

SERCA-mediated calcium uptake is impaired in the prefrontal cortex of Alzheimer's disease patients

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Introduction

- Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects over 55 million people worldwide.
- Excess calcium (Ca^{2+}) in the cytosol promotes amyloid beta ($\text{A}\beta$) production in neuronal cell cultures and promotes the hyperphosphorylation of tau¹⁻⁵.
- The sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) pump facilitates the removal of Ca^{2+} from the cytosol into the endoplasmic reticulum⁶, however, the SERCA pump is thought to be impaired in neurodegenerative diseases^{7,8}.
- Pharmacological activation of SERCA in rodent models of AD improve memory and motor function⁹, **however, whether SERCA is dysfunctional in humans with AD is unknown.**

Purpose

To determine if the SERCA pump is dysfunctional in the brain of AD patients, and whether it might be a viable therapeutic target.

Methods

- Postmortem control (n = 20, 10 per sex) and AD (n = 20, 10 per sex) prefrontal cortex samples (aged 68-95 years old) were obtained from the Douglas Bell Canada Brain Bank (Montreal, Quebec, Canada).
- Samples were homogenized in 1:10 w/v PMSF.
- Maximal SERCA activity was performed using an enzyme-linked spectrophotometric assay.
- SERCA-mediated Ca^{2+} uptake was performed using an Indo-1 supported assay.
 - To determine $\text{A}\beta$ involvement, control samples were incubated with 500 nm $\text{A}\beta$ -42, then subjected to a second Ca^{2+} uptake assay.
- Western blotting was performed for: SERCA2, presenilin-1, HSP70, 4-HNE, and ryanodine receptor.

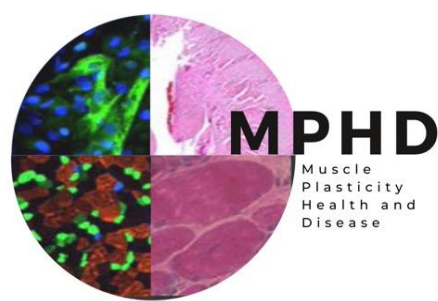
Conclusion

Results indicate that the prefrontal cortex of postmortem AD patients exhibit impaired SERCA-mediated calcium uptake, but not SERCA activity, compared to controls but this is not due to $\text{A}\beta$ interference.

References

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Results

Table 1. Patient Characteristics. Average age in years, brain weight in grams, and postmortem delay of tissue collection in hours for male and female subjects.

	Males (n = 10/group)			Females (n = 10/group)		
	Age (Years)	Brain Weight (g)	Postmortem Delay (Hours)	Age (Years)	Brain Weight (g)	Postmortem Delay (Hours)
Healthy Control	78.6 ± 8.05	1241.46 ± 131.16	16.84 ± 4.88	85.1 ± 7.77	1062 ± 125.83	17.36 ± 5.98
Alzheimer's Disease	79.6 ± 5.87	1251.36 ± 133.49	16.86 ± 4.04	81.4 ± 6.80	977.37 ± 190.24	14.77 ± 5.13

All data presented as means ± SD.

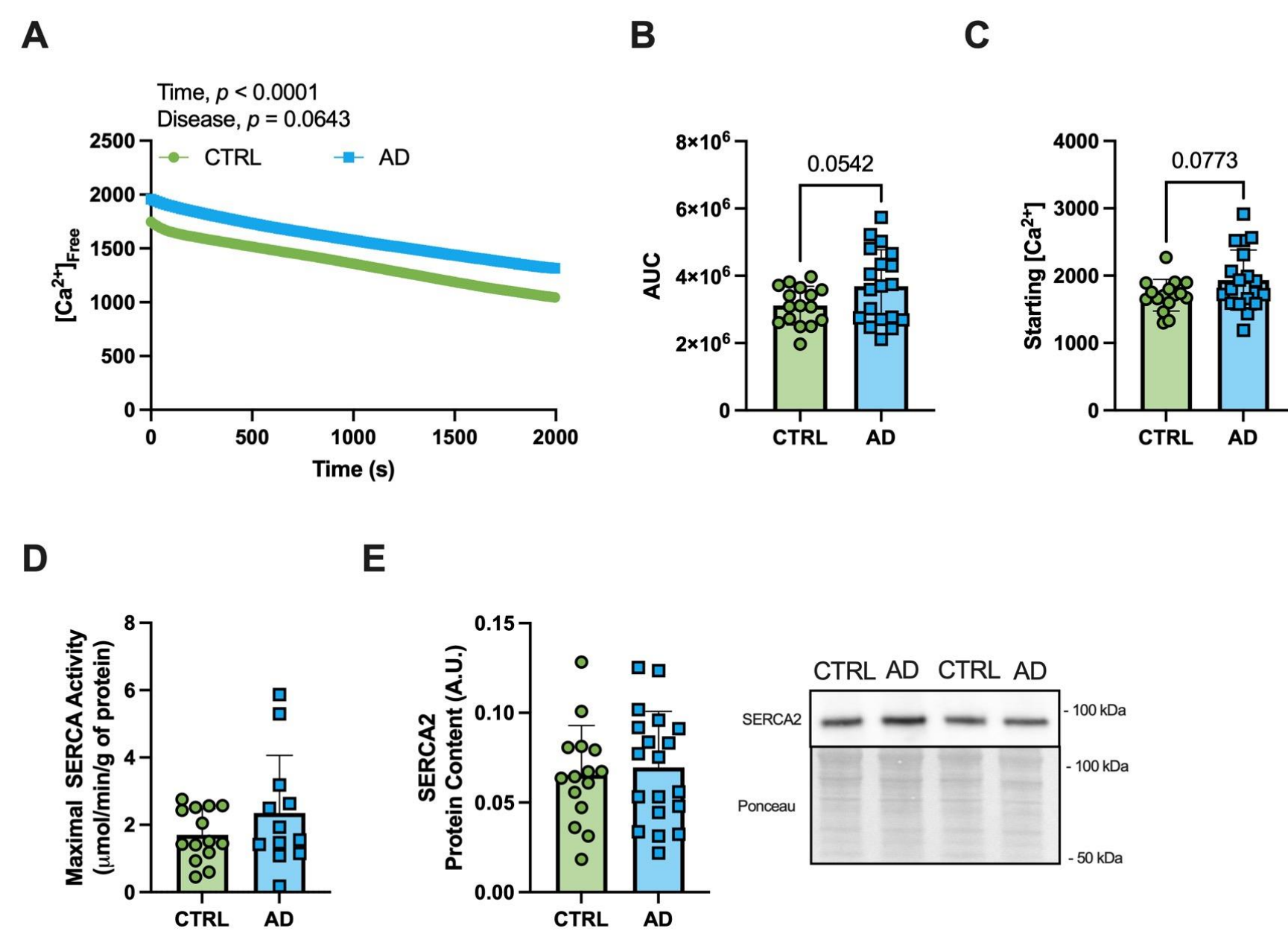


Figure 1. SERCA-mediated Ca^{2+} uptake and activity in the prefrontal cortex of postmortem human brain tissue. A) Representative trace of SERCA-mediated Ca^{2+} uptake over time. A two-way ANOVA was performed and main effects with exact p values are expressed above the graph; n = 19-20 per group. B) Area under the curve. C) Starting calcium concentration. D) Maximal SERCA activity. E) SERCA2 protein expression and representative blot. For B-E, a Student's t-test was run, and exact p values are shown for statistically significant results; n = 12-19 per group. All values are represented as means ± SEM.

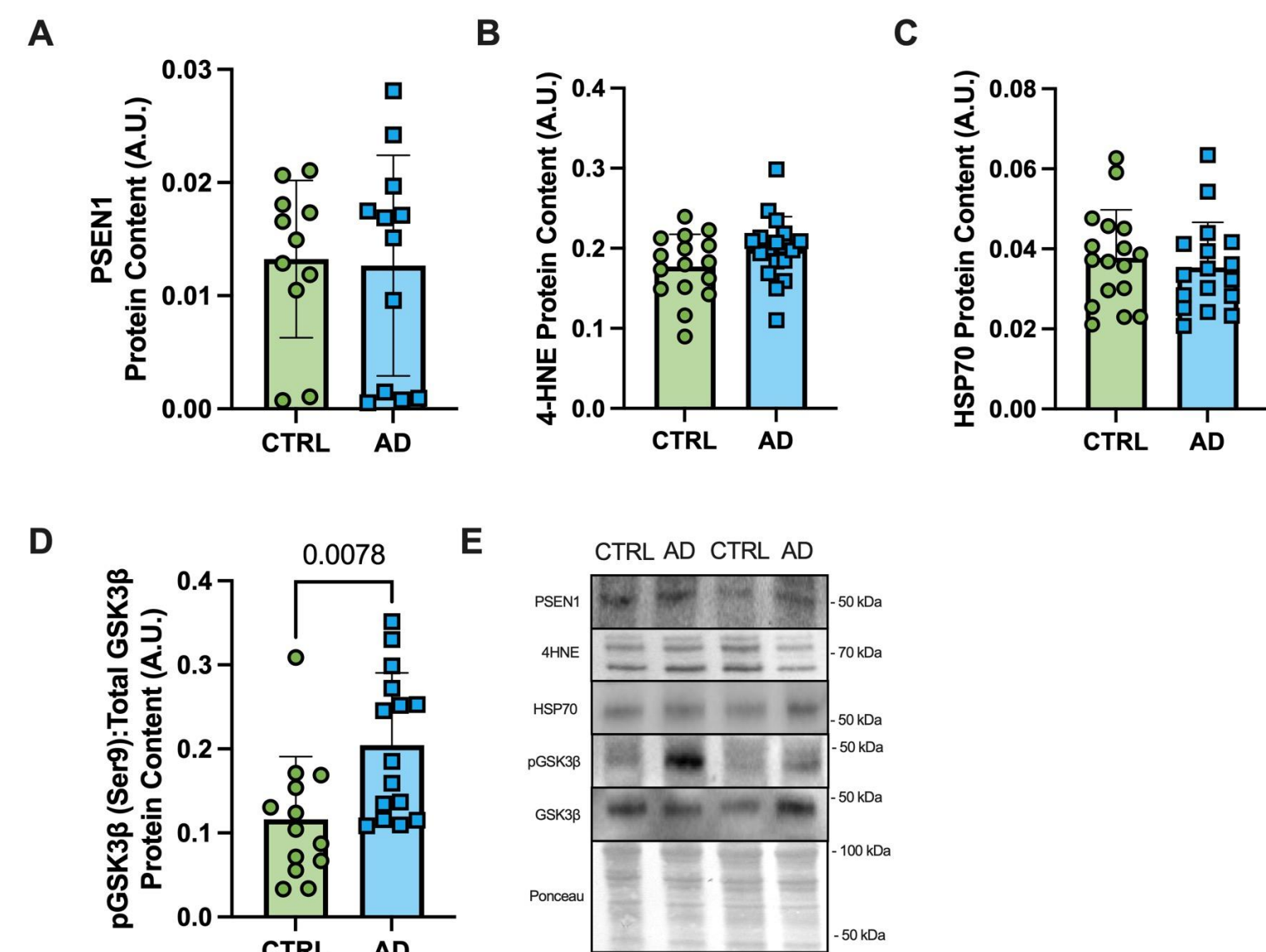


Figure 2. Protein expression of SERCA mediators in the prefrontal cortex of postmortem human brain tissue. A) Presenilin 1. B) 4-HNE. C) HSP70. D) Ratio between phosphorylated and total GSK3 β . E) Representative blots. A Student's t-test was run, and exact p values are shown for statistically significant results; n = 17-19 per group. All values are represented as means ± SEM.

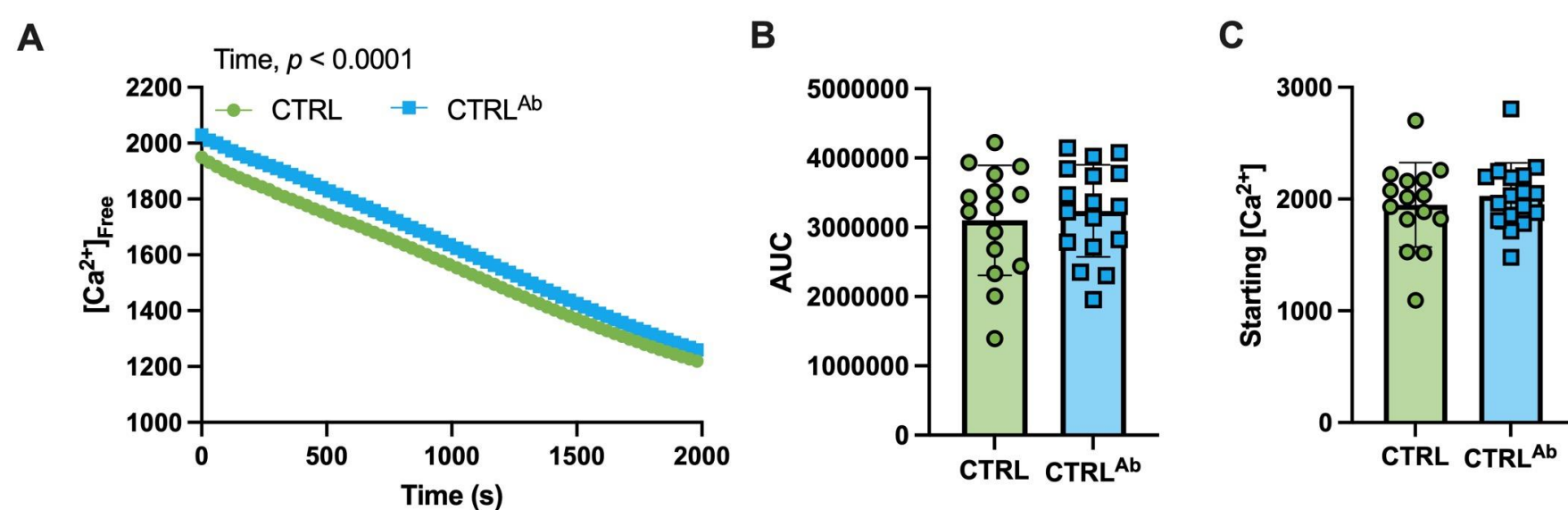


Figure 3. $\text{A}\beta$ -42 Incubation SERCA-mediated Ca^{2+} uptake in the prefrontal cortex of postmortem human brain tissue. A) Representative trace of SERCA-mediated Ca^{2+} uptake over time. A two-way ANOVA was performed and main effects with exact p values are expressed above the graph. B) Area under the curve. C) Starting calcium concentration. For B-C, a Student's t-test was run, and exact p values are shown for statistically significant results; n = 16-18 per group. All values are represented as means ± SEM.

