

Letter to the Editor

Answer to letter sent by Professor A.G. Renwick (University of Southampton, UK) related to Simintzi et al. report published in Food and Chemical Toxicology 2007;45(12):2397–401

Dear editor,

Our report on acetylcholinesterase (AChE) activity in frontal cortex homogenates from suckling rat brain in relation to various concentrations of aspartame (ASP) metabolites is an *in vitro* study on an immature tissue.

Furthermore, please, find the answers, point to point, to the interesting comments by Prof. Renwick.

1. Symptoms related to ASP intake were reported by many authors. Especially, CNS symptoms corresponding to ASP consumption such as seizures, memory loss, etc. were reported previously (Camfield et al., 1992; Moser, 1994). Recently, Rico et al. (2006) reported that methanol (aspartame metabolite) alters ecto-nucleotidases and AChE in zebrafish brain, suggesting its implication in neurodegenerative events. From this point of view it was important to investigate AChE activity in a sensitive tissue, such as frontal cortex of suckling rat brain in relation to ASP intake. The used concentrations of ASP metabolites were taken from the literature (Stegink, 1987; Stegink et al., 1988). So the comment by Prof. Renwick about CSF concentration cannot be accepted.
2. In addition, the concentrations of ASP metabolites used in this study were corresponded to those measured after a single dose of ASP ingestion (200 mg/kg) (Stegink, 1987; Stegink et al., 1988). This is correct and it is a common way of sweetened food intake (natural and/or artificial). Also, we cannot ignore that the consumption of a sweet food (i.e. cake, ice-cream) is a kind of loading test with the sweetener. Obviously, the concentrations of metabolites used in the incubation mixture of our experiments strongly mirrored into the way of sweetened food consumption of the individuals (Oyama et al., 2002; Tsakiris et al., 2006).
3. Definitely, the procedure of AChE activity measurements in tissues was the right one. This is further supported by the great number of our reports presented in PubMed,

showing the laboratory experience of the group of researchers in Athens University. Moreover, if we used the commonly found concentrations of ASP metabolites [phenylalanine (Phe), aspartate, etc.] in the blood of individuals who never used ASP as a sweetener in the incubation mixture of the control tissue, as suggested by Prof. Renwick, then we should add the above amount of ASP metabolites to those corresponding to toxic ASP ingestion in the incubation mixture of the tested tissue: e.g. tissue + (control values Phe, aspartate, methanol) vs tested tissue + [(control values Phe, aspartate, methanol) + (metabolites corresponding to toxic doses of the sweetener)]. Consequently, the results of AChE inhibition would be expected worse or similar to those related to higher concentrations of ASP degradation products (Oyama et al., 2002). The nature of inhibition of the membrane as well as pure enzyme AChE is described in details in our study. The interesting comment by Prof. Renwick about the same results caused by the sum and/or each metabolite of the same concentration (e.g. methanol, phenylalanine) could be related to the occupation of the active sites of the enzyme by one metabolite, the latter being a common observation in enzymology.

4. It is well-known that all *in vitro* studies have disadvantages, when they come in *in vivo* reality.
5. Finally, there is no natural food containing toxic amounts of methanol, aspartate and phenylalanine simultaneously, as far as we know.

In conclusion, the presented *in vitro* findings in the mentioned study may be a basis for further *in vitro* and *in vivo* investigations aiming to explain even the very recently reported symptoms related to ASP consumptions (Humphries et al., 2007).

The co-authors and I thank Prof. Renwick for the honour to study our report and make interesting comments. We hope that we gave enough explanation for the presented results.

References

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