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The Case for Manganese Interaction with Mitochondria

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In "Manganese accumulates primarily in nuclei of cultured brain cells", Kalia et al (Kalia *et al*., 2008) report that upon fractionation of neuronal cells, labeled Mn was found primarily in nuclei, with virtually none in the mitochondrial fraction and therefore, that mitochondria may play an insignificant role in subcellular Mn distribution. They also say that "there has been no direct evidence --- on subcellular distribution of Mn," and that the recent report by Morello et al (Morello *et al*., 2008) concluded that nuclear components may represent the "preferential targets for Mn accumulation and toxicity" (Kalia *et al*., 2008).

In fact, a number of other studies (Ayotte and Plaa, 1985; Lai *et al*., 1999; Liccione and Maines, 1988; Maynard and Cotzias, 1955; Miller *et al*., 1975) have determined the subcellular distribution of Mn after tissue fractionation following treatment with Mn *in vivo*, and all demonstrated treatment-related increases in intramitochondrial Mn. In addition, other workers have found that Mn decreases energy metabolism *in vivo* and *in vitro*, including decreases in the activities of mitochondrial enzymes, in membrane potential, and ATP production (Brouillet *et al*., 1993; Du *et al*., 1997; Galvani *et al*., 1995; Gavin *et al*., 1992; Malecki, 2001; Malthankar *et al*., 2004; Roth *et al*., 2000; Roth *et al*., 2002; Wolters *et al*., 1989; Zwingmann *et al*., 2003). Using electron energy-loss spectroscopy with electron microscopy, Morello et al (Morello *et al*., 2008) reported that although nuclei contained more Mn than mitochondria, treatment-related increases were greater in the mitochondria. They concluded that "the relevant distribution of Mn is *not limited* to the mitochondria." Consideration of why the results of Kalia et al differ from those of other distribution studies requires an understanding of mitochondrial ion transport and its response to fractionation.

Mitochondrial sequestration of Ca^{2+} or Mn^{2+} does not represent simple binding but weak binding within a steady state controlled by both influx and efflux of these ions (Gunter and Pfeiffer, 1990). Mn^{2+} is sequestered by the mitochondrial Ca^{2+} uniporter, primarily energized by the internally negative membrane potential ($\Delta\Psi$), and effluxed by the Na^{+}-independent mechanism, primarily energized by the pH gradient (Gavin *et al*., 1990; Gunter and Pfeiffer, 1990; Gunter and Sheu, 2008). Both are maintained by energy-dependent proton pumping across an intact inner membrane. If $\Delta\Psi$ falls, uptake velocity decreases precipitously – by over 83% as $\Delta\Psi$ falls from 180 to 160 mV, for example (Gunter and Sheu, 2008). If $\Delta\Psi$ falls near zero, the weakly bound ions rush out by reverse uniport (Gunter et al., 1975; Gunter et al., 1978)

The "isolated mitochondria" produced by fractionation, whether by mechanical action or by detergents, represent resealed fragments of the original cellular mitochondrial network (Gunter and Sheu, 2008; Chan, 2006). In these resealed mitochondria, $\Delta\Psi$ is dissipated during fractionation, then rebuilt by proton pumping energized by endogenous substrate -- e.g., pyruvate, a product of glycolysis that in the intact cell is transported continually into mitochondria for use in the TCA cycle. However, the fractionation procedure greatly dilutes glycolytic enzymes and substrates, and the amount of endogenous substrate within isolated, resealed mitochondria is greatly reduced and no longer replenished. It has been well known since the 1960's that appreciable Ca^{2+} or Mn^{2+} uptake by these resealed mitochondria requires addition of mitochondrial substrate; however, none was added in the fractionation studies cited

above. Why, then, did all except Kalia et al find Mn in the mitochondria? Maynard and Cotzias (1955) stressed that they treated animals with less Mn than that present in the food. Other *ex vivo* Mn distribution studies (Lai *et al*., 1999; Liccione and Maines, 1988) examined Mn concentrations in brain fractions; since brain Mn uptake is limited both by the blood-brain barrier and by rigorous homeostatic control of absorption and excretion, the amounts reaching mitochondria were probably not large. In contrast, Kalia et al exposed their cells for 24 hours to 100 μM Mn^{2+}. Based on the uptake that we measured in PC12 cells at 100 μM for 24 hours (11.7 nmoles/mg cell protein) (Gunter *et al*., 2005), we estimate that the mitochondria of Kalia et al were exposed to [Mn^{2+}]s over 100 times higher than those of Maynard and Cotzias and much higher than in the other fractionation studies. Following fractionation, the resealed mitochondria would begin to resequester and cycle the surrounding Mn^{2+}, using energy from endogenous substrate. However, in the presence of large amounts of Mn^{2+}, as in the experiments of Kalia et al, Mn cycling would quickly dissipate the endogenous substrate, ΔΨ would fall, and the Mn^{2+} would be released again from the mitochondria to bind to available sites such as nuclei. (For example, Mn^{2+} binds to DNA with an affinity of about 33 μM (Kennedy and Bryant, 1986)). In the presence of small amounts of Mn^{2+}, as with the other fractionation studies, ion cycling would dissipate the available energy much more slowly, and the mitochondria would likely retain measurable amounts of Mn.

While conducting earlier experiments (Gunter *et al*., 2004), we determined the medium [Mn^{2+}] to which isolated mitochondria would pump varying amounts of added Mn^{2+} (Fig. 1). Notice that if 2μM Mn^{2+} is added, the mitochondria will pump the external [Mn^{2+}] to around 80 nM, while if 142 μM Mn^{2+} is added, the mitochondria will only pump the external [Mn^{2+}] to around 3.4 μM. This is because the more extensive Mn^{2+} cycling in the latter case lowers ΔΨ, shifting the steady state toward less uptake. These concentrations represent the levels at which energized mitochondria compete with other cellular binding sites, such as those in the nucleus. These observations suggest that mitochondrial substrates should be added in fractionation studies to minimize redistribution of Mn^{2+}.

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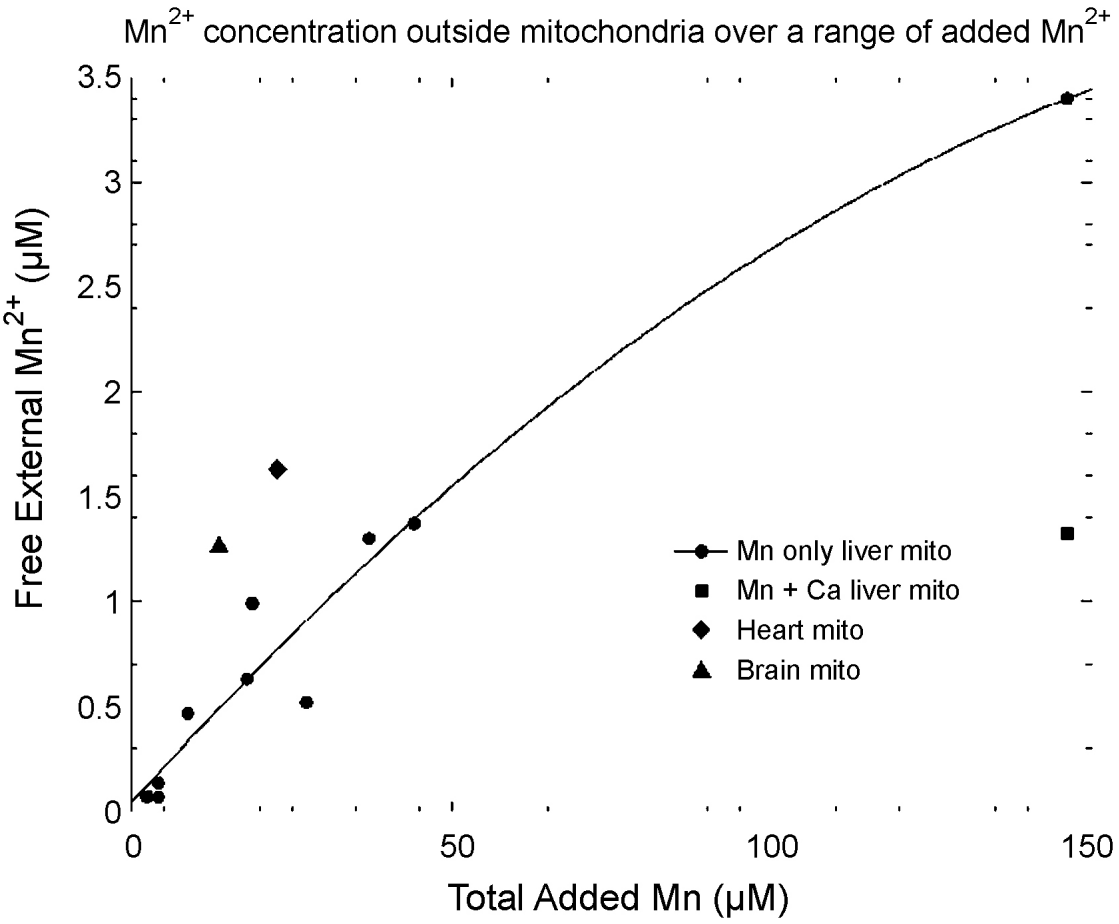

Figure 1.

Concentration of free Mn^{2+} in the medium in the presence of several preparations of energized mitochondria (4 mg/ml) as a function of the total concentration of Mn^{2+} added. 160 μM Ca^{2+} was also added to the data indicated by the filled square to activate the uniporter.