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Effects of vanadium treatment on the alterations of cardiac glycogen phosphorylase and phosphorylase kinase in streptozotocin-induced chronic diabetic rats

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Supersensitivity to isoproterenol (ISO) induced activation of cardiac phosphorylase in diabetic rat heart has been previously demonstrated and was also reproduced in this study. To explore further the nature of this supersensitivity, we examined the activity of phosphorylase kinase and the level of cyclic AMP (cAMP) in this tissue. We observed a significantly enhanced activation of phosphorylase kinase but no increase in cAMP levels in response to ISO stimulation in diabetic rat heart, suggesting that the supersensitivity of phosphorylase activation in diabetic heart may result from an enhanced activation of phosphorylase kinase that does not involve the cAMP pathway. On the other hand, perfusion of diabetic rat heart with verapamil (5×10^{-8} M) prior to ISO stimulation abolished the enhanced cardiac phosphorylase activation, suggesting a role for calcium in the supersensitivity of phosphorylase activation. Furthermore, treatment of the diabetic rats with an insulin-like compound, vanadyl sulphate, completely abolished the enhanced cardiac phosphorylase activation and restored the increase in ISO-induced cAMP elevation in diabetic heart. The present study has provided further information on the changes of phosphorylase activation in the diabetic rat heart and demonstrated beneficial effects of vanadyl sulphate on the pathway leading to phosphorylase activation in diabetic rat heart.

Key words: phosphorylase, phosphorylase kinase, catecholamines, vanadium.

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L'hypersensibilité de l'activation de la phosphorylase cardiaque, induite dans le coeur de rat diabétique par l'isoprotérénol (ISO), a déjà été démontrée et est reproduite à nouveau ici. Pour étudier davantage la nature de cette hypersensibilité, nous avons examiné l'activité de la phosphorylase kinase et le taux d'AMP cyclique (AMP_c) dans ce tissu. Nous avons observé une augmentation significative de l'activation de la phosphorylase kinase, mais aucune augmentation des taux d'AMP_c en réponse à une stimulation par ISO dans le coeur de rat diabétique; ceci suggère que l'hypersensibilité de l'activation de la phosphorylase dans le coeur diabétique pourrait résulter d'une augmentation de l'activation de la phosphorylase kinase qui n'implique pas la voie de l'AMP_c. Par ailleurs, la perfusion du coeur de rat diabétique avec du vérapamil (5×10^{-8} M), avant la stimulation par ISO, a supprimé l'augmentation de l'activation de la phosphorylase cardiaque, suggérant un rôle pour la calcium dans l'hypersensibilité de l'activation de la phosphorylase. De plus, le traitement des rats diabétiques avec un composé de type insuline, le sulfate de vanadyle, a totalement supprimé l'augmentation de l'activation de la phosphorylase cardiaque et rétabli l'augmentation des taux d'AMP_c induite par ISO dans le coeur diabétique. Cette étude nous a éclairé davantage sur les variations de l'activation de la phosphorylase dans le coeur de rat diabétique, en plus de démontrer les effets bénéfiques du sulfate de vanadyle sur la voie menant à cette activation.

Mots clés : phosphorylase, phosphorylase kinase, catécholamines, vanadium.

[Traduit par la Rédaction]

Introduction

Cardiovascular abnormalities associated with diabetes may result from atherosclerosis as well as from intrinsic defects in the cardiac muscle, a condition that has been referred to as cardiomyopathy (Dhalla et al. 1985; Fein and Sonnenblick 1985; Zarich and Nesto 1989; Fein 1990; Fisher and Frier 1990). Increasing evidence suggests that multiple factors are responsible for the pathogenesis of diabetic cardiomyopathy. These include the reduced β-adrenoceptors (Savarese and Berkowitz 1979; Nishio et al. 1988); an impaired Ca^{2+} ATPase of sarcoplasmic reticulum (SR) (Lopaschuk et al. 1982) and a defect in the Ca^{2+} pump in sarcolemma (SL) of the diabetic heart have also been observed (Heyliger et al. 1987). In addition, it has been repeatedly observed that the activity of glycogen phosphorylase, a key enzyme in the regulation of glycogen metabolism and glucose utilization, is increased in cardiac tissues from diabetic animals and that an enhanced phosphorylase activation occurs in response to catecholamine stimulation (Miller et al. 1981; Miller 1983, 1984; Vadlamudi and McNeill 1983). In view of the important role of glycogen phosphorylase in maintaining normal metabolism of glycogen in the heart, it is possible that alterations in its activity may contribute to the development of cardiac abnormalities. Studies on the mechanisms responsible for the supersensitivity of isoproterenol-induced cardiac phosphorylase activation in diabetes are still controversial. Whereas one study indicated that phosphorylase kinase was involved, another showed that there was no change in the activity of phosphorylase kinase during diabetes (Miller et al. 1981; Miller 1984). An increased level of intracellular calcium in diabetic rat heart due to impaired calcium transport has been suggested as a possible cause of the enhanced phosphorylase activation (Vadlamudi and McNeill 1983), but there is no solid evidence to support this speculation. Therefore, the alterations of glycogen phosphorylase in diabetic rat heart remain to be further defined.

Vanadium has a variety of complicated biological effects, including inhibition of Na^+-K^+ ATPase and phosphotyrosine phosphatase (Macara 1980; Boyd and Kustin 1984; Jandhyala and Hom 1983; Nechay 1984). Interestingly, vanadium produced insulin-like effects on glucose metabolism and corrected diabetes-induced changes in cardiac performance when given

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orally to diabetic animals (Heyliger et al. 1985; Ramanadham et al. 1989; Shechter et al. 1990). However, it is not known whether vanadium treatment can correct the alterations of phosphorylase in diabetic rat heart. Therefore, this study was undertaken not only to further characterize cardiac phosphorylase activation but also to examine the effects of vanadium treatment on the alterations of phosphorylase in diabetic rat heart. Our results suggest that Ca^{2+} may be involved in the enhanced activation of phosphorylase in diabetic rat heart via enhanced activation of phosphorylase kinase, and that treatment with vanadium reversed the enhanced isoproterenol activation of glycogen phosphorylase in diabetic rat heart.

Materials and methods

Materials

Streptozotocin (STZ), phosphorylase *b*, protein assay kit, glucose-1-phosphate (G-1-P), bovine serum albumin fraction V (BSA), glycogen, L-isoproterenol (ISO), ethylenediaminetetraacetic acid (EDTA), caffeine, Tris 7-9, phenylmethylsulfonyl fluoride (PMSF), glycerol phosphate, 2-mercaptoethanol, adenosine 5′-monophosphate (5′-AMP), and magnesium acetate ($Mg(Ac)_2$) were obtained from Sigma (St. Louis, Mo.). 1-Amino-2-naphthol-4-sulfonic acid (Fiske–Subbarow reagent) and vanadyl sulphate ($VOSO_4 \cdot H_2O$) were purchased from Fisher Scientific (Nepean, Ont.); [γ-^{32}P]ATP and the assay kit for cyclic AMP (cAMP) were obtained from Amersham (Oakville, Ont.). The insulin assay kit was obtained from Immunocorp (Montréal, P.Q.). Sodium fluoride was obtained from Mallinckrodt Inc. (Mississauga, Ont.).

Animal preparations

Diabetes was induced in male Wistar rats weighing 175–200 g by a single tail vein injection of STZ (60 mg/kg). Control rats received an injection of the saline vehicle. Blood glucose levels were measured using a Glucometer II (Miles Laboratories, Elkhart, Ind.) 3 days after STZ injection. Animals with glucose values greater than 15 mM were considered diabetic.

In the vanadium experiment, half of the rats received treatment with vanadyl sulphate in drinking water (1 mg/mL) for 5 weeks. Blood samples were collected at the time of sacrifice, and the serum was obtained by centrifugation. Body weight, serum glucose, and insulin were measured in each group of animals. All animals were killed 6 weeks after STZ or saline injection.

Serum glucose and insulin determination

Serum glucose concentration was determined with the Peridochrom^R Glucose GOD-PAP assay kit (Boehringer, Laval, P.Q.). Serum insulin was measured by a radioimmunoassay using the Immunocorp insulin radioimmunoassay kit. The assay was carried out following the standard procedures.

Preparation of hearts for perfusion

Hearts from control and diabetic rats were removed at the end of the 5-week vanadyl sulfate treatment, and were perfused via a Langendorff heart apparatus at a filling pressure of 55 cmH_2O (1 cmH_2O = 98.1 Pa). The perfusate in the two reservoirs was constantly bubbled with a mixture of 95% O_2 – 5% CO_2. Hearts were perfused with Chenoweth–Koelle (CK) buffer (containing (in mM) NaCl, 120; KCl, 5.6; $CaCl_2$, 2.0; $MgCl_2$, 2.1; $NaHCO_3$, 25; and glucose, 10) for 20 min to measure basal activity of glycogen phosphorylase, phosphorylase kinase, or the concentration of cAMP. These parameters were also determined after stimulation with ISO for another 30 s at a concentration of 5×10^{-9} M. For verapamil perfusion, the hearts were first perfused with CK buffer containing 5×10^{-8} M verapamil (basal) and subsequently stimulated with ISO. During this period, EDTA and ascorbic acid (both 10 mg/L) were added to the perfusion buffer to prevent the oxidation of ISO. After perfusion and drug administration, the hearts were freeze clamped, pulverized, and stored at −80°C until further analysis.

Phosphorylase assay

Cardiac glycogen phosphorylase activity was measured by determining the release of inorganic phosphate from G-1-P as previously described (McNeill and Brody 1966). Briefly, about 50 mg of frozen powdered tissue was homogenized in 2 mL of cold Tris buffer (pH 6.8) containing 50 mM Tris, 1 mM EDTA, and 10 mM NaF, and the homogenate was centrifuged at 10 000 × *g* for 10 min at 0–4°C. The reaction mixture contained 20 μL supernatant and 0.5 mL reaction medium containing 50 mM Tris buffer (pH 6.8), 0.48% glycogen, 0.3% BSA, and 1 μM G-1-P. In addition, the medium for the measurement of total phosphorylase activity (glycogen phosphorylase *T*, the sum of phosphorylase *a* and phosphorylase *b*) contained 1 mM 5′-AMP. The reaction was conducted at 37°C for 30 min and was terminated by adding 1 mL 10% trichloroacetic acid (TCA). The color development was carried out at room temperature with 0.8 mL of TCA mixture, 0.1 mL of Fiske–Subbarow reagent, and 1.6 mL of shelf molybdate solution (containing H_2SO_4 and ammonium molybdate) for 30–45 min. The concentration of phosphate was determined with a spectrophotometer, using sodium biphosphate (Na_2HPO_4) as a standard. Activities of both glycogen phosphorylase *a* and glycogen phosphorylase *T* were expressed in nanomoles of inorganic phosphate released per minute per milligram protein. Glycogen phosphorylase *a* was also expressed as a phosphorylase *a* ratio (%), i.e., (phosphorylase *a* activity/phosphorylase total activity) × 100.

Cardiac phosphorylase kinase assay

The activity of cardiac phosphorylase kinase was determined by a method modified from Cooper et al. (1980). Approximately 150 mg of heart tissue was suspended in 0.75 mL of extraction buffer containing 30 mM Tris (pH 7.5), 30 mM KCl, 5 mM EDTA, 100 mM NaF, and 1 mM PMSF. The tissue was homogenized and immediately centrifuged at 39 000 × *g* for 15 min at 4°C, and the supernatant was diluted with an equal volume of dilution buffer (pH 6.8) containing 10 mM β-glycerolphosphate, 5 mM EDTA, 125 mM NaF, and 45 mM 2-mercaptoethanol. The assay was carried out at pH 6.8 and 8.2, and the final concentrations in the reaction mixture were 41.67 mM Tris, 41.67 mM β-glycerol phosphate, 16.7 μM $CaCl_2$, 3 mM [γ-^{32}P]ATP (30 μCi/mmol; 1 Ci = 37 GBq), 10 mM magnesium acetate, 5 mg/mL phosphorylase *b*, and 10 mg/mL glycogen. The reaction was allowed to proceed for 5 min and terminated by spotting 50 μL of the mixture onto 1.5-cm^2 Whatman ED-31 filter-paper squares, which were immediately washed with 10% TCA for 30 min. This was followed by several continuous washes with 5% TCA and 95% ethanol. The filter papers were dried and counted in a liquid scintillation counter.

Inactive phosphorylase kinase from resting skeletal muscle has minimal activity at physiological pH (pH 6.8–7.0), but considerable activity at pH greater than 7.6 (Krebs et al. 1964). It has been shown that phosphorylation of phosphorylase kinase caused a large increase in the activity at pH 6.8, but little increase in the activity at pH 8.0 (Cohen 1988). Therefore, activation of the enzyme can be expressed either as activity at pH 6.8, or as the ratio of activity at pH 6.8 to the activity at pH 8.2.

Determination of protein concentration

The concentration of protein in the enzyme solutions of both phosphorylase and phosphorylase kinase assays was determined by a modified Lowry assay method using the Sigma protein assay kit. The analytical wavelength used was 750 nm, and BSA was used as a standard.

Cardiac cAMP assay

cAMP levels in the frozen heart tissues were determined by a radioimmunoassay kit supplied by Amersham (Oakville, Ont.). Briefly, about 20 mg of frozen samples was homogenized in 1 mL 6% TCA. After removal of TCA by washing three times with ether, the ether was evaporated with nitrogen gas and radioimmunoassays were performed on the ether-free aqueous extracts. Results were expressed as picomoles of cAMP per milligram wet tissue.

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Statistical analysis

Results are presented as means ± SEM. Statistical analysis was performed using a one-way ANOVA followed by a comparison of group means with Duncan's test. When only two group means were compared, statistical analysis was performed using an unpaired Student's t test. A value of $p < 0.05$ was taken as the level of statistical significance.

Results

General features of the experimental animals

The diabetic rats exhibited a lower body weight (302 ± 7 versus a control of 442 ± 5 g, $p < 0.05$), a higher level of fasting blood glucose (22.4 versus a control of 9.6 mM, $p < 0.05$), and a decreased level of insulin in the serum (30.5 ± 2.2 versus a control of 97.8 ± 9.0 μU/mL, $p < 0.05$). Treatment with vanadyl sulphate did not affect glucose levels in the control rats (6.8 ± 0.78) but lowered the glucose concentration in the diabetic rats (10.7 ± 0.8 mM). The response of diabetic rats to vanadium treatment was variable, with 41.7% of the rats being euglycemic, 33.3% with lowered glucose levels that were still higher than the controls, and 25% remaining hyperglycemic.

Supersensitivity of ISO-induced activation of cardiac phosphorylase and phosphorylase kinase in diabetic rats and the effects of verapamil perfusion

The basal activity of cardiac phosphorylase *a* in the diabetic rats was not different from that in the control rats. However, ISO-induced activation of phosphorylase was much greater in the diabetic rat, as indicated by a higher phosphorylase *a* activity as well as a higher activity ratio (Figs. 1A, 1B). ISO stimulation did not alter the total phosphorylase activity, which was significantly higher in the diabetic rat hearts (Fig. 1C).

We next measured the activity of cardiac phosphorylase kinase. It was activated by ISO to a greater extent in the diabetic rats than in the controls, as indicated by a higher activity of phosphorylase kinase at pH 6.8, and a higher activity ratio of pH 6.8/8.2 (Figs. 2A, 2B). These changes were similar to that of phosphorylase in that both displayed an enhanced sensitivity to ISO stimulation (compare with Fig. 1). In contrast to the changes of phosphorylase in diabetic rat heart, however, the basal phosphorylase kinase activity was higher in the diabetic rats (Fig. 2B), while the total phosphorylase kinase activity was the same in control and diabetic rats.

Since the activity of phosphorylase kinase is mainly regulated by intracellular concentrations of Ca^{2+} and cAMP, we investigated the role of these two intracellular second messengers in the alteration of cardiac phosphorylase and phosphorylase kinase in diabetic rats. We used a Ca^{2+} channel blocker, verapamil, to examine the possible involvement of Ca^{2+} in the supersensitivity of phosphorylase activation. Perfusion of rat heart with verapamil (5×10^{-8} M) for 20 min prior to ISO administration resulted in a similar magnitude of activation of cardiac phosphorylase in both control and diabetic rats, i.e., verapamil abolished the supersensitivity of phosphorylase activation by ISO in diabetic hearts (Fig. 1A). However, this effect appeared to result from a significant increase in the basal phosphorylase *a* activity after perfusion of diabetic heart with verapamil (Fig. 1B), although the total activity of phosphorylase was not affected (Fig. 1C). Similar results were obtained when phosphorylase kinase activity was determined. After verapamil pretreatment, the ISO-induced activation of phosphorylase kinase in the diabetic rat heart was not different from that of the controls (Fig. 2A). Again, the basal phosphorylase kinase activity (at pH 6.8) was higher in the diabetic rats (Fig. 2B), while the total activity was not changed (Fig. 2C). Thus, the effects of verapamil on phosphorylase may be most likely mediated through phosphorylase kinase.

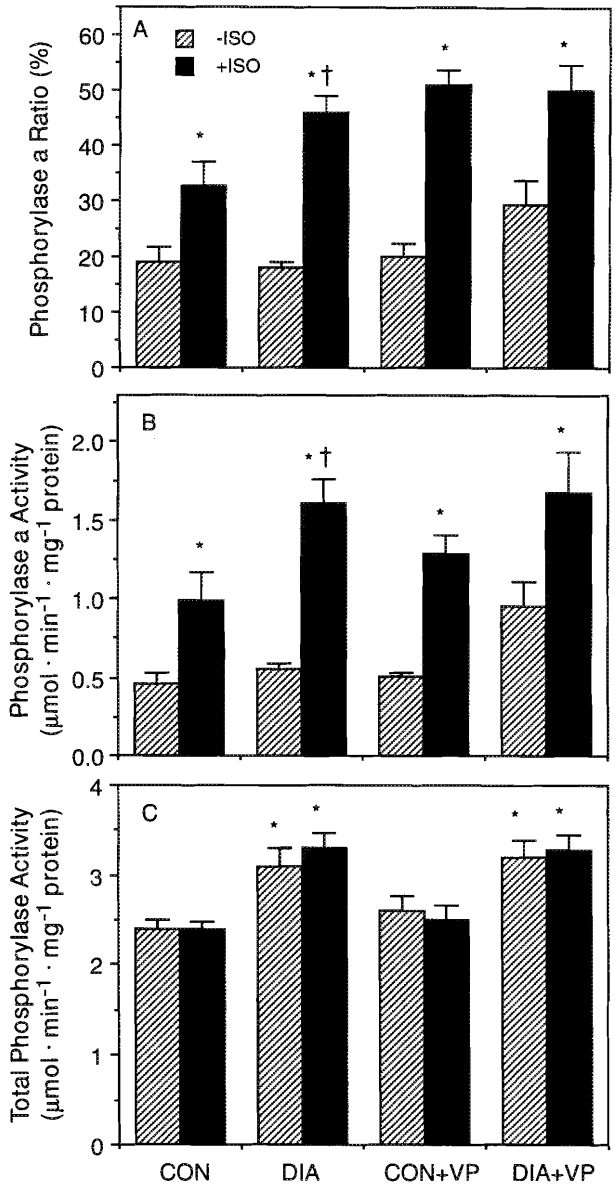

FIG. 1. Effects of ISO and verapamil (VP) perfusion on phosphorylase in control (CON) and diabetic (DIA) rats. The hearts were perfused for 20 min or for 20 min with an additional 30-s perfusion with ISO (5×10^{-9} M). In some experiments, the hearts were perfused with verapamil (5×10^{-8} M) for 20 min prior to freeze clamping or prior to a 30-s perfusion with isoproterenol (ISO). Phosphorylase was assayed as described in Materials and methods. (A) Phosphorylase *a* ratio; (B) phosphorylase *a* activity; (C) total phosphorylase activity. Bars represent means of observations made with 6–8 rats. *$p < 0.05$ vs. CON (−ISO); †$p < 0.05$ vs. CON (+ISO).

Vanadyl sulphate treatment normalized cardiac phosphorylase and phosphorylase kinase in diabetic rats

We examined the cardiac glycogen phosphorylase activity in rats that were treated with oral vanadyl sulphate (~100 mg ·

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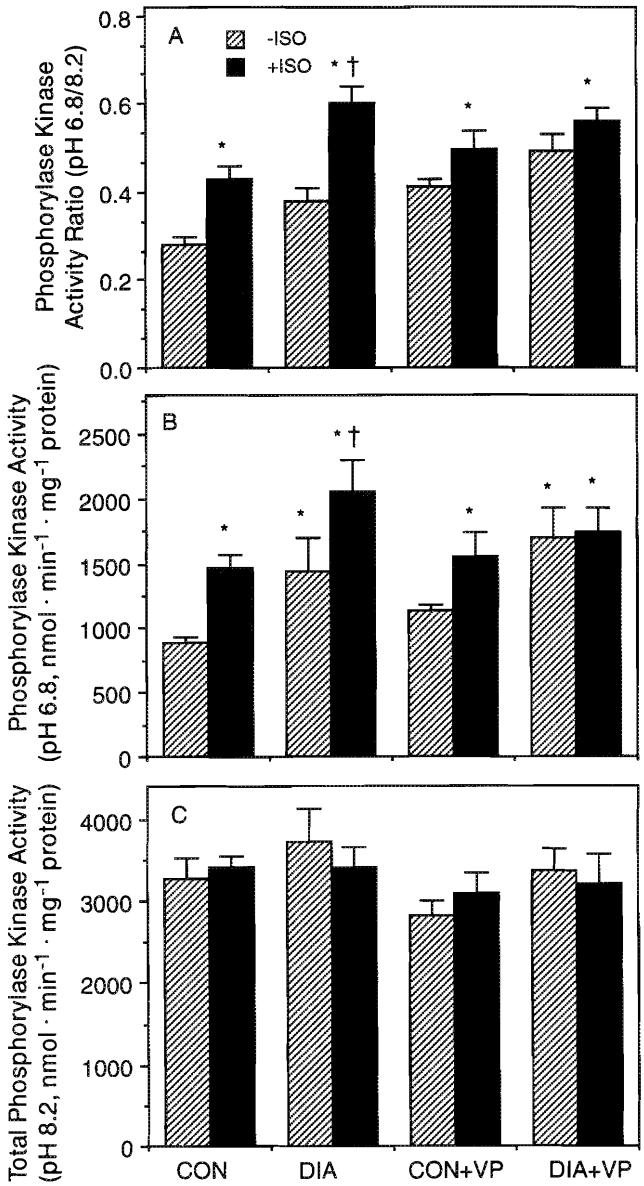

FIG. 2. Effects of ISO and verapamil (VP) perfusion on phosphorylase kinase in control (CON) and diabetic (DIA) rats. Phosphorylase kinase was assayed as described in Materials and methods, using samples that were used for phosphorylase assays in Fig. 1. The activities at pH 6.8 and 8.2 are shown in Figs. 2B and 2C, respectively, and Fig. 2A shows the activation ratio of phosphorylase kinase. Bars represent means of observations made with 4–6 rats. $*p < 0.05$ vs. CON (−ISO); $^{\dagger}p < 0.05$ vs. CON (+ISO).

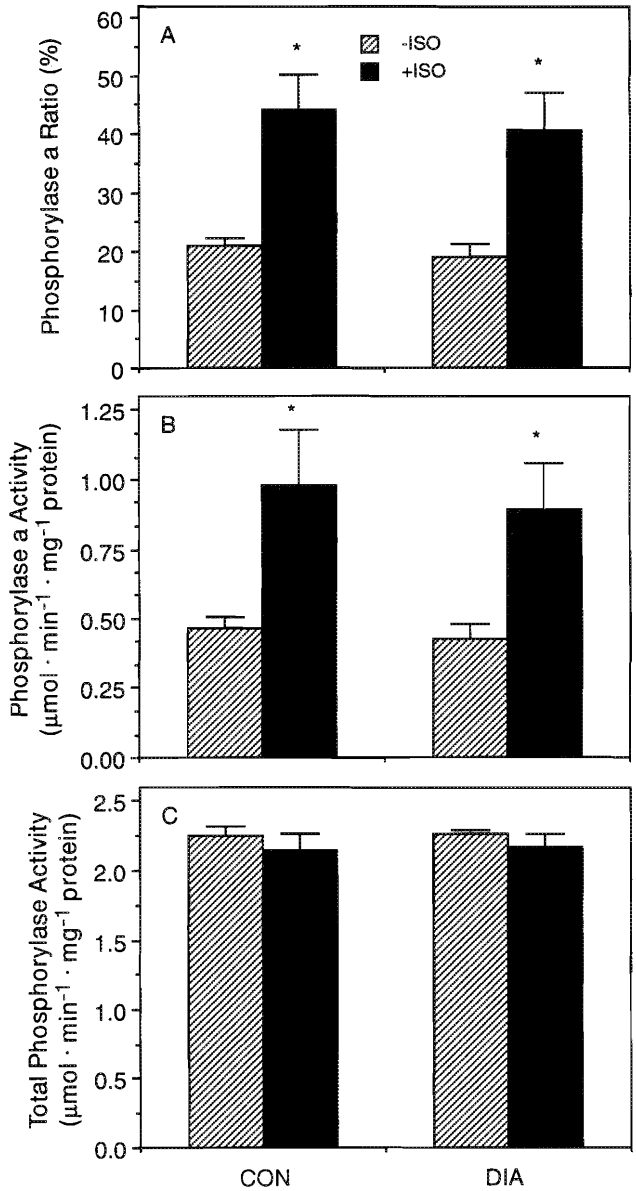

FIG. 3. Effects of vanadyl sulphate treatment on cardiac phosphorylase in control (CON) and diabetic (DIA) rats. Phosphorylase activity was determined in hearts from control and diabetic rats that were treated with vanadyl sulphate for 5 weeks. The hearts were perfused for 20 min before freeze clamping or before exposure to ISO for 30 s. Phosphorylase was assayed as described in Materials and methods. Figs. 3B and 3C show the phosphorylase *a* activity and total activity, respectively, and Fig. 3A shows the activation ratio of phosphorylase. Bars represent means of observations made with 6 rats. $*p < 0.05$ vs. CON (−ISO).

$kg^{-1} \cdot day^{-1}$) for 5 weeks. Following the treatment, no difference in phosphorylase *a* activity or ratio was apparent between the control and the diabetic rats (Figs. 3A, 3B). The treatment lowered the basal phosphorylase *a* activity to a normal level, and also normalized the activation of cardiac phosphorylase activation by ISO in diabetic rat hearts (Figs. 3A, 3B). In addition, the treatment reduced the total phosphorylase activity in diabetic rat heart to the control levels (Fig. 3C). Thus, vanadyl sulfate treatment completely restored phosphorylase to normal.

Similarly, vanadyl sulphate treatment also normalized the profile of phosphorylase kinase in the diabetic rats. The basal phosphorylase kinase activity at pH 6.8 and the activation by ISO were the same between the control and the diabetic rats (Figs. 4A, 4B). However, it should be noted that the total activity of phosphorylase kinase in the vanadyl sulphate treated animals (Fig. 4C) was significantly lower than that of the untreated rats for both control and diabetic groups (compare the activity values with those in Fig. 2C), although the total activity was not different between the control and the diabetic rat heart.

Effects of verapamil perfusion and vanadyl sulphate treatment on cardiac cAMP levels

To determine the role of cAMP and its interaction with intracellular Ca^{2+} in the alteration of phosphorylase in dia-

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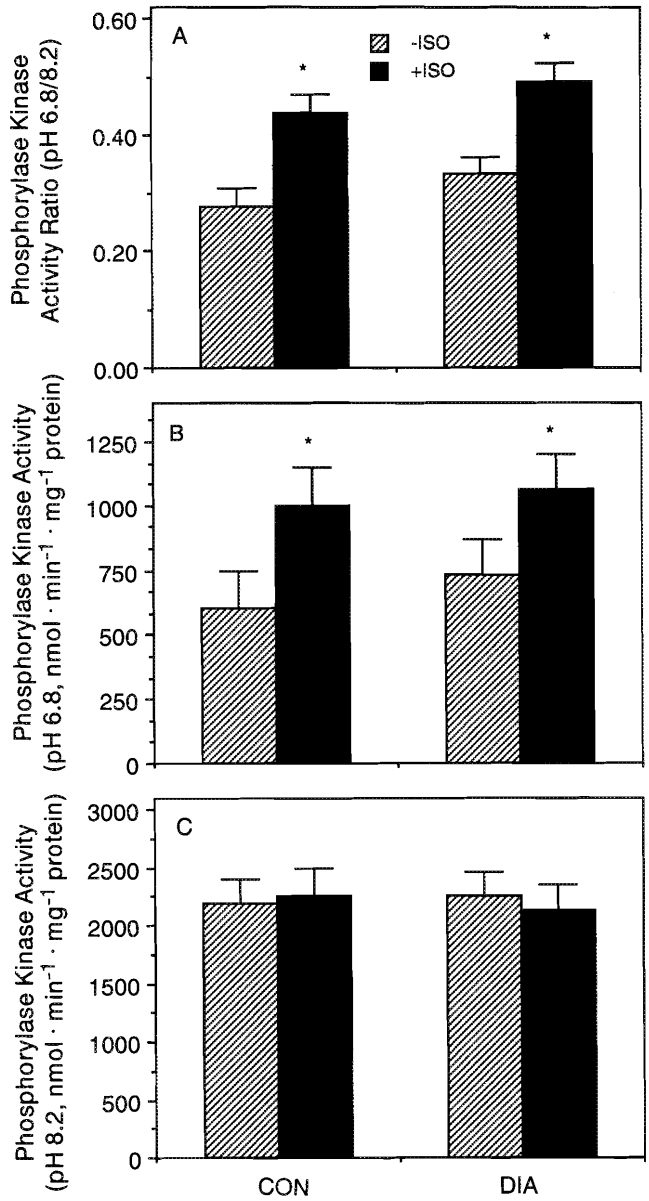

FIG. 4. Effects of vanadyl sulphate treatment on cardiac phosphorylase kinase in control (CON) and diabetic (DIA) rats. Phosphorylase kinase activity was determined in hearts from control and diabetic rats that were treated with vanadyl sulphate for 5 weeks. The hearts were perfused for 20 min before freeze clamping or before exposure to ISO for 30 s. Phosphorylase kinase was assayed as described in Materials and methods. Figs. 4B and 4C show the phosphorylase kinase activity at pH 6.8 and 8.2, and Fig. 4A shows the activation ratio of phosphorylase kinase. Bars represent means of observations made with 5 or 6 rats. *$p < 0.05$ vs. CON (−ISO).

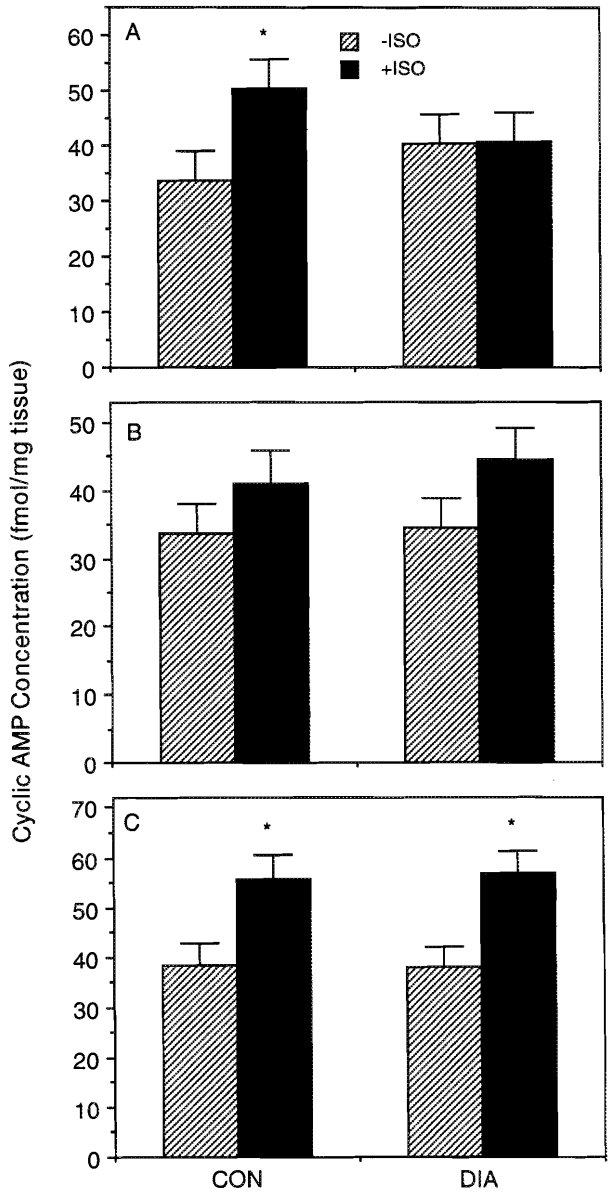

FIG. 5. Effects of verapamil (VP) perfusion and vanadyl sulphate (VS) treatment on cardiac cAMP in control (CON) and diabetic (DIA) rats. Cardiac cAMP was assayed as described in Materials and methods. The levels of cAMP in the control and the diabetic rat heart are shown in Fig. 5A. Some of the hearts were perfused with VP (5×10^{-8} M) for 20 min before freeze-clamping for cAMP determination (B). The heart samples from VS-treated rats were also used for cAMP analysis (C). Bars represent means of observations made with 4–6 rats. *$p < 0.05$ vs. CON (−ISO).

betic rat heart, we chose to measure basal and ISO-stimulated levels of cAMP in the absence and presence of verapamil. The basal level of cardiac cAMP was similar between the control and the diabetic rats, but the ISO-stimulated increase in cAMP levels was observed only in the control rats (Fig. 5A). This is in direct contrast to the changes of phosphorylase and phosphorylase kinase, both of which were activated by ISO to a higher extent in the diabetic rat heart. Interestingly, the increase in cAMP following ISO stimulation was much lower in the control rats after verapamil perfusion, and the difference between the control and the diabetic rats was eliminated (Fig. 5B).

Treatment with vanadyl sulphate also produced interesting effects on cardiac cAMP levels. While the treatment did not alter the increase of cAMP by ISO in the control rats, it did restore the response of diabetic rat heart to ISO stimulation. As shown in Fig. 5, the ISO-induced increase in the cAMP levels was the same in the control and the diabetic rat heart (Fig. 5C).

Discussion

The supersensitivity of catecholamine-induced activation of glycogen phosphorylase in diabetic animals was first observed

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by Miller et al. in acute alloxan-induced diabetic rats (Miller et al. 1981) and spontaneously diabetic Bio-Breeding/Worcester (BB/W) rats (Miller 1983). It was subsequently confirmed in STZ-induced chronically diabetic rats (Vadlamudi and McNeill 1983) as well as in isolated cardiomyocytes from diabetic rats (Miller and Jasper 1991). The mechanism for the supersensitivity could result from an increase in the activity of phosphorylase kinase or a decrease in the activity of phosphorylase phosphatase. Since phosphorylase phosphatase has been shown to be unchanged in diabetic heart (Miller et al. 1981; Miller 1983, 1984; Miller and Jasper 1991), it is more likely that the upstream activating components for phosphorylase, such as phosphorylase kinase, cAMP, or Ca^{2+}, may be responsible for the alterations of phosphorylase activation in diabetic animals.

A previous study demonstrated that the activation of phosphorylase kinase in response to epinephrine was two-fold higher in the diabetic rat heart (Miller et al. 1981), similar to the results in our present study, suggesting that an increase in the sensitivity of ISO-induced activation of cardiac phosphorylase kinase may be involved in the supersensitivity of glycogen phosphorylase activation. However, there have also been reports that phosphorylase kinase was not changed in diabetic rat heart (Miller 1984). This is in direct contrast to our experimental results as well as to an earlier report (Miller et al. 1981). The reasons for the discrepancies are not clear, and a direct comparison of these studies is difficult since no details were presented on phosphorylase kinase activities in the previous studies. A possible explanation may be that the duration of diabetes is different. A short-term diabetes model was used by Miller et al. (1981, 1984), while we used a long-term diabetes model in this study.

Phosphorylase kinase is covalently regulated by cAMP-dependent protein kinase (PKA) and allosterically controlled by intracellular calcium (Cohen 1988). Previous studies have demonstrated a significant decrease in the stimulation of the cAMP–PKA system in diabetic rats (Ingebretsen et al. 1981; Miller et al. 1981; Miller 1984; Miller and Jasper 1991). Our results confirm that the ISO-stimulated increase in cAMP content is markedly reduced in diabetic rat hearts. Thus, the alterations in the cAMP–PKA system could not explain the supersensitivity of phosphorylase activation in diabetic rat heart. We also explored the involvement of Ca^{2+} with the use of a calcium channel blocker, verapamil, which was shown to abolish the supersensitivity of phosphorylase activation in the diabetic rat heart without affecting the activation of phosphorylase in the control rat heart. This suggests that the cAMP–PKA system plays a dominant role in ISO-induced activation of phosphorylase kinase and phosphorylase under normal conditions, so that the effect of a calcium blocker is minimal in the control rats. However, in the diabetic rat heart, Ca^{2+} may play a dominant role in ISO-induced activation of phosphorylase kinase and phosphorylase, presumably as a result of defects in the cAMP–PKA system. Consequently, verapamil abolished the supersensitivity of phosphorylase and phosphorylase kinase activation in the diabetic rats. This notion is consistent with previous studies showing inhibition of major calcium transporters during diabetes (Smith et al. 1984; Heyliger et al. 1987; Makino et al. 1987), blockade of supersensitivity of glycogen phosphorylase activation to epinephrine by a low calcium medium perfusion of diabetic rat hearts (Miller 1984), and a decrease in the severity of diabetic cardiomyopathy by verapamil treatment (Ingebretsen et al. 1981; Michel et al. 1985; Afzal et al. 1988, 1989). Thus, we suggest that the enhanced glycogen phosphorylase activation by catecholamines in diabetic rat heart may be at least partially related to an increase in the activation of phosphorylase kinase due to defective calcium homeostasis in diabetic rat heart.

To investigate whether the insulin-mimetic agent vanadium could affect the changes of phosphorylase in diabetic rats, vanadyl sulphate was administered to some diabetic rats. A 5-week treatment with vanadyl sulphate had no effect on the basal level or the ISO-stimulated levels of cAMP, glycogen phosphorylase, or phosphorylase kinase in the control rats, nor did it affect the basal levels of cAMP or activity ratio of glycogen phosphorylase and phosphorylase kinase in diabetic rat hearts. The treatment did, however, eliminate the enhanced activation of both glycogen phosphorylase and phosphorylase kinase by ISO. The treatment also reduced the higher level of total cardiac glycogen phosphorylase, and normalized the ISO-stimulated cAMP production in the diabetic rats. These effects could be attributed to a direct effect of the treatment on cardiac glycogen phosphorylase or to an overall effect of the treatment on the various components in the cascade leading to the activation of glycogen phosphorylase, such as the level of cAMP and phosphorylase kinase. While the basal level of cAMP and the activation of phosphorylase kinase were not altered by treatment, the impaired response of cAMP production and the sensitivity of phosphorylase kinase activation by ISO were restored after vanadyl sulphate treatment. Similar effects were observed in hepatocytes where the cAMP level was increased by vanadate treatment (Almira and Misbin 1989). Since an increase in cAMP would generally lead to activation of glycogen phosphorylase, the effect of vanadyl sulphate on cAMP level apparently did not contribute to abolishing the supersensitivity of glycogen phosphorylase activation, which is consistent with our hypothesis that cAMP was not involved in supersensitivity of glycogen phosphorylase activation to ISO. Therefore, the effectiveness of vanadyl sulphate treatment to abolish the supersensitivity suggests that the treatment might affect intracellular Ca^{2+}, presumably through normalization of the cellular metabolism, rather than directly acting on the glycogen phosphorylase per se.

In summary, our results suggest that changes in intracellular calcium homeostasis may be implicated in the enhanced activation of cardiac phosphorylase kinase, leading to an altered response of phosphorylase to ISO in diabetes. We also demonstrated the beneficial effects of vanadium compounds in normalizing phosphorylase activity and its activation by catecholamines in diabetic rats, which may contribute to the improved cardiac function in vanadium-treated diabetic rats.

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