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Feedback Control of Second Messengers Signaling Systems in White Adipose Tissue Adipocytes in Healthy State and Its Loss at Adiposity

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Abstract

Second messengers Ca^{2+} , IP₃, cAMP, NO, cGMP, and cADP ribose are incorporated as obligatory elements into multivariable Ca^{2+} -signaling system, which integrates incoming signals of hormones and neurotransmitters in white adipocytes. This cross-controlled system includes two robust generators (RGs) of rhythmic processes, involving phospholipase C- and NO-synthase-dependent signaling networks (PLC-RG and NOS-RG). Multi-loop positive feedback control of both RGs provides their robustness, multistability, signaling interplay, and extreme sensitivity to the alterations of incoming signals of acetylcholine, norepinephrine, insulin, cholecystokinin, atrial natriuretic peptide, bradykinin, and so on. Hypertrophy of cultured adipocytes and of mature cells, isolated from epididymal white adipose tissue (eWAT), results in the loss of rhythmicity and development of general hormonal signaling resistance. Preadipocytes isolated from eWAT of obese mice cannot grow and accumulate lipids in the media devoid of fatty acids. However, even low concentrations of palmitoylecarnitine in the media (1 μM) may result in drastic suppression of mRNA expressions of the proteins of Ca^{2+} -signaling system, especially of NOS-RG. Similar alterations of gene expression are observed in eWAT and liver at adiposity. All this may indicate on universal background pathogenic mechanisms. Treatment modalities, which may help to restore deregulation of Ca^{2+} -signaling system and corresponding tissues dysfunction, are discussed briefly.

Keywords: adiposity, adipocyte dysfunction, second messengers, NO, PKG, feedback and cross-control, loss of rhythmicity

1. Introduction

Adipose tissue dysfunction (“adiposopathy”) is considered as one of the primary drivers of multifactorial pathological process, ranging from systemic insulin resistance and hypertension to cardiovascular diseases, liver and pancreas dysfunction, and type 2 diabetes (T2D) [1, 2]. Obviously, observed gradual dysfunction of various tissues at T2D is due to the deregulation of metabolic and signaling systems providing the fulfillment of these functions. All these processes of deregulation, especially of signaling systems, may have some universal features, which are being based on the loss of feedback control mechanisms in the systems studied. The identification of these control mechanisms, including crosstalk of signaling pathways, may create new opportunities to identify real targets and develop new options of various diseases treatment.

“Adiposopathy” is characterized by: lipid metabolism deregulation, development of oxidative stress and mitochondrial dysfunction, death of hypertrophied adipocytes, tissue remodeling, loss of fatty acids buffering, and endocrine and immune functions [2–6]. However, the existing data on external hormonal and autonomous feedback control of white adipose tissue (WAT) lipid metabolism (triglycerides—fatty acids turnover) are insufficient to answer the question on the mechanisms providing uncontrolled hypertrophy of adipocytes. Later, based on own results obtained in animal experiments and known literature data, we will try to represent (at first level of approximation) the structures and mechanisms of autoregulation of second messengers signaling systems, which might be functioning in the adipocytes and other types of nonexcitable cells.

2. Calcium, cAMP, and cGMP-related signaling systems, operating in adipocytes of healthy animals

2.1. Brief survey of existing models of adipocyte triglyceride metabolism control

It is known that, acting via lipid kinase (PI3K)/PKB-signaling pathway, insulin may stimulate adipogenesis and triglyceride (TAG) synthesis, by phosphorylating rate-limiting enzymes Acyl-CoA: glycerol-3-phosphate acyltransferases (GPAT1, 4) and phosphatidic acid phosphatase (Lipin) [7, 8]. On the contrary, norepinephrine (NE) promotes dephosphorylation of lipin [8]. Modern viewpoints on the control of TAG hydrolysis to free fatty acids (FFA) are mainly focused on the regulation (phosphorylation) of hormone sensitive lipase (HSL), adipocyte triglyceride lipase (ATGL), and perilipin by PKA and are being based on opposite influence of NE (β -adrenoreceptors; β -AR) and of insulin on cAMP concentration and PKA activity [9–14]. Supposed mechanisms of antilipolytic action of insulin include the activation of cAMP phosphodiesterases PDE3,4 and inhibition of PKA activity through Insulin/PI3K/PKB-pathway. In addition to insulin, antilipolytic action may be provided through G-protein-coupled receptors by NE (α 2-AR), adenosine, prostaglandins, neuropeptide Y, and so on [11–14].

Phosphorylation of key lipases and perilipin by PKG1 is considered as a separate mechanism, involved in the activation of TAG hydrolysis [10, 13, 14]. This signaling pathway, which is

based on the activation of PKG1 via atrial natriuretic peptide receptor A (NPR-A/mGC/cGMP/PKG1-pathway), does not involve nitric oxide (NO) and soluble guanylate cyclase (sGC).

This widely admitted model of TAG-FFA turnover (futile cycle) control describes the regulation of lipid metabolism as external hormonal adjustment, realized through PI3K/PKB, cAMP/PKA, and cGMP/PKG1, that is, as a model devoid of self-control and crosstalk of functioning second messenger signaling systems. Moreover, NO and calcium are not included into consideration as possible messengers, involved in the control of WAT metabolism. Though the results of the last decade indicate that the activation of endothelial NO-synthase (eNOS), NO bioavailability, and recruitment of eNOS/NO/sGC/cGMP/PKG1-signaling chain may protect against obesity, by influencing differentiation and mitochondrial biogenesis in brown fat cells, adipogenesis and lipolysis in white cells, and so on. [15–20]. Besides that, controversial results on the role of Ca^{2+} and calcium-sensing receptors in the regulation of body fat depots [21–23] point on the important role of Ca^{2+} in the mechanisms of self-control of second messengers signaling systems.

2.2. Two Ca^{2+} -dependent signaling systems and rhythmic processes in adipocytes of WAT

Like most of other nonexcitable types of cells, adipocytes possess two types of intracellular Ca^{2+} release channels, located on the membrane of endoplasmatic reticulum: inositol-1,4,5-triphosphate (IP3) receptors (IP3R) and ryanodine receptors (RyR). Both types of receptors are controlled by numerous signaling molecules, including PKA PKG, Ca^{2+} -dependent kinases, various isoforms of PKC, and so on [24–26]. This versatility of control defines the shaping of intracellular Ca^{2+} dynamics, which plays a primary role in the regulation of numerous cellular processes [24]. Ubiquitous oscillations of intracellular Ca^{2+} concentration, which are observed in most of nonexcitable cells [27–30], are often considered as the basic dynamic mechanisms involved in the control of cellular metabolic processes [27–29]. However, the role of Ca^{2+} oscillations and of triggering phenomena is not understood and evaluated yet properly [27, 30]. However, the analysis of such dynamic processes may be very instrumental for the determination and evaluation of operating feedback mechanisms.

Both types of Ca^{2+} release channels possess a fundamental property, called Ca^{2+} -induced Ca^{2+} release (CICR), which may provide Ca^{2+} -sparks, fast oscillations, and spatial waves [24–26]. Gaiting of IP3R is reinforced by IP3, which facilitates binding of Ca^{2+} and channel opening [24, 25]. In other words, Ca^{2+} and IP3 represent crosscoupled messengers targeted to IP3R [31].

As for RyR, according to generally accepted point of view, the regulation of this receptor lacks this kind of symmetry. Cyclic ADP-ribose (cADPr), which is formed from NAD by ADP-ribosyl cyclase (ARC) or ectoenzyme CD38, is not considered as an obligatory coagonist of RyR [26], in spite of existing data on its modulatory role in RyR-channels gaiting and CIRC control [32–37]. Really, in striated muscles RyR-channels gaiting and CIRC are determined mainly by plasmalemmal membrane depolarization [26]. In nonexcitable cells, the primary role in Ca^{2+} homeostasis is supposed to be realized via IP3R [24, 27–30], while modulatory role is delegated to RyR, which may amplify Ca^{2+} -signals produced by IP3-dependent CICR [24–26].

2.2.1. Ca^{2+} /phospholipase C/IP₃/IP₃R/ Ca^{2+} positive feedback signaling system

Numerous external signals, by stimulating Gq proteins and tyrosine kinase (TK) coupled receptors, result in the formation of IP₃ by various isoforms of phospholipase C (PLC) [24, 25, 38] with subsequent activation of IP₃R-channels and rise of Ca^{2+} in the cytoplasm via CICR mechanism:

Realization of IP₃-dependent CICR represents short positive feedback loop (PFL) in the system:

Being activated by Ca^{2+} , Ca^{2+} -dependent isoforms of PLC may provide functioning of long PFLs [31]:

Therefore, IP₃R-dependent Ca^{2+} -signaling system represents two loops' generator (**Figure 1**), in which short PFL (shown as broken arrow 1) is embedded by long PFL (arrow 2). This duplicating loop may provide the robustness with respect to the alteration of systems parameters [39, 40]. Released by IP₃R-channel intracellular Ca^{2+} may provoke RyR-dependent CICR, which, in turn, might further amplify initial signals and support generation of Ca^{2+} oscillations and/or wave propagation. Inhibition of IP₃R, due to phosphorylation of IP₃R by Ca^{2+} -activated CaM-kinases II (CaMKII), represents stabilizing negative feedback loop (NFL) (dotted line 4 with sign T).

This Ca^{2+} /PLC/IP₃/IP₃R/ Ca^{2+} -robust generator (PLC-RG) is cross-activated by adenylate cyclase (AC)/cAMP/PKA-signaling pathway, owing to phosphorylating of IP₃R (and RyR) by PKA (arrows 7, 8). Inhibition of AC and activation of PDE3, produced by the phosphorylation of both enzymes by PKA [42], may provide the functioning of two stabilizing NFLs in this pathway (dotted line 5 and arrow 6). And finally, PI3K/PKB/NO/cGMP/PKG1-signaling pathway participates in negative crosstalk with both systems, by inhibiting IP₃R via PKB (dotted line 10) and PKG1 (dotted line 12) and by activating PDE3 through PKB (arrow 9) [10, 11]. Well-known inhibition of PDE3B by cGMP (dotted line 11) [9] contradicts this logic of system's self-control and, apparently, may be realized at high concentrations of cGMP.

Cross-inhibition of eNOS, based on its phosphorylation by PKC [41], is shown at the bottom of **Figure 1** (dotted line 14). Cross-inhibition of PLC β activity, which may be realized with the involvement of PKG1 and PKA [42], is omitted for the simplicity.

It ought to outline that, besides combined action of IP₃ and Ca^{2+} on IP₃R, PLC-RG has second point of regulatory symmetry. The entry into the system through PLC is carried out by combined activation of PLC by $\text{G}_{\alpha q}$ -proteins (or TKs) and Ca^{2+} (**Figure 1**).

PLC-RG represents a highly nonlinear dynamic system, which incorporates the family of two nested PFLs. Due to that, this system possesses the properties of multistable generator, which

Figure 1. PLC/IP₃/IP₃R/Ca²⁺-signaling system and its cross-control by AC/cAMP/PKA and PI3K/PKB/NO/cGMP/PKG-signaling pathways. All abbreviations and explanations are given in the text. Various types of activation and inhibition (direct regulations or covalent modifications) are indicated as broken arrows and dotted lines (with symbol T), correspondingly. Positive and negative feedback loops are marked with white or black circles and have the numbers 1–3 and 4–6, correspondingly. Crosstalk loops are marked by the squares and have the numbers 8–12 and 14. Various hormones and neurotransmitters (with corresponding receptors), activating G-proteins and TKs, are placed in the boxes.

may produce: steady state regimes with different concentrations of intracellular Ca²⁺ (and of other second messengers), triggering phenomena, Ca²⁺ spikes, ordinary and complex (multi-periodic or chaotic) oscillations, and waves propagation. Realization of described regimes depends on the system's parameter values, that is, on the set of enzyme and channel activities and agonist affinities. All such regimes were observed experimentally in various types of cells [43–51] and were reproduced in mathematical models [52–54]. Apparently, all ranges of these dynamic regimes were observed for the first time on isolated hepatocytes [45]. In most of the published experiments registered Ca²⁺ oscillations and waves were attributed to the functioning of PLC-RG, including regimes elicited by NE [45], ACh [47, 49], histamine [44], glutamate [48], and so on.

In cultured adipocytes of epididymal WAT, periodic Ca²⁺ signals and spikes, which depend on PLC-RG activity, may be evoked by fetal bovine serum [50, 51], NE [55–57], Angiotensin II (Ang II) [58, 60], cholecystokinin (CCK) and ANP [58], insulin [59], and bradykinin (BK) (Turovsky et al., submitted for publication).

2.2.2. Ca²⁺/eNOS/NO/sGC/cGMP/PKG/ARC/cADPr/RyR/ Ca²⁺ positive feedback signaling system

In contrast to all abovementioned agonists, ACh may elicit Ca²⁺ oscillations in WAT adipocytes (9DIV) by involving another mechanism, which does not implicate PLC and IP₃R [61].

The effect of ACh is realized via *m3*-muscarinic receptors (*m3*-MR) and G $\beta\gamma$ subunits of corresponding Gq-proteins. This kind of rhythmic activity is characterized by Ca²⁺ and NO oscillations with phase shift about 180°. Remarkably, insulin, Ang II, CCK, and BK may also evoke Ca²⁺ and NO oscillations by activating second oscillatory mechanism [59, 60].

Earlier works, performed by several groups, depicted that NO, cGMP, and cADPr may induce Ca²⁺ mobilization and oscillations in hepatocytes [64, 65], smooth muscle cells [66, 67], and T-cells [68], involving RyR. The model, proposed for first time to explain mobilization of intracellular Ca²⁺ via NO/cADPr-dependent signaling pathway [33, 34], was based on known phosphorylation and activation of ARC by PKG1 [36, 37] and on the facilitation of RyR-channels gating by newly formed cADPr [32, 33]. This model include following signaling chain:

Well-known activation of eNOS by Ca²⁺ [69–71] transforms this linear chain into long PFL, which creates basic loop of NOS-dependent robust generator (NOS-RG):

Speaking on the math language, formation of long PFL(5) represents necessary conditions for the functioning of NOS-RG. PFL(5) is very sensitive to any input in it. The application of ANP (input of cGMP), 8-br-cGMP, SNAP (influx of NO), NAD (substrate in the synthesis of cADPr) [61], or activation of Ca²⁺-influx (by low concentrations of arachidonic acids via store-independent Orai channels) [63], may bring oscillations and triggering regimes in adipocytes.

ACh and all abovementioned hormones, activating TK or/and G-protein-coupled receptors, provide sufficient conditions for stable functioning of NOS-RG, activating eNOS via axis:

Incubation of cultured adipocytes with the inhibitors of the proteins of this axis prevents the activation of NOS-RG by ACh, insulin, CCK, and Ang II [61–63].

Thereby, NOS-RG also has both kinds of symmetries, including: (a) activation of RyR by cross-coupled second messengers Ca²⁺ and cADPr and (b) combined activation of eNOS by Ca²⁺ and PKB (via axis (6)).

The model of self-control of NOS-RG is presented in **Figure 2**. Besides short and long PFLs, based on cADPr-dependent CICR and activation of eNOS by Ca²⁺ [69–71] (broken arrows 1, 2), this model incorporates six PFLs (arrows 3–8) and three NFLs (dotted lines 10, 12, and broken arrow 11). Group of PFLs (arrows 3–6), based on the phosphorylation and activation of eNOS [70, 71] and of PKB by CaMKIV and AMPK [72, 73], provide the amplification of basic PFLs and robustness of NOS-RG.

PKG1 occupies a central position in the autoregulation of NOS-RG. Feedforward activation of RyR [26] and feedback activation of eNOS [74–76] and PKB [72, 77] by PKG1 (arrows 7–9)

raise the reliability of this system. Feedback inhibition of sGC [78] and activation of PDEV [79] by PKG1 are directed to lower the level of cGMP in NOS-RG (line 12 and arrow 11). The inhibition of external Ca^{2+} influx, realized via inhibition of TRP channels by PKG1 [80–82] (line 10), may reinforce these NFLs.

The reliability and low sensitivity to noise and parameters alterations of technical systems is primarily attained by multiple negative feedback control and duplication of operating elements [39, 40, 83, 84]. PFLs, in contrast to NFLs, may enhance system's sensitivity to changes of internal parameters and noise by amplifying incoming signals.

Basic structures of autoregulation of PLC-RG and NOS-RG involve the families of nested PFLs. Such unusual structures may create new properties of analyzed system: combination of extreme sensitivity to the alterations of incoming signals and the reliability, provided by the redundancy of PFLs. Due to that, both systems may be considered as robust but sensitive integrators of multiple hormonal signals.

Positive cross-control of NOS-RG, fulfilled by AC/cAMP/PKA-signaling pathway, is indicated by broken arrows 13–15 in **Figure 2**. This control is directed to internal elements of main PFLs (arrows 5, 6), being addressed to RyR, ARC, and PKB. Owing to that, NOS-RG might have high sensitivity to this kind of cross-control. Corresponding examples will be

Figure 2. PI3K/PKB/eNOS/NO/sGC/cGMP/PKG1/ARC/cADPr/RyR/ Ca^{2+} -signaling system with its system of autoregulation and cross-control by AC/cAMP/PKA-signaling pathways. All abbreviations and explanations are given in the text. Various types of activation and inhibition are indicated as broken arrows and dotted lines (with symbol T), correspondingly. The family of nested positive feedback loops (arrows with white circles) has the numbers from 1 to 8. Positive feedforward loop is numbered as 9. Negative feedbacks (marked by black circles) have the numbers 10 through 12. Crosstalk loops, describing positive impact of AC/cAMP/PKA-signaling pathway, have the numbers 13 through 15. Various hormones and neurotransmitters (with corresponding receptors), activating G-proteins and TKs, are placed in the boxes.

presented later. However, AC/cAMP/PKA-signaling pathway is under negative cross-control of NOS-RG (Figure 1). Robustness of NOS-RG and complexity of its crosstalk with AC/cAMP/PKA-signaling pathway represent serious problems in experimental studies and mathematical modeling of such systems.

2.3. Oscillatory and triggering regimes registered in adipocytes

Table 1 summarizes our earlier results, characterizing action of several hormones and neurotransmitters on Ca²⁺-signaling systems of cultured epididymal adipocytes (9DIV) of white healthy mice. ACh, activating m3-muscarinic receptors (m3-MR), may elicit periodic Ca²⁺ oscillations in 70–80% of the cells. About 10–15% of the cells respond by spikes. Rest of the cells is silent. Applied inhibitory analysis indicates the implication of NOS-RG [61]. In contrast to Ach, NE by activating PLC-RG via α1A-AR evokes Ca²⁺ oscillations in 30–40% of the cells. Subsequent application of NE, after washing of cultured cells of Ach, may induce Ca²⁺ oscillations in the same percentage of cells. Fast monomodal or complex multimodal oscillations may be observed in dependence of cell size [57]. Two lines of numbers at the fifth row describe these two limits of oscillations periods, which were registered in cultures studied.

In comparison with ACh, ANP, and NE, peptide hormones insulin, Ang II, and BK (Table 1) may evoke rhythmic activity by involving first or second oscillatory mechanisms (PLC-RG or NOS-RG) in dependence of cellular culture used. Besides that, insulin, CCK, BK, and ANP may often elicit complex multiperiodic and chaotic Ca²⁺ oscillations.

Agonists	ACh [61]	NE [51]	Ins [59]	Ang II [60]	ANP [58, 61]	CCK-4 [58]	BK **
Receptors involved and concentrations of agonists used	m3 1-5 μM	α1 1-5 μM	TK 3-5 nM	AT-1 300-500 nM	NPR-A 1-5 μM	CCK-B 1-10 nM	B2R 0.3-10 μM
PLC-RG, % of cells with rhythmic activity	—	30–40	20–30	20–30	—	20–30	30–40
NOS-RG, % of cells with rhythmic activity	70–80	—	15–25	30–35	30–40	20–40	25–30
Periods of oscillations (s) *	5–60	20–75	20–30	20–50	20–50	25–30	10–30
	100–300	100–300	50–150	75–200	200–300	300–500	200–500

In the table, second and third rows describe, which of two Ca²⁺ signaling systems is turned on by corresponding agonist. Numbers, presented at these rows, show average percentage of all cells in the cultures tested, which generate mono and multimodal oscillatory regimes, or chaotic oscillations. *Periods of minimal and maximal modes of oscillations, observed in the cells of different size, are presented in fourth row. In each experiment 5–10% of all cells were nonresponsive. Rest of the cells was characterized by Ca²⁺ spikes. From 10 to 15 experiments were used for each agonist applied. The number of monitored cells in each culture was 80–100 cells. References are indicated in the square brackets in top row. **Taken from: Turovsky et al., submitted to publisher.

Table 1. Involvement of two Ca²⁺-signaling systems: PLC and eNOS-dependent robust generators (PLC-RG and NOS-RG) in rhythmic processes evoked by hormones and neurotransmitters in cultured epididymal adipocytes (9DIV) of white 4–6 weeks old male mice.

2.4. Some elements of the control of both Ca^{2+} -signaling systems

2.4.1. Control of IP3R by PKG1

Preliminary results, obtained with fluorescent antibodies staining, indicate smooth dense distribution of IP3R in adipocytes in comparison with smooth but thin distribution of RyR (Turovsky et al., submitted to publisher). This difference corresponds to substantial difference in mRNA expression of the subtypes of IP3R and RyR-receptor proteins (see below). Due to the expression of both types of Ca^{2+} -channels in adipocytes, we might expect their tandem operation under the action of ACh. However, preincubation of cultured cell with PLC or IP3R inhibitors does not alter ACh effects [61]. Moreover, combined application of PLC inhibitors and IP3R antagonists added after ACh may only diminish the amplitude of Ca^{2+} oscillations by 10–15% [61]. All this may indicate that expected tandem operation of RyR and IP3R, or supportive role of IP3R [24, 26], is not realized due to inhibitory action of PKG1 on IP3R (**Figure 1**, dotted line 12) and, possibly, on PLC β . Recent data, demonstrating endothelium-dependent suppression of AVP-evoked Ca^{2+} oscillations in microvessel's myocytes by ACh, support this conclusion [85]. Taken together, these results may indicate universal role of the control of IP3R by PKG1. To stress the question, we might also speculate that, in spite of low protein content of NOS-RG in adipocytes, high activity of this system is supported (reinforced) by unusual multiloop feedback control.

2.4.2. Robustness of NOS-RG: Impact of PFLs, based on activation of several targets by CaMKII and AMPK

CaMKII may be involved in the activation of RyR, eNOS, and PKB (arrows 3, 5, 6 at **Figure 1**). AMPK, being activated by Ca^{2+} -dependent CaMKIV, may also promote further activation of eNOS (arrows 4 at **Figure 1**). To break corresponding PFLs, we applied the inhibitors of both enzymes. To avoid nonspecific effects, we used low concentrations of the inhibitors, equal to their *K_d*. Our preliminary studies have shown that the applications of KN-63 (inhibitor of CaMKII) and of Compound C (inhibitor of AMPK) altered the shape of Ca^{2+} -oscillations and even suppressed rhythmic activity in part of the cells (**Figure 3**). Combined effect of both inhibitors was statistically significant ($p \leq 0.02$). Rather weak effect of Compound C on NOS-RG might be explained by the fact that the activation of AMPK by CaMKIV (at the conditions of our experiments) is insufficient to keep required gain of PFL(4) (arrow 4 at **Figure 2**). Besides CaMKIV, the activity of AMPK is controlled by AMP, sirtuins (SIRT1), liver kinase B1(LKB1), and several other kinases [86].

2.4.3. On the involvement of $\alpha 1,2$ -AR and $\beta 1$: 3- AR in the activation of PLC-RG and NOS-RG

Agonist of $\alpha 1$ -AR phenylephrine (5–10 μM), like to NE (**Table 1**), evokes Ca^{2+} oscillations in 25–30% of the cells, implicating PLC-RG. Sustainability of these oscillations depends on store-operated Ca^{2+} entry into the cells [63].

Agonists of $\alpha 2$ -AR guanabenz (10 μM) and L-arginine (5–10 mM) may elicit Ca^{2+} spikes in 35–50% of cultured cells, involving NOS-RG [55].

Figure 3. Robustness of NOS-RG. Impact of CaMKII and AMPK on Ca²⁺- oscillations elicited by ACh in cultured adipocytes (9DIV). Bars represent average number of the cells, which respond to added ACh and the inhibitors of CaMKII (KN-63) and of AMPK (compound C). The inhibitors were added 10 min after the application of ACh. Details are given in the text. N = 6. Data presented as mean ± SD. *denotes statistically significant difference p ≤ 0.02. Results are taken from: Turovsky et al., submitted to publisher.

Activation of $\beta 1-3$ - AR by isoproterenol (3–5 μ M) is characterized by slow rise of Ca²⁺ in 40–50% of cells. The antagonists of IP3R and RyR suppress Ca²⁺ responses in 75–80% and 15–20% of activated cells, respectively [62]. Observed difference in this suppression may characterize the impact of PKA on the phosphorylation and activation of IP3R and RyR.

2.4.4. *Signaling interplay and sensitivity: Synergistic action of low concentrations of ACh and NE*

Low concentrations of ACh, NE, phenylephrine, and L-arginine cannot induce Ca²⁺ responses in adipocytes. However, sequential or combined application of ACh and these agonists display synergistic effects, promoting diverse oscillatory regimes (**Figure 4A–C**) and triggering oscillatory transitions from one stable steady state with low Ca²⁺ level to the second steady state with high Ca²⁺ concentration in the cell (**Figure 4B**, record 2) (Turovsky et al., in publication). This kind of synergy may be explained by combined action of various G $\beta\gamma$ - proteins on signaling axis (6): G $\beta\gamma$ → PI3K γ → PKB → eNOS.

Combined action of low concentration of ACh and of isoproterenol may elicit complex oscillations (**Figure 4D**), triggering transition from one stable state to another (**Figure 4E**) and Hopf bifurcation, that is, transition from stable steady state to stable oscillatory regime (**Figure 4F**). The mechanism of synergistic action of ACh (*m3-MR*) and of isoproterenol ($\beta 1-3$ - AR) may be based on the activation of axis (6) by ACh, reinforced by the activation of PKB (in this axis) and of ARC and RyR (in NOS-RG) by PKA (arrows 13–15 at **Figure 2**).

2.4.5. *cADPr and RyR may play a supportive role in the operation of IP3R and PLC-RG*

Nicotinamide (NAM), product and inhibitor of cADPr synthesis by ARC (or CD38), has some influence on Ca²⁺ oscillations evoked by NE. Added NAM (10 mM) may change the shape of oscillations in 20–30% of oscillating cells and suppress rhythmic activity in 15–20% of cells (Turovsky et al., submitted to publisher). This may indicate tandem operation of IP3R and RyR in some part of the cells, having rhythmicity evoked by NE.

Figure 4. Signaling interplay and sensitivity. Synergistic action of low concentrations of ACh and NE. A–C: Interplay of Gβγ-subunits of G-proteins of GPCR. Ca²⁺-oscillations and triggering regime (4B, record 2) produced by combined action of low concentrations of ACh and NE, ACh and phenylephrine, ACh and L-arginine, correspondingly. Record 1 at B represents an example of relaxation oscillations. D–F: Interplay of Gβγ-subunits of Gq-proteins (ACh) and of β1–3-AR (isoproterenol). Complex oscillations (D), triggering regimes (E) and Hopf bifurcation (F) produced by combined action of low concentrations of ACh and isoproterenol. Description in the text. Results from: Turovsky et al., submitted to publisher.

3. Loss of rhythmic activity and suppression of mRNA expression for the proteins of Ca²⁺-signaling systems at obesity

3.1. Influence of cell size on rhythmic activity of adipocytes of healthy animals

Preadipocytes isolated from healthy male mice, growing on high-glucose DMEM medium, become differentiated to the ninth day of culture (9DIV). Mature adipocytes represent heterogeneous populations of cells, which is characterized by different cell size, in dependence of number of lipid droplets inclusion [57]. We evaluated cell size by measuring the area (S) occupied by the cell. Small adipocytes, having $S \approx 300\text{--}400 \mu\text{m}^2$, generated fast regular monomodal Ca²⁺-oscillations with periods from 5 to 75 s in response to ACh or NE [57]. Such cells accounted for 10–15% of all monitored cells in culture and had few small lipid droplets. More than 50% of the cells had cellular size $S \approx 500\text{--}900 \mu\text{m}^2$. Rest of cells (15–20%) with $S \leq 1100 \mu\text{m}^2$ had several big lipid droplets or one lipid inclusion, which might occupy from 70 to 90% of the cell volume. ACh and NE elicited complex multimodal Ca²⁺ oscillations with periods from 100 to 300 s in the cells with $S \geq 600 \mu\text{m}^2$. Similar results, characterizing correlation of cell size with the shape and period of oscillations, have been registered for insulin [59]. Results presented in lower part of **Table 1** (fifth row) indicate that, independently of agonist used, cultured cells may generate Ca²⁺ oscillations in the ranges of periods from 5–60 s to 400–500 s.

Rhythmicity disappeared in hypertrophied adipocytes with $S \geq 1200 \mu\text{m}^2$, which may respond to the application of high doses of ACh or NE (20–30 μM) only by Ca^{2+} spikes or slow Ca^{2+} accumulation [57]. These observations may indicate that uncontrolled hypertrophy and corresponding cytoplasm shortage might predispose to loss of rhythmic processes in adipocytes and to the development of general hormonal resistance in WAT cells.

3.2. Impact of obesity on Ca^{2+} signaling systems of adipocytes

3.2.1. Model of obesity

We used 6 to 8 month course of high-fat feeding, based on the addition of pork lard (200–300 mg/day/animal) to standard chow of rodents, taking in experiments 7–8 month old mice. This model is described briefly in Appendix. Obese 6–7-month-old mice had elevated levels of glucose in blood in a fasted state (7–9 mM), raised arterial pressure (130–150 mm Hg), and macromolecular liver steatosis (Grishina et al., submitted to publisher).

3.2.2. Ca^{2+} signaling in hypertrophied primary adipocytes and cultured cells

Isolated primary epididymal adipocytes of medium size ($S \approx 6000\text{--}7500 \mu\text{m}^2$) had approximately 1–5% of cytoplasm (**Figure 5B** and **C**), which looks like bright oreol around of adipocyte. Most of these cells, being attached to cover glass by Cell-Tak adhesive, cannot generate Ca^{2+} signal in response to added high concentrations of ACh (**Figure 5D**). However, most of hypertrophied cells, having spots of cytoplasm, still preserve the ability to respond to added Ca^{2+} (**Figure 5E** and **F**) or ionomycin (**Figure 5D**). This kind of nonresponsiveness to ACh might characterize general hormonal resistance of hypertrophied eWAT cells in obese state.

Preadipocytes isolated from fat pads of obese animals cannot grow on glucose, being adapted to use long-chain fatty acids (LCFA) incoming from the blood. Incubation of preadipocytes with 100 nM of L-palmitoylcarnitine (PC) and standard high-glucose DMEM provides required conditions for cell differentiation and lipid accumulation. ACh, NE, and insulin may evoke weak Ca^{2+} signals in some part of cultured cells. Oscillations and standard amplitude Ca^{2+} spikes have never been observed in this kind of cell (Turovsky et al., submitted to publisher). These radical alterations in Ca^{2+} -signaling and hormonal sensitivity, registered in cultured cells of obese animals, may depend on radical alterations of mRNA expression for the enzymes and channels of both Ca^{2+} signaling systems (PLC-RG and NOS-RG).

3.2.3. Impact of LCFA on mRNA expression of cultured cells

Our preliminary results, obtained with the application of real-time PCR analysis (see Appendix), revealed significant depression of mRNA expression in cultured adipocytes of obese animals in comparison with the cells, which have been isolated from healthy animals. Results presented in **Figure 6** (gray columns) demonstrate that cultures grown on the medium containing 100 nM of PC have 3–5 fold lowering of the expression of: Ca^{2+} -dependent genes (of NFAT, NF κ B); genes of proteins involved in energy and lipid metabolism (citrate synthase (CS) and HSL); marker genes of SIRT1, AMPK, PI3K γ , and of eukaryotic translation initiation factor 2 alpha kinase 3 (PERK). As for NOS-RG and PLC-RG, the expression of mRNA of eNOS (NOS3), CD38, and RyR3 had fallen 10 times or more. The gene of IP3R isoform 3 (IP3R3) was

Figure 5. The shape and Ca^{2+} responses of primary hypertrophied adipocytes of obese mice. (A) confocal image of 3D reconstruction of primary hypertrophied adipocyte of obese mice (front view of one cell). The projection describes general uneven distribution of the cytoplasm in the cell loaded by Fluo-4. Fluorescent space corresponds to the part of cell occupied by the cytoplasm (up to 1–3% of total cell volume), while the bulk of the cell is engaged by fat droplet. (B, C) bright fluorescent areas around hypertrophied cells visualize the cytoplasm by fura-2 dye and are numbered as 1. Intracellular Ca^{2+} responses (Fura-2, ratio) from these areas, which were elicited by external Ca^{2+} , are presented in panels E and F, correspondingly. Panel D describes rare type of Ca^{2+} response evoked by ACh. Panel D also demonstrates ACh resistance of hypertrophied cell. Though, the response to ionomycin is preserved. From Sergeev et al., submitted for publisher.

most resistant to toxic action of very low dose of PC. The expression of inducible NOS (NOS2) mRNA was measured at the level of detection (Grishina et al., submitted to publisher).

Incubation of preadipocytes (isolated from obese animals) with 1 μM of PC revealed dramatic suppression of mRNA expression for all genes analyzed in cultured cells (9DIV). The expression of marker genes of NOS3 and RyR3 was not observed (marked in **Figure 6** as *). These results support widely distributed viewpoint on LCFA toxicity and indicate an important role of LCFA in the development of “adiposopathy”. Observed radical alterations in mRNA expression, especially for the proteins involved in the functioning of NOS-RG and PLC-RG, may mean that earlier discussed mechanisms of autoregulation and cross-control of both Ca^{2+} signaling systems are being lost under chronic toxic action of low concentrations of LCFA. This state of both systems may be characterized as absolutely deregulated state.

3.2.4. Expression of mRNA in eWAT of obese animals

In comparison with fat pads of age-matched healthy animals, eWAT of obese male mice is characterized by considerable down-regulation of the expression for all genes examined

(Figure 7). These alterations have some qualitative similarities with the results observed in cultured cells, especially with respect to the changes of expression of marker genes for the proteins of NOS-RG. Fat pads of obese animals have more than 8–12 times lowered expression of PKG1, PKG2, and eNOS genes. The expression of genes for RyR2 and RyR3 was under the level of detection. Observed significant down-regulation of IP3R1 and IP3R2 genes may mean that both Ca^{2+} signaling systems are in deregulated states. Considering NOS-RG as the system, which integrates hormonal signals involved in the control of NO bioavailability, we may conclude that the application of insulin, NE, ANP, and so on might be ineffective to rise NO level and PKG1 activity in “sick” fat depots of obese animals.

3.2.5. Benefit and disadvantages of physical activity in the treatment of obesity

Taking into account the benefit of physical activity in the treatment of obesity, we have applied a very low-intensity treadmill running program (6 weeks, 10 min/day) to treat diabetic overweight mice (56.2± 5.7 g. wt) in combination with animals treatment with NaCl (control group) or complex preparation, addressed for the treatment of liver diseases and hepatic encephalopathy [88]. However, in a control group, 8 of 20 diabetic mice treated with NaCl have died within first week of adaptation period due to exercise intolerance. Survivors were characterized by marked improvement in blood glucose and lipid profiles and in liver mRNA expression of all genes examined (of PLC-RG and NOS-RG). In comparison with control group, all animals treated with complex preparation tolerated exercise program well and showed further improvement in blood lipid profiles and mRNA expression [89]. Taking all this into account, we might speculate that application of various exercise programs to treat obese patients should be combined with the use of some performance-enhancing drugs, or drugs addressed to support liver and cardiovascular system, and so on.

Figure 6. Down regulation of marker genes expression in cultured adipocytes of obese male mice grown in presence of L-palmitoylcarnitine. On the plot are resented: Ca^{2+} -dependent genes NFAT and NFκB, genes of PERK and proteins of NOS-RG, IP3R (IP3R1,2 - subtypes 1, 2), CamKIIβ, AMPKα, and of energy and lipid metabolism (citrate synthase (CS), GPAT1, HSL), β- actin and of inducible NOS (NOS2). Gene of GAPDH is used as reference gene. Mean expression in control adipocytes from healthy animals was set as 100%. Error bars indicate SD. Gray and black columns correspond to cultured adipocytes grown in presence of 100 nM and 1 μM of L-palmitoylcarnitine, correspondingly.* indicate the absence of mRNA expression for eNOS (NOS3) and of subtype 3 of RyR (RyR3). Horizontal line marked at the level 1 indicates baseline gene expression. N = 4, number of cultures in each group.

Figure 7. Down regulation of marker genes expression in eWAT of obese mice. Presented Ca^{2+} -dependent genes NFAT and $\text{NF}\kappa\text{B}$, genes of PERK and proteins of NOS-RG, IP3R (IP3R- subtypes 1, 2), glutathione reductase (GR), uncoupling protein 1 (UCP1), tumor necrosis factor α ($\text{TNF}\alpha$), acetyl coenzyme a carboxylase (ACC), $\text{CamKII}\beta$, AMPK, and of energy and lipid metabolism (citrate synthase (CS), GPAT1, HSL), β -actin and NOS2. Gene of GAPDH is used as reference gene. Mean expression in control adipocytes from healthy 8 month old animals was set as 100%. Error bars indicate SD. N = 5, number of animals in each group.* indicate the absence of mRNA expression for both subtypes of RyR (RyR2, 3).

4. Conclusions

The main aim of our chapter was to reconstruct core elements of the Ca^{2+} signaling system of adipocytes and to demonstrate that this complex multivariable system cannot be divided on separate parts, independently controlled by various hormones and/or neurotransmitters. Having multiple feedbacks and cross-controls (**Figures 1** and **2**), this system makes interdependent the concentrations of all second messengers and the activities of various kinases. For example, considering lipogenic and antilipolytic action of insulin [7, 8], we have to take into account its lipolytic action. Insulin increases NO bioavailability and PKG1 activity by activating NOS-RG. The same effect may be produced by CCK, BK, Ang II (see **Table 1**). Reliability of NOS-RG, receptors' signaling interplay, and amplification of signals create important properties of Ca^{2+} signaling system, providing integration of hormonal signals at their low concentrations (**Figure 4**). This is especially important with respect to ACh.

Parasympathetic control of WAT is still under the question [90]. However, in our experiments ACh evokes marked Ca^{2+} and NO responses in cultured cells, implicating NOS-RG [61]. Some sensitivity to ACh is preserved in primary hypertrophied adipocytes ([57] and **Figure 5D**). Due to that, possible role of ACh in the control of WAT metabolism requires further investigations.

Gradual loss of rhythmic activity and the appearance of hormonal resistance, which are observed in hypertrophied cultured cells and in primary adipocytes isolated from obese animals, may be considered as markers of cell viability in the progress of pathologic process. Similar alterations in rhythmicity and resistance to ACh and NE have been registered in aorta rings isolated from obese and diabetic rats [87, 89].

Loss of rhythmicity in adipocytes is based on the alterations in enzyme activities and loss of feedback control of PLC-RG and NOS-RG, due to marked alterations in mRNA expression of corresponding genes (**Figures 6 and 7**). Our preliminary results indicate that qualitatively similar alterations in gene expression are observed in the liver of obese and diabetic mice [90].

All this may indicate universal mechanisms resulting in deregulation of metabolic and signaling systems in various organs and tissues.

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Conflict of interest

Authors declare no conflict of interests.

A. Appendix

Gene expression analysis

Gene expression in cultured adipocytes and in eWAT was performed using real-time PCR (Applied Biosystems 7300) with TagMan Universal Master Mix II, no UNG (Applied Biosystems). Total RNA was isolated with TRIzol (Invitrogen). RNA was quantified by Qubit $\circ$ RNA BR Assay Kit (Molecular Probes, Eugene, OR) and cDNA was synthesized from 5 μ g of total RNA using a reverse transcription system with random primers (Sileks, Russia). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as reference gene. All results were normalized to GAPDH mRNA expression. Fold difference in each gene expression was calculated as $2^{-\Delta\Delta C_t}$. $\Delta\Delta C_t$ were calculated relative to corresponding gene of control adipocytes grown on glucose, or of control healthy age-matched white male mice.

Animal model of obesity and type 2 diabetes

Animal model of obesity and type 2 diabetes (T2D), described for rats previously [87], was used in present experiments. This model is heterogeneous, like those presented by Duval et al. [5]. We used 6–8 month course of high-fat feeding, based on the addition of pork lard

(200–300 mg/day/animal) to standard chow of rodents, taking in experiments 7–8-week-old mice. Obese 6–7-month-old fat-responsive mice had elevated level of glucose in blood in fasten state (7–9 mM), raised arterial pressure (AP = 130–150 mm Hg) and macromolecular liver steatosis (Grishina et al., submitted for publisher). The animals with advanced T2D (9–10 month) have been characterized by: AP = 140–170 mm Hg, fasting glucose level of 12.1 ± 1.8 mM (SD), insulin of 2.9 ± 1.3 ng/ml (SD) and venous blood ammonia higher than 100–140 μ M, liver fibrosis or even cirrhosis. Dysfunctional preadipocytes, isolated from “sick” epididymal fat depots of diabetic mice, were characterized by inability to proliferate (Turovsky et al., submitted for publisher).

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References

- [1] Bays HE, González-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, Rodbard HW, Henry RR. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Review of Cardiovascular Therapy*. 2008;**6**(3):343-368. DOI: 10.1586/14779072.6.3.343
- [2] Bays H. Adiposopathy, “sick fat,” Ockham's razor, and resolution of the obesity paradox. *Current Atherosclerosis Reports*. 2014;**16**(5):409. DOI: 10.1007/s11883-014-0409-1
- [3] Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of Lipid Research*. 2005;**46**(11):2347-2355. DOI: 10.1194/jlr.M500294-JLR200
- [4] Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW 2nd, DeFuria J, Jick Z, Greenberg AS, Obin MS. Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes* 2007;**56**(12):2910-2918. DOI: 10.2337/db07-0767
- [5] Duval C, Thissen U, Keshtkar S, Accart B, Stienstra R, Boekschoten MV, Roskams T, Kersten S, Müller M. Adipose tissue dysfunction signals progression of hepatic steatosis towards nonalcoholic steatohepatitis in C57BL/6 mice. *Diabetes*. 2010;**59**(12):3181-3191. DOI: 10.2337/db10-0224 Epub 2010 Sep 21

- [6] Sanyal A, Naumann J, Hoffmann LS, Chabowska-Kita A, Ehrlund A, Schlitzer A, Arner P, Blüher M, Pfeifer A. Interplay between obesity-induced inflammation and cGMP signaling in white adipose tissue. *Cell Reports*. 2017;**18**(1):225-236. DOI: 10.1016/j.celrep.2016.12.028
- [7] Shan D, Li JL, Wu L, Li D, Hurov J, Tobin JF, Gimeno RE, Cao J. GPAT3 and GPAT4 are regulated by insulin-stimulated phosphorylation and play distinct roles in adipogenesis. *Journal of Lipid Research*. 2010;**51**(7):1971-1981. DOI: 10.1194/jlr.M006304
- [8] Harris TE, Huffman TA, Chi A, Shabanowitz J, Hunt DF, Kumar A, Lawrence JC Jr. Insulin controls subcellular localization and multisite phosphorylation of the phosphatidic acid phosphatase, lipin 1. *The Journal of Biological Chemistry*. 2007;**282**(1):277-286. DOI: 10.1074/jbc.M609537200
- [9] Degerman E, Belfrage P, Manganiello VC. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). *The Journal of Biological Chemistry*. 1997;**272**(11):6823-6826. DOI: 10.1074/jbc.272.11.6823
- [10] Lafontan M. Advances in adipose tissue metabolism. *International Journal of Obesity*. 2008;**32**:S39-S51. DOI: 10.1038/ijo.2008.237
- [11] Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. *Annual Review of Nutrition*. 2007;**27**:79-101. DOI: 10.1146/annurev.nutr.27.061406.093734
- [12] Luo L, Liu M. Adipose tissue in control of metabolism. *Journal of Endocrinology*. 2016;**231**:R77-R99. DOI: 10.1530/JOE-16-0211
- [13] Schlueter N, de Sterke A, Willmes DM, Spranger J, Jordan J, Birkenfeld AL. Metabolic actions of natriuretic peptides and therapeutic potential in the metabolic syndrome. *Pharmacology & Therapeutics* 2014Oct;**144**(1):12-27 DOI: 10.1016/j.pharmthera.2014.04.007
- [14] Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. *Biochimie*. 2016 Jun;**125**:259-266. DOI: 10.1016/j.biochi.2015.10.024
- [15] Hoffmann LS, Larson CJ, Pfeifer A. cGMP and Brown adipose tissue. *Handbook of Experimental Pharmacology*. 2016;**233**:283-299. DOI: 10.1007/164_2015_3
- [16] Haas B, Mayer P, Jennissen K, Scholz D, Berriel Diaz M, Bloch W, Herzig S, Fässler R, Pfeifer A. Protein kinase G controls brown fat cell differentiation and mitochondrial biogenesis. *Science Signaling*. 2009;**2**(99):ra78. DOI: 10.1126/scisignal.2000511
- [17] Sanyal A, Hoffmann LS, Etzrodt J, Pfeifer A. Effects of obesity on sGCb1 mediated signaling in white adipose tissue. In: 7th International Conference on cGMP Generators, Effectors and Therapeutic Implications. *BMC Pharmacology and Toxicology*. 2015;**16**(1): A83. <https://doi.org/10.1186/2050-6511-16-S1-A83>
- [18] Hoffmann LS, Etzrodt J, Willkomm L, Sanyal A, Scheja L, Fischer AW, Stasch JP, Bloch W, Friebe A, Heeren J, Pfeifer A. Stimulation of soluble guanylyl cyclase protects against obesity by recruiting brown adipose tissue. *Nature Communications*. 2015;**6**:7235. DOI: 10.1038/ncomms8235

- [19] Frigolet ME, Thomas G, Beard K, Lu H, Liu L, Fantus IG. The bradykinin-cGMP-PKG pathway augments insulin sensitivity via upregulation of MAPK phosphatase-5 and inhibition of JNK. *American Journal of Physiology. Endocrinology and Metabolism*. 2017;**313**(3):E321-E334. DOI: 10.1152/ajpendo.00298.2016
- [20] Sansbury BE, Hill BG. Regulation of obesity and insulin resistance by nitric oxide. *Free Radical Biology & Medicine*. 2014;**73**:383-399. DOI: 10.1016/j.freeradbiomed.2014.05.016
- [21] Zemel MB, Sun X. Calcitriol and energy metabolism. *Nutrition Reviews*. 2008;**66**(10):139-146. DOI: 10.1111/j.1753-4887.2008.00099.x
- [22] Bravo-Sagua R, Mattar P, Díaz X, Lavandero S, Cifuentes M. Calcium sensing receptor as a novel mediator of adipose tissue dysfunction: Mechanisms and potential clinical implications. *Frontiers in Physiology*. 2016;**7**:395. DOI: 10.3389/fphys.2016.00395
- [23] Greenberg HZE, Carlton-Carew SRE, Khan DM, Zargaran AK, Jahan KS, Vanessa Ho WS, Albert AP. Heteromeric TRPV4/TRPC1 channels mediate calcium-sensing receptor-induced nitric oxide production and vasorelaxation in rabbit mesenteric arteries. *Vascular Pharmacology*. 2017;**96-98**:53-62. DOI: 10.1016/j.vph.2017.08.005
- [24] Berridge MJ. The inositol trisphosphate/calcium signaling pathway in health and disease. *Physiological Reviews*. 2016;**96**(4):1261-1296. DOI: 10.1152/physrev.00006.2016
- [25] Prole DL, Taylor CW. Inositol 1,4,5-trisphosphate receptors and their protein partners as signalling hubs. *The Journal of Physiology*. 2016;**594**(11):2849-2866. DOI: 10.1113/JP271139
- [26] Fill M, Copello JA. Ryanodine receptor calcium release channels. *Physiological Reviews*. 2002 Oct;**82**(4):893-922. PMID: 12270947. DOI: 10.1152/physrev.00013.2002
- [27] Berridge MJ, Galione A. Cytosolic calcium oscillators. *The FASEB Journal*. 1988;**15**:3074-3082 doi.org/10.1096/fasebj.2.15.2847949
- [28] Cobbold PH, Cuthbertson KS. Calcium oscillations: Phenomena, mechanisms and significance. *Seminars in Cell Biology*. 1990;**4**:311-321 PMID:2103516
- [29] Thomas AP, Bird GS, Hajnóczy G, Robb-Gaspers LD, Putney JW. Spatial and temporal aspects of cellular calcium signaling. *FASEB Journal*. 1996;**3**:1505-1517. Review. PMID:8940296
- [30] Uhlén P, Fritz N. Biochemistry of calcium oscillations. *Biochemical and Biophysical Research Communications*. 2010;**396**(1):28-32. DOI: 10.1016/j.bbrc.2010.02.117
- [31] Meyer T, Stryer L. Molecular model for receptor-stimulated calcium spiking. *Proceedings of the National Academy of Sciences of the United States of America*. 1988;**85**(14):5051-5055 PMID:2455890
- [32] Galione A, Lee HC, Busa WB. Ca(2+)-induced Ca²⁺ release in sea urchin egg homogenates: Modulation by cyclic ADP-ribose. *Science*. 1991;**253**(5024):1143-1146. DOI: 10.1126/science.1909457

- [33] Willmott N, Sethi JK, Walseth TF, Lee HC, White AM, Galione A. Nitric oxide-induced mobilization of intracellular calcium via the cyclic ADP-ribose signaling pathway. *The Journal of Biological Chemistry*. 1996;**271**(7):3699-3705. DOI: 10.1074/jbc.271.7.3699
- [34] Reyes-Harde M, Empson R, Potter BV, Galione A, Stanton PK. Evidence of a role for cyclic ADP-ribose in long-term synaptic depression in hippocampus. *Proceedings of National Academy of Sciences USA*. 1999;**96**(7):4061-4066. PMID:10097163
- [35] White TA, Kannan MS, Walseth TF. Intracellular calcium signaling through the cADPR pathway is agonist specific in porcine airway smooth muscle. *The FASEB Journal*. 2003;**17**(3):482-484. DOI: 10.1096/fj.02-0622fje
- [36] Deshpande DA, White TA, Dogan S, Walseth TF, Panettieri RA, Kannan MS. CD38/cyclic ADP-ribose signaling: Role in the regulation of calcium homeostasis in airway smooth muscle. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2005;**288**(5):L773-L788. DOI: 10.1152/ajplung.00217.2004
- [37] de Toledo FG, Cheng J, Liang M, Chini EN, Dousa TP. ADP-Ribosyl cyclase in rat vascular smooth muscle cells: Properties and regulation. *Circulation Research* 2000; **86**(11):1153-1159. <https://doi.org/10.1161/CIRCRESAHA.117.311307>
- [38] Nalli AD, Kumar DP, Al-Shboul O, Mahavadi S, Kuemmerle JF, Grider JR, Murthy KS. Regulation of G $\beta\gamma$ i-dependent PLC- β 3 activity in smooth muscle: Inhibitory phosphorylation of PLC- β 3 by PKA and PKG and stimulatory phosphorylation of Gai-GTPase-activating protein RGS2 by PKG. *Cell Biochemistry and Biophysics*. 2014;**70**(2):867-880. DOI: 10.1007/s12013-014-9992-6
- [39] Safonov M.G. *Stability and Robustness of Multivariable Feedback Systems*. Boston, Massachusetts: MIT Press; 1980. 171 p. ISBN: 9780262191807
- [40] Carlson JM, Doyle J. Complexity and robustness. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;**99**(Suppl 1):2538-2545. DOI: 10.1073/pnas.012582499
- [41] Michell BJ, Zp C, Tiganis T, Stapleton D, Katsis F, Power DA, Sim AT, Kemp BE. Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. *The Journal of Biological Chemistry*. 2001; **276**(21):17625-17628. DOI: 10.1074/jbc.C100122200
- [42] Murthy KS, Zhou H, Makhoul GM. PKA-dependent activation of PDE3A and PDE4 and inhibition of adenylyl cyclase V/VI in smooth muscle. *American Journal of Physiology. Cell Physiology*. 2002;**282**(3):C508-C517. DOI: 10.1152/ajpcell.00373.2001
- [43] Woods NM, Cuthbertson KS, Cobbold PH. Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. *Nature*. 1986;**319**(6054):600-602
- [44] Jacob R, Merritt JE, Hallam TJ, Rink TJ. Repetitive spikes in cytoplasmic calcium evoked by histamine in human endothelial cells. *Nature*. 1988;**335**(6185):40-45. DOI: 10.1038/335040a0
- [45] Rooney TA, Sass EJ, Thomas AP. Characterization of cytosolic calcium oscillations induced by phenylephrine and vasopressin in single fura-2-loaded hepatocytes. *The Journal of Biological Chemistry*. 1989;**264**(29):17131-17141 PMID: 2793847

- [46] Harootunian AT, Kao JP, Paranjape S, Adams SR, Potter BV, Tsien RY. Cytosolic Ca^{2+} oscillations in REF52 fibroblasts: Ca^{2+} -stimulated IP_3 production or voltage-dependent Ca^{2+} channels as key positive feedback elements. *Cell Calcium*. 1991;**12**(2-3):153-164 PMID:1647875
- [47] Fritz N, Mironneau J, Macrez N, Morel JL. Acetylcholine-induced Ca^{2+} oscillations are modulated by a Ca^{2+} regulation of InsP_3R_2 in rat portal vein myocytes. *Pflügers Archiv*. 2008;**456**(2):277-283. DOI: 10.1007/s00424-007-0379-z
- [48] De Pittà M, Goldberg M, Volman V, Berry H, Ben-Jacob E. Glutamate regulation of calcium and IP_3 oscillating and pulsating dynamics in astrocytes. *Journal of Biological Physics*. 2009;**35**(4):383-411. DOI: 10.1007/s10867-009-9155-y
- [49] Bai Y, Edelmann M, Sanderson MJ. The contribution of inositol 1,4,5-trisphosphate and ryanodine receptors to agonist-induced Ca^{2+} signaling of airway smooth muscle cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2009;**297**(2):347-361. DOI: 10.1152/ajplung.90559.2008
- [50] Hu R, He ML, Hu H, Yuan BX, Zang WJ, Lau CP, Tse HF, Li GR. Characterization of calcium signaling pathways in human preadipocytes. *Journal of Cellular Physiology*. 2009;**220**(3):765-770. DOI: 10.1002/jcp.21823
- [51] Turovsky EA, Kaimachnikov NP, Turovskaya MV, Berezhnov AV, Dynnik VV, Zinchenko VP. Two mechanisms of calcium oscillations in adipocytes. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology*. 2012;**6**(1):26-34. DOI: 0.1134/S199074781106016X
- [52] Keizer J, Li YX, Stojilković S, Rinzel J. InsP_3 -induced Ca^{2+} excitability of the endoplasmic reticulum. *Molecular Biology of the Cell*. 1995;**6**(8):945-951. DOI: 10.1091/mbc.6.8.945
- [53] Schuster S, Marhl M, Höfer T. Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signalling. *European Journal of Biochemistry*. 2002;**269**(5):1333-1355. DOI: 10.1046/j.0014-2956.2001.02720
- [54] Thore S, Dyachok O, Gylfe E, Tengholm A. Feedback activation of phospholipase C via intracellular mobilization and store-operated influx of Ca^{2+} in insulin-secreting beta-cells. *Journal of Cell Science*. 2005;**118**(19):4463-4471. DOI: 10.1242/jcs.02577
- [55] Turovsky E, Turovskaya M, Berezhnov A, Tolmacheva A, Kaimachnikov N, Dolgacheva L, Zinchenko V, Maevskii E, Dynnik V. Convergence of Ca^{2+} signaling pathways in adipocytes. Role of L-arginine and protein kinase G in the generation of transient and periodic Ca^{2+} signals. *Biochemistry (Moscow), Series A: Membrane and Cell Biology*. 2012;**6**(1):35-43. DOI: <https://doi.org/10.1134/S1990747811060158>
- [56] Turovsky EA, Turovskaya MV, Tolmacheva AV, Dolgacheva LP, Zinchenko VP, Dynnik VV. Resistance of mouse adipocytes to noradrenaline at obesity and type 2 diabetes. *Fundamental Research*. 2013;**1-3**:595-599. https://elibrary.ru/download/elibrary18771743_53027627.pdf
- [57] Sergeev AI, Sirota NP, Turovsky EA, Turovskaya MV, Khoustova Ya V, Simonova MA, Grishina EV, Dolgacheva LP, Zinchenko VP, Dynnik VV. Dysregulation of Ca^{2+} signaling

- pathways of adipocytes in obesity and type 2 diabetes. *Fundamental Research*. 2013; **6-6**:1436-1441. https://elibrary.ru/download/elibrary_19088293_17181985.pdf
- [58] Turovsky EA, Turovskaya MV, Dolgacheva LP, Zinchenko VP, Dynnik VV. Peptide hormones CCK, ANP and ANGII as regulators of intracellular calcium in adipocytes of white fat. *Fundamental Research*. 2013;**8**:905-908. http://elibrary.ru/download/elibrar19433424_60712563.pdf
- [59] Turovsky EA, Turovskaya MV, Zinchenko VP, Dynnik VV, Dolgacheva LP. Insulin induces Ca^{2+} oscillations in white fat adipocytes via PI3K and PLC. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology*. 2016;**10**(1):53-59. DOI: 10.1134/S1990747815050189
- [60] Dolgacheva LP, Turovskaya MV, Dynnik VV, Zinchenko VP, Goncharov NV, Davletov B, Turovsky EA. Angiotensin II activates different calcium signaling pathways in adipocytes. *Archives of Biochemistry and Biophysics*. 2016;**593**:38-49. DOI: 10.1016/j.abb.2016.02.001
- [61] Turovsky EA, Turovskaya MV, Dolgacheva LP, Zinchenko VP, Dynnik VV. Acetylcholine promotes Ca^{2+} and NO-oscillations in adipocytes implicating $\text{Ca}^{2+} \rightarrow \text{NO} \rightarrow \text{cGMP} \rightarrow \text{cADP-ribose} \rightarrow \text{Ca}^{2+}$ positive feedback loop - modulatory effects of norepinephrine and atrial natriuretic peptide. *PLoS One*. 2013;**8**(5):e63483. DOI: 10.1371/journal.pone.0063483
- [62] Turovsky EA, Turovskaya MV, Tolmacheva AV, Dolgacheva LP, Zinchenko VP, Dynnik VV. β -Adrenoreceptors as regulators of intracellular calcium in adipocytes of white fat. *Fundamental Research*. 2012. № 11-5. pp. 1059-1062. https://elibrary.ru/download/elibrary_18318794_83005196.pdf
- [63] Turovsky EA, Kaimachnikov NP, Zinchenko VP. Agonist specific participation of SOC and ARC channels and of iPLA 2 in the regulation of Ca^{2+} entry during oscillatory responses in adipocytes. *Biochemistry (Moscow) supplement series a. Membrane and Cell Biology*. 2013;**30**(5):491-498. DOI: 10.7868/S0233475513050204
- [64] Rooney TA, Joseph SK, Queen C, Thomas AP. Cyclic GMP induces oscillatory calcium signals in rat hepatocytes. *The Journal of Biological Chemistry*. 1996;**271**(33):19817-19825. DOI: 10.1074/jbc.271.33.19817
- [65] Rooney TA, Renard DC, Sass EJ, Thomas AP. Oscillatory cytosolic calcium waves independent of stimulated inositol 1,4,5-trisphosphate formation in hepatocytes. *The Journal of Biological Chemistry*. 1991;**266**(19):12272-12282. DOI: 10.1016/0143-4160(91)90013-5
- [66] Kannan MS, Prakash YS, Brenner T, Mickelson JR, Sieck GC. Role of ryanodine receptor channels in Ca^{2+} oscillations of porcine tracheal smooth muscle. *The American Journal of Physiology*. 1997;**272**(4 Pt 1):L659-L664. DOI: 10.1152/ajplung.1997.272.4.L659
- [67] Fritz N, Macrez N, Mironneau J, Jeyakumar LH, Fleischer S, Morel JL. Ryanodine receptor subtype 2 encodes Ca^{2+} oscillations activated by acetylcholine via the M2 muscarinic receptor/cADP-ribose signalling pathway in duodenum myocytes. *Journal of Cell Science*. 2005;**118**(Pt 10):2261-2270. DOI: 10.1242/jcs.02344
- [68] Kunerth S, Langhorst MF, Schwarzmann N, Gu X, Huang L, Yang Z, Zhang L, Mills SJ, Zhang LH, Potter BV, Guse AH. Amplification and propagation of pacemaker Ca^{2+}

signals by cyclic ADP-ribose and the type 3 ryanodine receptor in T cells. *Journal of Cell Science*. 2004;**117**(Pt 10):2141-2149. DOI: 10.1242/jcs.01063

- [69] Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proceedings of National Academy of Science USA*. 1990;**87**(2): 682-685. PMID: PMC53329
- [70] Fleming I. Molecular mechanisms underlying the activation of eNOS. *Pflügers Archiv*. 2010;**459**(6):793-806. DOI: 10.1007/s00424-009-0767-7
- [71] Förstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. *European Heart Journal*. 2012;**33**(7):829-837. DOI: 10.1093/eurheartj/ehr304
- [72] Bozulic L, Hemmings BA. PIKKing on PKB: Regulation of PKB activity by phosphorylation. *Current Opinion in Cell Biology*. 2009;**21**(2):256-261. DOI: 10.1016/j.ceb.2009.02.002
- [73] Gocher AM, Azabdaftari G, Euscher LM, Dai S, Karacosta LG, Franke TF, Edelman AM. Akt activation by Ca²⁺/calmodulin-dependent protein kinase kinase 2 (CaMKK2) in ovarian cancer cells. *The Journal of Biological Chemistry*. 2017;**292**(34):14188-14204. DOI: 10.1074/jbc.M117.778464
- [74] Butt E, Bernhardt M, Smolenski A, Kotsonis P, Fröhlich LG, Sickmann A, Meyer HE, Lohmann SM, Schmidt HH. Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases. *The Journal of Biological Chemistry*. 2000;**275**(7):5179-5187 PMID:10671564
- [75] Gebaska MA, Zhang M, Stevenson BK, Bivalacqua TJ, Strong TD, Hemnes AD, Halushka MK, Krongkaew N, Murray KI, Zeineh N, Kass DA, Champion HC. Endothelial cGMP-dependent protein kinase (PKG1) directly activates eNOS and plays a key role in modulation of vascular tone. Abstract 3634. *Circulation*. 2008;**118**:S_455
- [76] John TA, Ibe BO, Raj JU. Regulation of endothelial nitric oxide synthase: Involvement of protein kinase G 1 beta, serine 116phosphorylation and lipid structures. *Clinical and Experimental Pharmacology & Physiology*. 2008;**35**(2):148-158. DOI: 10.1111/j.1440-1681.2007.04801.x
- [77] Lee SH, Byun JS, Kong PJ, Lee HJ, Kim DK, Kim HS, Sohn JH, Lee JJ, Lim SY, Chun W, Kim SS. Inhibition of eNOS/sGC/PKG pathway decreases Akt phosphorylation induced by Kainic acid in mouse hippocampus. *Korean Journal of Physiology and Pharmacology*. 2010;**14**(1):37-43. DOI: 10.4196/kjpp.2010.14.1.37
- [78] Zhou Z, Sayed N, Pyriochou A, Roussos C, Fulton D, Beuve A, Papapetropoulos A. Protein kinase G phosphorylates soluble guanylyl cyclase on serine 64 and inhibits its activity. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2008;**28**(10):1803-1810. DOI: 10.1161/ATVBAHA.108.165043
- [79] Rybalkin SD, Yan C, Bornfeldt KE, Beavo JA. Cyclic GMP phosphodiesterases and regulation of smooth muscle function. *Circulation Research*. 2003;**93**(4):280-291. DOI: 10.1161/01.RES.0000087541.15600.2B
- [80] Schröder F, Klein G, Fiedler B, Bastein M, Schnasse N, Hillmer A, Ames S, Gambaryan S, Drexler H, Walter U, Lohmann SM, Wollert KC. Single L-type Ca(2+) channel regulation

- by cGMP-dependent protein kinase type I in adult cardiomyocytes from PKG I transgenic mice. *Cardiovascular Research*. 2003;**60**(2):268-277 PMID:14613856
- [81] Koitabashi N, Aiba T, Hesketh GG, Rowell J, Zhang M, Takimoto E, Tomaselli GF, Kass DA. Cyclic GMP/PKG-dependent inhibition of TRPC6 channel activity and expression negatively regulates cardiomyocyte NFAT activation. Novel mechanism of cardiac stress modulation by PDE5 inhibition. *Journal of Molecular and Cellular Cardiology*. 2010;**48**(4):713-724. DOI: 10.1016/j.yjmcc.2009.11.015
- [82] Nishida M, Watanabe K, Sato Y, Nakaya M, Kitajima N, Ide T, Inoue R, Kurose H. Phosphorylation of TRPC6 channels at Thr69 is required for anti-hypertrophic effects of phosphodiesterase 5 inhibition. *The Journal of Biological Chemistry*. 2010;**285**(17):13244-13253. DOI: 10.1074/jbc.M109.074104
- [83] Freeman M. Feedback control of intercellular signalling in development. *Nature*. 2000;**408**(6810):313-319. DOI: 10.1038/35042500
- [84] Kitano H. Computational systems biology. *Nature*. 2002;**420**(6912):206-210. DOI: 10.1038/nature01254
- [85] Tertyshnikova S, Yan X, Fein A. cGMP inhibits IP₃-induced Ca²⁺ release in intact rat megakaryocytes via cGMP- and cAMP-dependent protein kinases. *The Journal of Physiology*. 1998;**512**(1):89-96. DOI: 10.1111/j.1469-7793.1998.089bf.x
- [86] Steinberg GR, Kemp BE. AMPK in health and disease. *Physiological Reviews*. 2009;**89**(3):1025-1078. DOI: 10.1152/physrev.00011.2008
- [87] Andreeva LA, Grishina EV, Sergeev AI, Lobanov AV, Slastcheva GA, Rykov VA, Temyakov AV, Dynnik VV. Emergence of acetylcholine resistance and loss of rhythmic activity associated with the development of hypertension, obesity, and type 2 diabetes. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology*. 2016;**10**(3):199-206. DOI: 10.1134/S1990747816020033
- [88] Dynnik VV, Grishina EV, Kononov AV, Zinchenko VP, Lobanov AV, Uvarova OV, Macievich MV, Bogomolov PO. Multi target treatment of hepatic encephalopathy, possible mechanisms of synergistic action of the substances incorporated into complex formulations. In: Abstracts of Drug Discovery and Therapy World Congress, August 22-25, 2016, Boston, MA, USA, track: Drug Metabolism, SL-107, 2016, p. 36. www.ddtwc.com
- [89] Andreeva LA, Sirota NP, Grishina EV, Sergeev AI, Lobanov AV, Murashev AN, Temyakov AV, Dynnik VV. On the mechanisms of hormonal resistance and loss of rhythmic activity in rat aorta under the development of obesity and type 2 diabetes. In: Abstracts of Global Biotechnology Congress, August 22-25, 2016, Boston, MA, USA, Track: Pharmaceutical Biotechnology, PO-22, 2016, pp. 180-181. www.ddtwc.com/www.globalbiotechcongress.com
- [90] Bartness TJ, Liu Y, Shrestha YB, Ryu V. Neural innervation of white adipose tissue and the control of lipolysis. *Frontiers in Neuroendocrinology*. 2010;**35**(4):473-493. DOI: 10.1016/j.yfrne.2014.04.001