In Vivo JNK Activation in Pancreatic β-Cells Leads to Glucose Intolerance Caused by Insulin Resistance in Pancreas

Jordi Lanuza-Masdeu,[1,2] M. Isabel Arévalo,[1,2] Cristina Vila,[2] Albert Barberà,[3,4] Ramon Gomis,[3,4] and Carme Caelles[1,2]

Insulin resistance is a key condition in the development of type 2 diabetes. It is well established that exacerbated Jun NH_2-terminal kinase (JNK) activity is involved in promoting insulin resistance in peripheral insulin-target tissues; however, this involvement is less documented in pancreatic β-cells. Using a transgenic mouse model, here we show that JNK activation in β-cells led to glucose intolerance as a result of impaired capacity to increase insulinemia in response to hyperglycemia. Pancreatic islets from these mice showed no obvious morphostructural abnormalities or decreased insulin content. In contrast, these islets failed to secrete insulin in response to glucose or insulin but were competent in succinate-, ketoisocaproate-, 3-isobutyl-1-methylxanthine (IBMX-), KCl-, and tolbutamide-induced insulin secretion. At the molecular level, JNK activation in β-cells inhibited insulin-induced Akt phosphorylation, pancreatic and duodenal homeobox 1 nucleocytoplasmic shuttling, and transcription of insulin-target genes. Remarkably, rosiglitazone restored insulin secretion in response to hyperglycemia in mice and insulin-induced insulin secretion and signaling in isolated islets. In conclusion, the mere activation of JNK suffices to induce insulin resistance in pancreatic β-cells by inhibition of insulin signaling in these cells, but it is not sufficient to elicit β-cell death. In addition, we provide the first evidence that thiazolidinediones exert insulin-sensitizing action directly on pancreatic β-cells. *Diabetes* 62:2308–2317, 2013

The incidence of type 2 diabetes is increasing, especially as a result of the tremendous increase in human obesity, a major risk factor for the development of this disease. Type 2 diabetes is characterized by defects in both insulin sensitivity and insulin secretion—either of which may predominate—that result in glucose intolerance and hyperglycemia with severe consequences. Obesity is often associated with a low-grade, chronic inflammatory state. In fact, the increased levels of proinflammatory cytokines have a major role in promoting peripheral insulin resistance (1). Furthermore, other conditions found in obesity, namely, the induction of endoplasmic reticulum stress caused by adipocyte

hypertrophy and the increased plasma level of free fatty acids (FFAs), also contribute to peripheral insulin resistance (2,3). At the molecular level, several serine/threonine protein kinases are activated by these stimuli, including the c-Jun NH_2-terminal kinase (JNK) and the inhibitor of nuclear factor-κB kinase, which both target the insulin receptor (InsR) substrate (IRS)-1 for serine phosphorylation. As a result, IRS-1 recruitment to the hormone-bound InsR is inhibited, thereby preventing activation of the insulin-signaling cascade (4). In contrast, in normal conditions, activated InsR triggers IRS-1 tyrosine phosphorylation, thus allowing the recruitment and activation of downstream effectors, such as the phosphatidylinositol 3-kinase–Akt cascade (5).

JNK comprises a group of serine/threonine kinases that belong to the mitogen-activated protein kinase family (6). In both dietary and genetic mouse models of obesity, there is a significant increase in JNK activity in peripheral insulin-target tissues, such as skeletal muscle, adipose tissue, and liver, which promotes insulin resistance (7). Consistently, the administration of the JNK inhibitory peptide JNKi-1 to these mice markedly improves insulin signaling in these tissues by reducing IRS-1 serine phosphorylation and in doing so increasing Akt phosphorylation (8). Likewise, the hypoglycemic action of thiazolidinediones (TZDs), a group of synthetic peroxisome proliferator–activated receptor (PPAR)γ ligands with insulin-sensitizing activity, is mediated by the inhibition of JNK (9).

In addition to the negative interaction of JNK with InsR signaling in peripheral insulin-responsive tissues, this kinase has also been implicated in promoting insulin resistance in pancreatic β-cells. In particular, FFA treatment results in sustained JNK activation in these cells concomitantly with the inhibition of the autocrine insulin action as a result of JNK-mediated IRS-1/2 phosphorylation at serine residues that interfere with their binding to activated InsR (3). Accordingly, treatment with JNKi-1 or *jnk1* deficiency relieves the inhibition of glucose-induced insulin transcription by FFAs and enhances obesity-inhibited and glucose-induced insulin secretion (GIIS) in pancreatic islets (3,8,10,11).

JNK is involved not only in the inhibition of insulin secretion but also in the loss of pancreatic β-cells induced by proinflammatory stimuli such as interleukin (IL)-1β (12,13). In this regard, treatment of insulin-secreting cell lines or pancreatic islets with JNK inhibitors prevents IL-1β–induced apoptosis (9,10,14). In addition, *jnk1*-deficient islets are more resistant to cytokine-induced cell death than wild-type or *jnk2*-deficient islets (11). Finally, activation of the JNK pathway may also be relevant for islet transplantation, since it is induced during islet isolation (15);

moreover, JNK inhibitors have been shown to preserve whole-islet mass and prevent islet apoptosis and graft loss (16–19).

Hence, compelling evidence supports that exacerbated JNK activity in type 2 diabetes mediates defects in peripheral insulin sensitivity by interfering with InsR signaling. Furthermore, several reports substantiate the involvement of JNK in insulin resistance in pancreatic β-cells. In addition, JNK also participates in pancreatic β-cell death. Nonetheless, in vivo data supporting these roles of JNK have been obtained using mainly genetic or chemical inhibition of the kinase—tools that allow the assessment of JNK requirement but not JNK sufficiency to achieve all these effects. Therefore, we generated a transgenic mouse model that allows JNK activation in a Cre recombinase expression–dependent manner and used it to study the effects of JNK activation in pancreatic β-cells regarding glucose homeostasis and β-cell function. Our data indicate that while JNK activation suffices to induce insulin resistance in β-cells as a result of the inhibition of InsR signaling, it is not sufficient to promote β-cell death.

RESEARCH DESIGN AND METHODS

Animals and in vivo studies. Transgenic C57BL/6J-Tg(Gfp[loxP]-MKK7D)Ccf mice were generated by microinjection of oocytes with the transgene GFP[loxP]-MKK7D (Fig. 1A), and two independent lines were selected for this study. All of the experiments described in this study were performed in both transgenic mouse lines, and comparable results were obtained. The RIP-Cre [C57BL/6-Tg (Ins2-Cre)25Mgn] and RIP-CreER [STOCK-Tg(Ins2-cre/ERT)1Dam/J] mouse strains were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice used in this study came from crosses of heterozygous Cre recombinase or Cre recombinase/estrogen receptor (ER)-expressing male mice with homozygous GFP[loxP]-MKK7D female mice. For activation of the Cre recombinase/ER fusion protein, 10 mg tamoxifen (T5648; Sigma) diluted in corn oil was administered to 8-week-old mice by oral gavage every other day for 5 days. Experiments were performed 2 weeks after tamoxifen treatment. The glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed in mice fasted for 6 h before injection of glucose (2 mg/g body wt i.p.) or insulin (0.5 units/g body wt i.p.), respectively. Blood was collected from the tail at the indicated time points, and plasma insulin and glucose were determined by ELISA (10-1249; Mercodia) and with an automatic glucometer (Elite;
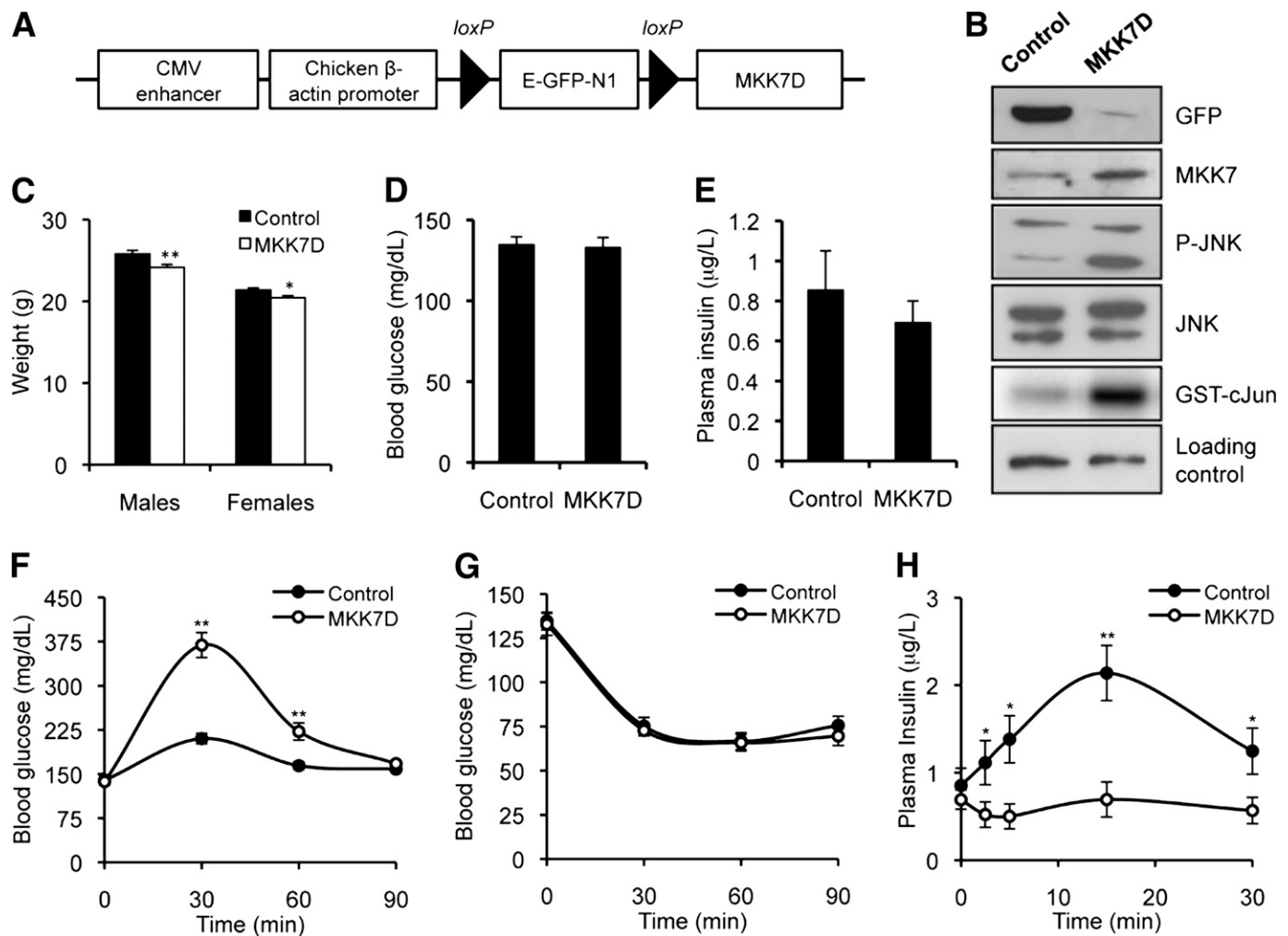

FIG. 1. JNK activation in pancreatic β-cells leads to glucose intolerance as a result of an impaired capacity to increase insulinemia in response to hyperglycemia. *A*: Diagram of the GFP[loxP]-MKK7D transgene. Transgenic mice constitutively express GFP under the control of the CMV enhancer/ chicken β-actin promoter (control mice). When crossed with RIP-Cre mice, the GFP cassette is floxed in pancreatic β-cells, leading to the constitutive expression of MKK7D in this cell type (MKK7D mice). E-GFP-N1 (enhanced green fluorescent protein) encodes a red-shifted variant of wild-type GFP. *B*: Immunoblot analysis of GFP, MKK7, phospho-JNK (P-JNK), and JNK in extracts from isolated pancreatic islets of control and MKK7D mice (first to fourth panel). Fifth panel shows JNK activity assessed by immunocomplex assay. *C*: Body weight comparison of control and MKK7D mice. Plasma glucose (*D*) and insulin (*E*) level in 6-h-fasted control and MKK7D mice. GTT (*F*), ITT (*G*), and glucose-stimulated insulin secretion (*H*) in control and MKK7D mice. Assays were performed in 3-month-old mice with at least 10 animals per group. *$P < 0.05$, **$P < 0.01$ with respect to the control group. GST, glutathione S-transferase.

Bayer), respectively. Rosiglitazone (1 mg/kg body wt) or vehicle (PBS) was administered to 2-month-old mice by oral gavage once a day for 10 consecutive days. All animal procedures were approved by the animal care research committee of the University of Barcelona.

Islet isolation and insulin secretion. After pancreas digestion by intraductal injection of collagenase, isolated islets were purified by centrifugation in ficoll gradients. They were then washed twice with Hank's balanced salt solution and cultured in RPMI 2% FBS for 3 h. Afterward, islets were handpicked, transferred to secretion vials in groups of six, and incubated for 60 min with a HEPES Krebs-Ringer bicarbonate buffer with 2.8 mmol/L glucose unless otherwise indicated. Supernatants were recovered, and insulin secretion was analyzed by ELISA. JNKi-1, 3-isobutyl-1-methylxanthine (IBMX), KCl, succinate, ketoisocaproate (KIC), rosiglitazone, and tolbutamide were used at 10 μmol/L, 50 μmol/L, 40 mmol/L, 10 μmol/L, 10 μmol/L, 10 μmol/L, and 250 mg/mL, respectively.

Immunoprecipitation and immunoblotting. Dual specificity mitogen-activated protein kinase kinase 7 (MKK7), JNK, green fluorescent protein (GFP), Akt, phospho-Akt, caspase 3, phospho-JNK, Pdx1, and GLUT2 were detected using the antibodies sc-7104, sc-474 (Santa Cruz Biotechnology), 11814460001 (Roche), 9272S, 4060P, 9662 (Cell Signaling), 36-9300 (Invitrogen), 07-696 (Upstate), and AB1342 (Chemicon), respectively. Immunoblots were performed using the enhanced chemiluminescence detection system. Immunodetection of Nek-9 was used as loading control.

JNK immunocomplex assay. JNK was immunoprecipitated with the antibody sc-474, and the JNK activity associated with the immunoprecipitates was determined as previously described (20).

Immunohistochemistry. When relevant, 6-h-fasted mice were injected with glucose (2 mg/g body wt i.p.) or insulin (0.5 units/g body wt i.p.), respectively. The pancreas was surgically removed, fixed in 4% paraformaldehyde in PBS, dehydrated, and embedded in paraffin. Sections (5 μm) were made with a manual microtome and stained with hematoxylin-eosin or immunostained with insulin and Pdx1 or glucagon antibodies (Dako A0564, Upstate 07-696, and Dako A0565, respectively). Slides were analyzed by fluorescence or confocal microscopy (Upright Microscope Nikon E1000 or Leica TCS SPE, respectively).

Quantitative real-time PCR. Islets were isolated as described above, and RNA was extracted with TRIzol reagent (Invitrogen). RNA was reverse transcribed with M-MLV reverse transcriptase (Invitrogen) and quantified by quantitative RT-PCR using SYBR Green (Applied Biosystems). The following pairs of primers were used: glyceraldehyde-3-phosphate dehydrogenase (aagggctcatgaccaca and gatgcagggatgatgttctg), Ins1 (tgaccagctataatcagagacca and aggtggggaccacaaagat), Ins2 (aggcttttgtcaagcagcac and ggtctgaaggtcacctg ctc), and GLUT2 (aatggtcgcctcattctttg and caagagggctccagtcaatg).

Caspase 3/7 activity assay. Caspase 3/7 activity was measured in extracts of pancreatic islets (10 μg) with the Caspase-Glo 3/7 assay (Promega) according to the manufacturer's instructions.

Statistical analysis. Data were analyzed with a two-tailed unpaired Student test. Values are presented as means + SE.

RESULTS

MKK7D overexpression in pancreatic β-cells leads to JNK activation. To gain insight into the effects of JNK activation on pancreatic β-cells and glucose homeostasis, we generated a transgenic mouse model that allows JNK activation in a Cre recombinase expression–dependent manner. We constructed a transgene (GFP^loxP-MKK7D) in the context of the pCAG vector, which contains the cytomegalovirus enhancer and chicken β-actin promoter sequences driving the constitutive expression of the GFP gene, which was flanked by two loxP sites and followed by an MKK7D-expression unit (Fig. 1A). The MKK7D sequence encodes the mitogen-activated protein kinase (MAP2K) of JNK, MKK7, which carries two mutations (S271D and T275D) that mimic the active form (21). For activation of JNK in pancreatic β-cells, mice harboring this transgene were crossed with RIP-Cre mice to obtain animals expressing either GFP (control mice) or, due to the Cre recombinase–dependent excision of the GFP-expression cassette, MKK7D (MKK7D mice). Immunoblot analysis of extracts from pancreatic islets isolated from these animals demonstrated that Cre recombinase expression drastically reduced GFP expression while, concomitantly, increasing

MKK7D expression, thereby resulting in augmented JNK phosphorylation and activity (Fig. 1B). MKK7D mice were born and developed normally, although they showed a slight decrease in body weight that was sex independent (Fig. 1C).

JNK activation in pancreatic β-cells disrupts glucose homeostasis. The JNK pathway has emerged as a main player in glucose homeostasis not only because of its physiological role as a negative-feedback mechanism of the insulin-signaling pathway (22) but also because of its contribution to insulin resistance in diverse pathological scenarios. Therefore, we focused our attention on the effects of pancreatic β-cell–specific activation of JNK on glucose homeostasis in our MKK7D mice. Although we found no significant differences in glycemia and insulinemia between 6-h fasted control and MKK7D mice (Fig. 1D and E), the GTT showed that the latter were glucose intolerant (Fig. 1F). Furthermore, this defect in glucose homeostasis was due not to decreased peripheral insulin sensitivity, as shown by the ITT (Fig. 1G), but to an impaired capacity to increase plasma insulin level in response to hyperglycemia (Fig. 1H). Overall, these results indicated that MKK7D mice suffered from pancreatic dysfunction. This glucose-intolerant phenotype was repeated to a similar extent in males and females of both transgenic lines and was observed in animals as early as 1 month of age (Supplementary Fig. 1).

JNK activation in pancreatic β-cells does not cause major morphostructural changes in islets. The success of JNK inhibitors in increasing the survival of pancreatic islets or insulin-secreting cell lines subjected to transplantation protocols or exposed to proinflammatory cytokines, respectively, has led to the notion that the JNK pathway plays a fundamental role in mediating β-cell death (9,14,16–19). This role might be consistent with the phenotype that we observed in the MKK7D mice. Therefore, we performed a histological analysis of the pancreatic islets by hematoxylin-eosin staining and immunostaining using antibodies against insulin and glucagon. In contrast to what we expected, the islet shape, number, and size as well as distribution and number of α- versus β-cells did not differ between control and MKK7D mice (Fig. 2A–D), and the overall size of the pancreas did not differ either (Fig. 2E). In addition, no differences regarding pancreas or islet insulin content were observed (Fig. 2F and G). Data from the literature demonstrate that JNK-induced β-cell apoptosis is mediated by caspase 3 (13). In this regard, analysis of caspase 3 cleavage (Fig. 2H) and activity (Fig. 2I) in isolated islets showed that MKK7D-triggered activation of JNK did not lead to caspase 3 activation. These results do not support the notion that major morphological or structural defects of pancreatic islets caused by massive β-cell death were responsible for the glucose-intolerant phenotype observed in the MKK7D mice.

JNK activation in pancreatic β-cells impairs insulin signaling. As the morphology and structure of the pancreas and islets were apparently unaffected by the activation of JNK in β-cells, next we performed a series of functional assays addressed to analyze insulin secretion and signaling in response to glucose in isolated islets. First, we determined GIIS. In agreement with the studies in mice, we observed an impaired capacity of isolated islets from the MKK7D mice to secrete insulin in response to glucose compared with control mice (Fig. 3A). Moreover, this impairment was overcome when islets were incubated with JNKi-1 (Fig. 3B). Afterward, we confirmed that this
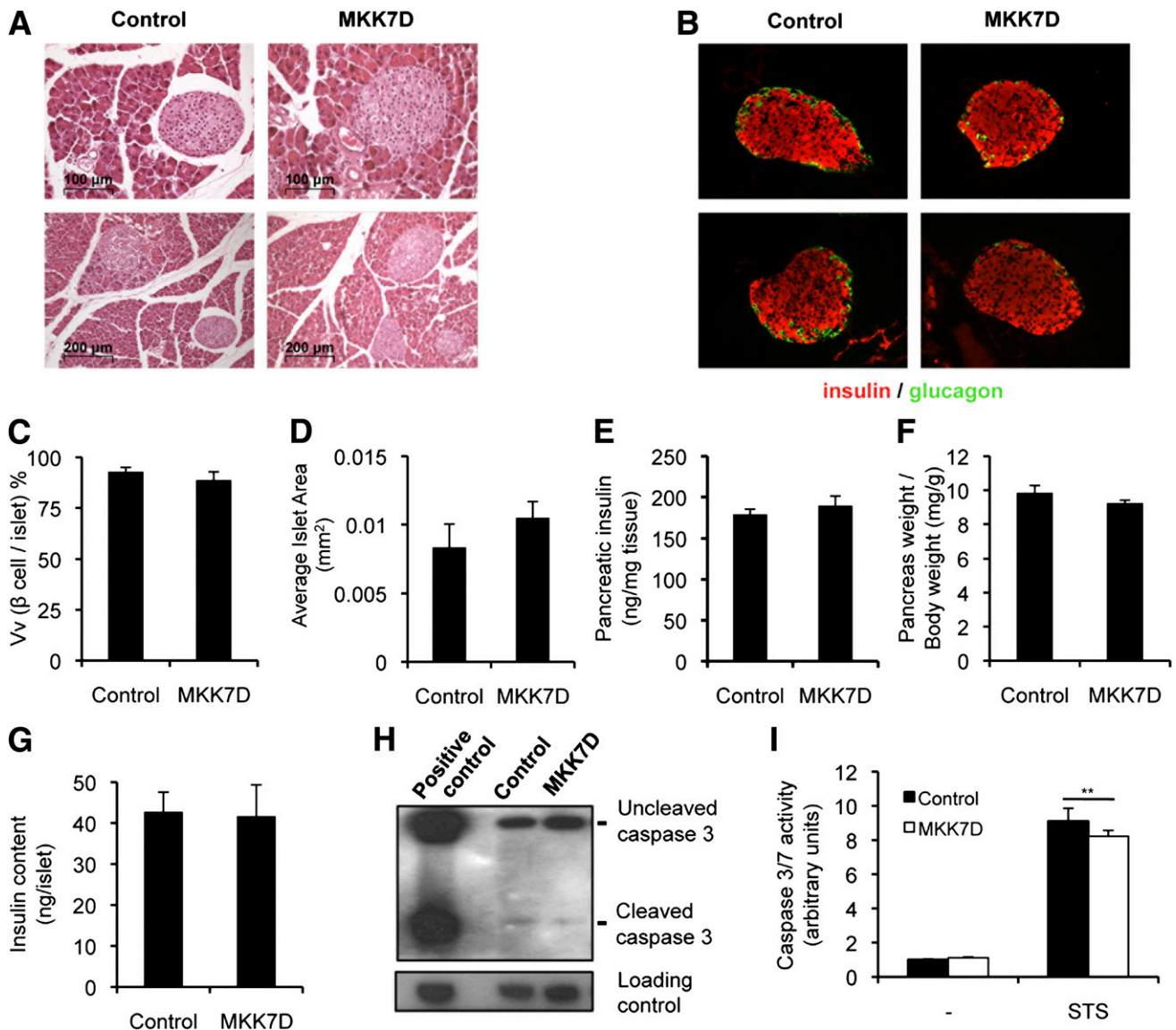
FIG. 2. JNK activation in pancreatic β-cells affects neither the morphology nor the insulin content of the islets. Pancreatic histological sections of control and MKK7D animals stained with hematoxylin-eosin (*A*) or analyzed by immunohistochemistry with antibodies against insulin and glucagon (*B*). Average β-cell volume to islet volume (Vv) (*C*) and islet area (*D*), insulin content of pancreas (*E*), pancreas weight relative to body weight (*F*), insulin content in isolated islets (*G*) from control and MKK7D mice. *H*: Immunoblot analysis of caspase 3 in extracts from isolated islets of control and MKK7D mice. Positive control was an extract from neurons treated overnight with staurosporine. *I*: Caspase 3 activity in extracts from isolated islets untreated or incubated for 20 h with 10 µmol/L staurosporine (STS) of control and MKK7D mice. Assays were performed in 3-month-old mice with at least 10 animals or 200 islets per group. **$P < 0.01$ with respect to the basal condition in islets from control animals.

defect was not caused by a reduction in overall amount or a subcellular mislocalization of GLUT2 (Fig. 3*C* and *D*) or defects in the glucose-signaling pathway, since insulin secretion of islets from MKK7D and control mice was equally increased by a range of compounds, such as IBMX, KCl, succinate, KIC, and tolbutamide, which activate this pathway at different steps (Fig. 3*E*).

As insulin induces its own release, next we studied insulin-induced insulin secretion in isolated islets. We found that this autocrine action of insulin was impeded in islets derived from MKK7D mice and, again, this defect was overcome when these islets were incubated with JNKi-1 (Fig. 4*A*). These results strongly pointed to a JNK activation–dependent interference with insulin signaling. To test this hypothesis, we analyzed insulin-induced phosphorylation of Akt, a key protein in the InsR pathway (23). We

found that this process was impaired in islets from MKK7D mice (Fig. 4*B*). Accordingly, insulin-induced expression of insulin target genes was also inhibited in these islets (Fig. 4*C*), regardless of their increased content in the transcriptional activator Pdx1 (Fig. 4*D*). Comparable results were observed in the induction of these genes by glucose (Supplementary Fig. 2). Immunohistochemistry analysis of pancreatic sections of control and MKK7D mice showed that Pdx1 is equally located in the nucleus in basal conditions, but in contrast to control animals, MKK7D mice failed to induce Pdx1 nucleocytoplasmic shuttling in response to glucose or insulin (Fig. 4*D*)

Transient JNK activation in adulthood recapitulates the glucose-intolerant phenotype shown by MKK7D mice. The experiments described above using the JNK inhibitory peptide showed that the JNK interference with
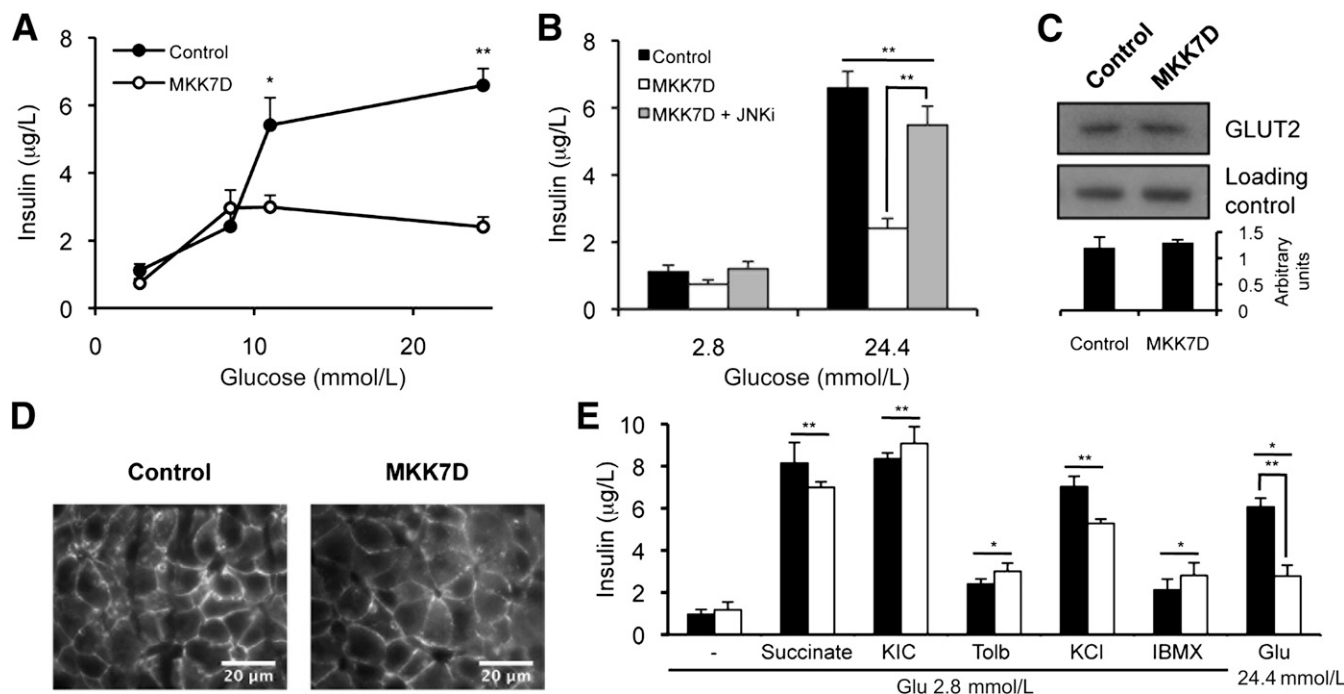
FIG. 3. JNK activation in pancreatic β-cells impairs glucose-stimulated insulin secretion. Insulin secretion of pancreatic islets isolated from control and MKK7D mice stimulated with the indicated concentrations of glucose (*A*) and in the presence or absence of JNKi-1 (JNKi) as indicated (*B*). Analysis of GLUT2 expression by immunoblot in extracts from pancreatic islets (*C*) and subcellular localization by immunohistochemistry in pancreatic sections (*D*) of control and MKK7D mice. *E*: Insulin secretion of islets isolated from control and MKK7D mice in response to 2.8 mmol/L (−) and 24 mmol/L glucose (Glu), succinate, KIC, tolbutamide (Tolb), KCl, and IBMX, as indicated. Assays were performed in 3-month-old mice and repeated at least 3 times. *$P < 0.05$, **$P < 0.01$ with respect to control group (*A*) or to the 2.8 mmol/L glucose condition in islets from control animals unless otherwise indicated (*B* and *E*).

the insulin-induced insulin secretion was reversible. Next, we addressed whether JNK activation is required early during development in order to achieve the phenotype or whether it occurs immediately after JNK activation, independently of the developmental stage. Thus, transgenic mice were crossed with mice expressing a tamoxifen-inducible Cre recombinase/ER in pancreatic β-cells to obtain control and MKK7DTam mice. Despite the overexpression of Cre-recombinase/ER in the β-cells, none of these mice were intolerant to glucose (Fig. 5A). The Cre-dependent recombination of the transgene was induced in adulthood by the administration of tamoxifen, and efficiency and concomitant JNK activation were confirmed (Fig. 5B). Tamoxifen-treated MKK7DTam mice (and control animals) were subjected to a GTT and ITT. These tests revealed that these animals were glucose intolerant (Fig. 5C), preserved peripheral insulin sensitivity (Fig. 5D), and were unable to increase insulinemia in response to hyperglycemia (Fig. 5E). Moreover, islets isolated from these mice did not secrete insulin in response to glucose or insulin (Fig. 5F). Therefore, upon tamoxifen treatment, MKK7DTam mice mimicked the phenotype previously observed in MKK7D mice.

Rosiglitazone reestablishes the glucose homeostasis and insulin signaling disrupted by JNK activation in pancreatic β-cells. We have previously reported that TZDs, in a PPARγ-dependent manner, are able to inhibit obesity- and proinflammatory cytokine–induced activation of JNK and, in doing so, alleviate insulin resistance (9). In fact, JNK inhibition by TZDs has been documented in a variety of cell types and circumstances (9,24–26). Particularly relevant to this study is the reported inhibition of IL-1β–induced activation of JNK by rosiglitazone in

insulin-secreting cell lines (9). Therefore, we tested the effect of rosiglitazone treatment on the phenotype exhibited by MKK7D mice. For this purpose, MKK7D and control mice were treated with rosiglitazone for 10 consecutive days, and afterward GTTs were performed. Rosiglitazone treatment significantly improved glucose tolerance in the MKK7D mice (Fig. 6A and B) and also restored normal insulin secretion in response to hyperglycemia in these animals (Fig. 6C and D), while no changes were observed in peripheral sensitivity to insulin in any of the animals tested, as assessed by ITT (Fig. 6E). Moreover, the direct action of rosiglitazone on β-cells was further assessed by confirming the ability of this drug to restore insulin secretion in response to glucose and insulin in islets from rosiglitazone-treated MKK7D mice (Fig. 6F). As a result of JNK mediating the feedback inhibition of the InsR cascade (22), rosiglitazone treatment of islets from control mice further augmented insulin-induced insulin secretion (Fig. 6F). This effect was not observed in response to glucose because experiments were performed at a saturating dose (Fig. 3A). At the molecular level, rosiglitazone inhibited MKK7D-induced activation of JNK and, accordingly, restored insulin-induced Akt phosphorylation (Fig. 6G).

DISCUSSION

Insulin resistance is an early trait in the development of type 2 diabetes; afterward, pancreatic β-cell failure caused by an increased insulin demand is the major determinant of progression to hyperglycemia, the hallmark of diabetes. Compelling evidence has demonstrated that exacerbated JNK activity participates in promoting insulin resistance in
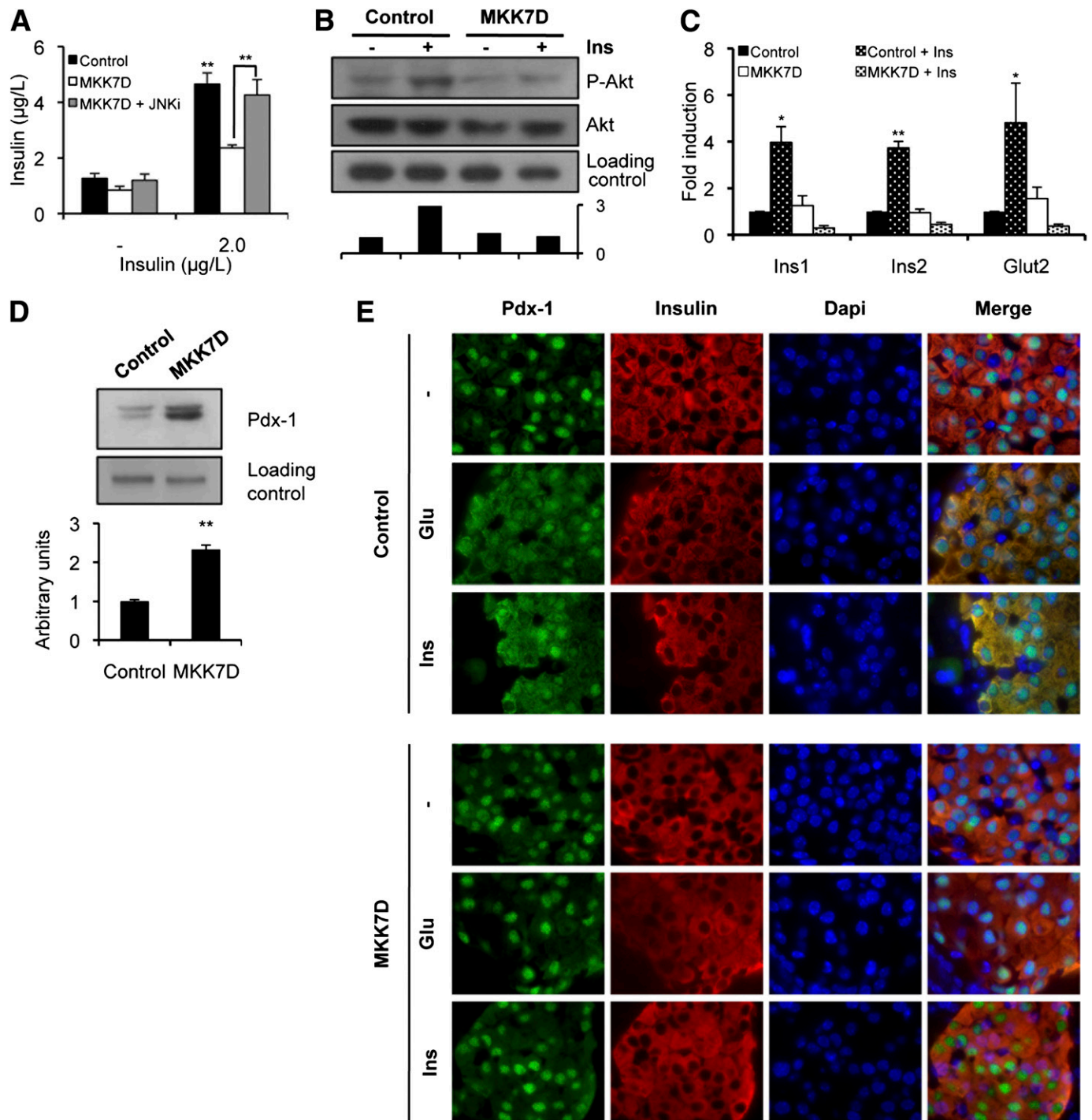
FIG. 4. JNK activation in pancreatic β-cells impairs insulin signaling. *A*: Insulin-induced insulin secretion of pancreatic islets isolated from control and MKK7D mice in the presence or absence of JNKi-1 (JNKi) as indicated. Secretions were performed in basal glucose concentration (2.8 mmol/L). *B*: Immunoblot analysis of phospho-Akt (P-Akt) and Akt in extracts of islets from control and MKK7D mice incubated with or without insulin (Ins), as indicated, for 20 min. The ratio of phospho-Akt to total Akt is represented in the *bottom panel*. *C*: Quantitative RT-PCR analysis of the indicated insulin target genes in islets from control and MKK7 mice incubated with or without insulin, as indicated, for 3 h. *D*: Immunoblot analysis of Pdx1 in extracts from pancreatic islets of control and MKK7D mice. The ratio of Pdx1 to loading control is represented in the *bottom panel*. *E*: Immunohistochemistry analysis of Pdx1 and insulin in pancreatic sections from control and MKK7D mice untreated (−) or treated with glucose (Glu) or insulin (Ins) for 30 min. Assays were performed in 3-month-old mice and repeated at least 3 times. *P < 0.05, **P < 0.01 with respect to the basal condition in islets from control animals unless otherwise indicated.

peripheral insulin-target tissues (7,27–32). In addition, a few studies indicate that the JNK pathway is also involved in the negative regulation of insulin sensitivity in pancreatic β-cells. In this regard, *jnk1*-deficient pancreatic islets show increased GIIS (11) and protection against

FFA-induced inhibition of glucose-triggered insulin gene transcription (3). Nonetheless, in vivo data supporting a regulatory role of JNK in insulin sensitivity in pancreatic β-cells have been obtained using genetic or chemical inhibition of JNK (3,8,10,11,33)—approaches that allow the
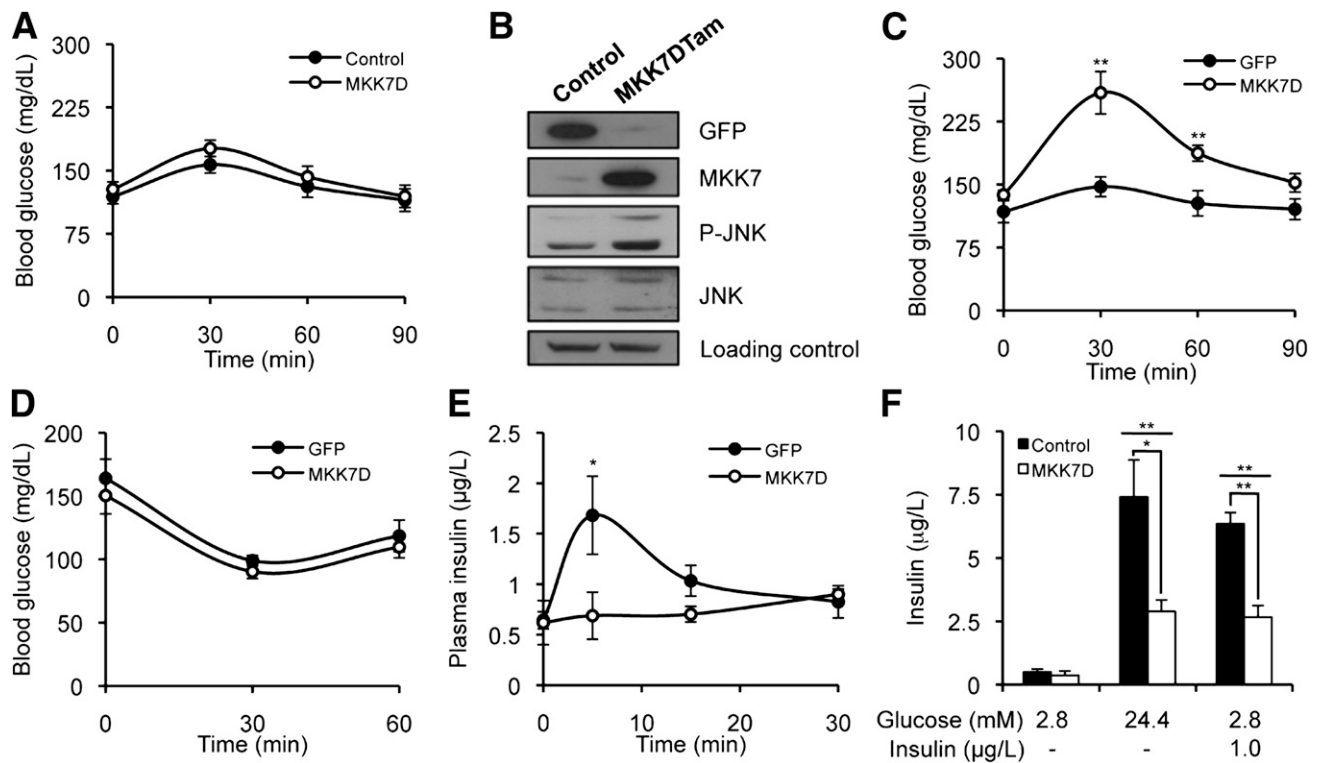
FIG. 5. Tamoxifen-inducible JNK activation in adult mice causes glucose intolerance. *A*: GTT of control and MKK7DTam 8-week-old mice before treatment with tamoxifen. *B*: Immunoblot analysis of GFP, MKK7, phosphorylated JNK (P-JNK), and JNK in extracts from isolated pancreatic islets of tamoxifen-treated control and MKK7DTam mice. *C*, *D*, and *E*: GTT, ITT, and glucose-stimulated insulin secretion, respectively, in tamoxifen-treated control and MKK7DTam mice. *F*: Insulin secretion of pancreatic islets isolated from tamoxifen-treated control and MKK7DTam mice stimulated with glucose and insulin as indicated. Unless indicated, assays were performed 2 weeks after tamoxifen treatment with 10 animals per group. *$P < 0.05$, **$P < 0.01$ with respect to control group (*A* and *C–E*) or the basal condition in islets from control animals (*F*) unless otherwise indicated.

assessment of JNK requirement but not competence of this kinase to perform a particular role.

With this aim, we generated a transgenic mouse model that allows JNK activation in a Cre recombinase expression–dependent manner, and using appropriate strains of mice we achieved β-cell–specific activation of JNK. Our results show that JNK activation in pancreatic β-cells produces a glucose-intolerant phenotype caused by impaired capacity to increase insulinemia in response to hyperglycemia. Although in vivo JNK activation by expression of a constitutively activated version of its immediately upstream activator MKK7D is a strategy previously shown to specifically induce JNK (21), we confirmed that the observed defects were dependent on JNK activity, as they were reverted by JNKi-1 and by rosiglitazone, a drug known to inhibit JNK activation in vivo and in several cell types, including insulin-secreting cell lines (9). Moreover, the glucose intolerance phenotype shown by MKK7D mice was exhibited independently of sex and the postnatal age of the animals and by all the animals tested so far (~200 mice). These findings suggest that the penetrance of this phenotype is close to 100%. In addition, it is likely to be achieved immediately after JNK activation and independently of the developmental stage, as comparable results were obtained in MKK7D and tamoxifen-treated MKK7DTam mice, and reversible, as it was reverted in vivo by treatment with rosiglitazone and in isolated islets by incubation with JNKi-1. Finally, although in both mice strains used the Cre recombinase expression is under the Ins2 promoter sequences, known to also drive expression to certain

regions of the brain (34,35), the finding that isolated pancreatic islets show defects in GIIS strongly suggests that the described phenotype is a β-cell–autonomous effect caused by JNK activation specifically in this cell type.

According to our results, JNK activation in pancreatic β-cells results in glucose intolerance as a result of impaired capacity to increase blood insulin level in response to hyperglycemia. However, this phenotype does not correlate with any obvious morphological or structural abnormality in the pancreas or the islets or with lower insulin content. Moreover, basal glycemia and insulinemia were normal in MKK7D and tamoxifen-treated MKK7DTam mice. In contrast, static insulin secretions of pancreatic islets isolated from these animals were markedly impaired in response to glucose and insulin, a defect that was reversed by incubation of the islets with the JNKi-1 or with rosiglitazone. In contrast, these same islets were not defective for IBMX-, KCl-, succinate-, KIC-, or tolbutamide-induced insulin release. Overall, these results indicate that insulin secretion in response to insulin, but not to glucose metabolism (36), was affected by JNK activation. Accordingly, insulin-induced Akt phosphorylation and the induction of expression of downstream insulin-target genes were impaired in islets from MKK7 mice. Defective insulin signaling in MKK7D mice was also supported by their inability to induce Pdx1 nucleocytoplasmic shuttling in response to glucose or insulin. In this regard, Pdx1 translocation from nucleus to cytoplasm has been reported to occur upon inhibition of InsR signaling by oxidative stress (37). Despite no differences being observed between
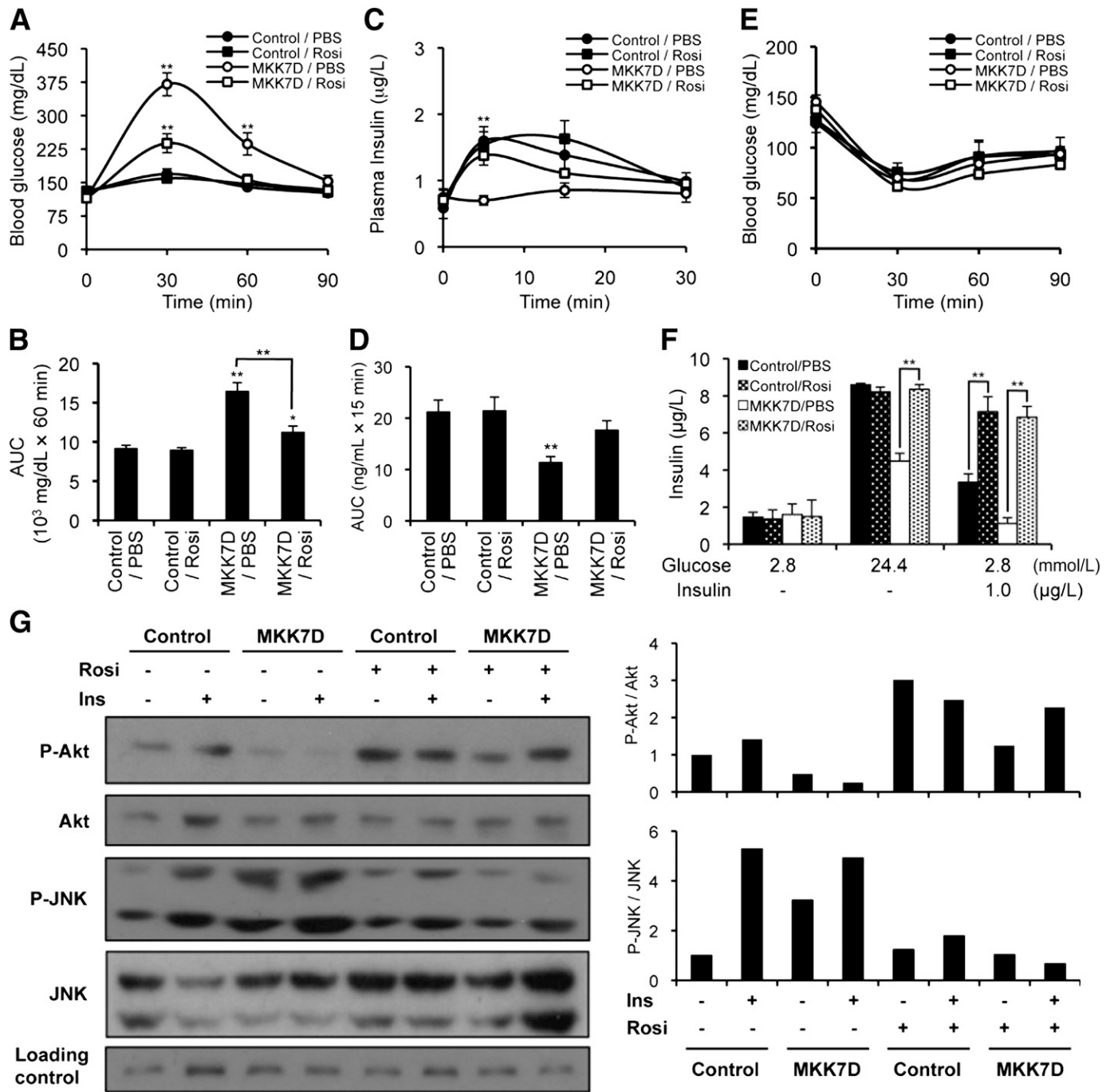
FIG. 6. Rosiglitazone counteracts JNK-induced β-cell dysfunction. GTT (*A*) and the corresponding area under the curve (AUC) for the first 60 min (*B*), glucose-stimulated insulin secretion (*C*), the corresponding AUC for the first 15 min (*D*), and ITT (*E*) performed in control and MKK7D 2-month-old mice treated for 10 days with rosiglitazone (Rosi) or PBS as indicated. *F*: Insulin secretion of pancreatic islets isolated from control and MKK7D mice treated with PBS or rosiglitazone. Islets were stimulated with glucose and insulin as indicated. *G*: Immunoblot analysis performed in extracts of pancreatic islets from control and MKK7D mice treated with rosiglitazone and insulin (Ins) (20 min) as indicated. The ratios of phospho-Akt (P-Akt) and phospho-JNK (P-JNK) to AKT and JNK, respectively, are represented in the *right panel*. Assays were performed with at least 10 animals per group and repeated at least 3 times. *P < 0.05, **P < 0.01 with respect to control group (*A–E*) or the basal condition in islets from control animals (*F* and *G*) unless otherwise indicated.

control and MKK7D mice in Pdx1 subcellular location in basal conditions, the latter maintained Pdx1 nuclear localization even 30 min after stimulation with glucose or insulin: a time point by which InsR signaling might be in the process of being turned off, as insulinemia almost returned to basal level, whereas in control mice Pdx1 shuttled from nucleus to cytoplasm suggesting that deactivation of the InsR signaling pathway was taking place.

Moreover, the increased Pdx1 content in MKK7D islets might be also indicative of defective InsR signaling, as this pathway regulates the steady-state level of Pdx1 (38). Overall, these results are consistent with the role of JNK in blocking InsR signaling through IRS-1/2 phosphorylation (3,39).

TZD treatment improves the pancreatic condition in type 2 diabetes by alleviating insulin resistance in

peripheral tissues in a PPARγ-dependent manner (40,41). In this regard, we had shown that the TZD antidiabetes action relies on the inhibition of the obesity-induced activation of the JNK pathway (9). Nonetheless, an insulin-sensitizing action of these drugs directly on the pancreas has not previously been reported. Supporting this notion is the documented expression of PPARγ in β-cells, where it regulates proliferation (42). Additionally, we had shown that rosiglitazone treatment improves the survival of insulin-secreting cells lines exposed to the proinflammatory cytokine IL-1β concomitantly with the inhibition of the cytokine-induced activation of JNK (9). Therefore, we considered that the MKK7D mouse was a suitable animal model in which to test whether rosiglitazone ameliorates pancreatic insulin resistance independently of its action on peripheral tissues. Remarkably, rosiglitazone almost completely reverted the in vivo (glucose intolerance) and in vitro (GIIS and insulin-induced insulin secretion) phenotype induced by JNK activation in pancreatic β-cells. These actions correlated with the ability of rosiglitazone to inhibit MKK7D-induced JNK activation and, concomitantly, the recovery of insulin-induced Akt phosphorylation. In addition, since none of the animals exhibited peripheral insulin resistance, the ITTs were not affected by rosiglitazone treatment in any group of mice. These results indicate that in addition to their insulin-sensitizing action in peripheral tissues, TZDs also ameliorate pancreatic insulin resistance and that in both actions the inhibition of exacerbated JNK activity plays a fundamental role.

JNK is involved in the loss of pancreatic β-cells induced by proinflammatory cytokines (12,13), and *jnk1* deficiency or treatment with JNK inhibitors prevents IL-1β–induced apoptosis of islets and insulin-secreting cell lines (9–11,14). Moreover, JNK inhibition enhances the survival of islets subjected to transplantation protocols (16,18). While all of these data support a role of JNK in promoting β-cell death, they do not distinguish whether JNK is sufficient or merely required to attain this process. In this regard, our results support the notion that JNK activation is not sufficient to promote β-cell death, as, contrary to what would be expected if JNK activation were sufficient, no morphostructural abnormalities or caspase 3 activation was observed in the pancreas or islets of MKK7D mice. Therefore, involvement of additional pathways deregulated in obesity and type 2 diabetes due to the chronic inflammatory state, increased FFAs, or induction of endoplasmic reticulum stress (1,2), such as the nuclear factor-κB cascade (43), might be required to effectively induce β-cell death.

In conclusion, our data support the relevance of JNK in the regulation of insulin sensitivity in pancreatic β-cells by showing that mere activation of this kinase is sufficient to inhibit InsR signaling and, hence, promote insulin resistance in pancreatic β-cells in vivo. However, JNK activation does not elicit β-cell death, thereby suggesting that JNK activity may be required but is not sufficient for this process to occur. Finally, we also provide the first evidence of insulin-sensitizing action of TZDs directly on pancreatic β-cells. Our observations therefore contribute to a better understanding of the mechanisms that mediate the antidiabetes action of these drugs.

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