Insulin/adenosine axis linked signalling

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ABSTRACT

Regulation of blood flow depends on systemic and local release of vasoactive molecules such as insulin and adenosine. These molecules cause vasodilation by activation of plasma membrane receptors at the vascular endothelium. Adenosine activates at least four subtypes of adenosine receptors (A1AR, A2AR, A2BAR, A3AR), of which A2AAR and A2BAR activation leads to increased cAMP level, generation of nitric oxide, and relaxation of the underlying smooth muscle cell layer. Vasodilation caused by adenosine also depends on plasma membrane hyperpolarization due to either activation of intermediate-conductance Ca2+-activated K+ channels in vascular smooth muscle or activation of ATP-activated K+ channels in the endothelium. Adenosine also causes vasoconstriction via a mechanism involving A1AR activation resulting in lower cAMP level and increased thromboxane release. Insulin has also a dual effect causing NO-dependent vasodilation, but also sympathetic activity- and increased endothelin 1 release-dependent vasoconstriction. Interestingly, insulin effects require or are increased by activation or inactivation of adenosine receptors. This is phenomenon described for D-glucose and L-arginine transport where A2AAR and A2BAR play a major role. Other studies show that A1AR activation could reduce insulin release from pancreatic β-cells. Whether adenosine modulation of insulin biological effect is a phenomenon that depends on co-localization of adenosine receptors and insulin receptors, and adenosine plasma membrane transporters is something still unclear. This review summarizes findings addressing potential involvement of adenosine receptors to modulate insulin effect via insulin receptors with emphasis in the human vasculature.

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1. Introduction

A proper regulation of the vascular tone is essential to maintain vascular and systemic homeostasis compatible with life in humans. Several diseases associate with alterations in the vascular response to vasodilators or vasoconstrictors, including hypertension, diabetes mellitus, and obesity (De Boer et al., 2012; Charkoudian, 1985; Escudero et al., 2014; Higashi and Yoshizumi, 2004; Hink et al., 2003; Jonk et al., 2007; King et al., 1994; Lamireau et al., 2002; Versari et al., 2016). These vascular reactivity complications associate with disorders of the heart and blood vessels, referred as cardiovascular disorders (CVDs), including coronary heart, cerebrovascular, and peripheral arterial disease (World Health Organization (WHO), 2016). Vascular endothelial and smooth muscle cells play crucial roles in the efficiency of the vessels to dilate or contract in

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response to circulating or locally released molecules. Among a large
variety of these molecules, are the endogenous nucleoside adeno-
sine (Antonioli et al., 2015; Headrick et al., 2013; Westermeier et al.,
2011) and the hormone insulin (Baumgard et al., 2016; Manrique
et al., 2014; Westermeier et al., 2016; Zaykov et al., 2016), both of
which act on plasma membrane receptors of relative high selec-
tivity and specificity triggering differential signalling mechanisms
according to the type of receptor(s) activated (Burnstock, 2016;
Fredholm et al., 2011; Fredholm, 2014; Westermeier et al., 2016).

The biological effects of adenosine depend on its extracellular
concentration and binding to plasma membrane adenosine re-
ceptors (ARs) (Fredholm, 2014; Headrick et al., 2013; Riksen and
Rongen, 2012). ARs are coupled to stimulatory or inhibitory G
proteins, which, among other things, lead to changes in the level of
the adenyl cyclase (AC)-generated cyclic AMP (cAMP), thus
modulating cell function and metabolism (Fredholm et al., 2011;
Fredholm, 2014). ARs are four subtypes expressed in most cell
types, including the human umbilical cord vessels and placenta
vasculature, i.e., foetoplacental vasculature (Wyatt et al., 2002;
Salsoso et al., 2015). Activation or blockage of ARs could result in
greater risk to develop diabetes mellitus, hypertension, or cancer
(Fredholm, 2010). Equally, ARs are essential in gestational diabetes
mellitus (GDM) (Vásquez et al., 2004; Martín and Sobrevia,
2006; Guzmán-Gutiérrez et al., 2016) and early or late pre-
eclampsia (Escudero et al., 2008; Salsoso et al., 2015)-associated
human umbilical vein endothelial dysfunction.

ARs are also critical in the biological effects of insulin in the
human vasculature (Guzmán-Gutiérrez et al., 2012, 2016; Salsoso
et al., 2015), and other cell types, including skeletal muscle (Figler
et al., 2011; Han et al., 1998; Law et al., 1988; Sacramento et al.,
2015; Thong et al., 2007) and adipocytes (Ciaraldi, 1988; Green,
1987; Lönnroth et al., 1988; Martin and Bockman, 1986; Wong
et al., 1984). Interestingly, different levels of expression of insulin
receptors (IRs), as well as triggering of their corresponding asso-
ciated signalling mechanisms, is reported in human umbilical vein
endothelial cells (HUVECs) from GDM pregnancies compared with
cells from normal pregnancies (Guzmán-Gutiérrez et al., 2016;
Westermeier et al., 2011, 2015). This condition results in endo-
thelial cell activation increasing the expression and activity of nitric
oxide synthases (NOS) in HUVECs (Westermeier et al., 2011) and
human placental microvascular endothelial cells (hPMecs)
(Salomon et al., 2012). Thus, a close relationship between adeno-
sine and ARs, and insulin and IRs is a mechanism that modulates
cell function, including vascular endothelial and smooth muscle
cells, in health and disease.

This review addresses potential cellular and molecular mecha-
nisms behind the biological actions of adenosine via ARs as modu-
lator of insulin effect via IRs with emphasis in the human vasculature.

2. Adenosine

Adenosine is an endogenous purine nucleoside that results from
the β-N-glycosidic bond between adenine and d-ribose, and is
synthesized, released, and taken up by most, if not all the cells
(Pelleg and Porter, 1990), including human foetoplacental vascular
endothelial (Ho et al., 2016; Vásquez et al., 2004; Westermeier et al.,
2011, 2016) and smooth muscle cells (Aguayo et al., 2001; Aguayo
and Sobrevia, 2000; Ho et al., 2016). This nucleoside is widely
recognized for being a local regulator of cellular function, medi-
ating autocrine and paracrine mechanisms in response to acute
alterations meeting the associated energy demands of cells (Chen
et al., 2013; Headrick et al., 2011). These physiological processes
include the local regulation of vascular tone in adults (Ballard,
2014; Kaufmann et al., 2007) and newborns (Westermeier et al.,
2015)}
Adenosine generation at the extracellular space results from ATP and ADP phosphohydrolysis mediated by a two-step process involving ectonucleoside triphosphate diphosphohydrolase (ecto-NTPDase-1) to generate AMP, and the activity of ecto-5'-nucleotidase to generate adenosine (Fernández et al., 2013). Adenosine degradation to inosine is mediated via adenosine deaminase (ADA). Cytoplasmic adenosine kinase regulates intracellular adenosine concentration forming AMP. Since ADA has lower affinity ($K_m$ ~20 μmol/L) for this nucleoside compared with adenosine kinase ($K_m$ ~2 μmol/L) (de Fazio et al., 1980; Masino and Boison, 2013; Tavenier et al., 1995), inosine formation from adenosine via ADA is not a preferential, but adenosine phosphorylation is a preferential pathway to maintain physiological intracellular level of this nucleoside.

2.2. Plasma membrane nucleoside transporters

Extracellular and intracellular concentration of adenosine is also regulated by the capacity of cells to take up this nucleoside via plasma membrane transport mechanisms (Baldwin et al., 2004; Young, 2016). The most well described transport mechanisms include the Na$^+$–independent equilibrative (ENTs) and Na$^+$–dependent concentrative (CNTs) nucleoside transporters (Young, 2016). At least two ENTs isoforms mediate adenosine transport across the plasma membrane, i.e., ENT1 and ENT2, thus regulating extracellular and intracellular adenosine concentration in mammalian cells. Transport activity and its contribution to this

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**Fig. 2. Adenosine synthesis and catabolism.** The metabolic activity of the cells (Intracellular metabolism) generates ATP, which is then exported to the extracellular space via different mechanisms including the possibility of hemichannels (Hc). Extracellular ATP concentration is increased due to the release from different phenomena in cells and tissues including platelet aggregation, neurotransmission, vascular shear-stress, and from damaged cells. ATP is converted to ADP via the ecto-NTPDase-2 (CD39L1) and to AMP via the ecto-NTase-1 (CD73) activity. AMP is also generated via the activity of the adenosine kinase (AdK). AMP is then converted into adenosine (Adenosine) via the ecto-5'-nucleotidase (CD73) activity. Adenosine extracellular level is also maintained by a potential direct release of this nucleoside from tissues and cells to the extracellular space. Adenosine removal from the extracellular space results from its conversion to inosine via ecto-adenosine deaminases (ADA) and the uptake mediated by nucleoside transporter (NTs) at the plasma membrane. Once adenosine is in the intracellular space it is phosphorylated to generate AMP via adenosine kinase (AK), which is then hydrolysed by AMP deaminase (AMPD) generating inosine monophosphate (IMP). Adenosine is also metabolized to inosine by intracellular ADA. An increase in the intracellular level of adenosine also results from the hydrolysis of S-adenosyl-$\cdot$homocysteine (SAH) via the activity of SAH hydrodrolase (SAH) to generate $\cdot$homocysteine ($\cdot$Hcy). Additionally, the activity of cytosolic 5'-nucleotidases (c5'NT) generates adenosine from AMP. The increase of adenosine in the extracellular space could leads to activation of adenosine receptors (ARs) to trigger signalling mechanisms increasing the synthesis, release, or activity of cyclic AMP (cAMP), nitric oxide (NO), phosphatidylinositol 3 kinase (PI3K), protein kinase $\beta$ (Akt), endothelin 1 (ET-1), tromboxanes (TXA), ATP-activated $K^+$ channels ($K_Ar$). However, the precise role of these molecules in the synthesis and catabolism of adenosine is not well described (7). Light blue arrows show reactions to increase adenosine formation. Red arrows show reactions to decrease adenosine formation. Composed from references addressed in the text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
2.3. Adenosine receptors

Several excellent and detailed reviews addressing the biochemistry, and biophysics and functionality of ARs are currently available (Eltzschig, 2009; Burnstock et al., 2010; Fredholm et al., 2011; Fredholm, 2014; Gutiérrez-de-Terán et al., 2016; Guo et al., 2016). Biological effects of adenosine are mediated by activation of ARs coupled either to G inhibitory (Gi) protein for adenosine receptor A2A (A2AAR) and A2B (A2BAR) subtypes, or stimulatory (Gs) protein for adenosine receptor A1 (A1AR) and 3 (A3AR) subtypes. These ARs present with different affinities for adenosine being in the range of ~100–310 nmol/L for A1AR, A2AAR, and A2AR, but in the range of ~5000 μmol/L for A2BAR. A1AR is ubiquitously expressed throughout the body, coupled to Gi/o-dependent signals inhibiting AC activity, activating K⁺, but inhibiting Ca²⁺ channels. A1AR activation also increases Ca²⁺ mobilization via a pertussis toxin-sensitive, G protein βγ subunit dependent mechanism by activating phospholipase Cβ (PLCβ) (Dickenson and Hill, 1998; Borea et al., 2015; Eltzschig, 2009; Fredholm et al., 2011; Lindorfer et al., 1998). A2AAR activates Gs and Gαolf (olfactory G protein, first identified in the olfactory epithelium) proteins (Herve et al., 1993) increasing cAMP generation and protein kinase A (PKA) activity, and is mainly associated with NO-dependent vasodilation. A2BAR is coupled to Gq protein, activates mitogen-activated protein kinases (MAPKs) (Linden et al., 1998), and is involved in NO-dependent vasodilation. The Gi protein coupled-A2AR reduces AC activity and is deamino-lysylating making this ARs subtype susceptible to desensitization (Gao et al., 1999; Palmer et al., 1996).

3. Insulin

3.1. Synthesis and release of insulin

Insulin is the major controller of glucose homeostasis and other functions in the human body (Fig. 3). It is an endocrine peptidic hormone synthesized and secreted by pancreatic β-cells. Human insulin is a 51-amino acid residues structure containing two peptide chains (A and B) joined by disulphide bonds (Fu et al., 2013; Rorsman and Braun, 2013). Insulin release by pancreatic β-cells results in response to high extracellular concentration of glucose (Rorsman and Braun, 2013; Schmitz et al., 2008; Suckale and Solimena, 2008), glutamine, and leucine (Giddings et al., 1982), and high intracellular level of cAMP (Knoch et al., 2006). Since glucose–induced insulin secretion depends on the uptake and degradation of glucose in the pancreatic β-cells (Rorsman and Braun, 2013), insulin release from these cells relies on the availability of glucose from the vasculature surrounding the pancreatic islets (Fu et al., 2013; Suckale and Solimena, 2008).

3.2. Insulin receptors

Insulin activates receptors of insulin (IRs) at the plasma membrane (Baumgard et al., 2016; Westermeier et al., 2016; Zaykov et al., 2016; De Meyts, 2016). Insulin signalling occurs by activation of at least two isoforms of IRs, i.e., insulin receptor A (IR-A) and B (IR-B) (Westermeier et al., 2016). Physiological plasma level of insulin activates IR-A ending in a preferential activation of p44 and p42 MAPKs (p42/44mapk) rather than protein kinase B (Akt) (i.e., activated p42/44mapk/activated Akt > 1), a phenomenon referred as mitogenic phenotype (Westermeier et al., 2015, 2016). However, preferential activation of IR-B results in a ratio for activated p42/44mapk/activated Akt < 1 that is referred as metabolic phenotype (Westermeier et al., 2015, 2016). A differential mRNA expression of IR-A and IR-B, as well as their associated signalling mechanisms, is reported in HUVECs and hMECs from GDM pregnancies compared with cells from normal pregnancies (Guzmán-Gutiérrez et al., 2016; Salomón et al., 2012; Westermeier et al., 2011, 2015). Thus, target cells will respond to insulin depending on the IRs type that is available at the plasma membrane in the human foetoplacental vascular endothelium (Sobrevia et al., 2016). Insulin shows two surfaces contact sites composed of hormone dimerizing (contact surface 1) or hormone-hexamerizing (contact surface 2) residues. These contact surfaces are thought to interact with IRs contact sites 1 and 2, respectively (De Meyts, 2004; De Meyts et al., 2016). A dynamics between the exposure of insulin surfaces and their binding kinetics to IRs contact sites could be determinant in the responsiveness of cells to insulin. Whether this is happening for IR-A and IR-B types is not yet reported. However, this phenomenon could be determinant in diseases where cells are less responsive to this hormone such as in insulin-resistant associated diseases including diabetes mellitus and obesity, or where insulin binding could be under modulation by other factors, including adenosine (Guzmán-Gutiérrez et al., 2012, 2016; Sobrevia et al., 2016).

4. Vascular effects of adenosine

4.1. Vasodilation

Readers are guide to review these initial findings and recent
excellent original studies and reviews on adenosine vascular action (Berne, 1963; Ballard, 2014; Collis, 1989; Kaufmann et al., 2007; Sobrevia et al., 2016). Since approximately 90 years from now adenosine was reported to cause vasodilation in humans and animal experimental models (see Collis, 1989). Adenosine caused dilation of human pial arteries in vitro, a phenomenon that likely depended on the nature of the vessel since this nucleoside did not alter extracranial arteries tone (Hardebo and Edvinsson, 1979). Studies performed in coronary vessels in dogs show increased blood flow in response to intravenous injection of adenosine (Holtz et al., 1983). Similar findings were reported in studies where adenosine was infused in patients undergoing cerebral aneurysm surgery causing hypotension due to a decrease in the peripheral arterial resistance with a parallel increase in the plasma adenosine concentration from 0.15 to 2.5 μmol/L (Sollevi et al., 1984). In the latter study, the use of dipyridamole, a general inhibitor of adenosine uptake (Young, 2016), caused a pronounced vasodilation in response to adenosine likely due to reduced removal of extracellular adenosine, thus leading to higher concentrations activating the relevant ARs. Dipyridamole was also shown to potentiate (2–5 fold) the adenosine-increased forearm blood flow increase in normal human subjects (Gamboa et al., 2005). These studies are demonstrations of the dynamics between adenosine uptake and ARs activation by adenosine in the human vasculature.

4.1.1. Role of nitric oxide on adenosine effect

Vascular endothelial cells exposed to adenosine respond with an increase in the activity of endothelial NOS (eNOS) and synthesis of NO (Vásquez et al., 2004; Guzmán-Gutiérrez et al., 2012, 2016), a phenomenon seen in several endothelial cell types and tissues (El-Gowelli et al., 2013; Rubio and Ceballos, 2003; Sobrevia et al., 2015, 2016) (see Table 1). Adenosine is used to estimate coronary flow reserve to adenosine in normal subjects and in patients with coronary artery disease (Kaufmann et al., 2007). However, studies in patients are restricted to the systemic use of general NOS inhibitors. NO-dependent vasodilation caused by adenosine is shown to result from activation of A2AR leading to increased cAMP in hPMECs (Escudero et al., 2008) and p44/42MAPK phosphorylation (i.e. activation) in HUVECs (Vásquez et al., 2004). This effect of adenosine was blocked by the A2AR antagonist ZM241385 and the sequence of signalling mechanisms involved was adenosine – A2AR activation – increased cAMP/PKA/ PKC – higher eNOS expression and activity – higher NO level – p44/42MAPK activation. This signalling pathway ended in increased expression of Slc7a1 gene (for human cationic amino acid transporter 1 (hCAT-1)) and hCAT-1 mediated l-arginine transport (San Martín and Sobrevia, 2006; Sobrevia et al., 2015, 2016). Activation of ARs causing increased NO synthesis and l-arginine transport was referred as ALANO (standing for Adenosine/L-Arginine/NO) signalling pathway in HUVECs (Pandolfi and Di Pietro, 2010; San Martín and Sobrevia, 2006; Vásquez et al., 2004). Thus, activation of A2AR and A2BAR leads to vasodilation dependent on NO synthesis and other mechanisms involving increased cAMP synthesis and PKA activation in the human foeto-placental vasculature (Fig. 4). It is also reported that adenosine could cause a NO-independent vasodilation in several organs and vascular beds, including human forearm skeletal muscle (Casey and Joyner, 1985), human resistance vessels (Gamboa et al., 2005), and kidney circulation in hypertensive patients (Wierema et al., 2005). However, ARs subtype and associated signalling mechanisms involved in this response to adenosine is unclear. On the other hand, there is little

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<tr>
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<td>Ren et al., 2001</td>
</tr>
<tr>
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<td>JXM afferent arteriole</td>
<td>Constriction</td>
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<td>Gutierrez et al., 1999</td>
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</tbody>
</table>

HUVECs, human umbilical vein endothelial cells; BMDCs, bone marrow-derived endothelial cells; IOPÉ, late-onset preeclampsia; JXM, juxtaglomerular; STZ, streptozotocin; hCAT-1, human cationic amino acids transporter 1; NO, nitric oxide; A<sub>1</sub>AR, A<sub>1</sub> adenosine receptor subtype; A<sub>2</sub>AR, A<sub>2</sub> adenosine receptor subtype; A<sub>2</sub>BAR, A<sub>2</sub>B adenosine receptor subtype; A<sub>3</sub>AR, A<sub>3</sub> adenosine receptor subtype; Ca<sup>2+</sup>, calcium; K<sub>ATP</sub>, ATP activated K<sup>+</sup> channels; PG<sub>2</sub>, prostaglandin I<sub>2</sub>; cAMP, cyclic AMP.
evidence that A3AR is involved in blood pressure changes (Nishat et al., 2016; Tabrizchi and Bedi, 2001). A role of A3AR as vasodilator was shown in rat coronary vessels (Ho et al., 2016; Jenner and Rose’Meyer, 2006), a phenomenon that is likely mediated by activation of PKC and ATP-activated K channels (KATP) channels in vascular smooth cells (Jenner and Rose’Meyer, 2006; Wan et al., 2008; Zucchi et al., 2001). Additionally, the A3ARi splice variant of A3AR detected in rat hearts was proposed to contribute to the coronary vasodilation in these animals (Jenner and Rose’Meyer, 2006).

4.1.2. Role of oxidative stress on adenosine effect

Oxidative stress is a condition that affects the vasculature where NADPH oxidase (Nox) activity plays crucial roles. Activation of Nox generates reactive oxygen species (ROS) in primary cultures of HUVECs incubated with high extracellular D-glucose (Gonzalez et al., 2015; Taye et al., 2010). The main ROS species generated under this environmental condition (~80%) was superoxide anion (O2•-). The increase in O2•- generation was a phenomenon associated with higher hCAT-1–mediated L-arginine transport in this fetoplacental endothelium (Gonzalez et al., 2015). Recent studies also proposed that Nox generates hydrogen peroxide (H2O2) in this cell type (Cabrera et al., 2016). H2O2 causes vasodilation in mice cerebral arteries (Gebremedhin et al., 2010) likely via a mechanism that was independent of A1AR, A3AR, or A2BAR activation (El-Awady et al., 2013; Zhou et al., 2015), but dependent on A2AAR activation (Zhou et al., 2015). Thus, ROS-dependent vasodilation caused by adenosine is highly specific for this type of ARs. The role of A2AAR activation in vascular reactivity and the involvement of ROS in this phenomenon are also suggested from studies in A2AAR knockout mouse (Shariﬁ-Sanjani et al., 2013). Adenosine–caused coronary reactive hyperemia requiring A2AAR activation resulted from higher H2O2 generation leading to activation of KATP channels in the vascular smooth muscle (Brayden, 2002; Murphy and Brayden, 1995).

The dependency of ARs (particularly A1AR, A2AAR, and A2BAR) on the generation of ROS has also been suggested in studies where the use of an A1AR and A2AR non-specific antagonist caused hypotension in rats (Sousa et al., 2008). The authors concluded that antagonizing these ARs result in increased Nox and generation of...
hydrogen peroxide (H₂O₂) from the O₂⁻. Thus, activation of A₁AR, A₂AAR, and A₂BAR will maintain a normotensive vascular tone by keeping low the Nox-generated O₂⁻ in these animals.

Interestingly, adenosine-increased rat coronary blood flow involves A₂AAR activation requires p44/42mapk phosphorylation (El-Awady et al., 2013). Since exposure of HUVECs from normal pregnancies to high extracellular β-glucose result in higher p44/42mapk phosphorylation (Montecinos et al., 2000; González et al., 2015) and increased extracellular concentration of adenosine (Vázquez et al., 2004), it is likely that A₂BAR activation by adenosine leading to increased l-arginine transport and NO synthesis (San Martín and Sobrevia, 2006; Guzmán-Gutiérrez et al., 2012) may result from increased generation of ROS in this type of endothelium. In fact, supporting this possibility are the findings showing that high extracellular β-glucose causes an increase in the hCAT-1-dependent l-arginine transport in parallel with Nox-generated O₂⁻ in this cell type (González et al., 2015). Furthermore, β-glucose effect on l-arginine transport and p44/42mapk phosphorylation was blocked by the Nox-inhibitor apocynin and the O₂⁻ scavenger tempol in HUVECs.

It is reported that β-adrenergic preconditioning in rat hearts was dependent on A₁AR activation and mediated by ROS generation involving activation of p44/42mapk and Akt (Salie et al., 2012). These findings complement those suggesting that activation of A₁AR with specific agonists results in ROS generation leading to cell death in a cell line of human glioma cells via a similar signalling mechanism (Kim et al., 2012). However, the involvement of A₁AR activation in cancer cells is still controversial since reports in AT6.1 rat prostate cancer cells show that A₁AR-activation dependent reduced proliferation and metastasis result from inhibition of Nox and p44/42mapk activity (Jajo et al., 2009). Thus, A₁AR involvement in the response of cancer cells due to changes in Nox-generated ROS will depend on the type of cancer. In addition, these findings could reflect a response in cancer cells rather than in non-cancer cells since A₁AR are not involved in the modulation of l-arginine transport and NO synthesis in HUVECs (Guzmán-Gutiérrez et al., 2012, 2016; Salsoso et al., 2015).

4.1.3. Role of K⁺ channels on adenosine effect

Assays in human coronary arteries under a pharmacological appraoch suggest that intermediate-conductance calcium-activated potassium (I_KCa) channels were involved in the response of this type of vascular smooth muscle to adenosine (Sato et al., 2005). Activation of I_KCa channels leads to plasma membrane hyperpolarization, probably due to activation of A₂AAR, A₂BAR, or both, and perhaps a parallel depolarization of the plasma membrane via activation of A₁AR. K_ATP channels may also be involved in the response of vascular smooth muscle to activation of ARs receptors (Brayden, 2002; Murphy and Brayden, 1995). Increased NO synthesis associates with K_ATP activation leading to plasma membrane hyperpolarization in primary cultures of HUVECs from normal pregnancies exposed to elevated extracellular concentrations of β-glucose (25 mmol/L for 24 h) (Flores et al., 2003). Activation of K_ATP channels with glibenclamide (a general K⁺ channels activator) also increased the maximal transport capacity (defined as the ratio between V_max/K_M for transport kinetics) (Devis and Boyd, 1998; Mann et al., 2003) of l-arginine transport in this cell type. The latter suggests a connection between the membrane potential sensitive transport of the cationic amino l-arginine, K_ATP, and NOS activity in this type of human foetal endothelium. Interestingly, since high β-glucose also increased the extracellular accumulation of adenosine in this cell type in vitro reaching ~1.5 μmol/L (Muñoz et al., 2006), and increased p42/44mapk phosphorylation (González et al., 2015; Montecinos et al., 2000) and expression of hCAT-1 isoform (González et al., 2011, 2015), ARs activation was likely mediating these effects of extracellular β-glucose in HUVECs. Considering that A₂AAR and A₂BAR signal through increased NO synthesis and all ARs subtypes signal increasing p42/44mapk and PKA activation (San Martín and Sobrevia, 2006), any of these receptors could be responsible for high β-glucose effect in HUVECs. More recently, it was shown that adenosine and nitrobenzylthioinosine (NBtI)-increased extracellular adenosine result in stimulation of l-arginine transport and the transcriptional activity of SLC7A1 coding for hCAT-1 in HUVECs (Guzmán-Gutiérrez et al., 2016).

4.2. Vasoconstriction

Adenosine also causes vasoconstriction in several vascular beds including the human placenta (Donoso et al., 2005), human and animal kidney (Bell and Welch, 2009; Hansen and Schmermann, 2003; Marraccini et al., 1996; Nishiyama et al., 2001; Persson and Carlström, 2015; Vallon et al., 2008), sheep lung (Biaggioni et al., 1989), and human lung (Saadjian et al., 1999) (see Fig. 4). Adenosine infusion causes constriction in dog kidney afferent and efferent arteriole via activation of A₁AR, a finding less pronounced when higher doses of this nucleoside were used (Nishiyama et al., 2001). Thus, under conditions where all the ARs subtypes are activated by adenosine concentrations overcoming their Kᵢ for this nucleoside, a vasodilator effect mediated by activation of A₂BAR and A₂BAR could mask a vasoconstrictor effect by A₁AR activation. Adenosine was thought to cause partial endothelium-dependent vasoconstriction via A₂BAR activation in human choriocarcinoma cells and veins, a response that also seems mediated by the release of thromboxane rather than the expected vasodilator effect of the cAMP-classical activation cell signalling mediated by the activation of these ARs (Donoso et al., 2005). However, since an A₁AR agonist also caused vasoconstriction it is likely that this type of ARs subtype is involved in the response to adenosine in these human placenta vessels. Additionally, the vasoconstriction caused by adenosine in endothelium-denuded vessels was partially reduced. Thus, vascular smooth muscle is likely to play a role in the response of these vessels to adenosine.

The role of A₁AR in vasoconstriction is scarcely known. In a recent study in A₁AR knockout mice subjected to nephrectomy and a diet high in salt did not develop hypertension (Yang et al., 2016). Thus, it is likely that this subtype of ARs is also involved in causing vasoconstriction. Since A₁AR activation also leads to inhibition of adenylyl cyclase activity, thus lowering cAMP level (Fredholm, 2014), it is likely that hypertension could result from reduced cAMP-signalling associated mechanisms. The cell signalling mechanisms resulting from A₁AR activation to cause vasoconstriction in the human vasculature is not available, and stays as a future research field to develop.

5. Vascular effect of insulin

5.1. Vasodilation

As mentioned at very early times peripheral vasodilation was described in human subjects that received insulin (Abramson et al., 1939). It was initially believed that insulin causes a decrease in vascular resistances as a consequence of this hormone’s induced systemic hypoglycaemia. However, insulin in a dose that is not causing hypoglycaemia increased the forearm blood flow and reduced the forearm vascular resistance in human subjects (Creager et al., 1985). Further studies showed that insulin reduced the sympathetic-induced vasoconstriction in humans (Lembo et al., 1996), reinforcing its role as vasodilator or as modulator of the vascular response.
Vasodilation in human skeletal muscle caused by insulin intravenous injection (Steinberg et al., 1994) or in subjects with hyperinsulinemia (Vollenweider et al., 1993) is a NO-dependent phenomenon (Fig. 5). Insulin causes vasodilation in at least two steps, i.e., first causing a rapid (lasting few minutes) dilation of terminal arterioles with no changes in the capillary blood flow, but requiring capillary recruitment (increase in the number of perfused capillaries), and a second step (lasting several minutes to hours) that comprises dilation of larger resistance vessels resulting in increased capillary blood flow (Muniyappa et al., 2007). Since NO generation in response to insulin is rather a rapid (few minutes) mechanism in the human microvasculature and macrovasculature (Sobrevia et al., 2015), NO-mediated signalling for insulin effect is a first response, which is followed by activation of NO-dependent secondary associated mechanisms. Indeed, in isolated human umbilical vein rings from normal pregnancies insulin causes rapid (2–3 min) endothelium-derived, NO-dependent dilation, NO-dependent dilation requiring p44/p42MAPK and PKB/Akt activity (Guzmán-Gutiérrez et al., 2012, 2016). Since the latter was measured in vessels rings mounted a wire myograph, the relevance of these findings is of importance, but they must be taken with caution since the setup in vitro is clearly far from observations described for systemic vasodilator effect of insulin. However, in healthy young adults insulin infusion in the legs caused an increase in blood flow and capillary recruitment, an effect that was suggested to be dependent on endothelium activation since L-NMMA blocked insulin vasodilation (Timmerman et al., 2010). Interestingly, when insulin was infused together with L-NMMA activation of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1), which is promoting translation initiation and accelerating muscle protein synthesis (Proud, 2002), was reduced. Thus, NO (likely derived from the endothelium in response to insulin) could sustain mTORC1 activation in humans to cause vasodilation. The results agree with findings in normal subjects where insulin was administered into the brachial artery (van Veen and Chang, 1997). The results suggest that insulin caused a reduction in the forearm vascular resistance that was dependent on NO synthesis since it was inhibited by L-NMMA, and was independent of locally released prostaglandins since the cyclooxygenase inhibitor indomethacin did not alter the vasodilation caused by insulin. Thus, most of the studies addressing vasodilation caused by insulin regards with the generation of NO from the vascular bed studied. The potential source of NO in these assays in unclear since inhibitors of NOS activity act indistinctly on the vascular endothelium and vascular smooth muscle.

Assays in vitro using vascular endothelial and smooth muscle cells show that the response of these cell types to insulin includes increased hCAT-1-mediated L-arginine transport and expression and increased NO synthesis (Table 2). This phenomenon results from IR-A activation by insulin triggering of ARs-dependent ALANO signalling pathway due to the extracellular accumulation of adenosine as a consequence of reduced hENT1/hENT2-mediated adenosine transport (Guzmán-Gutiérrez et al., 2012, 2016; Sobrevia et al., 2015, 2016).
### Table 2

Biological effects of insulin in health and disease.

<table>
<thead>
<tr>
<th>Specie (pathology)</th>
<th>Cell type</th>
<th>Biological effect of insulin</th>
<th>Proposed mechanisms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>HUVECs</td>
<td>Increases VCAM-1 expression</td>
<td>Activation of p38&lt;sup&gt;max&lt;/sup&gt; and PI3K inhibition</td>
<td>Madonna et al., 2004</td>
</tr>
<tr>
<td>Human</td>
<td>HUVECs</td>
<td>Increases NO synthesis</td>
<td>Activation of PI3K</td>
<td>Zeng and Quon, 1996</td>
</tr>
<tr>
<td>Human</td>
<td>HUVECs</td>
<td>Increases hCAT-1 and hCAT-2 mRNA expression</td>
<td>Activation of PI3K/PI3K/IRS-1 pathways</td>
<td>González et al., 2004</td>
</tr>
<tr>
<td>Human</td>
<td>HUVECs</td>
<td>Membrane hyperpolarization</td>
<td>NO-dependent activation of K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>González et al., 2004</td>
</tr>
<tr>
<td>Human</td>
<td>HUVECs</td>
<td>Increases α-arginine transport, and hCAT-1 expression</td>
<td>Reduced adenosine uptake leading to adenosine extracellular accumulation and A&lt;sub&gt;2A&lt;/sub&gt;AR activation resulting in higher SLC7A1 promoter activity</td>
<td>Guzmán-Gutiérrez et al., 2012</td>
</tr>
<tr>
<td>Human (GDM)</td>
<td>hPMECs</td>
<td>Restores GDM-reduced adenosine transport</td>
<td>Restoration of GDM-altered IR-A and IR-B expression and p42/44&lt;sub&gt;max&lt;/sub&gt;/Akt ratio to normalize hENT2 expression and activity</td>
<td>Salomón et al., 2012</td>
</tr>
<tr>
<td>Human (LOPE)</td>
<td>HUVECs</td>
<td>Reverses LOPE-increased α-arginine transport</td>
<td>Reverses LOPE-increased hCAT-1 expression and transport</td>
<td>Salsoso et al., 2015</td>
</tr>
<tr>
<td>Human (diabetic retinopathy)</td>
<td>HMECs</td>
<td>Stimulates growth and tube formation</td>
<td>Increased insulin receptors tyrosine phosphorylation to activate NF-κB- and VEGF mRNA upregulation</td>
<td>Yamagishi et al., 1999</td>
</tr>
<tr>
<td>Human</td>
<td>hAVSMCs</td>
<td>Activates vascular endothelial growth factor in vascular smooth muscle cells</td>
<td>Increased VEGF protein expression and secretion</td>
<td>Doronzio et al., 2004</td>
</tr>
<tr>
<td>Human</td>
<td>hAVSMCs</td>
<td>Activates HIF-1α</td>
<td>Activation of PI3K/Akt and MAPKs activation pathways</td>
<td>Doronzio et al., 2006</td>
</tr>
<tr>
<td>Human (GDM)</td>
<td>HUVECs</td>
<td>Reverses GDM-increased α-arginine transport</td>
<td>Reversed GDM-increased hCAT-1 transport activity</td>
<td>Guzmán-Gutiérrez et al., 2016</td>
</tr>
<tr>
<td>Human (GDM)</td>
<td>HUVECs</td>
<td>Restores GDM-reduced SLC7A1 expression and hENT1-mediated transport</td>
<td>Normalization of IR-A mRNA expression and hENT1-mediated transport activity</td>
<td>Westermeier et al., 2015</td>
</tr>
<tr>
<td>Human (GDM)</td>
<td>fpECs</td>
<td>Increases MT1-MMP generation.</td>
<td>Activation of PI3K/Akt signalling activation</td>
<td>Hiden et al., 2012</td>
</tr>
<tr>
<td>Human</td>
<td>HAECs</td>
<td>Increases α-arginine transport</td>
<td>Activation of PI3K/Akt signalling activation</td>
<td>Kohlihas et al., 2011</td>
</tr>
<tr>
<td>Human</td>
<td>HUVECs</td>
<td>Prevents apoptosis</td>
<td>Increased basal Akt activity and reduced TNF-α-induced Akt phosphorylation</td>
<td>Hermann et al., 2000</td>
</tr>
<tr>
<td>Bovine</td>
<td>bAVSMCs</td>
<td>Activates eNOS</td>
<td>Activation of MAPKs signalling pathway</td>
<td>Wang et al., 2003</td>
</tr>
<tr>
<td>Bovine</td>
<td>BAEcs</td>
<td>Promotes VSMCs migration</td>
<td>Activation of MAPKs signalling pathway</td>
<td>Takahashi and Mendelsohn, 2003</td>
</tr>
<tr>
<td>Mouse (atherosclerosis)</td>
<td>BAEcs</td>
<td>Increases generation and release of ET-1</td>
<td>Requires kinase activity</td>
<td>Hu et al., 1993</td>
</tr>
<tr>
<td>Mouse</td>
<td>mAVSMCs</td>
<td>Increases cell proliferation</td>
<td>Activation of IR-A and IGF-IR—increased TNF-α</td>
<td>Gómez-Hernández et al., 2013</td>
</tr>
<tr>
<td>Mouse</td>
<td>mAVSMCs</td>
<td>Increases VEGF expression</td>
<td>Requires IRS-1/Pi3K/Akt signalling cascade and Ras-MAPKs signalling pathways</td>
<td>Jiang et al., 2003</td>
</tr>
<tr>
<td>Rat</td>
<td>rAVSMCs</td>
<td>Relaxation</td>
<td>Reduces intracellular Ca&lt;sup&gt;2+&lt;/sup&gt; and reactivity to vasoconstrictors</td>
<td>Han et al., 1995</td>
</tr>
<tr>
<td>Rat</td>
<td>rAVSMCs</td>
<td>Inhibits cell migration</td>
<td>Requires NO/cGMP/cGK-I induction of MKP-1 and MAPKs inactivation</td>
<td>Jacob et al., 2002</td>
</tr>
<tr>
<td>Rat (hypertension)</td>
<td>rAVSMCs</td>
<td>Alters MKP-1 activity and increases MKP-1 expression</td>
<td>Increased Pi3K-induced iNOS and cGMP generation</td>
<td>Begum et al., 1998</td>
</tr>
<tr>
<td>Rat (T2DM)</td>
<td>rAVSMCs</td>
<td>Prevents apoptosis</td>
<td>Activation of PI3K and PKB/Akt</td>
<td>Nakazawa et al., 2005</td>
</tr>
</tbody>
</table>

HUVECs, human umbilical vein endothelial cells; hPMECs, human placental microvascular endothelial cells; HMECs, human microvascular endothelial cells; bAVSMCs, human aorta vascular smooth muscle cells; fpECs, fetal-placental endothelial cells; HAECs, human aortic endothelial cells; bBAECs, bovine aortic endothelial cells; mAVSMCs, mouse aorta VSMCs; rAVSMCs, rat aorta VSMCs; GDM, gestational diabetes mellitus; TIDM, type 1 diabetes mellitus; hENT1, human equilibrative nucleoside transporter 1; hENT2, human equilibrative nucleoside transporter 2; hCAT-1, human cationic amino acid transporter 1; hENT1, human equilibrative nucleoside transporter 1; hENT2, human cationic amino acid transporter 2; hCAT-1, human cationic amino acid transporter 1; hCAT-2, human cationic amino acid transporter 2A/B; NO, nitric oxide; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; IRS-1, insulin receptor substrate 1; SLC7A1, solute like carrier 7A1 gene; Akt, protein kinase B; AP-1, activator protein 1; cGK-I, cGMP-dependent protein kinase; cGMP, cyclic guanosine monophosphate; ERKs, extracellular signal-regulated kinases; ET-1, endothelin 1; HIF-1α, hypoxia-inducible factor-1α; HSP90, heat shock protein 90; TNF-α, tumour necrosis factor α; UCP-2, uncoupling protein 2; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.
5.2. Vasoconstriction

Insulin causes vasoconstriction via mechanisms involving activation of the sympathetic nervous system (Fig. 5), a phenomenon that is proposed to oppose to NO-mediated vasodilation caused by this hormone. Additionally, endothelin release from the endothelial cells is a mechanism that also mediates vasoconstriction. Excellent and detailed reviews describing this phenomenon are available (see Muniyappa et al., 2007; Muniyappa and Yavuz, 2013; Sartori and Scherrer, 1999).

Insulin increases the catecholamine levels and sympathetic activity in doses that caused massive fall in plasma D-glucose concentration (Anderson et al., 1991; Lembo et al., 1992; Rowe et al., 1981). Interestingly, a more efficient NO-dependent vasodilation in response to insulin was reported in patients undergoing sympatheticctomy (Sartori et al., 1999), suggesting the possibility that a mechanism other than insulin-induced vasodilation that was independent of NO was functional in humans. It was shown that β-adrenergic or cholinergic signals may not be involved in the vaso-dilator actions of insulin to increase calf blood flow in human (Randin et al., 1994). However, this is uncertain since involvement of these modulatory mechanisms of blood flow in humans is still controversial (Muniyappa et al., 2007; Muniyappa and Sowers, 2013). Indeed, insulin causes dilation of distal arterioles, but constriction of proximal arterioles, thus making clear that different mechanisms will result from insulin action in a same or different vascular bed. Interestingly, insulin activates MAPKs and PI3K in rat hypothalamus (Rahmouni et al., 2004). Since this effect was differential in several regions of the hypothalamus, and because MAPKs and PI3K signalling pathways are preferentially activated by IR-A and IR-B, respectively (Westemeier et al., 2016), it is likely that insulin via differential activation of these IRS subtypes will result in the control of the vascular tone starting with sympathetic activation at the central nervous system. The general accepted proposal is that insulin vasoconstriction due to sympathetic activation is masked and overpassed by the dilatory effect of this hormone. However, in obesity and hypertension, insulin effect is favoured in the sense of a sympathetic pressor action (Grassi et al., 2015; Laakso et al., 1990; Lembo et al., 1992). It is now clearer that other pathologies or conditions associated with defects in insulin signalling, such as GDM (Westermeier et al., 2016; Guzmán-Gutiérrez et al., 2016), preeclampsia (Salsoso et al., 2015; Mate et al., 2012), or hyperglycaemia (De Nigris et al., 2015; Florez et al., 2003; Vásquez et al., 2004), show with lower triggering of cell signalling mechanisms including those mediated by NO, p42/44MAPK, Akt, and PI3K, in the human endothelium (Leiva et al., 2016). Whether these mechanisms at the endothelial cell level are in parallel with a central sympathetic control of the vascular tone is a phenomenon not fully uncovered (Grassi et al., 2015; Leiva et al., 2016; Sobrevia et al., 2015, 2016).

Insulin also increases the synthesis and release of the vasoconstrictor endothelin-1 (ET-1) at the vascular endothelium (Hu et al., 1993; Mahmoud et al., 2016; Oliver et al., 1991; Younk et al., 2014). Additionally, hyperinsulinemia increases ET-1 synthesis and release resulting in reduced vasodilation in human skeletal muscle arterioles (Mahmoud et al., 2016). Thus, the insulin resistance or a less responsiveness of the vasculature to insulin results in this phenomenon, or alternatively increased vasoconstriction. ET-1 increases blood pressure depending on its circulatory concentration, a response proposed to counteract the insulin vasodilator effect in humans (Cardillo et al., 1999). Indeed, insulin increases the expression of ET-1 mRNA in the endothelium (Hu et al., 1993) suggesting a potential long lasting, and not only a rapid, local effect of insulin in this type of cells. ET-1 acts in the endothelium to activate either endothelin receptor A (ET_{A}) or B (ET_{B}), both of which are expressed in these cells. ET_{B} activation by ET-1 leads to increased synthesis and release of endothelial derived relaxing factors (EDRFs) resulting in relaxation of vascular smooth muscle cells and subsequent vasodilation. However, ET_{A} activation results in vasoconstriction due to the release of endothelial derived contracting factors (EDCFs). A general agreement is that endothelial cells will also release cyclooxygenase-derived vasoconstrictor prostaglandins, thus contributing to other molecules-induced contraction of blood vessels (for informative reviews see Younk et al., 2014; Vanhoutte et al., 2016).

6. Insulin and adenosine signalling are interdependent

Tonic adenosine action, probably via A1AR, facilitates insulin-dependent D-glucose transport in rat soleus muscle (Thong et al., 2007). Since the response of this tissue to insulin was reduced in ~50% when extracellular adenosine was removed by ADA or α,β-methylene adenosine diphosphate (AOPCP, which inhibits the extracellular membrane-bound 5’-ectonucleotidase for conversion of AMP to adenosine) a large component of the insulin stimulation of 3-O-methyl-D-glucose uptake is likely to depend on extracellular adenosine. This phenomenon may results from altered protein abundance of GLUT4 at the plasma membrane as shown in rat epitrochlears and soleus muscle in response to insulin (Han et al., 1998). Since expression, plasma membrane availability, and activity of GLUT4 are regarded as essential in diseases coursing with insulin resistance, adenosine and activation of ARs form part of the scenario of a lower tissue response to insulin or insulin resistance. However, the potential beneficial effects of adenosine and activation of ARs on insulin biological effects is variable. For example, in terms of regulation of D-glucose transport and transporters (expression and activity) some studies show no effect of adenosine (Vergauwen et al., 1994), others show an increase (Han et al., 1998; Law et al., 1988) or a decrease (Budohoski et al., 1984; Challiss et al., 1992) in response to insulin. Thus, nothing is still definitive regarding a potential adenosine modulation of insulin action on D-glucose uptake and expression of GLUT4 in human tissues.

Studies in vitro using primary cultures of HUVECs from normal pregnancies reported that insulin-increased l-arginine transport was blocked in the presence of A_{2A}AR antagonists (Guzmán-Gutiérrez et al., 2012, 2016). In addition, in the absence of insulin l-arginine transport was higher following activation of A_{2A}AR, but not A_{2B}AR. The mechanisms involved in the insulin or adenosine-increased l-arginine transport seems to be highly specific for hCAT-1 compared with hCAT-2B in terms of increasing its maximal transport activity. This phenomenon was also seen for the promoter transcriptional activity of SLC7A1 (for hCTA-1), but not SLC7A2 (for hCAT-2A/B) expression. The regulatory region at these promoters was delimited between the ~600 bp from the potential starting transcriptional point in these genes (González et al., 2011). Thus, upregulation of l-arginine transport is a phenomenon that requires differential ARs subtype activation depending on the absence or presence of insulin. The latter is a phenomenon also reported for D-glucose transport in perfused rat hearts where, as for skeletal muscle, A_{1}AR activation was required (Angello et al., 1993; Wyatt et al., 1989). Thus, it is suggested that adenosine will activate not only the transport of amino acids, but also other crucial metabolic substrates by mechanisms that could involve differential ARs activation. In addition, different tissues will require different ARs subtype activation to modulate plasma membrane transport of these nutrients. Since ARs expression is differential in the body (Fredholm et al., 2011; Fredholm, 2014; Lu et al., 2004), a preferential requirement for a certain ARs subtype could results from preferential expression of these receptors. Interestingly, in HUVECs from GDM pregnancies insulin restores the hCAT-1-mediated
increase of L-arginine transport to values in cells from normal pregnancies requiring activation of A1AR instead of A2AAR (Guzmán-Gutiérrez et al., 2016). Thus, insulin biological actions are also selective for the ARs subtype depending on a physiological or pathophysiological state (see reviews, Antonioli et al., 2015; Sobrevia et al., 2016).

An early finding in rat adipocytes showed that adenosine could also be acting as a modulator of the kinetics of insulin actions (Ciaraldi, 1988). The insulin-increased uptake of 2-deoxyglucose was shown to be less effective following the removal of adenosine by ADA, an effect reflected in a higher EC50 value (~5 fold at 37 °C). These results agree with those for 3-O-methyl-D-glucose uptake in this cell type (Lönroth et al., 1988; Wong et al., 1984) and in rat cardiac myocytes (Shanahan et al., 1986). It is likely that following treatment of cells with ADA, a potential residual low concentration of adenosine could still be found and may be enough to stimulate A1AR or A2AAR since the $K_d$ for adenosine varies between 1 and 30 nmol/L for these ARs subtypes. Since addition of

![Fig. 6. Insulin and adenosine linked signalling in the regulation of vascular tone.](image)

Insulin

- Activation of IR-A/IR-B
  - $p44/44^{mapk}/Akt > 1$
  - $p44/44^{mapk}/Akt < 1$
- Increased NO synthesis
- Reduced ENT1/2 activity
- High extracellular adenosine
- $A_{2a}AR/A_{2b}AR$ activation
  - Higher cAMP
  - Higher prostaglandins
- $A_{1}AR/A_{3}AR$ activation
  - Lower cAMP
  - Higher thromboxanes
  - Higher prostaglandins
- CNS-activation of P13K
- CNS-activation of $p44/44^{mapk}$
- CNS-prostaglandins

Vasodilation

Vasoconstriction

Fig. 6. Insulin and adenosine linked signalling in the regulation of vascular tone. Insulin causes activation of insulin receptors A (IR-A) or B (IR-B) increasing the cell signalling mediated by $p44/42$ mitogen-activated protein kinases ($p44/42^{mapk}$) in a preferential manner compared with protein kinase B (Akt) activation ($p44/42^{mapk}/Akt > 1$) to cause vasodilation (Vasodilation). However, a ratio $p44/42^{mapk}/Akt < 1$ leads to vasoconstriction (Vasoconstriction). Activation of IR-A and IR-B increases the nitric oxide (NO) synthesis, which is known to reduce the expression of human equilibrative nucleoside transporters 1 and 2 (hENT1/2) activity in the vascular endothelium. This phenomenon leads to extracellular accumulation of adenosine (High extracellular adenosine) and activation of adenosine receptors subtypes $A_{2a}$, $A_{2b}$, $A_{1}$, or $A_{3}$ (Adenosine receptors). Activation of these adenosine receptors increase the response of vascular cells to insulin causing either vasodilation or vasoconstriction. Insulin vasodilation requires increased cyclic AMP (cAMP) level, activation of $p44/42^{mapk}$, and increased synthesis and release of vasodilator prostaglandins. Equally, activation of $A_{2a}AR$ and $A_{2b}AR$ could result in insulin-induced vasoconstriction involving activation of $p44/42^{mapk}$ and increased synthesis and release of vasoconstrictor prostaglandins. Accumulation of extracellular adenosine caused by insulin-increased NO bioavailability also results in activation of adenosine receptors subtypes $A_{1}$ ($A_{1}AR$) or $A_{2a}$ ($A_{2a}AR$). This phenomenon leads to lower cAMP level, but increased thromboxanes and vasodilator prostaglandins synthesis and release causing vasoconstriction. The role of adenosine receptors in the response of the central nervous system (CNS), particularly at the hypothalamus, is not addressed. However, since the cell signalling molecules include PI3K, $p44/42^{mapk}$, and prostaglandins, a role for adenosine receptors in the response to insulin by the SNC is expected. Composed from references addressed in the text and Tables 1 and 2.
dibutyryl cAMP (dbcAMP), but not ADA, reduced basal 2-deoxyglucose uptake (Ciardelli, 1988), and because A2AAR activation increases adenyl cyclase activity, it is feasible that exposure of rat adipocytes to ADA results in reduced adenosine concentration to values < 1 nmol/L (a possibility not addressed in these studies).

Adenosine also support an early signalling step of insulin action following insulin binding, likely the insulin receptor tyrosine kinase activity (Ciardelli, 1988). Thus, adenosine would not interfere with insulin binding to IRS, but will modulate its activity. Moreover, lack of action of adenosine on the insulin binding to its receptors is likely not to differentiate between the higher affininity contact site 1 and the lower affininity contact site 2 of the IRS (Menting et al., 2013).

Thus, adenosine will facilitate insulin-increased 2-deoxyglucose uptake via a mechanism other than altering the dynamics between the exposure of insulin surfaces and their binding kinetics to IRS in rat adipocytes. A similar conclusion is feasible for the results showing that A1AR activation is required to assure a normal sensitivity to insulin to increase 2-deoxyglucose uptake in rat adipocytes (Green, 1987). This phenomenon was not due to changes in the binding kinetics of insulin to its receptors, but Gi protein activation likely associated with A1AR activation are necessary in this phenomenon. Changes in the kinetics of insulin binding have not yet been described in humans, thus, we cannot rule out this possibility as an explanation for insulin resistance or lower responsiveness in diseases such as T1DM, T2DM (Antonioli et al., 2015), GDM (Antonioli et al., 2015; Guzmán-Gutiérrez et al., 2016; Sobrevia et al., 2015, 2016; Westermeier et al., 2011, 2015, 2016), obesity (Pardo et al., 2015), or preeclampsia (Mate et al., 2012; Salsoso et al., 2015).

It is extensively reported that adenosine already in concentrations below 10 μmol/L can depress insulin release induced by high intracellular D-glucose concentration in rat pancreatic β-cells (for example see Ismail et al., 1977; Szukdelski and Szukdelska, 2015). This phenomenon was shown to be due to adenosine activation of A1AR in this cell type since reduction in insulin release was blocked by the antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Zywert et al., 2011). A1AR mediate adenosine modulation of insulin release in response to D-glucose is well established in the literature for mouse and rat islets, but nothing is reported for human pancreatic islets. The involvement of adenosine and ARs on the release of insulin is also determinant in diabetes mellitus (Antonioli et al., 2015; Cieslak and Roszek, 2014). Thus, further research clarifying the mechanisms behind adenosine effects is required.

Alternatively, one of the potential mechanisms that could account for a modulation of insulin biological effects by activation of ARs is a co-localization of these receptors and IRS (Westermeier et al., 2016). Unfortunately, there are not clear findings in the literature addressing this possibility. However, a dependency of IRS activation-associated signalling has been proposed for ENTS proteins modulating extracellular concentration of adenosine. To date, regional expression of A1AR and A2AAR was demonstrated in the human brain, with overexpression of these ARs (A1AR > A2AAR) in association with preferential expression of hENT1 (cortex and hippocampus) and hENT2 (thalamus), respectively (i.e., hENT1 > hENT2) (Armentero et al., 2011; Arroyo et al., 2013; Jennings et al., 2001). The latter could result in a fine regulation of ARs activation due to ENTs activity. Similar interaction could account for ARs and IRS (Guzmán-Gutiérrez et al., 2014; Sobrevia et al., 2016).

7. Concluding remarks

Vascular tone is under modulation by factors synthesized and released locally, including NO and adenosine. However, vascular tone also responds to modulation by circulating factors such as the hormone insulin. Plasma adenosine concentration is shown to be elevated in pathologies associated with abnormal catabolism of D-glucose that result in hyperglycaemia and subsequent hyperinsulinemia. Both hyperglycaemia and hyperinsulinemia leads to lower uptake of adenosine and extracellular accumulation of this vasactive nucleoside. Adenosine causes vasodilation by increasing the synthesis and release of NO and by KATP activation-dependent membrane hyperpolarization following A2AAR/A2BAR-increased cAMP level (Fig. 6). However, adenosine also increases the release of thromboxane and prostaglandins following activation of A1AR, or alternatively, we proposed that this nucleoside may reduce cAMP following activation of A2AR to cause vasoconstriction. On the other hand, insulin is a well-described vasodilator whose effect is mediated by endothelium-derived NO. This hormone also causes a dual effect in the vasculature and leads to vasoconstriction by releasing ET-1 from the endothelium. A role for IR-A and IR-B for insulin action activating MAPKs and PI3K/Akt is proposed for vasoconstriction starting with sympathetic activation at the central nervous system.

A potential link between insulin and adenosine vascular effects is not fully addressed, but several lines of evidence show that insulin biological effects are increased or reduced following activation of ARs. Several mechanisms include A1AR activation leading to an increase in insulin-stimulated 3-O-methyl-D-glucose or 2-deoxyglucose uptake. On the other hand, A2AAR activation seems involved in the increase of NO synthesis and 1-arginine transport in human endothelium. A2AAR activation is also required for IRS sensitivity to insulin in adipocytes, something that is unknown in human vascular tissues in health or disease.

Interestingly, few reports could also be read as a potential influence of insulin signalling pathway modulating adenosine biological effects. To date, in streptozotocin-induced diabetic rats the sensitivity of hippocampal slices to adenosine is reduced (Morrison et al., 1992). This reduced sensitivity to adenosine is also reported in human platelets from patients with T1DM, where the cAMP level in response to the adenosine general analogue NECA is reduced, but not in response to other molecules that increase cAMP formation (Gasser et al., 1993). Thus, not only a modulation of insulin biological effects by adenosine and ARs is evident, but adenosine biological effects could also be under modulation of insulin in mammalian cells.

Insulin biological effects modulated by activation ARs (and potentially insulin modulating adenosine effects) is a phenomenon of importance for a better understanding of the aetiology of diseases associated with insulin resistance or reduced responsiveness to insulin including diabetes mellitus, GDM, or obesity. Additionally, a potential linked signalling between insulin and adenosine biological effects could be determinant for the proposal of therapeutic protocols for patients affected by these abnormal physiological conditions (Antonioli et al., 2015; Guzmán-Gutiérrez et al., 2014; Sobrevia et al., 2015).

Conflict of interest

The authors confirm that there are no conflicts of interest.

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