J Physiol Sci (2014) 64:411–420
DOI 10.1007/s12576-014-0335-6

ORIGINAL PAPER

High dose of aspirin moderates diabetes-induced changes of heart glycogen/glucose metabolism in rats

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Received: 26 April 2014 / Accepted: 14 August 2014 / Published online: 3 September 2014

Abstract Aspirin (ASA), as a multifunctional drug has been used as a hypoglycaemic agent in the treatment of diabetes and severe hyperglycaemia and has been established as a secondary strategy which may prevent many cardiovascular events. In this study we investigated high dose (100 mg/kg b.w./i.p) and time-dependent (2, 7 and 14 days) effects of ASA on the heart key enzymes and substrates from glycogen/glucose metabolism in control and diabetic rats. The results accomplished demonstrated that ASA significantly potentiates glycogen accumulation, as well as decreased blood glucose level and heart glycolytic potential in control rats. The treatment of diabetic rats with ASA caused moderation of the diabetic complication—significant inhibition of glycogen accumulation, lowering of blood glucose, as well as elevation of glycolytic potential. In conclusion, we propose that use of high-dose of ASA has anabolic effects in control rats and reduces heart glycogen glucose complications in diabetic rats. The moderation of diabetes-induced changes is time-dependent and involves reduction of glycogenogenesis and inhibited depression of glycolysis, with a tendency to maintenance control values.

Keywords Diabetes · Aspirin · Heart · Glycogen/glucose metabolism · Rats

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Introduction

Diabetes mellitus, as a complex and heterogenic metabolic syndrome, is characterized by intensive metabolic disturbances in different metabolic pathways, with special respect to carbohydrate metabolism in most tissues in the organism. The association of metabolic diseases with increased risk for heart failure raises the distinct possibility that alterations in myocardial metabolic pathways play a critical role in the development and progression of the disease [1]. In this sense, previous date indicated that in the rat's heart, STZ-induced diabetes mellitus promote glycogenogenesis and impaired glycolysis as a result of decreased phosphofructokinase activity [2, 3].

Aspirin–acetyl salicylic acid (ASA) is one of the most worldwide used nonsteroidal anti-inflammatory drugs (NSAIDs), and as a multifunctional drug affects different systems and metabolic pathways in the body with various mechanisms. In the past, it was found that in intact animals single large dose of ASA have caused significant reduction of liver glycogen [4–6], muscle glycogen [5, 7] and decreased hepatic gluconeogenesis [6]. The hypoglycemic effect of ASA while diabetes is through decrease in the intestinal absorption of glucose [8], reduction in the hepatic gluconeogenesis, hepatic glucose production and the decrease in the insulin clearance [9]. Recently, since it was discovered the effect of aspirin for inhibition of the two

cyclooxygenase enzymes (COX-1 and COX-2) [10, 11], there was more precise data about its mechanism of action. Namely, it was found that diabetic hyperglycemia has been reported to increase human β-cell interleukin-1β and that this cytokine can induce COX-2 expression [12]. It is known that interleukin-1β and prostaglandin E2 (PGE2) both inhibit glucose-induced insulin secretion from the pancreatic islet [13, 14]. In this sense, according to Tran et al. [15], the sites of action through which sodium salicylate inhibits these negative effects of IL-1β on β-cell function include activation of NF-kB as well as generation of prostaglandin E2 (PGE2) by COX-2. ASA inhibition of COX-2 in human islets could be one route through which NSAID can moderate diabetic hyperglycemia.

As to our knowledge, the effect of ASA over the heart glycogen/glucose metabolism in diabetes mellitus has not studied yet. Thus, the objective of this study was to assess the impact of the high dose (100 mg/kg b.w./i.p) and time dependent (2, 7 and 14 days) use of ASA on heart key enzymes and substrates from glycogen/glucose metabolism in intact and diabetic rats. Taking in consideration fact that ASA has hypoglicemic and antidyslipidemic properties, here, we hypothesized that this drug may play great role in the moderation of diabetes-induced alteration at a level of heart carbohydrate metabolism, beside the more estimated influence on liver and muscle metabolism.

Materials and methods

The experimental study was performed on adult (3–4 months old), male Wistar rats ($n = 80$, 8 in each group), with an estimated weight of 250–300 g, kept at 12 h lightening regime (06–18 p.m. light) and fed on laboratory chow and water ad libitum.

The animals were divided into two general groups: control and diabetic. Control group was divided into four subgroups: intact animals (C) and three groups of animals treated with ASA (once daily) for a period of 2, 7 and 14 days, (CA_2, CA_7 and CA_{14}). ASA was given always at 08–09 h. The diabetic animals were divided into six subgroups: three control diabetic groups, (D_2, D_7 and D_{14}) and three diabetic groups treated with ASA (once daily) in the same period (DA_2, DA_7 and DA_{14}). Animals were sacrificed at the 2nd, 7th and 14th day, respectively. We propose this experimental period according to two different criteria: minimal period for development of hyperglycemia after STZ administration (48 h) and optimal period for manifestation of diabetes complications (in our experiment 14 days). Seven days after STZ administration is an intermediate period for following the continuation of the changes.

The induction of the experimental diabetes mellitus was performed by a single intraperitoneal injection of streptozotocin (STZ, 55 mg/kg body weight), freshly dissolved in 0.1 M citrate buffer, pH 4.5. All animals with clear diabetic symptoms (fasting glycemia levels higher than 15 mmol/L) 24–48 h after the induction of the experimental diabetes, were used for the purpose of this experiment.

Aspirin (ASA–acetylsalicylic acid, Sigma-Aldrich) was freshly dissolved in water (as 100 mg/kg b.w. solution). Subsequently, sodium carbonate crystals were slowly added, until the ASA crystals had dissolved (the pH of the solution remained just below 7.0 [16] and administrated intraperitoneally in a 0.5 mL volume [17, 18]. The first ASA treatment in diabetic animals started 15 min after STZ injection and continued in the following 2, 7 and 14 days, always in the same period of the day.

All experimental animals were anesthetized with a Na-thiopental narcosis (45 mg/kg) and sacrificed using a standard laparothomic procedure. Immediately after the opening of the abdominal cavity, the isolated heart was washed with cold saline solution and immersed in liquid nitrogen. The tissues were kept at −80 °C until the analyses and were finally prepared into tissue powder (at liquid N_2-temperature). The tissue powder was homogenized with an ultrasonic homogenizator (Cole-Parmer Instrument-4710) in a period of 7–10 s. The whole procedure was performed at a temperature of 0–4 °C (in ice).

Serum glucose (Glc) concentration was determinate using commercially available enzymatic colorimetric test (GOD-PAP method, Human, Germany). Heart glycogen (Glk), heart glucose (Glc) and glucose-6-phosphate (G6P) concentration were determined in perchlorate homogenates and neutralized with 5 M K_2CO_3. We measured the production of NADPH at 340 nm in a reaction catalyzed by glucose-6-phoshate dehydrogenase [19]. The activity of the glycogen phosphorylase (GPa) [20] was determined in a nuclear fraction, indirectly through the quantity of the produced inorganic phosphate [21]. We used a mitochondrial fraction for determination of activity of hexokinase (HK) [22] and phosphofructokinase (PFK) [23], by measuring the production of NADH and NAD^+ respectively at 340 nm.

For the interpretation of the activity as a specific enzyme activity (nmol P_i/min/mg proteins) for the GFa and (U/mg proteins) for the HK and PFK the total quantity of the proteins was determined by Lowry method [21].

Statistics

Results are presented as mean ± SD. To examine the statistical differences between each group we used one-way ANOVA with Neuman–Keuls post hoc test. Correlation analyses for each parameter, depending on

duration of acclimation, as well as between the parameters, were also assessed. Only significant coefficients of correlation are presented in the figures. In all tests, a probability level of $p < 0.05$ was used as a significant difference.

Results

Serum glucose concentration

The obtained results (Fig. 1) confirmed that STZ-induced diabetes mellitus caused significant increase of serum Glc concentration by around 3-fold (D_2:DA_2, D_7:DA_7, D_{14}:DA_{14}, $p < 0.05$), independently of duration of diabetes (Table 1). From another hand, is evident that ASA treatment caused slight, but significant lowering of serum Glc level in control for about 0.6-fold, as well as diabetic rats for about 0.8-fold, compared with untreated control and diabetic animals respectively.

Heart glucose concentration

Figure 2 and Table 2 are presenting the changes in heart Glc concentration depending on diabetic state and aspirin treatment.

We found that diabetes and ASA treatment in control animals significantly decrease tissue Glc level. Namely, it is evident that in control rats ASA administration cause decrease of tissue Glc level for about 4–5-fold. STZ-induced diabetes also cause progressive and significant decrease of Glc for about 1.3–2.5-fold (C:D_2, C:D_7 and C:D_{14}, $p < 0.05$). It is interesting to note that in diabetic state ASA rebounds heart Glc level especially after 7 and 14 days treatments with a tendency of normalization up to control values.

Glycogen content

It could be seen that STZ-induced diabetes as well as ASA treatment in control animals significant augmented Glk stores by 5.5–6-fold and 2.2–3-fold respectively (Fig. 3), independently of duration of diabetes (Table 3). From another hand is evident that ASA treatment given in diabetic rats caused progressively significant decreasing of Glk stores tending to normalize in DA14 group (D_2:DA_2 −16 %, D_7:DA_7, −38 %, D_{14}:DA_{14}, −63 %, $p < 0.05$).

Glucose-6-phosphate concentration

The obtained results (Fig. 4 and Table 4) in our study showed that ASA treatment in control rats as well as STZ-diabetic rats resulted with significant increase of the G6P

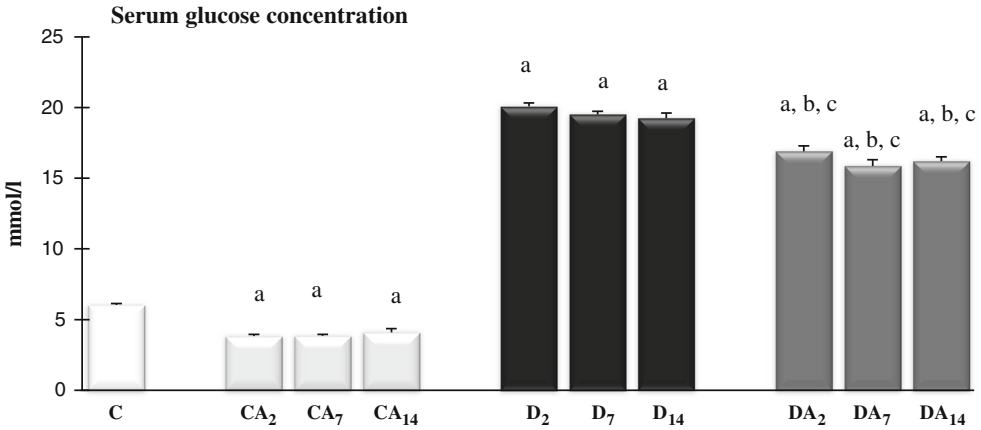

Fig. 1 Serum glucose concentration depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 1

Table 1 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to glycogen content in the heart. Legend as in Fig. 1

Serum glucose concentration								
Ratio	$p <$	%	ratio	$p <$	%	ratio	$p <$	%
CA2:CA7	n.s.	−0.8	D2:D7	n.s.	−2.8	DA2:DA7	n.s.	−6.2
CA2:CA14	n.s.	7.1	D2:D14	n.s.	−4.2	DA2:DA14	n.s.	−4.1
CA7:CA14	n.s.	6.2	D7:D14	n.s.	−1.4	DA7:DA14	n.s.	2.2

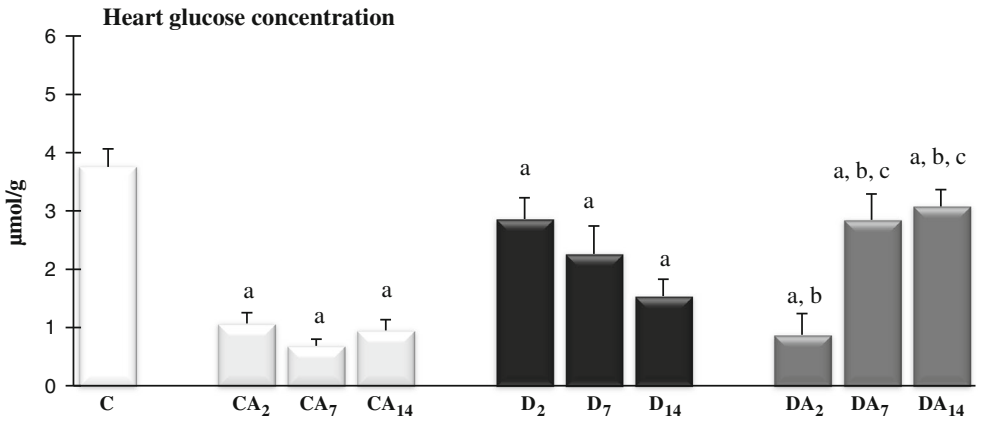

Fig. 2 Heart glucose concentration depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 2

Table 2 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to glucose concentration in the heart. Legend as in Fig. 2

Glucose								
Ratio	$p <$	%	Ratio	$p <$	%	Ratio	$p <$	%
CA2:CA7	0.050	−35.9	D2:D7	0.050	−20.9	DA2:DA7	0.050	224.3
CA2:CA14	n.s.	−11.0	D2:D14	0.050	−46.2	DA2:DA14	0.050	250.9
CA7:CA14	0.050	38.8	D7:D14	0.050	−32.0	DA7:DA14	n.s.	8.2

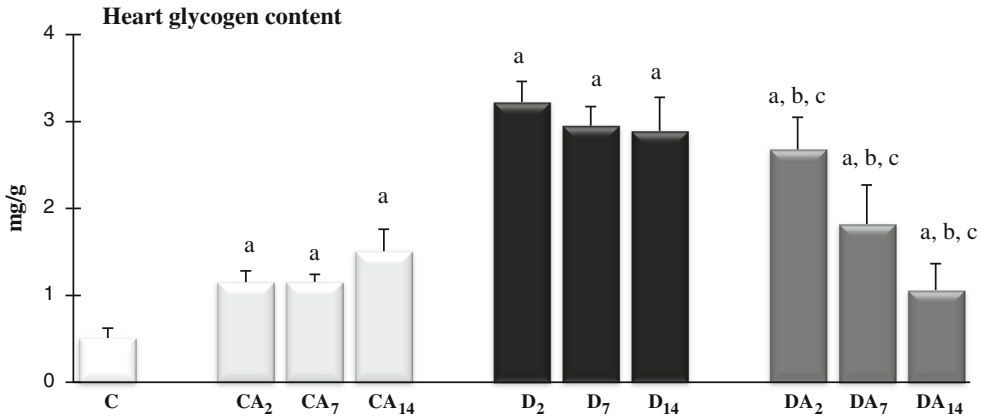

Fig. 3 Heart glycogen content depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 3

concentration for about 1.3–1.7-fold and 2.4-fold respectively. Opposite, ASA treatment in diabetic rats decreased G6P concentration only in 7- and 14-days treated groups (D_7:DA_7, D_{14}:DA_{14}, $p < 0.05$). Likewise, we found no significant changes in G6P concentration between control and diabetic animals treated with ASA after 7 and 14 days (C:DA_7, C:DA_{14}, p = n.s.).

Glycogen phosphorylase *a* activity

The obtained results (Fig. 5 and Table 5) showed that ASA treatment in control and diabetic animals significantly increase GPa activity in the rat heart. We found that this increase of enzyme activity in control rats treated with ASA was for about 1.2–1.6-fold, in

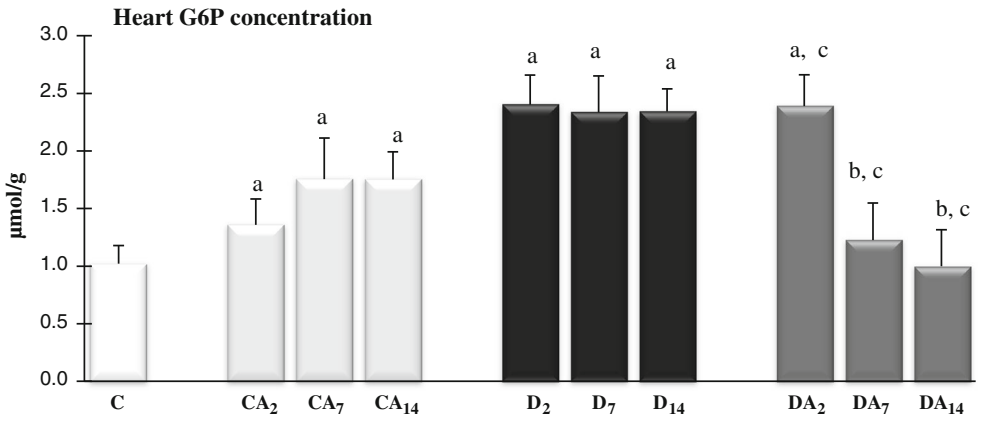

Fig. 4 Heart glucose-6-phosphate concentration depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 4

Table 3 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to serum glucose concentration. Legend as in Fig. 3

Glycogen								
Ratio	$p <$	%	Ratio	$p <$	%	Ratio	$p <$	%
CA2:CA7	n.s.	0.1	D2:D7	n.s.	−8.4	DA2:DA7	0.050	−32.0
CA2:CA14	0.050	30.9	D2:D14	0.050	−10.3	DA2:DA14	0.050	−60.4
CA7:CA14	0.050	30.8	D7:D14	n.s.	−2.1	DA7:DA14	0.050	−41.7

Table 4 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to glucose-6-phosphate concentration in the heart. Legend as in Fig. 4

Glucose-6-phosphate								
Ratio	$p <$	%	Ratio	$p <$	%	Ratio	$p <$	%
CA2:CA7	0.050	29.3	D2:D7	n.s.	−2.8	DA2:DA7	0.050	−48.7
CA2:CA14	0.050	29.1	D2:D14	n.s.	−2.5	DA2:DA14	0.050	−58.2
CA7:CA14	n.s.	−0.1	D7:D14	n.s.	0.3	DA7:DA14	n.s.	−18.5

Table 5 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to glycogen phosphorylase a activity in the heart. Legend as in Fig. 5

Glycogen phosphorylase *a*								
Ratio	$p <$	%	Ratio	$p <$	%	Ratio	$p <$	%
CA2:CA7	0.050	26.1	D2:D7	n.s	6.9	DA2:DA7	0.050	−25.0
CA2:CA14	0.050	22.1	D2:D14	n.s	−4.9	DA2:DA14	0.050	−16.0
CA7:CA14	n.s	−3.1	D7:D14	n.s	−11.0	DA7:DA14	n.s	12.0

comparison with diabetic rats where we found increased enzyme activity for about 1.8–2-fold. From another hand, treatment with ASA for a period of 7 and 14 days after STZ administration, significantly decrease GPa activity compared with untreated diabetic animals (D_7:DA_7, D_{14}:DA_{14}, $p < 0.05$) and control rats treated with ASA (CA_7:DA_7, CA_{14}:DA_{14}, $p < 0.05$), with a tendency to maintenance control value.

Table 6 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to hexokinase activity in the heart. Legend as in Fig. 6

Hexokinase								
Ratio	$p <$	%	Ratio	$p <$	%	Ratio	$p <$	%
CA2:CA7	n.s.	−7.5	D2:D7	0.050	85.4	DA2:DA7	0.050	−49.0
CA2:CA14	n.s.	−10.3	D2:D14	0.050	99.6	DA2:DA14	0.050	−79.5
CA7:CA14	n.s.	−3.0	D7:D14	n.s.	7.6	DA7:DA14	0.050	−59.8

Table 7 Statistical and percentage analysis of differences between experimental groups depending on duration of ASA treatment as well as duration of diabetes with respect to phosphofructokinase activity in the heart. Legend as in Fig. 7

Phosphofructokinase								
Ratio	$p <$	%	Ratio	$p <$	%	Ratio	$p <$	%
CA2:CA7	n.s	19.6	D2:D7	n.s	−20.0	DA2:DA7	0.050	130.1
CA2:CA14	n.s	25.8	D2:D14	0.050	−34.9	DA2:DA14	0.050	104.0
CA7:CA14	n.s	5.2	D7:D14	0.050	−25.2	DA7:DA14	n.s	−11.3

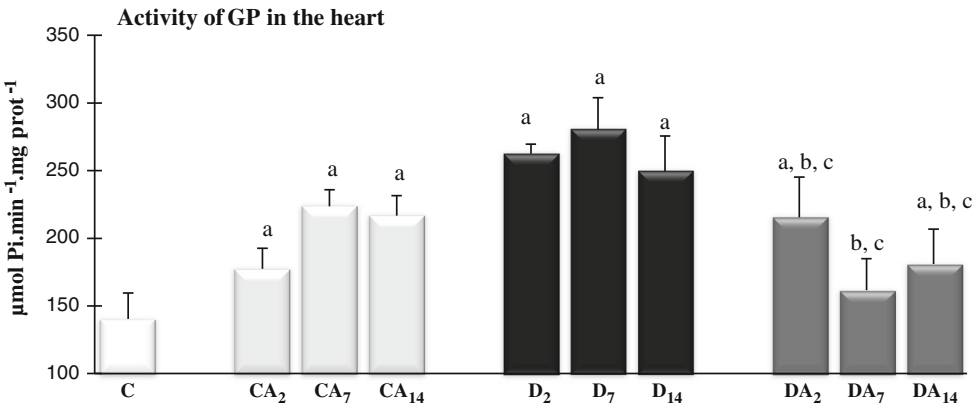

Fig. 5 Activity of glycogen phosphorylase a in the heart depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 5

Hexokinase activity

The obtained results (Fig. 6 and Table 6) showed that ASA treatment caused significant increase of HK activity in all three groups of control animals. STZ-induced diabetes also resulted with significant increase of HK activity but only in the 7 and 14 days diabetic animals. Treatment of diabetic animals with ASA is manifested with significantly high HK activity only in DA_2 group. In another two group (DA_7 and DA_{14}), we found gradually decreased enzyme activity with a tendency to maintenance control values.

Phosphofructokinase activity

Figure 7 and Table 7 show the changes in the specific activity of the heart PFK, depending on diabetic state and ASA treatment.

Activity of PFK was decreased in control rats treated with ASA for about 1.5–1.8-fold ($C:CA_2$, $C:CA_7$, $C:CA_{14}$, $p < 0.05$). More intensive decrease of PFK activity was observed in diabetic animals (2.8–4.3-fold, $p < 0.050$), while treatment of diabetic animals with ASA tended to reverse and increase PFK activity, even though still lower than CA groups.

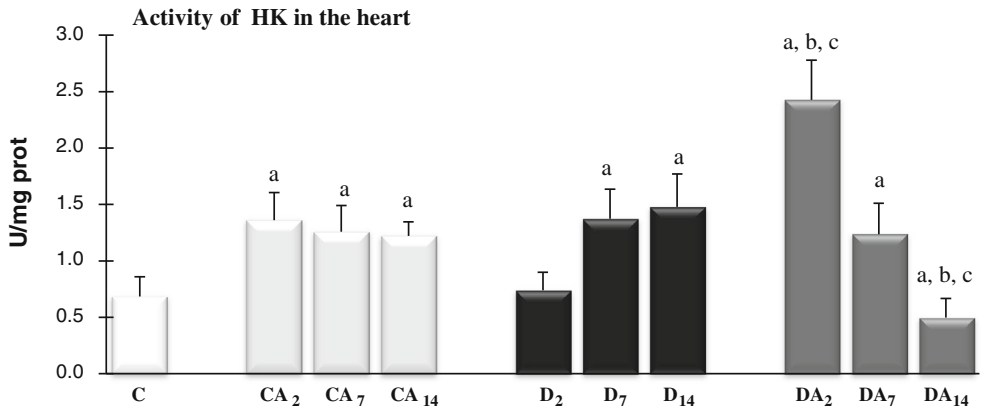

Fig. 6 Activity of hexokinase in the heart depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 6

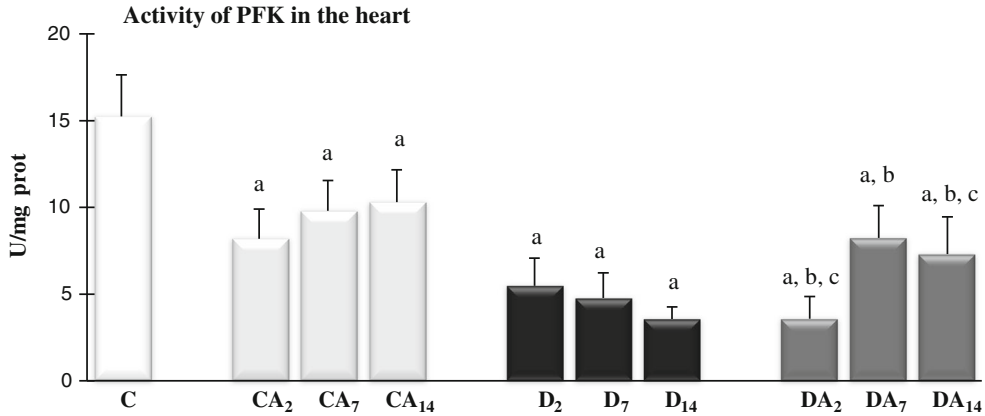

Fig. 7 Activity of phosphofructokinase in the heart depending on diabetic state and aspirin treatment C, control animals; CA_2, CA_7 and CA_{14}, control treated with ASA for 2, 7, and 14 days; D_2, D_7 and D_{14}, diabetic animals with duration of diabetes for 2, 7 and 14 days; DA_2, DA_7 and DA_{14}, diabetic animals treated with ASA for 2, 7 and 14 days. Significant differences ($p < 0.05$): **a** in comparison with C; **b** in comparison with D_2, D_7 and D_{14}, respectively; **c** in comparison with CA_2, CA_7 and CA_{14}, respectively. Additional statistical analysis are given in the Table 7

Discussion

Concerning the main aims of these investigations, we estimated the effects of high dose ASA treatment on the heart glycogen/glucose metabolism in control as well as in STZ-diabetic animals. Notably, the study does not give an insight over the COX inhibition by ASA.

The present study indicated that ASA treatment has some anabolic effect on heart glycogen/glucose metabolism in control rats, regardless of duration of treatment, but progressive with prolonging of the treatment. Importantly we found that ASA causes progressive increase heart Glk content (for about 2.2–3-fold, Fig. 3), accompanied with similar changes in G6P concentration (from 1.3 to 1.7-fold, Fig. 4) and GPa activity (for about 1.2–1.6-fold, Fig. 5). These results indicated that Glk itself is an important regulator of its own rate of catabolism [25], as well as important determinant of GPa activity and rate of glycogenolysis, both in skeletal muscles [26] and the heart [26, 27]. We found that glycogenogenic effect of ASA is probably due to increased HK activity (Fig. 6), which resulted with significant depletion of heart Glc level (Fig. 2), followed with impaired glycolysis as a result of decreased PFK activity (Fig. 7) during the all period of treatment.

However, accumulation of heart Glk caused by ASA in whole experimental period might be outcome of the insulin secretory effect knowing that insulin promote Glk synthesis [28]. It is possible that some NSAIDs [29], and ASA [30], enhanced the insulin secretion which in our results is

manifested with significant decreased serum Glc concentration (Fig. 1). Concerning effects of ASA administration, some literature date indicated that in normal patients ASA (10 g/daily) given in a period of four days decreased fasting glycaemia and Glc response to oral Glc changes associated with increased levels of serum insulin [31].

It is important to stress that in a normal heart, 70 % of ATP generation is through fatty acids oxidation, whereas glucose and lactate account for 30 % of energy provided to the cardiac muscle [32, 33]. It should be noted that acetylsalicylic acid promote fatty acid oxidation and reduce the plasma FFA level [34]. Taking in consideration that the heart can rapidly switch its substrate selection to accommodate different physiological and pathphysiological conditions [3, 35–37], accumulation of glycogen in our results probably is due to increased fatty acids oxidation which is mediated by ASA. From another hand, it has been suggested that effects of salicylates on depletion of liver glycogen is associated with stimulation of oxygen consumption, presumably through the uncoupling of oxidative phosphorylation [38].

The current findings confirmed that STZ-induced diabetes mellitus has an increasing effect on the heart Glk stores, associated with increased activation of the glycogenolytic enzyme GPa as well as increase of G6P concentration. It is important to note that our results showed that these effects are more evident with the prolonging of diabetes. Namely, the shift of cardiac energy substrate utilization from carbohydrate to lipids increases the intracellular Glk pool, probably through augmented Glk synthesis, or impaired glycogenolysis, or a combination of both processes [39, 40]. Some findings indicated that high levels of G6P stimulate heart Glk synthesis and inhibit Glk breakdown, resulting in an increase in Glk content [41, 42]. From another hand, increased GPa activity in our results probably is a kind of defense mechanism which protects cells from excessive augmentation of endogenous Glk [26, 27].

Streptozotocin-induced diabetes decreased heart Glc concentration as a result of increased phosphorylation by HK activity. Very evident and significant changes were observed with the duration of diabetes on both parameters (Figs. 2, 6). One of the most noted effects during diabetes is impaired myocardial Glc transport accompanied with decreased HK activity, which is partially due to a decreased myocardial concentration of GLUT 1 and GLUT 4 proteins and mRNA levels [3, 43, 44]. Increased HK activity in ours result indicated that Glc input in the cell probably is effectuated through some alternative mechanisms. Nevertheless, some studies indicated that transmembrane Glc gradient is determined by the interstitial and intracellular free Glc concentration [45], which in hyperglycemia, accompanying STZ-induced diabetes are helping to compensate for the decreased capacity for sarcolemmal Glc transport as a result of decreased GLUT transporters [3, 46]. Therefore, taking in consideration these changes, the increased HK activity that we found in the heart of diabetic animals, probably is important mechanism which facilitates Glc input into the cell and regulates intracellular free Glc level.

The Glk accumulation in our results was associated with decreased glycolytic potential in STZ-diabetic heart, as a result of significant decreased PFK activity (Fig. 7). Namely the increased fatty acid oxidation in diabetic animals is accompanied with increased myocardial citrate concentration [3, 47], which probably inhibit PFK and lead to increased levels of fructose-6-phosphate and G6P. This sequence is consistent with Randle's findings, that fatty acids inhibit Glc oxidation more than glycolysis, and inhibits glycolysis more than Glc uptake [2, 3, 46, 48, 49].

The data presented here demonstrate that high dose of ASA has some properties to moderate diabetes-induced changes at a level of the heart glucose/glycogen metabolism. In addition, compared with diabetic animals, ASA treatment during diabetes leads to a progressive reduction of heart Glk and G6P accumulation, accompanied with decreased GPa activity (Figs. 3, 4, 5), depending on the duration of treatment. On the other hand, we found reduction of HK activity, followed with increase of heart Glc level (Figs. 2, 6), both of them reaching the values of untreated control animals (C) after 14-days ASA treatment. All these metabolic changes are associated with higher PFK activity (Fig. 7), especially manifested after 7 and 14-days treatment. It is important to notice that all of these changes that we found in ASA-treated diabetic rats indicated that diabetic alterations (increased Glk synthesis and decreased glycolysis) were improved by ASA treatment, meaning depression of glycogenogenesis and inhibited depression of glycolysis, with a tendency to maintenance control values. All these changes finally are demonstrated with slight, but significant lower serum glucose level (Fig. 1).

Some studies indicated that sodium salicylate has been used as a hypoglicaemic agent in treatment of diabetes [50] and that the hypoglicemic effects of salicylates is mediated at least in part by enhanced insulin secretion, or changes in insulin sensitivity [51]. According to Hundal [9], high dose ASA treatment improved both fasting and postprandial hyperglycemia in patients with noninsulin-dependent diabetes mellitus (NIDDM), an effect that could be attributed to decreased basal rates of hepatic glucose production, enhanced peripheral insulin sensitivity and decreased insulin clearance.

Taking in consideration fact that we performed STZ-induced insulin-dependent diabetes mellitus (IDDM), the hypoglycemic effects of ASA and salicylates in diabetic

animals may be mediated by mechanism other than enhanced insulin secretion [52, 53]. In addition, elevated Glc metabolism that we found in diabetic rats during ASA treatment probably is due to impaired fatty acids oxidation. Numerous studies suggested that ASA primarily improve blood lipids and showed both preventive and therapeutic potential. Namely, ASA have a direct antilipolytic effect on adipocytes, the latter resulting in a reduced state of lipolysis leading to reduced serum cholesterol and triglycerides in STZ-diabetic rats [54], hypercholesterolemic rats [55], obese rodents [56], normal rats [57] and in human subject with type 2 diabetes [9]. These means that the influence of salicylate is multifactorial especially in high doses, and involves both beneficial and deleterious effects depending on the species and experimental model studied [58].

Finally, knowing that aspirin is an inhibitor of the two cyclooxygenase enzymes (COX-1 and COX-2) [10, 11] and that hyperglycemia has been reported to increase human β-cell production of interleukin-which can induce COX-2 expression, it could be one route through which hyperglycemia may contribute to β-cell dysfunction [12].

In conclusion, this study demonstrates that in intact animals, high dose of ASA stimulate heart Glk accumulation and has pronounced anabolic effect. Concerning diabetic animals, these findings give evidence that ASA treatment in vivo could lead to a moderation of heart carbohydrate-related complications caused by experimental diabetes: reduction of glycogenogenesis and inhibited depression of glycolysis, with a tendency to maintenance control values.

Conflict of interest We confirm that there is no conflict of interest between authors.

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