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Obesity Is Associated With Impaired Ventricular Protein Kinase C-MAP Kinase Signaling and Altered ANP mRNA Expression in the Heart of Adult Zucker Rats

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ABSTRACT

Background: **In the obesity model of the Zucker rat, myocardial protein kinase C (PKC) activation by phorbol ester is impaired. The influence of obesity on myocardial cell signaling was investigated by studying the activation of PKC isozymes and MAP kinases (MAPK) p38 and p42/44 as well as the induction of ANP mRNA.**

Methods: **Isolated hearts obtained from 17-week-old lean and obese Zucker rats were perfused with 200 nM phorbol 12-myristate 13-acetate (PMA) at different time periods. Immunodetectable PKC isozymes, phosphorylated-MAPK, and ANP mRNA were determined by Western and Northern blots, respectively.**

Results: **PMA promoted a marked transient translocation of ventricular PKCα from the cytosol to the membranes within 10 minutes in lean rats, whereas it had a much weaker effect in obese rats. Moreover, PMA induced a significant activation of PKCδ in lean but not in obese rat hearts. After PKC activation, increases in phosphorylation levels of myocardial p38 and p42 MAPK were approximately 3-fold higher in lean rats than in obese animals. Concerning the induction of ANP, PMA transiently tripled ANP mRNA within 60 minutes in lean but not in obese rats.**

Conclusions: **In the genetically obese Zucker rat, the myocardial signal transduction cascade PKC-MAPK-ANP mRNA seems to be markedly impaired. It can be speculated that this abnormal cardiac cell signaling in obese rats reflects an early phase in the cardiac pathogenesis accompanying obesity. (J Investig Med 2001;49:310–318) Key Words: isolated heart perfusion • phorbol 12-myristate 13-acetate • PKCα, δ, ϵ, ζ • p38-MAPK • p42/44- MAPK**

INTRODUCTION

Obesity is a major risk factor for cardiovascular complications such as coronary heart disease and congestive heart failure, but there is scant knowledge regarding the cellular mechanisms leading to cardiomyopathies associated with obesity.[1,2]

Of the several protein kinases involved in cell signaling, the protein kinase C (PKC) has been shown to play a key role in the regulation of signal transduction in many cell types. PKC is a family of serine-threonine kinases that can be classified into three subclasses on the basis of their domain structure, which dictates their requirements for Ca^{++} and *sn*-1,2-diacylglycerol (DAG). The conventional PKC (cPKCα, cPKC$\beta_{I/II}$, cPKCγ) are regulated by Ca^{++} and DAG, whereas the novel PKC (nPKCδ, nPKCϵ, nPKCη, nPKCθ) require DAG but not Ca^{++} for activation. The atypical PKC (aPKCλ, aPKCζ), the regulation of which has not been clearly established, are insensitive to both Ca^{++} and DAG (for reviews, see refs[3,4]). Phorbol esters such as phorbol 12-myristate 13-acetate (PMA) can substitute for DAG and produce a powerful activation of cPKC and nPKC, resulting in their translocation from the cytosolic to the membrane fraction of the cell.[5,6] The specific role of the different isozymes is not yet known, and it is also not clear whether multiple isoforms are at least in part redundant.

In cardiomyocytes, PKC has been shown to be implicated in cellular growth,[7] contraction[8] and regulation of

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gene expression.[9] More specifically, activation of myocardial PKC by endothelin-1, phenylephrine, or PMA has been found to induce phosphorylation of p38[10,11] and/or p42/44 MAP kinases (MAPK).[11–13]

The MAPK p42/44[14] and p38[15,16] subfamilies have been shown to participate in the regulation of *c-fos* expression. Heterodimers of the c-Fos and c-Jun proteins bind to activator protein regulatory sites present in the promoter regions of many genes such as the atrial natriuretic peptide (ANP) gene.[17,18] Moreover, activation of p42/44,[19] as well as p38 MAPK,[20] has been shown to induce the expression of ANP, recognized to be a marker of cardiac ventricular hypertrophy.[21,22]

In the obesity model of the Zucker rat, myocardial PKC activation by phorbol ester was found to be impaired in obese rats (fa/fa) when compared with that of lean animals (Fa/?).[23] In this context, it is interesting to note that the soleus muscle and the liver of obese Zucker rats contain significantly higher concentrations of the physiological PKC activator DAG than those of lean animals.[24–26] However, there is no information concerning the influence of obesity on the induction of ANP through the PKC-MAPK signaling pathway.

In view of these facts and to investigate the influence of obesity on myocardial cell signaling, we studied the effects of myocardial PKC activation by PMA in 17-week-old lean and obese Zucker rats on the subcellular localization of the PKC isozymes α, δ, ϵ, and ζ and the phosphorylation levels of p38 and p42/44 MAPK, as well as on the mRNA expression of ANP.

METHODS

Animals

Female 17-week-old Zucker rats were graciously furnished by Prof. B. Jeanrenaud (Medical Research Foundation, Geneva, Switzerland). Obese (fa/fa) rats weighed 380±2 g (n=101), and age-matched lean controls (Fa/?) rats weighed 220±3 g (n=118). Animals were fed a standard laboratory chow ad libidum and had free access to tap water.

Isolated Heart Preparation and Perfusion

Rats were anaesthetized with thiopental sodium (0.2 mL/100 g intraperitoneally; 5% Pentothal, Abbott Laboratories, Chicago, Ill). A thoracotomy was performed, 1000 IU of heparin (Liquaemin, Roche, Basel, Switzerland) was injected into the inferior vena cava, and the heart was quickly excised, placed in ice-cold saline, blotted, and weighed. The aorta was mounted into a steel canula, and retrograde perfusion was initiated at a constant flow (7 mL/min^{-1}) in a nonrecirculating system. The flow in the system was controlled by a roller pump (model IPN-8, Ismatec, Glattbrugg, Switzerland). Perfusate was Krebs-Henseleit buffer (KH buffer) containing 123 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1.4 mM KH_2PO_4, 1.2 mM $MgSO_4$, 20 mM $NaHCO_3$, and 11 mM glucose. KH buffer was equilibrated with 95% O_2/5% CO_2 and warmed to 37°C. At the end of the perfusion protocol, the ventricles were cut and immediately homogenized in buffer A (see next paragraph for more details) or immediately frozen in liquid nitrogen, powdered with a pestle in a mortar filled with liquid nitrogen, and stored up to 2 weeks at −70°C for subsequent analysis. For technical reasons (ie, availability of Zucker rats), both frozen and fresh tissues were used, resulting in similar values for all parameters tested.

To ensure consistency in comparing data of lean and obese rats, we always perfused hearts in the presence and the absence of PMA from both animal groups the same day. After an equilibration time of 10 minutes, control hearts were perfused with KH buffer during different time periods, while stimulated hearts were perfused with 200 nM phorbol 12-myristate 13-acetate (PMA; Alexis, San Diego, Calif) during the same time periods.

Preparation of Cytosolic and Particulate Fractions and of Homogenate

Subcellular fractionation was performed according to the modified methods of Rybin and Steinberg[27] and Halsey et al.[28] Minced cardiac ventricles were homogenized in 2 volumes of ice-cold buffer A (pH 7.45, containing 20 mM Tris base, 2 mM EDTA Titriplex, 10 mM EGTA, 0.25 M saccharose, 10 mM DTT, 55 μM leupeptin) with a Dounce homogenizer. Homogenates were centrifuged at 100,000*g* for 1 hour. The supernatant was used as the cytosolic fraction. The pellet was resuspended in ~200 μL of buffer A containing 1% (vol/vol) Triton X-100 and incubated for 30 minutes at room temperature, before being diluted with buffer A to obtain a final concentration of 0.2% of Triton X-100. The homogenate was centrifuged at 100,000*g* for 1 hour, and the supernatant obtained was used as the particulate fraction. This procedure resulted in cytosolic and membranous fractions containing 21.8±1.2 and 2.6±0.25 μg protein/mg of wet tissue, respectively. The cytosolic and membrane fractions were analyzed within 24 hours for the presence of the four PKC isozymes (Western blot analysis).

For Western blot analysis of MAPK, pulverized ventricles were homogenated with 60 strokes of Dounce homogenizer, in 14 μL/μg powder of ice-cold lysis buffer (containing: 50 mM Tris base, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA Titriplex, 2 mM EGTA, 40 mM β-glycerophosphate, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 200 μM sodium

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orthovanadate, 10 μg/mL leupeptin, 0.3 TIU/mL aprotinin, 1 μM pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 100 nM calyculin A). Homogenates from hearts of both animal groups, perfused in the presence and absence of PMA, were analyzed the same day for phosphorylation levels of MAPK.

Protein Assay

Protein content of homogenate or cytosolic and particulate fractions was determined using a Lowry Protein Assay detergent compatible kit (Bio-Rad, Hercules, Calif). Bovine serum albumin was used as protein standard.

Western Blot Analysis for PKC and MAPK Detection

Homogenate, cytosolic, and membrane fractions were diluted and boiled 5 minutes with 0.33 vol of sample buffer containing 10% (wt/vol) SDS, EDTA Titriplex, 2.1% (wt/vol) DTT, 40% (vol/vol) glycerol, 30 mM Tris HCl, 0.01% bromophenol blue, pH 7.9. We always charged 20 μg protein/lane as measured by photometric determination before charging the gel. Proteins were separated by SDS-PAGE using 10% (wt/vol) acrylamide resolving gel (150 V for 1 hour, in a Mini-protean II tank [Bio-Rad]) and were transferred to 0.45 μm reinforced nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany, or Porablot, Machery-Nagel, Düren, Germany). Electroblotting was performed in a Trans-blot cell (Bio-Rad Laboratories, Richmond, Calif) in 25 mM Tris base, 190 mM glycine, 20% (vol/vol) methanol, at 120 V for 2 hours.

Nitrocellulose membranes were incubated in a washing buffer (5% nonfat dried milk in 50 mM Tris/HCl, 200 mM NaCl, 0.2% (vol/vol) Tween 20, pH 7.5) for 1 hour at room temperature. Thereafter, nitrocellulose membranes were incubated for 2 hours at room temperature or overnight at 4°C with antibodies raised against PKCα, PKCζ (Transduction Laboratories, Lexington, Ky), PKCδ, PKCε (Santa Cruz Biotechnology, Santa Cruz, Calif), phosphorylated p42/44 and p38 MAPK (New England Biolabs, Beverly, Mass) as well as total p38-MAPK (Santa Cruz Biotechnology) or total p42/44-MAPK (Transduction Laboratories) recognizing both phosphorylated and nonphosphorylated MAPK.

After being washed three times with blocking buffer containing nonfat-milk powder, nitrocellulose membranes were incubated with the appropriate horseradish peroxidase-linked secondary antibody, either antirabbit (CovalAb, Oullins, France) or antimouse (Santa Cruz Biotechnology) and then washed six times for 10 minutes. Immunoreactive bands were visualized by the enhanced chemiluminescent method with exposure to Hyperfilm ECL (Amersham, Buckinghamshire, United Kingdom) and quantified with a Molecular Dynamics scanner using ImageQuant 3.3 Version Software (Molecular Dynamics, Queensland, Australia). To ensure consistency in the data analysis, the cytosolic and particulate fractions of the PMA-stimulated heart and the control-heart of lean and obese rats were run on the same gel.

Isolation of RNA and Northern Blot Analysis

Total RNA from homogenate powder was isolated with Promega RNA extraction kit (Madison, Wis) according to the manufacturer's instructions. After ethanol precipitation, RNA pellets were resuspended in H_2O and quantitated by absorption at 260 nm.

Northern blot analysis was used to quantify mRNA. Fifteen micrograms of total RNA was denatured and separated on 1% agarose gel and blotted on 0.45-μm nylon membrane (Schleicher & Schuell). After the transfer, the Nytran membranes were prehybridized in Rapid-hyb buffer (Amersham) with 0.07 μg/μL of herring sperm for 1 to 2 hours at 65°C and the appropriate probe was added to the same buffer for an overnight hybridization (at 65°C). The following probes were used for Northern blotting hybridization: a 0.77-kb fragment of mouse 18S ribosomal cDNA and a 0.62-kb fragment of rat ANP cDNA (gifts of Prof. R. Lerch, Division of Cardiology, University Hospital, Geneva, Switzerland). The probes were labeled with 50 μCi [α-^{32}P]deoxycytidine triphosphate (Hartmann Analytic GmbH, Braunschweig, Germany) using a random-prime labeling kit (Amersham) and the unincorporated nucleotides were removed with QIAquick nucleotide removal kit (Qiagen GmbH, Hiden, Germany) according to the manufacturer's instructions.

After hybridization, blots were washed serially, for 5 and 15 minutes with 2×SSC (1×SSC: 150 mM NaCl, 15 mM tri-sodium citrate dihydrate, pH 7) 0.1% SDS, and 1×15 minutes with 1×SSC 0.1% SDS at room temperature, followed by three washings at 65°C with 1×SSC, 0.5×SSC and 0.2×SSC 0.1% SDS.

After autoradiography (Hyperfilm MP, Amersham), membranes were stripped and reprobed with the 18S probe. Blots were autoradiographed (~2–7 days for ANP and ~20 hours for 18S) with intensifying screen (Kodak X-Omatic cassette, Eastman Kodak, Rochester, NY), and relative amount of mRNA was determined by densitometric scanning. To correct for differences in sample loading and transfer, the ANP mRNA was normalized in relation to the corresponding hybridization signal obtained with the 18S probe. All chemical reagents, unless otherwise specified, were purchased from Sigma Chemical Co (Zürich, Switzerland).

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Statistical Analysis

Quantitative data were described as mean±SEM, and statistical analyses were performed using an unpaired Student's *t* test or a one-way analysis of variance test, with $P < 0.05$ defined to be significant.

RESULTS

Time-Dependent Activation of Cardiac PKC Isozymes in Obese and Lean Zucker Rats

The subcellular distribution of ventricular PKCα, δ, ϵ, and ζ was determined in hearts of lean and obese rats perfused for 10 to 120 minutes with either KH buffer alone or with 200 nM PMA. Perfusing isolated heart from lean rats with PMA concentrations from 10 to 300 nM, we observed a maximal translocation of PKCα from the cytosol to the membranous fraction with 200 nM PMA (data not shown).

As shown in Figure 1, immunoblotting experiments indicate that adult heart ventricles from both lean (Fa/?) and obese (fa/fa) Zucker rats contain PKCα, δ, ϵ, and ζ. No significant difference was observed in the immunoreactive levels and subcellular distribution of these isozymes between the two animal groups. As is illustrated in Figure 2, the content of PKCα was markedly higher in the cytosolic than in the membranous fraction of unstimulated (KH buffer–perfused) heart tissue, whereas PKCδ and ϵ were found to be mostly in the membranous fraction. Indeed, the ratio of membrane:cytosolic PKC was approximately 3:1 for PKCδ and ϵ, whereas it was 0.5:1 and 1:1 for PKCα and ζ, respectively.

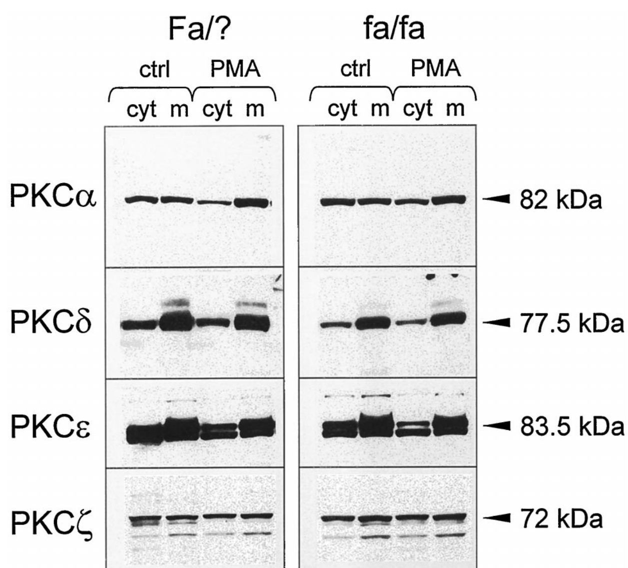

Figure 1. PMA-induced translocation of PKCα, δ, ϵ, and ζ in heart ventricles of lean and obese Zucker rats. Hearts from lean (Fa/?) and obese (fa/fa) Zucker rats were perfused with 200 nM PMA for 10–120 minutes (PMA) or KH buffer (ctrl), subfractionated and analyzed by Western blots, loading 20 μg of protein on each lane as described in Methods. Shown are representative blots illustrating the subcellular localization of ventricular PKCα, PKCδ, PKCϵ, and PKCζ after 20 minutes of perfusion. Results are representative of 4–7 separate experiments. Left panel: data obtained with lean (Fa/?) rats; right panel: data obtained with obese (fa/fa) rats. cyt: cytosolic fraction; m: membranous fraction.

Densitometric scanning of immunoblots (Figures 1 and 2A, upper panels) revealed that perfusion of hearts from lean rats with 200 nM PMA led to a marked transient increase in membranous PKCα with maximal values at 10 and 20 minutes of 293±47% and 287±32% of control values, respectively, as well as a persistent decrease in cytosolic PKCα. The PMA-induced increase in membranous PKCα was markedly diminished in the heart of obese rats (170±24% and 199±26% of control values at 10 and 20 minutes, respectively) when compared with lean animals (Figure 2A, upper left panel). As expected, the subcellular distribution of the atypical PKCζ was not influenced by 200 nM PMA, neither in the heart of lean rats nor in that of obese animals (Figure 2A, right panels, n=4–8).

Similar to PKCα, cytosolic ventricular PKCδ and ϵ were decreased after perfusion of hearts from lean and obese rats with 200 nM PMA (Figure 2B, lower panels), whereas membranous PKCδ and ϵ remained unchanged after PMA perfusion (Figure 2B, upper panels). PMA-induced decreases in cytosolic ventricular PKCδ were similar in lean and obese rats up to 60 minutes. After 120 minutes of perfusion with PMA, cytosolic PKCδ was significantly reduced to 17±6% of control values in lean rats, whereas it was decreased to 59±27% of control values in obese rats. Concerning cytosolic ventricular PKCϵ, we observed similar but not significant decreases in both lean and obese rats (Figure 2B, lower right panel).

PMA-Induced Phosphorylation of Cardiac p38 and p42/44 MAPK in Lean and Obese Rats

Because PKC activation has been shown to induce stimulation of MAPK,[11,29,30] we determined the phosphorylation levels of ventricular p38 and p42/44 MAPK from hearts of lean and obese rats perfused with 200 nM PMA for 10 to 120 minutes.

As is shown in Figures 3 and 4, perfusion of hearts with 200 nM PMA led to a significant increase in phosphorylation levels of ventricular p38 as well as p42 and p44 MAPK within 10 minutes in both animal groups, reflecting rapid activation of these kinases. Using highly specific antibodies against the total and phosphorylated forms of MAPK, we showed that in KH buffer–perfused hearts there is no significant difference in the ventricular amount

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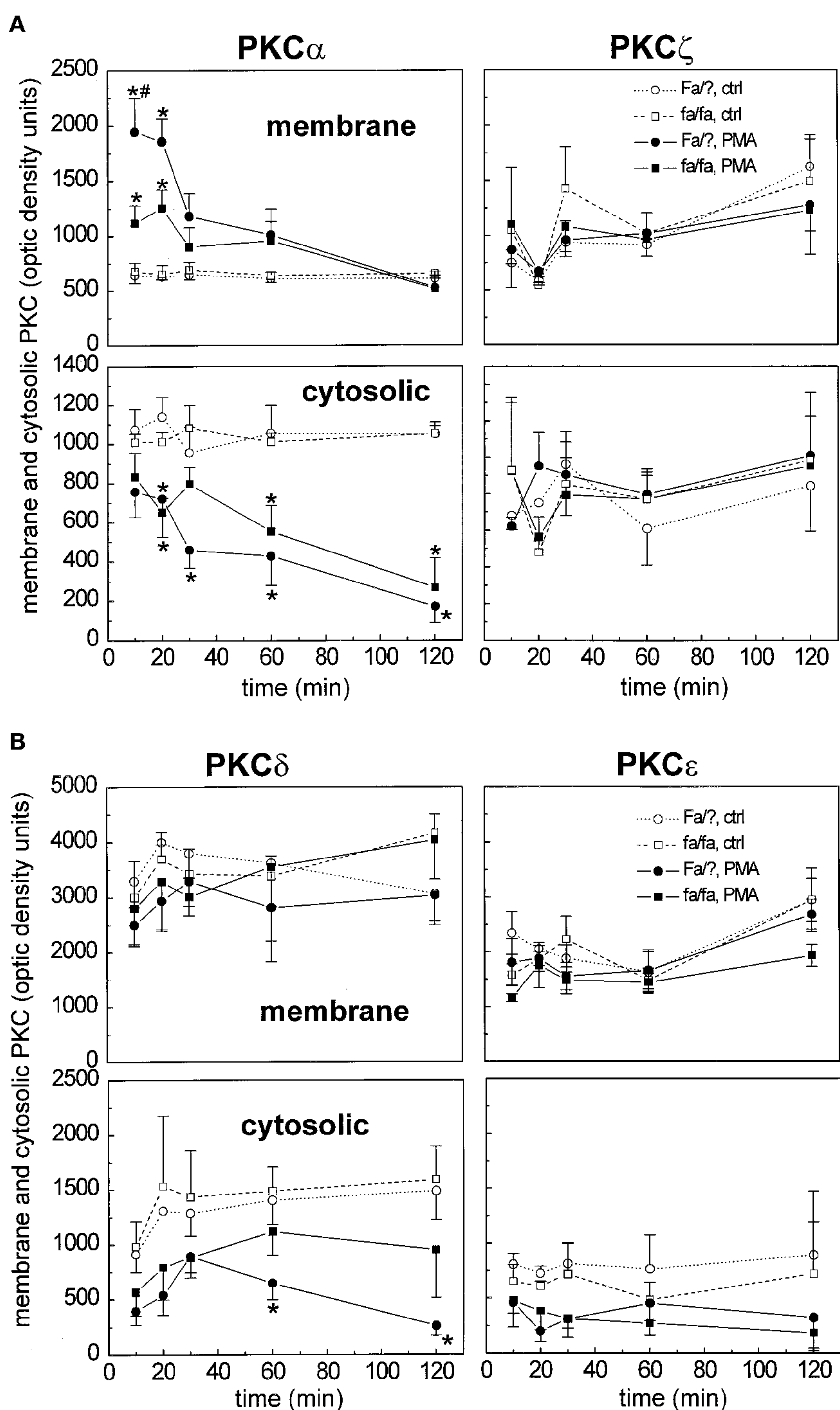

Figure 2. Time-dependency of PMA-induced activation of PKCα and ζ (A) and PKCδ and ϵ (B) in heart ventricles of lean and obese Zucker rats. Hearts from lean (Fa/?) and obese (fa/fa) Zucker rats were perfused with 200 nM PMA or KH buffer for 10–120 minutes. Western blots of membranous and cytosolic PKCα, δ, ϵ, and ζ were quantified by laser scanning densitometry. Results represent mean±SEM of 2–10 separate experiments for each time point of perfusion. Each value represents ventricular PKC in membrane or cytosolic fractions of control (KH buffer–perfused) and of PMA-perfused hearts expressed as optical density units. *$P<0.05$*, PMA-perfused hearts vs control hearts; #$P<0.05$, PMA-perfused hearts of Fa/? rats vs PMA-perfused hearts of fa/fa rats. Fa/? rat heart (circle), fa/fa rat heart (square), control hearts (open symbol), PMA-perfused hearts (closed symbol).*

or phosphorylation level of these kinases between lean and obese rats (Figures 3 and 4).

Figure 4A illustrates that, in heart ventricles of lean rats, the PMA-induced increase in the phosphorylation level of p38 MAPK was 15-fold and 12-fold at 10 and 30 minutes, respectively, decreasing rapidly thereafter. This PMA-induced increase in p38 MAPK phosphorylation was strongly reduced in the heart of obese rats. Indeed, in obese animals, PMA induced only a 5-fold increase in the phosphorylation level of ventricular p38 MAPK after 10

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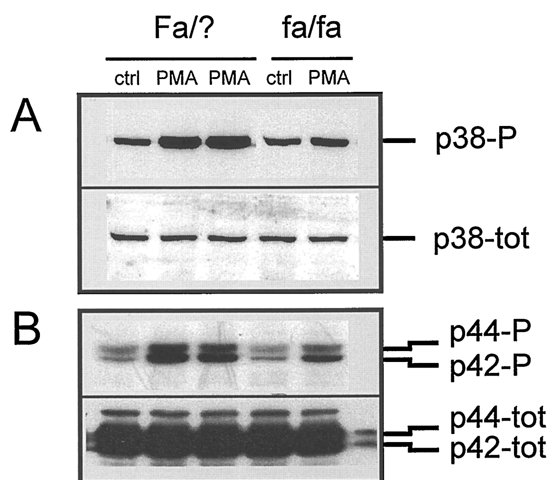

Figure 3. PMA-induced phosphorylation of p38 (A), p42, and p44 (B) MAPK in heart ventricles of lean and obese Zucker rats. Ventricular homogenates from hearts perfused with 200 nM PMA for 10–120 minutes or KH buffer (ctrl) were analyzed by Western blots, loading 20 μg of protein on each lane, as described in Methods. This figure is representative for the 30-minute perfusion time point in 7–11 experiments, each lane representing a single heart preparation. A and B, upper rows: Membranes were incubated with an antibody raised against the phosphorylated forms of p38 or p44/42 (-P). To ensure equal loading and protein transfer, duplicate blots were performed for the same samples and probed with an antibody raised against total p38 or p44/42 (phosphorylated plus not phosphorylated forms; A and B, lower rows [-tot]).

minutes of perfusion, with the level decreasing rapidly thereafter.

As shown in Figure 4B, perfusion of hearts from lean rats with PMA resulted in a 16-fold increase in the phosphorylation level of p42 MAPK after 10 minutes, with the level decreasing rapidly after 30 minutes. In obese rats, this PMA-induced phosphorylation was significantly reduced to a 6-fold increase in the phosphorylation level of p42 MAPK (Figure 4B). Conversely, the phosphorylation level of myocardial p44 MAPK was 4-fold enhanced in both lean and obese rats (Figure 4C).

Effect of PKC Activation on ANP mRNA Expression

Because it has been shown that activation of p38[20] and/or p42/44 MAPK[19] promoted by hypertrophic stimuli is involved in the expression of ANP, we evaluated the expression of ventricular ANP mRNA in hearts of lean and obese rats perfused with 200 nM PMA for 10 to 120 minutes. As is shown in Figure 5A and B, in lean rat hearts, perfusion with 200 nM PMA induced a significant, transient 3-fold increase in the expression of ventricular ANP mRNA at 60 minutes. By contrast, in obese rat hearts, PMA did not affect the ventricular level of ANP

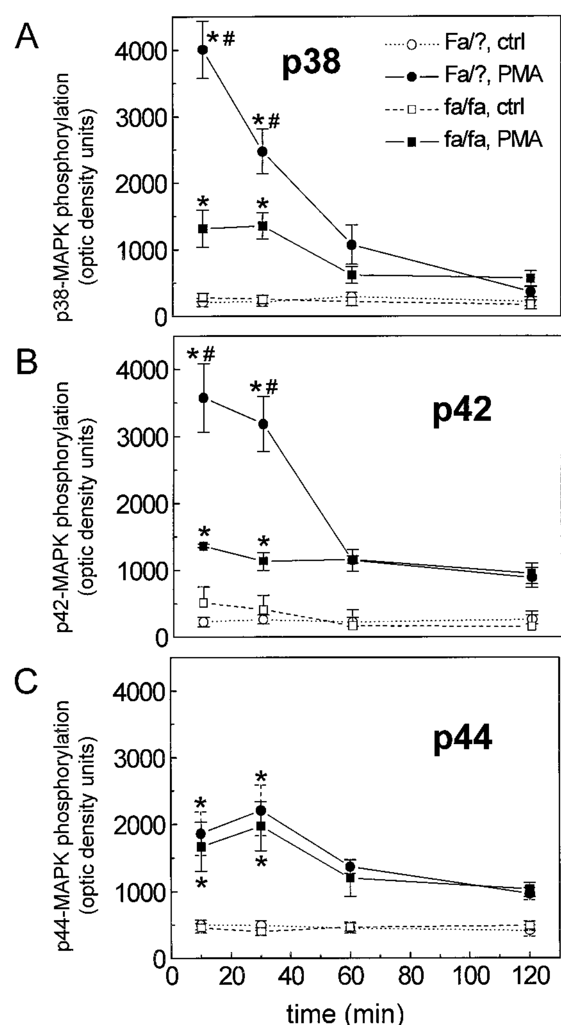

*Figure 4. Time-dependency of PMA-induced phosphorylation of p38 (A), p42 (B), and p44 (C) MAPK in heart ventricles of lean and obese Zucker rats. Immunoblots of phosphorylated p38, p42, and p44 MAPK from ventricular homogenates were quantified by laser scanning densitometry. The data (mean±SEM, n=4–11) are expressed as optical density units. Each value represents phosphorylated ventricular MAPK in control (KH buffer–perfused) and PMA-perfused hearts. *P<0.05, PMA-perfused hearts vs control hearts. #P<0.05, PMA-perfused hearts of Fa/? rats vs PMA-perfused hearts of fa/fa rats. Fa/? rat heart (circle), fa/fa rat heart (square), control hearts (open symbol), PMA-perfused hearts (closed symbol).*

mRNA. Basal levels of ventricular mRNA were found to be approximately 2-fold higher in lean than in obese rats, but the difference was statistically not significant (P=0.088).

DISCUSSION

The present study demonstrates for the first time that the PKC-MAP kinase signaling system is significantly

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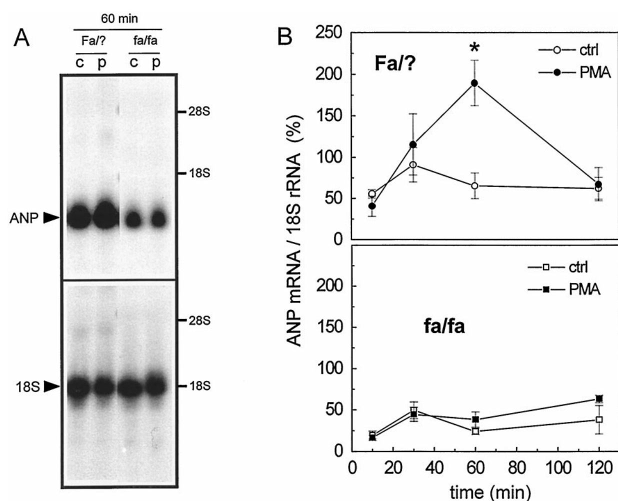

*Figure 5. Time-dependent expression of ANP mRNA induced by PMA in heart ventricles of lean and obese Zucker rats. Hearts were perfused for the times indicated in the presence (closed symbol) or absence (open symbol) of 200 nM PMA. Total RNA was extracted from ventricles for Northern blotting analysis as described in Methods. 15 μg of total RNA was loaded on each lane. Blots were autoradiographed for 3–7 days on films with enhancing screen. Thereafter, membranes were stripped and hybridized to a ^{32}P-labeled 18S rRNA probe. (A) Representative autoradiograms for the gene ANP and for the housekeeping gene 18S, with each lane representing a single heart preparation after 60 minutes of perfusion. (B) Corresponding time-course experiment as line graph. Results have been normalized using 18S rRNA as internal invariable control (100%) and are the mean±SEM of 3–5 independent experiments for each time point of perfusion. *P<0.05 PMA perfused heart vs untreated heart. Fa/? rat heart (circle), fa/fa rat heart (square).*

impaired in the heart of obese Zucker rats when compared with that of lean animals. In obese Zucker rats, the PMA-induced translocation of myocardial PKCα from the cytosolic to the membrane fraction observed in lean animals was markedly decreased. Similarly, ventricular cytosolic PKCδ was significantly less decreased in obese rats than in lean animals after PMA perfusion, whereas in both animal groups membranous PKCδ, as well as PKCε, seemed not to be affected by PMA. Because membrane-bound PKC is known to be more sensitive to degradation than cytosolic PKC and because PKCδ and ε are present in approximately three times greater quantity in the membrane fraction in unstimulated heart ventricles than in cytosolic, we can speculate that degradation processes as of yet unspecified of these membranous PKC isozymes do not allow us to observe an increase in the membranous form of PKCδ and ε.

In contrast to the observed impaired activation of myocardial PKCα and δ in obese rats, we found no difference in the basal levels and in the subcellular distribution of PKCα, δ, or ε between lean and obese animals. Our results are in agreement with an earlier study by van de Werve et al,[23] who observed that the translocation of myocardial PKC activity from the cytosolic to the membrane fraction promoted by PMA was diminished in the heart of obese Zucker rats when compared with lean animals. The same study revealed that basal membranous PKC activity was significantly lower in the heart of obese rats. Our work indicates that this decreased activity is not a result of a decreased level of PKCα, δ, or ε.

Conversely, Considine et al[31] found that membranous and cytosolic PKC activities, as well as membrane levels of PKCα, ε, and ζ, were the same in the liver of obese and lean Zucker rats. In contrast to our results and those of Considine et al, Cooper et al[32] found that levels of PKCα, β, and ε were diminished in the soleus muscle of obese Zucker rats when compared with lean rats. Avignon et al[25] observed that, relative to lean Zucker rats, the levels of PKCα and ε in the soleus muscle of obese animals were increased in the membrane fraction and decreased in the cytosolic fraction, whereas PKCβ levels were decreased in both fractions and PKCδ levels were not significantly changed. Clearly, further studies are necessary to determine whether the impairment of the PKC system in the obese Zucker rat is tissue- and/or isozyme-specific.

There is increasing evidence that PKC activation promotes the phosphorylation of p42 and p44 in neonatal ventricular cardiomyocytes[13,33,34] as well as in the adult heart.[12,35] Recently, Zu et al[36] showed that phosphorylation of p38 MAPK is mediated by PKC activation in the rat cardiac myoblast cell line H9c2. In the present study, we show that PMA induces a spectacular phosphorylation of the myocardial MAPK p42/44 and p38 in lean Zucker rats and that this phosphorylation is impaired in obese animals. Indeed, perfusion of hearts from Zucker rats with PMA leads to an increase in the phosphorylation levels of myocardial MAPK, which were 3- and 2-fold lower for p38 and p42, respectively, in obese rats compared with those of their lean counterparts and which were identical for p44 in both groups. Our results are in agreement with those of Lazou et al,[12] which reported that perfusion of Wistar rat hearts with PMA led to a 10-fold increase in the phosphorylation level of p42 MAPK. The perfusion of Sprague-Dawley rat hearts with phenylephrine resulted in a similar strong, 12-fold increase in the phosphorylation level of myocardial p38 MAPK.[11]

Interestingly, the two ventricular MAPK p38 and p42, the phosphorylation levels of which were highly enhanced (12- to 16-fold) by PMA in lean rats, were much less activated in obese rats (5- to 6-fold increases). In contrast, the phosphorylation level of p44 MAPK was increased in both animal groups 4-fold after PKC activation. Thus, our observations indicate that an impaired PKC activation in

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obese heart ventricles results in a strongly diminished activation of p42 and p38 MAPK but had no effect on PMA-induced phosphorylation of p44 MAPK.

In the present study, we observed no significant difference in the levels of ANP mRNA between lean and obese Zucker rats, suggesting that the obese rat heart is not hypertrophic according to the ANP expression criterion. Concerning ANP mRNA expression, we also found that activation of PKC resulted in the induction of myocardial ANP mRNA with a transient maximum at 60 minutes in lean Zucker rats. Because it has been shown that in the adult heart ventricles PMA had no effect on ANP secretion,[37,38] it can be speculated that the transient increase in ANP mRNA observed in heart ventricles from adult lean Zucker rats results from a possible negative feedback mechanism resulting from ANP accumulation. With a finding similar to our own, Cornelius et al[39] reported a transient increase in ANP mRNA 120 minutes after imposition of elevated wall stress and 90 minutes after forskolin stimulation. In contrast to the lean Zucker rat, myocardial ANP mRNA expression was not increased by PMA in the obese rat, suggesting that the impaired activation of the myocardial PKC-MAPK signaling cascade in the obese animal is not sufficient to promote ANP mRNA expression.

The sequential timing of ventricular PKC and MAPK activation and induction of ANP mRNA in PMA-perfused hearts, as well as the impairment of each of these cellular events in obese rat hearts, confirms the hypothesis that obesity affects the whole signal transduction cascade PKC-MAPK-ANP mRNA in adult Zucker rats. Taken together, our results indicate that in genetically obese adult Zucker rats the myocardial PKC-MAPK signaling cascade is impaired, which is in line with the absence of any effect of PMA on ANP mRNA in the heart of obese rats. We propose that this abnormal cell signaling in heart ventricles of obese rats reflects an early phase in the cardiac pathogenesis accompanying obesity.

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