are linked to the increased vasodilatation, vascular permeability and hyperalgesic responses of inflammation [2]. In other models, induction of iNOS by these same agonists to generate nitric oxide contributes to the nociceptive processing of inflammatory pain [19]. Therefore, we suggest that chronic urothelial β-AR stimulation leading to increased levels of prostaglandins and NO is one potential mechanism of inflammatory pain in IC. Moreover, higher levels of prostanoids and NO may also contribute to the symptomatic increases in urinary frequency and urgency diagnosed in patients with IC [4,8].

Support of this hypothesis has been reported using a mouse model of bladder inflammation in which genes encoding for iNOS and the β_2-AR subtype were upregulated when compared to control [11]. Interestingly, a significant increase in genomic expression of the β_2-AR subtype was only observed in a chronic and not an acute bladder inflammation model. Conversely, other investigators have shown using transient application of β-AR agonists that an increase in cAMP is sufficient to generate maintenance levels of NO in primary rat urothelial cells [20]. However, our studies using a human urothelial cell model, demonstrates that cAMP-dependent PKA activation is not necessary to induce inflammatory mechanisms for generating NO. Moreover, generation of homeostatic levels of NO in the rat model was sensitive to Ca^{2+} indicating that the responsible enzyme was eNOS, although transcriptional message (mRNA) for iNOS was well documented in this same report [20]. This suggests that chronic β-AR stimulation may induce expression of iNOS, which would generate higher levels of NO contributing to the production of inflammatory pain and increased micturition associated with IC.

In our human urothelial cell model we document a β-AR stimulated, PKA-independent signaling pathway that simultaneously increases the expression of two mediators of inflammation, COX-2 and iNOS. In addition, the pathophysiology linked to increased prostaglandin and NO production correlate well with the clinical manifestations associated with chronic inflammatory diseases like IC. Consequently, we believe that UROtsa cells serve as a readily accessible model for studying the β-AR-effector system associated with inflammation in IC. Nonsteroidal anti-inflammatory drugs (NSAIDs), which block the synthesis of prostaglandins by inhibiting COX, are commonly prescribed to relieve discomforts associated with IC. Moreover, NSIADs have been shown to decrease the amount of NO *in vivo* indicating the importance of COX-2 activity in regulating NO production during inflammation [21]. Furthermore, combined pharmacological inhibition of COX-2 and iNOS in a rat model of tonic pain, produces a synergistic antinociceptive effect [22]. This data suggests a common mechanism of action between these two drug classes, however, the associations between COX-2 and iNOS effector systems are currently unknown. Therefore, UROtsa cells represent a unique cell model whereby signal-transduction pathways common to the induction of both COX-2 and iNOS can be investigated. These studies not only may reveal novel targets of inflammatory pain that could be exploited therapeutically, but would increase our understanding of the etiology for general bladder inflammation and hyperexcitability in IC.

Conclusion

Stimulation of β-ARs expressed on cultured human urothelial cells leads to ERK phosphorylation and production of the pro-inflammatory enzymes. While cAMP levels rise in these cells after β-AR activation, production of COX-2 and iNOS are not dependent upon an increased cAMP regulated PKA activity. Continual initiation of AR function documented for patients diagnosed with IC would likely stimulate urothelial cell inflammatory responses thereby contributing to the etiology of this disease. Our results suggest that by focusing on common urothelial β-AR mediated inflammatory signaling pathways, reasonable pathophysiological mechanisms and potential therapeutic strategies could be developed for chronic inflammatory diseases like IC.

Methods

Cell Culture

The immortalized human urothelial (UROtsa) cell line was a gift from Donald Sens (University of North Dakota) and was propagated as previously reported [12]. Briefly, undifferentiated UROtsa cells were grown to confluence in serum-containing Dulbecco's Modified Eagle's Medium (DMEM) under standard cell culture conditions. Confluent UROtsa cells were washed in serum-free DMEM and pre-incubated with or without inhibitors protein kinase A (PKA) inhibitors 1 hr before addition of the selective β-AR agonist, isoproterenol. The PKA inhibitors H-89 (Sigma, St. Louis, MO) was used at a final concentration of 100 nM, while Rp-cAMPS (BioLog, Bremen, Germany) was used at 10 μM. Unless noted otherwise, isoproterenol (Sigma, St. Louis, MO) was added to cells at a final concentration of 100 nM. As a nonspecific initiator of inflammation control, cells were incubated with 300 nM lipopolysaccharide (CalBioChem, La Jolla, CA).

Membrane Preparation

A crude cell membrane preparation was prepared as previously described [23]. Briefly, UROtsa membranes were prepared by transferring suspended cells to a 50 mL conical tube and twice washing by centrifugation at 1000 × *g* using cold Hank's balance salt solution (HBSS). The intact cell pellet was resuspended in 10 mL of 0.25 M sucrose containing 10 μg/mL bacitracin, 10 μg/mL benza-