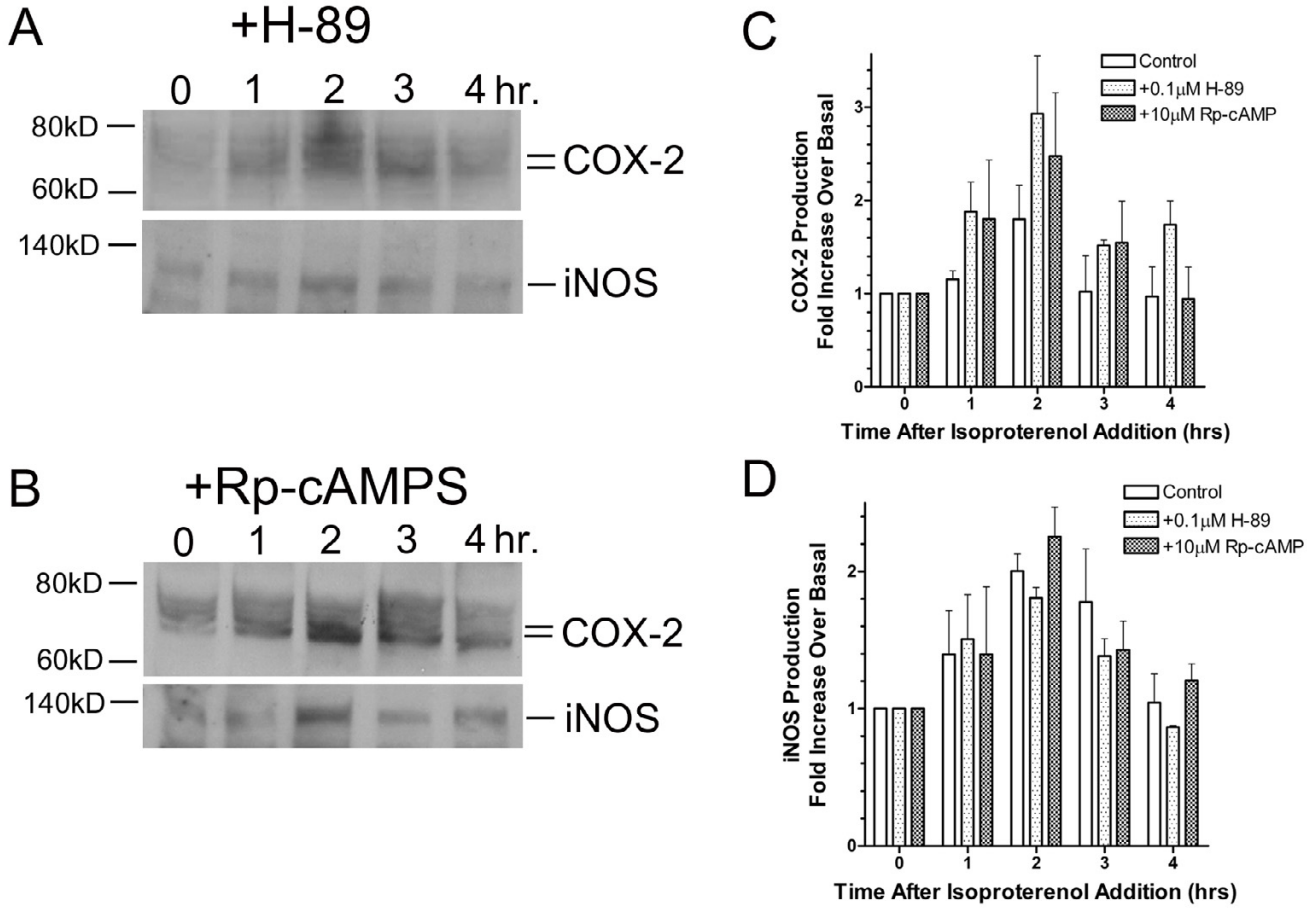

Figure 6

β-AR stimulated expression of pro-inflammatory mediators occurs through PKA-independent mechanisms. After a 30 min pre-incubation with 100 nM of H-89 (*panel A*) or 10 μM of Rp-cAMPS (*panel B*), UROtsa cells were stimulated with 100 nM isoproterenol in serum-free DMEM for the indicated times and immunoblotted with anti-COX-2 or anti-iNOS antibody to determine β-AR mediated changes in protein expression. Peak UROtsa cell expression of the pro-inflammatory mediators COX-2 and iNOS was observed 2 hrs after addition of isoproterenol even after pre-incubation with selective PKA inhibitors (*panels C and D*). Levels of COX-2 generated in the presence of H-89 or Rp-cAMPS were significantly increased 3.0 ± 0.4 and 2.5 ± 0.7 fold over basal, respectively. However these isoproterenol induced levels of COX-2 were not significantly different from cells pretreated in the absence of inhibitor (1.9 ± 0.5 fold over basal). Likewise, levels (fold over basal) of iNOS production generated by isoproterenol after H-89 (1.8 ± 0.3) or Rp-cAMPS pretreatment (2.2 ± 0.6) were significantly greater than basal. However these responses were not significantly different from levels observed for isoproterenol induced iNOS production in the absence of PKA inhibitors (2.0 ± 0.7). Values are presented as the mean ± S.E. and the autoradiographs are representative immunoblots of n = 3–5 independent UROtsa cell treatments.

of β-AR mediated iNOS production after H-89 (1.8 ± 0.3 fold over basal; n = 5) or Rp-cAMPS pretreatment (2.2 ± 0.6 fold over basal; n = 3) were not significantly different from levels seen in the absence of PKA inhibitors (2.0 ± 0.7 fold over basal; n = 3). These results provide evidence for selective production of inflammatory mediators in UROtsa cells through activation of β-ARs that is independent of PKA.

PKA-Dependent Phosphorylation of the Cyclic AMP-Responsive Element Binding Protein

Since our results reveal no changes in the amount of ERK phosphorylation or induction of COX-2 and iNOS after pretreatment with selective concentrations of H-89 or Rp-cAMPS, it was necessary to confirm that these PKA inhibitors were being used effectively in our system. Therefore, we examined the isoproterenol mediated phosphoryla-