Biochimica et Biophysica Acta 1838 (2014) 1332–1337

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Insulin and IGF-I actions on IGF-I receptor in seminiferous tubules from immature rats

Gustavo Monteiro Escott [a], Alexandre Luz de Castro [a], Ana Paula Jacobus [b], E.S. Loss [a,*]

[a] Laboratório de Endocrinologia Experimental e Eletrofisiologia, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil
[b] Centro Universitário Metodista do IPA, Brazil

ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form 14 January 2014
Accepted 1 February 2014
Available online 11 February 2014

Keywords:
Sertoli cell
Insulin-like growth factor 1
Membrane potential
Calcium uptake
Glucose transport
Amino acid transport

ABSTRACT

Insulin and insulin-like growth factor 1 (IGF-I) are capable of activating similar intracellular pathways. Insulin acts mainly through its own receptor, but can also activate the IGF-I receptor (IGF-IR). The aim of this study was to investigate the involvement of the IGF-IR in the effects of insulin and IGF-I on the membrane potential of immature Sertoli cells in whole seminiferous tubules, as well as on calcium, amino acid, and glucose uptake in testicular tissue of immature rats. The membrane potential of the Sertoli cells was recorded using a standard single microelectrode technique. In calcium uptake experiments, the testes were pre-incubated with $^{45}Ca^{2+}$, with or without JB1 (1 μg/mL), and then incubated with insulin (100 nM) or IGF-I (15 nM). In amino acid and glucose uptake experiments, the gonads were pre-incubated with or without JB1 (1 μg/mL) and then incubated with radiolabeled amino acid or glucose analogues in the presence of insulin (100 nM) or IGF-I (15 nM). The blockade of IGF-IR with JB1 prevented the depolarising effects of both insulin and IGF-I on membrane potential, as well as the effect of insulin on calcium uptake. JB1 also inhibited the effects of insulin and IGF-I on glucose uptake. The effect of IGF-I on amino acid transport was inhibited in the presence of JB1, whereas the effect of insulin was not. We concluded that while IGF-I seems to act mainly through its cognate receptor to induce membrane depolarisation and calcium, amino acid and glucose uptake, insulin appears to be able to elicit its effects through IGF-IR, in seminiferous tubules from immature rats.

1. Introduction

Insulin and insulin-like growth factor 1 (IGF-I) are essential in testis development [1,2]. Although several factors are related to the proliferation and differentiation of Sertoli cells, insulin and IGF-I signalling is highly involved in regulating the final number of Sertoli cells during the pre-pubertal period, which is crucial for future male fertility [1]. The insulin receptor tyrosine kinase family, which comprises insulin receptor (IR) and IGF-I receptor (IGF-IR), plays an essential role in regulating Sertoli cell proliferation, and is crucial to the reproductive function [1,2]. After binding to their cell-surface receptors, insulin and IGF-I generate several biological effects that are essential for metabolic regulation and growth stimulation [1–8].

The nutritional support of germ cells is one of the most important functions of Sertoli cells [9]. These cells can use glucose to produce lactate, and secrete lactate in order to provide adequate nutritional support for spermatogenesis [10,11]. Sertoli cells also use amino acids for energy production, such as alanine and valine, which have an important role in Sertoli cell metabolism [12]. In immature Sertoli cells, both insulin and IGF-I are capable of stimulating energetic substrate transport through the plasma membrane [4,5]. Jacobus et al. [5] found that IGF-I induced amino acid transport, as well as a rapid depolarisation of the membrane potential of immature Sertoli cells. Similarly, Escott et al. [4] showed that insulin also stimulated a rapid membrane depolarisation in Sertoli cells, as well as calcium, amino acid and glucose uptake. In these cells, the membrane depolarising effect and the transport of glucose were stimulated by insulin only at 580 ng/mL (100 nM). Lower or higher doses were ineffective. Likewise, the depolarising effect of IGF-I was not observed at doses lower than 100 ng/mL (15 nM) [4].

Both insulin and IGF-I and their receptors are closely related and their signalling pathways are largely overlapping [13,14]. Hence, when both receptors are expressed in the same cell, it is difficult to distinguish between the effects of insulin and IGF-I [13]. In many different cell types

Abbreviations: IGF-IR, insulin-like growth factor 1 receptor; IR, insulin receptor; α-[1-^{14}C], [^{14}C]MeAIB, methylaminoisobutyric acid; 2-[1-^{14}C], [^{14}C]2DG, deoxy-D-glucose; PI3K, phosphatidylinositol 3-kinase; IRS-1, insulin receptor substrate 1; IRS-2, insulin receptor substrate 2; GBq, gigabecquerel; HBSS, Hank's balanced salt solution; DMSO, dimethyl sulfoxide; GLUT4, glucose transporter 4

* Corresponding author at: Laboratório de Endocrinologia Experimental e Eletrofisiologia, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite 500, sala 212, Porto Alegre, RS, CEP: 90050-170, Brazil. Tel.: +55 51 3308 3302.
E-mail address: eloss@ufrgs.br (E.S. Loss).
URL: http://www.ufrgs.br/labenex (E.S. Loss).

http://dx.doi.org/10.1016/j.bbamem.2014.02.002
0005-2736

[4,13,15,16], insulin stimulates the phosphorylation of insulin receptor substrates 1 (IRS-1) and 2 (IRS-2) and activates the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signalling pathway. However, in preadipocytes insulin-stimulated phosphorylation of IRS-2 also occurs in the absence of IR, indicating that insulin binding to IGF-IR can support the generation of these signals [13]. In foetal brown adipocyte, insulin also acts through IGF-IR to induce cell differentiation [17]. Like insulin, IGF-I is capable of phosphorylating both IRS-1 and IRS-2 and activate PIK3/Akt signalling pathways, but only in the presence of IGF-IR [17]. In immature Sertoli cells, the stimulatory effects of IGF-I and insulin on substrate transport and calcium uptake were blocked by wortmannin [4], thereby demonstrating a relationship between the PI3K/Akt pathway and the effects of these hormones.

Despite the evidences that insulin can act through the IGF-IR in several tissues [13,17,18], it is not clear if the effects of insulin on Sertoli cell membrane depolarisation and on calcium, amino acid and glucose uptake [4] occur through this receptor. In view of that, this study aimed to investigate the involvement of the IGF-IR, using JB1 (a highly selective IGF-IR antagonist [19]), in the effects of these hormones on calcium, amino acid, and glucose uptake in immature testicular tissue, as well as in the electrophysiological effects of these hormones in immature Sertoli cells in whole seminiferous tubules.

2. Methods

2.1. Animals

The experimental animals were 12-day-old Wistar rats. The animals were bred in our animal facility and housed in an air-conditioned room (approximately 24 °C) with controlled lighting (lights on from 07:00 to 19:00). Pelleted food (Purina, Nutripal, Porto Alegre, RS, Brazil) and tap water were made available to the mothers ad libitum. The suckling rats were kept with their mothers until required for the experiments and were sacrificed by cervical dislocation. The study and animal care procedures were reviewed and approved by the Ethics Committee for animal research at this University (Universidade Federal do Rio Grande do Sul — UFRGS, www.ufrgs.br), protocol number: 18097.

2.2. Chemicals and solutions used

Insulin and IGF-I were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). JB1 (an inactive analogue of IGF-I) was purchased from Bachem Bioscience, Inc. (King of Prussia, PA, USA). $^{45}Ca^{2+}$ (specific activity 444 GBq/g), methylaminoisobutyric acid, α-[1-^{14}C] ([^{14}C]MeAIB, specific activity 1.85 GBq/mmol) and deoxy-D-glucose, 2-[1-^{14}C] ([^{14}C]2-DG, specific activity 1.66 GBq/mmol) were acquired from PerkinElmer NEN® (Waltham, MA, USA).

Hank's Balanced Salt Solution (HBSS) contained: NaCl (145 mM), KCl (4.6 mM), $NaHCO_3$ (5 mM), $MgCl_2$ (1.6 mM), $CaCl_2.2H_2O$ (1.6 mM), glucose (5 mM) and HEPES (10 mM) at pH 7.4. Stock solution of JB1 was prepared in dimethylsulphoxide (DMSO) and stored at −20 °C until required. The stock solution was diluted in HBSS to achieve the final concentration required at the time of use. The final concentration of DMSO did not exceed 0.1% or affect the analysed parameters. The drug concentrations used in each experiment are included in the figures and legends.

2.3. Electrophysiological experiments

In each experiment, whole testes were decapsulated and carefully stretched with two tweezers, exposing three to 10 undisrupted seminiferous tubules. The tubules were then fixed to the bottom of a superfusion chamber, incubated with 1 mL/min of HBSS buffer, pH 7.4, at 34 °C. Standard single microelectrode recording was performed according to the method described in von Ledebur et al. [20]. The electrophysiological characteristics of immature Sertoli cells were analysed using whole seminiferous tubules, without Leydig cells. The functional integrity of the tubular cells was preserved in order for the tubular environment to remain as close as possible to the physiological conditions. The bulk of the recorded cells were most likely to be Sertoli cells based on the following factors: age of the animals [21], microelectrode impalement depths [22]; membrane potential stability; and membrane potential range according to previously published data [21].

Microelectrode borosilicate pipettes were filled with KCl (3 M) and had a tip resistance of 15 to 25 MΩ. This tip resistance is appropriate for the impalement of cells of similar size to Sertoli cells. This tip diameter helps to eliminate the impalement of slim cells such as peritubular cells [20]. Intracellular recording was amplified using an intracellular amplifier, Intra 767 (World Precision Instruments Inc., USA). Square current pulses of 0.5 nA, 0.5 Hz, and 250 ms duration were applied through the intracellular electrode to estimate membrane resistance using the S48 stimulator (Grass Instrument, West Warwick, RI, USA). An oscilloscope (Tektronix, 2 Channel Digital Oscilloscope TDS 210, Beaverton, OR, USA) and Wavestar Lite Version 1.0.10 software were used to record the variation in membrane potential. Insulin (100 nM) and IGF-I (15 nM) were topically administered in the bath after the resting potential had stabilised for at least 5 min. JB1 (1 μg/mL) solution was superfused for 10 min before the topical application of insulin and IGF-I. Each treatment was repeated at least five times with different cells from different animals, and variations in membrane potential were recorded. The results are presented as mean ± standard error of the mean (SEM).

2.4. $^{45}Ca^{2+}$ uptake experiments

Testes were removed and one gonad from each rat, alternately the left or right, was used as the experimental gonad; the contralateral gonad was used as the control. The testes (n = 5 in each group) were weighed, decapsulated, and pre-incubated in HBSS with $^{45}Ca^{2+}$ (0.2 μCi/sample) for 60 min with or without JB1 (1 μg/mL) in a Dubnoff metabolic incubator to equilibrate intra- and extracellular $^{45}Ca^{2+}$ levels until they reached a plateau. This equilibration was carried out at 34 °C and pH 7.4. Following equilibration, the gonads were incubated for 2 min in HBSS with $^{45}Ca^{2+}$, with or without insulin (100 nM) or IGF-I (15 nM). To end the experiment and stop calcium flux, 1 mL of cold buffer (0 °C) containing lanthanum chloride ($LaCl_3$) (10 mM) was added to the samples. This solution was calcium-free containing (in mM): NaCl, 127.5; KCl, 4.6; $MgCl_2$, 1.2; $LaCl_3$, 10; HEPES, 10; and glucose, 5 [23]. The supernatant was preserved, and the testes were removed into screw-cap tubes containing 1 mL of distilled water and stored at −20 °C for further analysis, as described in Section 2.7. The results are expressed as pmol of $^{45}Ca^{2+}$/g tissue.

2.5. [^{14}C]MeAIB transport experiments

The testes were removed and one gonad from each rat, alternately the left or right, was used as the experimental gonad; the contralateral gonad was used as the control. The samples (n = 5 in each group) were weighed, decapsulated, and pre-incubated in HBSS buffer for 60 min in a Dubnoff metabolic incubator at 34 °C and pH 7.4. The gonads were then incubated for 45 min in HBSS with [^{14}C]MeAIB (0.2 μCi/sample), with or without insulin (100 nM) or IGF-I (15 nM). In experiments utilising JB1 (1 μg/mL), the testes were pre-incubated for 60 min with JB1 and then incubated for 45 min in the presence of the hormones. The supernatant was preserved, and the testes were removed into screw-cap tubes containing 1 mL of distilled water and stored at −20 °C until further analysis, as described in Section 2.7. The results are expressed as the tissue/medium (T/M) ratio.

2.6. [^{14}C]2-DG transport experiments

The testes were removed and one gonad from each rat, alternately the left or right, was used as the experimental gonad; the contralateral gonad was used as the control. The samples (n = 5 in each group) were weighed, decapsulated and pre-incubated in HBSS buffer for 30 min in a Dubnoff metabolic incubator at 34 °C and pH 7.4. The gonads were then incubated for 60 min in HBSS with [^{14}C]2-DG (0.15 μCi/sample), with or without insulin (100 nM) or IGF-I (15 nM). In experiments utilising JB1 (1 μg/mL), the testes were pre-incubated for 60 min with JB1 and then incubated for 60 min in the presence of the hormones. The supernatant was preserved, and the testes were removed into screw-cap tubes containing 1 mL of distilled water and stored at −20 °C until further analysis, as described in Section 2.7. The results are expressed as the tissue/medium (T/M) ratio.

2.7. Sample processing for radioactivity count

For $^{45}Ca^{2+}$, [^{14}C]MeAIB, and [^{14}C]2-DG experiments, the testes were frozen for 24 h and subsequently boiled. Aliquots of 100 μL were taken from each sample and placed in OptiPhase HiSafe 3® (Perkin Elmer, Inc, USA) for the measurement of radioactivity using an LKB rack beta liquid scintillation spectrometer, model 1215 (LKB — Producer AB, Bromma, Sweden). The counting efficiency was 85–90%.

2.8. Statistics

The following statistical analyses were performed: one-way analysis of variance (ANOVA) with Bonferroni's post test or an ANOVA of repeated measures with Bonferroni's post test. The analyses were carried out using GraphPad InStat version 3.01, 32 bit for Windows 95/NT (GraphPad Software, San Diego, California, USA, www.graphpad.com). Differences were considered significant if $p < 0.05$.

3. Results

3.1. Basal electrophysiological values of Sertoli cells

The basal electrical characteristics were assayed. As expected, in our experimental conditions, the membrane potential of impaled Sertoli cells from 12-day-old rats were −46.56 ± 2.3 mV (n = 11). The hormonal treatment was performed after these basal conditions remained stable for at least 5 to 10 min.

3.2. Effect of the blockade of IGF-I receptor on insulin and IGF-I depolarising action on membrane potential of Sertoli cells in seminiferous tubules

Membrane potential depolarisation of Sertoli cells in whole seminiferous tubules from 12-day-old rats was induced by insulin (100 nM) and IGF-I (15 nM), with the basal potential ranging from −45.63 ± 1.2 to −33.77 ± 3.04 mV and from −44.38 ± 0.5 to −41 ± 1,7 mV, respectively. Both responses were significant at 180 s after application of the hormone. Fig. 1A and B shows, respectively, that the blockade of IGF-IR with JB1 (1 μg/mL) prevented the depolarising effect of both insulin and IGF-I on membrane potential.

3.3. Effect of the blockade of IGF-I receptor on insulin stimulating action on calcium uptake in testicular tissue

The radioisotope $^{45}Ca^{2+}$ was used as a marker to investigate calcium uptake. Insulin (100 nM) increased $^{45}Ca^{2+}$ uptake in testicular tissue from 12-day-old rats within 2 min of incubation (Fig. 2). To verify the involvement of the IGF-IR in the effects of insulin on calcium uptake, testes were pre-incubated with $^{45}Ca^{2+}$ and JB1 (1 μg/mL), and then incubated with insulin (100 nM). In the presence of JB1, the insulin-stimulated effect was completely blocked (Fig. 2).

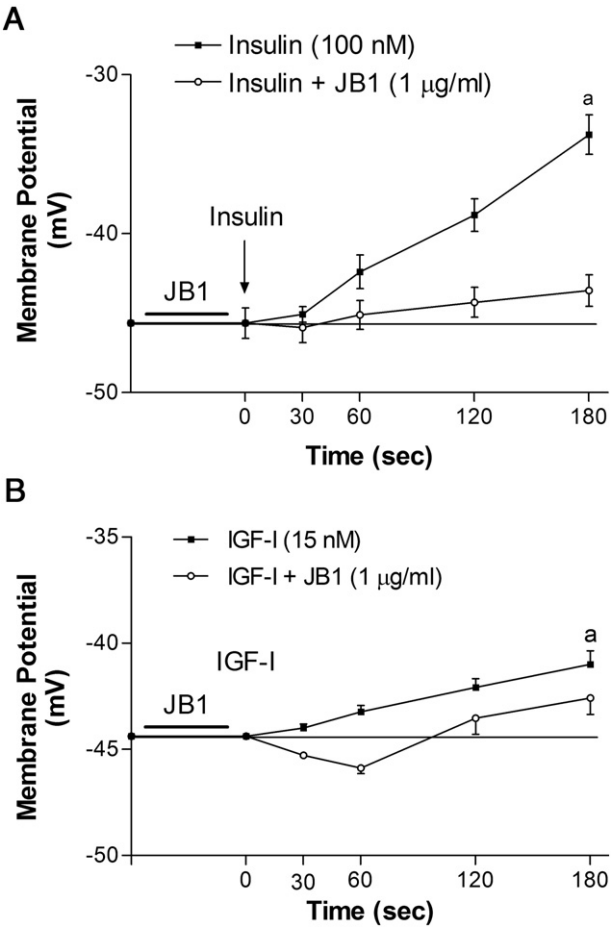

Fig. 1. Effect of JB1 on the depolarising action of insulin and IGF-I on the membrane potential of Sertoli cells from 12-day-old rats. A. Depolarising effect of insulin (100 nM) on the membrane potential of Sertoli cells. Effect of JB1 (1 μg/mL) on the action of insulin on Sertoli cell membrane (n = 8). B. Depolarising effect of IGF-I (15 nM) on the membrane potential of Sertoli cells. Effect of JB1 (1 μg/mL) on the action of IGF-I on Sertoli cell membrane (n = 5). Repeated measures ANOVA $^{a}p < 0.05$ compared with resting potential.

3.4. Effect of the blockade of IGF-I receptor on insulin and IGF-I stimulating action on amino acid transport in testicular tissue

In amino acid transport experiments, [^{14}C]MeAIB was used. This is a non-metabolisable alanine analogue that is frequently used to investigate system A neutral amino acid transporter activity in Sertoli

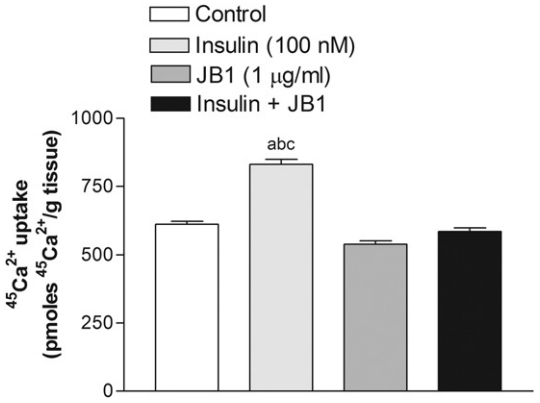

Fig. 2. Effect of JB1 on the action of insulin on Ca^{2+} uptake in whole testis from 12-day-old rats. Effect of insulin (100 nM) on $^{45}Ca^{2+}$ uptake in whole testis from 12-day-old rats. One-way ANOVA $^{a}p < 0.05$ compared with control (n = 16), $^{b}p < 0.01$ compared with JB1 group (n = 14), $^{c}p < 0.05$ compared with insulin + JB1 group (n = 15).

cells. Insulin (100 nM) (Fig. 3A) and IGF-I (15 nM) (Fig. 3B) stimulated [^{14}C]MeAIB transport in testicular tissue within 45 min of incubation. The blockade of IGF-IR with JB1 (1 μg/mL) did not inhibit the effect of insulin on amino acid transport, while the effect of IGF-I was completely blocked (Fig. 3A and B).

3.5. Effect of the blockade of IGF-I receptor on insulin and IGF-I stimulating action on glucose transport in testicular tissue

In glucose transport experiments, [^{14}C]2-DG, a non-metabolisable glucose analogue, was used to measure glucose transport. Insulin (100 nM) and IGF-I (15 nM) stimulated [^{14}C]2-DG transport in testicular tissue, with a significant response within 60 min of incubation (Fig. 4A and B). The blockade of the IGF-IR with the IGF-I antagonist JB1 (1 μg/mL) completely blocked the effects of both insulin and IGF-I (Fig. 4A and B).

4. Discussion

The indispensable role of insulin and IGF-I in testis development, testis size, sperm production, and in the total number of Sertoli cells, which occur at pre-pubertal period, is well established. The lack of insulin receptor family in Sertoli cells prevents testis growth and maturation but does not prevent germ cell development [1,2].

Pitteti et al. [1] demonstrated that the contribution of the IGF-IR to regulating Sertoli cell number and testis size is more important than that of the IR. In addition, the concomitant ablation of both receptors resulted in a much more severe reduction in testis weight implying in an important redundancy and suggesting that the IR and IGF-IR act in a synergistic manner to regulate Sertoli cell number and testis size [1]. Although the IR is the main physiological receptor for insulin, at higher concentrations this peptide hormone can also bind and exert effects through the IGF-I receptor [14], which is more abundant than IR in immature Sertoli cells [24]. Versteyhe et al. [14] demonstrated that the saturating concentrations of insulin and IGF-I for the IGF-IR were 5168 nM and 20 nM, respectively. Similar results were found by Oonk and Grootegoed [24] in Sertoli cells. In a previous study from our research group, the effect of 100 nM insulin on membrane potential and on calcium, amino acid and glucose transport in immature Sertoli cells was demonstrated [4]. Once it has been demonstrated that testicular cells can produce insulin [25–27], and then the testicular levels of insulin might be eventually higher than the plasma levels, the involvement of the IGF-IR in the electrophysiological response of Sertoli cells exposed to this concentration of insulin was investigated, using a highly selective antagonist of the IGF-IR, JB1.

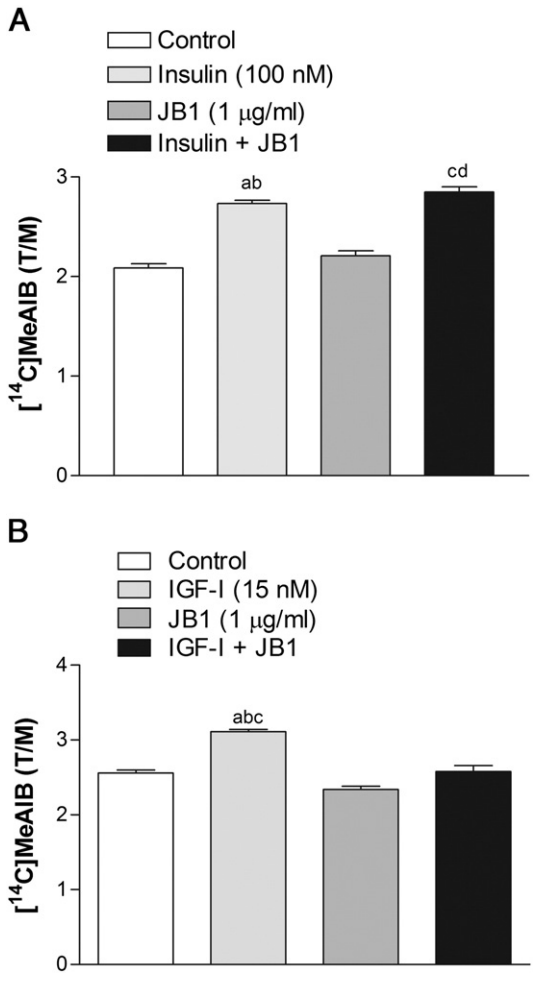

Fig. 3. Effect of JB1 on the action of insulin and IGF-I on neutral amino acid transport in whole testis from 12-day-old rats. A. Effect of insulin (100 nM) on [^{14}C]MeAIB transport in whole testis from 12-day-old rats. One-way ANOVA $^{a}p < 0.05$ compared with control ($n = 9$), $^{b}p < 0.05$ compared with JB1 group ($n = 10$), $^{c}p < 0.05$ compared with control ($n = 8$), $^{d}p < 0.05$ compared with control ($n = 9$). B. Effect of IGF-I (15 nM) on [^{14}C]MeAIB transport in whole testis from 12-day-old rats. One-way ANOVA $^{a}p < 0.05$ compared with control ($n = 10$), $^{b}p < 0.001$ compared with JB1 group ($n = 10$), $^{c}p < 0.05$ compared with insulin + JB1 group ($n = 6$).

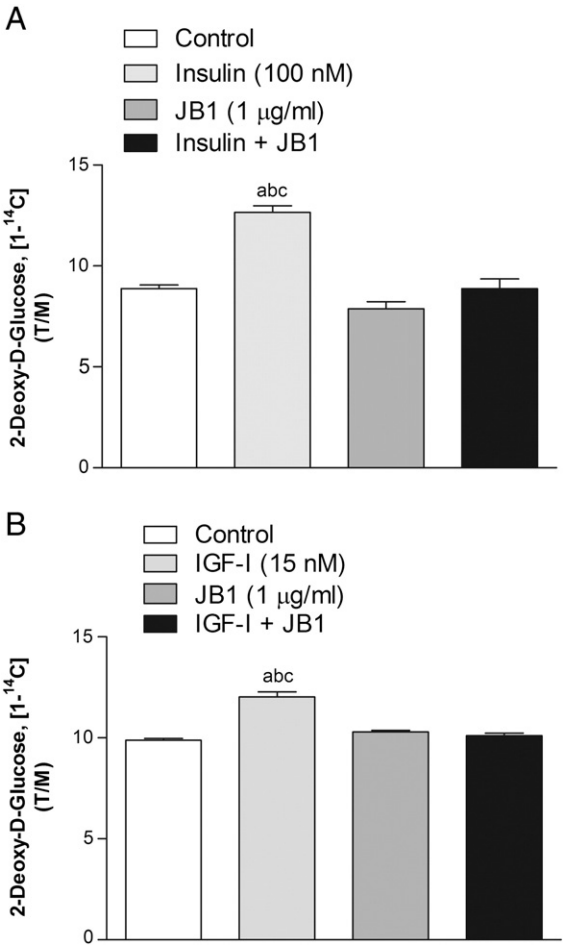

Fig. 4. Effect of JB1 on the action of insulin and IGF-I on glucose transport in whole testis from 12-day-old rats. A. Effect of insulin (100 nM) on [^{14}C]2-DG transport in whole testis from 12-day-old rats. One-way ANOVA $^{a}p < 0.05$ compared with control ($n = 8$), $^{b}p < 0.05$ compared with JB1 group ($n = 8$). B. Effect of IGF-I (15 nM) on [^{14}C]2-DG transport in whole testis from 12-day-old rats. One-way ANOVA $^{a}p < 0.01$ compared with control ($n = 6$), $^{b}p < 0.05$ compared with JB1 group ($n = 9$), $^{c}p < 0.05$ compared with insulin + JB1 group ($n = 9$).

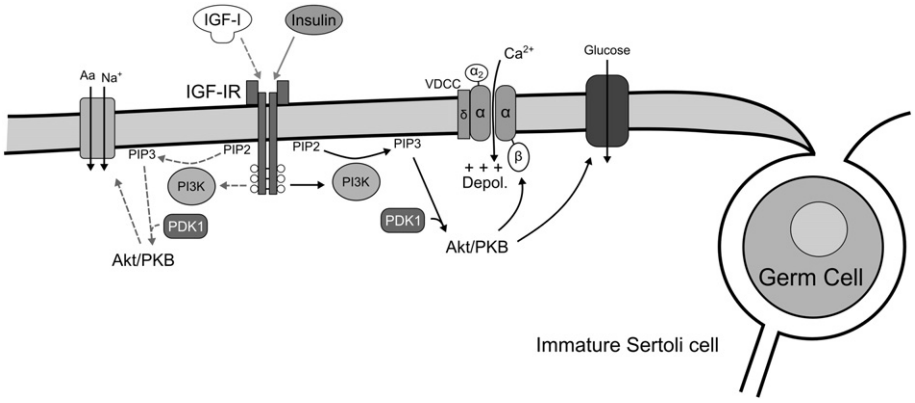

Fig. 5. Schematic representation of the mechanism of action of insulin and insulin-like growth factor 1 (IGF-I) through the IGF-I receptor (IGF-IR) in immature Sertoli cells. Both hormones bind to the IGF-IR inducing the activation of phosphatidylinositol 3-kinase (PI3K)/Akt downstream signalling. The activation of Akt induces Ca^{2+} influx through the L-type voltage-dependent calcium channel (VDCC) and consequent membrane depolarisation, probably via direct phosphorylation of the β-subunit. This signalling pathway induced by insulin and IGF-I also stimulates glucose transport, but only IGF-I is capable of inducing neutral amino acid (Aa) transport through the IGF-IR. Dashed gray arrows: effects of the IGF-I; Solid black arrows: effects of both hormones. PIP2: phophatidylinositol biphosphate; PIP3, phosphatidylinositol triphosphate; PDK1: phosphoinositide-dependent kinase 1; PKB: protein kinase B.

The electrophysiological recording of immature Sertoli cells from whole seminiferous tubules has enabled several observations, introducing new concepts regarding the mechanisms of action of hormones, especially follicle-stimulating hormone (FSH) and testosterone [21] and more recently, insulin and IGF-I [4,5]. In order for the tubular environment to remain as close as possible to the physiological conditions, whole seminiferous tubules were used to preserve the functional integrity of the tubular cells, the gap junctions and the paracrine factors, as reviewed in Loss et al. [21]. Considering the electrode insertion depth (a few micrometres deep) and the membrane potential stability we are sure that peritubular cells were not registered [22]. Working with the same technique, Eusebi et al. [28] observed two distinct peaks of resting potential from cells of whole seminiferous tubules from immature rats: one with values around −20 to −25 mV and the other with values around −40 to −45 mV. This last peak is similar to the resting potential found in cells from Sertoli cell-enriched (SCE) tubules. In a previous study by our group, it was found that the average resting potential in SCE seminiferous tubules (irradiated in utero with 1 Gy of ^{60}Co in order to eliminate germinal epithelium) from 15-day-old rats was -44 ± 0.5 mV (n = 48), ranging from −35 mV to −50 mV, similar to the second peak observed by Eusebi et al. [28]. To ensure that the registered cells were indeed Sertoli cells, all cells with a resting potential less negative than −35 mV and cells with a resting potential more negative than −50 mV were excluded. Furthermore, Sertoli cells are predominant along the basement membrane during the proliferative phase (up to 15 days of age) [29].

The effects of both insulin and IGF-I on the membrane potential of immature Sertoli cells were blocked by JB1 (Fig. 1). These results demonstrate that the depolarising effect elicited by these hormones at this stage of the development of Sertoli cells depends on the IGF-IR. Likewise, the involvement of the IGF-IR in the well-known effect of insulin and IGF-I on calcium uptake in testicular tissue [4,5] was investigated. The effects of both hormones on calcium uptake were blocked by JB1 (Fig. 2). It has also been demonstrated by Jacobus et al. [5] that JB1 inhibits IGF-I-stimulated calcium uptake. These data corroborate the existence of a relationship between activation of the IGF-IR by insulin and IGF-I and the calcium-dependent depolarising effect (Figs. 1 and 2).

The effect of IGF-I on amino acid transport was completely blocked by this IGF-IR antagonist (Fig. 3B); however, the amino acid transport stimulated by insulin was not blocked by JB1 (Fig. 3A). These results show that it is not only the IGF-IR that is involved in the effects of insulin on amino acid uptake. The IR probably has an important role in the regulation of this physiological mechanism mediated by insulin. The fact that the effect of insulin on amino acid transport was not blocked by JB1 (Fig. 3A) indicates that insulin-induced amino acid transport, even at high concentrations, is independent of IGF-IR activation, and possibly occurs through the IR.

On the other hand, the stimulating effect of insulin on glucose transport was completely blocked by JB1 (Fig. 4A), which demonstrates an involvement of the IGF-IR in this action by insulin. Likewise, IGF-I seems to act only through its cognate receptor to stimulate glucose transport, as this effect was also completely blocked by JB1 (Fig. 4B). Altogether, these results demonstrate that while the effects of IGF-I on amino acid and glucose transport occur only through the IGF-IR, the effects of insulin on the transport of these substrates occur through different receptors.

Although insulin and IGF-I have a similar structure and thus the ability to bind to both IR and IGF-IR, several studies have demonstrated that the IR and IGF-IR are not redundant molecules and one receptor cannot functionally compensate when the other receptor is absent [1,2,13]. A previous study from Entingh-Pearsall and Khan [13] has demonstrated in adipose cells that insulin and IGF-I induce different signalling pathways when they bind to one or the other receptor. The authors showed that insulin induced IRS-1 and Akt phosphorylation only through the IR, while phosphorylation of IRS-2 and mitogen-activated protein kinase (MAPK) could be induced by insulin in the absence of the IR, indicating that the IGF-IR might be involved in the generation of these signals [13]. Versteyhe et al. [14] showed that insulin, IGF-I, and IGF-II promoted different gene expression responses when stimulating the IGF-I receptor. Thus, it could be postulated that insulin and IGF-I can induce different signalling pathways binding to its cognate or a non-cognate receptor. However, although it has been demonstrated that both insulin and IGF-I induce their actions on calcium, amino acid and glucose uptake through the PI3K/Akt pathway in Sertoli cells from immature rats [4], the activated pathway by itself does not completely explain the different actions of insulin on distinct receptors.

It has been suggested that the spatial organisation of different signalling molecules at specific intracellular locations is a determinant for signalling specificity [30]. In adipocytes, both platelet-derived growth factor (PDGF) and insulin stimulate PI3K activity, but only insulin promotes the translocation of the glucose transporter 4 (GLUT4) to the cell surface. The ability of insulin, but not PDGF, to stimulate GLUT4 translocation has been associated with the preferential recruitment of PI3K from intracellular compartments and plasma membrane by insulin, whereas PDGF recruits only from the plasma membrane [31]. In immature Sertoli cells, both insulin and IGF-I activate the PI3K/Akt signalling pathway [5]; however, different effects were observed in amino acid and glucose transport when the IGF-IR was blocked (Fig. 5). The insulin action on amino acid transport was not affected by JB1, but IGF-I-stimulated amino acid transport was

completely blocked (Figs. 3 and 5). These discrepancies could be explained by the nature of the ligand or even by the compartmentalisation of these downstream molecules and substrate transporters [30]. A better understanding of the compartmentalisation and redistribution of these molecules may help to explain how shared signalling components can elicit specific and unique downstream events for these hormones [31,32].

However, studies have correlated different biological responses after IR stimulation by different ligands with ligand binding kinetics and internalisation properties of the hormone–receptor complex [33,34]. Furthermore, Versteyhe et al. [14] demonstrated that insulin and IGF-I specifically regulated different groups of transcripts when binding to the IGF-IR. These results are in accordance with the data presented here which, in turn, corroborate the hypothesis that it is not just the hormone concentration and affinity but also the nature of the ligand bound to a receptor that determines the downstream cellular response [13,14,33]. Further investigations are necessary to clarify the differences in the downstream events upon insulin and IGF-I binding to their cognate and non-cognate receptors in immature Sertoli cells.

5. Conclusions

The results presented here demonstrate that in our experimental conditions while the effects of IGF-I occur mainly through the IGF-IR, the effects of insulin seem to occur through different receptors. The IGF-I receptor seems to be involved in insulin-induced membrane depolarisation, calcium uptake, and glucose transport, but not in amino acid transport, which probably occurs through the cognate receptor.

Acknowledgements

This work was supported by Conselho Nacional de Pesquisa (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and PROPESQ-UFRGS. We thank Dr. Luiz Carlos Kucharski for kindly donating deoxy-D-glucose, 2-[^{14}C]. We are also indebted to Dr. Guillermo F. Wassermann for his full assistance.

References

[1] J.-L. Pitetti, P. Calvel, C. Zimmermann, B. Conne, M.D. Papaioannou, F. Aubry, et al., An essential role for insulin and IGF1 receptors in regulating Sertoli cell proliferation, testis size, and FSH action in mice, Mol. Endocrinol. 27 (2013) 814–827.
[2] S. Nef, S. Verma-Kurvari, J. Merenmies, J.D. Vassalli, A. Efstratiadis, D. Accili, et al., Testis determination requires insulin receptor family function in mice, Nature 426 (2003) 291–295.
[3] A.S. Andersen, T. Kjeldsen, F.C. Wiberg, H. Vissing, L. Schaffer, J.S. Rasmussen, et al., Identification of determinants that confer ligand specificity on the insulin receptor, J. Biol. Chem. 267 (1992) 13681–13686.
[4] G.M. Escott, A.P. Jacobus, E.S. Loss, PI3K-dependent actions of insulin and IGF-I on seminiferous tubules from immature rats, Pflugers Arch. — Eur. J. Physiol. 465 (2013) 1497–1505.
[5] A.P. Jacobus, E.S. Loss, G.F. Wassermann, Pertussis toxin nullifies the depolarization of the membrane potential and the stimulation of the rapid phase of Ca entry through L-type calcium channels that are produced by follicle stimulating hormone in 10- to 12-day-old rat Sertoli cells, Front. Physiol. 1 (2010) 138.
[6] L. Rato, M.G. Alves, S. Socorro, A.I. Duarte, J.E. Cavaco, P.F. Oliveira, Metabolic regulation is important for spermatogenesis, Nat. Rev. Urol. 9 (2012) 330–338.
[7] T.R. Dias, L. Rato, A.D. Martins, V.L. Simões, T.T. Jesus, M.G. Alves, et al., Insulin deprivation decreases caspase-dependent apoptotic signaling in cultured rat Sertoli cells, ISRN Urol. 2013 (2013) 970370.
[8] M.G. Alves, S. Socorro, J. Silva, A. Barros, M. Sousa, J.E. Cavaco, et al., In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17β-estradiol and suppressed by insulin deprivation, Biochim. Biophys. Acta 1823 (2012) 1389–1394.
[9] C. Petersen, O. Soder, The Sertoli cell — a hormonal target and "super" nurse for germ cells that determines testicular size, Horm. Res. 66 (2006) 153–161.
[10] P.F. Oliveira, M.G. Alves, L. Rato, S. Laurentino, J. Silva, R. Sa, et al., Effect of insulin deprivation on metabolism and metabolism-associated gene transcript levels of in vitro cultured human Sertoli cells, Biochim. Biophys. Acta 1820 (2012) 84–89.
[11] R.M. Sharpe, C. McKinnell, C. Kivlin, J.S. Fisher, Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood, Reproduction 125 (2003) 769–784.
[12] M.G. Alves, L. Rato, R.A. Carvalho, P.I. Moreira, S. Socorro, P.F. Oliveira, Hormonal control of Sertoli cell metabolism regulates spermatogenesis, Cell. Mol. Life Sci. 70 (5) (2012) 777–793.
[13] A. Entingh-Pearsall, C.R. Kahn, Differential roles of the insulin and insulin-like growth factor-I (IGF-I) receptors in response to insulin and IGF-I, J. Biol. Chem. 279 (2004) 38016–38024.
[14] S. Versteyhe, B. Klaproth, R. Borup, J. Palsgaard, M. Jensen, S.G. Gray, et al., IGF-I, IGF-II, and insulin stimulate different gene expression responses through binding to the IGF-I receptor, Front. Endocrinol. 4 (2013) 98.
[15] G. Li, E.J. Barrett, S.-H. Ko, W. Cao, Z. Liu, Insulin and insulin-like growth factor-I receptors differentially mediate insulin-stimulated adhesion molecule production by endothelial cells, Endocrinology 150 (2009) 3475–3482.
[16] A.R. Saltiel, C.R. Kahn, Insulin signalling and the regulation of glucose and lipid metabolism, Nature 414 (2001) 799–806.
[17] C. Mur, M. Arribas, M. Benito, A.M. Valverde, Essential role of insulin-like growth factor I receptor in insulin-induced fetal brown adipocyte differentiation, Endocrinology 144 (2003) 581–593.
[18] B. Lamothe, A. Baudry, C.T. Christoffersen, P. De Meyts, J. Jami, D. Bucchini, et al., Insulin receptor-deficient cells as a new tool for dissecting complex interplay in insulin and insulin-like growth factors, FEBS Lett. 426 (1998) 381–385.
[19] M.W. Elmlinger, M.S. Sanatani, M. Bell, G.E. Dannecker, M.B. Ranke, Elevated insulin-like growth factor (IGF) binding protein (IGFBP)-2 and IGFBP-4 expression of leukemic T-cells is affected by autocrine/paracrine IGF-II action but not by IGF type I receptor expression, Eur. J. Endocrinol. 138 (1998) 337–343.
[20] E.I. Von Ledebur, J.P. Almeida, E.S. Loss, G.F. Wassermann, Rapid effect of testosterone on rat Sertoli cell membrane potential. Relationship with K + ATP channels, Horm. Metab. Res. 34 (2002) 550–555.
[21] E.S. Loss, A.P. Jacobus, G.F. Wassermann, Rapid signaling responses in Sertoli cell membranes induced by follicle stimulating hormone and testosterone: calcium inflow and electrophysiological changes, Life Sci. 89 (2011) 577–583.
[22] A.L. de Castro, F.C. Cavalari, M.V. Diello, B.M. Fracasso, E.S. Loss, Epitestosterone and testosterone have similar nonclassical actions on membrane of Sertoli cells in whole seminiferous tubules, Horm. Metab. Res. 45 (2013) 15–21.
[23] S. Batra, C. Sjogren, Effect of estrogen treatment on calcium uptake by the rat uterine smooth muscle, Life Sci. 32 (1983) 315–319.
[24] R.B. Oonk, J.A. Grootegoed, Insulin-like growth factor I (IGF-I) receptors on Sertoli cells from immature rats and age-dependent testicular binding of IGF-I and insulin, Mol. Cell. Endocrinol. 55 (1988) 33–43.
[25] E.L. Schoeller, G. Albanna, A.I. Frolova, K.H. Moley, Insulin rescues impaired spermatogenesis via the hypothalamic–pituitary–gonadal axis in Akita diabetic mice and restores male fertility, Diabetes 61 (2012) 1869–1878.
[26] S. Aquila, M. Gentile, E. Middea, S. Catalano, S. Andò, Autocrine regulation of insulin secretion in human ejaculated spermatozoa, Endocrinology 146 (2005) 552–557.
[27] O. Gómez, B. Ballester, A. Romero, E. Arnal, I. Almansa, M. Miranda, et al., Expression and regulation of insulin and the glucose transporter GLUT8 in the testes of diabetic rats, Horm. Metab. Res. 41 (2009) 343–349.
[28] F. Eusebi, E. Ziparo, G. Fratamico, M.A. Russo, M. Stefanini, Intercellular communication in rat seminiferous tubules, Dev. Biol. 100 (1983) 249–255.
[29] L.R. Franca, R.A. Hess, Structure of the Sertoli cell, in: M.D. Griswold, M.K. Skinner (Eds.), The Sertoli Cell Biology, Elsevier Academic Press, San Diego, California, 2004, pp. 19–40.
[30] G. Inoue, B. Cheatham, R. Emkey, C.R. Kahn, Dynamics of insulin signaling in 3T3-L1 adipocytes. Differential compartmentalization and trafficking of insulin receptor substrate (IRS)-1 and IRS-2, J. Biol. Chem. 273 (1998) 11548–11555.
[31] P.A. Staubs, J.G. Nelson, D.R. Reichart, J.M. Olefsky, Platelet-derived growth factor inhibits insulin stimulation of insulin receptor substrate-1-associated phosphatidylinositol 3-kinase in 3T3-L1 adipocytes without affecting glucose transport, J. Biol. Chem. 273 (1998) 25139–25147.
[32] X. Gao, P.R. Lowry, X. Zhou, C. Depry, Z. Wei, G.W. Wong, et al., PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 14509–14514.
[33] A. Morcavallo, M. Genua, A. Palummo, E. Kletvikova, J. Jiracek, A.M. Brzozowski, et al., Insulin and insulin-like growth factor II differentially regulate endocytic sorting and stability of insulin receptor isoform A, J. Biol. Chem. 287 (2012) 11422–11436.
[34] M. Jensen, B. Hansen, P. De Meyts, L. Schäffer, B. Ursø, Activation of the insulin receptor by insulin and a synthetic peptide leads to divergent metabolic and mitogenic signaling and responses, J. Biol. Chem. 282 (2007) 35179–35186.