

## Effects of a New Hypoglycemic Agent A-4166 on Glucose Metabolism in Rat Adipocytes and Muscle Tissues

L. MACHO, M. FICKOVÁ, Š. ZÓRAD, E. ŠEBÖKOVÁ AND I. KLIMEŠ

*Institute of Experimental Endocrinology, Slovak Academy of Sciences,  
Bratislava, Slovakia*

**Abstract.** The effects of the oral administration of a non-sulfonylurea hypoglycemic agent, the phenylalanine derivative A-4166, on serum insulin and glucose levels and glucose metabolism in isolated rat adipocytes and slices of muscle tissues were studied. An increase in serum insulin and a decrease in glucose levels were observed 30 minutes after A-4166 administration to rats fed basal or high fat diet. No changes in basal glucose transport in isolated fat cells were observed after the administration of A-4166. The effect of *in vitro* added insulin was, however, stronger in rats fed basal diet and treated with A-4166. An elevation of the membrane glucose transporter GLUT 4 was observed in rats treated with A-4166. An increase of basal lipogenesis, measured by incorporation of radiocarbon labeled glucose into lipids, was noted in adipocytes from rats fed high fat diet. The addition of insulin was followed by stimulation of lipogenesis in rats fed basal diet, however, this hormone had no effect in rats fed high fat diet. The administration of A-4166 did not affect the basal or insulin stimulated lipogenesis. Basal glucose oxidation in the diaphragm was not influenced by high fat diet or by A-4166 treatment. In the soleus muscle, basal glucose oxidation was decreased in rats fed high fat diet, and treatment with A-4166 increased the glucose oxidation up to values observed in the control basal diet fed rats. These results indicate that the administration of A-4166 can affect glucose metabolism in muscle tissue and the sensitivity of adipocytes to insulin.

**Key words:** Hypoglycemic agent — Insulin — Glucose — Adipocytes — Muscle tissue

### Introduction

The new class of non-sulfonylurea hypoglycemic agents, *d*-phenylalanine derivatives (e.g. nateglinide, SDZ DJN 608, A-4166) are compounds stimulating early insulin release important for the regulation of glycemia after glucose load and meal

---

Correspondence to: Ladislav Macho MD, DSc, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárská 3, 833 06 Bratislava, Slovakia.  
E-mail: ueenmach@savba.savba.sk

intake (Sato et al. 1991). Experimental studies in mice, rats and dogs *in vivo* have demonstrated that A-4166 is able to stimulate insulin secretion with a rapid onset and short duration of action (Shinkai and Sato 1990; Fujitani and Yada 1994). The rapid stimulatory effect of A-4166 on insulin release was also proven by incubation of isolated rat pancreas islets, and the stimulation was related to the threshold of glucose concentration (Tsukuda et al. 1998). Studies of the mechanism of A-4166 action on insulin secretion demonstrated that this hypoglycemic substance mediates its effect by binding to the sulfonylurea receptors located in beta-cells very close to the ATP-sensitive  $K^+$  channel (Panten et al. 1992; Fujita et al. 1996; Tsukuda et al. 1998). The possible presence of a specific receptor or binding sites for A-4166 in pancreatic beta-cells has also been suggested. The early insulin release is followed by a decrease of the blood glucose concentration, inhibition of hepatic glucose production and promotion of hepatic glucose uptake (Shinkai et al. 1989; Shinkai and Sato 1990; Sato et al. 1991). However, the influence of A-4166 administration on glucose metabolism in peripheral tissues has not been studied as yet. The possible effect of A-4166 on extrapancreatic tissues is supported by the observation that the duration of the glucose lowering effect extends beyond the duration of elevated insulin levels in plasma (Sato et al. 1991). Therefore, in the present experiments the metabolism of glucose in isolated fat cells and slices of muscle tissues was studied by using *in vitro* incubation of tissues after an oral load of A-4166. The extrapancreatic effects of *in vivo* administered A-4166 on glucose metabolism were investigated in normal rats and in animals with dietary induced insulin resistance (by feeding a high fat diet, Klimes et al. 1998).

## Materials and Methods

Adult male Wistar rats, SPF colony (Anlab, Prague, Czech Rep.), with a body mass  $340 \pm 15$  g, were divided into 4 groups, (10 animals each): **1**-basal, laboratory chow fed rats with administration of vehiculum (CB, containing 12 cal % of fat), **2**-basal diet with administration of A-4166 (CBA), **3**-high fat diet with administration of vehiculum (HF, containing 70 cal % of fat), **4**-high fat diet with administration of A-4166 (HFA). The composition of the diets was described elsewhere (Klimes et al. 1998). The hypoglycemic agent A-4166 was a gift from Ajinomoto Co., Tokyo, Japan. The animals were housed in fours in mesh wire cages in temperature and light controlled rooms and fed ad libitum the above described diets for 3 weeks. The animals were not fasting before the experiment. The active substance A-4166, (100 mg per kg body mass as a suspension in 0.5% methylcellulose in water or the same amount of 0.5% methylcellulose without the active substance) were administered intragastrically by gavage 30 minutes before decapitation (at the time interval when maximal elevation of serum insulin levels and significant decrease of glycemia were noted, Shinkai and Sato 1990). Blood was collected into ice cooled tubes and serum was separated for analysis of insulinemia (Rat Insulin Ria Kit, Linco Res. INC, St. Charles, MO, USA), and glycemia (GLU, Boehringer, Mannheim, Germany). The

biochemical serum analyses were made using a BM Hitachi 704 analyzer (Japan).

The epididymal fat pads, diaphragm and the soleus muscle from both hind legs were rapidly removed. Isolated adipocytes were prepared from adipose tissue according to Rodbell (1964). The size of adipocytes was determined on an aliquot of the isolated cell suspension by measurement of the diameter of 100 cells from each sample under light microscope.

### *Glucose transport*

The basal and insulin stimulated glucose transport were determined by incubating the suspension of isolated adipocytes with 2-deoxy [ $1\text{-}^3\text{H}$ ] glucose in the presence of insulin ( $1 \times 10^{-9}$  and  $1 \times 10^{-7}$  mol/l, Porcine monocomponent insulin, Novo, Denmark) or in the absence of the hormone as described by Fickova et al. (1997). The glucose uptake was corrected for nonspecific transport and extracellular trapping determined in the presence of 25 mmol/l cytochalasine B. The glucose uptake was expressed in pmol of glucose per  $10^5$  cells or per cell surface unit (square micrometers).

### *Western blot analysis of GLUT4 protein*

Plasma membranes were prepared from isolated adipocytes according to Simpson et al. (1983). Isolated plasma membranes were solubilized in a solution containing 2.5% SDS, 75 mmol/l DTT, 12.5% bromphenol blue and 12.5 mmol/l Tris-HCl, pH 7.4. Solubilized membrane protein (5  $\mu\text{g}$  in each sample) was subjected to SDS gel electrophoresis according to Laemli (1970). Protein from gel was transferred on Hybond-C nitro-cellulose membrane (Amersham, UK). The membrane sheets were incubated successively with rabbit anti-GLUT 4 antibodies (East Acres Biologicals, Southbridge, Ma, USA) and developed with secondary antibody linked to horseradish peroxidase (anti-rabbit IgG, Pierce, USA). Labeled bands were revealed by the ECL procedure (Amersham, UK) and the images were scanned using a Kodak DS DC40 camera. The results are expressed in arbitrary units of signal intensity.

### *Lipogenesis*

Two milliliters of 10% suspension of isolated fat cells in Krebs-Ringer bicarbonate solution, pH 7.4, containing 5.88 mmol/l glucose and 2% bovine serum albumin were incubated at  $37^\circ\text{C}$  with two doses of insulin ( $10^{-9}$  and  $10^{-7}$  mol/l) and with  $^{14}\text{C}$ -U-glucose (7.4 kBq per sample, Amersham, UK) for two hours. After the incubation, lipids were extracted 3 times with 5 ml of chloroform:methanol (3:1) according to Folch et al. (1957). The lipid extracts were dried, weighed and dissolved in 0.5 ml of chloroform:methanol mixture (3:1) and two aliquots (0.2 ml) were used for liquid scintillation counting and determination of radioactivity of  $^{14}\text{C}$  of glucose incorporated into lipids. The incorporation of glucose into lipids was expressed in nmols of glucose per mg of lipids or per  $10^5$  cells.

### Glucose oxidation

Pieces of the diaphragm (60–115 mg) or the soleus muscle (50–80 mg) were incubated in Warburg flasks in 2 ml of Krebs Ringer bicarbonate solution containing 2% bovine serum albumin, 20 mmol/l of  $^{14}\text{C}$ -U-glucose (18.5 kBq/flask) either with 5 and 25 mU of insulin or without addition of the hormone (control samples) according to Goldberg et al. (1975). The  $^{14}\text{CO}_2$  was trapped into hyamine hydroxide moistened filter paper and the radioactivity was counted in a liquid scintillation counter. Glucose oxidation to  $\text{CO}_2$  was expressed in nmol of glucose per 1 mg of tissue protein. Protein was determined in tissue homogenates according to Lowry et al. (1951).

Statistical differences between the two groups were tested using Student's *t*-test. Comparisons between more than two groups were done by one-way analysis of variance (ANOVA).

## Results

### Body mass

The mean body mass of 3 month-old Wistar rats at the beginning of the experiments was  $340 \pm 15$  g, this increased at the end of the 3 week period to  $438 \pm 15$  g. No significant differences were observed in the body mass between rats fed basal or high fat diet (Table 1).

**Table 1.** Body mass, serum levels of insulin and glucose and size of adipocytes in rats fed basal (CB) or high fat diet (HF) after the administration of A-4166 (A)

	BODY MASS g		INSULIN $\mu\text{U}/\text{ml}$		GLUCOSE mmol/l		ADIPOCYTES <i>d</i> in $\mu\text{m}$	
	CB	HF	CB	HF	CB	HF	CB	HF
O	$438 \pm 15$	$454 \pm 20$	$82 \pm 10$	$130 \pm 16^{\S}$	$8.1 \pm 0.1$	$9.2 \pm 0.2^{\S}$	$77 \pm 3$	$89 \pm 4^{\S}$
A	$446 \pm 15$	$432 \pm 14$	$148 \pm 25^*$	$158 \pm 17$	$5.1 \pm 0.4^*$	$5.3 \pm 0.3^*$	$78 \pm 3$	$81 \pm 3$

$\S = p < 0.05$  for CB to HF,  $* = p < 0.05$  for O to A. O without and A after A-4166 treatment, *d* = adipocyte diameter in  $\mu\text{m}$ .

### Insulinemia

Increased serum insulin values were found in rats fed high fat diet as compared with those on basal diet (Table 1). The data suggest the presence of insulin resistance due to the intake of high fat diet (elevated insulinemia and hyperglycemia). The administration of A-4166 induced a significant increase of serum insulin levels in rats fed basal diet. A similar tendency towards elevated serum insulin concentrations after the administration of A-4166 was observed also in rats fed high fat diet;

however, this increase was not significant due to great intra-individual variations of insulin levels and also high basal insulinemia in animals fed high fat diet (Table 1).

### *Glycemia*

Significant increases in serum glucose levels were observed in rats fed high fat diet (Table 1). The administration of A-4166 caused a significant diminution of glycemia in rats fed both basal and high fat diet. These results are in full agreement with previous observations on the plasma glucose reducing effect of A-4166. The above data illustrate that this model of dietary induced insulin resistance is suitable to demonstrate the beneficial effects of A-4166 administration on serum insulin and glucose levels.

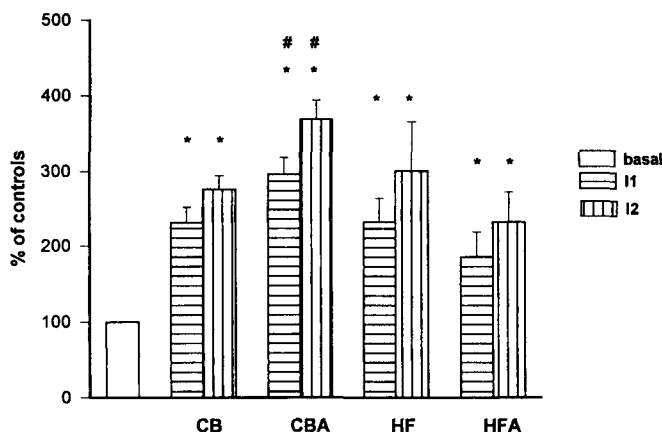
### *Glucose transport*

There were no significant differences in basal glucose transport (expressed per  $10^5$  fat cells) into adipocytes of rats fed control or high fat diet (Table 2). Because an increase of the size of adipocytes was noted in rats fed high fat diet (Table 1), the glucose transport was also calculated per surface unit of fat cell. The lower values of basal glucose transport observed in rats fed high fat diet were not significantly different from those found in controls (Table 2). The administration of A-4166 did not affect the basal glucose transport in isolated cells from either dietary group. The addition of insulin to isolated adipocytes increased the glucose transport in a dose dependent manner and the stimulatory effect of insulin was similar in fat cells of rats fed both basal and high fat diet (Fig. 1). A small increase in the effect

**Table 2.** Values of basal glucose transport and lipogenesis in isolated adipocytes, and glucose oxidation in slices of the diaphragm and soleus muscle in rats fed standard diet (CB) and high fat diet (HF) after the administration of A-4166 (A)

DIET DRUG	0	CB A	0	HF A
<b>GLUCOSE TRANSPORT</b>				
(pmol/ $10^5$ IFC)	38.08 $\pm$ 8.55	38.15 $\pm$ 2.46	42.83 $\pm$ 5.57	42.90 $\pm$ 5.36
(fmol/ $10^4$ $\mu\text{m}^2$ )	0.205 $\pm$ 0.033	0.201 $\pm$ 0.020	0.180 $\pm$ 0.017	0.204 $\pm$ 0.028
<b>LIPOGENESIS</b>				
(nmol/ $10^5$ IFC)	3.19 $\pm$ 0.66	3.78 $\pm$ 0.93	6.04 $\pm$ 1.48*	4.39 $\pm$ 1.49
<b>GLUCOSE OXIDATION – diaphragm</b>				
(nmol/mg P)	10.01 $\pm$ 1.13	10.27 $\pm$ 0.95	10.35 $\pm$ 1.23	11.42 $\pm$ 0.89
<b>GLUCOSE OXIDATION – soleus muscle</b>				
(nmol/mg P)	5.48 $\pm$ 0.79	4.29 $\pm$ 0.33	3.57 $\pm$ 0.5*	5.48 $\pm$ 0.52 <sup>#</sup>

CB – control basal diet, HF – high fat diet, 0 – without administration of A-4166, A – after the administration of A-4166, glucose transport was expressed per number of IFC – isolated fat cells or per surface unit of cell in  $\mu\text{m}^2$ , P = proteins, means  $\pm$  S.E.,  $n = 10$ , \* CB: HF  $p < 0.05$ , <sup>#</sup> OHF: AHF  $p < 0.05$ .



**Figure 1.** Stimulation of glucose transport by insulin in isolated adipocytes expressed as percentage of control values CB – controls fed basal diet, HF – animals fed high fat diet, CBA or HFA animals after administration of A-4166 I1 – insulin concentration  $10^{-9}$  mol/l and I2 – insulin concentration  $10^{-7}$  mol/l Means  $\pm$  S E,  $n = 10$  Statistical significance \* C I1 or I2  $p < 0.05$ , # CB CBA  $p < 0.05$

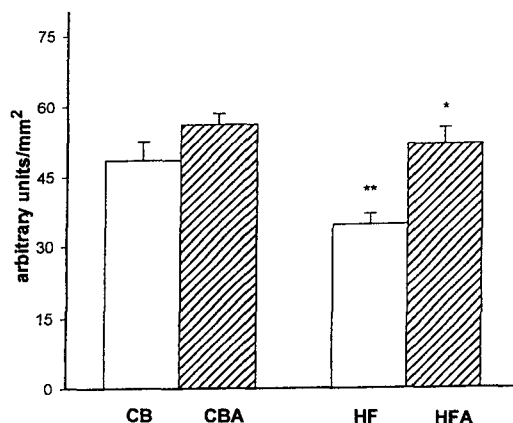
of *in vitro* added insulin was observed in rats on the basal diet treated with A-4166 (Fig. 1), when the stimulatory effect of insulin was expressed as percentage of basal values without the addition of the hormone. These results suggest that A-4166 probably ameliorates the effect of insulin on the first steps of glucose utilization in tissues in normal rats and is not effective in rats fed high fat diet.

#### *GLUT 4 protein*

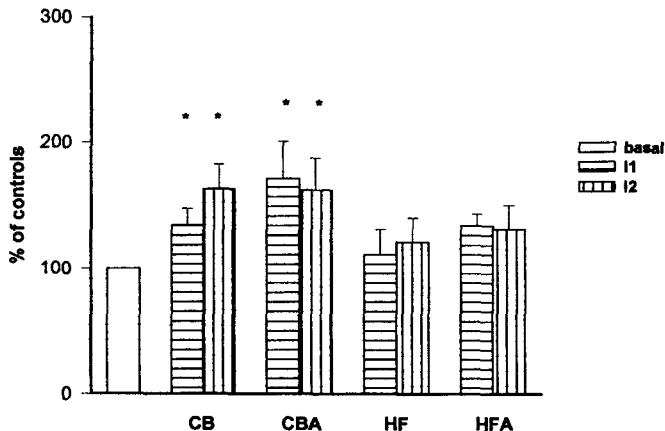
Intake of high fat diet induced a reduction of the glucose transporter (GLUT4) protein in adipocyte plasma membrane fraction (Fig. 2). The administration of A-4166 mildly, insignificantly elevated GLUT4 in adipocytes from rats fed basal diet, while approximately a 50% increase was observed in rats fed high fat diet. The augmentation achieved values very similar to those observed in control group.

#### *Lipogenesis*

In isolated adipocytes the metabolic conversion of radiocarbon labeled glucose is straightened into lipids, mainly into triglycerides (Ficková and Macho 1983; Macho et al 1984). The feeding of Wistar rats with high fat diet resulted in an increase of basal lipogenesis (Table 2). The addition of insulin stimulated lipogenesis in isolated adipocytes (Fig. 3) from rats on the basal diet only. However, insulin *in vitro* failed to have a significant stimulatory effect on lipogenesis in adipocytes of animals fed the high fat diet indicating the presence of insulin resistance at the adipose tissue level. The administration of A-4166 did not affect the basal or insulin stimulated lipogenesis in rats fed high fat diet. (Fig. 3).



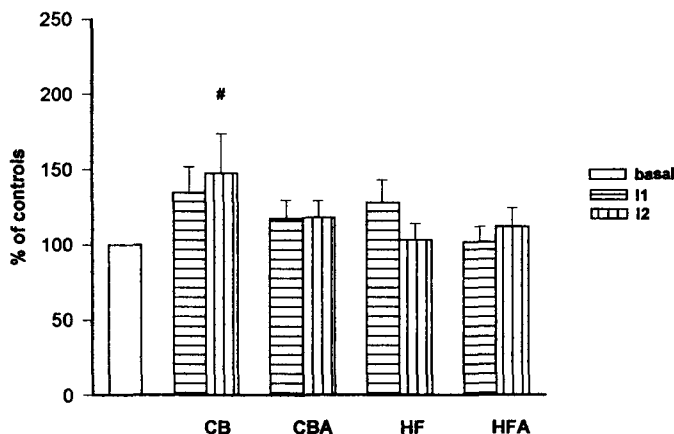
**Figure 2.** Glucose transporter protein GLUT 4 content in plasma membranes of isolated adipocytes from rats fed basal (CB) or high fat diet (HF) without and after the administration of A-4166 (CBA, HFA). Quantification of GLUT 4 protein in plasma membrane was done by densitometric analysis and is represented in arbitrary units per mm<sup>2</sup>. Statistical significance \* HF HFA  $p < 0.05$ , CB HF \*\*  $p < 0.01$ .



**Figure 3.** Stimulation of lipogenesis by insulin in isolated fat cells expressed as percentage of basal values. C-controls, I1 – insulin concentration 10<sup>-9</sup> mol/l, I2 – insulin concentration 10<sup>-7</sup> mol/l, CB rats fed basal diet, HF – rats fed high fat diet, CBA, HFA 0 – animals after administration of A-4166. Statistical significance \* C I1 or I2  $p < 0.05$ .

### Glucose oxidation

The glucose oxidation to CO<sub>2</sub> was measured in muscle slices. In the diaphragm the basal oxidation of glucose was not influenced by the high fat diet or by the admin-



**Figure 4.** Effect of insulin on glucose oxidation in the diaphragm expressed as percentage of control values without addition of the hormone to incubation medium I1 – 5 mU and I2 – 25 mU of insulin per sample. Statistical significance 0 I2 #  $p < 0.05$ .

istration of A-4166 (Table 2). The basal glucose oxidation was slightly lowered in the soleus muscle of rats fed high fat diet as compared to controls on basal diet (Table 2). In this experimental group the administration of A-4166 increased basal glucose oxidation (in the absence of insulin) up to values similar to those for the control group. The small stimulatory effect of insulin added *in vitro* was noted only in the muscle tissue (data from the diaphragm in Fig. 4, for the soleus muscle, the stimulation with insulin was  $+38 \pm 14\%$ ,  $p < 0.05$ , data not shown) from rats fed the basal diet.

## Discussion

Oral hypoglycemic agents, widely used in the therapy of NIDDM, affect glucose metabolism in different ways. Sulfonylurea derivatives stimulate insulin secretion from the islets of Langerhans and, after long-term administration, they also show extrapancreatic influence by increasing insulin effects in target tissues (Bak et al. 1989). Biguanide derivatives (e.g. metformin) reduce hyperglycemia by increasing glucose utilization without stimulation of insulin secretion (Bailey 1992). New non-sulfonylurea hypoglycemic agents (e.g. repaglinide, A-4166, KAD-1229) show a potent stimulatory effect on insulin secretion from the  $\beta$ -cells of the pancreatic islets and also increase glucose induced insulin release (Malaisse 1995). However, their influence on glucose metabolism in peripheral tissues has not yet been studied in detail. Therefore, in the present experiments the metabolism of glucose in adipose and muscle tissues under *in vitro* conditions was studied 30 minutes after the administration of A-4166, at the time interval when maximal elevation of serum insulin levels and a significant decrease of glycemia had been noted (Shinkai and Sato 1990; Sato et al. 1991).



In agreement with previous observations (Sato et al 1991) the results of the present work show that the administration of A-4166 is followed by an elevation of blood insulin levels and a diminution of glycemia. These changes were observed in rats fed basal diet and in rats with insulin resistance induced by feeding a high fat diet. The glucose reducing effect of A-4166 could be a result of the reduction of hepatic glucose production or increased utilization of glucose in peripheral tissues. The changes of glucose metabolism in peripheral tissues could be the consequence of higher plasma insulin levels and a possible direct effect of A-4166 on glucose utilization in tissues.

No significant changes in basal glucose transport were observed in adipocytes after the administration of A-4166 to rats despite a significant increase in insulin levels *in vivo*. The data indicate that the effect of elevated endogenous insulin on glucose transport measured *in vitro* was probably eliminated during the procedure used to isolate the adipocytes. The addition of insulin *in vitro* showed a stimulatory effect on glucose transport and the administration of A-4166, 30 minutes before examination of the animals, increased this insulin effect only in animals fed basal diet. This elevation of the insulin effect on glucose transport in A-4166 treated rats fed basal diet can not be explained only by changes in the amount of GLUT 4 membrane protein in the adipocytes, but also an activation of glucose transport proteins could be involved.

The feeding of high fat diet did not change the total glucose transport in rat adipocytes despite the reduction of GLUT 4 protein in the cell membrane. When glucose transport was expressed per surface unit of fat cell, the lower values of glucose transport in adipocytes from rats fed high fat diet were noted, but they were not significantly different as compared to controls. The addition of insulin to adipocytes from rats fed high fat diet and treated with A-4166 stimulated the glucose transport similarly as in rats on basal diet, however, when the stimulatory effect of insulin was expressed as a percentage over the basal value without the hormone, the effect in adipocytes from high fat diet fed rats was weaker as compared to the controls. This diminution of the insulin effect in high fat diet fed animals after A-4166 administration was observed despite the fact that the GLUT 4 protein content was similar to that in rats on basal diet. It seems possible that not all the transport proteins were fully activated by the *in vitro* addition of insulin in the adipocytes.

The determination of lipogenesis in isolated fat cells from rats fed basal diet showed that the administration of A-4166 did not affect the basal lipogenesis and did not significantly change the effect of insulin added *in vitro*. The animals fed high fat diet failed to respond significantly to insulin and the administration of A-4166 did not influence this resistance of adipocytes to insulin.

The administration of A-4166 did not significantly affect basal glucose oxidation in the diaphragm and the soleus muscle in rats fed basal diet. The addition of insulin to tissue slices from muscle showed an increase in glucose oxidation in the diaphragm. Insulin action was not significantly changed by the administration of A-4166. The failure of insulin to affect glucose oxidation was probably due to the

high body mass and age of the animals used, as it was noted in previous experiments that the stimulatory effect on glucose oxidation rapidly decreases with age (Macho et al. 1977). The administration of A-4166 increased basal glucose oxidation in the soleus muscle of rats fed high fat diet, but did not change the effect of insulin

The results of these studies showed that the hypoglycemic agent A-4166 administered *in vivo* 30 minutes before the tests enhanced the effect of insulin on fat cells in control animals fed basal diet (see glucose transport). Treatment with A-4166 also ameliorated basal glucose oxidation in the soleus muscle in rats fed the high fat diet. We believe that the blood glucose lowering effect, observed in the A-4166 treated animals either on basal or high fat diet, is mainly due to the insulinotropic effect of this drug increasing the secretion of insulin from islets, and only partially due to changes in glucose metabolism in peripheral tissues. The increased insulin levels have an inhibitory effect on hepatic glucose production resulting in a lowered concentration of glucose in the peripheral circulation. The results of our experiments do not exclude direct effects of A-4166 on glucose metabolism in tissues as was observed after the addition of some sulfonylurea, thiazolidinedione or biguanide derivatives (Farese et al. 1991, Ciaraldi et al. 1995; Tsiani et al. 1995). Such direct effects probably need long-term treatment with A-4166 as was demonstrated for the influence of troglitazone on cultured muscle cells (Ciaraldi et al. 1995).

**Acknowledgements.** We are grateful for the competent technical assistance of Mrs M Škultetyová, E Vízstová, A Mitková and S Kuklová. This research was supported by an Ajinomoto Co, Tokyo, Japan, research grant and partially by GAV SAS VEGA-2 /4130/97

## References

- Bailey C J (1992) Biguanides and NIDDM. *Diabetes Care* **15**, 755—772
- Bak J F, Schmitz O, Schwartz N, Pedersen O (1989) Postreceptor effects of sulfonylurea on skeletal muscle glycogen synthase activity in type II diabetic patients. *Diabetes* **38**, 1343—1350
- Ciaraldi T P, Huber-Knudsen K, Hickman M, Olefsky J M (1995) Regulation of glucose transport in cultured muscle cells by novel hypoglycemic agent. *Metabolism* **44**, 976—982
- Farese R V, Ishizuka T, Standaert M K, Cooper D R (1991) Sulfonylureas activate glucose transport and protein kinase C in rat adipocytes. *Metabolism* **40**, 196—200
- Ficková M, Macho L (1983) The lipogenic effect of insulin on glucose metabolism in isolated adipocytes of rats with different neonatal nutrition. *Hormone Metab Res* **15**, 354—355
- Ficková M, Zorad S, Macho L (1997) The effect of *in vivo* thyroxine treatment on insulin receptors, glucose transport and GLUT4 in rat adipocytes. *Hormone Metab Res* **29**, 16—19
- Folch J, Ascoli I, Less M, Mesth M, Le F H (1957) Preparation of lipid extracts from brain tissue. *J Biol Chem* **226**, 833—842
- Fujita T, Seto Y, Kondo N, Kato R (1996) Studies on N-[(trans-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine (A-4166) receptor in HIT T15, Displacement of [<sup>3</sup>H]-glibenclamide. *Biochem Pharmacol* **52**, 407—411

- Fujitani S, Yada T (1994) A novel D-phenylalanine-derivative hypoglycemic agent A-4166 increases cytosolic free  $\text{Ca}^{2+}$  in rat pancreatic  $\beta$ -cells by stimulating  $\text{Ca}^{2+}$  influx Endocrinology **134**, 1395—1400
- Goldberg A L, Martel S B, Kushmerick M J (1975) *In vitro* preparations of the diaphragm and other skeletal muscles In Methods in Enzymology (Eds J G Hardman and B W O Mallaey) **39**, pp 82—94, Academic Press, New York
- Klimes I, Sebkova E, Gasperikova D, Mitkova A, Kuklova S, Bohov P, Stanek J (1998) Search for extrapancreatic effects of new oral hypoglycemic agent A—4166 1 Oral glucose tolerance tests in normal and hereditary insulin resistant rats Endocrine Regulations **32**, 115—123
- Laemli U K (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4 Nature **227**, 580—685
- Lowry O H, Rosebrough N J, Farr A L, Randall R J (1951) Protein measurement with the Folin phenol reagent J Biol Chem **193**, 265—275
- Macho L, Ficková M, Hupková A (1977) Effect of insulin on glucose metabolism in isolated adipocytes and in diaphragm Effect of age and neonatal nutrition Endocrinol Exp **11**, 75—84
- Macho L, Kvetňanský R, Ficková M (1984) The effect of hypokinesia on lipid metabolism in adipose tissue Acta Astronautica **11**, 735—738
- Malaisse W J (1995) Stimulation of insulin release by non-sulfonylurea hypoglycemic agents The meglitinide family Hormone Metab Res **27**, 263—266
- Panten U, Schwanstecher M, Schwanstecher C (1992) Pancreatic and extrapancreatic sulfonylurea receptors Hormone Metab Res **24**, 549—554
- Rodbell M (1964) Metabolism and lipolysis J Biol Chem **239**, 375—380
- Sato J, Nishikawa M, Shinkai H, Sukegawa E (1991) Possibility of ideal blood glucose control by new oral hypoglycemic agent, N-[(trans-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine (A-4166), and its stimulatory effect on insulin secretion in animals Diabetes Res Clin Pract **12**, 53—60
- Shinkai H, Sato Y (1990) Hypoglycemic action of phenylalanine derivatives In New Antidiabetic Drugs (Eds C J Bailey and P R Flatt) pp 249—254, Smith-Gordon, Ltd London
- Shinkai H, Nishikawa M, Sato Y, Toi K, Kumashiro I, Seto Y, Fukuma M, Dan K, Toyoshima S (1989) N-(Cyclohexylcarbonyl)-D-phenylalanines and related compounds A new class of oral hypoglycemic agents 2 J Med Chem **32**, 1436—1441
- Simpson I A, Yver D R, Hissin P J, Wardzala I J, Karnieli E, Salans I B, Cushman S W (1983) Insulin stimulated translocation of glucose transporters in isolated rat adipose cells characterization of subcellular fractions Biochim Biophys Acta **763**, 393—407
- Tsiani E, Ramlal T, Leiter L A, Klip A, Fantus I G (1995) Stimulation of glucose uptake and increased plasma membrane content of glucose transporters in L6 skeletal muscle cells by the sulfonylureas Glyclazide and Glyburide Endocrinology **136**, 2505—2512
- Tsukuda K, Sakurada M, Niki I, Oka Y, Kikuchi M (1998) Insulin secretion from isolated rat islets induced by the novel hypoglycemic agent A-4166, a derivative of D-phenylalanine Hormone Metab Res **30**, 42—49