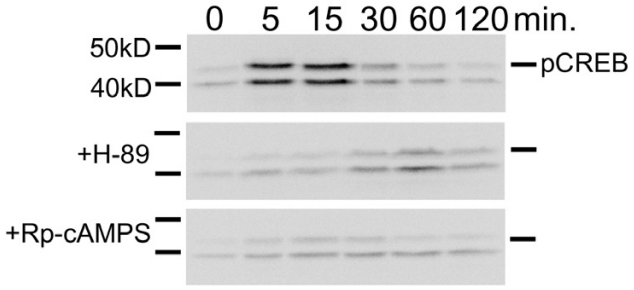

Figure 7
PKA inhibitors H-89 and Rp-cAMPS block phosphorylation of CREB. After a 30 min pre-incubation with serum-free DMEM alone or with 100 nM of H-89 or 10 μM of Rp-cAMPS, UROtsa cells were stimulated with 100 nM isoproterenol in serum-free DMEM for the indicated times and immunoblotted with an antibody specific to CREB phosphorylated at S129 and S133 (pCREB). Without inhibitor pretreatment, isoproterenol induced CREB phosphorylation within 5 min. Significant phosphorylation was still observed 15 min. after drug addition with banding intensity patterns similar to basal after 60 min. Preincubation with H-89 or Rp-cAMPS significantly decreased the phosphorylation of CREB at the 5 and 15 min time points in UROtsa cells, demonstrating the effectiveness of PKA inhibition for these compounds. As reported by the antibody suppliers, pCREB is identified as a band running at 43 kD. The second band seen on the autoradiographs represents a previously-reported alternative splice variant of CREB. Autoradiographs are representative immunoblots of n = 3 independent cell treatments.

tion state of the cAMP responsive element binding protein (CREB) in the absence and presence of selective concentrations of H-89 or Rp-cAMPS. CREB is a well characterized transcriptional factor, which is activated by cAMP-dependent PKA phosphorylation of specific serine residues [16]. In our experiments, the isoproterenol induced phosphorylation state of CREB was observed over a 2 hr interval (Figure 7). In the absence of PKA inhibitors, isoproterenol increased the phosphorylation state of CREB within 5 min returning to basal after 60 min (n = 3). This time course for CREB phosphorylation is similar to what has been described by others [17]. Pretreatment of UROtsa cells with 100 nM H-89 or 10 μM Rp-cAMPS significantly blocked the isoproterenol mediated CREB phosphorylation (n = 3). These results confirm that selective concentrations of H-89 and Rp-cAMPS used for this study can effectively block a cAMP-dependent PKA phosphorylation process. Furthermore, this finding supports our previous observations that the selective production of inflammatory mediators through induction of β-ARs is unrelated to the cAMP-dependent activation of PKA.

Discussion

This study characterizes a novel role of β-AR signaling in urothelial cells that leads to selective induction of protein products associated with inflammatory responses. Our results demonstrate that a previously described human urothelial cell line expressing functional β-ARs increases production of cAMP, phosphorylated ERK and heightened translation of COX-2 and iNOS in response to agonist activation. β-AR stimulation classically precedes cAMP accumulation, which regulates the activity of PKA leading to phosphorylation of PKA-sensitive substrates. However, phosphorylation of ERK and selective production of inflammatory mediators in UROtsa cells occurs independently of PKA activation, as similar results were observed in the presence of two analogous inhibitors specific for this cAMP-dependent kinase. Effective use of these compounds was confirmed by documenting the inhibition of PKA dependent protein phosphorylation in our same model system. Therefore, functional β-ARs present on these human urothelial cells elicit pro-inflammatory responses by a PKA-independent mechanism.

Previous studies by others have demonstrated the link between activation of MAPK pathways and the induction of inflammatory mediators [14]. In these studies, receptor regulated expression of COX-2 and iNOS was dependent upon the intermediary phosphorylation of ERK. Moreover, β-AR activation, although classically linked to generation of cAMP, has been shown in other studies to influence MAPK activation in a PKA-independent manner [18]. These PKA-independent mechanisms associated with β-AR mediated phosphorylation of ERK have been shown to involve β-arrestin scaffolding complexes [18]. In our studies we show that β-AR mediated ERK phosphorylation in UROtsa cells is independent of active cAMP-dependent PKA. Whether other scaffolding complexes caused by β-AR stimulation in these cells are associated with ERK phosphorylation is currently under investigation by our laboratory.

Despite the fact that a specific etiology has yet to be identified, inflammatory pain is a common mechanism associated with the symptoms of IC [1]. With reference to our human urothelial cell model, we demonstrate an induction of mediators associated with inflammatory pain and bladder hyperexcitability in response to β-AR activation. Clinical correlations have recognized an increased sympathetic innervation as well as elevated catecholamine levels in IC patients when compared to controls [9,10]. Our studies suggest that chronic urothelial β-AR stimulation in these patients may induce COX-2 and iNOS leading to the increased progression of inflammatory pain and bladder hyperexcitability associated with this disease. Induction of COX-2 by bacterial lipopolysaccharide or endogenous cytokines has been shown to elevate prostanoid levels that