Nicotinic receptor signaling in nonexcitable epithelial cells: paradigm shifting from ion current to kinase cascade. Focus on “Upregulation of nuclear factor-κB expression by SLURP-1 is mediated by α7-nicotinic acetylcholine receptor and involves both ionic events and activation of protein kinases”

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In this issue, Dr. Grando’s research group reports an agonist-like upregulation of nuclear factor-κB (NF-κB) expression by SLURP (secreted mammalian Ly-6/urokinase plasminogen activator receptor-related protein)-1, which is mediated by α7-nicotinic acetylcholine (ACh) receptors (nAChRs) and which involves both ionic events and activation of protein kinases (10). The elucidation of the mechanism for SLURP-1 signaling in the nonexcitable cells opens a door for novel approaches to pharmacologic regulation of the cellular functions controlled by autocrine/paracrine actions of ACh.

Nonneuronal Cholinergic System

ACh, an important neurotransmitter, is synthesized and degraded in a regulated fashion by many, if not all types, of nonneuronal cells and plays a role as an autocrine/paracrine hormone and/or cytotoxin (20, 34). These nonneuronal cells, including keratinocytes (KCs), express nicotinic and muscarinic receptors, activation of which by ACh or other ligands affects many cellular functions that include proliferation, differentiation, apoptosis, adhesion, and migration (20, 34). This nonneuronal cholinergic system is an example of more general neuroendocrine-like mechanisms that mediate peripheral responses to environmental factors (31) and of evolutionary conservation of neuroendocrine systems in the periphery (32).

SLURP-1

 SLURP-1 belongs to the Ly-6 protein superfamily that is divided into two subfamilies based on the presence of a glycosphatidyl inositol (GPI)-anchored signal sequence, of which secreted forms, such as SLURP-1, lack the GPI anchor and include snake neurotoxins (18). SLURP-1 was isolated from a variety of biological materials including human blood, saliva, sweat, tears, and urine (1, 4, 16), and its gene and/or protein expression has been identified in many tissues, including skin, bladder (17), lung, trachea, esophagus, stomach, immune system, uterus, and cornea (21, 24, 26, 27). SLURP-1 has been shown to alter expression of cell cycle regulators and differentiation markers and to activate caspases in human KCs (7). SLURP-1 can also protect respiratory and oral epithelial cells from nitrosamine-induced malignant transformation (2, 3). Notably, nAChRs in KCs regulate SLURP-1 production, suggesting a physiologic interplay between the ACh and SLURP-1 signaling (4), which is further substantiated by homology in its amino acid composition to that of single domain frog cytotoxin and snake venom neurotoxins, such as α-bungarotoxin.

Statement of the Problem

In this issue, Dr. Grando and colleagues continue their studies on SLURP-1 and Ach and demonstrate that the mode of interaction of SLURP-1 with α7-nAChR is agonist-like, and that activation of α7-nAChR by SLURP-1 triggers the two-component signaling systems, coupling the ionic events and protein kinase signaling cascades to upregulation of NF-κB expression (10). This study has exciting clinical and biological implications on the nonneuronal activity of the peripheral cholinergic system. These include the following two major points and other considerations (Fig. 1).

The nAChRs mediate signaling mechanisms of novel noncanonical nicotinic ligands of the Ly-6 protein family. Specifically, a novel paradigm of regulation of KCs by nAChRs has been discovered, indicating that SLURP-1 interacts with α7-nAChR expressed in KCs as an allosteric agonist (15). This was further substantiated by radioligand binding studies that showed that SLURP-1 interacted with the ligand-binding site of KC nAChRs, showing a higher affinity to the [3H]nicotinoid-sensitive receptors, such as α7, compared with the [3H]epibatidine-sensitive non-α7-nAChRs (7). A drawback of the studies, however, was a lack of experiments employing labeled SLURP-1 peptide to confirm that it can be precipitated or colocalized with the α7-subunit.

In relation to the SLURP-1 mechanisms of signaling and biologic effects, the authors show that the SLURP-1 signal emanates from kinases physically associated with α7, leading to activation of kinase cascades. The authors of this study could have done more whole cell patch-clamp recordings, but this might not have been an appropriate approach for measuring SLURP-1 signaling in nonexcitable cells.

A new paradigm of signaling by nAChR is presented that does not require membrane depolarization as occurs in neurons and muscles. Earlier, the authors identified a novel paradigm of nAChR-mediated coordination of the ionic and metabolic signaling events that allows a nicotinic agonist to simultaneously alter gene expression and induce reciprocal changes in the cytoskeleton and contractile system of KCs (13). In the current study, the authors show that a nonionic signaling mechanism mediates upregulation of the NF-κB gene expression by SLURP-1 downstream of α7-nAChR via coupling of ionic events to protein kinase signaling cascades upon α7-nAChR activation. Other pathways (e.g., Akt) that can regulate
NF-κB downstream of nAChR were not explored, and the signal transduction pathways of SLURP-1 regulation of expression of transglutaminase type I cytokeratin 10, p21, and caspase-3 in KCs (7) were also not addressed in this study.

It is expected that such issues will be assessed in future studies, since the authors have previously demonstrated that downstream of KC nAChRs, the signaling pathways can involve elevation of intracellular Ca\(^{2+}\), activation of the protein kinase C isoforms, CaMKII, Jak2, phosphatidylinositol 3-kinase (PI3K), Akt, and Rho as well as the Ras/Raf-1/MEK/ERK pathway (5, 6, 11, 12, 14). Of note, the Raf/MEK/ERK cascade can be activated independently of Ras in a PKC-dependent manner (19) and PI3K is positively regulated by Ca\(^{2+}\) via CaMKII (23).

**Other considerations.** NF-κB is considered to be a master regulator of the inflammatory, proliferative, differentiation, and cell survival processes (reviewed in Ref. 22). NF-κB is also important in the development, prevention, and therapy of cancer (33). NF-κB activity is stimulated by many pathways that converge on IkB kinases, including the signaling pathways activated by various cytokines, lipopolysaccharide (LPS), and tumor necrosis factor-α (8, 9). In this context, the identification of NF-κB as a downstream target of SLURP-1/nAChR is of considerable interest. These studies may also serve as an example of interaction of different signaling pathways at a common effector.

**Applicability of This Work To Other Cell Types**

The authors’ findings have important implications to other cell types in which SLURP-1 has been detected: neurons (25), epithelia (respiratory, digestive, and mucocutaneous), cornea, fibroblasts, lymphocytes, uterus, bone, blood, saliva, tears, sweat, and urine (4, 7, 15, 16, 21, 24, 26, 27, 30), and in addition, its enhancement of survival of periodontal ligament fibroblasts (30), and its contribution to the maintenance of bronchial epithelial cell homeostasis (21) and immune functions and motility (28). Furthermore, nAChRs can regulate the production and secretion of SLURP-1 in several cell types (4, 29), thus reciprocally arranged signaling that involves SLURP-1 and nAChR could operate in cells that express both components.

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**DISCLOSURES**

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