



OPINION ARTICLE

REVISED **Insulin Receptors and Intracellular Ca²⁺ Form a Double-Negative Regulatory Feedback Loop Controlling Insulin Sensitivity [version 2; peer review: 3 approved]**Igor Pomytkin ¹, Vsevolod Pinelis²¹Department of Advanced Cell Technologies, Sechenov First Moscow State Medical University (Sechenov University), Moscow, Moscow, 119991, Russian Federation²National Medical Research Center for Children's Health, Russian Ministry of Health, Moscow, 119991, Russian Federation**v2** First published: 12 Jun 2020, 9:598
<https://doi.org/10.12688/f1000research.24558.1>Latest published: 13 Jan 2021, 9:598
<https://doi.org/10.12688/f1000research.24558.2>**Abstract**

Since the discovery of insulin and insulin receptors (IR) in the brain in 1978, numerous studies have revealed a fundamental role of IR in the central nervous system and its implication in regulating synaptic plasticity, long-term potentiation and depression, neuroprotection, learning and memory, and energy balance. Central insulin resistance has been found in diverse brain disorders including Alzheimer's disease (AD). Impaired insulin signaling in AD is evident in the activation states of IR and downstream signaling molecules. This is mediated by A β oligomer-evoked Ca²⁺ influx by activating N-methyl-D-aspartate receptors (NMDARs) with A β oligomers directly, or indirectly through A β -induced release of glutamate, an endogenous NMDAR ligand. In the present opinion article, we highlight evidence that IR activity and free intracellular Ca²⁺ concentration [Ca²⁺]_i form a double-negative regulatory feedback loop controlling insulin sensitivity, in which mitochondria play a key role, being involved in adenosine triphosphate (ATP) synthesis and IR activation. We found recently that the glutamate-evoked rise in [Ca²⁺]_i inhibits activation of IR and, vice versa, insulin-induced activation of IR inhibits the glutamate-evoked rise in [Ca²⁺]_i. In theory, such a double-negative regulatory feedback loop predicts that any condition leading to an increase of [Ca²⁺]_i may trigger central insulin resistance and explains why central insulin resistance is implicated in the pathogenesis of AD, with which glutamate excitotoxicity is a comorbid condition. This model also predicts that any intervention aiming to maintain low [Ca²⁺]_i may be useful for treating central insulin resistance.

KeywordsInsulin, insulin receptor, glutamate, NMDA receptor, Ca²⁺, double-negative feedback loop, mitochondria, ATP**Open Peer Review****Reviewer Status**

	Invited Reviewers		
	1	2	3
version 2			
(revision)			
13 Jan 2021	report		report
version 1			
12 Jun 2020	report	report	report

1. **Kevin N. Hascup** , Center for Alzheimer's Research and trEatment (CARE), Springfield, USA
2. **Zhen Deng** , Southern Medical University, Guangzhou, China
3. **Venkatesh V. Kareenhalli**, Indian Institute of Technology Bombay, Mumbai, India

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding authors: Igor Pomytkin (ipomytkin@mail.ru), Vsevolod Pinelis (pinelis@mail.ru)

Author roles: **Pomytkin I:** Conceptualization, Writing – Original Draft Preparation; **Pinelis V:** Conceptualization, Writing – Review & Editing

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First published: 12 Jun 2020, 9:598 <https://doi.org/10.12688/f1000research.24558.1>

REVISED Amendments from Version 1

The manuscript has been revised in accordance with reviewer's notes that (a) bistability is not a necessary consequence of a double negative regulatory feedback loop and (b) **Figure 1** will be more useful when signal transduction pathways will be shown. Sentences related to "bistability" have been removed from the abstract, main text, and reference list. **Figure 1** has been revised and includes now pathways described in the text of the first version.

Any further responses from the reviewers can be found at the end of the article

Introduction

Since the discovery of insulin¹ and insulin receptors (IR)² in the brain in 1978, numerous studies have revealed a fundamental role of IR in the central nervous system (CNS). IR-mediated signaling is implicated in the regulation of diverse functions in the CNS, including synaptic plasticity, long-term potentiation and depression, neuroprotection, learning and memory, and energy balance³. Central insulin resistance has been found in neurodegenerative diseases such as Alzheimer's disease (AD)^{4,5} and Parkinson's disease (PD)⁶, stroke, and traumatic brain injury (TBI)⁷. Impaired insulin signaling in AD is evident in the activation states of IR and downstream signaling molecules⁵. Compared with control cases, insulin in AD brains induced 24–58% less activation at the level of IR and 90% less activation of insulin receptor substrate 1 (IRS-1)⁵. It has been presumed⁵ that the inhibition of IR activation is mediated by A β oligomer-triggered Ca²⁺ influx, in part by activating N-methyl-D-aspartate receptors (NMDARs)⁸, followed by a rise in Akt1 pS⁴⁷³⁹, which can inhibit insulin-induced IR activation through Thr phosphorylation of the IR β subunit¹⁰. A β oligomers may activate the NMDAR-gated Ca²⁺ influx directly¹¹ or indirectly through the intermediate release of glutamate, a ligand of NMDAR^{11–15}. This suggests that the rise in intracellular free Ca²⁺ concentration ([Ca²⁺]_i), evoked by either A β oligomers or glutamate, leads to dysfunctional activation of IR in AD. In the present opinion article, we highlight evidence that IR and [Ca²⁺]_i form a double-negative regulatory feedback loop controlling insulin sensitivity, and mitochondria have a key role in this feedback loop, being involved in adenosine triphosphate (ATP) synthesis and IR activation.

Glutamate-evoked rise in [Ca²⁺]_i causes inhibition of IR activation

Glutamate serves as the major excitatory neurotransmitter in the CNS. Its excessive accumulation in a synaptic cleft can trigger excitotoxicity, a pathologic process leading to neuronal cell death. Glutamate-induced activation of the NMDAR-gated Ca²⁺ influx is generally considered central to the development

of excitotoxicity¹⁶. Prolonged glutamate exposure causes a rapid initial increase in the [Ca²⁺]_i, followed by a larger secondary [Ca²⁺]_i increase concomitant with a decrease in the mitochondrial inner membrane potential ($\Delta\Psi_m$)^{17–19}. We recently found that on Ca²⁺-induced mitochondrial depolarization, insulin induced 48% less activation of IR (assessed by pY^{1150/1151}) compared with control²⁰. Earlier, we showed that a decrease in $\Delta\Psi_m$ can abrogate IR activation¹⁸, since the $\Delta\Psi_m$ -dependent hydrogen peroxide (H₂O₂) mitochondrial signal at complex II is critically involved in the activation of IR in neurons^{21–23}. Thus, the glutamate-evoked increase in [Ca²⁺]_i, followed by the drop in $\Delta\Psi_m$, leads to the inhibition of insulin-induced activation of IR (**Figure 1a**).

Insulin prevents glutamate-evoked rise in [Ca²⁺]_i

Normally, the NMDAR-gated Ca²⁺ influx is counterbalanced with Ca²⁺ efflux, which is governed by plasma membrane Ca²⁺ ATPase (PMCA) and the Na⁺/Ca²⁺ exchanger (NCX)^{24,25}. NCX-mediated Ca²⁺ efflux is also ATP-dependent, since NCX exchanges one Ca²⁺ for three Na⁺, and the three Na⁺ are then pumped out by the Na⁺/K⁺ ATPase at the expense of one ATP. In excitotoxicity, prolonged stimulation with glutamate leads to ATP depletion and an abnormal rise in [Ca²⁺]_i, since the massive Ca²⁺ influx is no longer counterbalanced by Ca²⁺ efflux²⁶. Therefore, maintenance of ATP production is crucial for preventing the rise in [Ca²⁺]_i in excitotoxicity. We found recently that pre-treatment with insulin prevents neurons from glutamate-evoked ATP depletion due to its protective effect on spare respiratory capacity (SRC), a measure that relates to the amount of extra ATP that can be produced via oxidative phosphorylation in case of increased energy demand¹⁹. The effect of insulin on SRC relates to its action on mitochondrial metabolism. It has long been known that the tricarboxylic acid cycle is the intracellular site of insulin action and that insulin acutely stimulates succinate oxidation at mitochondrial complex II^{26,27}. Succinate oxidation at mitochondrial complex II has been identified recently as the main source of SRC²⁸. In line with this, insulin prevented the glutamate-evoked rise in [Ca²⁺]_i in our experiments with glutamate excitotoxicity¹⁹ (**Figure 1b**).

IR and [Ca²⁺]_i form a double-negative feedback loop controlling insulin sensitivity

Collectively, this evidence suggests that a double-negative regulatory feedback loop exists between IR activity and [Ca²⁺]_i. The glutamate-evoked rise in [Ca²⁺]_i inhibits activation of IR and, vice versa, insulin-induced activation of IR inhibits the glutamate-evoked rise in [Ca²⁺]_i (**Figure 1c**).

This double-negative feedback loop model predicts that any condition leading to an increase in [Ca²⁺]_i may trigger insulin resistance. It appears to explain why central insulin resistance

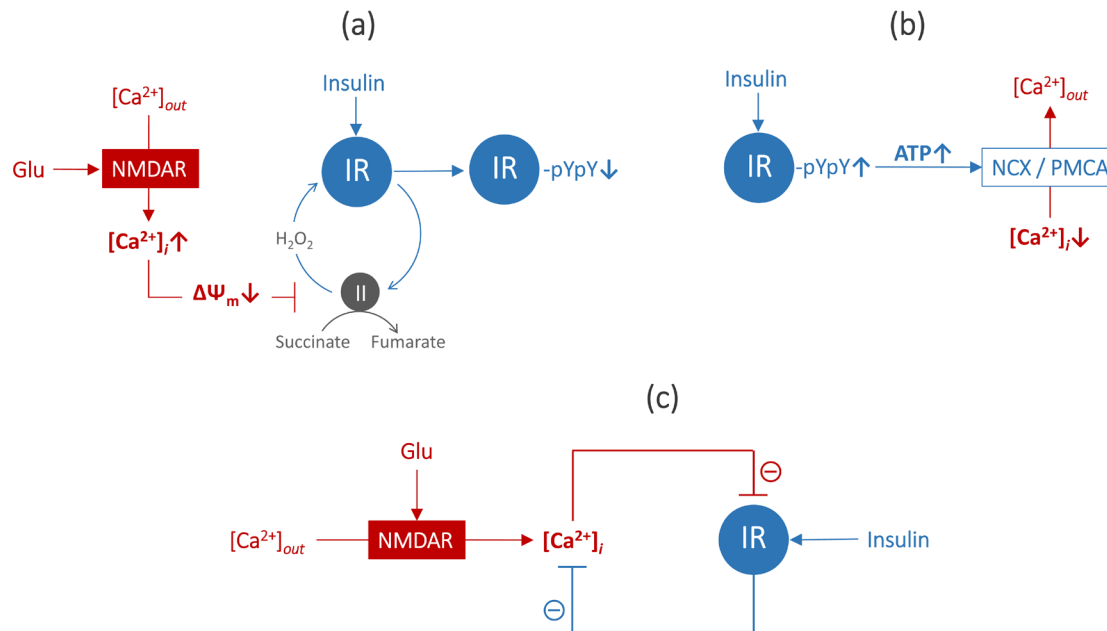


Figure 1. A double-negative feedback loop between insulin receptors (IR) activity and intracellular free Ca^{2+} concentration $[Ca^{2+}]_i$. (a) glutamate-evoked increase in $[Ca^{2+}]_i$, followed by drop in $\Delta\Psi_m$, leads to inhibition of insulin-induced H_2O_2 signal at mitochondrial complex II, thereby inhibiting IR activation (IR-pYpY↓)^{20,21}; (b) insulin-triggered increase in ATP levels enhances ATP-dependent Ca²⁺ efflux via PMCA and NCX, thereby lowering $[Ca^{2+}]_i$ ¹⁹; (c) glutamate triggers NMDA receptor-gated Ca²⁺ influx, inhibiting IR activation, and insulin triggers activation of IR, inhibiting $[Ca^{2+}]_i$ rise. Abbreviations: $\Delta\Psi_m$, mitochondrial inner membrane potential; H_2O_2 , hydrogen peroxide; pY, phosphotyrosine; ATP, adenosine triphosphate; PMCA, plasma membrane Ca²⁺ ATPase; NCX, Na⁺/Ca²⁺ exchanger; NMDA, N-methyl-D-aspartate.

is implicated in the pathogenesis of disorders such as AD^{4,5}, PD⁶, stroke, and TBI⁷, with which glutamate excitotoxicity is a comorbid condition²⁹. The model also predicts that any intervention aiming to prevent Ca²⁺ influx of or enhance efflux of Ca²⁺ from neurons, thereby maintaining low $[Ca^{2+}]_i$, may be useful for treating central insulin resistance. Given that Ca²⁺

efflux is ATP-dependent, any intervention directed to enhance ATP production in neurons may be especially useful to improve insulin sensitivity in the brain.

Data availability

No data are associated with this article.

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Version 2

Reviewer Report 29 January 2021

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Kevin N. Hascup 

Department of Neurology, Center for Alzheimer's Research and trEatment (CARE), Springfield, IL, USA

Although the authors have adequately presented point-by-point responses to my initial concerns, they have taken a minimalistic approach to incorporating some of these concepts into the actual manuscript. It would have been interesting to read the authors ideas on how this double feedback loop is challenged in different disease states considering this forms the premise of the introduction and the reasoning behind the opinion piece. Regardless, in situations of extreme deviations from homeostatic calcium concentrations (stroke and traumatic brain injury) concerns about cerebral insulin resistance is minimal compared to the rapid loss of nervous tissue. This double feedback loop postulate may be better suited for slower progressing neurodegenerative disorders.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: glutamate signaling, Alzheimer's disease, Parkinson's disease, insulin signaling, gerontology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 27 January 2021

<https://doi.org/10.5256/f1000research.52891.r77181>

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Venkatesh V. Kareenhalli

Indian Institute of Technology Bombay, Mumbai, Maharashtra, India

The manuscript can now be accepted as all concerns have been addressed.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Systems biology, Liver metabolism, modeling signaling pathways

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 02 November 2020

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Venkatesh V. Kareenhalli

Indian Institute of Technology Bombay, Mumbai, Maharashtra, India

The authors propose an existence of double negative feedback loop which may result in bistable response. It is clear that Ca signaling and insulin signaling play a role in the CNS and the related pathogenesis of neurodegenerative diseases. The mitochondrial function and thereby ATP synthesis also plays a role in the stated phenotype. The proposed dual negative feedback on each other can toggle between two states, but it may not be bistable. Bistable is with respect to an input variation. It is unclear what is the input that the hypothesis is being discussed. At a given input, the existence of both steady state, with different history, yields bistability. A figure that demonstrates all the molecular components and action including mitochondrial state and metabolites is more helpful than what is illustrated.

Is the topic of the opinion article discussed accurately in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Are arguments sufficiently supported by evidence from the published literature?

Yes

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

No

Competing Interests: No competing interests were disclosed.**Reviewer Expertise:** Systems biology, Liver metabolism, modeling signaling pathways**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 08 Jan 2021

Igor Pomytkin, Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation

The manuscript has been revised in accordance with notes that (a) bistability is not a necessary consequence of a double negative regulatory feedback loop and (b) figure 1 will be more useful when signal transduction pathways will be shown. Sentences related to "bistability" have been removed from the abstract, main text, and reference list. Figure 1 has been revised and includes now pathways described in the text of the first version.

Competing Interests: no coming interests

Reviewer Report 03 August 2020

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**Zhen Deng** 

Department of Neurology, Nanfang Hospital, Southern Medical University, Guangzhou, China

This model predicts that any disease that causes elevated $[Ca^{2+}]_i$ may trigger central insulin resistance, and explains why central insulin resistance is related to the pathogenesis of AD, and glutamate excitotoxicity is a comorbidity. The model also predicts that any intervention aimed at maintaining low $[Ca^{2+}]_i$ can be used to treat central insulin resistance.

It is an interesting theory between brain insulin resistance and AD. Although not much experimental data support the theory directly, it is worth following.

Is the topic of the opinion article discussed accurately in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Are arguments sufficiently supported by evidence from the published literature?

Yes

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cerebral vascular disease

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 July 2020

<https://doi.org/10.5256/f1000research.27092.r64837>

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Kevin N. Hascup 

Department of Neurology, Center for Alzheimer's Research and trEatment (CARE), Springfield, IL, USA

The authors put forth an opinion article in regard to a double negative feedback loop that controls cerebral insulin receptor signaling during times of either high glutamate or insulin levels. They briefly go onto hypothesize that any disease state resulting in elevated intracellular calcium will result in reduced cerebral insulin resistance, particularly when excitotoxicity is a comorbid factor. The authors attempt to simplify an extremely complicated phenomenon. However, this oversimplification neglects to take into account several factors that need to be addressed. Reduced insulin signaling as a result for glutamate induced calcium influx has been discussed in other literature (see Zhao *et al.*, 2008¹). This work should be taken into consideration when authors discuss their model.

Although insulin receptors are ubiquitously expressed in different cell types of the CNS, these cell types rely on different mechanisms for ATP production. Glial cells predominantly use glycolytic pathways in the cytoplasm whereas neurons rely on oxidative phosphorylation in the mitochondria. (For review see Pellerin and Magistretti, 2012²). Accordingly, the cellular localization of ATP production should be taken into consideration. Furthermore, do the authors believe different cell type specific mechanisms exist that their model should take into consideration? In several places, the authors discuss the role of amyloid on activation of NMDA receptors and the

subsequent intracellular Calcium increase. However, the authors have not taken into account how amyloid binding can also elicit glutamate release from either $\alpha 7$ nAChRs (see Hascup and Hascup, 2016³ and Mura *et al.*, 2012⁴) or mGLUR5 receptors (Renner *et al.*, 2010⁵). In the latter case, several laboratories have shown mGLUR5 acts as a scaffolding complex for amyloid accumulation causing receptor clustering at the membrane surface and results in elevated intracellular calcium levels.

Additionally, the manuscript could be improved if a discussion on how their model might vary across different CNS disorders such as AD, Parkinson's TBI, etc. in relation to healthy functional activity.

The authors neglect to take into account factors that also play a role in modulating insulin signaling. These include:

- Inflammation. This is prominent in numerous disease states and can negatively impact insulin signaling, while exacerbating mechanisms associated with multiple neurodegenerative disorders.
- Inhibitory feedback regulation. Insulin signaling is tightly controlled to prevent perturbations in metabolism as well as control the specificity of the signal on multiple downstream effectors. Several phosphatases are responsible for this, not just at the receptor, but also on effector enzymes. The current model does not take into consideration this tightly controlled feedback loop.
- Receptor internalization. Upon insulin binding, the insulin receptor becomes internalized as another means to control the strength and duration of the signal. This internalization is more prominent in hyperinsulinemia and may account for the resulting insulin resistance. How would this model change during stages of insulin resistance, which are hypothesized to initiate the cognitive decline observed in AD?
- Peripheral insulin signaling. Insulin produced in the pancreas is able to enter the CNS. How does the proposed model take into consideration fluctuations during normal periods of food consumption?

Are the authors proposing a similar mechanism for the structurally analogous insulin-like growth factor-1?

Minor concerns:

- The glutamate pathway in Figure 1 should have a different color scheme to make it easier to differentiate from Calcium concentration.
- Figure 1B is slightly confusing. The red line makes it seem that low levels of glutamate give rise to high levels of Calcium instead of showing just the rise in calcium. A way to incorporate glutamate activation of NMDA receptors in Fig 1B & C might help to conceptualize the model.
- The abstract is lengthy in relation to the article. I would suggest this is shortened and made more succinct.

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- of neuronal insulin receptors. *FASEB J.* 2008; **22** (1): 246-60 [PubMed Abstract](#) | [Publisher Full Text](#)
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Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Yes

Are arguments sufficiently supported by evidence from the published literature?

No

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: glutamate signaling, Alzheimer's disease, Parkinson's disease, insulin signaling, gerontology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 10 Jul 2020

Igor Pomytkin, Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation

Our opinion article relates only to relationship between intracellular Ca^{2+} concentrations [Ca^{2+}]_i and insulin receptor activation state, and not between [Ca^{2+}]_i and insulin signaling system as a whole. Insulin resistance is the term that currently applied to any of the biological actions of insulin and, therefore, is too broad to be discussed in terms of models that may predict the system behavior. The proposed model in the opinion article is not oversimplified, but takes into consideration only link between [Ca^{2+}]_i and the stage of activation of insulin receptor kinase (i.e. Tyr1150/1151 phosphorylation), the earliest step in

insulin action that precedes all other signaling events and effects of insulin.

In our opinion article we selected only two measurable parameters, namely $[Ca^{2+}]_i$ and insulin receptor activation state defined as Tyr1150/1151 phosphorylation, but not downstream molecules of IR signaling pathway such as IRS-1 or others. Therefore, our opinion relates only to the activation of insulin receptor, and not to downstream molecules or events. This approach relates directly to insulin sensitivity, since the activation of the receptor with insulin is the only stage at which insulin sensitivity can be measured directly.

The opinion about existence of regulatory double-negative feedback loop between $[Ca^{2+}]_i$ and insulin receptor activation state is based on our experimental results, obtained at the same experimental conditions in two studies that have already been published [references 19 and 20].

Results of Zhao et al. do not contradict our opinion about relationship between Ca^{2+} and insulin receptor activation state. Moreover, results of Zhao et al. support our opinion. Zhao et al. have found that glutamate stimulation reduced the insulin-stimulated tyrosine phosphorylation of the receptor beta-subunit at Tyr1150/1151 and this glutamate effect was completely inhibited by the cell-permeable Ca^{2+} chelator BAPTA-AM. Although authors did not measure intracellular Ca^{2+} concentrations, they concluded that IR inhibition is dependent on elevated intracellular Ca^{2+} , given a well-known link between glutamate and Ca^{2+} . It is the same conclusion that we made. The difference is only that our conclusion is made on our direct measurements of intracellular Ca^{2+} .

In our studies [references 19 and 20] that underlie the our opinion we used glia-free cortical neurons and directly measured ATP levels.

Our opinion relates to link between $[Ca^{2+}]_i$ and insulin receptor activation state, and amyloid-NMDA relationship are out of scope of our opinion.

Our opinion is limited to only insulin receptor activation, but not to more broad "insulin signaling". According to current knowledge, inflammation affects insulin signaling, but downstream of insulin receptor at IRS-1 level. This is out of scope of our opinion.

Our opinion does not relate to any signaling events downstream of insulin receptor, such phosphatase action and receptor internalization.

The insulin regulated internalization of insulin receptors has been shown to require autophosphorylation of all three regulatory tyrosines 1146, 1150, and 1151. Carpentier JL et al. Two steps of insulin receptor internalization depend on different domains of the beta-subunit. *J Cell Biol.* 1993 Sep;122(6):1243-52. doi: 10.1083/jcb.122.6.1243. Thus, the internalization occurs after the activation of IR and, therefore, is out of the scope of our opinion.

According to the model, insulin inhibits rise of $[Ca^{2+}]_i$ independently of the insulin source. Therefore, during periods of insulin transport to the brain, the rise of $[Ca^{2+}]_i$, e.g. glutamate-evoked, would be diminished, independently on whether it comes from pancreas

or administered intranasally.

We did not propose the same mechanism for IGF-1 in our opinion article, since we have no supportive evidence. However, it is likely that there is a link between $[Ca^{2+}]_i$ and activation state of IGFR1.

In a conclusion, the model proposed in the opinion article relates only to relationship between two measurable parameters, namely intracellular Ca^{2+} concentrations and insulin receptor activation state. Therefore, all other downstream elements of insulin signaling system and factors affecting the downstream elements are out of scope of this opinion. The opinion is completely based on our experimental results previously published, references 19 and 20 of the opinion article.

Competing Interests: there is no competing interests

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