Hypertrophic agonists induce the binding of c-Fos to an AP-1 site in cardiac myocytes: implications for the expression of GLUT1

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Abstract

Objectives: Serum is among the agents known to induce hypertrophy of cardiac myocytes, which occurs concomitant with an increase in AP-1-mediated transcription. We have examined if this effect correlates with changes in the relative abundance of particular AP-1 heterodimers, as their exact composition under these conditions is unknown. Furthermore, we obtained insight on the specific role of c-Fos from studying the induction of the glucose transporter GLUT1 by serum in fibroblasts. Methods: We characterised the AP-1 heterodimers expressed in neonatal cardiac myocytes by supershift electrophoretic mobility shift assay (EMSA) analysis. Quantitative changes in transcription were measured using a luciferase reporter vector, and we examined the expression of the glucose transporter GLUT1 in cardiac myocytes and a c-Fos knockout-derived fibroblast cell line by western blotting. Results: Transcriptionally active AP-1 heterodimers containing c-Fos were transiently induced in cardiac myocytes, and this was dependent on the ERK mitogen-activated protein kinase pathway and coincided with the activation of AP-1-mediated transcription and the induction of GLUT1. In fibroblasts, the induction of GLUT1 by serum required the specific expression of c-Fos. Conclusion: Our data suggest that induction of c-Fos containing AP-1 heterodimers may partly activate AP-1-mediated transcription in cardiac myocytes treated with hypertrophic agonists under conditions known to induce GLUT1. Data obtained in fibroblasts treated with serum lead us to hypothesise that c-Fos might play a major role in the regulation of GLUT1 expression.

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1. Introduction

The treatment of cultured neonatal rat ventricular cardiac myocytes with serum, TPA (a phorbol ester that activates protein kinase C), hormones such as endothelin-1 (ET-1) and angiotensin II, or phenylephrine (PE), a pharmacological agonist of the α-adrenergic receptor, constitutes a well-established in vitro model for studying the pathways that lead to cardiac hypertrophy \cite{1}. The signal transduction pathways elicited by ET-1 and PE via their G\textsubscript{q} protein-coupled receptors activate multiple genes required for hypertrophic growth, and among those, genes that are regulated by the transcription factor AP-1. PE and ET-1 cause the rapid induction of the expression of c-Fos, c-Jun, and Jun B \cite{2–4}, protein constituents of AP-1; this has been proposed as a mechanism that may mediate the actions of PE on AP-1-mediated transcriptional regulation of certain target genes in cardiac myocytes \cite{5,6}.

AP-1 exists as a heterodimer of proteins from the Jun and Fos families and binds to TPA-responsive elements in target genes (TRE \cite{7}). Jun also heterodimerizes with members of the ATF family of transcription factors to bind c-AMP responsive elements \cite{8}, and forms homodimers; however, these only bind to TRE with low affinity \cite{9}. All these proteins share a basic-leucine zipper structure which

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mediates DNA-binding and dimerization [10]. The Jun family is composed of c-Jun, JunD, and Jun B, and the Fos family consists of c-Fos, FosB, Fra1 and Fra2. c-Jun and c-Fos have been defined as immediate early response genes as their abundance increases rapidly and transiently in response to mitogenic stimuli [11]. The half-life of c-Fos mRNA is very short, which ensures that the levels of expression of the factor return to basal upon suppression of the growth-promoting signal; this allows a finely tuned cellular response to such stimuli [12].

There is evidence that glucose metabolism and glucose transporter expression is altered in in vivo models of cardiac hypertrophy and heart failure patients [13,14]. The GLUT1 glucose transporter is one of the genes whose expression is increased by treatment with hypertrophic agonists in cardiac myocytes [15]. However, the cis- or trans-acting elements responsible for this effect have not been fully characterised. In a variety of cell types, mitogenic stimuli such as serum, PDGF and EGF rapidly induce the expression of the GLUT1 mRNA in parallel to the upregulation of Jun and Fos [16,17]. A series of putative TREs in two enhancers located at the 5′-flanking region and second intron, respectively, of the mouse GLUT1 gene have been proposed to mediate the effects of growth factors on GLUT1 transcription [18]. Nevertheless, no strong evidence has been provided that shows the direct role of AP-1 in mediating any of these effects.

In this study, we hypothesised that changes in the abundance of particular AP-1 heterodimers expressed in cardiac myocytes could correlate with the increase in AP-1-mediated transcription seen when these cells are exposed to hypertrophic agonists. Here we show that the binding of c-Fos-containing AP-1 to DNA is rapidly induced in cardiac myocytes by hypertrophic stimuli, and that this is accompanied by the upregulation of GLUT1 expression. We also show in a mouse fibroblast cell line lacking c-Fos that the induction of GLUT1 by serum is critically dependent on the expression of c-Fos, implying that the expression of this factor is crucial for the upregulation of GLUT1 transcription in fibroblasts. We hypothesise that this could also be the case in cardiac myocytes undergoing hypertrophy.

2. Methods

2.1. Cell culture

Neonatal rat ventricular cardiac myocytes in primary culture were isolated and cultured as described before [19]. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Wild type and c-fos −/− skin fibroblast cell lines were cultured as previously described [20].

2.2. Transfection and luciferase assays

Transfection of cardiac myocytes was carried out by the calcium phosphate precipitation method, essentially as previously described [19]. Briefly, neonatal rat cardiac myocytes cultured on six-well plates (6×10³ per well) were transfected with a total of 15 μg of DNA per well comprising 8 μg of 2×TRE PRL Luc reporter or PRL Luc control luciferase plasmids (a gift of Dr. Stephen Fuller, Imperial College London) [5] and pUC18 as carrier DNA. CaCl₂ was added dropwise to a 250 mM final concentration, followed by two-times concentrate 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES)-buffered saline, pH 7.12 [21]. A DNA co-precipitate in a final volume of 350 μl was allowed to form by incubating the solution for five min at room temperature. The precipitate was added dropwise onto the cells, followed by an overnight incubation. The cells were washed twice in medium and incubated for 4 h in the absence of serum before adding PE at 10 μM final concentration to designated wells. Cells were treated for 48 h and harvested in 250 μl of lysis buffer (Roche Diagnostics) according to the manufacturer’s instructions. All agonists were dissolved in culture medium. The mitogen-activated protein kinase (MAPK) inhibitors PD98059 and SB203580 were dissolved in dimethylsulfoxide (DMSO). Luciferase activity in the extracts was determined by using the luciferase assay system (Promega). Luciferase activity was normalized to total protein content of the extracts, determined using the bicinchoninic (BCA) system (Pierce).

2.3. Electrophoretic mobility shift assays

Nuclear extracts from neonatal rat cardiac myocytes and mouse fibroblasts were obtained as described [22]. For electrophoretic mobility shift assays (EMSAs), the oligonucleotides 5′-CGCGCCCTGACTCACTTAAGACCC-3′ and 5′-GGGTCTTAAGTGAGTCAGGGCGCG-3′, which contain a consensus AP-1 site (underlined) were annealed and radiolabelled. A 5 to 10-μg amount of protein from nuclear extracts was incubated with the probe as described [23] and run on 8% non-denaturing polyacrylamide gels. Samples were run for 2 h at 200 V. The gels were dried and exposed to an X-OMAT (Kodak) film or a phosphor-imager screen. Supershift experiments were performed the same way but the incubation of the extracts with the probe was followed by a 15-min incubation on ice with antibodies against the selected transcription factors. These antibodies were: K-25 (recognises all fos family members), 4 (c-fos specific), H-75 (FosB), R20 (Fra1), Q20 (Fra2), H-79 (c-jun), 329 (JunD), and N-17 (JunB) (Santa Cruz Biotechnologies).

2.4. Western blotting

For total protein extracts, neonatal rat cardiac myocytes
were plated at $1 \times 10^6$ cells per 60 mm plate, whereas wild type and c-fos -/- fibroblasts were plated at $2.5 \times 10^5$ and $4 \times 10^5$ cells per 60 mm plate, respectively. Fibroblasts were incubated for 24 h in growth medium. All cells were starved of serum overnight and subjected to treatment as indicated in the figure legends. To prepare the extracts, the cells were washed twice in phosphate-buffered saline (PBS), and lysed in 200 μl of RIPA buffer supplemented with 40 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF) and phosphatase inhibitor cocktails 1 and 2 (Sigma). The lysate was aspirated a few times with a 1-ml syringe fitted with a 25-gauge syringe tip to ensure complete lysis. Debris and nuclei were discarded after centrifuging the lysate for 5 min at 4,000 rpm on a microfuge at 4°C, and the supernatant was stored at −70°C until used.

A 10-μg amount of total protein [as quantitated by using the BCA reagent (Pierce)] was loaded on a 10% sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE) gel and analyzed by western blotting with a polyclonal antibody raised against rat GLUT1 (Diagnostic International). The same membranes were re-blotted with a monoclonal antibody against rat β-tubulin (Sigma) to provide a loading control.

3. Results

3.1. Activation of the ERK MAPK pathway in neonatal rat cardiac myocytes leads to the temporary induction of AP-1 binding to DNA

Nuclear extracts prepared from neonatal rat cardiac myocytes treated with or without 10 μM PE [24] for different times were incubated with a radiolabelled oligonucleotide probe containing a consensus AP-1 binding site (TGAGTCA) in EMSA experiments. One major specific band (Fig. 1A, arrow A) was detected both in the absence and presence of PE. Nevertheless, extracts from cells treated with PE showed a transient retardation complex with a slightly slower mobility, which was evident as early as 30 min (Fig. 1A, arrow B). The binding of this complex peaked after 1 h of treatment with PE and started to decrease afterwards, reaching basal levels after 8 h. The formation of the transient complex B was specific for the AP-1 probe since the complex formed on an unrelated oligonucleotide containing an Oct1 binding site remains constant when incubated with extracts from cells treated with PE (Fig. 1B). Competition experiments performed with nuclear extracts obtained from cells stimulated with PE for 1 h showed that the complexes A and B were due to the binding to the AP-1 site, as they were competed by a molar excess of the same oligonucleotide (Fig. 1C, self), but not with an excess of the oligonucleotide containing a mutant AP-1 binding site (Fig. 1C, mutAP-1).

The formation of complex B was not exclusively due to PE, since we observed that 10 nM TPA (Fig. 2A), 10% horse serum/5% fetal calf serum, or 10 nM ET-1 ([25]; Fig. 2B) were able to induce the temporary binding of complex B in nuclear extracts with a timing similar to PE. Therefore, it is likely these agonists trigger the activation of a common signal transduction pathway in cardiac myocytes that leads to the same qualitative change in the binding of AP-1 complexes.

We transfected cardiac myocytes with a reporter vector that expressed the luciferase gene under the control of the rat prolactin minimal promoter fused to two copies of the consensus TRE in order to test if the changes in binding to an AP-1 site would correlate with a change in transactivation properties. A vector lacking the two TREs was transfected in parallel as a control (see schematic diagram in Fig. 2C). In untreated cells, the presence of the TREs induced a 28-fold increase in the transcription of the luciferase reporter compared to that of the control vector that lacks the AP-1 sites (Fig. 2C). Treatment of cardiac myocytes with PE resulted in a further eightfold activation of transcription (Fig. 2C). This effect was due to the AP-1 sites because the control vector did not respond likewise (Fig. 2C). These results are in concordance with results described elsewhere [5], and indicate that the binding of AP-1 heterodimers in basal conditions may already confer an important transcriptional activity, which is further enhanced by the treatment with PE and perhaps by the changes in binding that this brings about. PE is known to activate the ERK MAPK pathway in cardiac myocytes [26]. In order to investigate if this pathway is involved in the induction of the AP-1 binding activity, we treated cardiac myocytes with the MEK1 inhibitor PD98059 (20 μM [27]) for 30 min prior to incubating the cells for 1 h with PE. This treatment completely abolished the formation of complex B (Fig. 2D). On the other hand, treatment with either the inhibitor on its own or with the p38 MAPK inhibitor SB203580 (10 μM [28]), failed to inhibit the formation of complex B under the same conditions (Fig. 2D). The PD98059 and SB203580 inhibitors were dissolved in DMSO, which did not affect the binding of proteins to the AP-1 probe on its own (data not shown). The PD98059 inhibitor also abolished the effects of ET-1 and TPA (data not shown), but only slightly affected the binding of AP-1 heterodimers containing c-Fos as a consequence of the treatment with serum, and required the addition of the p38 inhibitor SB203580 for the induction of c-Fos to be further reduced (data not shown). This suggests that due to the intrinsic complexity of serum, other signal transduction pathways besides the ERK MAPK pathway are being activated by serum and impinge on regulatory elements of the c-fos gene.

3.2. The PE-induced AP-1 binding activity is composed of heterodimers of the Jun family members and c-Fos

The composition of the AP-1 binding activities found in
Fig. 1. PE induces a temporary AP-1 binding activity in neonatal rat cardiac myocytes. (A) A 5-μg amount of total protein of nuclear extracts prepared from cardiac myocytes treated as indicated was incubated with 50,000 cpm of an oligonucleotide probe containing a consensus AP-1 site in EMSA assays. Arrows A and B indicate AP-1 specific complexes obtained in non-stimulated or PE-stimulated (10 μM) cells. NS, Non-specific bands, due to the binding of proteins elsewhere in the probe, FP, free probe. (B) Nuclear extracts obtained from cardiac myocytes incubated in the conditions indicated were used in EMSA experiments with an oligonucleotide probe containing a consensus Oct1 binding site, in order to show that complex B obtained with the AP1 probe in the presence of PE was specific for the TRE. (C) A competition EMSA experiment was carried out with nuclear extracts from cardiac myocytes incubated with PE for 1 h. Either the same oligonucleotide (self) as the probe or one containing a mutation in the AP-1 site that disrupted the consensus AP-1 binding site (mutAP-1) were used as competitors at a 200-fold molar excess.

nuclear extracts from cardiac myocytes was elucidated by performing supershift EMSA experiments using a panel of antibodies specific for Jun and Fos family members. In nuclear extracts from cells treated with PE for 1 h (Fig. 3A), each of the antibodies against c-Jun, JunD or JunB were able to partially supershift both complexes A and B to varying degrees, indicating that they could be composite complexes of AP-1 heterodimers containing all three Jun isoforms (Fig. 3A, left panel). In fact, each of the antibodies seemed to remove a particular fraction of complex B. The use of combinations of two of the antibodies at a time hinted at the position of the fraction containing JunB (Fig. 3A, lane 5), Jun D (Fig. 3A, lane 6) and c-Jun (data not shown). When all three antibodies were used at a time, practically all of complexes A and B were supershifted; the faint remainder may reflect perhaps one of the antibodies not removing all complexes bearing the corresponding epitope (Fig. 3A, lane 7).
Fig. 2. The induction of AP-1 binding by PE in cardiac myocytes depends on the ERK MAPK pathway and coincides with the activation of transcription of an AP-1 driven luciferase reporter. (A) Neonatal rat cardiac myocytes were treated for the indicated times with 10 nM TPA prior to obtaining nuclear protein extracts. A 5-μg amount of extract was incubated with the AP-1 consensus probe as in Fig. 1. Arrows A and B indicate complexes characteristic of non-stimulated or TPA-stimulated cells, respectively. (B) As in A, but cells were stimulated for 1 h in 10% horse serum (HS)/5% FBS (HS/FBS) or 10 nM ET-1 as indicated. (C) Neonatal rat cardiac myocytes were transfected with a luciferase reporter vector containing two consensus AP-1 binding sites (2×TRE PRL-LUC) cloned 5′ to the basal prolactin promoter (PRL-LUC), or with a control plasmid lacking the AP-1 sites. Cells were treated with or without PE for 48 h and luciferase activity was determined as described. Data represent the average of two experiments (each experiment performed in triplicate), normalized to protein in the extracts, and are represented relative to the activity of the control plasmid in basal conditions. (D) A 5-μg amount of nuclear protein extract was obtained from neonatal rat cardiac myocytes treated for 1 h with PE, in the absence or presence of 20 μM PD98059 (MEK1 inhibitor) or 10 μM SB203580 (p38 MAPK inhibitor), as indicated. Inhibitors were added to the cells 1 h prior to the addition of PE. The extracts were incubated with the AP-1 probe in EMSA experiments as indicated in Fig. 1.

We found that these juxtaposed complexes were heterodimers of Jun and Fos proteins, since an antibody that recognized an epitope common to all Fos isoforms was able to completely supershift both complexes A and B (Fig. 3A, lane 9). A more detailed study with Fos isoform-specific antibodies revealed that the AP-1 binding complex also resolved into three main bands or clusters, possibly due to the heterodimerization of a given Jun isoform with either Fra1, Fra2 or c-Fos (Fig. 3A, lanes 8 to 12). We found that the Fra1- and Fra2-containing complexes were in close proximity to each other, and the c-Fos-containing complexes displayed a slower mobility (Fig. 3A, lanes 10–12). Again, combinations of antibodies were used to make clearer the relative position of the Fos-family members in both complexes A and B (Fig. 3A, lanes 13–16). Once c-Fos-, Fra-1, and Fra-2-containing complexes were removed, a further fraction of the remainder of complex A was shifted with an antibody against FosB (Fig. 3A, lane 16). A faint persistent band was not removed by this antibody, possibly due to a poorer efficiency or tertiary structure/modifications masking epitopes for any of the Fos family antibodies used. Nonetheless, the complexes containing FosB overlap with those containing Fra1 and Fra2, but not with those containing c-Fos (Fig. 3A, lane 16).

Although the intensity of complex A in non-stimulated cell extracts is fainter than in PE-stimulated cells (see Fig. 1A), our supershift data show that it has a composition equivalent to the one found in PE-stimulated nuclear extracts (Fig. 3B). This includes the relative positioning of
Fig. 3. The PE-induced AP-1 binding activity is composed of heterodimers of members of the Jun family and c-Fos. (A) A 5-μg amount of protein from cardiac myocytes stimulated for 1 h with PE was used in supershift EMSA experiments with the AP-1 probe as described in Methods. The names above the lanes indicate the specificity of the antibodies used for the supershift reaction. The asterisks in lanes 10 and 11 indicate the positions vacated by complexes supershifted by the anti-Fra1 and anti-Fra2 antibodies, respectively. (B). As in A, but using extracts prepared from non-stimulated cardiac myocytes. (C) Schematic depiction of the composition of the complexes obtained with nuclear extracts from PE-treated or untreated cells. The relative positioning of the complexes is used to reflect the overlapping of bands that migrate closely in the EMSA.
the Fra1- and Fra2-containing complexes. However, none of the complexes shown in Fig. 3B contained c-Fos because they could not be supershifted by an anti-c-Fos specific antibody (Fig. 3B, lanes 12–13). We provide a schematic depiction (Fig. 3C) of the composition of complexes A and B, both in untreated and PE-treated nuclear extracts, which indicates the overlapping of bands due to the binding of different AP-1 heterodimers to the probe. In summary, treatment of cardiac myocytes with PE leads to the transient accumulation of AP-1 heterodimers containing c-Fos, which add to the already existing AP-1 heterodimers composed of Jun family members combined with Fra1, Fra2 and possibly FosB.

3.3. c-Fos is necessary for the induction of GLUT1 by serum in mouse fibroblasts

In the results described so far, the activation of the binding of AP-1 heterodimers containing c-Fos correlated with the activation of transcription of an AP-1-driven reporter vector. Thus, we investigated the possible role of this mechanism in the regulation of gene expression by hypertrophic agonists in cardiac myocytes. To this end, we studied the effect of these agonists on the expression of GLUT1 glucose transporter, as it has been reported that PE activates the transcription of this gene [15]. We found that treatment of cardiac myocytes in culture with PE, ET-1 or serum for 48 h induced the expression of GLUT1 in all cases, as deduced by western blotting (Fig. 4A). The effect of PE on GLUT1 expression was prevented by prior treatment of the cells with the MEK1 inhibitor PD98059 (Fig. 4B), which indicated that at least the activation of GLUT1 expression by PE is mediated by the ERK MAPK pathway. The fact that serum, a powerful mitogen for a variety of cell lines, was able to induce the expression of GLUT1 in cardiac myocytes, which only show hypertrophic growth under these conditions, was of interest since it is known that immediate-early response genes such as c-Fos are up-regulated in parallel to GLUT1 in 3T3 fibroblasts when treated with serum [29]. This allowed us to approach the contribution of c-Fos to the stimulation of GLUT1 transcription by serum in a simple cellular model using two cell lines derived from mouse skin fibroblasts, one obtained from a homozygous knockout mouse deficient for c-Fos (c-fos−/−), the other obtained from a wild type (WT) littermate. EMSA analysis of WT fibroblasts treated with serum for 1 h revealed a similar transiently-formed complex B as the one detected in rat cardiac myocytes in addition to the faster-migrating A complex (Fig. 5A, lane 2). However, treatment of these cells with TPA or ET-1 failed to induce the formation of complex B (Fig. 5A, lanes 3 and 4; compare with Fig. 2). In contrast, when c-fos−/− cells were treated with either serum, TPA or ET-1, no complex B appeared (Fig. 5A, lanes 5–8). This further supports our EMSA observations that complex B contains c-Fos heterodimers. Finally, we examined the effect of serum stimulation on the expression of GLUT1 in WT and c-fos−/− fibroblasts stimulated with serum. Cells were treated with 10% fetal bovine serum (FBS) for 48 h, or were left in serum-free medium for an identical period of time. Treatment of WT cells with serum caused an increase in the expression of GLUT1 (Fig. 5B, compare lanes 1 and 3), which was not observed when cells were treated for an identical period of time with 1 μM TPA (Fig. 5B, lane 2). On the other hand, treatment of c-fos−/− fibroblasts with 10% FBS failed to significantly induce the expression of GLUT1 (same figure, compare lanes 4 and 6). These data suggest that in fibroblasts, the expression of c-Fos is a pre-requisite for the induction of GLUT1 expression by serum, and that c-Fos may be a key regulator of GLUT1 transcription.

4. Discussion

We show that treatment of neonatal rat cardiac myocytes with PE induces the selective and transient DNA-binding of AP-1 heterodimers containing c-Fos, and that this correlates with an increase in the transcriptional activity of an AP-1-driven reporter vector in transient transfections in cardiac myocytes. Transcriptional activation by mitogenic or hypertrophic stimuli may partly depend on an increase in c-Fos expression triggered by the agonists, as suggested...
heterodimers in extracts from untreated cardiac myocytes and that they bind to the consensus AP1 site used in the EMSA experiments (Fig. 3). The different Jun isoforms possess different transactivation potential [33,34]. Thus, we think that the presence of the AP-1 sites exerts a positive transcriptional effect on the luciferase reporter as a result of the combined effects of the different AP-1 heterodimers that are being expressed in untreated cells, though we are unable to attribute such a property to a particular AP-1 combination at this stage. Nevertheless, the presence of heterodimers containing c-Fos in PE-treated cardiac myocytes seems to be associated with a further increase in the transcriptional output from the reporter. The modification of AP-1 heterodimers by phosphorylation induced by the treatment with PE may contribute to the increase in expression of Jun and Fos proteins [35,36], and potentiate their capacity as trans-activators [37,38].

Furthermore, we show how the pre-treatment of cardiac myocytes with the MEK1 inhibitor PD98059 impairs the induction of the expression of GLUT1 and of AP-1 containing c-Fos. The latter is likely to be due to the action of the inhibitor blocking the activation of transcription of c-Fos by the ERK MAPK pathway. The activation of this pathway by PE has been previously implicated in the induction of the c-Fos gene in cardiac myocytes [39].

Fig. 5. c-Fos is required for the induction of GLUT1 by serum in mouse fibroblasts. (A) Nuclear extracts prepared from wild type- (WT) or c-Fos homozygous knock-out-derived (c-fos −/−) skin fibroblasts treated for 1 h as indicated were used in EMSA experiments with the AP-1 probe. Arrows A and B show complexes observed after treating WT fibroblasts with 10% FBS and that are compatible with those obtained with nuclear extracts from cardiac myocytes treated with PE for 1 h. (B) Total protein extracts were obtained from WT or c-fos −/− fibroblasts treated for 48 h as indicated. The expression of GLUT1 in these extracts was analysed by western blotting. A loading control was obtained by re-blotting the membrane with an antibody against β-tubulin.

by experiments performed on a mouse fibroblast cell line lacking c-Fos (Fig. 5A and 5B).

Although the composition of AP-1 has been studied in different situations, including cell cycle progression for fibroblasts [30], no previous study has characterised so thoroughly the composition of AP-1 heterodimers in cardiac myocytes stimulated with PE or other hypertrophic agonists. We have not studied the binding expression of AP-1 heterodimers composed of ATF2 and Fos family members, as these do not bind to the consensus AP-1 site used in the EMSA experiments. Therefore, we cannot discount that the stimuli used on cardiac myocytes could be inducing changes in the binding of this particular AP-1 combination as well.

Any Jun protein seems capable of dimerising with any member of the Fos family [31]. This property may allow for the tuning of the activity of AP-1 depending on the isoforms expressed in a tissue at a given moment or in response to particular extracellular cues [32]. Our results show that c-Jun, JunD, JunB and Fra1 or Fra2 form
Our results show that c-Fos expression is essential for the induction of GLUT1 by serum in fibroblasts. Based on the evidence obtained, we hypothesise that this same factor could have a similar role in the activation of GLUT1 expression by PE in cardiac myocytes. Future work could explore the role of putative TPA-responsive elements described in the distal enhancers of the mouse GLUT1 gene that might mediate the effects of PE or serum on GLUT1 expression by allowing the binding of AP-1. As to the functional significance of our findings, a cardiac-specific transgenic mouse model of over-expression of GLUT1 shows an increased survival to hypertrophy and heart failure induced by aortic constriction [44]. In this paper, Liao et al. propose that an increased glucose availability is beneficial for the condition of hypertrophied or failing hearts, which could help in the generation of future pharmacological targets to improve the survival of heart failure patients. One such possibility could be the use of drugs that increase the expression of GLUT1 in myocardium. Since little is known yet about the mechanisms that control GLUT1 expression in cardiac myocytes, our study provides some preliminary insight into this matter.

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