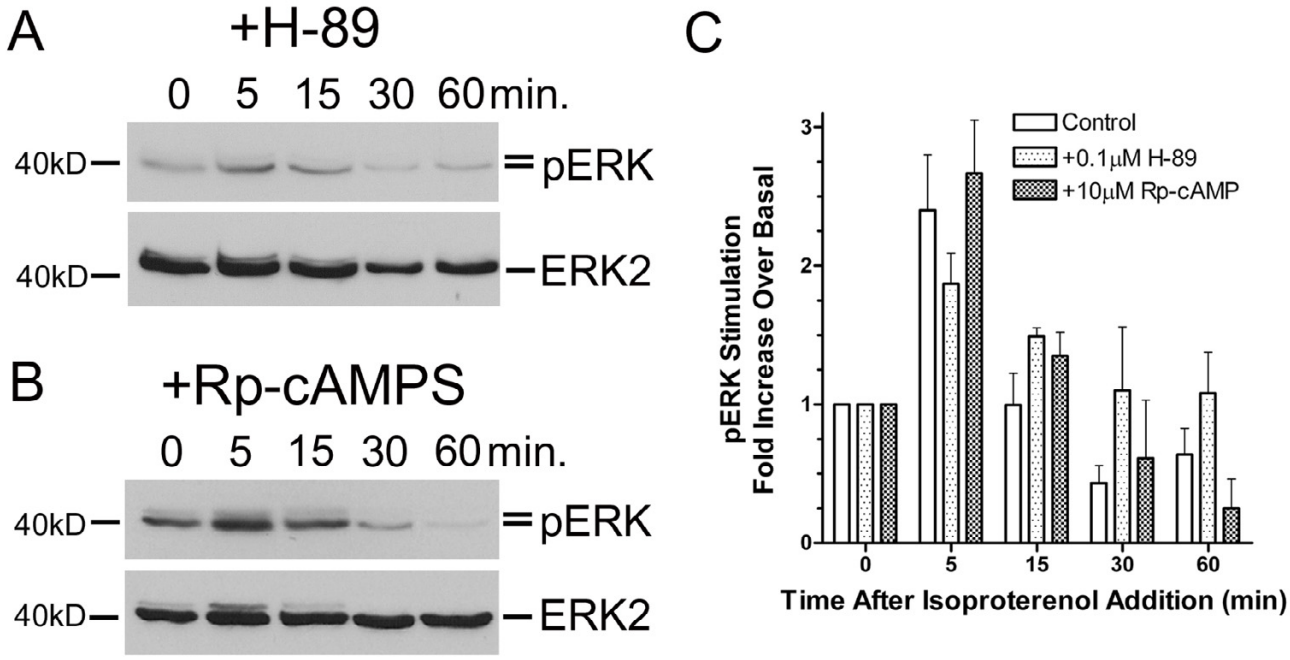

Figure 5

β-AR stimulation activates the MAPK pathway after treatment with selective PKA inhibitors. After a 30 min preincubation with 100 nM of the PKA inhibitors H-89 (*panel A*) or 10 μM of Rp-cAMPS (*panel B*), UROtsa cells were stimulated with 100 nM isoproterenol for the indicated times and immunoblotted with anti-pERK or anti-ERK2. Peak levels of pERK were observed within 5 min after the addition of isoproterenol with no changes in the total cell lysate levels of ERK2. Semi-quantitave analysis of the immunoblots revealed a time-dependent increase in ERK phosphorylation (*panel C*). Five minutes after treatment, isoproterenol significantly induced a 1.9 ± 0.4 and 2.6 ± 0.7 fold increase in pERK levels over basal after H-89 or Rp-cAMPS pretreatment, respectively. These peak values are not significantly different than the fold increase over basal for pERK measured in the absence of PKA inhibitor (2.6 ± 0.9). Values are presented as the mean ± S.E. and the autoradiographs are representative immunoblots of n = 3–4 independent UROtsa cell treatments.

cAMPS. Cell pretreatment in serum-free DMEM containing 100 nM of H-89 or 10 μM of Rp-cAMPS did not significantly alter levels of pERK after stimulation with isoproterenol when compared to cells preincubated in the absence of PKA inhibitors (Figure 5). Increases in pERK when compared to basal were still detected following H-89 or Rp-cAMPS pretreatment with peak levels generated at 5 min. Semi-quantitave analysis of the immunoblots revealed a 1.9 ± 0.4 and 2.6 ± 0.7 fold increase in pERK levels over basal after H-89 (n = 4) or Rp-cAMPS (n = 3) pretreatment, respectively, which are not significantly different than the fold increase for pERK observed without inhibitor pretreatment (2.6 ± 0.9; n = 4). Results of these experiments demonstrate that β-AR stimulated MAPK activation in UROtsa cells is not dependent upon generation of cAMP production and subsequent activation of PKA.

PKA-Independent Production of Inflammatory Mediators

Although previous results revealed that MAPK activation is not dependent upon PKA activation, we were interested in whether or not production of inflammatory mediators initiated by β-AR activation also occurred under PKA-independent mechanisms. Pretreatment of UROtsa cells with selective concentrations of H-89 (100 nM) or Rp-cAMPS (10 μM), again did not affect production of COX-2 or iNOS 2 hrs after addition of isoproterenol, when compared to cells pretreated in the absence of inhibitor (Figure 6). In these experiments, levels of COX-2 generated by β-AR activation in the presence of H-89 (n = 3) or Rp-cAMPS (n = 3) were increased 3.0 ± 0.4 and 2.5 ± 0.7 fold over basal, respectively. This level of COX-2 expression in response to isoproterenol was not significantly different from cells pretreated in the absence of inhibitor (1.9 ± 0.5 fold over basal; n = 5). Likewise, levels