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Amylin effect in extrapancreatic tissues participating in glucose homeostasis, in normal, insulin-resistant and type 2 diabetic state

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ABSTRACT

Amylin is co-secreted with insulin, responds to the same stimuli, is anorectic, lowers body weight by reducing fat mass, and is proposed for diabetes treatment. We examined the effect of a 3-day constant infusion of close to physiological doses of amylin in Wistar rats, on glucotransporter expression, glycogen content (G), glycogen synthase *a* activity (GS*a*) and glucose transport (GT), in liver, muscle and fat from insulin resistant (IR) and type 2 diabetic (T2D) models, compared to normal (N) animals; plasma glucose and insulin were measured. Plasma insulin in IR was higher than in N or T2D, and amylin normalized the value. In both, IR and T2D, liver G was lower than normal, accompanied by GLUT-2, mRNA and protein, higher and lower, respectively, than in N; amylin normalized G in both groups, without changes in GLUT-2, except for an mRNA increase in T2D. In IR and T2D, muscle GS*a* was reduced, together with respective over- and under-GLUT-4 expression; amylin induced only a trend toward GS*a* normalization in both groups. In isolated adipocytes, GT and GLUT-4 in IR and T2D were lower and higher, respectively, than in N; after amylin, not only GT was normalized in both groups but also the response to insulin was much more pronounced, including that in N, without major changes in GLUT-4. This suggests that the beneficial effect of amylin in states running with altered glucose homeostasis could occur by partially acting on the hexose metabolism of the liver and mainly on that of the adipose tissue.

1. Introduction

Amylin, also called islet amyloid polypeptide, is a 37 aminoacid peptide synthesized mainly in the pancreatic islet β-cell; it is co-secreted with insulin and responds to the same stimuli that evoke insulin release [21,33], despite the fact that there is a differential regulation of amylin and insulin genes. Apart from the fact that tissue-specific expression of the rat amylin gene were shown to occur only in pancreatic β-cell [27], detectable quantities of amylin have been localized in islet non-β-cells, lung and nervous system and, in a lower extent, in several sites of the gut [9].

Amylin is released after food intake, and shows the characteristics of an endocrine hormone that regulates fuel metabolism in association with other metabolic, endocrine and neural influences [9]; in addition, by primarily acting at the hindbrain, and also at the rostral central nervous system region, amylin reduces food intake at physiological concentrations [54]. The latter fulfills the criteria of a peripheral satiety signal [4,28,41], which occurs upon amylin binding to specific receptors within the area postrema [7]. Also, amylin decreases body weight gain and reduces adiposity, and this hormone has been proposed as a necessary co-adjuvant of leptin in the therapy of obesity [43]. Other actions of amylin have been described on the renal proximal tubular cells and the endothelial vasculature, which is considered in some states of impaired amylin regulation as a link in the pathogenesis of hypertension associated to diabetes [2,22].

It is well known that in men and other mammals, amylin can adopt a fibrillar structure to form amyloid deposits, an event that is linked to a reduction of β-cell mass and loss of signal-secretion coupling [6,15]. Based on previous results from *in vitro* studies, in which

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it was tested the effect of high concentrations of amylin on glucose transport and metabolism in rat extrapancreatic tissues [6,54] or cell lines [31], together with the fact that amylin is the major peptide in human islet amyloid deposits founded in the pancreas of type 2 diabetic patients [18], it was postulated that this peptide could have responsibility in the development of insulin resistance. Nevertheless, far from the adverse effects of amylin under pathological conditions, analogs of amylin are currently well considered as therapeutic agents in type 1 and type 2 diabetes [26,36] showing also potential benefits in the obesity syndrome [12].

In the present work we have searched for the action of physiological doses of amylin, in prolonged treatment, on the major corresponding glucotransporter expression in rat extrapancreatic tissues–liver, muscle and fat-, and also on parameters related with the glucose transport and/or metabolism, in normal, insulin-resistant and type 2 diabetic states.

2. Materials and methods

Animal housing and protocols were submitted to, and approved by, the Animal Use Committee of the IIS-Fundación Jiménez Díaz, Madrid, Spain.

2.1. Reagents

Rat amylin, insulin, GLP-1 (Bachem AG, Bubendorf, Switzerland); Amylin ELISA kit (Millipore Co., MA, USA); TRI REAGENT™, glycogen, UDP-glucose, anthrone reagent and anti-α-tubulin (Sigma Aldrich Quimica S. A., Madrid, Spain); Collagenase P of Clostridium hystolitium (Roche Diagnostics, Indianapolis, IN, USA); probes and primers for rat GLUT-4 (Rn00652597_m1), GLUT-2 (Rn00563565_m1), Eukaryotic 18s (4319413E) and rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 99999916_s1), High Capacity cDNA reverse transcription kit and Taqman Universal PCR master mix (Applied Biosystems, Woolston, Warrington Cheshire, UK); osmotic pumps (Alzet 1003D, Alza, Palo Alto, CA, USA); NuPage 12% Bis–Tris gels, Mops SDS running buffer, transfer buffer and iBlot™ dry blotting system (Invitrogen, Carlsbad, CA, USA); 2-deoxy-D-[1,2-3H(N)]glucose (40 Ci/mmol, Moravek Biochemicals, Brea, CA, USA); [U–^{14}C]sorbitol (320 mCi/mmol, American Radiolabeled Chemicals, Inc., St. Louis, MO, USA); uridine diphosphate glucose [glucose^{14}C (U)], 302 mCi/mmol, Perkin Elmer, Boston, MA, USA; dioctyl phthalate (Acros Organics, NJ, USA); rainbow molecular weight markers, ECL-Western blotting kit, Hyperfilm ECL, horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin and anti-mouse immunoglobulin (Amersham Pharmacia Biotech, Buckinghamshire, UK); anti-glucose transporter-4 rabbit PAb and anti-glucose transporter-2 rabbit PAb (Calbiochem, Darmstadt, Germany). All other commonly used chemicals were from Sigma or Merck (Merck Pharma Quimica, S.A., Barcelona, Spain).

2.2. Animals

Male Wistar rats, kept on a standard pellet diet (UAR Panlab, Barcelona, Spain) and tap water *ad libitum*, were used. The insulin-resistant model was prepared in normal rats (5 weeks old) by chronic feeding, during 8 weeks, with standard chow combined with D-fructose dissolved in the drinking water at 20% concentration [5]. The type 2 diabetic model was obtained in normal rats by a single dose of streptozotocin dissolved at 100 μg/g body weight, in saline solution (0.9% NaCl), intraperitoneally administered on the day of birth [40]; at the age of 8 weeks, those rats showing a glucose disappearance constant (K) below 2.5×10^{-2}/min during an *i.v.* glucose tolerance test (0.05 mg of glucose/g of body weight, in 30 s) were selected. All rats of the same three groups were having the same age (12–13 weeks old) by the time to apply the experimental protocol of the study.

2.3. Experimental design

Normal (N), insulin-resistant (IR) and type 2 diabetic (T2D) rats were subjected to a 3-day treatment with amylin at the dose of 0.03 nmol/kg/h dissolved in saline solution, by continuous infusion through a subcutaneously implanted osmotic pump. Treatment of the rats from the three groups was initiated at 9:00 am. As respective control, rats of the three groups were treated only with saline solution. Just before and by finishing the treatment (72 h), blood samples were collected from all rats, in fed condition, for plasma glucose and insulin determinations; afterwards, the animals were stunned and killed by a sharp blow to the head. Tissues–liver, muscle and epididimal fat – were collected and immediately frozen at −70 °C for the measurement of GLUT-2 and GLUT-4 expression – mRNA and protein – in solubilized membranes [51] or, in the case of the liver, also glycogen content, by the anthrone method [8]. In the muscle, we measured glycogen synthase *a* activity, as this is a key enzyme in the metabolic response to insulin leasing to increase glycogen synthesis. In epididimal fat pads from some rats of all experimental groups and conditions were used to isolated adipocytes by enzymatic digestion [42], to study their glucose uptake characteristics but not GS*a*, as the main role of glucose in the adipocytes is to be acting precursor for triglycerides synthesis [38]. In the three groups of rats, no changes in the body weight were detected after any treatment.

2.4. Plasma measurements

Glucose was determined by the glucose oxidase method (Glucose analyzer 2; Beckman, Galway, Ireland); insulin was measured by radioimmunoassay [16], using rat insulin as standard and a polyclonal anti-insulin serum (GP-25) developed in our laboratory [49], amylin was determined by ELISA.

2.5. Immunoblotting

Equal amounts of tissue lysate from each sample were subjected to SDS-PAGE [25], in parallel with molecular weight markers, on 12% NuPage bis–tris gel; the separated proteins were then transferred to a nitrocellulose membrane in a dry system. For immunodetection, a Western blotting kit was used following the manufacturer's instructions, using GLUT-2, GLUT-4 and tubulin respective antibodies, and a horseradish peroxidase-conjugated donkey anti-rabbit and anti-mouse immunoglobulin second antibody, with detection by the enhanced chemiluminiscence method and quantization by densitometric scanning of the autoradiography [51]. In all experiments, the densitometric value of the band corresponding to rats treated with saline was used as control value.

2.6. Isolation of total RNA and real time PCR

Total RNA was extracted from liver, muscle and fat by the TRI reagent isolation method, accordingly to the manufacturer's protocol. For quantitative real-time PCR, the first strand cDNA was synthesized from 4 μg of total RNA using the High Capacity cDNA Reverse Transcription Kit. Samples were subjected to quantitative amplification using the TaqMan probe and primer sets for rat GLUT-2 and GLUT-4, respectively. PCR amplification was carried out in triplicate for each sample, and performed in a total volume of 20 μl containing 400 ng of cDNA, 900 nM each primer, 500 nM of the respective probe, and 6 μl of TaqMan Master Mix. The conditions of amplification and detection were as described [3]. Gene expression

was normalized with that of the housekeeping gene 18s, and with that of GAPDH in the case of fat tissue.

2.7. Glucose transport

Isolated adipocytes were resuspended in Krebs–Ringer Bicarbonate buffer supplemented with HEPES, Trasylol, BSA and without or with D-glucose, pH 7.4, at a density of 10^6 cells/ml. Cells (10^5) were incubated for 15 min at 37 °C in 400 μl KRB, 10.9 mM HEPES, 500 U/ml Trasylol and 2% BSA, pH 7.4, in the absence (basal) and presence of 10^{-9} M insulin; this was followed by a 3-min incubation in the additional presence of 0.26 μCi (6.5 pmol) 2-deoxy-D-[1,2–3 H(N)]glucose (final concentration 16.3 nM 2-DOG). Adipocytes, after being separated at 12,000 rpm in 100 μl dioctyl phthalate, were added to 3 ml scintillation liquid for β-counting. The total D-glucose content was corrected for the unspecific D-glucose uptake value, obtained in cell samples from each experiment treated in parallel with 0.175 mM cytochalasin B [38].

2.8. Glycogen synthase a activity

The *soleus* muscle samples were homogenized in a medium containing 100 mM NaF, 35 mM EDTA, 50 mM glycilglycine and 0.5% glycogen (w/v) at pH 7.4, as described [50], and maintained at −70 °C until the enzymatic activity was assayed. The frozen tissue homogenates were thawed at 4 °C, and their enzymatic activity was assayed, at least in duplicate, as the incorporation of UDP-glucose into glycogen, during 15 min, as previously described in detail [50]. In each experimental rat, the mean value of the replicates corresponding to the muscle of the rat saline-treated was used as control value.

2.9. Statistical study

Results are expressed as mean ± SEM, together with the number of observations. The statistical significance ($p < 0.05$) of the increments was assessed either by one-way analysis of variance, followed by the least significant differences (LSD) test for post hoc multiple comparisons, using the Statistical Package for the Social Science (SPSS) software or the Student's *t*-test.

3. Results

3.1. Plasma measurements

Table 1 contains the glucose, insulin and amylin values in plasma of the three groups of rats – N, IR and T2D – before (basal) and after saline (control) or amylin treatment. As previously observed [35], basal insulin in IR rats was higher ($p < 0.05$) than that in N, and so it was the plasma glucose ($p < 0.05$); in the T2D group, insulin was lower ($p < 0.001$) than in N, while the glucose value was higher ($p < 0.001$). Three days treatment with amylin did not affect the plasma glucose values in any group, but significantly reduced ($p < 0.001$) those of insulin in IR and, although slightly, also in T2D rats. Basal plasma amylin values in the three groups were undistinguishable from each other, and amylin infusion significantly increased three times the basal values in all of them, reaching circulating amylin levels close to 10^{-10} M.

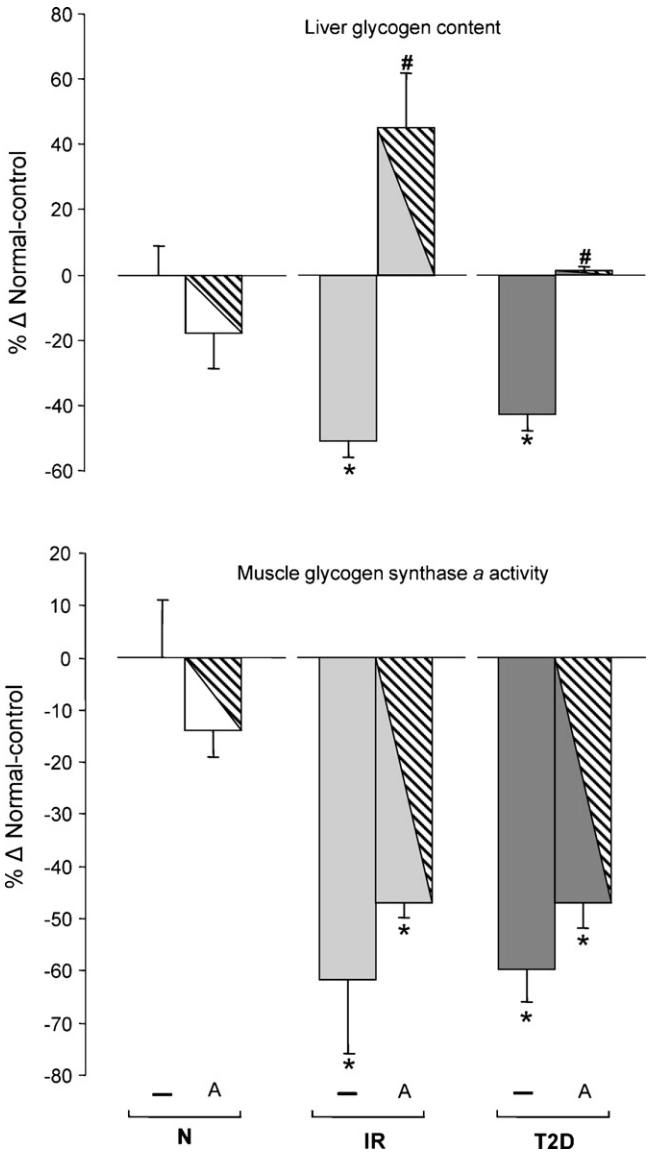

Fig. 1. Liver glycogen content (upper panel) and muscle glycogen synthase *a* activity (lower panel) in normal (N, ▭), insulin-resistant (IR, ▭) and type 2 diabetic (T2D, ▭) rats, three days treated with saline (control, –) or amylin (A, ▨). Results are expressed as mean ± SEM; $^{*}p < 0.05$ or lower vs N; $^{\#}p < 0.05$ or lower vs respective control saline treated.

Table 1
Plasma values in fed rats normal, insulin resistant (IR) and Type 2 diabetic (T2D) models, before (basal) and during 3-day (24 and 70 h) treatment with saline (control) or amylin [mean ± SEM, number of rats in parenthesis].

	Normal			IR			T2D		
	Basal (18)	Saline (9)	Amylin (9)	Basal (20)	Saline (11)	Amylin (9)	Basal (24)	Saline (10)	Amylin (14)
Glucose (mg/dl)	134 ± 2	141 ± 4	139 ± 7	140 ± 2*	138 ± 4	147 ± 6	169 ± 5*	160 ± 12	156 ± 5
Insulin (ng/ml)	0.88 ± 0.04	0.91 ± 0.11	0.92 ± 0.13	0.96 ± 0.05	0.80 ± 0.04	0.57 ± 0.08#	0.63 ± 0.04*	0.66 ± 0.11	0.52 ± 0.05
Amylin (fmol/ml)	14 ± 2	18 ± 5	36 ± 6#	14 ± 2	16 ± 2	31 ± 4#	17 ± 4	18 ± 7	36 ± 6#

* $p < 0.05$ vs normal basal value.
\# $p < 0.05$, or lower, vs its respective basal value.

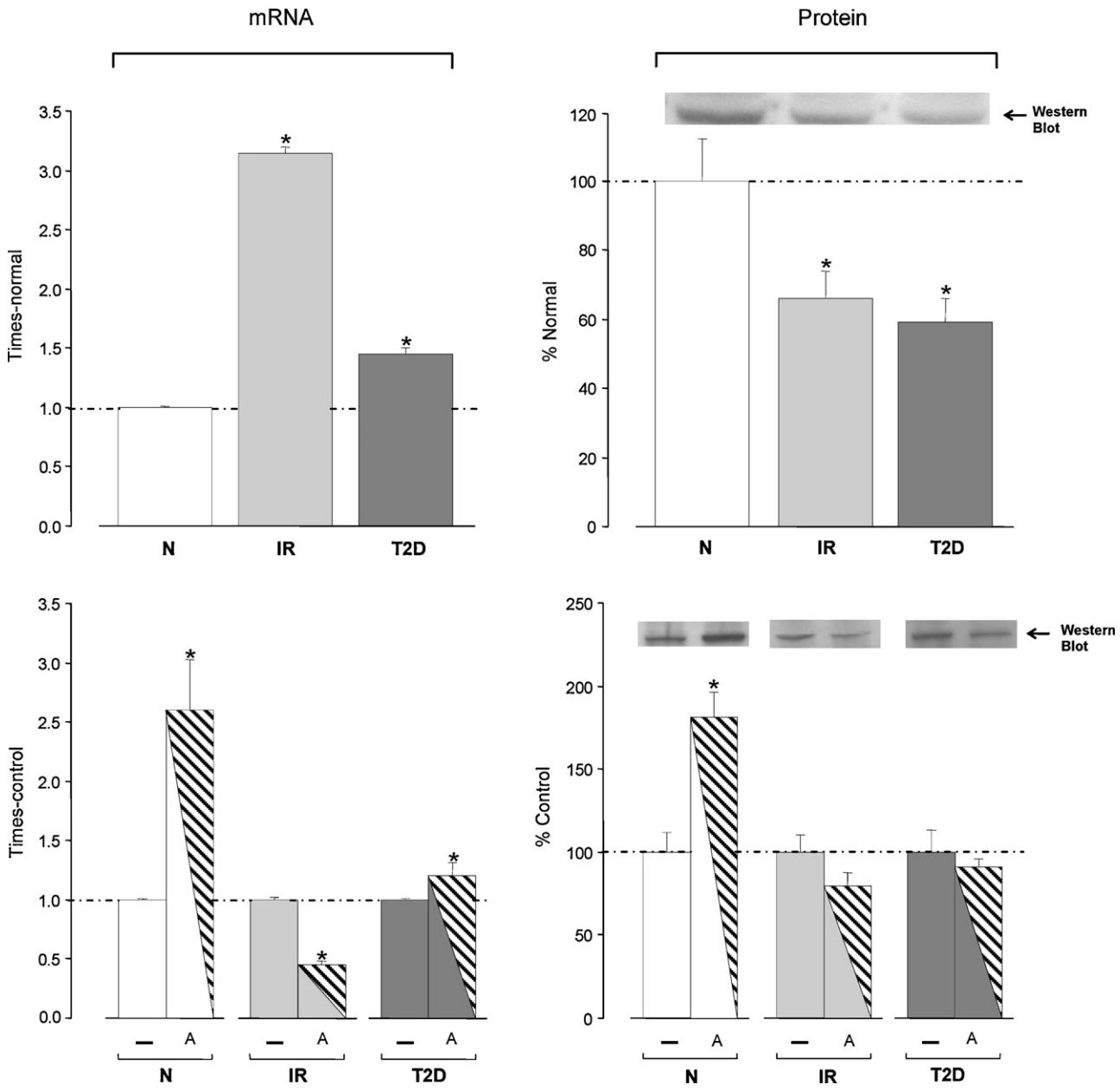

Fig. 2. Liver GLUT-2 expression – mRNA and protein – in normal (N, ▭), insulin-resistant (IR, ▭) and type 2 diabetic (T2D, ▭) rats, three days treated with saline (control, –) or amylin (A, ▨). Results are expressed as mean ± SEM; $^{*}p < 0.05$ or lower vs N; $^{\#}p < 0.05$ or lower vs respective control saline treated.

3.2. Liver glycogen and GLUT-2 expression

In IR-control rats (Table 2 and Fig. 1), liver glycogen content (193 ± 20 μg/mg protein, $n = 8$ rats) was much lower ($p < 0.001$) than that in N-control (400 ± 22 μg/mg, $n = 6$), and so it was that in T2D-control (220 ± 15 μg/mg, $n = 6$, $p < 0.001$ vs N-control), in the latter case as previously observed [2]. Amylin treatment did not modify the liver glycogen concentration in N (322 ± 45 μg/mg, $n = 5$), but highly increased the value in IR (573 ± 66 μg/mg, $n = 5$, $p < 0.01$ vs IR-control) and also, although moderately, in T2D (398 ± 4 μg/mg, $n = 5$, $p < 0.001$ vs T2D-control), amylin in both groups a normalizing effect in liver glycogen content.

In the IR-control rats, GLUT-2 mRNA value (Table 2 and Fig. 2) was higher ($p < 0.001$) than that in N (3.14 ± 0.05 times N-control, $n = 11$), and so it was, although in a lower extent, in T2D-control (1.45 ± 0.05 times N-control, $n = 8$, $p < 0.001$); amylin treatment increased the gene expression in N (2.60 ± 0.43 times N-control, $n = 9$, $p < 0.01$) and T2D (1.21 ± 0.10 T2D-control, $n = 7$, $p < 0.05$), while reducing that in the IR group (0.45 ± 0.03 times IR-control, $n = 9$, $p < 0.001$). GLUT-2 protein in IR was much lower than that in N (66 ± 8% N-control, $n = 8$, $p < 0.01$), and so it was that in the T2D group (59 ± 7% N-control, $n = 11$, $p < 0.01$) as previously observed [2]; amylin treatment increased the value in N (182 ± 15% N-control, $n = 7$, $p < 0.001$), but did not apparently affect that in IR (80 ± 8% IR-control, $n = 5$) or T2D (92 ± 5% T2D-control, $n = 5$).

3.3. Muscle glycogen synthase a and GLUT-4 expression

In IR-control rats (Fig. 1 and Table 2), glycogen synthase a activity (0.39 ± 0.14 U/g protein, $n = 4$) was lower ($p < 0.05$) than that in N-control (1.03 ± 0.11 U/g protein, $n = 4$), and, as previously observed [32], the same lower than normal value ($p < 0.05$) was detected in the T2D group (0.40 ± 0.06 U/g protein, $n = 4$); amylin treatment did not modify the synthase a activity in N rats, but

Table 2
GLUT-2 and GLUT-4 expression, and glucose metabolism parameters, in insulin resistant (IR) and type 2 diabetic (T2D) rats respect normal (N) animals, and effect of 3-day treatment with amylin (A) relative to their respective control (c) value (saline treated).

		Normal		IR		T2D	
		N-c vs N-c	A vs N-c	IR-c vs N-c	A vs IR-c	T2D-c vs N-c	A vs T2D-c
Liver	Glut-2, protein/mRNA	–/–	↑/↑	↓/↑	↓/↓	↓/↑	–/↑
	Glycogen content	–	↓	↓	↑	↓	↑
Muscle	Glut-4, protein/mRNA	–/–	–/–	↑/↑	↑/–	↓/↓	↓/↓
	Glycogen synthase *a*	–	↓	↓	↑	↓	–
Fat	Glut-4, protein/mRNA	–/–	↓/↓	–/↓	↑/↓	↓/↓	↑/–
	Glucose transport	–	↓	↓	↑	↑	↑

–, indistinct from control; and significantly higher and lower, respectively, than control; ↑ and ↓, slightly higher and lower, respectively, than control.

significantly stimulated that in IR (140 ± 8% IR-control, $n = 8$, $p < 0.01$) and, apparently, in T2D (138 ± 12% T2D-control, $n = 4$), amylin showing a trend to normalize the deleterious enzyme activity value in the muscle of both experimental group.

Muscle GLUT-4 mRNA (Fig. 3, Table 2) in IR-control rats (2.53 ± 0.35 times N-control, $n = 5$) was higher ($p < 0.01$) than that in N-control, whereas that in the T2D group showed to be lower (0.53 ± 0.13 times N-control, $n = 4$, $p < 0.01$); while amylin

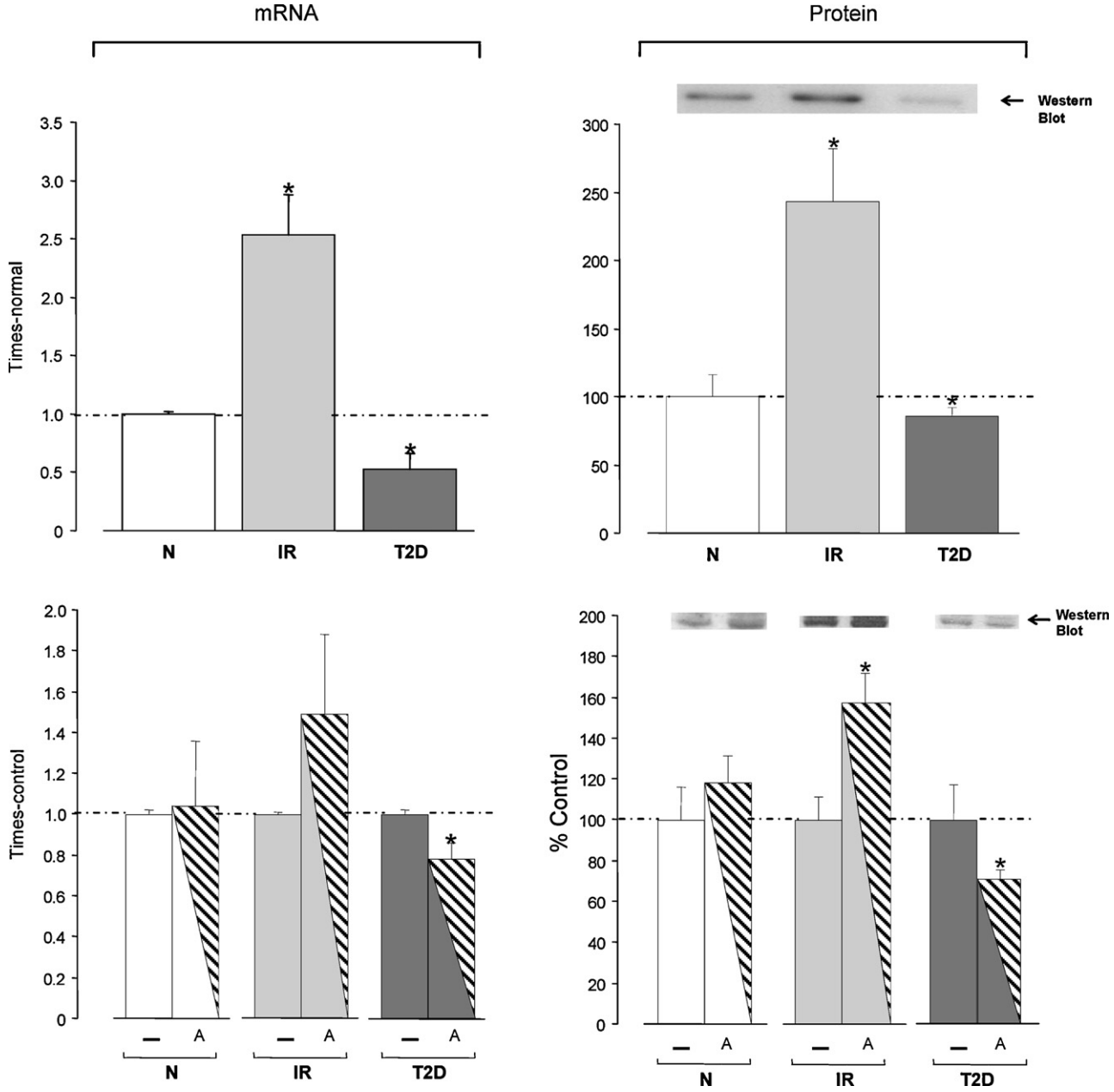

Fig. 3. Muscle GLUT-4 expression – mRNA and protein – in normal (N, ☐), insulin-resistant (IR, ▨) and type 2 diabetic (T2D, ■) rats, three days treated with saline (control, –) or amylin (A, ▨). Results are expressed as mean ± SEM; *$p < 0.05$ or lower vs N; #$p < 0.05$ or lower vs respective control saline treated.

treatment apparently increased GLUT-4 mRNA in the IR group, the value in T2D became slightly reduced ($p < 0.05$ vs T2D-control). GLUT-4 protein levels in IR-control were much higher than those in N (243 ± 39% N-control, $n = 4$, $p < 0.05$), whereas in T2D, as previously observed [2], a small reduction was detected ($p < 0.05$ vs N-control); amylin treatment further increased the GLUT-4 protein level in IR (157 ± 15% IR-control, $n = 4$, $p < 0.05$) while slightly reducing that in T2D (72 ± 4% T2D-control, $n = 7$, $p < 0.001$).

3.4. Fat glucose transport and GLUT-4 expression

In isolated adipocytes from IR-control rats (Fig. 4 and Table 2), glucose uptake (8.1 ± 0.5 fmol/10^5 cells, $n = 6$) was lower ($p < 0.01$) than that in N-control (15.1 ± 1.6 fmol/10^5 cells, $n = 12$), while that in cells from T2D-control, and accordingly with previous observation [45], it showed to be much higher (45.1 ± 2.7 fmol/10^5 cells, $n = 23$, $p < 0.001$); amylin treatment slightly reduced the value in N (85 ± 6% N-control, $n = 4$) and T2D (22 ± 3% T2D-control, $n = 4$, $p < 0.001$), and increased that in IR (136 ± 10% IR-control, $n = 5$, $p < 0.02$). In both experimental groups, amylin treatment induced a trend toward normalization of the altered adipocyte glucose transport (IR and T2D overall mean: 70 ± 5% N-control).

In a separate set of experiments, we meant to search for the *in vitro* glucose uptake response to 10^{-9} M insulin, in adipocytes from the three groups and conditions, the value obtained in the absence of insulin being considered as basal value (Fig. 4). In cell from N-amylin treated, insulin induced an increase in glucose transport (513 ± 41% basal, $n = 4$ rats, $p < 0.001$) which was even higher ($p < 0.001$) than that previously detected in N-control rats [45]; the same higher increasing effect of insulin ($p < 0.001$) was detected in cells from the IR-amylin treated rats (226 ± 21% basal, $n = 4$, $p < 0.001$) respect that in adipocytes from the IR-control group (135 ± 9% basal, $n = 7$, $p < 0.01$), and so it was the insulin-induced increment in T2D-amylin treated (343 ± 32% basal, $n = 4$, $p < 0.001$), respect that previously detected [45] by insulin in cells from untreated T2D rats ($p < 0.001$).

GLUT-4 mRNA level in IR-control rats (Fig. 5 and Table 2) was lower than in N (0.52 ± 0.05 times N-control, $n = 8$, $p < 0.001$), and so it was that in T2D (0.26 ± 0.09 times N-control, $n = 4$, $p < 0.02$); amylin treatment did not apparently modify the gene expression in N or T2D, but further reduced that in IR (0.67 ± 0.09 times IR-control, $n = 6$, $p < 0.02$). GLUT-4 protein value in IR rats was unaltered, but in the T2D group it showed to be lower than normal (72 ± 6% N-control, $n = 6$, $p < 0.05$); amylin treatment, while reducing ($p < 0.01$) the protein level in N, exerted an increasing effect in IR (168 ± 17% IR-control, $n = 5$, $p < 0.02$) and, apparently, also in T2D rats (129 ± 13% T2D-control, $n = 6$).

4. Discussion

In order to evaluate the short-term action of amylin in the glucose metabolism of extrapancreatic tissues involved in the regulation of glucose homeostasis, in the present study we have analyzed the effect of a 3-day constant infusion close to physiological doses of amylin in normal rats and in two well characterized experimental rats models, insulin-resistant and type 2 diabetic [5,40]. During amylin treatment, this hormone reached concentrations in plasma slightly over physiological levels, lower though than those being used for therapeutic purpose [19], but similar to those observed in some pathological conditions such as obesity, pregnancy [23] and type 2 diabetes carrying polymorphism in the amylin gene [34].

As expected, the IR model of the present study showed a higher basal plasma insulin levels than those in both T2D and normal rats, which were significantly reduced though after

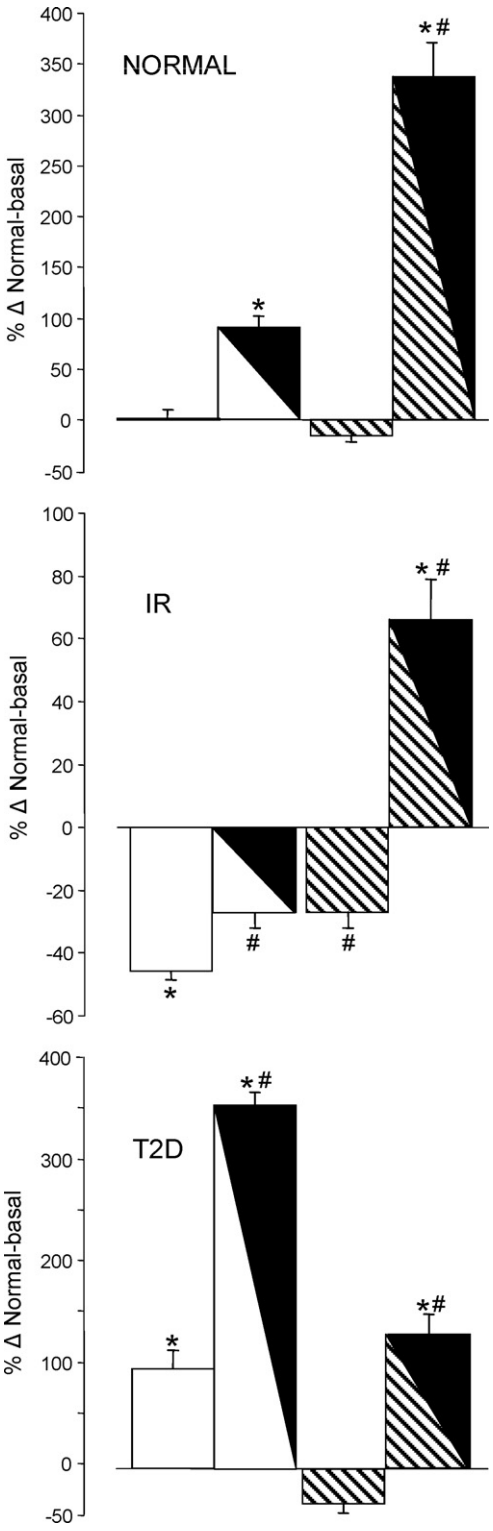

Fig. 4. *In vitro* effect of 10^{-9} M insulin ( ) on glucose transport in isolated adipocytes from normal, insulin-resistant (IR) and type 2 diabetic (T2D) rats, saline treated (control) and treated with amylin ( ). All results (mean ± SEM) are expressed as percent increment of value obtained in cells from normal control rats, incubated in the absence (basal) or presence of 10^{-9} M insulin. $^{*}p < 0.05$ or lower vs normal basal; $^{\#}p < 0.05$ or lower vs its respective basal amylin treated.

amylin infusion; however, no changes were detected in the latter groups after amylin. This effect of amylin in IR is in accordance with results from previous reports in which suppression of insulin secretion by amylin was demonstrated in perfused pancreas [11] and isolated islets [48], and also in experimental models of

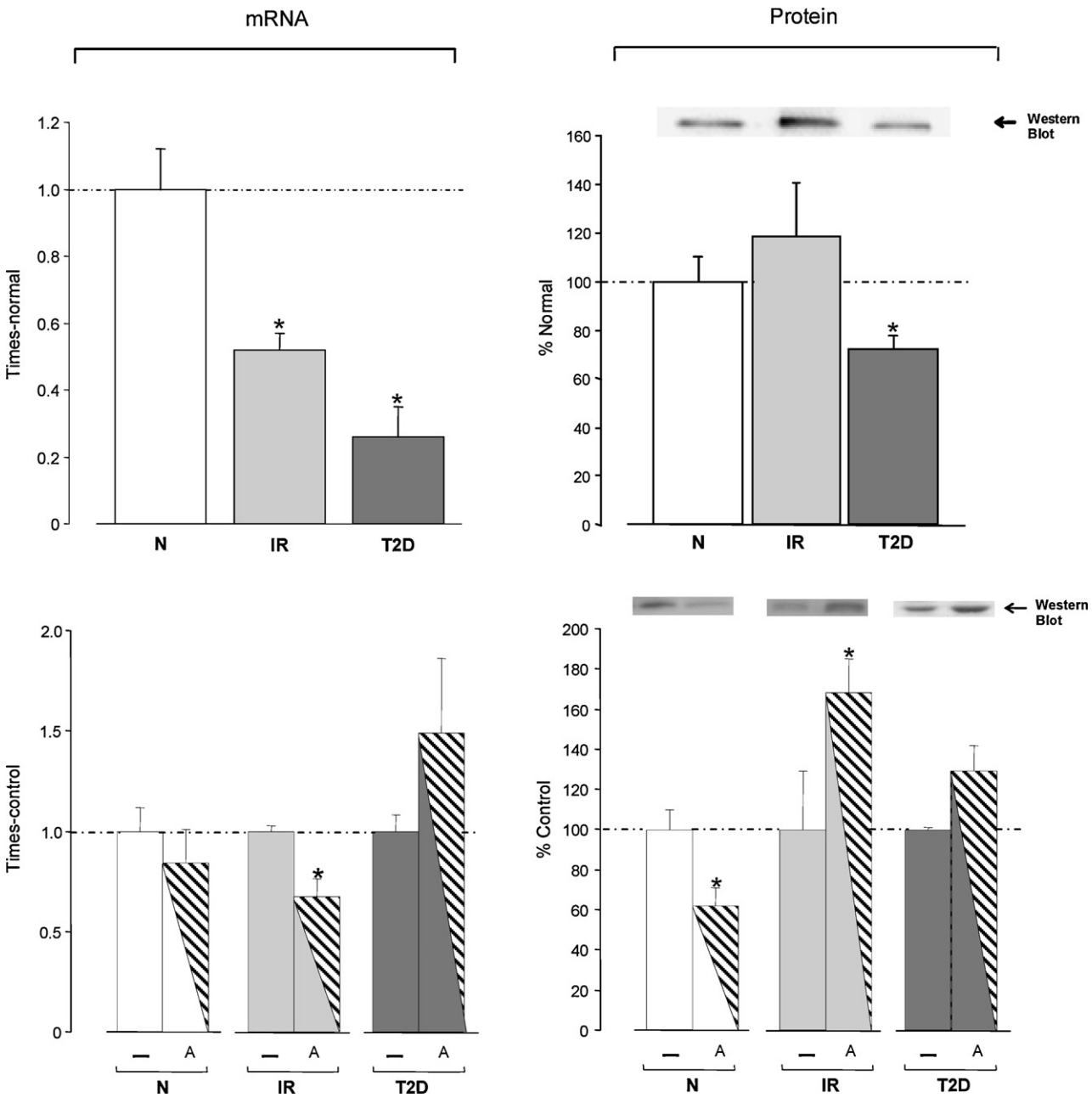

Fig. 5. Fat GLUT-4 expression – mRNA and protein – in normal (N, ☐), insulin-resistant (IR, ▭) and type 2 diabetic (T2D, ▬) rats, three days treated with saline (control, –) or amylin (A, ▨). Results are expressed as mean ± SEM; $^{*}p < 0.05$ or lower vs N; $^{\#}p < 0.05$ or lower vs respective control saline treated.

intracellular overproduction of amylin [1]; yet, the mechanism by which this occurs remains to be elucidated.

The liver glycogen content in the IR and T2D groups of the present study were lower than normal, confirming previous results in the case of T2D [2], and after amylin treatment the values increased up to normal in both pathological states. It is well known that amylin exerts a glucagonostatic effect [14], suppressing glucagon secretion during pancreas perfusion [47]; it was also demonstrated that suppression of glucagon secretion after amylin treatment ameliorates postprandial hyperglycemia in diabetic patients [13]; nevertheless, others have shown that amylin stimulates the endogenous glucose production *in vivo*. However, it has been reported that amylin has no effect in the intermediary carbohydrate metabolism in rat isolated hepatocytes or perfused liver, the authors speculating that augmentation of endogenous glucose production was secondary to the release of lactate from the muscle, thereby stimulating gluconeogenesis by increasing the availability of the substrate [52].

Data from a work meant to examine the *in vivo* effects of amylin on insulin-mediated carbohydrate metabolism in conscious rats, and by using the euglucemic/hyperinsulinemic clamp technique combined with [3-^{3}H]-glucose infusion [24], have shown that supraphysiological levels of amylin antagonize only the insulin action on liver, resulting in a 100% increase in hepatic glucose output; from that study, in which a very high concentrations of amylin were applied, it seemed that amylin causes *in vivo* insulin resistance, and that the liver is the predominant organ regulated by this hormone. Based on the results from the present study, in which different experimental conditions have been used, it could be speculated that suppression of glucagon by low doses of amylin infusion in the IR rats could favor the observed increase in liver glycogen content and so helping to reverse the insulin-resistant state. This dual effect

has also been shown in other extrapancreatic tissues: in vessels, slightly supraphysiological concentrations of amylin induce non-endothelial dependent vasodilatation but endothelial-dependent vasoconstriction [34]. Similarly to the effect of amylin in the liver, this hormone induced, mainly in the IR group (present study), an increase in the initially reduced muscle glycogen synthase *a* activity, together with a positive effects on GLUT-4 mRNA and protein levels.

One of the biological actions of amylin early described was its ability to inhibit the insulin-induced stimulation of glycogen synthase *a* activity and glycogenesis in rat isolated soleus muscle [10]. Based on those results, for which amylin was tested at high doses (close to 10^{-7} M), the authors proposed that this hormone is involved in the pathogenia of insulin-resistance and type 2 diabetes, as its lowering action on insulin-stimulated glycogen accumulation could be due to an increase in glycogenolysis, through a stimulation of glycogen phosphorylase a activity via cAMP, protein kinase C-dependent signaling pathway. Also, in normal rats, amylin secreted in response to meal absorption seems to mobilize carbon from the skeletal muscle, the amylin-inducing glycogenolysis resulting in an intramuscular accumulation of glucose-6-phosphate and release of lactate from tissue beds [9]. The present data in normal and T2D rats, in which no changes in this enzymatic activity was detected, are in accordance with the above observations; however, in the IR model of the present study, an increasing action of amylin on the muscle glycogen synthase a activity is shown, indicating that amylin, in these metabolic conditions, would be stimulating glycogen synthesis in this tissue.

Finally, in fat, which is an important tissue because of its role in the generation of signals involved in the cross-talk between obesity and diabetes, we have observed that GLUT-4 expression was lower than normal in the T2D model of the present study, confirming previous results [51], and that this was accompanied by a higher than normal adipocyte glucose transport value, the latter confirming previous observations [45]; yet, in the IR group, fat GLUT-4 mRNA was down regulated, accompanied by no changes detected in the protein levels. Amylin treatment induced a further decrease in the mRNA and significantly stimulated the protein value in the IR group, while in the T2D rat, a trend toward normalization was detected in both parameters. Apart from this, it is of interest the changes observed after amylin treatment in the glucose uptake characteristics of the adipocyte, tested *in vitro*. In fact, in normal rats, amylin did not modify the basal glucose transport basal value (adipocytes incubated in the absence of hormones), but the cells responded to insulin in a much higher degree than those from normal control animals; also, in both experimental groups, IR and T2D, the *in vitro* response to insulin after amylin treatment was more effective than that of cells from their respective control group (saline treated); and in both cases, it was also higher than that to insulin in normal control adipocytes, observation which was much more evident in adipocytes from the amylin-treated IR model.

As far as we know, no information has been provided about a short-term effect of a treatment with a moderate dose of amylin in the adipocyte glucose uptake parameter. It is known, though, that amylin not only shows characteristics of satiation signals, like CCK or GLP-1, but also adiposity signals, like leptin and insulin [17,46,53]. In fact, and although information about amylin levels in individual animals throughout the development of obesity is lacking, it is known that basal plasma amylin is higher in obese than in lean individuals [39]. Also, it has been reported that chronic peripheral [30] or central [44] amylin infusion decreases body weight gain by specifically reducing fat mass, and this effect has been associated with a detected increasing action of amylin on energy expenditure [20,36]. In addition, other data have suggested that the lowering effect of amylin on body adiposity occurs through its stimulating action on lipid metabolism, as indicated by a lower respiratory quotient [29]. Nevertheless, the results from the present study show that 3-day constant infusion of a moderate dose of amylin exerts a normalizing effect in the deleterious glucose uptake by adipocytes from these experimental IR and T2D rat models; not only that, the data also show that, after amylin, the cells normally respond to insulin in the case of T2D, and in a much higher degree in that of IR, than adipocytes from normal control animals.

In summary, the results from the present study suggest that the reported beneficial effect of amylin in states running with an altered glucose homeostasis could occur by amylin partially acting on the hexose metabolism of the liver and mainly on that of the adipose tissue.

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