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MINIREVIEW

Intracellular Mediators in Regulation of Leptin Secretion from Adipocytes

T. SZKUDELSKI

Department of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, Poznan, Poland

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Summary

Leptin is a hormone primarily secreted by adipocytes and participating in the regulation of food intake and energy expenditure. Its blood levels usually correlate with adiposity. The secretion of this hormone is affected, among others, by food consumption, insulin, fasting and cold exposure. Regulation of leptin secretion depends on many intracellular events. It is known that the activation of mTOR (the mammalian target of rapamycin) as well as increase in ATP and malonyl-CoA content in adipocytes enhance secretion of leptin. The rise in intracellular cAMP and fatty acids is thought to evoke the opposite effect. Moreover, the undisturbed action of endogenous adenosine in adipocytes and the proper intracellular Ca^{2+} concentration in these cells were also found to have an important function in leptin release. The role of mTOR, ATP, cAMP, fatty acids, malonyl-CoA, adenosine and Ca^{2+} in the regulation of leptin secretion from adipocytes is discussed.

Key words

Leptin secretion • Adipocytes • mTOR • ATP • cAMP • Fatty acids • Malonyl-CoA • Adenosine • Calcium

Leptin is a product of the *ob* gene secreted predominantly by white adipocytes (Zhang *et al.* 1994) and is thought to be an important element in the "lipostat" theory. Its concentration in the plasma reflects the total amount of adipose tissue in the body. This correlation may, however, be disturbed after energy deprivation, e.g. during fasting (Kim and Scarpace 2003). Leptin acts within the hypothalamus regulating energy expenditure and food consumption (Friedman and Halaas 1998). However, leptin receptors are present not only in the hypothalamus and the physiological role of this hormone involves many effects which are not directly related to the energetic status of the organism (for review see Janečková 2001). Under physiological conditions, plasma leptin levels are affected by several factors (Table 1). Food consumption and the postprandial rise in insulinemia increase the concentration of this hormone in the plasma. This effect is, however, observed after a relatively long time after a meal (several hours) (Koopmans *et al.* 1998, Lynch *et al.* 2006), which indicates that leptin is not a short-term satiety signal. The stimulation of β-adrenergic receptors (Gettys *et al.* 1996),

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

fasting (Boden *et al.* 1996, Szkudelski *et al.* 2004) and cold exposure (Rayner and Trayhurn 2001) exert an opposite effect and decrease blood leptin values. Moreover, it was observed that leptin secretion has a strong circadian rhythmicity with a nadir in the morning and a maximum in the middle of the night (Licinio *et al.* 1997, Mastronardi *et al.* 2000, Nagatani *et al.* 2000).

Changes in leptin secretion from adipocytes result from many alterations in these cells. These regulatory events are not yet fully understood and some discrepancies in the literature are still found. However, several investigations have provided convincing findings about intracellular mediators engaged in the regulation of leptin secretion. It is well established that mTOR, ATP, cAMP, fatty acids, malonyl-CoA, adenosine and Ca^{2+} participate in this regulation (Table 2).

mTOR

Insulin is an important physiological factor activating mTOR (the mammalian target of rapamycin) in adipocytes. Activation of this pathway mediates different effects of insulin such as stimulation of adipogenesis or lipogenesis (Cho *et al.* 2004) and potentiation of leptin secretion (Bradley and Cheatham 1999). The incubation of adipocytes with insulin was found to increase leptin secretion after 30 min (Gettys *et al.* 1996) or even earlier (Barr *et al.* 1997). The use of confocal microscopy allowed to reveal the enhanced transport of leptin from the endoplasmic reticulum towards the plasma membrane already 15 min upon insulin stimulation. It was also found that the insulin-induced rise in leptin secretion is accompanied by an initial decrease in the intracellular leptin content, probably due to its augmented release from fat cells (Barr *et al.* 1997). The involvement of mTOR in the insulin-induced secretion of leptin was proved by Bradley and Cheatham (1999). They observed that adipocytes incubated for 2 h with insulin release more leptin in spite of unchanged leptin mRNA content. Moreover, insulin stimulation of leptin secretion was not disturbed by actinomycin D, an inhibitor of transcription. However, the inhibition of protein synthesis by cyclohexamide substantially depressed this process, which indicates that the regulation at a posttranscriptional level is pivotal for the stimulation of leptin secretion by insulin (Bradley and Cheatham 1999). The important role of mTOR in this process was further confirmed in experiments demonstrating that rapamycin, an inhibitor of mTOR, diminishes the insulin-induced (but not basal) secretion of leptin (Bradley and Cheatham 1999). Recently, it has been well established that the insulin-induced activation of mTOR is mediated *via* phosphatidylinositol 3-kinase and protein kinase B (PKB, AKT) (Hinault *et al.* 2004).

It is noteworthy that, apart from insulin, there is another well recognized agent activating mTOR in different kinds of cells, including adipocytes, namely leucine (Xu *et al.* 2001, Lynch 2001, Roh *et al.* 2003, Lynch *et al.* 2006, Norton and Layman 2006). Roh *et al.* (2003) revealed that 5 mM leucine incubated with isolated rat adipocytes substantially augmented the concentration of leptin in the medium. This effect was not accompanied by an increase in the amount of leptin mRNA in adipocytes. Moreover, the inhibitor of transcription failed to attenuate the release of leptin induced by leucine. This process was, however, restricted by rapamycin. It is known that the pathway whereby insulin and leucine activate mTOR is not the same. Leucine, conversely to the pancreatic hormone, activates mTOR by a mechanism independent of protein kinase B (Lynch 2001, Hinault *et al.* 2004). Insulin and leucine were found to have an additive effect on mTOR activation (Hinault *et al.* 2004). On the other hand, Cammisotto *et al.* (2005) failed to demonstrate the stimulatory effect of 5 mM leucine alone or in combination with insulin on leptin secretion from freshly isolated rat adipocytes. However, these authors observed that leucine effectively enhanced leptin release in the presence of glucose (with or without insulin). This indicates that, under physiological conditions, leucine may exert an additive action with other agents increasing secretion of leptin. It has been postulated that activation of mTOR by this amino acid is partially responsible for the postprandial rise in blood leptin level (Roh *et al.* 2003). The stimulatory effect of orally administered leucine on protein synthesis in rat adipose tissue supports this assumption (Lynch *et al.* 2002). The most recent studies have confirmed the role of exogenous leucine in the activation of mTOR and stimulation of leptin secretion after a meal (Lynch *et al.* 2006).

ATP

Glucose seems to be the most important source of ATP in adipocytes during leptin secretion. The increment in hormone release elicited by glucose requires the transport of this hexose into fat cells. This process is achieved *via* glucose transporter GLUT1 (basal glucose

Table 1. Factors regulating leptin secretion from adipocytes and known intracellular mediators involved in this action.

Stimulation	Mediator	References
Food consumption	ATP mTOR	Thompson (1996), Weigle *et al.* (1997), Lynch *et al.* (2006)
Insulin	mTOR, Adenosine	Barr *et al.* (1997), Cheng *et al.* (2000)
Leucine	mTOR	Roh *et al.* (2003), Lynch *et al.* (2006)
Glucose	ATP	Mizuno *et al.* (1996), Mueller *et al.* (1998), Levy *et al*. (2000)
Glucocorticoids	Transcriptional factors	De Vos *et al.* (1995), Slieker *et al.* (1996), Bradley and Cheatham (1999)
Inhibition	**Mediator**	**References**
Fasting	cAMP	Boden *et al.* (1996), Hardie *et al*. (1996), Szkudelski *et al.* (2004)
Cold exposure	cAMP	Rayner and Trayhurn (2001), Korhonen and Saarela (2005)
β-adrenergic stimulation	cAMP	Gettys *et al.* (1996), Slieker *et al.* (1996), Donahoo *et al.* (1997), Szkudelski *et al.* (2005b)
Exercise	cAMP	Zheng *et al.* (1996), Bramlett *et al.* (1999)

Table 2. Changes in adipocytes leading to the stimulation or inhibition of leptin secretion.

Stimulation	Inhibition	References
ATP↑	ATP ↓	Mueller *et al*. (1998), Levy *et al.* (2000), Mueller *et al.* (2000)
cAMP ↓	cAMP ↑	Gettys *et al.* (1996), Cammisotto and Bukowiecki (2002), Szkudelski *et al.* (2005b)
Malonyl-CoA↑	Malonyl-CoA ↓	Shirai *et al.* (2004)
mTOR↑	mTOR ↓	Bradley and Cheatham (1999), Lynch (2001), Hinault *et al.* (2004), Roh *et al.* (2003)
Adenosine ↑	Adenosine ↓	Rice *et al.* (2000), Cheng *et al.* (2000)
	Fatty acids ↑	Shintani *et al.* (2000), Arai *et al*. (2002), Cammisotto *et al*. (2003)
	Ca^{2+} ↓, Ca^{2+} ↑	Levy *et al.* (2000), Cammisotto and Bukowiecki (2004)

↑ - increase or activation, ↓ - decrease or inactivation

uptake) in the absence of insulin, and *via* GLUT4 (stimulated transport) in the presence of this hormone. Insulin promotes the translocation of GLUT4 from the intracellular pool to the plasma membrane and thereby effectively accelerates glucose transport into adipocytes (Smith *et al.* 1991, Khan and Pessin 2002). The rise in the transport of this sugar is, however, not sufficient to potentiate hormone secretion (Mueller *et al.* 2000).

Another prerequisite is the metabolism of glucose providing ATP. Insulin not only accelerates glucose transport but also shifts its metabolism from anaerobic to mitochondrial oxidation. This important feature of insulin results in decreased formation of lactate, increased glucose utilization and ATP generation and, finally, augmented secretion of leptin (Mueller *et al.* 1998, Levy *et al.* 2000, Levy and Stevens 2001). Compounds enhancing glucose uptake, but potentiating its metabolism to lactate (*e.g.* metformin) were found to restrict secretion of leptin (Mueller *et al.* 2000). Similarly, inhibition of glucose transport or glycolysis and the presence of substrates depleting ATP in adipocytes (*e.g.* 2-deoxyglucose) abate the release of this hormone (Mueller *et al.* 1998, Levy *et al.* 2000).

The stimulatory effect of glucose and insulin on

leptin secretion is time-dependent (Mueller *et al.* 1998, Levy *et al.* 2000, Levy and Stevens 2001). It was found that within 2 hours of stimulation the rise in hormone release induced by glucose and insulin was proportional to the increase in the ATP content in adipocytes. After this time, leptin secretion still increased, but no further rise in its concentration was observed (Levy *et al.* 2000). Some authors demonstrated that glucose- and insulin-induced secretion of leptin depends on glucose concentration (Levy *et al.* 2000). However, the others failed to ascertain such dependency – insulin potentiated leptin secretion induced by 5 or 25 mM glucose, but there was no substantial difference in the amount of hormone released by cells incubated with low or high glucose concentrations (Cammisotto *et al.* 2005, Szkudelski *et al.* 2005a). Earlier studies with adipocytes incubated for 96 hours revealed that the effect of glucose and insulin on leptin secretion correlated better with the glucose uptake than with insulin concentration (Mueller *et al.* 1998). One can suppose that the stimulatory effect of higher glucose concentrations on leptin secretion is partially restricted as a result of the ability of this sugar to induce lipolysis – an effect abating secretion of leptin (see below). This is possible since the elevation of glucose concentrations in the medium with adipocytes substantially enhanced lipolysis even in the presence of insulin (Szkudelski and Szkudelska 2000). Moreover, inhibition of protein kinase A (PKA) slightly restricted lipolysis in fat cells and simultaneously augmented leptin secretion induced by glucose and insulin (Szkudelski *et al.* 2005b).

Apart from glucose, other substrates potentially generating ATP in adipocytes such as fructose, alanine, and pyruvate are also able to increase secretion of leptin in the absence and presence of insulin. However, the clear-cut stimulatory effect was demonstrated at high concentrations of these compounds (25 mM) (Levy *et al.* 2000). In another study, 5 mM alanine had no effect on leptin secretion, whereas 5 mM fructose and pyruvate potentiated this process only in the presence of insulin (Cammisotto *et al.* 2005). It is, however, possible that under physiological conditions combination of different metabolizable and ATP-generating compounds may exert synergistic action, especially in the presence of insulin, and already enhance secretion of leptin at lower concentrations. Therefore, the increase in ATP formation in adipocytes seems to be an important factor increasing leptin gene expression and enhancing leptin secretion after a meal.

cAMP

cAMP is a factor participating in the regulation of pivotal processes in adipocytes, including leptin secretion. There is no doubt that a rise of its concentration in these cells decreases leptin secretion, whereas a decrease in cAMP content exerts the opposite effect. It seems that different factors decreasing leptin secretion such as fasting, cold exposure or exercise exert this effect, among others, *via* the increase in cAMP content in adipocytes. It was demonstrated that the incubation of fat cells with a lipolytic hormone – epinephrine, CL316,243 (a selective β_3-adrenergic receptor agonist) (Gettys *et al.* 1996) or isoprenaline (Hardie *et al.* 1996) substantially restricted the insulin-stimulated leptin secretion. Similar effect was evoked by forskolin (a compound directly activating adenylate cyclase), by different cAMP analogues and inhibitors of cAMP phosphodiesterase (Cammisotto and Bukowiecki 2002). Therefore, the attenuation of the insulin-induced leptin secretion is caused by a wide spectrum of agents augmenting, *via* distinct pathways, the content of cAMP in fat cells. It was, however, found that basal, i.e. non-stimulated hormone release, is not significantly influenced by compounds increasing cAMP in adipocytes (Cammisotto and Bukowiecki 2002). The inhibitory action of agents raising intracellular cAMP on leptin secretion is not limited to the process stimulated exclusively by insulin. Recent experiments revealed that dibutyryl-cAMP, a non-hydrolysable cAMP analogue, substantially abated the release of leptin induced by glucose and insulin, alanine and insulin and leucine with insulin (Szkudelski *et al.* 2005b).

A physiological factor exerting an opposite effect and decreasing cAMP in adipocytes is insulin. This effect is predominantly due to an activation of cAMP phosphodiesterase 3B in fat cells (Eriksson *et al.* 1995). Since insulin is able to abate lipolysis (reflecting cAMP content in adipocytes) induced by epinephrine (Eriksson *et al.* 1995), it can be supposed that, under physiological conditions, the reduction of cAMP caused by insulin contributes to the rise in leptin secretion. A pharmacological agent which is postulated to enhance leptin secretion *via* decreasing cAMP content in adipocytes is nicotinic acid and its longer-acting analogue Acipimox. Wang-Fisher *et al.* (2002) revealed that both these compounds potentiated secretion of leptin from adipocytes of normal and insulin-resistant rats. The stimulatory action of these compounds was time- and

dose-dependent. Nicotinic acid and its analogue at concentrations 100 μM elicited secretory responses similar to those evoked by 10 nM insulin. Leptin release enhanced by these compounds was antagonized by dibutyryl-cAMP. The known action of nicotinic acid in adipocytes is preceded by its binding to a G-protein-coupled receptor resulting in reduced cAMP content and restriction of lipolysis (Karpe and Frayn 2004). It is therefore postulated that nicotinic acid enhances leptin release due to its antilipolytic action (Wang-Fisher *et al.* 2002). However, this hypothesis does not seem to be fully convincing since nicotinic acid induced hormone secretion under basal conditions, i.e. in adipocytes non-treated by any agent augmenting intracellular cAMP concentration. Moreover, leptin secretion enhanced by Acipimox was suppressed by epinephrine (Wang-Fisher *et al.* 2002). It was also shown that the inhibition of lipolysis is insufficient to increase leptin secretion from adipocytes since antilipolytic agents do not mimic the stimulatory action of insulin on leptin release in spite of the suppression of norepinephrine-induced lipolysis (Cammisotto and Bukowiecki 2002). These findings suggest that the reduction of cAMP is not the sole effect whereby nicotinic acid stimulates the release of leptin.

The inhibitory action of cAMP on leptin secretion is PKA-dependent (Szkudelski *et al.* 2005b) and is achieved by different ways. Scott and Lawrence (1998) provided an evidence that increased concentration of this nucleotide in 3T3-L1 adipocytes counteracted the phosphorylation and activation of mTOR by insulin. Moreover, in experiments with isolated fat cells cAMP analogue used in a high concentration (1 mM) was found to inhibit glucose transport *via* GLUT4 (Kelada *et al.* 1992). It is also well established that the restrictive action of cAMP on leptin secretion is accompanied by a concomitant rise in lipolysis (Gettys *et al.* 1996, Cammisotto and Bukowiecki 2002, Szkudelski *et al.* 2005b), whereas the use of a specific PKA inhibitor attenuates lipolysis and simultaneously restores hormone release (Szkudelski *et al.* 2005b). These observations raise a question whether lipolytic products – glycerol and/or fatty acids – are involved in the inhibition of leptin release due to increased cAMP content in adipocytes. Experimental data confirmed this assumption.

Fatty acids

24-h incubation of murine adipocytes with 2-bromopalmitate, a non-metabolizable palmitate analogue, has been reported to reduce leptin mRNA content in 3T3-L1 adipocytes, however, the release of leptin was not examined (Rentsch and Chiesi 1996). Another study revealed that both palmitate and 2-bromopalmitate restrict not only leptin gene expression, but also deteriorate hormone secretion from fat cells (Shintani *et al.* 2000). Moreover, the inhibition of acyl-CoA synthetase by triacsin C, leading to intracellular fatty acid accumulation, also resulted in reduced leptin release and leptin mRNA content in rat adipocytes (Shintani *et al.* 2000) and 3T3-L1 cells (Arai *et al.* 2002). Cammisotto *et al.* (2003) provided further evidence that fatty acids generated in adipocytes during triglyceride breakdown diminish the insulin-induced leptin secretion. The inhibitory effect already appeared after 2 h of adipocyte incubation with fatty acids, was specific for medium- and long-chain acids and did not depend on the degree of their saturation. It was also shown that the attenuation of hormone secretion evoked by fatty acids was not suppressed by the inhibitors of their mitochondrial oxidation (Cammisotto *et al.* 2003). These outcomes and observations with 2-bromopalmitate indicate that the restriction of leptin secretion caused by fatty acids results from the rise in their intracellular concentration (Shintani *et al.* 2000, Arai *et al.* 2002) – a process which normally occurs during lipolysis. Therefore, fatty acids generated during lipolysis seem to constitute one of the important intracellular signals inhibiting leptin secretion. It is noteworthy that the restriction of hormone secretion is brought about by fatty acids arising exclusively in adipocytes during lipolysis. Free fatty acids circulating in the blood do not directly affect hormone secretion because of their binding to albumin. This conclusion is based on experiments demonstrating that the ability of fatty acids to abate leptin release from isolated cells is suppressed by albumin present in the incubation medium at concentrations similar to those in the plasma (Cammisotto *et al.* 2003). Other studies also revealed that exogenous fatty acids failed to affect pivotal aspects of insulin action in fat cells (Lundgren and Eriksson 2004). Moreover, plasma leptin concentrations were not affected by circulating free fatty acids (Stumvoll *et al*. 2000). However, the results of the most recent studies indicate that some exogenous fatty acids may exert the opposite influence on leptin secretion. Such an effect was observed in the case of eicosapentaenoic fatty acid. Incubation of adipocytes with this n-3 polyunsaturated acid enhanced both basal and insulin-stimulated leptin release. This effect was

accompanied by increased basal glucose uptake, enhanced oxidation and utilization of this sugar and its decreased metabolism to lactate (Perez-Matute *et al.* 2005).

Malonyl-CoA

Shirai *et al.* (2003) demonstrated that changes in leptin release evoked by some compounds are related to their ability to affect the concentration of malonyl-CoA, an intermediate of fatty acid synthesis, in adipocytes. The inhibition of malonyl-CoA formation from acetyl-CoA (catalyzed by acetyl-CoA carboxylase) was found to decrease the release of leptin stimulated by glucose and insulin, whereas restricted conversion of malonyl-CoA to palmitate (catalyzed by fatty acid synthase) intensified this process. The importance of malonyl-CoA in leptin secretion was additionally confirmed by the demonstration that exogenous malonyl-CoA incubated with fat cells (in the presence of glucose, insulin and NaF) enhanced secretion of this hormone. Good examples supporting this theory are i) glucose, which increases malonyl-CoA content in adipocytes and simultaneously potentiates leptin secretion, and ii) palmitate, which inhibits acetyl-CoA carboxylase and attenuates release of this hormone (Cammisotto *et al.* 2003, Shirai *et al.* 2003). Since palmitate is one of the main fatty acids stored in adipocytes (Raclot and Oudart 2000), the increased triglyceride breakdown upon stimulation by lipolytic hormones (e.g. epinephrine) may contribute to the inhibition of acetyl-CoA carboxylase, leading to decreased content of malonyl-CoA and reduced secretion of leptin. Conversely, the stimulatory effect of insulin on acetyl-CoA carboxylase in adipocytes (Haystead and Hardie 1986), especially in the presence of glucose as a source of acetyl-CoA, may contribute to the increased formation of malonyl-CoA and enhanced secretion of leptin (Shirai *et al.* 2003).

Adenosine

Endogenous adenosine formed in adipocytes is another factor participating in the regulation of leptin secretion. In rats receiving cyclopentyladenosine (CPA) – a pharmacological activator of adenosine A_1 receptor – serum leptin concentration was elevated (Rice *et al.* 2000). The observed effect was dose-dependent and the maximal increase in leptinemia was found 8-16 h after CPA treatment. Studies with isolated adipocytes confirmed the direct stimulatory influence of this compound on leptin secretion – the fat cells incubated for 24 h with the adenosine analogue released significantly more leptin in comparison to non-stimulated cells. The effect of CPA was greater *in vivo* than *in vitro*, probably due to its interaction with other factors affecting leptin secretion *in vivo*. Further experiments revealed that the adenosine A_1 receptor agonist substantially enhances both basal and insulin-induced leptin secretion from isolated adipocytes in a dose-dependent manner (Cheng *et al.* 2000). The opposite effect was evoked by deprivation of endogenous adenosine by adenosine deaminase and by an antagonist of adenosine A_1 receptor – in both cases insulin-stimulated secretion of leptin was attenuated. However, basal hormone release was not affected by adenosine decomposition. These results imply that endogenous adenosine is an important adipocyte-derived factor potentiating leptin secretion and point to the synergism between adenosine and insulin not only in relation to the inhibition of lipolysis but also in relation to the secretion of leptin. It seems that adipocyte-derived adenosine is involved in the stimulation of leptin secretion caused by insulin. This assumption is supported by results demonstrating that insulin increases the release of adenosine from fat cells (Cheng *et al.* 2000). The mechanism thereby endogenous adenosine enhances leptin secretion may be related to the inhibition of lipolysis. It is known that adenosine A_1 receptor activation results in diminished adenylyl cyclase activity, cAMP content and restricted triglyceride breakdown. Adenosine released from adipocytes activates its receptor and already exerts these effects at very low concentrations (Liang *et al.* 2002). The important feature of adenosine is that this compound tonically restrains lipolysis. Incubation of cells with adenosine deaminase or adenosine A_1 receptor antagonists results in a substantial increase in triglyceride breakdown (Honnor *et al.* 1985, Szkudelski *et al.* 2004). Some observations also indicate that phospholipase C is involved in leptin release evoked by adenosine. Inhibition of this enzyme was found to decrease CPA-induced leptin secretion, whereas basal process was unchanged (Cheng *et al.* 2000). The stimulatory influence of adenosine on leptin release seems to be without influence on leptin gene expression since neither CPA-induced rise in serum leptin concentration nor increased secretion of this hormone by isolated cells was accompanied by changes in leptin gene expression in epididymal adipocytes (Rice *et al.* 2000).

Calcium

Experiments with isolated rat adipocytes pointed to the involvement of Ca^{2+} in the process of leptin secretion. Levy et al. (2000) observed that fat cells incubated for 4 h in the medium deprived of these ions secreted less leptin in comparison to adipocytes incubated with Ca^{2+}. This difference was less marked during non-stimulated leptin release, but secretion elicited by glucose and insulin was substantially decreased by calcium deprivation. In the other experiment, the insulin- and glucose-induced rise in intracellular leptin content and leptin secretion were restricted by calcium deprivation in the medium already after 2 h of incubation, whereas basal hormone release was preserved. It is interesting that the enhancing effect of insulin on leptin release was not accompanied by changes in calcium uptake by adipocytes. Moreover, L-type calcium channel inhibitors did not disturb leptin secretion induced by the pancreatic hormone. Surprisingly, this process was attenuated by the increase in the intracellular calcium concentration (Cammisotto and Bukowiecki 2004). These results demonstrate that basal intracellular Ca^{2+} concentration is required for proper leptin synthesis and secretion. Under physiological conditions, different factors affect the intracellular calcium concentration in adipocytes (Kelly *et al.* 1989). However, it is not known whether some of them are able to regulate leptin secretion as a result of changes in the intracellular calcium. The role of these ions in leptin release seems to be indirect, resulting predominantly from their participation in glucose transport *via* GLUT4 (Cammisotto and Bukowiecki 2004). All these observations point to the clear-cut difference in the role of calcium in the process of leptin secretion from adipocytes in comparison to the secretion of hormones from some other endocrine cells such as pancreatic B cells. In the latter case, a rise in intracellular Ca^{2+} suddenly triggers insulin secretion and is required for its exocytosis (Rorsman *et al.* 1988, Henquin 2000). This distinction arises, among others, from specific features of leptin-containing membrane compartment in adipocytes which differs from secretory granules present in other kinds of endocrine cells (Roh *et al.* 2000).

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Corresponding author

Tomasz Szkudelski, Department of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, 60-637 Wolynska 35, Poznan, Poland, Fax: +48 61 8487197. E-mail: tszkudel@jay.au.poznan.pl