables were
entered one after another, depending on their respective contribution to the
explained variance, until the remaining factors failed to reach the 30%
significance level of the F statistics. A similar procedure was followed to examine
the relationship between these potential confounders and placental
boron levels.

Univariate analysis between enzyme activities and \(B_{PL}\), \(Pb_{PL}\), and \(Pb_{C}\)
measurements used Spearman correlations. ALA-D\(_A\) was adjusted for the optimal
subset of isolated potential confounders.

RESULTS

Population and Biological Variables Characteristics

Mothers’ mean age at delivery was 29.4 \(\pm 4.1\) years. Half of the
participants were primiparous. Fifty-four percent of newborns were of male gender. The mean birthweight was 3368 \(\pm 417\) g. Means and distributions of \(B_{PL}\), \(Pb_{PL}\), \(Pb_{C}\) levels and
ALA-D\(_C\), Na\(^+\)K\(^+\)-ATPase, and Ca-pump activities are shown in
Table 1. \(Pb_{PL}\) and \(Pb_{C}\) were available only on 195 and
175 samples respectively, due to lack of biological material. Nine measures of Na\(^+\)K\(^+\)-ATPase and Ca-pump activities were missing because of failure in analysis procedure.

Predictive Variables for \(B_{PL}\) and ALA-D, Na\(^+\)K\(^+\)-
ATPase, and Ca-Pump Activities

In linear regression models using a forward stepwise procedure,
mother’s tobacco consumption (\(p = 0.01\)) paternal

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<td>Biological Variables Characteristics</td>
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<tr>
<td>(B_{PL}) ((\mu g/g))</td>
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<td>(Pb_{PL}) ((\mu g/g))</td>
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<td>ALA-D(_C) ((\mu M min^{-1} l^{-1}))</td>
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<tr>
<td>Na(^+)K(^-)-ATPase ((nM mg^{-1} h^{-1}))</td>
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<tr>
<td>Ca-pump ((nM mg^{-1} h^{-1}))</td>
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Note. \(B_{PL}\), placental boron content; \(Pb_{PL}\) and \(Pb_{C}\), placental and cord blood lead levels; ALA-D\(_A\), cord blood ALA-D activity.
age (p = 0.08), gestational age (p = 0.24), and tap water consumption (p = 0.25) were positively related to BPl, whereas other variables were not related to BPl (p > 0.30).

In the same way, using a forward stepwise procedure, tobacco consumption (p = 0.02), maternal age (p = 0.18), and gestational age (p = 0.18) were negatively related to ALA-DC, while alcohol consumption (p = 0.26) was positively related to ALA-DC activity. Other variables taken into consideration in this study were not related to ALA-DC (p > 0.30).

Coffee consumption (p = 0.11), maternal age (p = 0.07), gestational age (p = 0.08), and newborn sex were related to Na\(^++\)K\(^+-\)ATPase activity while paternal age (p = 0.11), newborn sex (p = 0.11), tea consumption (p = 0.12), gestational age (p = 0.18), and coffee consumption (p = 0.18) were related to Ca-pump activity.

In the following analyses, ALA-DC values were adjusted for tobacco consumption, gestational age, maternal age, and alcohol consumption, while Na\(^++\)K\(^+-\)ATPase activity was adjusted for coffee consumption, maternal age, gestational age, and newborn sex, and Ca-pump activity was adjusted for newborn sex, tea consumption, gestational age, and coffee consumption. To avoid overmatching in the statistical analysis, BPl was adjusted only on tap water consumption. PbC and PbPl being considered as potential confounding variables in this study, no such adjustment was done for these variables.

Interrelationships between Adjusted BPl, PbC, and PbPl

A positive and statistically significant link was observed between BPl and PbPl (r = 0.193; p < 0.01, n = 195). BPl was not related to PbC levels (r = 0.041; p = 0.58, n = 195) while PbC and PbPl levels were highly correlated (r = 0.453; p < 0.001, n = 175).

Interrelationships between Adjusted ALA-D Activity and BPl, PbC, and PbPl

ALA-DC was negatively correlated with BPl, PbC, and PbPl although only PbC reached the 5% significant level (r = -0.133, p = 0.06, n = 197; r = -0.156, p = 0.03, n = 175; and r = -0.131, p = 0.06, n = 195, respectively).

Relationships between Adjusted ALA-D, Na\(^++\)K\(^+-\)ATPase, and Ca-Pump Activities

A negative relationship between ALA-D and Na\(^++\)K\(^+-\)ATPase was discovered (r = -0.165, p < 0.05, n = 188), whereas no other significant links appeared between ALA-D and Ca-pump activities (r = 0.017, p = 0.87, n = 169) nor between Ca-pump and Na\(^++\)K\(^+-\)ATPase activities (r = -0.054, p = 0.48, n = 169).

### TABLE 2

| Estimated Regression Coefficients, t and p Values for Variables ALA-D, Ca-Pump, and Na\(^++\)K\(^+-\)ATPase as Dependent Variables |
|--------------------------|--------------------------|--------------------------|
| Dependent variables      | Independent variables    | β  | ± STD | t   | p   |
| ALA-D                    |                          |   |       |     |     |
| Placental boron          | -0.112                   | ±0.051 | -2.19 | 0.03|
| Cord blood lead          | -0.085                   | ±0.056 | -1.51 | 0.13|
| Placental boron          | -0.106                   | ±0.046 | -2.30 | 0.02|
| Placental lead           | -0.043                   | ±0.039 | -1.10 | 0.27|
| Ca-Pump                  |                          |   |       |     |     |
| Placental boron          | 0.016                    | ±0.158 | 0.10  | 0.91|
| Cord blood lead          | 0.218                    | ±0.171 | 1.27  | 0.20|
| Placental boron          | -0.018                   | ±0.150 | -0.12 | 0.90|
| Placental lead           | 0.213                    | ±0.120 | 1.77  | 0.07|
| Na\(^++\)K\(^+-\)ATPase  |                          |   |       |     |     |
| Placental boron          | 0.009                    | ±0.141 | 0.06  | 0.94|
| Cord blood lead          | -0.193                   | ±0.153 | -1.26 | 0.20|
| Placental boron          | 0.075                    | ±0.133 | 0.57  | 0.57|
| Placental lead           | 0.053                    | ±0.109 | 0.49  | 0.62|

Note. Placental boron and cord blood lead, or placental boron and placental lead were independent variables.

### Relationships between Adjusted ALA-D, Na\(^++\)K\(^+-\)ATPase, and Ca-Pump Activities, and BPl after Taking into Account PbC or PbPl

Relationships between adjusted ALA-DC and BPl after taking into account PbC or PbPl were analyzed using linear regression models. The models were constructed with ALA-DC activity as the dependent variable (y). Placental boron contents and lead levels (in cord blood or placenta) were used as the independent variables (x1 and x2).

Estimated regression coefficients, t and p values are presented in Table 2 after taking BPl and PbC then BPl and PbPl as the independent variables. The relationships between ALA-DC activity and Pb exposure (assessed by PbC and PbPl) did not appear to be statistically significant while the relationship between BPl and ALA-DC activity maintained a significant statistical level taking into account lead exposure assessments. There was not any significant interaction between BPl and PbC or PbPl.

Figure 1 shows adjusted ALA-D activities following increasing boron placental content. At low values of BPl, no significant change for ALA-DC activity occurred, but after a threshold value of BPl (estimated graphically at about 0.08 µg/g), ALA-DC activity fell down significantly.

### DISCUSSION

The relationship between boron exposure and ALA-D activity remained statistically significant after taking into account lead exposure. These results suggest an inhibiting effect of an
environmental exposure to boron on whole cord blood ALA-D activity. A potential boron threshold, below which there was no inhibiting effect of boron on ALA-D activity, was observed, but could not be demonstrated. We also examined factors associated with ALA-D activity and BPL levels. Inhibiting effect of tobacco consumption was putative (Heinemann et al., 1982). Alcohol consumption and age effects on ALA-D activity also were suspected (Berny et al., 1994; Sieg et al., 1991). In our study, only tobacco consumption was statistically significant, whereas gestational age, maternal age, and alcohol consumption could be slightly predictive. On the other hand, BPL levels were related significantly to maternal tobacco consumption. Other parameters could play also a predictive role on BPL but at a lower extend: paternal age, gestational age, and tap water consumption. Because of a lack in epidemiological data, we could not attempt any comparison with other studies.

We adjusted ALA-D for tobacco and alcohol consumption, maternal age, boron levels in tap water, and paternal age. To avoid overmatching phenomenon we did not adjust for gestational age. Results were basically similar whether they were controlled or not for these potential confounders. Therefore, it is not likely that the negative relationship that we observed between placental boron and cord blood ALA-D activity at such a low exposure level is due to a confounding effect.

If the inhibiting effect of boron on ALA-D activity is real, then we expect to observe effects of boron exposure on the hematopoietic system, because of the well-known role of ALA-D on heme biosynthesis. Literature findings consolidate this hypothesis. In experimental studies, rats were administered weight-normalized doses of boron per kg of body weight per day in the diet as borax or boric acid. High-dosed animals had their hematocrit and hemoglobin significantly lower than in controls. In middle- and low-dose groups, no significant effects on hematological parameters, serum chemistry, or histopathology were observed (Weir and Fisher, 1972). In the field of clinical epidemiology, the effects of boron supplementation after boron depletion in experiments were multiple. Among these effects, changes in erythropoiesis and hematopoiesis were observed, and were related to an increase in blood hemoglobin and mean corpuscular hemoglobin content, but a decrease in hematocrit, platelet and erythrocyte numerations. Two hypotheses seemed to account for the multiple effects described in scientific findings (ATSDR, 1992; WHO, 1998). One hypothesis is that boron has a role in cell membrane function, stability, or structure, and it influences the response to hormone action, transmembrane signalling or trans-membrane movement of regulatory cations or anions (Nielsen, 1991). The second one is that boron is a negative regulator that influences a number of metabolic pathways by competitively inhibiting some key enzyme reactions (Hunt, 1994; Kelly, 1997).

It has been hypothesized that ionic lead (Pb²⁺) may replace ionic zinc (Zn²⁺) on ALA-D binding sites (Campagna et al., 1999; Simons, 1995), which leads to bridging of the sulphydryl functions and to ALA-D inhibition (Tsukamoto et al., 1979). Such a mechanism is not retainable for boron because of its chemical proprieties. Moreover the present study gave insight to the independent inhibiting mechanism of lead and boron given the absence of interaction between them on ALA-D activity. Nevertheless, there is evidence in both in vitro and in vivo studies that boric acid has an affinity for hydroxyl groups, and this may be the mechanism that explains the biological effects of boric acid.

The first logical hypothesis is that boron has a tendency to form double bonds and macromolecules (Gregory and Kelly, 1997; Nielsen, 1988). Boron, as boric acid, acts as a Lewis acid,
accepting hydroxyl ions (OH\(^-\)) and leaving an excess of protons (Loomis and Durst, 1992). Because boron complexes with organic compounds containing hydroxyl groups, it is capable of interacting with number of substances of biological interest (Zittle, 1951). Hence, boron is able to react with multiple steps involving the Lewis acid-base reactions. In this mechanism, a basic group, speculated as a zinc-bound hydroxide, is required to link the two ALA substrates in the final pyrrole product (Erskine et al., 1999, 2001). Given boron’s ability as a Lewis acid to complex with hydroxyl groups, we think that such a mechanism may explain the inhibiting effect of boron on ALA-D.

From a very general point of view, the collections of studies on metal ion toxicity seem to emphasize that inhibition occurs at many levels in many systems, and it is unlikely that any single event can be blamed for toxic effects (O’Halloran, 1993; Thiele, 1992).

In conclusion, in a population of newborns exposed to general environmental boron level through pregnancy, we found a negative relationship between whole blood ALA-D activity and placental boron levels, and a potential boron threshold for this relationship. An important implication of this study is that at low environmental exposure, boron is responsible for a demonstrable biochemical effect. Hence, this potential ALA-D inhibition may lead to neurotoxic and hematological effects. Studies are needed to confirm biochemical effects of boron exposure, and to assess potential effects on newborns and children.

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REFERENCES


