To poster P4-224, ICAD2008, Pomytkin et al.. Mitochondrial respiration plays an integral role in insulin receptor activation in neurons: a possible link between dysfunctional insulin receptor signaling and mitochondrial dysfunction.

Background. Insulin receptor is a tyrosine kinase regulated by reversible tyrosine phosphorylation of critical tyrosine residues 1158, 1162, and 1163 in the region of kinase domain. It is generally believed that insulin binding leads to major conformational changes, upon which insulin receptor undergoes fast phosphorylation and becomes fully active.

Results 1. Herein, we demonstrate that the process of insulin receptor phosphorylation (i.e. activation) is more complex than thought before and involved mitochondria as a key regulatory element:

Upon insulin stimulation, cerebellar granule neurons (CGN) produce an about 20-seconds-long nanomolar spike of hydrogen peroxide (see figure 1).

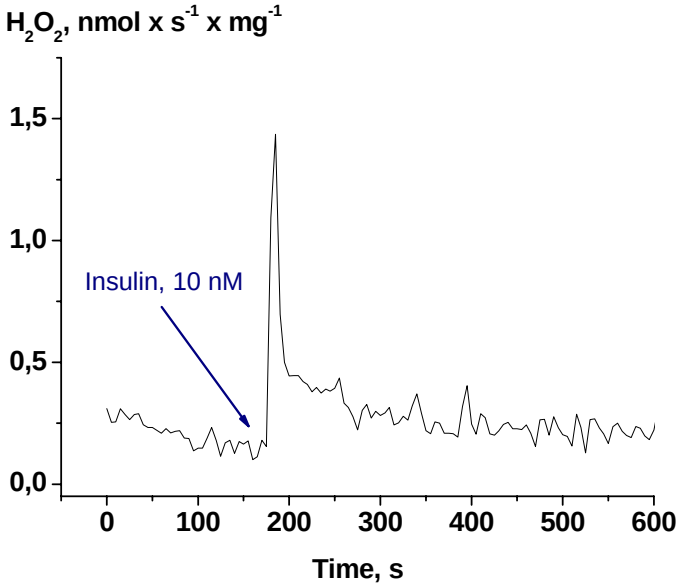

Figure 1. The rate of H_2O_2 release from CGN in response to insulin stimulation.

The source of insulin-stimulated H_2O_2 is mitochondria. *See attached pdf file 1471-2202-8-84.*

Removal of insulin-stimulated H_2O_2 by N-acetylcysteine, a H_2O_2 scavenger, almost completely prevents insulin receptor phosphorylation (i.e. activation). *See attached pdf file 1471-2202-8-84.* Thus, insulin-stimulated H_2O_2 is critical for optimal activation of insulin receptor.

Inhibiting of mitochondrial respiration with malonate, a competitive inhibitor of succinate dehydrogenase, or FCCP, an uncoupler that decreases mitochondrial potential, almost completely prevents insulin receptor phosphorylation (i.e. activation). *See attached pdf file 1471-2202-8-84*. Thus, mitochondria are critical element in the process of insulin receptor activation.

Signal that activates mitochondria in response to insulin stimulation is unknown.

Discussion. At first seconds of insulin stimulation, insulin activates mitochondria (+) and mitochondria, in turn, activate insulin receptor tyrosine phosphorylation, i.e. activation (+). Thus, there is a double positive regulatory feedback loop at early stage of insulin receptor activation. See figure 2.

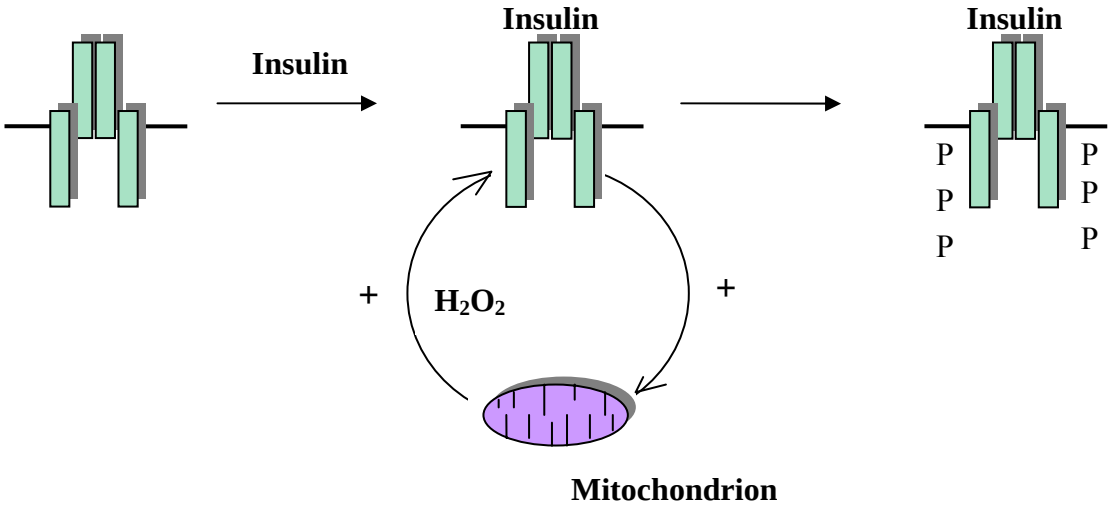

Figure 2. Double positive regulatory feedback loop at early stage of insulin receptor activation process. Insulin activates mitochondria (+), mitochondria activate insulin receptor activation (+).

Theoretically, double positive regulatory feedback loop indicates trigger-like regulation of a process. A trigger-like (or switch-like) process is characterized by a threshold level of a stimulus that activates such process. In relation to insulin-stimulated process, it means that:

(1) Activation of insulin receptor occurs when insulin reach to the threshold level necessary to activate the receptor. At concentrations below that threshold level, insulin can not activate the receptor.

(2) The threshold levels of insulin depends on functional state of mitochondria: any factor that will decrease mitochondrial potential, a parameter regulating H_2O_2 production, will increase the threshold level of insulin required for the activation of mitochondria; and contrary, any factor that will increase mitochondrial potential will decrease the threshold level of insulin required for the activation of mitochondria. It exact molecular mechanism of hyperinsulinemia and insulin resistance.

(3) Insulin receptor activation depends on two keys: 1) insulin level and 2) mitochondria. So, it is now possible to activate insulin receptor with suboptimal insulin concentrations acting on mitochondrial respiration. See figure 3. Although 5 nM insulin alone is not sufficient to produce activation of insulin receptor (first key) and 50 μM succinate (in form of choline salt) alone is not sufficient to produce activation of insulin receptor (second key), taken together they significantly activates the receptor.

(4) Mitochondrial respiration is a target for pharmaceutical interventions for the treatment of insulin resistant states, including Alzheimer's disease.

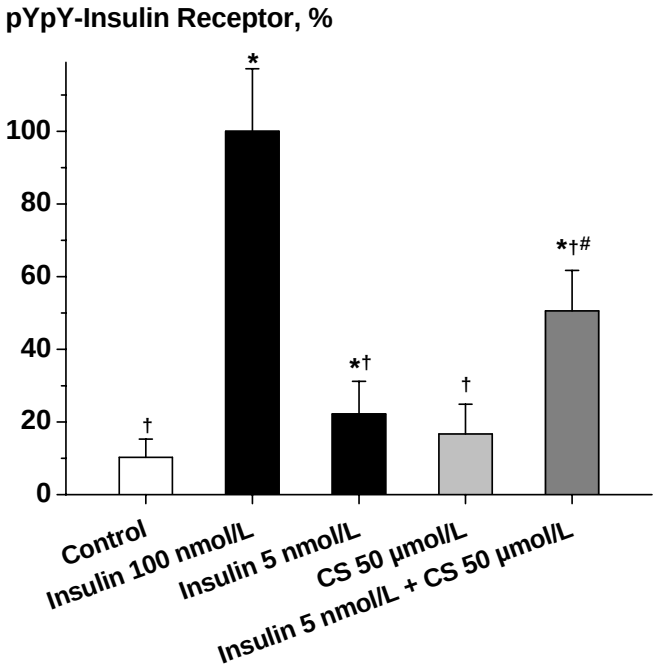

Figure 3. Tyrosine phosphorylation (pYpY) of insulin receptor in cerebellar granule neurons in response to optimal insulin concentration (100 nM) and suboptimal insulin concentration (5 nM) with or without choline salt of respiratory substrate succinate (CS, 50 µM). †Differs significantly of insulin 100 nM; *Differs significantly of control; #Differs significantly of insulin 50 nM. $P<0.05$.

The key idea: is to test the hypothesis whether succinate in form of choline salt, a neuronal insulin sensitizer, can treat cognitive dysfunctions in animal models.

Results 2. Results are presented in attached pdf file 1471-2210-8-1.

Conclusion. The results of the present study suggest that dicholine salt of succinic acid, the novel neuronal insulin sensitizer, ameliorates cognitive deficits and neuronal dysfunctions in animal models relevant to age-related cognitive impairments, vascular dementia, and Alzheimer's disease.

Thus, we identified a new target and new approach to the treatment of age-related cognitive disorders.