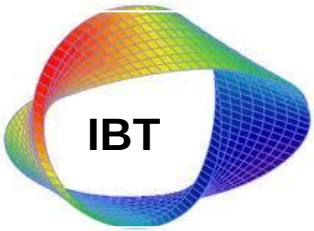
QUANTUM BIOLOGY



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EFFECT OF SILICA ON MEMBRANE TRANSPORT OF PHOSPHO-CHOLINE

Executive Summary

Using yeast cells as a model system for neuronal membrane transport, this study demonstrates that low concentrations of Silica stimulate membrane transport of choline into the cells. Although the effect is small in magnitude, it is highly statistically significant. Further study will be required to determine if this effect also occurs in mammalian neurons, to further substantiate Lifewave's claim that Theta Activate rapidly increases nutrient uptake.

Uptake of Nutrient Choline

Choline and its derivatives are major constituents of the active ingredients (citicholine and alpha GPC) in the Lifewave ThetaMind product. Choline is taken up into cells and either used in the synthesis of phospholipids in most prokaryotic and eukaryotic cells or the synthesis of acetylcholine in nerve cells. Choline uptake into cells occurs via a process called active membrane transport across the cell membrane.

Membrane transport of extracellular choline has been well characterized in neuronal cells grown in tissue culture (Quastel, 1970), where it has been shown to be energy (ATP) dependent and sodium dependent. Recently, a simple model system has been demonstrated to have similar membrane transport properties to those observed in mammalian cells. Sachromyces cerevisiae yeast cells, for example, have been shown to exhibit energy dependent membrane transport of choline (Hosaka, 1980).

Published Membrane Transport Methods

Membrane transport can be measured using radiolabelled choline or fluorescent-labeled choline derivates. When studying membrane transport in S. cerevisiae cells, various authors have measured the uptake of fluorescent labeled choline derivates by incubating the cells in various concentrations of choline (5-50uM), in various solvents (from pure DI water to DI supplemented with salts and proteins), for varying amounts of time (30 – 120 minutes) and at various temperatures (4 – 30° C) (Kean, 1997; Hanson, 2002; Hanson,2003; Elvington, 2005; Chen, 2011). Cell densities, which are determined by optical densities at 600nm, also varied from 0.1 to 0.6 absorption units.

After incubation of cells with chlorine derivates, all methods involve centrifugation and removal of the supernatant containing unincorporated labeled choline, followed by

washing three times to remove non-specifically bound choline from the plasma membrane. The amount of choline inside the cells is then determined either by measuring the absorption or emission of light from the fluorescent label on the choline.

Specific Methodology Used Here

In the experiments reported here, the basic method of Hanson (2003) was used. The fluorescent labeled choline used was NBD-glycero-phosphocholine (Life Technologies (Carlsbad, CA). The NBD label absorbs light at 463nm and emits light at 536nm. S cereviciae (strains WL090 and WLP001 from White Labs, San Diego, CA) were grown in standard dextrose broth suspension medium (from Midwest Supplies, St Paul MN) supplemented with peptone as a source of nitrogen and amino acids (Spectrum Chemical, New Brunswick, NJ) and grown at 30° C.

Standard Assay Conditions

Labeled choline was incubated with S. cereviciae (density range from 0.2 to 0.7 absorption at 600 nm) for 40 minutes at 20°C in the presence and absence of Silica from Theta Activate (Lifewave, San Diego, CA). The solvent for the incubation mixture was either distilled water (DI) or DI water supplemented with minerals (1/1000 of Ionic Sulfate Mineral solution from Adya Clarity, Coldwater, MI). Cells were then centrifuged at room temperature a standard bench-top clinical centrifuge. The supernatant was discarded and the cells were washed three time with cold DI water. Cells were then suspended in 1ml DI water and the amount of labeled choline taken up into the cells was monitored by measuring the amount of light absorption at 463nm using a standard UV-visible spectrophotometer (Spectrecology, Jasper, GA). Labeled choline absorption readings were normalized with respect to cell number which was measured as the absorption values at 600nm. This ratio is referred to as the Specific Activity which represents the amount of labeled choline taken up by the cells relative to the amount of protein. Statistical analysis was done using two sample t-tests (assuming equal variance) from 4-10 separate experimental determinations. “p” values less to 0.05 are considered statistically significant.

The standard procedure utilized the following experimental parameters:

Cell density (0.4 absorption units at 600nm) – synchronized WL090 cells
Labeled choline concentration – 20 uM
Incubation time – 40 minutes
Incubation solvent – DI water
Incubation temperature – 20° C

The following variations were made from the standard assay described above.

1. Synchronized and non-synchronized (starved) cells
2. Two different strains of S. Cerevisiae
3. Incubation time - 20 to 60 minutes
4. Incubation temperature - 4° C to 30° C

5. Incubation solvent – DI water with and without added minerals
6. Labeled choline concentration - 5uM to 50uM
7. Concentration of added Si – 5 to 4800 mg/L
8. Silica added to incubation mixture or pre-incubated
8. Pre-incubation exposure time to Si - 30 minutes to 6 hrs

RESULTS

A. Using the standard assay procedure, linearity of the assay with respect to time was demonstrated. The results are presented in Figure 1. The results indicate the assay is linear for the first 40 minutes and then gradually begins to saturate. This indicates the assay is working properly

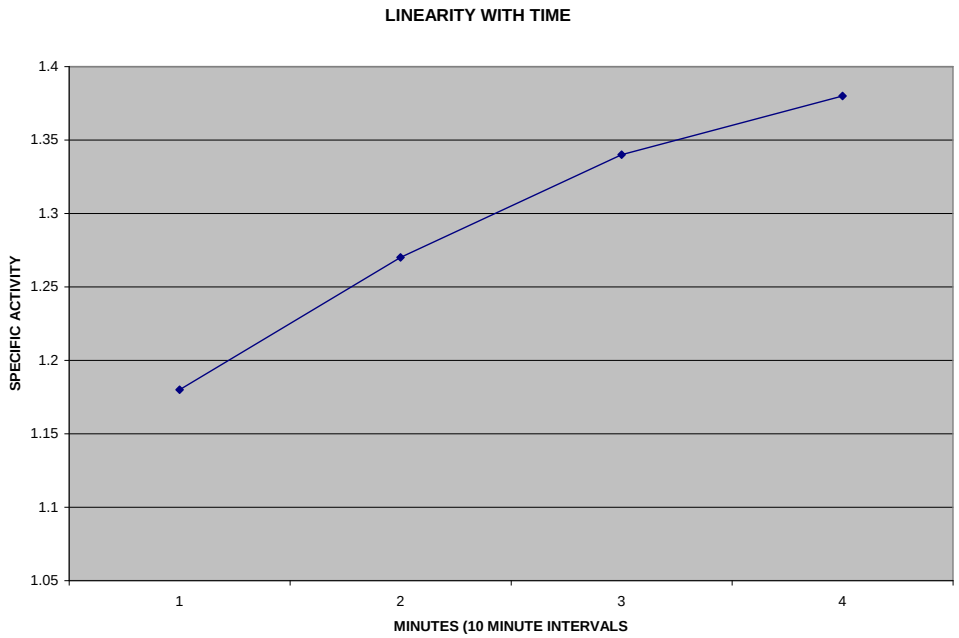

Figure 1 – Linearity with Time

Using the standard assay, Silica was added to the incubation mixture at varying concentrations from 5-4800 mg/L. Using the standard assay conditions and using most of the experimental variations, Silica had no effect on membrane transport.

B. Using the following experimental conditions Silica inhibited membrane transport by 15% (Table 1). The experimental parameters used are:

Cell density (0.7 absorption units) – synchronized WL090 cells
Labeled choline concentration – 20 uM
Incubation time – 40 minutes
Incubation solvent – DI water

Incubation temperature - 20° C
Silica concentration - 3200 mg/L – no preincubation

Exptl Condition	Specific Activity	Std Dev	Inhibition	n	p
Control	1.45	0.06		6	
+ Si	1.23	0.05	15 %	8	$4x10^{-6}$

Table 1: High Concentrations of Silica Inhibit Membrane Transport

C. Using the following experimental conditions Silica stimulated membrane transport by 10% (Table 2): The experimental parameters used are:

Cell density (0.5 absorption units) – synchronized WL090 cells
Labeled choline concentration – 20 uM
Incubation time – 40 minutes
Incubation solvent – DI water supplemented with minerals
Incubation temperature - 20° C
Silica concentration - 5 mg/L – preincubated for 4 hours

Exptl Condition	Specific Activity	Std Dev	Stimulation	n	P
Control	1.22	0.04		10	
+ Si	1.34	0.07	10	10	$3x10^{-4}$

Table 2: Low Concentrations of Silica Stimulate Membrane Transport

DISCUSSION

The results of the present study demonstrate that low concentrations of silica increase the absorption, uptake and delivery of choline derative nutrients into yeast cells. Since the nutrient-based neuro-actives in Lifewave's ThetaMind are taken internally, they are most likely absorbed by the stomach lining and migrate toward enteric neurons in the inner mucosal lining of the stomach. Once activated, enteric cholinergic neurons can communicate directly with the CNS where the stimulating effects of the product can be felt.

Nutrient absorption (in the stomach) occurs via transcellular transport, a type of membrane transport where the bio-active molecules get taken up into the cell across the plasma membrane. Transcellular transport is an active transport (requires chemical energy in the form of ATP) and is carrier-mediated (involve membrane proteins). This basic transport mechanism is universal and occurs in a wide variety of species from humans to yeast cells (Hosaka, 1980).

Due to the inherent difficulty in growing stomach enteric neurons in tissue culture, we chose using S. cereviciae yeast cells as an alternative model system for measuring the influence of Silica on membrane transport processes. Although both neurons and yeast share the same basic trans-membrane transport system, some of their regulatory mechanisms, like energy dependence, are not identical (Hosaka, 1980).

These difference could explain the biphasic dose response observed in the presence study – ie high concentrations of Silica have the opposite effect on membrane transport. Previous studies have demonstrated Silica is toxic to cells at high concentrations around 5000 mg/L (Oberdorster, 1996). Although biphasic responses are somewhat atypical, they have been well documented in the biomedical literature (Huang, 20009). The differences in membrane transport between mammalian and yeast cells could also explain the relatively small (10%), although statistically significant, stimulation of membrane transport by Silica. In one experiment there was a 15% stimulation, but this reduces to 10% when all five experiments were pooled for statistical analyses. It is likely that Silica will be more effective on human enteric neurons.

From a physiological point of view, although the concentration of Silica in the final ingestible solution of the Theta Mind product is relatively high (around 1600 mg/L), by the time the Silica is distributed through the small intestinal lining and migrates to the environment of the enteric neurons, its concentration would likely be diluted to approximately 10-100 mg/L. The lower end of this concentration range corresponds to the dose of Silica demonstrated here to stimulate membrane transport. It is also interesting to note that the lower end of this concentration range also corresponds to the dose of Silica previously shown to stimulate growth of aquatic diatoms (Penna, 2003).

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