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European Journal of Pharmacology 435 (2002) 269–276

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Streptozotocin diabetes protects against arrhythmias in rat isolated hearts: role of hypothyroidism

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Received 18 June 2001; received in revised form 10 September 2001; accepted 13 September 2001

Abstract

We examined the contribution of hypothyroidism to streptozotocin diabetes-induced alterations in the arrhythmia susceptibility of ex vivo hearts to regional zero-flow ischaemia. Diabetic rats received either protamine zinc insulin (10 IU/kg/day, s.c.) or triiodothyronine (10 μg/kg/day, s.c.) for 8 weeks commencing 72 h after injection of streptozotocin (60 mg/kg, i.p.). Arrhythmias were determined in ex vivo Langendorff-perfused hearts, subjected to a 30-min main left coronary artery occlusion, followed by 30-min reperfusion. Serum free thyroxine concentrations, rectal temperature and ex vivo heart rate were significantly decreased in the 8-week diabetic group ($P < 0.001$). These changes were prevented by administration of triiodothyronine or insulin. Ventricular fibrillation during reperfusion was abolished in hearts from diabetic rats. This protection was prevented by treatment with either triiodothyronine or insulin. Hearts from methimazole-hypothyroid rats also showed no ventricular fibrillation during reperfusion. The protection against ischaemia–reperfusion–arrhythmias observed in hearts from streptozotocin-diabetic rats may be due to diabetes-induced hypothyroidism.

Keywords: Streptozotocin; Diabetes; Heart, ex vivo; Hypothyroidism; Arrhythmias; Ischaemia–reperfusion, rat

1. Introduction

Ischaemic heart disease is an important cause of death in diabetic patients (Kannel et al., 1974; Schernthaner, 1996; Pickup and Williams, 1997). Whether or not the diabetic heart is more susceptible to ischaemia–reperfusion arrhythmias is uncertain (Feuvray and Lopaschuk, 1997) and results obtained from experimental diabetes are conflicting. Diabetic animals were reported to have more spontaneous arrhythmias (Navaratnam and Khatter, 1989; Hekimian et al., 1985). Other studies showed the diabetic heart to be either more vulnerable (Hekimian et al., 1985; Bhimji et al., 1986; Bakth et al., 1986; Beatch and McNeill, 1988; Tosaki et al., 1996) or more resistant to ischaemia–reperfusion arrhythmias (Kusama et al., 1992; Suzuki et al., 1993). Most of these studies have used streptozotocin- or alloxan-induced diabetes, which are models widely used to study all aspects of the disease. Hypothyroidism is associated with both models of diabetes (Sochor et al., 1987; Sundaresan et al., 1984; Rodgers et al., 1991; Rondeel et al., 1992; Schroder-Van Der Elst and Van Der Heide, 1992; Katovitch et al., 1993). As thyroid hormones are important in maintaining cardiac function, hypothyroidism could contribute to the cardiac dysfunction in diabetes. Indeed, hypothyroidism was shown to contribute significantly to the reduced cardiac contractility seen in the diabetic, renovascular hypertensive rat (Rodgers et al., 1991). However, other studies showed no effect of thyroid hormone treatment on diabetes-induced cardiac dysfunction (Barbee et al., 1988; Sato et al., 1989; Beenen et al., 1996). Moreover, cardiac dysfunction was also observed in spontaneous diabetic BB rats, in the absence of hypothyroidism (Ramanadham et al., 1989). Most of these studies have concentrated on measurement of cardiac contractility, and there is no information about the contribution

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0014-2999/02/$ - see front matter
PII: S0014-2999(01)01398-X

of hypothyroidism to altered susceptibility to ischaemia–reperfusion arrhythmias in diabetes. Apart from its negative cardiac inotropic and chronotropic effects, hypothyroidism is profoundly antiarrhythmic in dog (Venkatesh et al., 1991; Liu et al., 1996) and rat hearts (Chess-Williams and Coker, 1989) in vivo. Preliminary observations (Zhang et al., 1999a,b) indicated that streptozotocin-induced diabetes protected the ex vivo heart against ischaemia–reperfusion induced arrhythmias. This effect of diabetes may conceivably be secondary to hypothyroidism. Activation of protein kinase C has been implicated in the protective effect on the heart of ischaemic preconditioning (Downey and Cohen, 1997), although there is little work on the role of protein kinase C in the protective effect of preconditioning against arrhythmias. Increases in protein kinase C activity and expression have been found in the diabetic heart (Giles et al., 1998; Inoguchi et al., 1992; Xiang and McNeill, 1992). Therefore, we determined the effect of diabetes on ventricular levels of protein kinase C isoforms to see if these changes could be related to the effects on the heart.

2. Methods

2.1. Experimental diabetes

Diabetes was induced in male Sprague–Dawley rats (200–220 g) by a single streptozotocin injection (60 mg/kg, i.p.). Age- and weight-matched control rats received the same volume of vehicle. Animals were housed in pairs for 2–8 weeks with free access to normal laboratory diet and water. Diabetes was confirmed by glycosuria with reagent strips for urinalysis (Bayer, Berks) and serum glucose was assayed at the end of experiments (Beckman Glucose Analyzer). Any streptozotocin-injected rat not showing glycosuria at 72 h after injection was excluded.

2.2. Hormone replacement therapy

In one series of experiments control rats ($n=14$) were compared with streptozotocin-diabetic rats receiving either vehicle (saline, $n=12$), insulin (protamine zinc insulin, 10 IU/kg, s.c. daily, $n=9$) or triiodothyronine (10 μg/kg, s.c. daily, $n=8$) from 72 h after induction of diabetes for the duration of the experiment.

2.3. Experimental hypothyroidism

Rats were made hypothyroid using methimazole (0.03% in the drinking water, for 4 weeks).

2.4. Serum glucose, free thyroxine and free triiodothyronine concentrations assay

Serum glucose concentration (mmol/l) was measured using a Beckman glucose analyser (Beckman Instruments, Fullerton, USA). The assay kits were purchased from Beckman Instrument Espana (Madrid). Serum free thyroxine and free triiodothyronine levels were measured by competitive magnetic separation assay with kits from Bayer (NY, USA). These assays were carried out in the Biochemistry Institute, Glasgow Royal Infirmary by an investigator blind to the treatment of the animals.

2.5. Isolated perfused hearts

At the end of pretreatment, rats were anaesthetized with sodium pentobarbitone (60 mg/kg body weight, i.p.) and rectal temperature was recorded. The left jugular vein was cannulated and 1 ml blood collected, followed by injection of heparin (500 IU/kg). The thorax and pericardium were opened and a thread was put around the main left coronary artery close to its origin. The heart was excised, placed in ice-cold heparinised buffer and was then perfused (Langendorff mode) as described before (Song et al., 1994) via an aortic cannula with modified Krebs–Henseleit buffer (NaCl 118, KCl 3.2, $CaCl_2$ 2.52, KH_2PO_4 1.18, $MgSO_4$ 1.66, $NaHCO_3$ 26.88, glucose 5.55, sodium pyruvate 2 mM, pH 7.38, gassed with 95% O_2 and 5% CO_2, 37 °C). The perfusion pressure was measured through a side-tube above the aorta cannula. The perfusion pressure and epicardial electrocardiograph were monitored using a Gould 240 recorder (Gould Instrument, OH). The heart was perfused for 15–20 min for stabilization and the two ends of the thread were then passed through a small vinyl tube with a flat end, which formed a snare. Occlusion was produced by pushing the snare towards the heart and was confirmed by increase of perfusion pressure and regional cyanosis. The main left coronary artery was occluded for 30 min and reperfused by loosening the snare. After 30-min reperfusion, the hearts were blotted dry and their wet weights recorded.

All arrhythmias during both occlusion and reperfusion were counted and classified as single ectopic beats, salvos, ventricular tachycardia and ventricular fibrillation according to the Lambeth conventions (Walker et al., 1988). The total number of arrhythmias during occlusion and reperfusion were calculated. The incidences of ventricular tachycardia, ventricular fibrillation and sustained ventricular fibrillation (persisting for more than 5 min) were determined during both occlusion and reperfusion.

2.6. Protein kinase C detection

Protein kinase C isoforms were detected using 10 μg of protein per sample and Western blotting as described before (Wilson et al., 1996). Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham Life Science, UK) for 2.5 min. After enhanced chemiluminescence the blots were exposed to X-OMAT Kodak scientific imaging films (Eastman Kodak, Rochester, USA). Each blot was then incubated at 65–70 °C in stripping buffer (35 mM sodium dodecylsulphate, 63 mM Tris-hydrochloride, β-

mercaptoethanol, pH 6.7) for 1 h and then blocked overnight and reprobed with other isoform-specific antibodies. Each gel was reprobed a maximum of five times and the order of the antibodies was changed each time. Densitometry was performed for the density of each isoform (GS-69 image densitometer, Bio-Rad, CA, USA) and density was calculated using the Molecular Analyst program (Bio-Rad). For quantification to compare treatments, the densitometer readings of each band were divided by the densitometer reading of the control band on each gel and the corresponding ratios (referred to as densitometric ratios) used for statistical comparisons.

2.7. Statistics

All streptozotocin-injected rats with serum glucose concentration below 22 mmol/l (except insulin-treated ones) were excluded from the data analysis. Results are expressed as means ± S.E.M. or as percentages, for the incidences of arrhythmias. An unpaired, two-tailed Student's *t*-test was performed for comparison between two groups and one-way analysis of variance (ANOVA) was used for multiple groups. If ANOVA indicated significant differences, then either Dunnett's test or Tukey's test was used as appropriate. Fisher's exact test was performed to determine the statistical significance of difference in incidences of arrhythmias. Pearson's test was used for correlation between two sets of data and followed by linear regression. Statistical significant differences were accepted where $P < 0.05$.

2.8. Drugs and chemicals

Streptozotocin, sodium pyruvate, tridothyronine, methimazole and all chemicals for protein kinase C assay were purchased from Sigma (Dorset, UK). The other chemicals used for isolated heart perfusion (NaCl, KCl, $CaCl_2$, KH_2PO_4, $NaHCO_3$, $MgSO_4$, KCl) were from BDH Laboratory Supplies (Poole, UK). Sodium pentobarbitone (Sagatal) was purchased from Rhone Merieux (Tallaght, Dublin) and heparin from Leo Laboratories (Bucks, UK and Dublin, Ireland). Protamine zinc insulin was purchased from CP Pharmaceutical (Wrexham, UK).

3. Results

3.1. Effects of streptozotocin diabetes on body weight, heart weight, Q–T interval, ex vivo heart rate, rectal temperature and serum concentrations of glucose and thyroid hormones

Two weeks after induction of diabetes, the serum glucose concentration of diabetic rats was significantly increased and serum free thyroxine concentration was significantly decreased, compared to age-matched control rats (Table 1). There was a significant negative correlation between serum free thyroxine and glucose concentrations ($r = -0.76$, $n = 10$, $P < 0.05$). The weight gain was significantly lower in the diabetic rats. There was no significant difference in the rectal temperature, ex vivo heart rate or the heart weight/body weight ratio.

Eight weeks after induction of diabetes, rats showed significantly elevated serum glucose concentration, reduced weight gain and a significant increase in the heart weight/body weight ratio, relative to their age-matched controls (Table 1). Serum free tridothyronine and free thyroxine concentrations were significantly lower in diabetic rats and there was a significant negative correlation between both serum free triiodothyronine and free thyroxine concentrations and serum glucose concentrations (Fig. 1). Rectal temperature and ex vivo heart rate were significantly reduced and the Q–T interval was significantly increased, compared with control. Insulin-treated diabetic rats showed no statistically significant difference compared with control animals, in blood glucose, weight gain, heart weight/body weight ratio, serum free triiodothyronine and free thyroxine, ex vivo heart rate or Q–T interval (Table 2).

Administration of triiodothyronine to streptozotocin-diabetic rats did not modify the decrease in body weight or the hyperglycaemia but further increased the heart weight/body weight ratio (Table 2). However, it reversed the effects of

Table 1
Changes in weight gain, heart weight, rectal temperature, serum glucose, free thyroxine concentrations and ex vivo heart rate in 2- and 8-week diabetic rats and in respective control groups

	Controls for 2-week diabetes ($n = 12$)	2-week diabetes ($n = 10$)	Controls for 8-week diabetes ($n = 30$)	8-week diabetes ($n = 24$)
Weight increase/original weight (%)	37.8 ± 2.0	18.5 ± 4.6[a]	57.9 ± 2.8	−4.2 ± 3.0[a]
Heart weight/body weight (%)	0.39 ± 0.01	0.42 ± 0.02	0.38 ± 0.04	0.46 ± 0.01[a]
Rectal temperature (°C)	36.9 ± 0.1	36.6 ± 0.1	37.1 ± 0.1	36.5 ± 0.1[a]
Serum glucose (mmol/l)	8.3 ± 0.6	30.8 ± 2.1[a]	7.1 ± 0.3	35.8 ± 1.5[a]
Serum free thyroxine (pmol/l)	40.9 ± 4.8	24.7 ± 1.8[b]	30.8 ± 1.1	12.3 ± 1.0[a]
Ex vivo heart rate (beat/min)	250.5 ± 11.4	234.7 ± 8.7	272.4 ± 5.7	231.9 ± 7.5[a]

Each value is mean ± S.E.M.

[a] $P < 0.001$, indicates a significant difference between diabetic groups and their respective control groups.

[b] $P < 0.05$, indicates a significant difference between diabetic groups and their respective control groups.

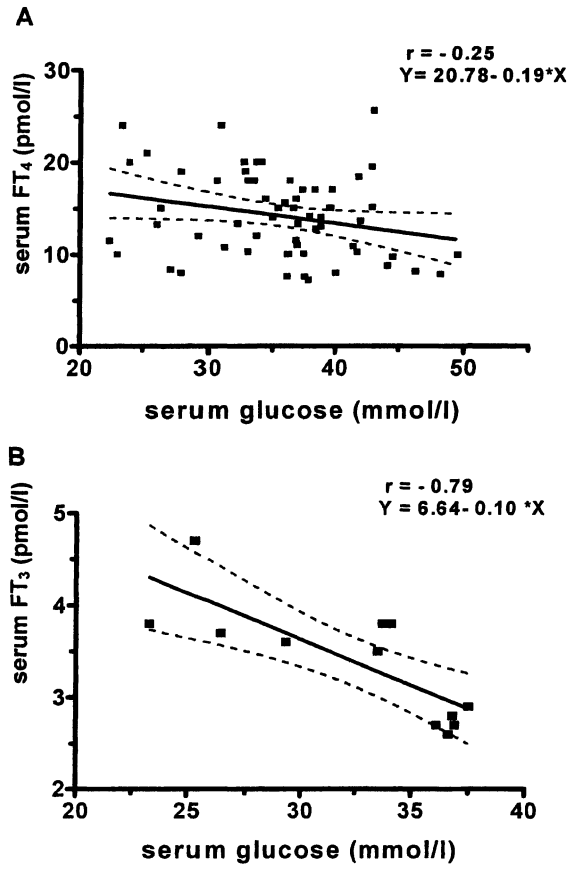

Fig. 1. Correlations in streptozotocin-diabetic rats (at 8 weeks after induction of diabetes) between (A) serum glucose and free thyroxine concentrations ($r = -0.25$, $P < 0.05$, $n = 61$), and (B) serum glucose and free triiodothyronine concentrations ($r = -0.79$, $P < 0.01$, $n = 12$).

diabetes on rectal temperature, Q–T interval and restored the ex vivo heart rate to control levels. Administration of triiodothyronine further reduced serum free thyroxine concentration to below the range of detection, without a significant elevation of free tridothyronine.

3.2. Effects of streptozotocin diabetes on the susceptibility to ischaemia–reperfusion arrhythmia

In hearts removed from 2-week streptozotocin-diabetic, the incidence of ventricular fibrillation and sustained ventricular fibrillation during reperfusion were significantly lower than those in the age-matched control group ($P < 0.0001$ and 0.01, respectively, Table 3). The total number of arrhythmias during occlusion in the diabetic hearts was also lower than in control hearts (368 ± 154 vs. 1519 ± 444, $P < 0.05$).

In hearts removed from 8-week streptozotocin-diabetic rats, the incidence of ventricular tachycardia during occlusion was significantly decreased compared to that seen in the control group ($P < 0.05$, Table 3). Both occlusion and reperfusion-induced ventricular fibrillation were abolished in diabetic hearts, whilst in the control group ventricular fibrillation occurred in all the hearts, and it was sustained ($P < 0.01$, Table 3). The total number of arrhythmias during occlusion in diabetic hearts tended to be lower than in control hearts (164 ± 54 vs. 990 ± 389). However, the difference was not statistically significant ($P = 0.07$).

3.3. Levels of protein kinase C isoforms in control and 8-week diabetic ventricles

Eight protein kinase C isoforms were identified in both cytosolic and particulate fractions of ventricles from hearts ($n = 3–6$) of control and diabetic rats. The majority of each protein kinase C isoform was localised in the high-speed cytosolic fraction (data not shown). The levels of protein kinase C isoforms β, θ, λ (Fig. 2) and ε, δ, ι (data not shown)

Table 2
Changes in weight gain, heart weight, rectal temperature, serum glucose, free thyroxine (FT_4) and triiodothyronine (FT_3) concentrations, ex vivo heart rate and Q–T interval in STZ-diabetic rats, age-matched control and STZ-diabetic rats receiving 8-week insulin or triiodothyronine treatment

	Control with vehicle ($n = 14$)	Diabetic with vehicle ($n = 12$)	Diabetic with T_3 ($n = 8$)	Diabetic with PZI ($n = 9$)
Weight increase/original weight (%)	65.2 ± 13.7	16.3 ± 3.7^a	14.5 ± 5.6	66.5 ± 4.4^b
Heart weight/body weight (%)	0.39 ± 0.01	0.42 ± 0.01^a	0.50 ± 0.1^b	0.37 ± 0.1^b
Rectal temperature (°C)	37.1 ± 0.1	36.6 ± 0.2^c	37.6 ± 0.3^d	36.9 ± 0.2
Serum glucose (mmol/l)	6.8 ± 0.3	32.4 ± 1.5^a	29.3 ± 1.4	8.1 ± 0.8^b
Serum FT_3 (pmol/l)	5.0 ± 0.2	3.4 ± 0.2^a	3.8 ± 0.4	5.4 ± 0.5^b
Serum FT4 (pmol/l)	28.4 ± 1.4	15.6 ± 1.5^a	$< 5.0^b$	27.4 ± 1.7^b
Ex vivo heart rate (beat/min)	257.1 ± 7.4	240.0 ± 9.8	292.5 ± 11.9^d	252.5 ± 10.5
Q–T interval (ms)	70.5 ± 2.7	86.3 ± 2.8^a	69.2 ± 3.5^d	75.0 ± 2.7^e

Each value is mean ± S.E.M. Age-matched control rats and streptozotocin-diabetic rats received vehicle injections daily. One group of diabetic rats received triiodothyronine (diabetes with T_3, 10 μg/kg/day, s.c.) from 72 h after induction of diabetes for the duration of the experiment. Another diabetic group received protamine zinc insulin (diabetes with PZI, 10 IU/kg/day, s.c.) from 72 h after induction of diabetes and for the duration of the experiment.

[a] $P < 0.01$ of vehicle-treated control group.
[b] $P < 0.001$ vs. diabetes with vehicle group.
[c] $P < 0.05$ of vehicle-treated control group.
[d] $P < 0.01$ vs. diabetes with vehicle group.
[e] $P < 0.05$ vs. diabetes with vehicle group.

Table 3
Incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and sustained VF during occlusion and reperfusion in 2- and 8-week diabetic rats and corresponding controls

Treatment	Occlusion			Reperfusion		
	VT (%)	VF (%)	Sustained VF (%)	VT (%)	VF (%)	Sustained VF (%)
Control ($n=6$)	100	50	30	100	100	71.4
2-week STZ-diabetes ($n=10$)	50	12.5	0	50	0[a]	0[a]
Control ($n=12$)	100	50	33.3	100	100	100
8-week STZ-diabetes ($n=6$)	16.7[b]	0	0	33.3	0[a]	0[a]

Each value is the percentage of animals showing the particular response.
[a] $P<0.01$ vs. corresponding controls.
[b] $P<0.05$ vs. corresponding controls.

Table 4
Effects in rats of triiodothyronine and insulin treatment (from 72 h after induction of diabetes for 8-week) on incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and sustained VF during both occlusion and reperfusion

Treatment	Occlusion			Reperfusion		
	VT (%)	VF (%)	Sustained VF (%)	VT (%)	VF (%)	Sustained VF (%)
Control with vehicle ($n=9$)	100	77.8	66.7	100	100	100
Diabetic + vehicle ($n=9$)	77.8	0[a]	0[a]	55.6	0[a]	0[a]
Diabetic + T_3 ($n=6$)	83.3	50[b]	33.3	100	100[c]	100[c]
Diabetic + PZI ($n=7$)	71.4	57.1[b]	42.9	75	75[b]	50

Each value is the percentage of animals showing the particular response.
[a] $P<0.01$ vs. vehicle-treated control.
[b] $P<0.05$ vs. vehicle-treated diabetic group.
[c] $P<0.01$ vs. vehicle-treated diabetic group.

were unaltered between control and diabetic ventricles. In contrast, there were increases in the levels of protein kinase Cγ (densitometric ratios diabetic/control 1.5 ± 0.1, $n=6$) and protein kinase Cα (densitometric ratios diabetic/control 1.7 ± 0.4, $n=3$) in ventricles from diabetic rats (Figs. 2 and 3).

3.4. Effects of triiodothyronine or insulin treatment, on changes produced by streptozotocin diabetes

Triiodothyronine supplementation in diabetic rats significantly increased the incidences of ventricular fibrillation during occlusion ($P<0.05$) and reperfusion ($P<0.01$), and sustained ventricular fibrillation during reperfusion ($P<0.01$, Table 4), relative to the hearts removed from vehicle-treated diabetic rats. There was no significant difference in the incidence of arrhythmias between this group and the non-diabetic group.

Insulin replacement in diabetic rats also significantly augmented the incidences of ventricular fibrillation during both occlusion and reperfusion ($P<0.05$, Table 4). There was no statistical difference in any parameters between the insulin treated diabetic group and the nondiabetic controls.

Fig. 3 shows that tridothyronine (densitometric ratio—diabetic + triiodothyronine/control 1.0 ± 0.2, $n=3$) and insulin treatment (densitometric ratio—diabetic + insulin/control 0.83 ± 0.1, $n=3$, $P<0.05$ vs. diabetic group) each prevented the diabetes-induced increase in ventricular protein kinase Cγ.

3.5. Effects of methimazole-induced hypothyroidism

Methimazole treated rats (controls $n=15$; methimazole $n=28$) also showed reduction in ex vivo heart rate (control 240 ± 9 beats/min; methimazole 187 ± 9 beats/min, $P<0.001$), rectal temperature (control 37.1 ± 0.1 °C; methimazole 35.7 ± 0.1 °C, $P<0.01$) and serum free thyroxine concentrations (control 29.6 ± 1.2 pmol/l; methimazole 2.4 ± 0.3 pmol/l, $P<0.001$) and prolongation in Q–T interval (control 87.8 ± 2.2 ms; methimazole 112 ± 7.6 ms, $P<0.01$). Serum glucose concentrations in methimazole-treated rats (9.9 ± 0.05 mmol/l) were not significantly different from controls (8.4 ± 1.1 mmol/l). Ventricular fibrillation and sustained ventricular fibrillation during occlusion and reperfusion were abolished ($P<0.01$) in hearts from methimazole-treated rats ($n=10$) (control incidence of ven-

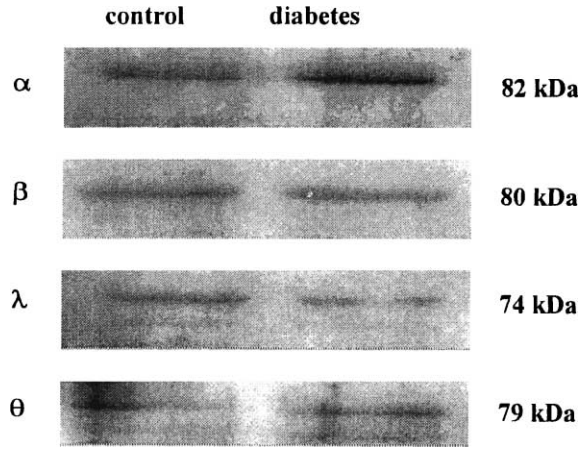

Fig. 2. Immunoblot showing PKC α, β, θ, λ in ventricles removed from control and streptozotocin diabetic rats 8 weeks after induction of diabetes.

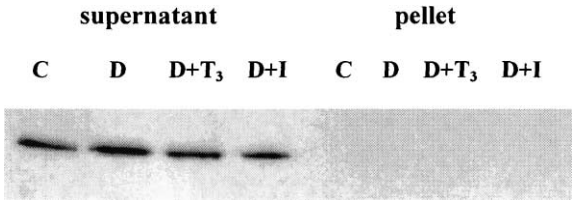

Fig. 3. Immunoblot showing PKCγ in ventricles from control, vehicle-treated diabetic, T_3-treated diabetic and insulin-treated diabetic rats. Ventricles were removed 8 weeks after induction of diabetes or after injection of saline.

tricular fibrillation ($n = 7$) during occlusion was 71% (14% sustained) and during reperfusion was 100%, which was sustained).

4. Discussion

4.1. Diabetes-induced hypothyroidism

The present study confirmed that streptozotocin diabetes in the rat produces hypothyroidism (Sundaresan et al., 1984; Rodgers et al., 1991; Rondeel et al., 1992; Schroder-Van Der Elst and Van Der Heide, 1992; Katovitch et al., 1993) as evidenced by the reduction of serum concentrations of thyroid hormone concentrations. The reduction in free thyroxine and free triiodothyronine concentrations, together with clinical signs of hypothyroidism (decreased heart rate, prolonged Q–T interval and decreased rectal temperature) suggest that the streptozotocin-induced diabetes produces true hypothyroidism rather than the "low T_3" or "euthyroid sick syndrome", which is associated with severe illness (Chopra, 1997). Although the rats were markedly hyperglycaemic, they were not severely ill, as evidenced by normal grooming and food intake. The degree of hypothyroidism was related to the duration of diabetes, as indicated by the further decrease in the serum free thyroxine concentration at 8 weeks compared with 2 weeks after injection of streptozotocin. Moreover, some characteristic changes associated with hypothyroidism (decreased body temperature and heart rate) were not significant in 2-week diabetes but clearly evident at 8-week diabetes. A relationship between the level of hypergly-caemia and the degree of hypothyroidism was shown by the negative correlation between serum free thyroxine and free tridothyronine and blood glucose concentrations. The fact that insulin treatment prevented all signs of hypothyroidism and restored both free thyroxine and free triiodothyronine levels to normal, whereas triiodothyronine normalized only body temperature, heart rate and Q–T interval but not serum glucose strongly suggest that the changes in body temperature, heart rate and Q–T interval in streptozotocin diabetes were secondary to hypothyroidism.

4.2. Susceptibility of experimental diabetic hearts to ischaemia–reperfusion arrhythmias

The significant protection against regional ischaemia–reperfusion arrhythmia, in hearts from both 2- and 8-week streptozotocin-diabetic rat hearts supports other observations in which sustained ventricular fibrillation was abolished in 3-week streptozotocin-diabetic hearts following both five and 10 min regional ischaemia (Suzuki et al., 1993; Zhang et al., 1999a,b). Very recently, Ravingerova et al. (2001) showed a reduction in ischaemia-induced ventricular tachycardia and ventricular fibrillation both in vivo and ex vivo in streptozotocin-diabetic rats. However, there are some contrary reports in rats (Hekimian et al., 1985) rabbits (Bhimji et al., 1986) and dogs (Bakth et al., 1986).

Paulson (1997) suggested that the protection found in diabetic hearts against ischaemia–reperfusion injury was related to the experimental models used. Most studies showing protection in diabetic hearts used mild diabetes and/or of short duration, undergoing zero-flow ischaemia whilst long-term, severe diabetes in low-flow ischaemia models usually showed increased vulnerability.

In the present study, the profound protective effect was seen in diabetes of long duration and associated with very marked hyperglycaemia. This substantial antiarrhythmic effect was prevented by insulin treatment; this implies that the protection resulted from hyperglycaemia, hypoinsulinaemia or some other metabolic changes of diabetes. However, even in the presence of hyperglycaemia, triiodothyronine treatment also completely abolished the protection. This, for the first time, disclosed the important role of hypothyroidism in the reduced susceptibility of streptozotocin-diabetic hearts to ischaemia–reperfusion arrhythmia. The dose of tridothyronine used in this experiment did not cause hyperthyroidism, which is proarrhythmic in normal rats. Substantial evidence shows that the hypothyroidism, whether produced by thyroidectomy or goitrogen treatment produces a profound antiarrhythmic effect (Chess-Williams and Coker, 1989; Venkatesh et al., 1991; Liu et al., 1996). In the present work, we showed that methimazole-induced hypothyroidism, while producing a much more marked reduction in serum free thyroxine concentrations, produced very similar effects to streptozotocin diabetes on ischaemia–reperfusion–induced arrhythmias in the subsequently isolated hearts. In other reports which demonstrated an antiarrhythmic effect of streptozotocin diabetes, signs of hypothyroidism, such as prolongation of Q–T interval (Kusama et al., 1992), decrease in basal heart rate (Navaratnam and Khatter, 1989) and reductions in serum free thyroxine and free triiodothyronine concentrations (Tosaki et al., 1996) had also been reported. However, these data were not discussed in the context of establishing a relationship between hypothyroidism and the antiarrhythmic effects of streptozotocin diabetes. On the other hand, in one canine model, which showed increased susceptibility to ventricular fibrillation in the diabetic heart (Bakth et al., 1986), no sign of hypothyroidism was present. In that model, there was also no significant increase in serum glucose concentration but only a reduction in glucose tolerance. The data strongly suggested that the antiarrhythmic effect demonstrated in the present study was produced by hypothyroidism.

The increase in protein kinase Cγ in the ventricles of diabetic rats has not been reported previously, although increased levels of protein kinase Cα have been reported by others (Liu et al., 1999; Kang et al., 1999). Previous studies showed increased levels of protein kinase Cβ, protein kinase Cε and protein Cς (Liu et al., 1999), although Kang et al. (1999) found a down-regulation of protein kinase Cς. The increased level of protein kinase Cγ was

probably secondary to the streptozotocin-induced hypothyroidism, since it was prevented by treatment with either insulin or triiodothyronine. Experimental hypothyroidism was found to increase protein kinase C levels in the liver (Meier et al., 1991) and expression in the myocardium (Rybin and Steinberg, 1996), although in the latter study the affected isoform was protein kinase Cε. The contribution of increased protein levels of protein kinase Cγ and protein kinase Cα to the reduction in ischaemia–reperfusion induced arrhythmias seen in the streptozotocin-diabetic rat is unknown. However, protein kinase C inhibitors were shown to prevent the increased resistance of streptozotocin-diabetic rat hearts to ischaemia–reperfusion-induced impairment of myocardial contractility (Moon et al., 1999). Nevertheless, there is little work on the role of protein kinase C in the antiarrhythmic effect of ischaemic preconditioning and, indeed, activation of certain protein kinase C isoforms may be pro-arrhythmic (Black et al., 1993). It is possible that other isoforms may be protective and further work is needed.

In conclusion, hypothyroidism is present in streptozotocin diabetes and its severity is correlated to the duration and severity of diabetes. Our data suggest that the hypothyroidism is responsible for the antiarrhythmic effect in the diabetic rat hearts observed in the present study. Hypothyroidism should be considered as a factor whenever experimental diabetes is used, especially when the model involves very marked hyperglycaemia.

Acknowledgements

LQZ was supported by an ORS award. We are grateful to the Henry Lester Trust and Great Britain-China Education Trust for financial support (LQZ) and to G. Walker, G. Watt and C. Whitehouse for their excellent technical support.

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