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Propofol mediates signal transducer and activator of transcription 3 activation and crosstalk with phosphoinositide 3-kinase/AKT

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We previously demonstrated that propofol, an intravenous anesthetic with anti-oxidative properties, activated the phosphoinositide 3-kinase (PI3K)/AKT pathway to increase the expression of B cell lymphoma (Bcl)-2 and, therefore the anti-apoptotic potential on cardiomyocytes. Here, we wanted to determine if propofol can also activate the Janus kinase (JAK) 2/signal transducer and activator of transcription (STAT) 3 pathway, another branch of cardioprotective signaling. The cellular response of nuclear factor kappa B (NFκB) and STAT3 was also evaluated. Cardiac H9c2 cells were treated by propofol alone or in combination with pretreatment by inhibitors for JAK2/STAT3 or PI3K/AKT pathway. STAT3 and AKT phosphorylation, and STAT3 translocation were measured by western blotting and immunofluorescence staining, respectively. Propofol treatment significantly increased STAT3 phosphorylation at both tyrosine 705 and serine 727 residues. Sustained early phosphorylation of STAT3 was observed with 25~75 μM propofol at 10 and 30 min. Nuclear translocation of STAT3 was seen at 4 h after treatment with 50 μM propofol. In cultured H9c2 cells, we further demonstrated that propofol-induced STAT3 phosphorylation was reduced by pretreatment with PI3K/AKT pathway inhibitors wortmannin or API-2. Conversely, pretreatment with JAK2/STAT3 pathway inhibitor AG490 or stattic inhibited propofol-induced AKT phosphorylation. In addition, propofol induced NFκB p65 subunit perinuclear translocation. Inhibition or knockdown of STAT3 was associated with increased levels of the NFκB p65 subunit. Our results suggest that propofol induces an adaptive response by dual activation and crosstalk of cytoprotective PI3K/AKT and JAK2/STAT3 pathways. Rationale to apply propofol clinically as a preemptive cardioprotectant during cardiac surgery is supported by our findings.

Introduction

Propofol (2,6-diisopropylphenol) is a widely used intravenous anesthetic agent that has been found to prevent myocardial ischemia–reperfusion injury in isolated heart models.[1-3] Propofol was used to attenuate cardiac injury in a porcine model of cardiopulmonary bypass and in low risk patients undergoing cardiac surgery.[4,5] The mechanism of cardioprotection is unclear. Initially this cardioprotection was attributed to antioxidant scavenging.[6] However, propofol has also been shown to inhibit mitochondrial permeability transition pore opening to confer cytoprotection.[7] Studies in models of simulated ischemia–reperfusion suggest drug-induced prosurvival signal transduction may play a role.[8,9]

Phosphoinositide 3-kinase (PI3K)/AKT signaling pathway is one of the key cellular prosurvival pathways central to conditioning and prevention of ischemia–reperfusion injury.[10] Phosphorylation and activation of AKT at both serine 473 (ser473) and threonine 308 (thr308) residues induces the activation of AKT's putative downstream effectors, such as endothelial nitric oxide synthase, glycogen synthase kinase 3 β, and anti-apoptotic B cell lymphoma (Bcl)-2, to prevent mitochondrial-directed cell death. We previously reported that propofol induced AKT phosphorylation and Bcl-2 gene expression, and protected cardiac H9c2 cells from apoptotic injury in response to oxidative stress.[11] Although AKT inhibition by wortmannin in propofol-treated cells decreased Bcl-2 protein expression and increased cell death, wortmannin treatment had no effects on propofol-induced Bcl-2 gene expression. This suggests that propofol may modulate Bcl-2 gene expression through an AKT-independent pathway.

The survivor activating factor enhancement (SAFE) signaling pathway acts independently of the activation of AKT to prevent cardiomyocyte injury and death.[12] Pathway activation involves

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phosphorylation and activation of the signal transducer and activator of transcription (STAT) 3 transcription factor at tyrosine 705 (tyr705) or serine 727 (ser727) residue by activated Janus kinase (JAK) 2. Phosphorylated STAT3 undergoes dimerization and subsequent translocation into the nucleus, where it stimulates the transcription of target genes, including anti-apoptotic B-cell lymphoma-extra large and Bcl-2.[13,14]

Nuclear factor k-light-chain-enhancer of activated B cells (NFκB) is another transcription factor involved in cardioprotection. Although it is activated in the presence of inflammatory stimuli such as tumor necrosis factor α (TNFα) to transcribe pro-inflammatory genes, it has also been shown to increase transcription of cell survival genes, including Bcl-2.[15] Inhibition of STAT3 has been shown to reverse the inactivation of the p65 subunit of NFkB.[16] NFκB functionally could ensure STAT3 nuclear retention or prevent sustained STAT3 activation to enhance cell survival. The PI3K/AKT and JAK2/STAT3 pathways may act alone or in concert to protect ischemic and reperfused myocardium.[12] Experimentally, crosstalk between these two pathways has been demonstrated in models of pharmacologic or ischemic preconditioning.[17,18]

It has yet to be determined if propofol-mediated signal transduction is drug induced or reflects an epiphenomenon associated with a generalized cellular defense against stress and injury. Of interest, Wickley et al.[19] found that propofol activated protein kinase C (PKC) ζ and increased its nuclear localization in rat ventricular cardiomyocytes. PKCζ is known to increase NFκB activity and may also do so under propofol stimulation. Whether propofol activates the JAK2/STAT3 pathway is unknown. Given our previous findings, the purpose of this study was to determine whether propofol can stimulate JAK2/STAT3 signaling in unstressed cardiac H9c2 cells and if crosstalk with the PI3K/AKT pathway can be identified. Based on their functional significance, the cellular response of NFκB and STAT3 was also evaluated in our model.

Results

Propofol induced STAT3 phosphorylation in H9c2 cells

We previously reported that propofol reduced TNFα-induced apoptosis in a dose-dependent manner in cultured human umbilical vein endothelial cells.[20] To determine if the effect of propofol on STAT3 phosphorylation is also dose-dependent, we initially treated H9c2 cells over a concentration range of propofol (10 to 100 μM) for 10 or 30 min.

As shown in **Figure 1A**, propofol treatment for 10 min or 30 min resulted in an increase in phospho-STAT3 expression. Sustained phosphorylation of STAT3 was seen with propofol treatment at doses from 25 to 75 μM. Based on these results and similar procedures described in scientific literature,[11,20] we chose 50 μM of propofol for use in our subsequent experiments. This concentration also represents the average blood propofol concentration during human cardiac surgery in our clinical project.[21]

Propofol-mediated STAT3 phosphorylation was time-dependent in H9c2 cells

To determine whether the effect of propofol on STAT3 phosphorylation is time-dependent, serum-starved H9c2 cells were stimulated with 50 μM of propofol for various times starting from 10 min up to 6 h. STAT3 phosphorylation and nuclear translocation were detected through western blotting and immunofluorochemistry, respectively.

As shown in **Figure 1B**, propofol significantly increased STAT3 phosphorylation at tyr705 at 10 min, 30 min, and 6 h relative to control. Propofol also significantly increased STAT3 phosphorylation at ser727 at 10 min and 30 min but not 6 h. Nuclear translocation of STAT3 (green) was observed by immunofluorescence staining at 4 h after propofol stimulation (**Fig. 1C**). STAT3 localization in the nucleus was highly specific as seen by the formation of punctate dots (yellow arrows).

Based on these observations, as well as our previous findings, where propofol significantly increased phosphorylation of AKT at 30 min,[11] we standardized a propofol stimulation time period of 30 min for further pathway inhibition experiments.

Inhibition of PI3K/AKT signaling decreased propofol-induced STAT3 phosphorylation

To distinguish crosstalk between JAK2/STAT3 and PI3K/AKT pathways under propofol stimulation, we first determined STAT3 phosphorylation in the presence of PI3K/AKT inhibitors (wortmannin and API-2). As shown in **Figure 2A and B**, STAT3 was not phosphorylated at neither ser727 nor tyr705 residues in propofol-stimulated cells in the presence of wortmannin or API-2. No significant changes were observed in protein expression of total STAT3 in all groups.

Inhibition of JAK2/STAT3 signaling decreased propofol-induced AKT phosphorylation

We then determined AKT phosphorylation in the presence of JAK2/STAT3 signaling inhibitors. AG490 (selective JAK2 inhibitor) and stattic (selective STAT3 inhibitor) were used in this study. As shown in **Figure 2C and D**, inhibition of JAK2/STAT3 signaling by either AG490 or stattic abolished propofol-induced AKT phosphorylation at both ser473 and thr308. No significant changes were observed in protein levels of total AKT in all groups.

Propofol increases IκBα degradation

Time-course treatment of serum-starved cells with propofol showed significant increase in IκBα degradation at 2 h (**Fig. 3**). Propofol-mediated IκBα degradation at 2 h was comparable to the positive control for IκBα degradation, TNFα.

STAT3 knockdown increases NFκB (p65)

To further investigate the inhibitory effect of STAT3 inhibition we used siRNA to knockdown STAT3 in H9c2 cells. No statistical analysis was done on the degree of siRNA mediated knockdown. Experiments with t-STAT3 knockdown of greater than 70% were considered for further analysis.

As seen in **Figure 4A**, STAT3 knockdown increased p65 levels. Although, these levels were significantly higher in H9c2 cells treated with EGF, there is a general trend toward higher p65 levels in cells with STAT3 knockdown.

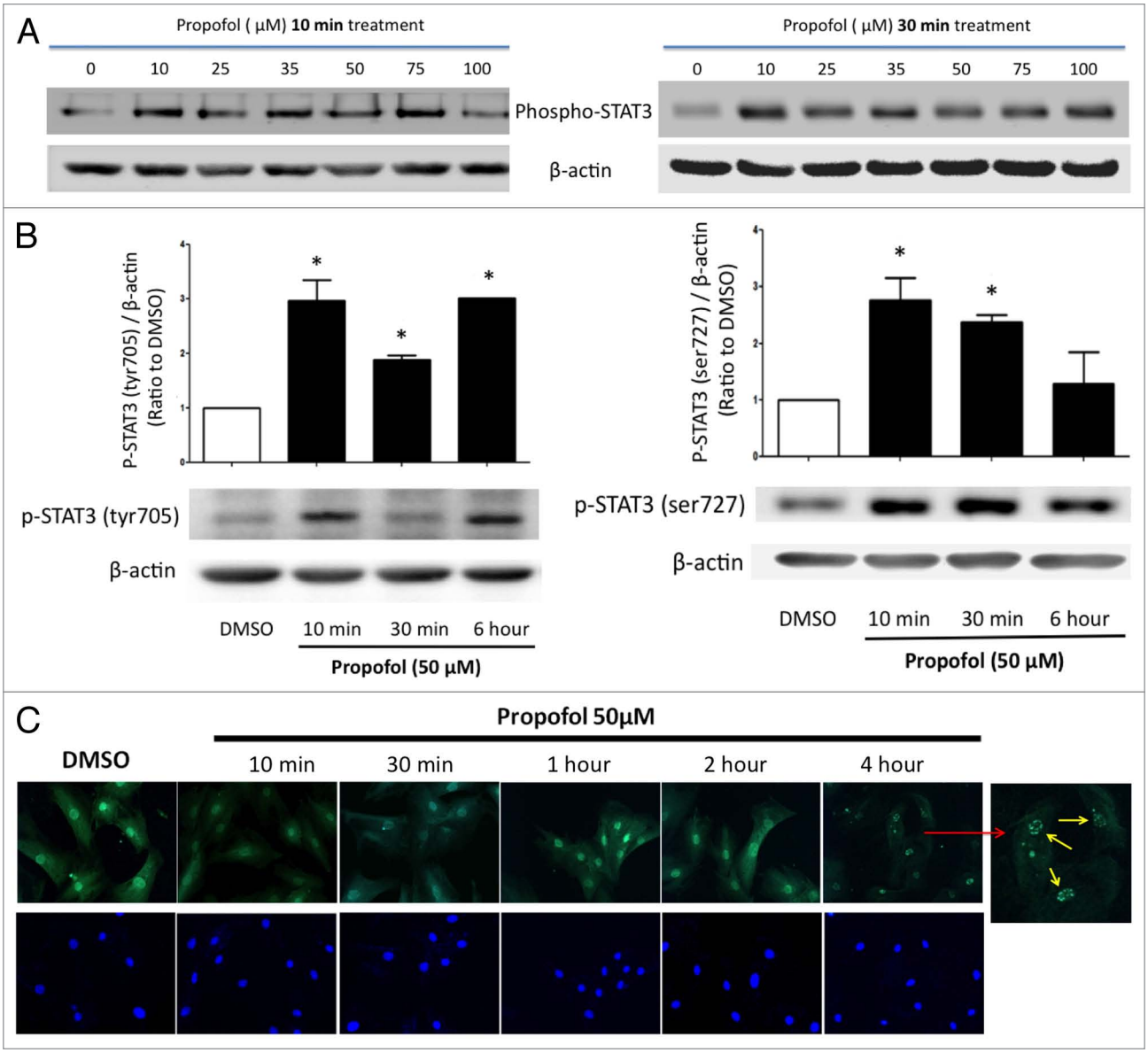

Figure 1. Propofol induced STAT3 phosphorylation in H9c2 cells. (**A**) Cells were treated with different concentrations of propofol for 10 min and 30 min. Cell lysates were harvested and western blots were performed to examine STAT3 phosphorylation. Propofol treatment at 10 μM and 100 μM resulted in an increase in phospho-STAT3 expression. Sustained phosphorylation of STAT3 was seen with propofol treatment at doses from 25 to 75 μM. (**B**) Cells were treated with propofol (50 μM) for various times (from 10 min up to 6 h). STAT3 phosphorylation at tyr705 and ser727 residues was detected by western blot analysis; Propofol significantly increased phosphorylation at tyr705 at 10 min, 30 min, and 6 h. Propofol also significantly increased phosphorylation at ser727 at 10 min and 30 min but not 6 h. (**C**) STAT3 nuclear translocation was detected by immunofluorescence staining. Nuclear translocation of STAT3 (green) was observed at 4 h after propofol stimulation. STAT3 localization in the nucleus was highly specific as seen by the formation of punctate dots (yellow arrows). *$P < 0.05$ vs. DMSO control.

STAT3 activity inhibition increases NFκB (p65)

Knockdown of STAT3 should reduce overall STAT3 activity in the cell similar to STAT3 inhibition by stattic. To check if stattic inhibition of STAT3 activity also resulted in increased p65 protein levels, cells were inhibited with stattic or DMSO control and then stimulated with vehicle control DMSO or propofol or EGF. This experimental set-up was similar as that in **Figure 4A**.

STAT3 activity inhibition by stattic did not increase p65 levels within the individual groups (data not shown). However there was a significant increase when data from the three groups (DMSO, propofol, and EGF) was pooled together and DMSO was compared with stattic only (**Fig. 4B**).

Propofol does not increase NFκB (p65) nuclear translocation

To assess NFκB (p65) nuclear translocation, serum starved H9c2 cells were treated with 50 μM propofol for 10 min, 30 min, 1 h, 2 h, and 4 h and stained for p65 and DAPI, a nuclear specific dye. As seen in **Figure 4C**, relative to DMSO control propofol-mediated IκBα degradation did not increase nuclear

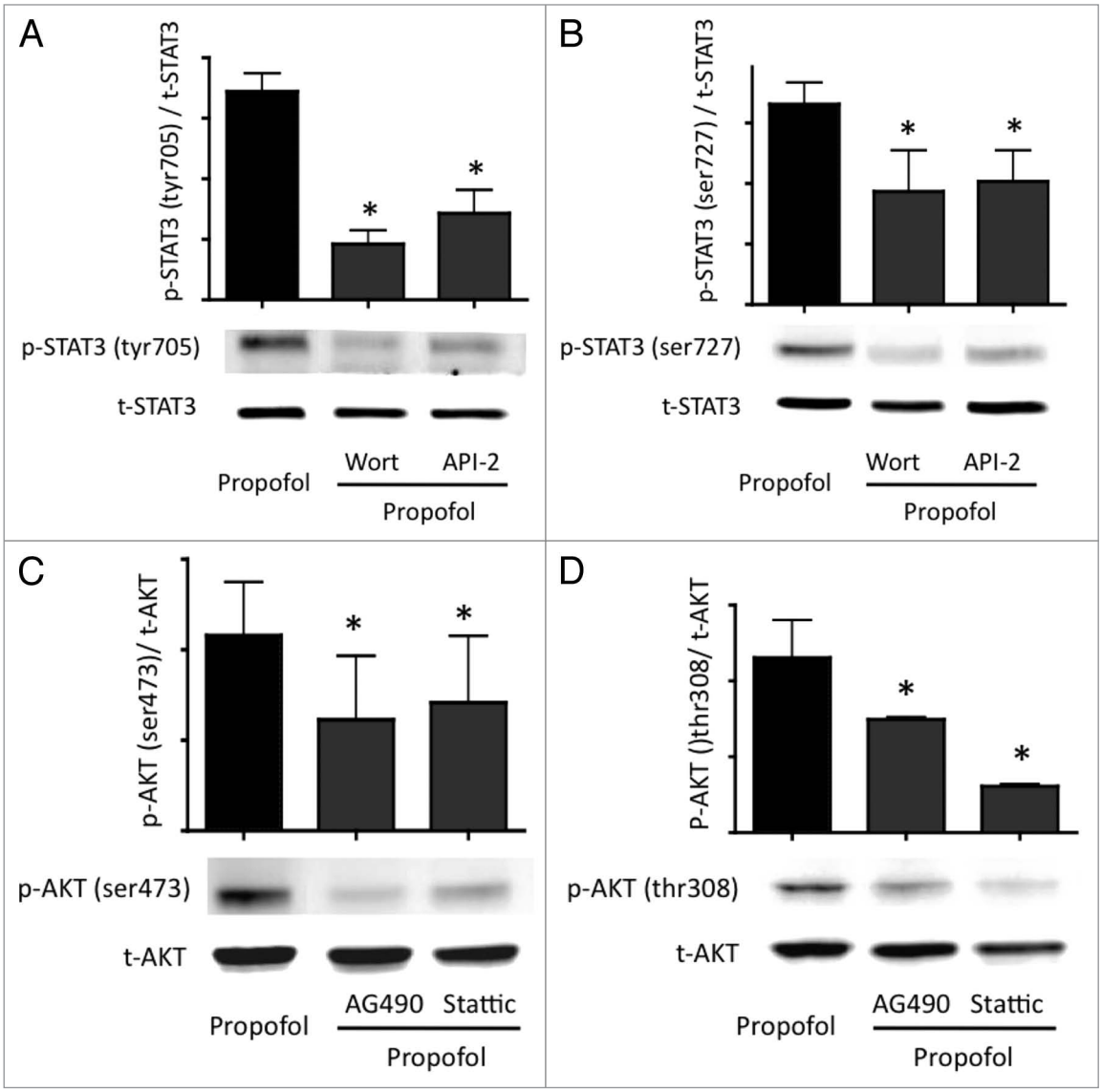

Figure 2. Effects of PI3K/AKT or JAK2/STAT3 signaling inhibition on propofol-induced STAT3 and AKT phosphorylation. Cells were pre-incubated with different inhibitors for 30 min, and then treated with propofol for another 30 min. Cell lysates were collected and AKT and STAT3 phosphorylation was detected. (**A**) Pretreatment with wortmannin (Wort) or API-2 reduced STAT3 phosphorylation at tyr705. (**B**) Wortmannin or API-2 pretreatment reduced propofol-induced STAT3 phosphorylation at ser727. (**C and D**) Pretreatment with AG490 or stattic inhibited propofol-induced AKT phosphorylation at ser473 and thr308. No changes in protein expression were observed with total STAT3 and total AKT in all groups (**A–D**).

translocation of p65 at up to 4 h of propofol exposure. However, there was increased peri-nuclear staining of p65 (yellow arrows in **Fig. 4D**) relative to DMSO control.

Discussion

In this study, we demonstrated that propofol induced IκBα degradation, NFκB p65 subunit perinuclear translocation, and STAT3 nuclear translocation. Inhibition or knockdown of STAT3 was associated with increased levels of NFκB p65 subunit. Propofol induced temporally distinct patterns of STAT3-tyr705 and -ser727 activation with persistence of STAT3-tyr705 phosphorylation. Reciprocal changes in PI3K/AKT or JAK2/STAT3 activation occurred when the opposite pathway was inhibited, indicative of propofol-mediated crosstalk.

Major efforts have focused on increasing the myocardial tolerance to ischemia by conditioning the heart, through physical or pharmacologic means. Conditioning in general involves diverse cell signaling mechanisms. It appears to be predicated on the direct and/or indirect activation of several key cellular pro-survival pathways, including the PI3K/AKT and JAK2/STAT3 pathways.

We previously demonstrated that propofol activated the PI3K/AKT signaling pathway in a time-dependent manner to avert apoptotic cell death from oxidative stress.[11] We determined that propofol upregulates Bcl-2 gene and protein expression in an AKT-dependent and -independent manner, to enhance cell

viability. Propofol has been shown to activate atypical PKCξ in cardiomyoctyes to induce NFκB activity.[19] NFκB and its p65 subunit inhibit the major negative regulator of the PI3K/AKT pathway, phosphatase, and tensin homolog on chromosome 10 (PTEN).[22] We have now determined that propofol induces degradation of IκBα and stimulates the perinuclear translocation of p65, a critical factor in NFκB-mediated gene transcription.

Both the NFκB and STAT3 pathways trigger gene expression responsible for cell proliferation and survival. Time course differences in the nuclear translocation of p65 and STAT3 in response to propofol, combined with the results of our STAT3 inhibitor or knockdown studies may be indicative of distinct roles for NFκB either in the preservation of nuclear levels of STAT3, or the prevention of sustained STAT3 activation in our model.[16,23] Whether propofol stimulates the NFκB

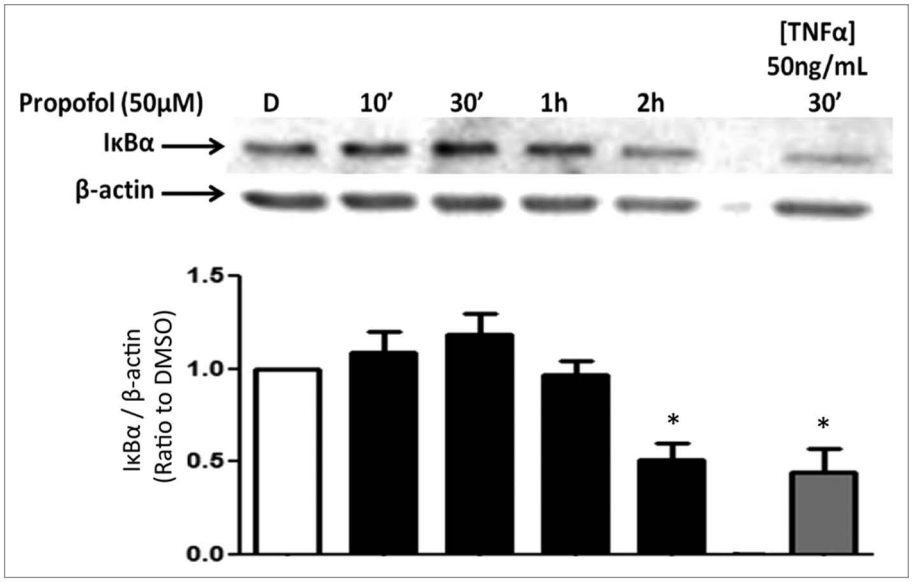

Figure 3. Propofol increases IκBα degradation. Serum-starved H9c2 cells were treated with 50 μM propofol for 10 min, 30 min, 1 h, and 2 h. DMSO was used as a vehicle control. TNFα at 50 ng/mL for 30 min was used as a positive control. Representative western blot and densitometric analysis of IκBα bands are displayed. Results are shown normalized to control (DMSO vehicle). *$P < 0.05$ vs. DMSO control.

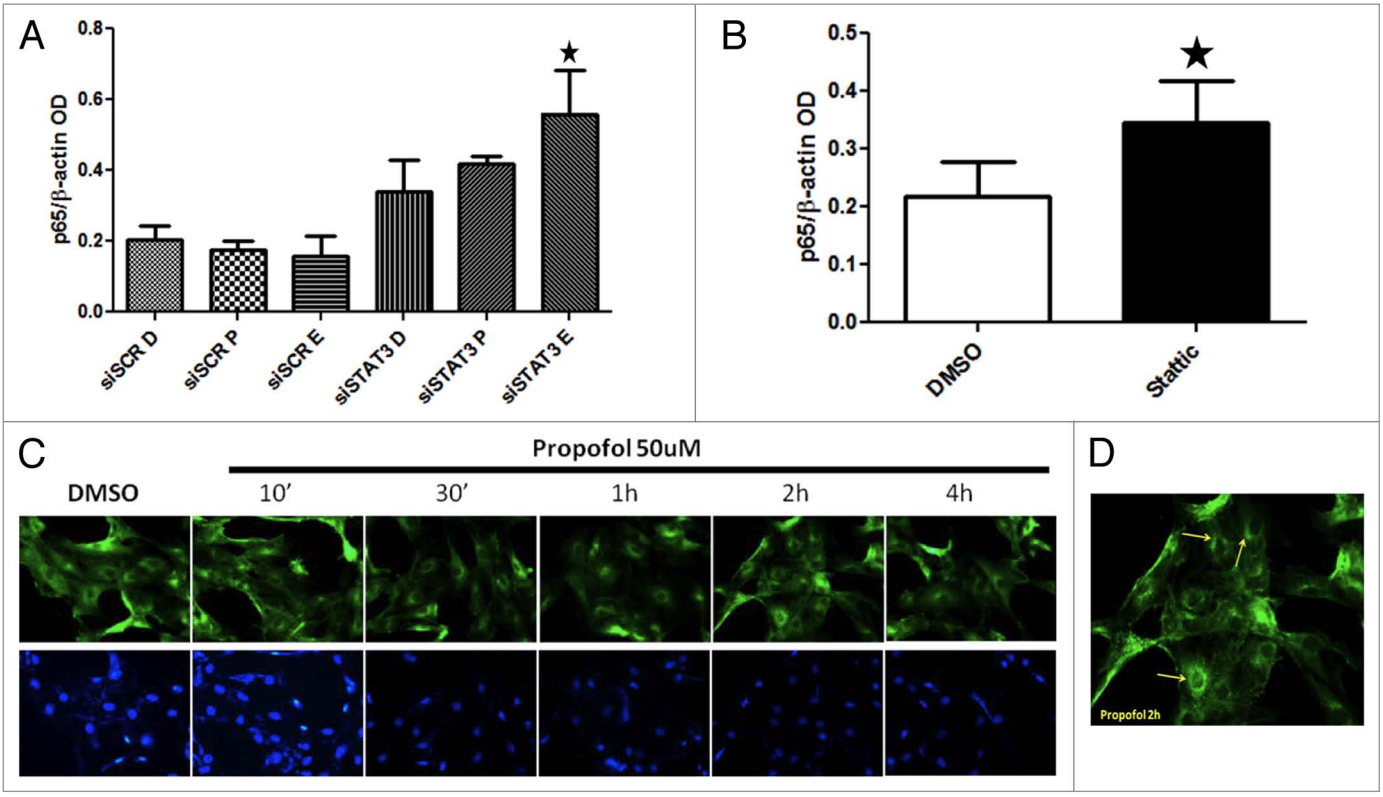

Figure 4. STAT3 knockdown or inhibition increases p65 subunit of NFκB. (**A**) After 48 h serum-starved H9c2 cells were transfected with 33 nM control Scramble siRNA (siSCR) or 33 nM of STAT3 siRNA (siSTAT3) overnight. Cells were then stimulated with DMSO (D), propofol (P), or EGF (E) for 1.5 h to induce p65; (**B**) 48 h serum-starved H9c2 cells were treated with stattic or DMSO for 30 min and then treated with DMSO or propofol or EGF for 1.5 h. Densitometric analysis for p65/β-actin; *$P < 0.05$ vs. siSCR E and DMSO, respectively. (**C**) Propofol mediated IκBα degradation did not increase nuclear translocation of p65 at up-to 4 h of propofol exposure. However, there was increased peri-nuclear staining of p65 (yellow arrows in [**D**]) relative to DMSO control.

and STAT3 pathways to act in synergism or antagonize each other's function remains unknown and requires clarification.

Propofol induces STAT3 nuclear translocation and temporal differences in the activation of STAT3-tyr705 and STAT3-ser727 residues in our model. Phosphorylation of STAT3 at tyr705 promotes STAT3 dimerization, nuclear translocation, and transcriptional activation of target genes.[12] Phosphorylation at tyr705 has been shown to reduce the rate of nuclear export and increase the nuclear content of STAT3.[24] Cell survival is enhanced by increasing transcription of anti-apoptotic proteins such as Bcl-2.[25] Therefore, propofol-mediated tyr705-STAT3 phosphorylation may increase cellular anti-apoptotic potential. Independent of tyr705 phosphorylation, phosphorylation at ser727 increases STAT3 mitochondrial localization for transcriptional activity, participation in the electron transport chain, and preservation of myocardial energetics.[23,26]

The STAT protein family is unique given their capacity to transmit their signal from the plasma membrane to the nucleus without involving a second messenger system.[27] Signal transduction is rapid, but not a simple process of diffusion. Recent research indicates that nuclear translocation and transcriptional activation of STAT3 depend on endosomes or signalosomes that traffic the prosurvival stimulus from plasma membrane to the nucleus.[28] Taken together, our results indicate that propofol-mediated STAT3 activation may not be restricted to canonical activation at the level of the cell membrane. Persistence of STAT3-tyr705 activation could reflect delayed receptor internalization to prevent further STAT3-ser727 activation. Alternatively, it may be linked to PI3K-AKT pathway signaling that may assemble due to the process known as crosstalk.

Lecour and colleagues have demonstrated the interrelationships between the PI3K/AKT and JAK/STAT3 pathways and their convergence at the level of the mitochondria to prevent ischemia–reperfusion injury in the murine heart.[29] Dual protective signaling is mediated, in part, by high density lipoprotein/sphingosine-1-phosphate. Of interest, adenosine and classical ischemic preconditioning have been shown to induce crosstalk.[30,31] Our results are in accordance with those of Tu et al. who showed that expression of a dominant-negative AKT or inhibition of PI3K with LY294002 decreased STAT3 phosphorylation at both tyr705 and ser727 residues in response to oncostatin M, a cytokine of the STAT3 activating interleukin-6 family.[32] Whether propofol activates STAT3 as a single event at level of plasma membrane or via receptor internalization that links with prosurvival PI3K-AKT signaling is the subject of ongoing investigation by our laboratory.

In conclusion, we report for the first time that propofol induces NFκB and STAT3 nuclear translocation. Propofol activates STAT3-tyr705 and STAT3-ser727 in a temporally distinct manner. Persistent STAT3-tyr705 activation suggests that drug action is not restricted to the plasma membrane but could involve receptor internalization critical in signal attenuation and/or crosstalk between the PI3K/AKT and JAK2/STAT3 pathways. Confirmatory studies in STAT3 knockout models of ischemia–reperfusion injury are required to validate these results, and to determine the functional significance of this cellular adaptation in cardioprotection.

Materials and Methods

Materials

Cell culture reagents, Alexa Fluor-568 goat anti-rabbit antibody, and 4,6-diamino-2-phenylindole (DAPI) were purchased from Life Technologies. Rabbit or mouse antibodies against STAT3, phospho-STAT3 (tyr705 and ser727), AKT, phospho-AKT (ser473 and thr308), p65 unit of NFκB, inhibitor of NFκB α (IκBα), β-actin, and horseradish peroxidase (HRP)-conjugated secondary antibodies were from Cell Signaling Technology. AG490 (selective JAK2 inhibitor) was purchased from Cayman Chemical. Stattic (selective STAT3 inhibitor) and API-2 (highly specific AKT inhibitor) were obtained from Tocris Bioscience. Wortmannin (selective PI3K inhibitor) and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich. Fluoromount-G was purchased from Southern Biotech. Endothelial growth factor (EGF) was purchased from Sigma Aldrich and was diluted to a working concentration of 200 ng/mL. TNFα was purchased from Invitrogen/Gibco and was diluted to a working concentration of 50 ng/mL. Propofol was purchased from Sigma Aldrich and dissolved in DMSO before application to cultured cells.

Cell culture

Cardiac H9c2 cells (CRL-1730) were obtained from the American Type Culture Collection. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 mg/mL streptomycin. The cells were maintained in a humidified incubator containing 95% air and 5% CO_2 at 37 °C. The medium was changed every 2 to 3 d until the cells reached confluence.

Experimental protocol

Before experimental intervention, cells were serum-starved for 48 h in DMEM supplemented with 0.5% FBS. The starved cells were then treated with propofol or with the same amount of vehicle (DMSO) for the times indicated in the figure legends. For pathway inhibition experiments, cells were pre-incubated with different inhibitors for 30 min, and then treated with propofol. The applied concentrations of inhibitors: AG490 (100 μM), stattic (20 μM), wortmannin (500 nM), and API-2 (10 μM) were determined from pilot studies by our group and based on similar methods described in the scientific literature.[11,30,31,33]

siRNA Transfection

H9c2 cells were plated in 0.5% FBS DMEM for 48 h followed by overnight incubation with 33nM small interfering RNA (siRNA). Media was changed the next day and experiments were performed. Scrambled siRNA, 5′-CUUCCUCUCU UUCUCUCCCU UGUGA-3′, was used as a control. The sequence for STAT3 siRNA was 5′-ACAUAGAAGC UAGGACUA-3′ (Integrated DNA technologies). Lipofectamine 2000-mediated transfection was performed as per merchant's instructions (Invitrogen).

Western blotting

Upon completion of the experiment, cells were harvested using radioimmunoprecipitation assay (RIPA) lysis buffer containing 20 mM TRIS-HCl (pH 7.4), 150 mM NaCl, 1% Nonider

P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.004% sodium azide, 1% phenylmethylsulfonyl fluoride (PMSF), 1% sodium orthovanadate, and 1% protease inhibitor cocktail.[11] The protein concentration was measured by Bradford protein assay. Protein samples separated by 10% SDS-PAGE (PAGE) gels were transferred to nitrocellulose membrane and processed further for western blotting as described previously.[11] The protein expression levels were determined by analyzing the signals captured on the nitrocellulose membranes using a FluorChem® image analyzer (ProteinSimple).

Immunofluorescence assays

H9c2 cells were plated on glass coverslips coated with poly-D-lysine in a 24-well plate. Following treatment, cells fixed in 4% paraformaldehyde were permeabilized with 0.2% Triton X-100 and then blocked in 5% normal goat serum. Primary antibody was added and incubated with permeabilized cells overnight at 4 °C. Then Alexa Fluor-568 goat-anti rabbit antibody was added and incubated at room temperature for 1 h. After incubation with DAPI for 5 min, coverslips containing stained cells were adhered to glass slides using Fluoromount-G and immunofluorescence was detected using an Olympus fluorescence microscope. Images were captured using the Q capture Pro 6.0 software.

Statistical analysis

GraphPad Prism was used for statistical analysis. One-way ANOVA with Tukey test was used to evaluate significance between more than two groups. The Student *t* test was used to evaluate significance between two groups. A P value of < 0.05 was seen as statistically significant. Data are presented as mean ± standard deviation (SD).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Kamada N, Kanaya N, Hirata N, Kimura S, Namiki A. Cardioprotective effects of propofol in isolated ischemia-reperfused guinea pig hearts: role of KATP channels and GSK-3beta. Can J Anaesth 2008; 55:595-605; PMID:18840589; http://dx.doi.org/10.1007/BF03021433
2. Kokita N, Hara A. Propofol attenuates hydrogen peroxide-induced mechanical and metabolic derangements in the isolated rat heart. Anesthesiology 1996; 84:117-27; PMID:8572324; http://dx.doi.org/10.1097/00000542-199601000-00014
3. Xia Z, Godin DV, Ansley DM. Application of high-dose propofol during ischemia improves postischemic function of rat hearts: effects on tissue antioxidant capacity. Can J Physiol Pharmacol 2004; 82:919-26; PMID:15573153; http://dx.doi.org/10.1139/y04-097
4. Lim KH, Halestrap AP, Angelini GD, Suleiman MS. Propofol is cardioprotective in a clinically relevant model of normothermic blood cardioplegic arrest and cardiopulmonary bypass. Exp Biol Med (Maywood) 2005; 230:413-20; PMID:15956771
5. Xia Z, Huang Z, Ansley DM. Large-dose propofol during cardiopulmonary bypass decreases biochemical markers of myocardial injury in coronary surgery patients: a comparison with isoflurane. Anesth Analg 2006; 103:527-32; PMID:16931656; http://dx.doi.org/10.1213/01.ane.0000230612.29452.a6
6. Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG. The antioxidant potential of propofol (2,6-diisopropylphenol). Br J Anaesth 1992; 68:613-8; PMID:1319189; http://dx.doi.org/10.1093/bja/68.6.613
7. Javadov SA, Lim KH, Kerr PM, Suleiman MS, Angelini GD, Halestrap AP. Protection of hearts from reperfusion injury by propofol is associated with inhibition of the mitochondrial permeability transition. Cardiovasc Res 2000; 45:360-9; PMID:10728356; http://dx.doi.org/10.1016/S0008-6363(99)00365-X
8. Pachori AS, Smith A, McDonald P, Zhang L, Dzau VJ, Melo LG. Heme-oxygenase-1-induced protection against hypoxia/reoxygenation is dependent on biliverdin reductase and its interaction with PI3K/Akt pathway. J Mol Cell Cardiol 2007; 43:580-92; PMID:17920074; http://dx.doi.org/10.1016/j.yjmcc.2007.08.003
9. Xu JJ, Wang YL. Propofol attenuation of hydrogen peroxide-mediated oxidative stress and apoptosis in cultured cardiomyocytes involves haeme oxygenase-1. Eur J Anaesthesiol 2008; 25:395-402; PMID:18289444; http://dx.doi.org/10.1017/S0265021508003542
10. Ansley DM, Wang B. Oxidative stress and myocardial injury in the diabetic heart. J Pathol 2013; 229:232-41; PMID:23011912; http://dx.doi.org/10.1002/path.4113
11. Wang B, Shravah J, Luo H, Raedschelders K, Chen DDY, Ansley DM. Propofol protects against hydrogen peroxide-induced injury in cardiac H9c2 cells via Akt activation and Bcl-2 up-regulation. Biochem Biophys Res Commun 2009; 389:105-11; PMID:19703415; http://dx.doi.org/10.1016/j.bbrc.2009.08.097
12. Lecour S. Activation of the protective Survivor Activating Factor Enhancement (SAFE) pathway against reperfusion injury: Does it go beyond the RISK pathway? J Mol Cell Cardiol 2009; 47:32-40; PMID:19344728; http://dx.doi.org/10.1016/j.yjmcc.2009.03.019
13. Nielsen M, Kaestel CG, Eriksen KW, Woetmann A, Stokkedal T, Kaltoft K, Geisler C, Röpke C, Odum N. Inhibition of constitutively activated Stat3 correlates with altered Bcl-2/Bax expression and induction of apoptosis in mycosis fungoides tumor cells. Leukemia 1999; 13:735-8; PMID:10374878; http://dx.doi.org/10.1038/sj.leu.2401415
14. Yang Y, Duan W, Jin Z, Yi W, Yan J, Zhang S, Wang N, Liang Z, Li Y, Chen W, et al. JAK2/STAT3 activation by melatonin attenuates the mitochondrial oxidative damage induced by myocardial ischemia/reperfusion injury. J Pineal Res 2013; 55:275-86; PMID:23796350; http://dx.doi.org/10.1111/jpi.12070
15. Galante JM, Mortenson MM, Schlieman MG, Virudachalam S, Bold RJ. Targeting NF-kB/BCL-2 pathway increases apoptotic susceptibility to chemotherapy in pancreatic cancer. J Surg Res 2004; 121:306-7; http://dx.doi.org/10.1016/j.jss.2004.07.130
16. Zhang ZH, Yu LJ, Hui XC, Wu ZZ, Yin KL, Yang H, Xu Y. Hydroxy-safflor yellow A attenuates $A\beta_{1-42}$-induced inflammation by modulating the JAK2/STAT3/NF-κB pathway. Brain Res 2014; 1563:72-80; PMID:24690200; http://dx.doi.org/10.1016/j.brainres.2014.03.036
17. Goodman MD, Koch SE, Fuller-Bicer GA, Butler KL. Regulating RISK: a role for JAK-STAT signaling in postconditioning? Am J Physiol Heart Circ Physiol 2008; 295:H1649-56; PMID:18708442; http://dx.doi.org/10.1152/ajpheart.00692.2008
18. Smith RM, Suleman N, Lacerda L, Opie LH, Akira S, Chien KR, Sack MN. Genetic depletion of cardiac myocyte STAT-3 abolishes classical preconditioning. Cardiovasc Res 2004; 63:611-6; PMID:15306216; http://dx.doi.org/10.1016/j.cardiores.2004.06.019
19. Wickley PJ, Ding X, Murray PA, Damron DS. Propofol-induced activation of protein kinase C isoforms in adult rat ventricular myocytes. Anesthesiology 2006; 104:970-7; PMID:16645449; http://dx.doi.org/10.1097/00000542-200605000-00013
20. Luo T, Xia Z, Ansley DM, Ouyang J, Granville DJ, Li Y, Xia Z-Y, Zhou Q-S, Liu X-Y. Propofol dose-dependently reduces tumor necrosis factor-alpha-Induced human umbilical vein endothelial cell apoptosis: effects on Bcl-2 and Bax expression and nitric oxide generation. Anesth Analg 2005; 100:1653-9; PMID:15920191; http://dx.doi.org/10.1213/01.ANE.0000150945.95254.D8
21. Raedschelders K, Hui Y, Laferlita B, Luo T, Zhang H, Chen DDY, Ansley DM. Target-achieved propofol concentration during on-pump cardiac surgery: a pilot dose-finding study. Can J Anaesth 2009; 56:658-66; PMID:19639375; http://dx.doi.org/10.1007/s12630-009-9145-3
22. Vasudevan KM, Gurumurthy S, Rangnekar VM. Suppression of PTEN expression by NF-kappa B prevents apoptosis. Mol Cell Biol 2004; 24:1007-21; PMID:14729949; http://dx.doi.org/10.1128/MCB.24.3.1007-1021.2004
23. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, et al. Function of mitochondrial Stat3 in cellular respiration. Science 2009; 323:793-7; PMID:19131594; http://dx.doi.org/10.1126/science.1164551
24. Sun W, Snyder M, Levy DE, Zhang JJ. Regulation of Stat3 transcriptional activity by the conserved LPMSP motif for OSM and IL-6 signaling. FEBS Lett 2006; 580:5880-4; PMID:17027757; http://dx.doi.org/10.1016/j.febslet.2006.09.054
25. Bhattacharya S, Ray RM, Johnson LR. STAT3-mediated transcription of Bcl-2, Mcl-1 and c-IAP2 prevents apoptosis in polyamine-depleted cells. Biochem J 2005; 392:335-44; PMID:16048438; http://dx.doi.org/10.1042/BJ20050465
26. Schuringa JJ, Schepers H, Vellenga E, Kruijer W. Ser727-dependent transcriptional activation by association of p300 with STAT3 upon IL-6 stimulation. FEBS Lett 2001; 495:71-6; PMID:11322950; http://dx.doi.org/10.1016/S0014-5793(01)02354-7
27. Levy DE, Darnell JE Jr. Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 2002; 3:651-62; PMID:12209125; http://dx.doi.org/10.1038/nrm909
28. German CL, Sauer BM, Howe CL. The STAT3 beacon: IL-6 recurrently activates STAT 3 from endosomal structures. Exp Cell Res 2011; 317:1955-69; PMID:21619877; http://dx.doi.org/10.1016/j.yexcr.2011.05.009

29. Lecour S, Suleman N, Deuchar GA, Somers S, Lacerda L, Huisamen B, Opie LH. Pharmacological preconditioning with tumor necrosis factor-alpha activates signal transducer and activator of transcription-3 at reperfusion without involving classic prosurvival kinases (Akt and extracellular signal-regulated kinase). Circulation 2005; 112:3911-8; PMID:16344382; http://dx.doi.org/10.1161/CIRCULATIONAHA.105.581058

30. Lu Y, Zhou J, Xu C, Lin H, Xiao J, Wang Z, Yang B. JAK/STAT and PI3K/AKT pathways form a mutual transactivation loop and afford resistance to oxidative stress-induced apoptosis in cardiomyocytes. Cell Physiol Biochem 2008; 21:305-14; PMID:18441519; http://dx.doi.org/10.1159/000129389

31. Neiva KG, Zhang Z, Miyazawa M, Warner KA, Karl E, Nör JE. Cross talk initiated by endothelial cells enhances migration and inhibits anoikis of squamous cell carcinoma cells through STAT3/Akt/ERK signaling. Neoplasia 2009; 11:583-93; PMID:19484147

32. Tu HJ, Lin TH, Chiu YC, Tang CH, Yang RS, Fu WM. Enhancement of placenta growth factor expression by oncostatin M in human rheumatoid arthritis synovial fibroblasts. J Cell Physiol 2013; 228:983-90; PMID:23042533; http://dx.doi.org/10.1002/jcp.24244

33. Shu L, Houghton PJ. The mTORC2 complex regulates terminal differentiation of C2C12 myoblasts. Mol Cell Biol 2009; 29:4691-700; PMID:19564418; http://dx.doi.org/10.1128/MCB.00764-09