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Effects of rosiglitazone and interactions with growth-regulating factors in ventricular cell hypertrophy

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Abstract

Chronic administration of thiazolidinediones might predispose to cardiac hypertrophy. The aim was to investigate direct effects of rosiglitazone in rat ventricular cardiomyocytes maintained in vitro (24 h). Rosiglitazone ($\leq 10^{-5}$ M) did not increase protein synthesis and produced small inconsistent increases in cellular protein. In the presence of serum (10% v/v), but not insulin-like growth factor (IGF-1, 10^{-8} M) or insulin (1 U/ml), an interaction with rosiglitazone to stimulate protein synthesis was observed. The hypertrophic responses to noradrenaline (5×10^{-6} M), PMA (10^{-7} M) and ET-1 (10^{-7} M) were not attenuated by rosiglitazone. Rosiglitazone (10^{-7} M) did not influence protein synthesis in response to insulin (1 U/ml) and elevated glucose (2.5×10^{-2} M) alone or in combination, but attenuated the increase in protein mass observed in response to elevated glucose alone. In re-differentiated cardiomyocytes, a model of established hypertrophy, rosiglitazone (10^{-8} M–10^{-6} M) increased protein synthesis. Together, these data indicate that rosiglitazone does not initiate cardiomyocyte hypertrophy directly in vitro. However, during chronic administration, the interaction of rosiglitazone with locally-derived growth-regulating factors may make a modest contribution to cardiac remodelling and influence the extent of compensatory hypertrophy of the compromised rat heart.

Keywords: Cardiac; Hypertrophy; Diabetes; PPARγ; Troglitazone; Protein kinase C

1. Introduction

The thiazolidinediones, troglitazone and rosiglitazone (BRL49653-C), are novel antiglycaemic agents that bind to nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), specifically the PPARγ subtype (Lehmann et al., 1995). Although long-term administration is not associated with cardiac mass increase or functional impairment in Type II diabetic patients (Ghazzi et al., 1997; St. John Sutton et al., 2002) or healthy Cynomolgus monkeys (Mayfield et al., 1993), there is evidence that chronic administration of thiazolidinediones is associated with development of cardiac hypertrophy in experimental animal models in vivo (Stephens et al., 1995; Ghazzi et al., 1997). In view of the pathophysiological complications in heart function caused by cardiac hypertrophy and because the target population for these anti-diabetic agents is at particular risk from the development of cardiovascular disease, it is of interest to assess the effective plasma concentration and temporal dependence of this hypertrophic response. Systemic haemodynamic effects have been attributed to the thiazolidinediones: troglitazone (i) increases cardiac output due to decreased after-load as a consequence of decreased peripheral resistance (Ghazzi et al., 1997) and (ii) promotes fluid retention leading to increased plasma volume in vivo (Hopkins, 1997) and hence increases preload. Given the potential of thiazolidinediones to affect haemodynamics, the use of in vitro cellular models is warranted to determine whether these compounds elicit their trophic effects in cardiomyocytes directly rather than as an indirect consequence of increased mechanical loading.

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Isolated adult mammalian ventricular cardiomyocytes, maintained in short-term serum-free culture, provide an appropriate experimental model in which to investigate the direct influence of these drugs on the initiation of cardiomyocyte growth; such cells are non-beating, therefore the contribution made by mechanical loading to the development of myocardial hypertrophy is eliminated (Schluter et al., 1995). At cellular level myocardial hypertrophy is based on increased mass, not number, of cardiomyocytes since adult cardiomyocytes do not undergo cell division (Jacobson and Piper, 1986; Vliegen et al., 1990). An alternative to freshly isolated cardiomyocytes is the use of re-differentiated cardiomyocytes, obtained after long-term culture in the presence of serum. These cells provide an experimental model of relevance to established cardiac hypertrophy (Pinson et al., 1993). During cellular re-differentiation, β_2-adrenoceptors become coupled to the hypertrophic process (Zhou et al., 1996).

We reported previously that troglitazone per se (Bell and McDermott, 2000) did not initiate cardiomyocyte growth directly in vitro, but inhibited protein kinase C (PKC)-mediated growth mechanisms. However, the interaction of troglitazone with serum growth factors was found to contribute modestly to the development of hypertrophy. The purpose of the present study was to clarify if these findings represented actions of troglitazone specifically, or were indicative of a general drug class effect, by determining if another thiazolidinedione, rosiglitazone (i) initiated a trophic response directly in freshly isolated ventricular cardiomyocytes or enhanced the underlying trophic response observed in re-differentiated cardiomyocytes; (ii) sensitised cardiomyocytes to the trophic effects of a general growth stimulus, serum, and, specifically, to the effects of insulin-like growth factor-1 (IGF-1) and insulin; (iii) elicited per se a more marked trophic response in the presence of elevated glucose concentration with and without insulin; (iv) attenuated PKC-mediated cardiomyocyte growth. The results obtained have been compared with those of troglitazone in order to establish cardiac effects that are characteristic of the thiazolidinediazone class of anti-diabetic agents, in general, from effects that can be attributed specifically to the properties of troglitazone.

2. Materials and methods

2.1. Isolation and culture of cardiomyocytes

The study was performed in accordance with Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty's Stationary Office, London. Ventricular cardiomyocytes were isolated from 12 week old male Sprague–Dawley rats by Langendorff perfusion of the excised heart with collagenase solution as described previously (Pinson et al., 1993). Following purification and restoration of Ca^{2+} tolerance, the cells were re-suspended in serum-free creatinine–carnitine–taurine supplemented glutamine-free Medium M199 with Earle's salts (CCT medium) at a concentration of 1.5×10^5 viable cardiomyocytes per milliliter. Aliquots (1 ml) were pipetted gently onto plastic Petri dishes (35 mm diameter), which had been pre-incubated for 2 h with foetal calf serum (4% v/v) in CCT Medium. After 1 h, viable cardiomyocytes had attached to the surface of the dish. The dishes were then washed with fresh CCT medium to remove non-attached cells and cell debris and the attached cells were subsequently incubated at 37 °C for 24 h in CCT medium (1 ml) containing the appropriate concentrations of various drugs as specified in the specific experimental protocols. Under all experimental conditions, cardiomyocytes remained mechanically quiescent.

For the establishment of cultures of re-differentiated cardiomyocytes, cells were first incubated for 6 days in CCT medium in the presence of foetal calf serum (20% v/v). The dishes were then washed with fresh CCT medium to remove non-attached cells and cell debris, and the attached cells were subsequently incubated at 37 °C for 24 h, in the absence of serum, in CCT medium (1 ml) containing the appropriate concentrations of various drugs as specified in the specific experimental protocols.

2.2. Incorporation of l-U-[^{14}C] phenylalanine, total mass of cellular protein and total content of cellular DNA

The extent of de novo synthesis of protein in the cell cultures was estimated by exposing the cells for 24 h to *l*-U-[^{14}C] phenylalanine (0.1 μC_i/ml culture medium). In each case, the incorporation of radioactivity into the acid-insoluble cell fraction was determined. At the end of the chosen period of incubation, cell viability was determined by light microscopy (Olympus CK2 microscope); the number of rod-shaped cells and non-viable rounded cells respectively were determined for each of 10 fields randomly selected for each Petri dish and viewed at a magnification of ×10; viability (%) was estimated from the mean values. Experiments were subsequently terminated by the removal of the supernatant medium from the dishes. The attached cells were washed with an aliquot (1 ml) of ice-cold PBS, prior to the addition of an aliquot (1 ml) of ice-cold trichloroacetic acid (10% w/v). After storage overnight at 4 °C, the acid, containing the intracellular precursor pool, was removed from the dishes and the attached cells were washed with an aliquot (1 ml) of PBS. The precipitate remaining on the Petri dishes was dissolved in an aliquot (1 ml) of NaOH (0.1 M)/ sodium dodecyl sulphate (0.01%w/v) by overnight incubation at 37 °C. In these samples, the concentration of protein was determined by the colorimetric method of Lowry, and the concentration of DNA in the neutralized sample was determined by a spectrophotometric method in which bisbenzamide dye was incorporated into DNA (Mullan et al., 1997), and the radioactivity was counted. The ratio of protein to DNA per dish was used as the

parameter of cell mass and the ratio of *l*-U-[^{14}C] phenylalanine incorporated to DNA per dish served as the parameter of de novo synthesis of protein, respectively.

2.3. Materials

Rosiglitazone (BRL49653-C) was a gift from GlaxoSmithKline Pharmaceuticals (Herts., UK). *l*-noradrenaline hydrochloride, isoprenaline hydrochloride, phorbol-12-myristate 13-acetate (PMA), *l*-carnitine, creatinine, taurine, cytosine-β-D arabinofuranoside, bovine serum albumin, trypsin, DNA (sodium salt, from calf thymus) and assay kits for the quantification of microprotein were obtained from Sigma Chemical Company (Poole, Dorset, UK). Insulin (porcine, highly purified) was obtained from Novo Nordisk Wellcome (England) and insulin-like growth factor-1 (human) from Peninsula Laboratories (Europe). Endothelin-1 and angiotensin II were purchased from Bachem (CA, USA). Bisbenzamide was supplied by Riedel-de-Haen (Germany). Collagenase was purchased from Serva Feinbiochemica (Heidelberg, Germany). *l*-U-[^{14}C] phenylalanine was obtained from Amersham International plc. (Buckinghamshire, UK). Medium M199 (glutamine free with Earle's salts), foetal calf serum and penicillin (5000 IU)/streptomycin (5 mg/ml) were purchased from Gibco (UK). Plastic Petri dishes were supplied by Falcon (Becton-Dickinson, UK). Liquid scintillation fluid was obtained from Zinsser Analytic (Berkshire, UK). All other chemicals used were of analytical grade and purchased from BDH Chemicals (UK).

Serum-free CCT medium for the culturing of cardiomyocytes consisted of modified glutamine-free Medium M199 with Earle's salts, HEPES (15 mM), creatinine (5 mM), *l*-carnitine (2 mM), taurine (5 mM), ascorbic acid (100 μM), penicillin (100 IU/ml) and streptomycin (100 μg/ml). Medium was also supplemented with cytosine β-D arabinofuranoside (10 μM) to prevent growth of non-myocytes. The composition of the calcium-free Krebs–Ringer (KRB) solution was as follows: NaCl (110 mM), KCl (2.6 mM), $NaHCO_3$ (25 mM), $MgSO_4$ (1.2 mM), KH_2PO_4 (1.2 mM), glucose (11 mM). This solution was gassed with 95% O_2/5% CO_2 and maintained at a pH of 7.4 at 37 °C. The composition of the phosphate-buffered saline (PBS) was as follows: NaCl (137 mM), KH_2PO_4 (1.5 mM), Na_2PO_4 (1.0 mM), pH 7.4. The composition of DNA assay solution was NaCl (1.985 M), Na_2HPO_4 (25 mM), pH 7.4. Bisbenzamide was dissolved in water (0.2 mg/ml). This stock solution was diluted 1:200 with DNA assay solution to give a working concentration of 1 μg/ml. Solutions of bisbenzamide are very sensitive to light and were therefore kept in the dark prior to use. The stock solution was stable for 6 months in the dark at 4 °C. Dilute solutions were prepared daily.

2.4. Statistical analysis

In each experiment, the total population of cells contained on the culture dishes was obtained from a pooled suspension prepared from 2 hearts. Under each condition (in the absence/presence of drug at various concentrations, with or without stimuli), the average value measured in 3 culture dishes was calculated for each parameter ([^{14}C]-phenylalanine incorporation or protein/DNA content). Replicate data were obtained for n preparations ($5 \leq n \leq 8$) and the mean value ± S.E.M. was calculated. Because of inter-assay variability of the basal values between experiments, which was 29% (c.v.) for measurement of incorporation of [^{14}C]-phenylalanine (n=46) and 33% (c.v.) for total protein mass (n=40), data were calculated as percent differences from the respective basal values. Data were analysed statistically using a one or two factor repeated measures analysis of variance (SPSS-PC, version 8.0). In experiments to examine drug concentration–response relationships in the presence of different stimuli, average tests of significance for within-subjects effects (concentration and the concentration-stimulus interaction) were adjusted if necessary to accommodate lack of constant variance using the Huynh–Feldt epsilon. If $P<0.05$ for the overall effect of concentration under a particular condition, differences between the mean values at a particular concentration (x_1) and at baseline (x_0) were tested by calculation of the t statistic as $(x_1-x_0)/\sqrt{}$ residual mean square (2/n). In experiments in which a single concentration of drug was applied, differences from the basal condition were analysed using the paired Student's t test.

3. Results

3.1. Cell viability and pH

Rosiglitazone, at the maximum concentration tested (10^{-5} M), did not exert any detrimental effects on the viability of cardiomyocytes maintained in short-term (24 h) culture: 68% ± 1.2% (n=6 experiments) of the cells were viable after 24 h in the absence of drug compared to 69% ± 1.4% (n=6 experiments) in the presence of rosiglitazone. The pH of the culture medium did not alter during the 24 h incubation with rosiglitazone.

3.2. Effect of rosiglitazone in the absence and presence of serum

Rosiglitazone (10^{-8} M–10^{-5} M) did not exert a hypertrophic effect per se on cardiomyocytes maintained in culture for 24 h since the incorporation of ^{14}C-phenylalanine into cellular protein was not increased above the basal value (843.1 ± 57.7 dpm/μg DNA; Fig. 1). Rosiglitazone produced small increases ($P<0.05$ at 10^{-6} M only) in the total mass of cellular protein above the basal value (43.7 ± 3.5 μg/μg DNA), although the changes were not concentration-dependent (data not shown).

Foetal calf serum (10% v/v) increased the incorporation of [^{14}C]-phenylalanine and total mass of cellular protein by

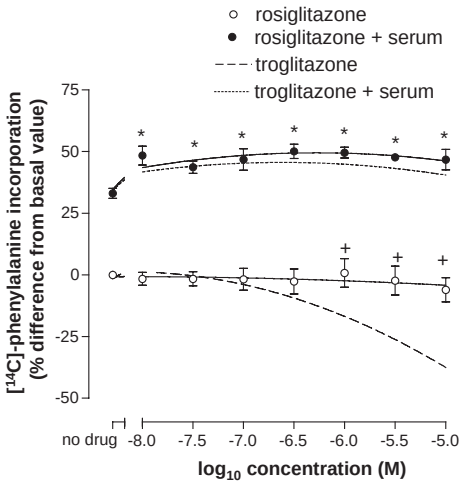

Fig. 1. Effect of rosiglitazone (10^{-8} M–10^{-5} M) alone or in the presence of serum (10% v/v) on incorporation of [^{14}C]-phenylalanine into de novo cellular protein (dpm/μg DNA) in cardiomyocytes isolated from the ventricles of adult rats and maintained in short-term (24 h) culture. Data are the mean+S.E. mean of 6 heart cell preparations. *Denotes difference between responses elicited with and without rosiglitazone ($P \leq 0.05$). Troglitazone response (10^{-8} M–10^{-5} M) is included for comparison. $^{+}$Denotes difference between basal response to rosiglitazone and that to troglitazone ($P<0.05$).

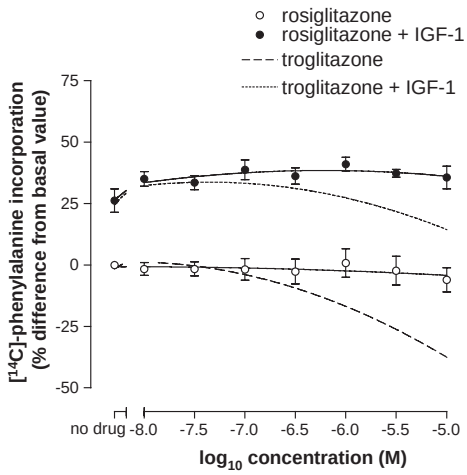

Fig. 2. Effect of rosiglitazone (10^{-8} M–10^{-5} M) alone or in the presence of IGF-1 (10^{-8} M) on incorporation of [^{14}C]-phenylalanine into de novo cellular protein (dpm/μg DNA) in cardiomyocytes isolated from the ventricles of adult rats and maintained in short-term (24 h) culture. Data are the mean+S.E. mean of 6 heart cell preparations. Troglitazone response (10^{-8} M–10^{-5} M) is included for comparison.

33% and 10% above the basal values (493.3±51.8 dpm/μg DNA and 41.2±6.5 μg/μg DNA), respectively. In the presence of serum, rosiglitazone (10^{-8} M–10^{-5}M) elicited an effect on the amount of [^{14}C]-phenylalanine incorporated, which at maximum was 50% greater than the basal value, and 13% greater than serum value, respectively (Fig. 1); the interaction with rosiglitazone was significant (P=0.05). Generally, rosiglitazone stimulated small increases in the total mass of cellular protein above that elicited by serum per se, but the drug did not exhibit a significant interaction with serum to increase protein mass (data not shown).

3.3. Effects of rosiglitazone in the presence of insulin-like growth factor-1 or insulin

IGF-1 (10^{-8} M) increased the incorporation of [^{14}C]-phenylalanine and total mass of cellular protein by 26% and 6% above the basal values (511.7±34.3 dpm/μg DNA and 44.1±2.4 μg/μg DNA), respectively. When combined with IGF-1, rosiglitazone (10^{-8} M–10^{-5} M) elicited a small hypertrophic effect, since the incorporation of [^{14}C]-phenylalanine into cellular protein was at maximum 41% above the basal value, which was 11% greater than the response to IGF-1 per se, but the interaction of drug with growth factor was not significant (Fig. 2). Rosiglitazone did not alter the total protein mass consistently from that observed in the presence of IGF-1 alone (data not shown).

Insulin (1 U/ml) increased the incorporation of [^{14}C]-phenylalanine and total mass of cellular protein by 57% and 7%, above the basal values (681.8±72.7 dpm/μg DNA and 45.7±3.2 μg/μg DNA), respectively. Rosiglitazone (10^{-8} M–10^{-5} M) did not exert hypertrophic effects when combined with insulin, since incorporation of [^{14}C]-phenylalanine into cellular protein was not increased above that elicited by insulin per se (Fig. 3). Indeed, decreases in the response to insulin occurred at 10^{-5} M in the presence of rosiglitazone. Furthermore, rosiglitazone (10^{-8} M–10^{-5} M) did not alter the total protein mass from that stimulated by insulin (1 U/ml; data not shown).

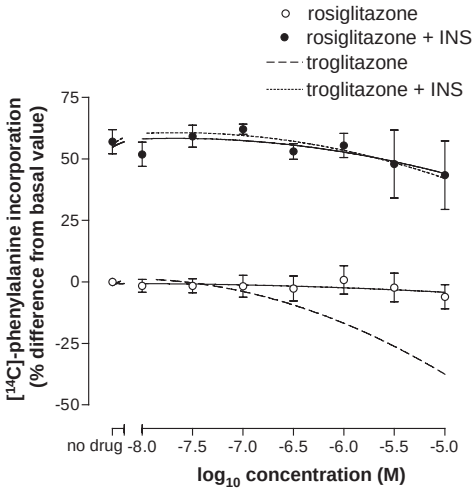

Fig. 3. Effect of rosiglitazone (10^{-8} M–10^{-5} M) alone or in the presence of insulin (1 U/ml) on incorporation of [^{14}C]-phenylalanine into de novo cellular protein (dpm/μg DNA) in cardiomyocytes isolated from the ventricles of adult rats and maintained in short-term (24 h) culture. Data are the mean+S.E. mean of 6 heart cell preparations. Troglitazone response (10^{-8} M–10^{-5} M) is included for comparison.

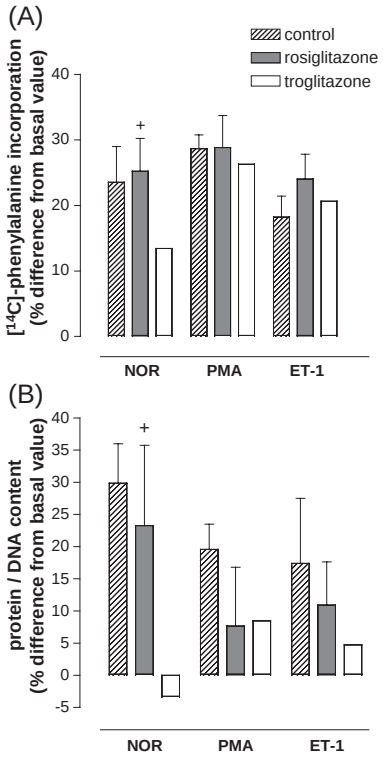

Fig. 4. Interaction of rosiglitazone (10^{-6} M) with activators of PKC: (A) incorporation of [^{14}C]-phenylalanine into de novo cellular protein (dpm/μg DNA); (B) total mass of cellular protein (μg/μg DNA) of cardiomyocytes isolated from the ventricles of adult rats and maintained in short-term (24 h) serum-free primary culture without stimuli (basal) or in the presence of noradrenaline (5×10^{-6} M; NOR), phorbol-12 myristate-13 acetate (10^{-7} M; PMA) or endothelin-1 (10^{-7} M; ET-1), in the absence (control) and presence of drug (10^{-6} M). Data are mean+S.E. mean of 5–8 heart cell preparations. Troglitazone response (10^{-6} M) is included for comparison. $^{+}$Denotes difference between responses to rosiglitazone and troglitazone ($P<0.05$).

3.4. Interaction of rosiglitazone with activators of protein kinase C

The activators of PKC, noradrenaline (5×10^{-6} M), PMA (10^{-7} M) and endothelin-1 (10^{-7} M), produced marked hypertrophic effects as evidenced by increased incorporation of [^{14}C]-phenylalanine by 23% and 29% above the basal value (603.5±35.7 dpm/μg DNA) and 18% above the basal value (867.7±79.2 dpm/μg DNA), respectively (Fig. 4A) and increased the total mass of cellular protein by 27% and 17% above the basal value (57.3±5.9 μg/μg DNA) and 11% above the basal value (57.4±9.4 μg/μg DNA), respectively (Fig. 4B). The hypertrophic responses to all of these stimuli were not altered in the presence of rosiglitazone (10^{-6} M).

3.5. Interaction of rosiglitazone with elevated glucose concentration

Elevated glucose concentration (2.5×10^{-2} M) per se did not influence the amount of [^{14}C]-phenylalanine incorporated into cellular protein above the respective basal values obtained in the presence of 5.5×10^{-3} M glucose with or without insulin (1 U/ml), which were 1017±108 and 700±65 dpm/μg DNA (Fig. 5A). Rosiglitazone (10^{-6} M) did not influence the amount of [^{14}C]-phenylalanine incorporated under basal conditions, or in the presence of insulin, elevated glucose, or their combination. Individually, elevated glucose and insulin concentrations increased the protein content of cardiomyocytes above the basal value of 75.3±5.1 μg/μg DNA (Fig. 5B). Insulin and glucose together increased total protein, but by no more than they did individually. Rosiglitazone (10^{-6} M) decreased protein content, significantly in the presence of elevated glucose alone, but not in the presence of insulin alone and with glucose.

3.6. Effects of rosiglitazone on re-differentiated cardiomyocytes

PMA (10^{-7} M) and isoprenaline (10^{-6} M), elicited marked hypertrophic effects as evidenced by increased incorporation of ^{14}C-phenylalanine, both by 26%, above the basal value (1110±108 dpm/μg DNA; Fig. 6A) and both

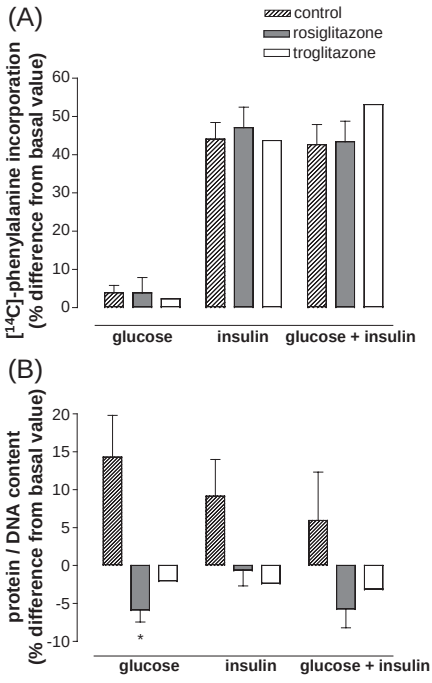

Fig. 5. Interaction of rosiglitazone (10^{-6} M) with elevated glucose concentration: (A) incorporation of [^{14}C]-phenylalanine into de novo cellular protein (dpm/μg DNA); (B) total mass of cellular protein (μg/μg DNA) of cardiomyocytes isolated from the ventricles of adult rats and maintained in short-term (24 h) serum-free primary culture without stimuli (basal, 5.5×10^{-3} M glucose) or in the presence of elevated glucose concentration (2.5×10^{-2} M; glucose), insulin (1 U/ml), or glucose (2.5×10^{-2} M) in combination with insulin (1 U/ml), in the absence (control) and presence of drug (10^{-6} M). Data are the mean+S.E. mean of 7 heart cell preparations. *Denotes difference for effects of rosiglitazone ($P\leq0.05$) by comparison with respective control response. Troglitazone response (10^{-6} M) is included for comparison.

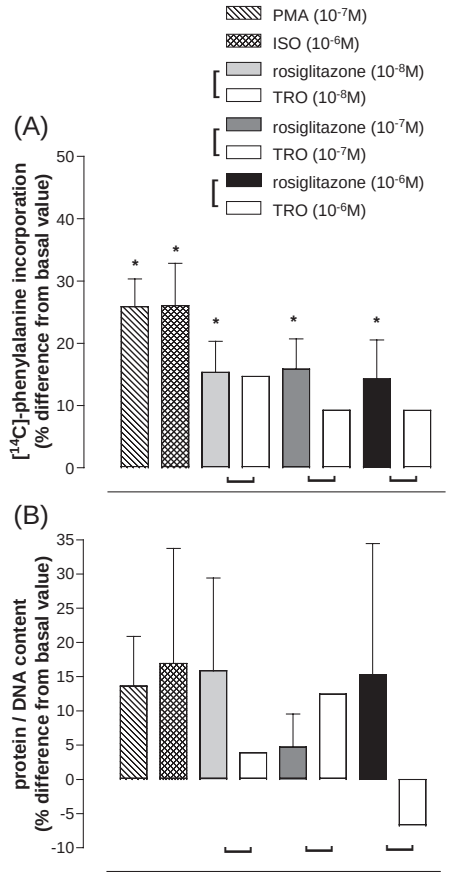

Fig. 6. Effect of rosiglitazone (10^{-8} M–10^{-5} M) on the (A) incorporation of [^{14}C]-phenylalanine into de novo cellular protein (dpm/μg DNA); (B) total mass of cellular protein (μg/μg DNA); in re-differentiated cardiomyocytes. Phorbol-12 myristate-13 acetate (10^{-7} M; PMA) and isoprenaline (10^{-6} M; ISO) were included as positive controls. Data are the mean+S.E. mean of 7 heart cell preparations. *Denotes significant difference for effects of stimulus ($P \leq 0.05$) by comparison with respective basal response. Troglitazone (10^{-8} M–10^{-5} M) response is included for comparison.

increased the total mass of cellular protein by 14% and 17%, respectively, above the basal value (43.6±10.8 μg/μg DNA; Fig. 6B). Rosiglitazone (10^{-8} M–10^{-6} M) at all concentrations increased the incorporation of [^{14}C]-phenylalanine and total mass of cellular protein, although responses were not concentration-dependent.

4. Discussion

It should be noted at the outset that in all of the experiments described in this paper, the actions of rosiglitazone and troglitazone were investigated simultaneously in the same heart cell preparation to ensure that there was no assay specific variability which would reduce the reliability of any comparisons made between the effects of the two drugs (delay in release of the rosiglitazone data by the *Avandia* publishing group, prevented the publication of these data alongside the troglitazone data-Bell and McDermott, 2000).

In the present study, rosiglitazone per se did not initiate a hypertrophic effect directly in cardiomyocytes maintained in short-term (24 h) serum-free primary culture. A minor attenuation of de novo protein synthesis observed in response to 10^{-5} M rosiglitazone, much smaller than that exerted by 10^{-5} M troglitazone (Bell and McDermott, 2000), could not be attributed to a cytotoxic effect of the drug at this concentration, since the viability of the cell population was not reduced relative to that of cells incubated in the absence of rosiglitazone for a similar period of time (24 h). In addition, rosiglitazone did not alter the pH of supernatant media from the cell cultures; the attenuation of protein synthesis by troglitazone had been attributed to the ability of this compound to increase the acidity of culture medium (Bell and McDermott, 2000).

Rosiglitazone displayed an interaction with serum, in relation to effects on de novo protein synthesis, which was much less in magnitude than that induced by the interaction of troglitazone with serum, although total responses to drug-serum combinations were similar (Bell and McDermott, 2000). As with troglitazone, the possibility that factors present in serum can sensitise the cells to the hypertrophic effect of rosiglitazone, by activating autocrine factors released from the cells themselves, is consistent with the data obtained in re-differentiated cardiomyocytes, a cellular model in which hypertrophy is already established (Pinson et al., 1993); rosiglitazone elicited more of a hypertrophic response in these cells than in freshly-isolated cells, which was comparable in magnitude to those of troglitazone. Sensitisation of the cells to the drug, as a consequence of activation by serum of autocrine growth factors, must have already occurred during the re-differentiation. It is unclear which autocrine factor(s) activated by serum might prime cardiomyocytes to the hypertrophic effects of rosiglitazone. At nanomolar concentrations, rosiglitazone produced a small hypertrophic response in the presence of IGF-1 although this was smaller than that obtained when cells were incubated with the thiazolidinedione in the presence of serum. Furthermore, the interaction of IGF-1 with rosiglitazone was not statistically significant, in contrast to that with troglitazone (Bell and McDermott, 2000), indicating that local synthesis of IGF-1 cannot account for the ability of serum to prime the myocardium to the effects of rosiglitazone.

Troglitazone has been shown to antagonise phorbol-ester mediated activation of protein kinase C in rat ventricular cardiomyocytes in vitro (Bahr et al., 1996), indicating that the drug might be expected to attenuate the hypertrophic responses to stimuli coupled to the activation of PKC, including angiotensin II (Asakawa et al., 2002), by an action independent of any growth promoting effects of troglitazone per se. The observation that the hypertrophic response to the α-adrenoceptor agonist, noradrenaline, which is coupled to the activation of PKC, is attenuated markedly by troglitazone (Bell and McDermott,

2000) is in contrast to the finding that rosiglitazone does not attenuate the hypertrophic responses to noradrenaline. The ability of troglitazone to attenuate selectively the hypertrophic response to noradrenaline may represent a unique property of this drug, not shared by rosiglitazone, and unrelated to the specific interaction of the thiazolidinediones with PPARγ receptors. One possibility is that troglitazone is able to interact with a particular isoform of PKC (Steinberg et al., 1995), activated specifically in response to stimulation of α-adrenoceptors by noradrenaline, which is not recognised by rosiglitazone.

Elevation of the extracellular glucose concentration to 2.5×10^{-2} M did not result in a hypertrophic effect per se: a hypertrophic effect might have been anticipated since elevated glucose concentration would lead to an elevated intracellular calcium ion concentration which has implications for the activity of kinase enzymes and the regulation of gene expression (Gupta and Wittenberg, 1993). In addition, elevated glucose concentration did not result in an alteration of the response to insulin, and the presence of rosiglitazone did not alter the extent of de novo protein synthesis in response to elevated glucose concentration or insulin alone or in combination; troglitazone tended to sensitise cardiomyocytes to the hypertrophic response to insulin and elevated glucose in combination, although not significantly so (Bell and McDermott, 2000).

In conclusion, these data indicate that rosiglitazone, like troglitazone, does not initiate significant cardiomyocyte hypertrophy directly in vitro. It is possible, however, that during chronic administration, the interaction of these compounds with growth-regulating factors may make a modest contribution to cardiac remodelling and influence the extent of established compensatory hypertrophy of the compromised heart. However, no cardiac mass changes have been observed in studies of Type II diabetic patients (Ghazzi et al., 1997) or primates (Mayfield et al., 1993) during chronic administration of thiazolidinediones at doses which achieved plasma concentrations of approximately 7 μM in vivo, comparable with those applied to the rat cardiomyocytes in vitro. It is possible therefore that the mild hypertrophic response to roziglitazone, elicited in the presence of serum, in rat ventricular cardiomyocytes in vitro, may be restricted to particular species and does not occur in human myocardium.

While many of the actions described in this study represent class effects of the thiazolidinediones, variation between troglitazone and rosiglitazone with regard to the basal effects on protein synthesis, the magnitude of the hypertrophic effects of the drugs per se in the presence of serum and IGF-1, and the interaction of the drugs with noradrenaline tends to support the conclusion that the cellular effects of these compounds cannot be explained exclusively by an (identical) interaction with the PPARγ receptor, and may provide evidence for additional perhaps non-PPARγ receptor-mediated effects. Indeed the amount of PPARγ expressed in cardiomyocytes is low (Glide and VanBilsen, 2003) and thiazolidinediones may not all interact similarly with such receptors to induce exactly the same set of genes, though there is some overlap (Camp et al., 2000). A large body of evidence is accumulating to support non-PPARγ dependent actions of the thiazolidinediones (Lennon et al., 2002; Glide and VanBilsen, 2003). At the level of the cardiomyocyte, troglitazone may exhibit a greater propensity for non-PPARγ receptor mediated side effects than rosiglitazone.

References

Asakawa, M., Takano, H., Nagai, T., Uozumi, H., Hasegawa, H., Kubota, N., Saito, T., Masuda, Y., Kadowaki, T., Komuro, I., 2002. Peroxisome proliferators-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy in vitro and vivo. Circulation 105, 1152–1154.

Bahr, M., Spelleken, M., Bock, M., von Holtey, M., Kiehn, R., Eckel, J., 1996. Acute and chronic effects of troglitazone (CS-045) on isolated rat ventricular cardiomyocytes. Diabetologia 39, 766–774.

Bell, D., McDermott, B.J., 2000. Troglitazone does not initiate ventricular cardiomyocyte hypertrophy in vitro but can sensitise cardiomyocytes to the hypertrophic effects of serum growth factors. Eur. J. Pharmacol. 390, 237–244.

Camp, H.S., Li, O., Wise, S.C., Hong, Y.H., Frankowski, C.L., Shen, X., Vanbogelen, R., Leff, T., 2000. Differential activation of PPARγ by troglitazone and rosiglitazone. Diabetes 49, 539–547.

Ghazzi, M.N., Perez, J.E., Antonucci, T.K., Driscoll, J.H., Haung, S.M., Faja, B.W., Whitcomb, R.W., The Troglitazone Study Group, 1997. Cardiac and glycaemic benefits of troglitazone treatment in NIDDM. Diabetes 46, 433–439.

Glide, A.J., VanBilsen, M., 2003. Peroxisome proliferators-activated receptors (PPARs): regulators of gene expression in heart and skeletal muscle. Acta Physiol. Scand. 178, 425–434.

Gupta, R.K., Wittenberg, B.A., 1993. Nuclear magnetic resonance studies of free calcium in heart cells. Biophys. J. 65, 2547–2558.

Hopkins, K.D., 1997. Heart mass not altered by troglitazone. Lancet 349, 781.

Jacobson, S.L., Piper, H.M., 1986. Cell cultures of adult cardiomyocytes as models of the myocardium. J. Mol. Cell. Cardiol. 18, 439–448.

Lehmann, J.M., Moore, L.B., Smith-Oliver, T.A., Wilkison, W.O., Wilson, T.M., Kliewer, S.A., 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J. Biol. Chem. 270, 12953–12956.

Lennon, A.M., Ramauge, M., Dessouroux, A., Pierre, M., 2002. MAP kinase cascades are activated in astrocytes and preadipocytes by 15-deoxy-delta 12–14 prostaglandin J2 and the thiazolidinedione ciglitazone through peroxisome proliferator activator receptor gamma-independent mechanisms involving reactive oxygenated species. J. Biol. Chem. 277, 29681–29685.

Mayfield, R., McLean, D., Crook, D., Buist, D.P., Gopinath, C., 1993. CS-045 toxicity to the Cynomolgus monkeys by repeated oral administration for 52 weeks. J. Clin. Ther. Med. 9 (Suppl. 3), 317–341.

Mullan, D.M., Bell, D., Kelso, E.J., McDermott, B.J., 1997. Involvement of ETA and ETB receptors in the hypertrophic effects of endothelin-1 in adult rabbit ventricular cardiomyocytes. J. Cardiovasc. Pharmacol. 29, 350–359.

Pinson, A., Schluter, K.D., Zhou, X.J., Schwartz, P., Kessler-Icekson, G., Piper, H.M., 1993. Alpha and beta adrenergic stimulation in cultured adult ventricular cardiomyocytes. J. Mol. Cell. Cardiol. 25, 477–490.

Schluter, K.D., Millar, B.C., McDermott, B.J., Piper, H.M., 1995. Regulation of protein synthesis and degradation in adult ventricular cardiomyocytes. Am. J. Physiol. 38, C1347–C1355.

Steinberg, S.F., Goldberg, M., Rybin, V.O., 1995. Protein kinase C isoform diversity in the heart. J. Mol. Cell. Cardiol. 27, 141–153.

Stephens, T.W., Bergman, J.A., Bue-Valleskey, J.M., DiMarchi, R.D., Slieker, L.J., Tinsley, F.C., Williams, G.D., 1995. Thiazolidinedione induced cardiac biochemical changes and increased IGF-1 action on cardiomyocytes. Diabetologia 38, A200.

St. John Sutton, M., Rendell, M., For the Rosiglitazone Clinical Trials Study Group, 2002. A comparison of the effects of rosiglitazone and glyburide on cardiovascular function and glycaemic control in patients with Type 2 diabetes. Diabetes Care 25, 2058–2064.

Vliegen, H.W., Bruschke, A.V.G., Van der Laarse, A., 1990. Different response of cellular DNA content to cardiac hypertrophy in human and rat heart myocytes. Comp. Biochem. Physiol. 95, 109–114.

Zhou, X.J., Schluter, K.D., Piper, H.M., 1996. Hypertrophic responsiveness to beta 2-adrenoceptor stimulation on adult ventricular cardiomyocytes. Mol. Cell. Biochem. 163–164, 211–216.