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TACHYKININS AND THEIR RECEPTORS: CONTRIBUTIONS TO PHYSIOLOGICAL CONTROL AND THE MECHANISMS OF DISEASE

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Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and Their Receptors: Contributions to Physiological Control and the Mechanisms of Disease. *Physiol Rev* 94: 265–301, 2014; doi:10.1152/physrev.00031.2013.—The tachykinins, exemplified by substance P, are one of the most intensively studied neuropeptide families. They comprise a series of structurally related peptides that derive from alternate processing of three *Tac* genes and are expressed throughout the nervous and immune systems. Tachykinins interact with three neurokinin G protein-coupled receptors. The signaling, trafficking, and regulation of neurokinin receptors have also been topics of intense study. Tachykinins participate in important physiological processes in the nervous, immune, gastrointestinal, respiratory, urogenital, and dermal systems, including inflammation, nociception, smooth muscle contractility, epithelial secretion, and proliferation. They contribute to multiple diseases processes, including acute and chronic inflammation and pain, fibrosis, affective and addictive disorders, functional disorders of the intestine and urinary bladder, infection, and cancer. Neurokinin receptor antagonists are selective, potent, and show efficacy in models of disease. In clinical trials there is a singular success: neurokinin 1 receptor antagonists to treat nausea and vomiting. New information about the involvement of tachykinins in infection, fibrosis, and pruritus justifies further trials. A deeper understanding of disease mechanisms is required for the development of more predictive experimental models, and for the design and interpretation of clinical trials. Knowledge of neurokinin receptor structure, and the development of targeting strategies to disrupt disease-relevant subcellular signaling of neurokinin receptors, may refine the next generation of neurokinin receptor antagonists.

I. INTRODUCTION

Substance P (SP), the first member of the tachykinin family of peptides, has been called a "pioneering neuropeptide," since knowledge gained from studies of tachykinins has informed our understanding of many neuropeptides. Indeed, the discovery of SP as an activity in extracts of horse brain and intestine with effects on intestinal contractility and blood pressure marked the identification of the first of many "brain-gut neuropeptides," which are present in enteric neurons and enteroendocrine cells as well as in neurons of the brain (341). SP belongs to a large family of structurally related peptides, the tachykinins, that derive from alternative processing of three *Tac* genes. The tachykinins interact with three neurokinin receptors (NKRs) encoded by three *Tacr* genes. Knowledge of the structure, function, signaling, and trafficking of these receptors has guided studies of other GPCRs and, in this sense, the NKRs may be considered "pioneering receptors."

The tachykinins are expressed throughout the nervous and immune systems, regulate an extraordinarily diverse range of physiological processes, and have been implicated in important pathological conditions. The realization that tachykinins mediate pathological processes that underlie important human disorders spurred enormous efforts by the pharmaceutical industry to develop NKR antagonists. These efforts have been highly successful. There are multiple NKR antagonists, with varying degrees of selectivity. Many antagonists are effective in preclinical studies of disease in experimental animals. Some have progressed to clinical trials, where the results have been generally disappointing. At present, there is but a single success: the approval of NK_1R antagonists to treat nausea and

vomiting after chemotherapy or surgery. However, there are many plausible explanations for these failures, including an inadequate understanding of disease mechanisms, the poor predictive value of animal models, and the inherent redundancy of the tachykinin system. Moreover, new information about the participation of tachykinins in disease processes, and a deeper understanding of the NKRs, has served to maintain interest in this field, and multiple clinical trials are still in progress.

In this review, we discuss the contributions of tachykinins and NKRs to pathophysiological control. We summarize the discovery, structure, and function of tachykinins and their receptors, review their roles in major organ systems (gastrointestinal, respiratory, urogenital, dermal, nervous, immune) and pathological processes (inflammation, pain, cancer), and discuss the successes and failures of NKR antagonists in clinical trials. Throughout, we highlight the challenges of defining functions of tachykinins in health and disease and identify key gaps in our understanding of this system. However, the tachykinin literature is vast, and some aspects are not discussed, including the development of antagonists and an in-depth discussion of tachykinins in the central nervous system (reviewed in Ref. 127).

II. TACHYKININ PEPTIDES AND GENES

A. Overview

The tachykinins are named for their ability to rapidly stimulate contraction of intestinal muscle, in contrast to the slower acting bradykinins. They possess a conserved COOH-terminal sequence (-Phe-X-Gly-Leu-Met-NH_2, X hydrophobic), which is required for receptor activation. The major mammalian tachykinins are SP, neurokinin A (NKA), and neurokinin B (NKB), together with NH_2-terminally extended forms of NKA, including neuropeptide K (NPK) and neuropeptide γ (NPγ) **(FIGURE 1)**. SP, NKA, NKB, and NPK were discovered as biological activities in tissue extracts and were subsequently identified by isolation, sequence, synthesis, and analysis of the *Tac* genes. Other tachykinins were first identified in the *Tac* genes, and were subsequently purified from tissues. These include NPγ, hemokinin-1 (HK-1) and the NH_2-terminally extended forms of HK-1, endokinin A (EKA), and EKB **(FIGURE 1)**. Three genes encode the tachykinins: *Tac1* (pre-pro-tachykinin-A, *Ppt-a*), *Tac3* (*Ppt-b*), and *Tac4* (*Ppt-c*) **(FIGURE 2)**. *Tac1* encodes SP, NKA, NPK, and NPγ; *Tac3* encodes only NKB; and *Tac4* encodes HK-1 and EKA, EKB, EKC, and EKD. Although *Tac2* was initially assigned to the gene encoding the NKA precursor, it was subsequently found to be identical to *Tac1*.

B. *Tac1*-Derived Tachykinins

1. SP

In the course of investigating the tissue distribution of acetylcholine, von Euler and Gaddum (341) reported the discovery of an activity in extracts of horse intestine and brain that induced atropine-resistant contraction of isolated rabbit jejunum and fall in blood pressure of anesthetized rabbits **(FIGURE 3A)**. Extraction of tissues with acid and alcohol allowed the preparation of a water-soluble powder that retained high activity and was called "preparation P" and later "substance P." Activity was highest in intestinal muscle, which contains enteric nerves, and in the brain, suggesting a neuronal origin. Incubation of extracts with trypsin

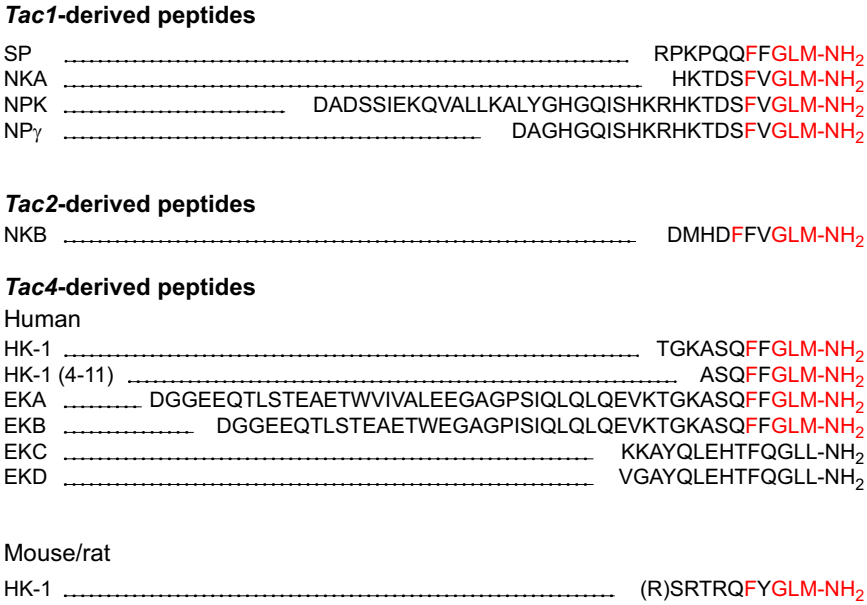


FIGURE 1. The amino acid sequences of the tachykinins and tachykinin gene-related peptides. Note the presence of the signature tachykinin sequence X-Phe-X-Gly-Leu-Met-NH_2 (red) in all of the tachykinins. This sequence is lacking in EKC and EKD, which are not tachykinins although they derive from the *Tac4* gene. HK-1 peptides are unique since they differ between mammalian species, whereas other tachykinins are conserved. The existence of an NH_2-terminal Arg residue of rat/mouse HK-1 is debatable.

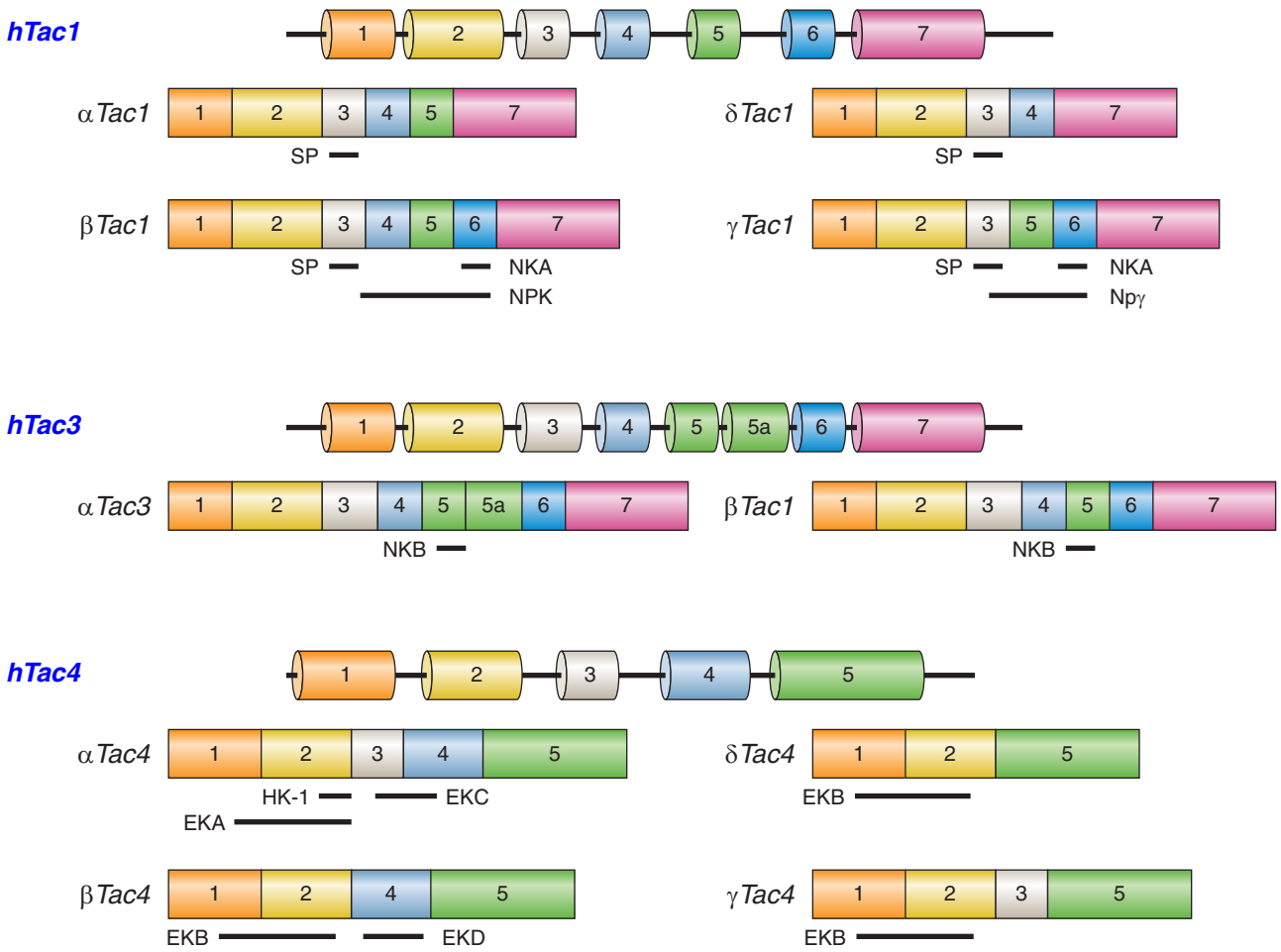


FIGURE 2. Structures of the human (h) *Tac* genes and the existence of mRNA splice variants. The peptide products are indicated by the horizontal black bars beneath the mRNA structures. [Adapted from Shimizu et al. (305).]

destroyed biological activity, leading von Euler to conclude that "the active principal is of protein nature."

Thirty years after its discovery, SP was identified as peptide from an unrelated line of investigation. While purifying corticotrophin-releasing factor from bovine hypothalamus, Leeman (180) identified fractions that stimulated atropine-resistant salivation in rats. This activity, named sialogen, was destroyed by proteases and had an estimated mass of 8,000–10,000 Da. Shortly thereafter, Lembeck (181) reported that preparations of SP also stimulated salivary secretion and suggested that sialogen and SP were the same. Starting with 20 kg of bovine hypothalami, Chang and Leeman isolated 0.15 mg of pure SP and determined its amino acid composition **(FIGURE 3*B*)** (60) and sequence (61), enabling synthesis of SP with the expected biological activity (337).

2. NKA

NKA (substance K, neurokinin α, neuromedin L), which is highly homologous to SP, was identified as an activity in extracts of porcine spinal cord that stimulated contraction of guinea pig ileum (151) **(FIGURE 1)**. The existence of NKA was predicted from the *Tac1* gene structure (233).

3. NPK

NPK, a 36-amino acid peptide with the COOH-terminal sequence of NKA **(FIGURE 1)**, was isolated from the porcine brain using methods for detecting COOH-terminal amides of bioactive peptides (332). NPK stimulated gall bladder contraction, plasma extravasation, hypotension, and bronchial smooth muscle spasm. The existence of NPK was predicted from the *Tac1* gene (233).

4. NPγ

NPγ, a 21-amino acid peptide, was predicted from the rat *Tac1* gene (168) **(FIGURE 1)**. NPγ was subsequently isolated from the rabbit intestine (142). Like NPK, NPγ is an NH_2-terminally extended form of NKA, but NPγ lacks residues 3–17 of NPK **(FIGURE 1)**.

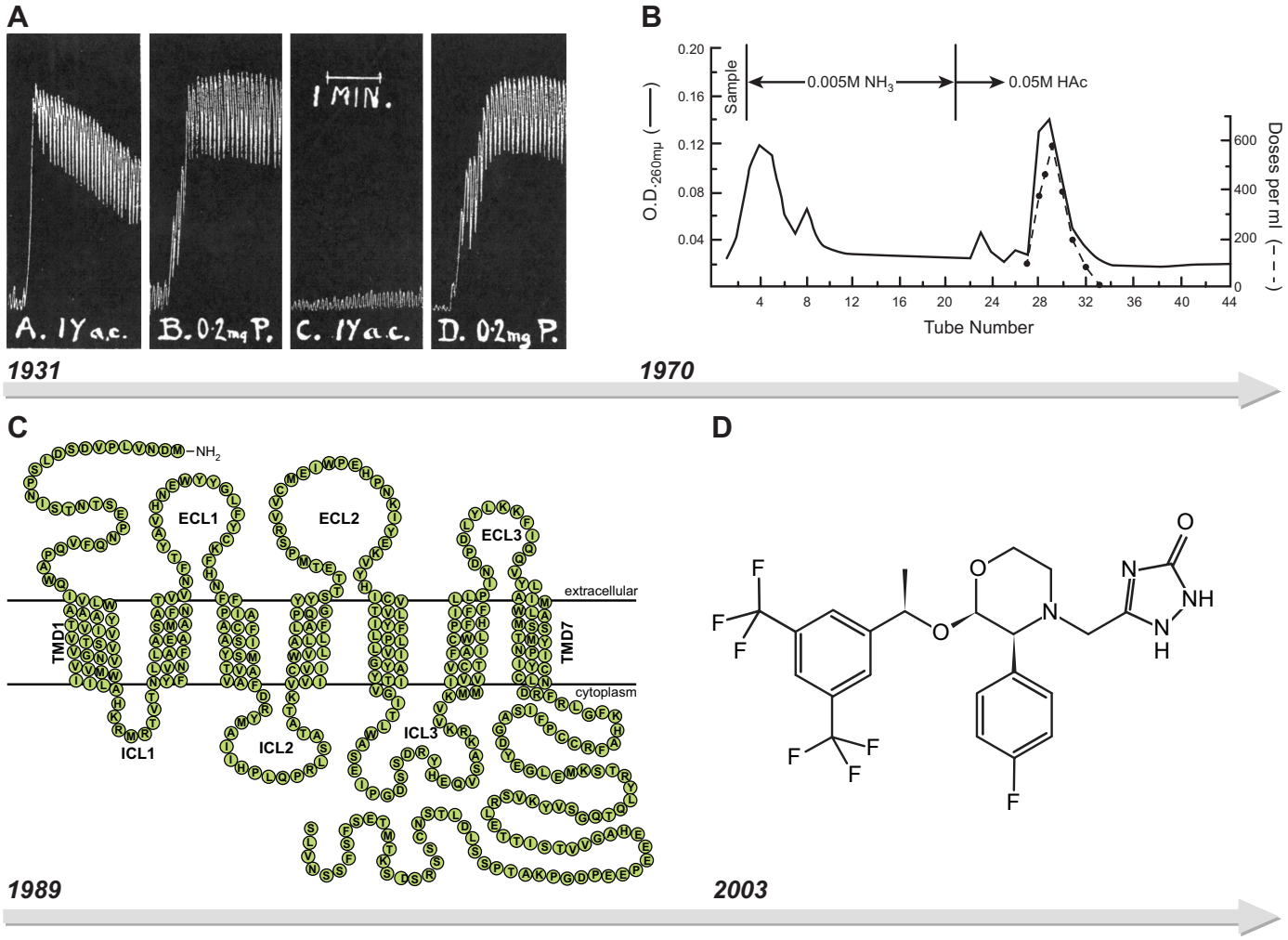


FIGURE 3. Landmark events in the history of tachykinins and neurokinin receptors. *A*: the discovery of substance P (SP). The kymograph tracings show contractions of the rabbit jejunum in response to acetylcholine (a.c.) (*A*, *C*) and SP (P) (*B*, *D*). The addition of atropine abolished the effects of acetylcholine (*C*) but not SP (*D*). [From von Euler and Gaddum (341). Copyright Wiley, Inc.] *B*: the isolation of SP. The trace of optical density (O.D.) shows the elution profile of extracts of bovine hypothalami, previously fractionated by gel filtration, from a column of carboxy-methyl cellulose. Fractions were assayed for their ability to stimulate salivary secretion in rats (dashed line). [From Chang and Leeman (60). Copyright American Society of Biochemistry and Molecular Biology.] *C*: a snake diagram of the human NK_1R. *D*: the structure of aprepitant, the first NK_1R antagonist to be approved for treatment of a human disorder (CINV).

5. *Tac1*

Two splice variants of *Tac1* (*αTac1*, *βTac1*) were first cloned from bovine striatum (233) **(FIGURE 2)**. Both mRNAs encoded SP. The COOH terminus of SP was followed by Gly, which donates the amide group of the COOH-terminal Met, and Gly was followed by a dibasic Lys-Arg processing site. An Arg-Arg sequence formed the NH_2-terminal processing site. Whereas *αTac1* encoded SP alone, *βTac1* encoded an additional peptide with the tachykinin signature sequence (-Phe-X-Gly-Leu-Met-NH_2) and appropriate residues for processing. The peptide was named substance K (NKA) due to homology with amphibian kassinin. *Tac1* has been cloned from several species, which confirmed existence of *αTac1* and *βTac1*, and identified the *γTac1* and *δTac1* splice variants (51, 168). These splice variants lack various exons; encode a combination of SP, NKA, NPK, and NPγ **(FIGURE 2)**; and are differentially expressed in various tissues.

C. *Tac3*-Derived Tachykinins

1. NKB

NKB (neurokinin β, neuromedin K), which resembles SP and NKA, was isolated from porcine spinal cord as an activity that stimulates contraction of the guinea pig ileum (143) **(FIGURE 1)**.

2. *Tac3*

Tac3 was first identified in the bovine as a gene that encoded neuromedin K (NKB) (164). There are two forms of *Tac3*, both of which encode a single tachykinin, NKB **(FIGURE 2)**.

D. *Tac4*-Derived Tachykinins

1. HK and EK

The HKs and EKs were discovered by analysis of the *Tac4* gene rather than by extraction and bioassays of tissue extracts **(FIGURE 1)** (248, 362).

2. Tac4

The appreciation that tachykinins control the myeloid lineage and regulate lymphoid differentiation led to the search for tachykinins in the hematopoietic system (362). A cDNA was identified that encoded an open reading frame of 128 amino acids, including a stretch of 11 amino acids with tachykinin signature sequence flanked by dibasic processing sites and with a Gly adjacent to the COOH-terminal Met. The gene was designated *Ppt-c* (*Tac4*), and the peptide named HK-1 to reflect its presence in hematopoietic cells **(FIGURE 1)**. Although mouse and rat *Tac4* are homologous, the human precursor is truncated to 68 amino acids, and human HK-1 differs from the mouse/rat form within the NH_2-terminal region. Thus HK-1 differs from the other tachykinins, which are conserved across mammals. Moreover, the human precursor contains two monobasic cleavage sites and has the potential to generate truncated HK-1(4–11) as well as full-length HK-1. Four splice variants of human *Tac4* have been identified (α, β, γ, δ), which are generated from a combination of five exons **(FIGURE 2)** (248). These splice variants are capable of encoding other peptides, named endokinins in view of their proposed role in endocrine tissues. EKA and EKB are NH_2-terminally extended forms of HK-1 and are true tachykinins. As expected, HK-1, EKA, and EKB have SP-like biological actions and can interact with NKRs (248, 362). EKC and EKD lack the tachykinin sequence, have minimal tachykinin-like actions, and show negligible affinity for the NKRs. They are tachykinin gene-related peptides, not tachykinins.

III. NEUROKININ RECEPTORS AND GENES

A. Overview

The first suggestion of multiple receptors for tachykinins came from comparisons of the potencies of mammalian and nonmammalian tachykinins in bioassays. Cloning of three NKRs (NK_1R, *Tacr1*; NK_2R, *Tacr2*; NK_3R, *Tacr3*) confirmed this proposal, representing a major advance in this field. The *Tacr* genes possess five exons and four introns, which interrupt the protein coding sequences. They encode GPCRs with seven membrane-spanning domains, three extracellular and intracellular loops, and extracellular NH_2 and intracellular COOH termini. The availability of the cloned receptors enabled studies of receptor structure, function, and regulation and facilitated the identification of selective antagonists. However, there is still much to learn about this family of receptors, especially the NK_2R and NK_3R, which have been less thoroughly studied. Importantly, to our knowledge, the crystal structures of the NKRs have not been reported, which would provide key information about receptor activation and signaling.

B. NKR Structure

1. NK_1R

The NK_1R (SP receptor) was cloned from a rat brain cDNA library by electrophysiological assessment of receptor expression in *Xenopus* oocytes and cross-hybridization to bovine NK_2R (substance K receptor) (355), which had been cloned earlier (203). A clone of 3,408 nucleotides was identified encoding a GPCR of 407 residues (**FIGURES 3*C* AND 4*A***). When expressed in monkey kidney COS cells, the clone conferred high-affinity binding for SP, that was displaced by SP, NKA, and NKB (IC_{50} SP > NKA > NKB). Although there is a high degree of similarity of NK_1Rs between different species (94.5% identity between rat and human), differences at key residues can affect interaction with antagonists.

A splice variant of the human and guinea pig NK_1R missing exon five encodes a COOH-terminally truncated NK_1R of 311 residues that lacks most of the intracellular C-tail **(FIGURE 4*B*)** (19, 98). The truncated human receptor has been detected in monocytes and macrophages (173), discrete brain regions (cortex, cerebellum) (172), and colonic epithelial cells of patients with colitis-associated cancer, where the short form is preferentially upregulated (108). Since the truncated receptor signals differently from the full-length NK_1R (see sect. IIIC), this differential expression is of probable functional importance.

2. NK_2R

The NK_2R (substance K receptor) was the first tachykinin receptor to be cloned. Nakanishi's group used the new approach of expression cloning, in which mRNAs from a bovine stomach cDNA library were expressed in *Xenopus* oocytes (203). From $\sim 3 \times 10^5$ clones, a single clone was identified that conferred an electrophysiological response to NKA. The 2,458-nucleotide sequence encoded a GPCR of 384 residues. Oocytes expressing this clone responded to tachykinins with a potency ranking of NKA>NKB>SP.

3. NK_3R

The NK_3R (NMB receptor) cDNA was cloned from rat brain by hybridization with bovine NK_2R cDNA, and predicted to encode a GPCR of 452 residues (304). When expressed in *Xenopus* oocytes, the clone conferred electrophys-

iological responses to tachykinins, and ligand-binding experiments on membranes from NK_3R-expressing COS cells revealed the rank order of potency of NKB > NKA > SP.

C. NKR Signaling

Although NKR signaling has been thoroughly studied, many aspects warrant further attention. First, NKR signaling has been studied mostly in cell lines rather than primary cells, where the intricacies of signaling depend on the level of NKR expression, the compliment of signaling proteins, and the cellular environment. Second, most information derives from studies of high-affinity ligands (SP for NK_1R). Since agonists can stabilize distinct GPCR conformations that transmit unique signals (biased agonism, reviewed in Ref. 281), individual tachykinins may signal differently via the same NKR, with diverse outcomes. Third, NKR signaling is usually studied by measuring total cellular levels

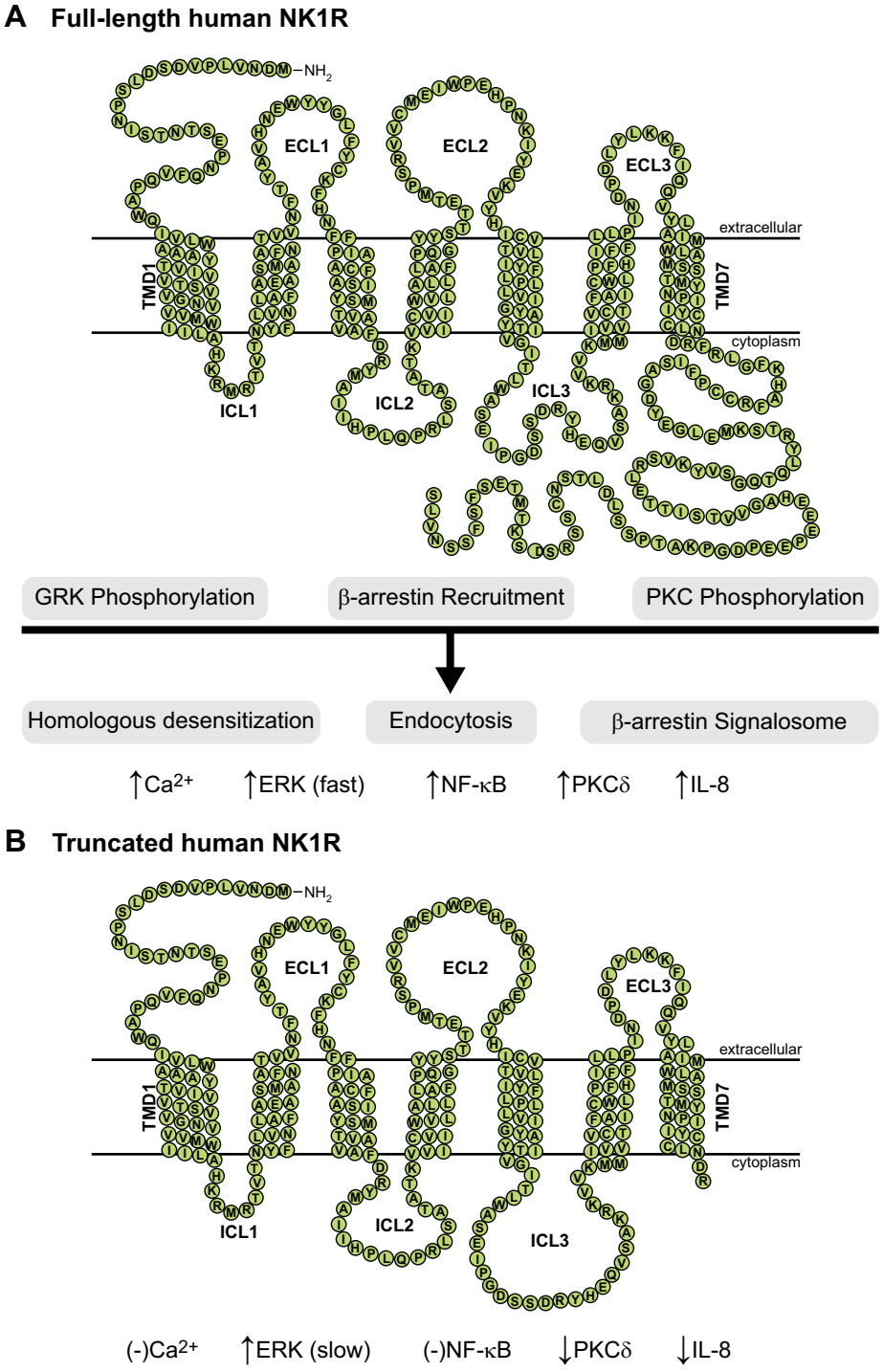


FIGURE 4. Comparison of regulation and signaling of the full-length and truncated human NK_1R. *A*: the full-length NK_1R is phosphorylated by GRKs and PKC within the COOH terminus and interacts with β-arrestins, which mediate desensitization, endocytosis, and endosomal signaling (75). The receptor activates Ca^{2+}, ERK1/2, NF-κB, and PKC-δ and stimulates IL-8 secretion (175). *B*: the truncated NK_1R lacks most of the COOH terminus, is not phosphorylated, and does not interact with β-arrestins. As a result, it is defective in desensitization and endocytosis and does not assemble β-arrestin signalosomes (75, 183). The truncated receptor does not couple robustly to Ca^{2+} and NF-κB and inhibits PKCδ phosphorylation and IL-8 secretion (175).

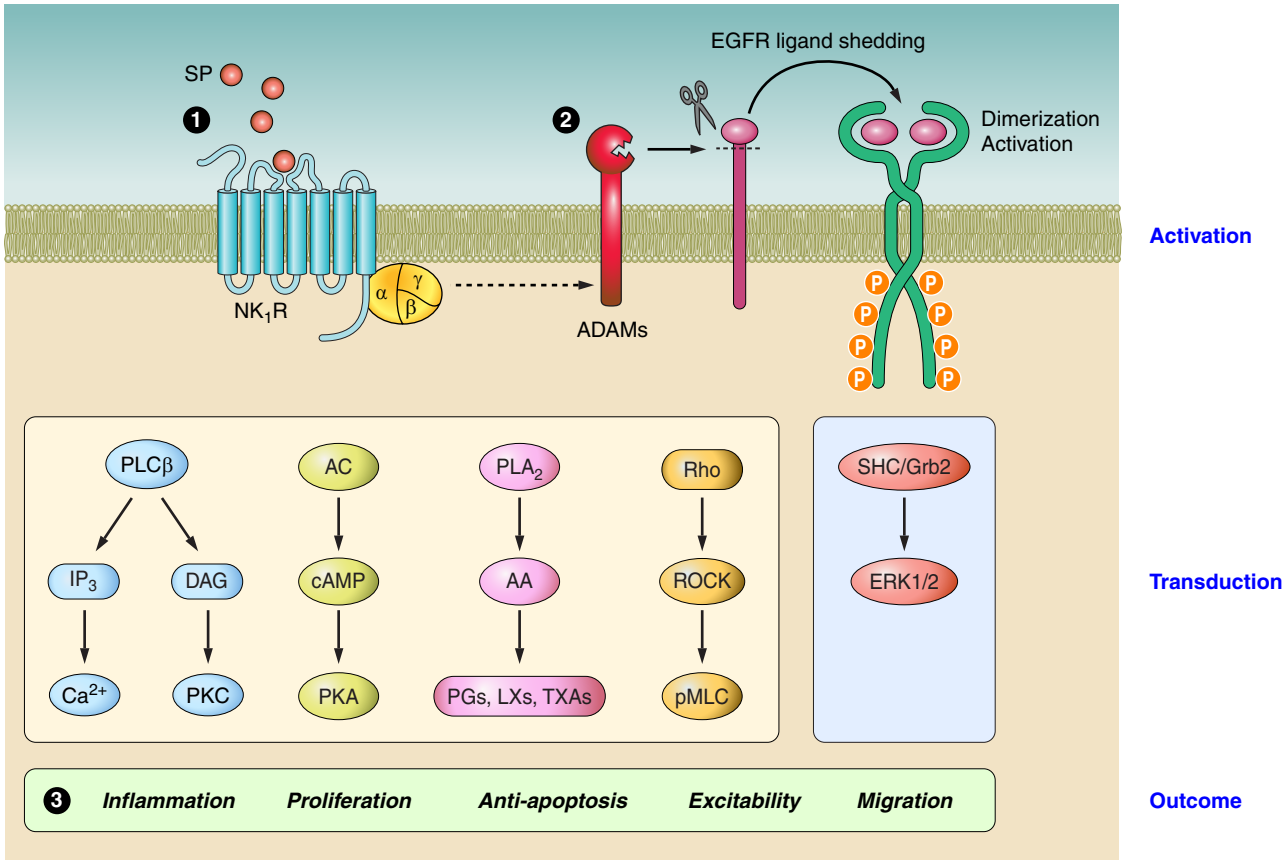


FIGURE 5. Mechanisms of neurokinin receptor signaling from the plasma membrane. *1*: SP activation of the NK_1R at the plasma membrane initiates G protein-mediated signaling events that include activation of phospholipase C (PLC), formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG), mobilization of intracellular stores of Ca^{2+}, and activation of PKC; activation of adenylyl cyclase (AC), formation of cAMP, and activation of PKA; activation of phospholipase A_2 (PLA_2), formation of arachidonic acid (AA), and generation of PGs, leukotrienes (LX), and thromboxane A_2 (TXA_2); and activation of ROCK and phosphorylation of myosin regulatory light chain (MLC). *2*: the NK_1R also transactivates the EGFR by a mechanism that involves G protein-dependent activation of members of the a disintegrin and metalloproteinase (ADAM) domain-containing proteases that cleave and liberate membrane-tethered EGFR agonists. The EGFR dimerizes, phosphorylates (P), and assembles a SHC/Grb2 complex that leads to activation of ERK1/2. There are many interactions between these pathways, and the precise details of activation vary between cell types. *3*: NK_1R signaling leads to diverse and sometimes cell type-specific effects that include inflammation, proliferation, anti-apoptosis, neuronal excitation, and migration.

of second messengers, rather than second messenger generation in subcellular compartments. Compartmentalized signaling can also lead to divergent outcomes, which explains how GPCRs that couple to the same G proteins can transduce specific signals (223). Finally, most studies have examined signaling by full-length unmodified receptors. Variants of the same receptor, which can be generated by alternative splicing or by posttranslational mechanisms, can signal by distinctly different mechanisms. This differential signaling may be important in pathophysiological situations where alternative receptor processing can occur.

1. Initiation of NKR signaling

The major proximal pathways that are activated by tachykinins in NKR-transfected Chinese hamster ovary (CHO) and rat kidney epithelial (KNRK) cells include the following: *1*) activation of phospholipase C, leading to formation of inositol triphosphate, which mobilizes intracellular stores of Ca^{2+}, and diacylglycerol, which activates protein kinase C (PKC); *2*) activation of adenylate cyclase, resulting in accumulation of cAMP and stimulation of protein kinase A (PKA); and *3*) activation of phospholipase A_2 and generation of arachidonic acid, a precursor of lipid inflammatory mediators **(FIGURE 5)**. SP-induced activation of the NK_1R in human embryonic kidney (HEK293) cells also causes a rapid change in morphology, including the formation of blebs in the plasma membrane, which involves the Rho-associated ROCK system and phosphorylation of the myosin regulatory light chain (212). SP-evoked blebbing is not related to apoptosis, which often accompanies blebbing, but rather to formation of microparticles, a mechanism of intercel-

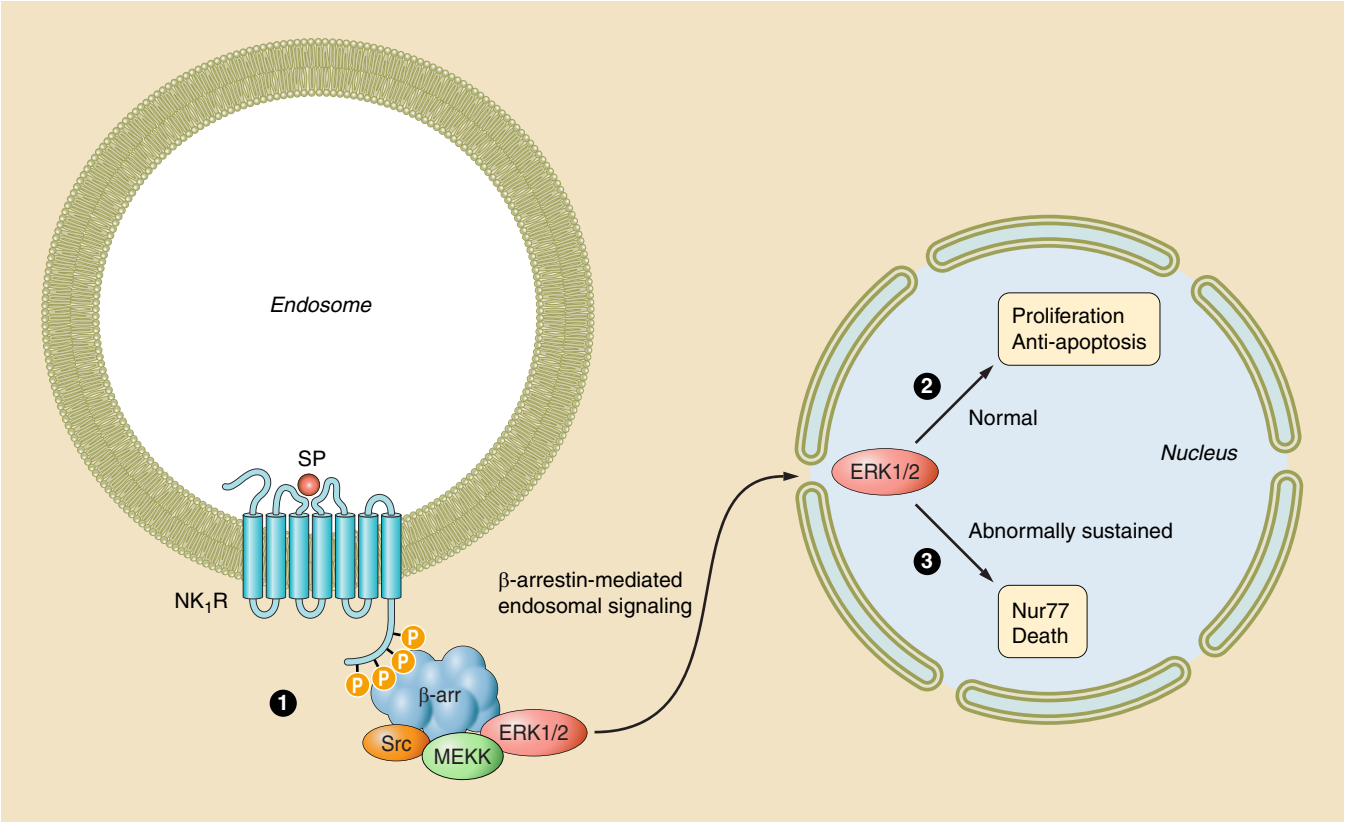


FIGURE 6. Compartmentalized signaling of the NK_1R from endosomal membranes. *1*: β-Arrestins (β-arrs) recruit the NK_1R, Src, MEKK, and ERK1/2 to endosomes and thereby assemble a signalosome that mediates ERK1/2 phosphorylation and activation. β-Arrestin-activated ERK1/2 translocate to the nucleus. *2*: Under normal circumstances, ERK1/2 mediate the proliferative and anti-apoptotic actions of SP. *3*: When ERK1/2 activation is abnormally sustained, as occurs in cells that lack active ECE-1, ERK1/2 phosphorylate and induce Nur77, which induces cell death.

lular communication that has been implicated in disease (63).

SP signaling has been extensively studied in U373MG human astrocytoma cells, which express endogenous NK_1R, and in NK_1R-transfected NCM460 human colonocytes. In both cell types, SP and the NK_1R transactivate the epidermal growth factor receptor (EGFR), which leads to activation of mitogen-activated protein kinases (MAPK), extracellular signal regulated kinases (ERK) 1 and 2, DNA synthesis, and proliferation **(FIGURE 5)** (54, 158). This mechanism partially mediates the ability of the NK_1R to promote healing of the inflamed colonic epithelium (52). Mucosal healing also depends on the anti-apoptotic effect of SP, which involves Janus kinase 2 (JAK-2) and phosphoinositide 3-kinase (PI3K)-mediated activation of the anti-apoptotic molecule Akt (protein kinase B) (159). SP also activates Akt in glioblastoma cells (3).

In view of the proinflammatory actions of SP (see sect. IV), NK_1R inflammatory signaling has been extensively studied. In U373MG cells, SP and the NK_1R activate p38 and generate proinflammatory cytokines, including interleukin (IL)-6 and IL-8 (79, 94). SP activates the master proinflammatory transcription factor nuclear factor kappa B (NF-κB) in NCM460 cells by mechanisms that involve activation of Rho family kinases and PKCδ, leading to formation of IL-6, IL-8 and tumor necrosis factor α (TNF-α) (161, 363). SP induces cyclooxygenase-2 expression and prostaglandin (PG) E_2 production in these cells *via* PKC-θ and JAK3/STAT3/5 pathways (160).

In addition to their role in GPCR desensitization and endocytosis, β-arrestins recruit signaling proteins to internalized GPCRs and mediate sustained signaling from endosomes (223). In NK_1R-transfected KNRK cells and dermal endothelial cells that naturally express the NK_1R, SP induces the assembly of an endosomal signaling complex (signalosome) comprising NK_1R, β-arrestin, Src, MEKK, and ERK1/2 **(FIGURE 6)** (75). This complex promotes the nuclear translocation of activated ERK1/2, which is necessary for the proliferative and anti-apoptotic effects of SP. The truncated NK_1R lacks phosphorylation sites that are necessary for high-affinity β-arrestin interactions, and thus cannot assemble the complex **(FIGURE 4*B*)**. Although protease-activated receptor-2 (PAR_2) also assembles β-arrestin-dependent signalosomes, activated ERK1/2 is retained in the cytosol (75). This difference in the subcellular fate of ERK1/2

depends on the affinities of the NK_1R and PAR_2 for β-arrestins, with PAR_2 exhibiting a higher affinity and assembling a more stable complex that retains activated ERK1/2 in the cytosol (251).

There are other differences in signaling of the full-length and truncated NK_1R. Whereas SP stimulates NF-κB activity and IL-8 expression in cells expressing full-length NK_1R, SP stimulation of the truncated receptor inhibits IL-8 expression **(FIGURE 4*B*)** (175). These differences may have implications for tachykinin signaling under pathological conditions where selective upregulation of the truncated receptor is observed (108).

Posttranslational modifications, including phosphorylation (see below) and glycosylation, can influence NK_1R signaling. The NK_1R has two potential sites for *N*-linked glycosylation: Asn-14 and Asn-18. The importance of glycosylation has been examined by expressing a mutated human NK_1R lacking these sites in NCM460 colonocytes (331). Mutation prevented NK_1R glycosylation, accelerated SP-induced NK_1R endocytosis, and suppresses SP-stimulated IL-8 secretion (331). Thus glycosylation stabilizes the NK_1R in the plasma membrane and controls proinflammatory signaling. Whether NK_1R glycosylation is regulated under pathophysiological conditions is unknown.

The endogenous NK_1R signals differently in primary neurons (190). Nociceptive dorsal root ganglia (DRG) neurons release SP, which mediates neurogenic inflammation and pain (see sect. IV*F*). However, SP can also act in a poorly understood autocrine fashion to activate the NK_1R on DRG neurons and sensitize transient receptor potential vanilloid 1 (TRPV1), leading to hyperalgesia but not spontaneous pain. Although SP activates PLC in DRG neurons, resulting in PKC-mediated sensitization of TRPV1, it does not robustly elevate intracellular Ca^{2+}. Instead, the NK_1R induces a $G_{i/o}$-dependent release of reactive oxygen species (ROS), leading to oxidation and activation of M-type K^+ channels and consequent neuronal hyperpolarization, which explains why SP does not cause spontaneous pain. Importantly, when overexpressed in neurons, the NK_1R induces a robust Ca^{2+} signal and inhibits M-currents. These results highlight the importance of understanding signaling by endogenous receptors in primary cells.

2. Termination of NKR signaling

GPCR signaling is terminated by mechanisms that remove agonists from the extracellular fluid (reuptake, degradation) and that restrict the capacity of the receptor to couple to signaling machinery (uncoupling, desensitization) **(FIGURE 7)**.

Neprilysin is a cell-surface metalloendopeptidase that degrades SP in the extracellular fluid and thereby terminates NK_1R activation. Neprilysin disruption suppresses SP degradation and causes widespread NK_1R-dependent plasma extravasation (195). Neprilysin-deficient mice are more susceptible to SP-dependent inflammation of the intestine (24, 325) and skin (296), which illustrates the importance of this mechanism of restricting SP signaling.

After stimulation with SP, subsequent responses usually fade (desensitize) and then recover (resensitize). G protein-coupled receptor kinases (GRKs) and β-arrestins mediate desensitization of the NK_1R **(FIGURE 7)**. GRK2, GRK3, and GRK5 interact with and can phosphorylate the NK_1R (23, 141, 170). The NK_1R is extensively phosphorylated, which promotes high-affinity interactions with β-arrestins at the plasma membrane and in endosomes. β-Arrestins sterically uncouple the NK_1R from G proteins and desensitize G protein-mediated signaling. The truncated NK_1R is resistant to desensitization because it lacks GRK phosphorylation sites and does not interact with β-arrestins (183).

Mechanisms that control NK_1R signaling from plasma membranes (SP degradation, NK_1R interaction with β-arrestins) similarly control NK_1R signaling from endosomal membranes **(FIGURE 7)**. Endothelin-converting enzyme-1 (ECE-1) is a membrane metalloendopeptidase that degrades SP in endosomes and thereby disassembles the SP/NK_1R/β-arrestin/Src signalosome, which attenuates ERK1/2 signaling (68, 259, 285). The importance of this mechanism is illustrated by the finding that ECE-1 inhibition causes sustained SP-induced ERK1/2 activation, leading to activation of the death receptor Nur77 and neurotoxicity **(FIGURE 6)** (68).

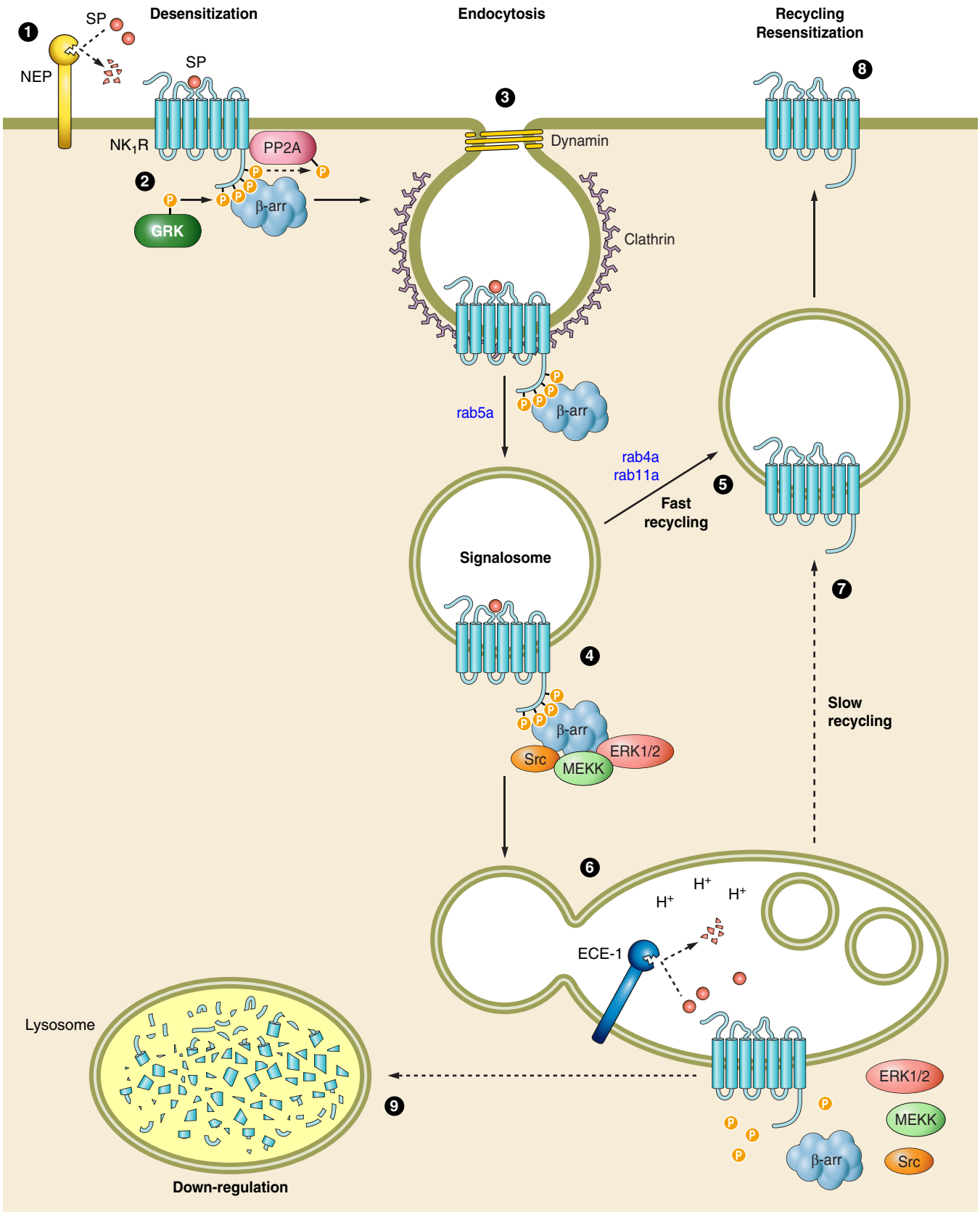
Resensitization of SP responses requires NK_1R endocytosis, dissociation of β-arrestins, and NK_1R recycling (see sect. III*D*). However, after stimulation with SP, a substantial proportion of desensitized NK_1Rs remain at the plasma membrane, where they resensitize by a mechanism that involves recruitment of protein phosphatase 2A, a β-arrestin binding partner that can dephosphorylate and resensitize the NK_1R **(FIGURE 7)** (224).

D. NKR Trafficking

NK_1R endocytosis is widely used to detect sites of SP release and receptor activation in the nervous system. Although endocytosis is also a key component of signal transduction (223, 342), the importance of NK_1R trafficking for signaling in the nervous system is not fully understood.

1. Pathways of NKR trafficking

In NK_1R-transfected KNRK and HEK293 cells, SP stimulates rapid NK_1R endocytosis **(FIGURE 7)** (103, 110, 210). However, the fate of the internalized NK_1R depends on the stimulation conditions (286). After brief exposure to low concentrations of SP, the NK_1R traffics to rab5a-positive endosomes. Endosomal acidification dissociates SP from


Desensitization
Endocytosis
Recycling
Resensitization
SP
NEP
SP
NK1R
PP2A
β-arr
GRK
Dynamin
Clathrin
rab5a
β-arr
rab4a
rab11a
Fast
recycling
Signalosome
Slow
recycling
β-arr
Src
MEKK
ERK1/2
H+
H+
H+
ECE-1
Lysosome
ERK1/2
MEKK
Src
β-arr
Down-regulation

the NK_1R, SP is degraded in endosomes by ECE-1, and the NK_1R recycles (259, 285). After sustained incubation with high SP concentrations, as may occur during inflammation, the NK_1R is ubiquitinated and degraded (67). Brief stimulation with SP evokes NK_1R endocytosis and recycling in myenteric (109, 316) and spinal (202) neurons.

SP also stimulates NK_1R trafficking in vivo. In rats, SP stimulates endocytosis and recycling of the NK_1R in endothelial cells of tracheal postcapillary venules, which coincides with desensitization and resensitization of plasma extravasation (41). Stimuli that promote SP release from the central projections of DRG neurons in the dorsal horn of the spinal cord, including electrical stimulation of dorsal roots and intraplantar injection of the TRPV1 agonist capsaicin, trigger NK_1R endocytosis in neurons in superficial laminae of the dorsal horn (199, 202). Stroking of the mucosa of the guinea pig ileum evokes NK_1R endocytosis in myenteric neurons, which suggests that stimulation of enteric afferent mechanoreceptors promotes SP release and NK_1R activation (317). Intestinal inflammation causes NK_1R endocytosis in myenteric and spinal neurons, which reflects sustained release of SP from primary spinal afferent neurons in the inflamed intestine and dorsal horn (198, 361). Thus NK_1R endocytosis occurs under pathophysiological conditions associated with SP release. The endocytosed NK_1R has been used to deliver toxins, allowing ablation of NK_1R-expressing spinal neurons and determination of their contribution to nociception (200). However, despite the extensive studies of NK_1R endocytosis in the nervous system, the functional importance of this trafficking is unknown.

Like the NK_1R, the NK_2R internalizes and recycles (102). However, in neurons of the paraventricular hypothalamic nucleus, endogenous NKB stimulates internalization and trafficking of the NK_3R to the nucleus (136). Importins mediate nuclear trafficking of the NK_3R, which then associates with transcriptionally active chromatin and thereby controls gene transcription (97, 137).

2. Mechanism and function of NKR trafficking

The NK_1R internalizes in cell lines and neurons by a clathrin-dependent mechanism that requires dynamin, rab5a, and β-arrestins **(FIGURE 7)** (109, 110, 210, 259, 294). Rab5a also mediates NK_1R trafficking to endosomes in a perinuclear location, and rab4a and rab11a mediate NK_1R recycling (286, 294). The NK_1R has also been detected in lipid rafts of HEK293 cells, suggesting the existence of nonclathrin-dependent trafficking mechanisms (169).

Endocytosis does not mediate NK_1R or NK_2R desensitization (102), which instead depends on β-arrestins. However, NK_1R resensitization requires endocytosis, intracellular processing, and recycling. Thus disruption of the mechanisms of NK_1R endocytosis (294) or recycling (286) blocks the resensitization of SP-induced Ca^{2+} signals. By degrading SP in endosomes, ECE-1 disassembles the NK_1R/β-arrestin signalosome, which allows the receptor, freed from β-arrestins, to recycle and resensitize (259, 285). By preventing the NK_1R resensitization, ECE-1 inhibitors attenuate SP-stimulated plasma extravasation, which illustrates the importance of NK_1R recycling for sustained inflammatory signaling of SP (55).

The NK_1R can regulate trafficking and signaling of other GPCRs by mechanisms that include competition of receptors for β-arrestins and physical interactions between receptors. In KNRK cells and enteric neurons, the activated NK_1R sequesters β-arrestins in endosomes and thereby impedes β-arrestin-dependent endocytosis and desensitization of the NK_3R, which has a low affinity for β-arrestins (293). The activated NK_1R similarly blocks agonist-stimulated trafficking of the μ-opioid receptor (356). The NK_1R and μ-opioid receptor can also form heterodimers in HEK293 cells, where NK_1R activation promotes μ-opioid receptor endocytosis, and delays μ-opioid receptor resensitization, presumably by causing its prolonged retention in endosomes with β-arrestins (261). Whether these complexes assemble in primary neurons is unclear. However, studies in living cells exclude the existence of NK_1R homodimers or oligomers of the NK_1R and suggest that NK_1Rs concentrate in microdomains at the plasma membrane (213).

Conversely, other receptors can control NK_1R trafficking. Transforming growth factor-β (TGF-β) delays SP-stimulated endocytosis of the NK_1R in T cells of the inflamed intestine (30). Although the mechanism of this effect is un-

FIGURE 7. The regulation and NK_1R signaling and trafficking. *1*: The cell-surface peptidase neprilysin (NEP) degrades and inactivates SP in the extracellular fluid and thereby limits NK_1R activation. *2*: The SP-occupied NK_1R is a substrate for GRK 2, 3, and 5, which phosphorylate Ser and Thr residues in intracellular loop 3 and the COOH-terminal tail. Phosphorylation increases affinity of the NK_1R for β-arrestins (βarrs), which translocate to the plasma membrane, interact with the NK_1R, and mediate G protein uncoupling and receptor desensitization. β-Arrestins also recruit protein phosphate 2A (PP2A), which can dephosphorylate and thereby resensitize the cell-surface receptor. *3*: β-Arrestins are adaptor proteins for clathrin and AP2 and thereby promote dynamin-dependent endocytosis of the SP and the NK_1R. *4*: By recruiting the NK_1R together with Src, MEKK, and ERK1/2 to endosomes, β-arrestins assemble signalosomes that allow the endocytosed NK_1R to continue to signal. Rab5a mediates trafficking to early endosomes. *5*: The NK_1R can rapidly recycle from superficially located endosomes by rab4a- and rab11a-dependent mechanisms. *6*: Alternatively, the NK_1R traffics to endosomes in a perinuclear location that contain ECE-1. ECE-1 degrades SP in acidified endosomes and thereby destabilizes the SP/NK_1R/β-arrestin/Src/MEKK and ERK1/2 signaling complex. *7*: The NK_1R then slowly recycles, which also mediate resensitization (8). *9*: After sustained stimulation with high concentrations of SP, the NK_1R is ubiquitinated and traffics to lysosomes, where degradation downregulates SP signaling.

known, it may be relevant to inflammation because TGF-β amplifies SP-induced inflammatory signaling, and the combination of TGF-β and SP stimulates release of interferon-γ and IL-17 from intestinal inflammatory T cells, whereas either agonist alone has no effect.

E. NKR Expression

The NKR system is remarkably plastic. Alterations in receptor expression influence responses to tachykinins and contribute to pathology, with implications for therapy with antagonists.

1. NK_1R

The NK_1R is upregulated in the inflamed organs, including in the intestine of patients with inflammatory bowel disease (IBD) (282), in the pancreas of mice with acute pancreatitis (33), and in mesenteric fat of mice with colitis (144), which may exacerbate the inflammatory effects of SP. However, the NK_1R is also upregulated in noninflammatory states, including in spinal neurons of rats during chronic stress (43) and the cingulate cortex of HIV-positive patients (84).

NF-κB is a major regulator of NK_1R expression during inflammation. A putative NF-κB binding site was noted in the promoter of the human NK_1R gene (327) and was subsequently confirmed experimentally (307). NF-κB mediates IL-12- and IL-18-induced NK_1R transcription in splenic T cells (347), IL-1β-stimulated NK_1R expression in astrocytes (117), and expression of the truncated NK_1R in breast cancer cells (275). SP and the NK_1R can in turn activate NF-κB, leading to transcription of proinflammatory genes (26, 161).

The NK_1R gene promoter contains putative binding sites for additional transcription factors, including AP-1, Sp1, and Oct-2, which regulate many inflammatory genes (327). Leukemia inhibitory factor, a cytokine of the IL-6 family, promotes NK_1R expression in airway epithelial cells by JAK/STAT and MAPK/ERK pathways (128), and JNK upregulates the NK_1R in acinar cells during pancreatitis (154). Notably, SP activates the JAK/STAT pathway in colonic epithelial cells leading to PGE_2 secretion (160).

2. NK_2R

The NK_2R is upregulated in the ileum of rats with acute necrotizing enterocolitis (303), and in inflammatory cells in the colon of patients with IBD (282), which may explain the anti-inflammatory effects of NK_2R antagonists. In fibroblasts, TGF-β1 and IL-1α stimulate NK_2R expression, whereas IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF) have the opposite effect (21). Two p53 consensus sequences in the NK_2R promoter are critical for the suppressive effects of NKA on the proliferation of hematopoietic progenitor cells (340).

3. NK_3R

There is a complex pattern of NK_3R regulation during inflammation. Whereas carrageenan-induced peripheral inflammation reduces responsiveness of spinal dorsal horn neurons to a NK_3R agonist (2), intraplantar injection of Formalin or adjuvant upregulates NK_3R mRNA expression in the spinal dorsal horn (209).

Alterations in the expression of the NK_3R and NKB in the uterus during pregnancy point to important roles in reproduction and reproductive disorders. In the rat placenta, normal late pregnancy is associated with downregulation of NKB and NK_3R (249). However, there are marked increases in the circulating concentrations of NKB in individuals with pre-eclampsia, NKB and NK_3R levels in the placentas of women with pre-eclampsia are elevated, and NK_3R expression in umbilical vein endothelial cells is upregulated in severe pre-eclampsia (193, 250). These results point to a major role of NKB and the NK_3R in hypertension and pre-eclampsia, where NK_3R blockade is a possible therapy.

Genetic studies suggest an important role for NKB and the NK_3R in the hypothalamic-pituitary-gonadal axis in humans. A search for genetic associations that may cause isolated hypogonadotropic hypogonadism identified four pedigrees of Turkish descent with severe congenital gonadotropin deficiency and pubertal failure, with all affected subjects homozygous for loss-of-function mutations in the NKB or NK_3R genes (335). A study of a larger and more ethnically diverse population revealed that mutations of NKB and NK_3R occur in ~5% of a normosmic isolated hypogonadotropic hypogonadism population (107). Thus NKB and NK_3R may be critical regulators of gonadal function.

IV. PATHOPHYSIOLOGICAL FUNCTIONS OF TACHYKININS

A. Overview

Although knowledge of the localization of tachykinins and NKRs and the availability of selective antagonists and mice lacking *Tac* and *Tacr* genes have contributed enormously to our understanding, there are formidable challenges to defining the pathophysiological functions of tachykinins. The observation that exogenous tachykinins exert an effect does not imply that endogenous tachykinins have the same actions. Altered tachykinin and NKR expression in disease, and the inherent redundancy of the tachykinin system, with multiple peptides and receptors, can complicate interpretations. There are obvious limitations in extrapolating find-

ings from inbred rodents under controlled conditions to diverse human subjects, and experimental animal models rarely recapitulate human diseases, especially those of unknown etiology. Despite these caveats, there is a wealth of information about the pathophysiological functions of tachykinins. This section discusses the functions of tachykinins in major organs systems (gastrointestinal, respiratory, urogenital, dermal, nervous, immune) and in important pathological processes (inflammation, pain, cancer).

B. Gastrointestinal Tract

The localization and function of tachykinins in the gastrointestinal tract have been reviewed (305). Although tachykinins are found in intestinal immune and enterochromaffin cells, the major sources in the gut are enteric neurons, followed by nerve fibers from dorsal root and vagal ganglia. Tachykinin-containing fibers surround enteric ganglia, ramify through muscle, form a perivascular mesh around submucosal arteries, and supply the mucosa. These fibers are in close proximity to cells expressing NKRs. The NK_1R is expressed by enteric neurons, interstitial cells of Cajal, epithelial cells, and lymphocytes and macrophages of the lamina propria; the NK_2R is expressed by myocytes, neuronal varicosities, and epithelial cells; and the NK_3R is mostly neuronal. The locations of tachykinins and NKRs are consistent with the regulation of neuro-neuronal transmission, motility, secretion, inflammation, and pain.

1. Neuro-neuronal transmission

Evidence for neuro-neuronal transmission, whereby neuronal tachykinins activate NKRs on enteric neurons, derives from studies of receptor trafficking and synaptic transmission. Stimulation of enteric nerves induces endocytosis of the NK_1R in myenteric neurons, which requires neuronal conduction, SP and NKA release, and NK_1R activation (109, 316). Tachykinins generate slow excitatory postsynaptic potentials in enteric neurons by activating NK_1R and NK_3R (5).

2. Motility

SP and NKA mediate excitatory transmission via NK_1Rs on interstitial cells of Cajal and NK_2Rs on smooth muscle (305). In interstitial cells of Cajal, SP activates a nonselective cation channel that controls pacemaker functions (69) and a Na^+-leak channel that mediates depolarization (149). Given the importance of interstitial cells of Cajal in motility, targeting these mechanisms could be treatment of motility disorders. HK-1, which is produced by immune cells, contracts circular muscle of mouse colon primarily by activating the neuronal NK_1R, with a minor contribution of NK_2Rs on myocytes (155). However, in segments of human colon, SP, NKA, and NKB all stimulate contraction of circular muscle by activating the NK_2R on colonic myocytes (228).

Mechanical stimulation of the mucosa evokes release of SP and NKA, which partly mediate ascending contraction of peristalsis by NK_1R- and NK_2R-dependent mechanisms (76). Glial cell line-derived neurotrophic factor augments SP release to enhances its contractile actions (114). Given the role of SP in peristalsis, defects in tachykinin innervation could contribute to abnormal motility. There is a reduced density of SP-positive fibers in colonic circular muscle of children with slow transit constipation, which could explain the dysmotility (152). Conversely, the density of tachykinin-positive nerve fibers is increased in ileal myenteric ganglia of diabetic guinea pigs, which correlates with enhanced release of acetylcholine and tachykinins, and increased sensitivity of smooth muscle (58).

3. Secretion

Tachykinins stimulate electrolyte and fluid secretion from the intestinal epithelium by activating NKRs on epithelial cells, enteric neurons, or immune cells. This mechanism facilitates propulsion and mediates protective secretory responses to infection. Cryptosporidiosis is a diarrheal disorder caused by the protozoan parasite *Cryptosporidium parvum*. Although self-limiting in healthy subjects, diarrhea can be severe in immunocompromised patients. SP and the NK_1R are upregulated in the jejunal mucosa of macaques infected with *C. parvum* and mediate increased Cl^- secretion and glucose malabsorption (124). Thus NK_1R antagonists may be useful to treat the symptoms of cryptosporidiosis and other infections of the intestine.

4. Inflammation

A wealth of evidence implicates tachykinins in intestinal inflammation, including plasma extravasation, granulocyte influx, generation of proinflammatory cytokines, and tissue damage **(FIGURE 8)**. Inflammation correlates with NK_1R activation (see sect. III*D*) and upregulation (see sect. III*E*), and SP and the NK_1R activate proinflammatory signaling pathways in colonocytes (see sect. III*C*). NK_1R blockade or deletion abrogates intestinal inflammation induced by *Clostridium difficile* toxin A (53), TNBS (80), and piroxicam in IL-10 knockout mice (348). Tachykinins also participate in the inflammatory responses to infection, including formation of granulomas, sites of chronic inflammation that prevent spread of infectious agents (346). Together, these findings suggest a role of NKR antagonists in intestinal inflammatory diseases (see sect. V*D*).

SP and the NK_1R also contribute to the aftermath of inflammation, including fibrosis and healing. The NK_1R is expressed by fibroblasts in the chronically inflamed mouse

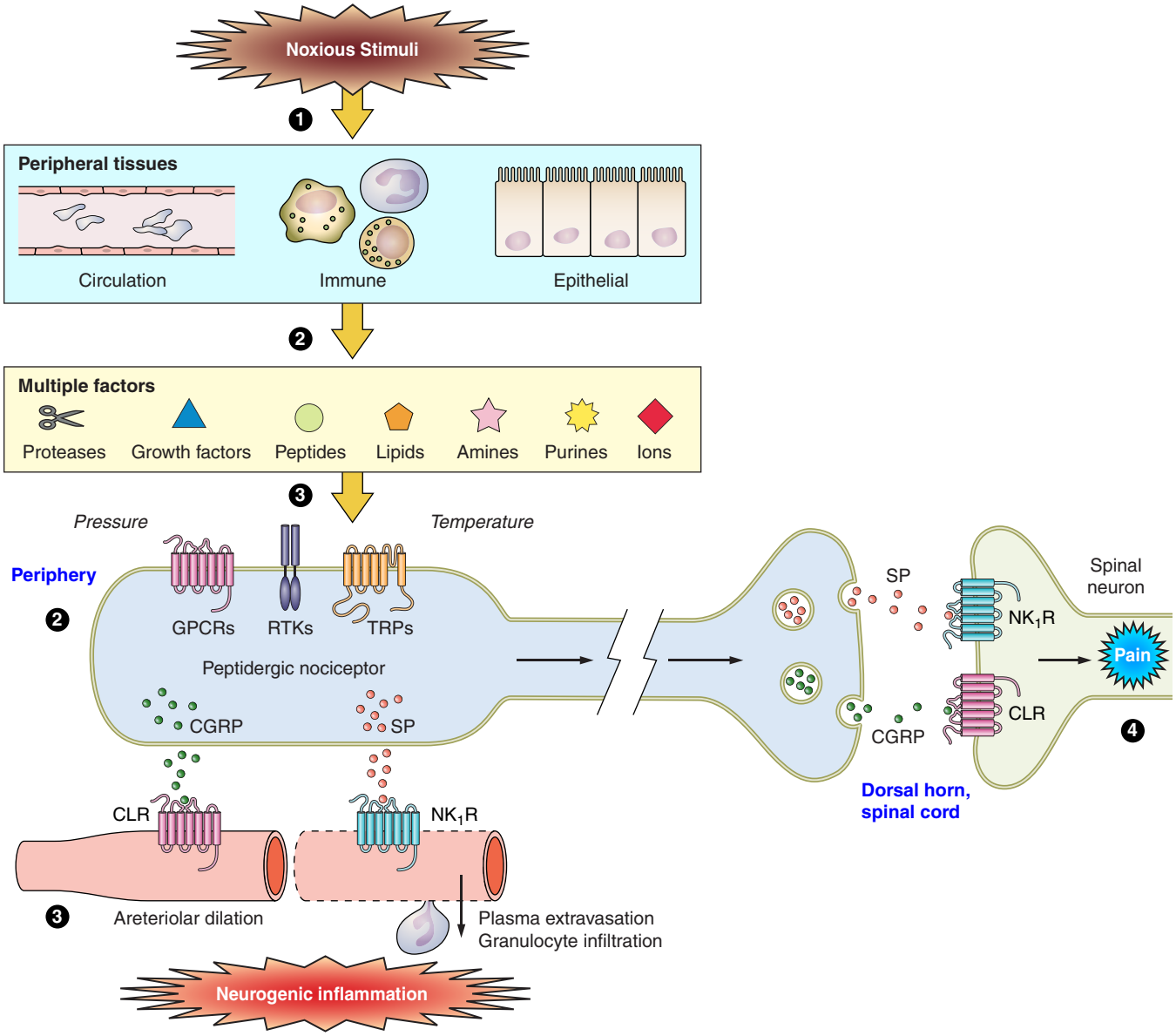


FIGURE 8. Contributions of tachykinins and neurokinin receptors to neurogenic inflammation and pain. *1*: Noxious stimulation of peripheral tissues leads to the release or generation of multiple factors that derive from the circulation, immune cells, and epithelial tissues. These can include proteases (e.g., mast cell tryptase), growth factors (NGF), peptides (bradykinin), lipids (prostaglandins), amines (5-hydroxytryptamine), purines (ATP), ions (protons), pressure, and elevated temperature. *2*: These factors can activate several classes of receptors and channels expressed by peptidergic nociceptors, including GPCRs, TRP channels, and receptor tyrosine kinases (RTKs). *3*: Activated nociceptors release neuropeptides in peripheral tissues, including SP and NKA, which stimulate NK_1Rs on endothelial cells of postcapillary venules and cause plasma extravasation and granulocyte infiltration, and CGRP, which stimulates the calcitonin receptor-like receptor (CLR) on arterioles to cause hyperemia. Together, these changes constitute neurogenic inflammation. *4*: If the factors excite nociceptors and generate action potentials, SP and CGRP are also released from the central projections of nociceptors in superficial laminae of the spinal cord dorsal horn, where neuropeptides activate receptors on spinal neurons to transmit painful stimuli centrally.

colon and in tissues from patients with Crohn's disease, where SP stimulates collagen synthesis (156). SP also protects colonocytes from apoptosis (159) and promotes expression of cysteine-rich angiogenic inducer 61 in the colonic epithelium, which contributes to healing (157). Consistent with these effects, NK_1R deletion hampers healing of the mucosa after chronic colitis (52). Thus SP and the NK_1R have dual roles in intestinal inflammation, orchestrating inflammation yet mediating repair. The potential beneficial effects of NK_1R antagonists in IBD could, therefore, be offset by disrupted healing of inflamed tissues.

Tachykinins mediate intestinal inflammation induced by activation of TRP channels of primary spinal afferent neu-

rons. The TRPV1 agonist capsaicin induces SP release and NK_1R-mediated plasma extravasation in the mouse intestine (96). Components of the inflammatory soup that activate TRP ankyrin 1 (TRPA1) include 4-hydroxy-2-nonenal, formed when ROS peroxidate membrane phospholipids (338), and cyclopentenone metabolites of PGD and PGE (204). TRPA1 is expressed by primary sensory nerves innervating the intestine and pancreas, where activation promotes the release of SP and inflammation (56, 59). TNBS, commonly used to evoke inflammation, covalently binds to and activates TRPA1, which stimulates SP release and neurogenic inflammation in the colon (88). Thus TRPA1 antagonists could be a new therapy for colitis.

Mesenteric fat accumulates at sites of intestinal inflammation. In human mesenteric preadipocytes, SP induces NK_1R-dependent proinflammatory signals and has proliferative and antiapoptotic actions that could contribute to development of the mesenteric fat that is characteristic of Crohn's disease (144, 146). SP activation of the NK_1R in adipose tissues may mediate pathologies that are associated with obesity, including glucose intolerance and insulin resistance, and NK_1R antagonism has anti-obesity effects in mice (145).

Fibrous adhesions within the abdominal cavity invariably occur after surgical manipulation of the intestine. Although usually benign, adhesions can impede transit, lead to visceral pain and female infertility, and complicate further surgery. In line with the proinflammatory actions of SP, NK_1R activation during surgical manipulation of the abdominal contents promotes formation of adhesions by limiting fibrinolytic activity, which allows fibrinous adhesions to persist (279). The NK_1R antagonist aprepitant reduces adhesion formation in rats, supporting its therapeutic potential (188).

5. Pain

Colitis induces NK_1R endocytosis in spinal neurons and nocifensive behavior that are blocked by intrathecal NK_1R antagonist, consistent with SP release in the dorsal horn and NK_1R activation on spinal neurons that transmit pain (361) **(FIGURE 8)** (see sect. III*D*). The NK_1R may also be activated in pain-processing areas of the brain of patients with IBD and irritable bowel syndrome (IBS) (135). A PET analysis of binding of the NK_1R-selective ligand [^{18}F]SPA-RQ revealed reduced NK_1R binding in cortical and subcortical regions in patients with IBD and IBS. Several processes may account for decreased binding of the NK_1R ligand, including release of endogenous SP and activation of the NK_1R, displacement of the ligand by endogenous SP, or reduced expression of the receptor. Whatever the mechanism, the results suggest a role for the NK_1R in the human brain during pain.

Stress-evoked activation of the NK_1R in the spinal cord and intestine contribute to hyperalgesia and motility disorders. The chronic psychological stress of water avoidance in rats upregulates NK_1R expression in neurons of superficial laminae in the spinal cord, and induces an NK_1R-dependent visceromotor reflex to colorectal distention (42). Spinal microglial cells, p38 MAPK, and NF-κB contribute to these stress-induced changes. Thus water avoidance stress in rats activates spinal microglial cells, as determined by phosphorylation of p38 MAPK in microglial cells in laminae I and II of the spinal cord (43). Intrathecal administration of minocycline (inhibitor of microglial cell activation) suppressed stress-evoked p38 phosphorylation, NF-κB activation, and NK_1R upregulation, whereas intrathecal SB203580 (inhibitor of p38 MAPK) blocked stress-evoked NF-κB activation but not NK_1R upregulation. Notably, both minocycline and SB203580 suppressed the visceral hyperalgesia in stressed rats. Considered together, these results support a major role for spinal microglial cells and p38 MAPK in NK_1R-dependent visceral hyperalgesia (43). Whether activated microglial cells release cytokines that upregulate the NK_1R on spinal neurons and cause central sensitization requires further investigation.

C. Respiratory Tract

The contributions of tachykinins to inflammation, hyperreactivity, and secretion of the respiratory system have been reviewed (231). This section reviews recent insights into the debatable importance of tachykinins in the airways.

1. Neurogenic inflammation and airway hyperreactivity

There is a sparse innervation of the airways by peptidergic C- and Aδ-fibers, although nerve terminals containing SP and NKA supply the vasculature, smooth muscle, epithelium, and secretory glands. Although neuropeptide-containing sensory neurons innervating the nose and larynx contribute to sneezing and cough, it is the proinflammatory actions of SP and NKA that provided a major impetus for the development of NKR antagonists to treat inflammatory diseases of the airways.

As in many tissues, SP/NKA stimulate plasma extravasation and granulocyte infiltration in the airways by activating the NK_1R in endothelial cells of postcapillary venules. Tachykinins also stimulate secretion from airway seromucous glands (283). Although these neurogenic inflammatory effects of tachykinins occur in the airways of most species, there are marked interspecies differences in the ability of tachykinins to alter tracheo-bronchial smooth muscle tone. In the isolated guinea pig and human bronchus, exogenous SP and NKA produce contraction that is mostly mediated by the NK_2R and partly by NK_1R, which are coexpressed by myocytes (7, 95). In guinea pigs, SP and NKA also induce relaxation due to activation of epithelial NK_1R and release of nitric oxide and prostaglandins, although this effect is masked by the contractile component (95). However, in rats and mice, where the contractile response is absent because NKRs are not expressed by airway smooth muscle,

NK_1R-mediated epithelium-dependent relaxation prevails (201). After two decades of intense study, a role for endogenous tachykinins and the NK_1R in airway hyperresponsiveness in allergen-stimulated mice has been proposed, providing evidence for a major role of SP and the NK_1R in allergic hyperreactivity of the airways (123).

In the isolated guinea pig bronchus, electrical field stimulation (EFS) or capsaicin causes nonadrenergic and noncholinergic (NANC) bronchoconstriction that is mediated by tachykinins from sensory nerve endings. However, the situation is completely different in mice and rats, where EFS or capsaicin provoke an epithelium- and tachykinin-dependent bronchodilatation (106). Moreover, in the isolated human bronchus, there is no evidence that capsaicin affects motor functions or that EFS stimulates a NANC contractile and tachykinin-mediated effect. Although inhaled capsaicin causes cough in healthy human subjects that is exaggerated in asthmatic patients (318), there is no evidence that endogenous tachykinins cause bronchoconstriction in humans.

Despite this lack of evidence, the robust observation that exogenous SP and NKA constrict the isolated human bronchus has strengthened the proposal that NKR antagonists are a therapy for asthma and chronic obstructive pulmonary diseases (COPD) (see sect. V*E*). However, the finding that exogenous tachykinins cause bronchoconstriction does not imply that endogenous tachykinins have the same effect. The failure of endogenous tachykinins to increase bronchomotor tone in humans may be due to inadequate release of tachykinins, release from nerve fibers supplying cells that are distant from myocytes, or release from immune cells that are resistant to EFS and capsaicin. Whatever the explanation, the findings obtained by using exogenous tachykinins continue to confound our comprehension of the role of endogenous tachykinins in airway diseases.

2. TRP channels

A major advance in our understanding of neurogenic mechanisms of acute inflammation was provided by the report that α,β-unsaturated aldehydes, which are found in cigarette smoke, can activate TRPA1 on peptidergic primary sensory neurons of rodent airways (11). Activated TRPA1 triggers the release of SP and NKA that mediate neurogenic inflammation in response to inhaled cigarette smoke. These results are corroborated by the observations that other components of cigarette smoke, including acetaldehyde and nicotine, can also stimulate TRPA1 (22, 330). Indeed, TRPA1 is a major target of reactive oxygen, nitrogen, and carbonyl species. This unique sensitivity, coupled to the selective expression of TRPA1 by peptidergic nociceptors, suggests that TRPA1 is a neuronal sensor of oxidative stress. Oxidative stress is not only increased in the respiratory tract by environmental agents, such as cigarette smoke, but also occurs at sites of inflammation during the development of asthma (38) and COPD (273). In ovalbumin-sensitized mice, ovalbumin challenge induces airway hyperresponsiveness and inflammation that is blunted by deletion of TRPA1 (48). Thus TRPA1 is a neuronal sensor for factors that contribute to asthma and COPD, where activation triggers the release of neuropeptides, including tachykinins, that mediate neurogenic inflammation.

When inhaled, certain volatile anesthetics, including isoflurane and desflurane, induce airway irritation, inflammation and cough that can precipitate laryngospasm during anesthesia. TRPA1 has been implicated in these life-threatening adverse reactions, as it mediates the irritant and inflammatory effects of isoflurane and desflurane (87, 207, 292). NK_1R and NK_2R antagonists block these inflammatory effects, which depend on TRPA1-dependent release of tachykinins from primary afferent neurons in the airways (87, 207, 292).

The increased prevalence of asthma in infants and children in the last 50 years is a mystery that cannot be explained by the hygiene hypothesis. However, large epidemiological studies have identified an association between the increased prevalence of asthma with the growing use of acetaminophen in pregnant women, infants, and children (28). Although the pathway that links acetaminophen use with asthma is unknown, the *N*-acetyl-*p*-benzo-quinoneimine metabolite of acetaminophen can activate TRPA1 by virtue of its electrophilic nature, thereby provoking neurogenic inflammation of the airways (230). Although clinical doses of acetaminophen may promote moderate and reversible neurogenic inflammation of the airways, repeated use, especially in susceptible individuals with reduced levels of the endogenous ROS scavenger glutathione, may favor the development of the asthmatic phenotype (230). Of relevance for this hypothesis is the suggestion that the ability of *N*-acetyl-*p*-benzo-quinoneimine to stimulate and desensitize TRPA1 is the major mechanism for the analgesic action of acetaminophen (10).

In addition to the established pathway by which reactive molecules initiate tachykinin-mediated neurogenic inflammation in the airways via TRPA1, SP released from sensory nerve terminals increases ROS generation by a NK_1R-dependent mechanism. Thus NK_1R antagonism reduces ROS formation, epithelial damage, and subsequent remodeling in allergen-challenged guinea pigs (319), and SP induces NK_1R-dependent neurogenic inflammation, oxidative stress, and proinflammatory responses in rats (185). Given the role of oxidative stress in thermal injury (221), it is of interest that an NK_1R antagonist prevents pulmonary inflammation evoked by local burn injury (311).

Tachykinins may also participate in abnormalities of cough. Coughing subjects have elevated levels of calcitonin gene-related peptide (CGRP) and SP in nasal secretions (187). TRPA1 may contribute to abnormal cough since

agonists cause cough in guinea pigs and humans (12, 36), and TRPA1 mediates the cough response to cigarette smoke (11). In common with other TRP channels, TRPA1 desensitizes after activation (290). The observations that smoking cessation leads to prompt enhancement of cough sensitivity, even after many years of smoking, and that suppression of cough reflex sensitivity is caused by resumption of cigarette smoking (312), may relate to the ability of cigarette smoke to activate and desensitize TRPA1. Although the role of tachykinins in these phenomena remains to be determined, an enhanced secretion of SP mediated by increased nitrosative stress could contribute to chronic cough hypersensitivity (18).

In addition to neurons, TRPA1 is expressed by airway epithelium, smooth muscle, and fibroblasts, which can contribute to inflammation by releasing cytokines (232). The possibility that tachykinins, which can also release cytokines from airway epithelial cells (165), synergize with TRPA1 to control cytokine production remains to be studied. Moreover, SP expression may not be restricted to sensory nerves, since airway epithelial cells express *Tac1* (323), and cells of the hematopoietic lineage can secrete tachykinins. Indirect evidence of this possibility derives from the observation that immune complex-mediated and stretch-induced lung injury are enhanced by the presence of the *Tac1* gene in hemopoietic-derived cells (62). Whatever their origin, tachykinins, acting on the NK_2R, can activate dendritic cell-mediated type 1 immune responses, thereby increasing the production of IFN-γ and IL-2 production by CD4(+) T cells (153). Overdistension of lung tissue during mechanical ventilation can also evoke cytokine release, and neuronal and nonneuronal SP contributes to ventilator-induced lung inflammation and injury by an NK_1R-mediated mechanism (45). In this context, it is not surprising that corticosteroids, the mainstream therapy of asthma, downregulate NK_1R expression in airway myocytes of asthmatic rats (184). Remodeling of the sensory innervation of the airways may also contribute to inflammatory diseases. A TNF-α-mediated increase in the levels of nerve growth factor (NGF) results in the proliferation of tachykinin-containing sensory nerve endings (329). NGF also contributes to the tachykinin-mediated responses in rodent airways after ozone inhalation (246), including during early life (131). Finally, in a mouse model of allergic asthma induced by house-dust mite antigen, NGF, primarily expressed in the bronchial and alveolar epithelium, mediates the enhanced release of SP, sensory innervation, and airway hyperresponsiveness (240).

3. Fluid secretion

The mortality of patients with cystic fibrosis is mainly due to chronic bacterial infections of the airway that are favored by the impaired reflex stimulation of mucus secretion from submucosal glands. SP mediates the local responses to capsaicinoids through a mechanism involving coordinated activation of cystic fibrosis transmembrane regulator and K^+ channels, which eventually results in secretion of fluid from seromucous glands (132). While seromucous glands from noncystic fibrosis patients respond to SP with increased secretion, glands from cystic fibrosis patients are unresponsive to SP (65). Similar findings were obtained in a pig model of cystic fibrosis, where SP was unable to cause glandular secretion (140). Thus defective secretory responses to SP may contribute to the abnormalities in airway secretion that underlie the pathology of cystic fibrosis.

D. Urogenital Tract

The role of tachykinins in urinary and reproductive tracts and the therapeutic potential of NKR antagonists have been reviewed (49, 247).

1. Urinary tract

Primary spinal afferent nerves containing SP and NKA innervate the renal pelvic wall, ureter, and urinary bladder, including the urothelium, muscle, and blood vessels (49). In the wall of the renal pelvis, endothelin 1 (350) and PGE_2 (163) stimulate SP release from sensory nerves, whereas angiotensin counteracts the effects of PGE_2 (162).

Tachykinins stimulate smooth muscle contraction of the human ureter, mostly by activating the NK_2R (138, 227, 255), suggesting the potential use of NK_2R antagonists for ureteral disease. In the urinary bladder, the NK_1R is found in blood vessels, the urothelium and muscle layers, with some inter-species differences, and the NK_2R is expressed by detrusor muscle of all mammalian species studied, including humans (49). In the rat, the NK_1R couples to Rho kinase, linking this receptor to smooth muscle contraction (349). Patients with multiple sclerosis have increased density of SP-containing fibers in the urinary bladder, which may contribute to detrusor overactivity in these patients (271). NK_1R antagonists are a potential therapy for overactive bladder syndrome in postmenopausal women (299) (see sect. V*F*), whereas NK_2R blockade controls neurogenic detrusor overactivity in rats with a spinal cord injury (1).

SP and NK_1R have proinflammatory effects in the bladder. In rats, bladder inflammation during early life induces an upregulation of SP that persists into adulthood and is associated with elevated micturition frequency, decreased micturition volume, and enhanced vascular permeability (74, 300). Other proinflammatory actions of SP in the bladder include stimulation of plasma extravasation and leukocyte infiltration, mast cell degranulation, generation of ROS, and expression of proinflammatory cytokines, chemokines, adhesion molecules, and cyclooxygenase-2 (49). SP stimulates expression of glucose-regulated protein 78, a receptor for activated α2-macroglobulin, and blockade of this receptor prevents SP-mediated bladder and urothelial inflamma-

tion (339). The NK_1R mediates the proinflammatory effects of tachykinins in the mouse urinary bladder, since NK_1R blockade or deletion attenuates antigen-induced cystitis (291). NK_1R antagonists also prevent stress-induced urothelial degeneration and mast cell degranulation in the urinary bladder (89). Thus tachykinins contribute to the clinical manifestations of interstitial cystitis, and NK_1R antagonists may be a treatment for inflammatory disorders of the bladder.

There is a high degree of comorbidity between genitourinary and gastrointestinal disorders that are characterized by chronic pelvic pain. For example, colitis in rats is associated with increased expression and release of SP and CGRP in the urinary bladder that requires activation of TRPV1 pathways (253).

2. Female reproductive system

Tachykinins and all NKRs are present in the uterus (57, 256). Their prevalence changes during pregnancy (256) and is regulated by ovarian steroids (263). The NK_2R is the predominant tachykinin receptor involved in uterine contraction, and its activation is under tight regulation during pregnancy (260). However, the NK_1R in the uterus can mediate inflammatory responses that may lead to abortion (93).

NKB is present in the human placenta, and increased placental levels of NKB and *Tac3* could contribute to preeclampsia and hypertension during late pregnancy (249, 250). Although placental levels of NKB increase during normal pregnancy and decline after delivery, they increase even more after preterm labor (336), suggesting that regulation of NKB during pregnancy is important for normal gestation. NKB may induce NK_1R-mediated vasodilation of the placenta during pregnancy and cause systemic NK_3R-mediated vasoconstriction that leads to hypertension. Elevated NKB levels may be a diagnostic marker for preeclampsia, and NK_3R antagonists could be a therapy for this common condition (247).

Within the ovary, tachykinins control steroid secretion and may have an ancient role in stimulating oocyte growth (14). Although tachykinins have been implicated in age-related decline in reproductive function (357), their contribution to control of reproductive capability remains to be fully defined.

3. Male reproductive system

Tac1, *Tac3*, and *Tac4* are expressed by human sperm, and tachykinins increase sperm motility by NK_1R- and NK_2R-dependent mechanisms (264). Sperm also express the SP-degrading enzyme neprilysin, and neprilysin inhibition promotes motility by inhibiting degradation of endogenous tachykinins (264). The epididymis produces SP, which stimulates sperm motility, and tachykinins potentiate contractility of the vas deferens (49). The importance of tachykinins in testes development is illustrated by the finding of severe testicular atrophy in dogs treated with a mixed NKR antagonist at a young age (194).

The seminal vesicles are innervated by SP-containing fibers, and SP induces contractions and facilitates neurally mediated responses in this system (49). SP and NKA are present at low levels in the rat and guinea pig prostate, abundant in the dog prostate, but absent in humans (49). However, *Tac1, Tac3*, and *Tac4* mRNAs have been detected in human prostate. The effects of tachykinins on prostate contraction involve the NK_1R in all species, but NK_2R is dominant in human (49).

SP is present in human erectile tissue and penile vessels and can contract human erectile tissue (49). However, its role in erection is unclear, since it has no effects in the cavernous artery and relaxes norepinephrine-induced contractions of corpus cavernosum and corpus spongiosum. NK_1R antagonism suppresses ejaculation in rats in response to intraventricular administration of a dopamine D3 receptor agonist (66).

E. Skin

The contribution of tachykinins to homeostasis and diseases of the skin have been reviewed **(FIGURE 9)** (287).

1. Neuronal tachykinins

SP and NKA are present in primary sensory nerves in the skin (70). Tachykinin-positive nerve fibers supply the dermis and epidermis as well as innervate dermal blood vessels, keratinocytes, mast cells, dendritic cells, and hair follicles. Many exogenous and endogenous factors can stimulate the release of tachykinins from peripheral nerves in the skin, including physical stimuli (heat, ultraviolet radiation, scratching), allergens, and inflammatory mediators (bradykinin, prostaglandins, proteases, cytokines). Other factors, for example, pituitary adenylate cyclase activating polypeptide-38, inhibit tachykinin release from cutaneous sensory nerves, in line with their anti-inflammatory effects (236).

2. Keratinocytes

Mouse and human keratinocytes express NK_1R and NK_2R **(FIGURE 9)** (315). The consensus of multiple studies is that SP and NKA control the capacity of keratinocytes to serve as cytokine factories by regulating production of proinflammatory cytokines (315). SP also upregulates NGF production by keratinocytes and may thereby control the regeneration of cutaneous nerves under normal conditions and during wound healing (47). In contrast to CGRP, SP has only a moderate effect on keratinocyte proliferation (284). Nepri-

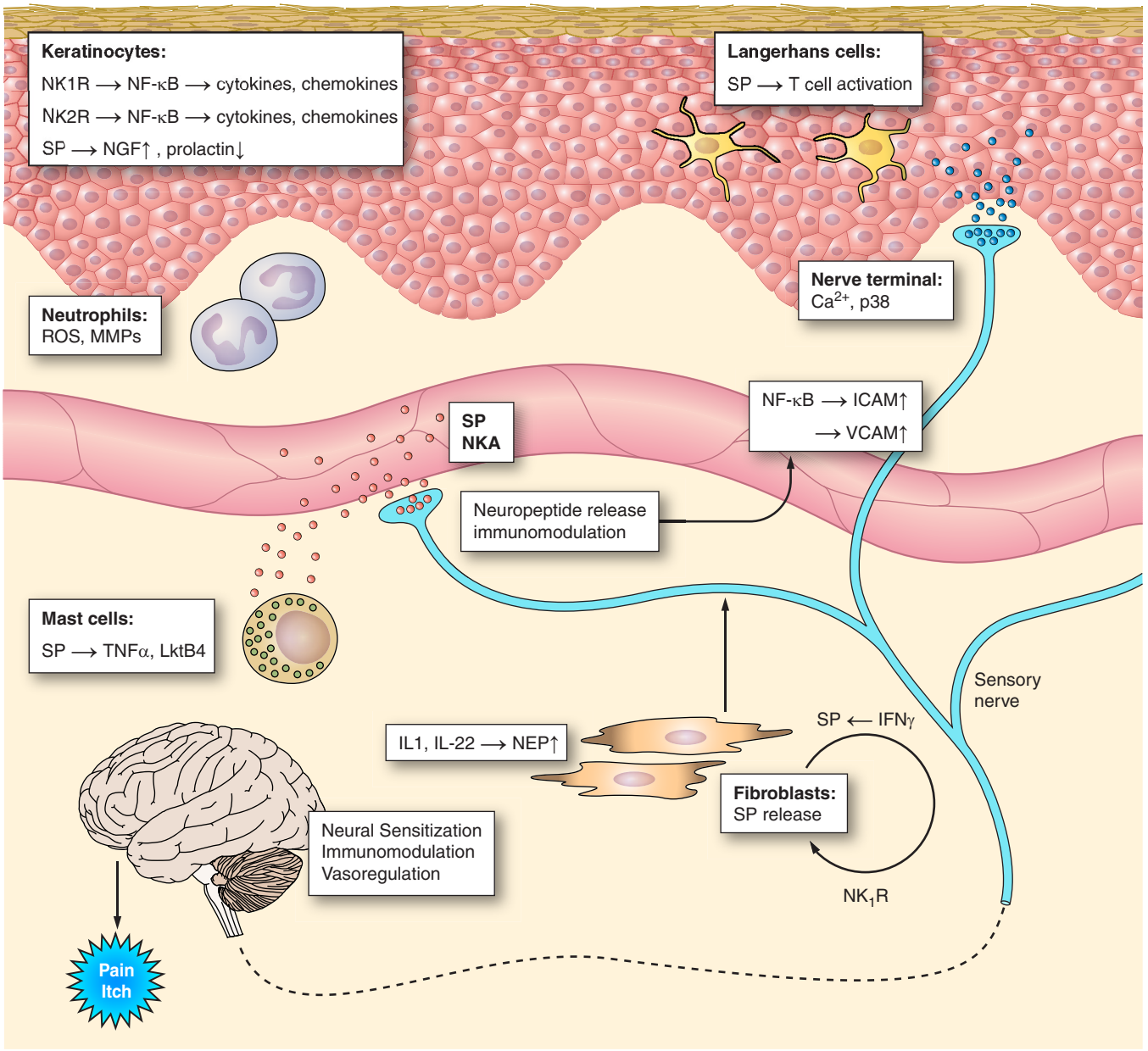


FIGURE 9. Functions of tachykinins in the skin. SP and NKA are released from the peripheral endings of primary sensory nerves in the skin. They act on keratinocytes via NK_1R and NK_2R to activate NF-κB and promote release of cytokines and chemokines. SP and NKA act within the vasculature to induce plasma extravasation and to upregulate adhesion factors that stimulate neutrophil adhesion and infiltration. During inflammation, SP and NKA activate mast cells, neutrophils, and Langerhans cells, which amplifies the inflammatory response. Centrally released tachykinins contribute to pain and itch transmission.

lysin is expressed by keratinocytes and endothelial cells, where it dampens the actions of tachykinins (245).

3. Cutaneous blood vessels

SP and NKA-positive nerve fibers innervate the vasculature of the superficial dermis, where tachykinins activate the NK_1R on endothelial cells of postcapillary venules to stimulate plasma extravasation, granulocyte infiltration, and release of proinflammatory mediators (neurogenic inflammation) **(FIGURE 8)**. However, endothelial cells can also produce tachykinins, and NGF upregulates SP expression and release from human dermal microvascular endothelial cells (215). Although SP and NKA promote plasma extravasation from postcapillary venules, resulting in edema, recent evidence suggests that while tachykinins maintain basal cutaneous microcirculation, pituitary adenylate cyclase activating polypeptide-38 mediates neurogenic inflammatory vasodilation of arterioles and neuropathic mechanical hyperalgesia (40). In human dermal microvascular endothelial

cells, SP induces an NK_1R-dependent upregulation of adhesion molecules through activation of the transcription factors NF-AT and NF-κB, which leads to the influx of inflammatory cells (269, 270).

4. Fibroblasts

Compared with other tissues, such as the airways, the role of tachykinins in dermal fibroblasts is poorly understood. Human dermal fibroblasts in culture express *Tac1*, which is upregulated by exogenous SP (17), as well as the NK_1R, which is upregulated by interferon-γ (192). Thus tachykinins may regulate fibroblasts by autocrine and neuronal mechanisms to regulate proliferation and wound healing. Indeed, SP induces an NK_1R-dependent proliferation of human dermal fibroblasts (129). Human dermal fibroblasts express neprilysin, which is augmented by IL-1β and IL-22 (351). Notably, neprilysin is upregulated in the skin and in ulcers of patients with diabetes, which, combined with the peripheral neuropathy, could contribute to impaired wound healing (13).

5. Dermatitis and pruritus

The peripheral nervous system has long been implicated in the pathophysiology of inflammation and itch in dermatitis. Tachykinins are upregulated in the lesional skin of humans and mice with atopic dermatitis (242). Studies in mice implicate tachykinins and NKRs in the sensitization and inflammatory phases of allergic contact dermatitis. In a model of allergic contact dermatitis in mice, NK_1R deletion or antagonism attenuates the sensitization and inflammatory responses to dinitrofluorobenzene (297). SP acting within lymph nodes mediates the sensitization phase (302). Surprisingly, NK_2R antagonists enhanced the inflammatory response, whereas NK_2R agonists had the opposite effect, suggesting a protective role of for the NK_2R. Similarly, repeated SP challenge resulted in an anti-inflammatory response by modulating T cell and dendritic cell function in a chronic stress-induced model of allergic contact dermatitis (258). Whereas neprilysin disruption exacerbates allergic contact dermatitis, it has no effect on irritant dermatitis in mice (296). In summary, the contributions of tachykinins to allergic contact dermatitis are not fully understood, with evidence for pro- and anti-inflammatory functions.

SP is a major mediator of pruritus of atopic dermatitis, and NKR antagonists have been proposed as a therapy for itch (see sect. VG). SP induces expression of artemin, a member of glial cell line-derived neurotrophic factors, by human dermal fibroblasts, and artemin evokes warmth-evoked scratching and thermal hyperalgesia in mice (222). As a novel therapeutic strategy, ointment containing the nerve repulsion factor semaphorin 3A reduces the density of innervation of mouse skin with SP-containing nerve fibers, attenuates inflammation, and suppresses pruritus in a mouse model of allergic dermatitis (234).

TRP channels of sensory neurons innervating the skin also contribute to inflammation and pruritus of contact dermatitis. Notably, TRPA1 mediates the inflammation and scratching behavior of mice exposed to haptens (oxazolone, urushiol) and the allergen of poison ivy, and SP-induced scratching behavior is not observed in *Trpa1* knockout mice (191).

6. Wound healing

Tachykinins have been implicated in wound healing, and deficits in tachykinin innervation (266) and upregulation of neprilysin (13) may contribute to the abnormal wound healing that occurs in patients and animals with diabetes. In a laser-induced wound healing model in rats, exogenous SP was found to promote neurite outgrowth and wound healing (77), and capsaicin also causes a NK_1R-dependent increase in NGF biosynthesis in the rat skin (8).

7. Stress-induced hair loss

The hair follicle apparatus expresses tachykinins, NKRs, and endopeptidases, and tachykinins have been implicated in stress- and autoimmune-evoked hair loss. Stress-induced premature induction of catagen and hair follicle apoptosis in mice requires expression of the NK_1R and the presence of mast cells (15). Observations of human skin biopsies and hair follicles in culture indicate that SP downregulates production of prolactin, which is important for hair growth (178). SP, NK_1R, and neprilysin regulate the inflammatory response in a murine model of alopecia areata, an autoimmune disorder of the hair follicle associated with inflammatory cell influx around growing hair follicles (306).

F. Neurogenic Inflammation and Nociceptive Transmission

Primary sensory neurons innervate most tissues and can release tachykinins from peripheral and central endings to induce neurogenic inflammation and pain transmission. This section discusses the mechanisms and importance of tachykinins in these processes.

1. Tachykinins in primary sensory neurons

The localization of tachykinins in a subset of primary sensory neurons of the trigeminal, dorsal root, and vagal ganglia has been a topic of great interest for the past 60 years since SP and NKA have been proposed to play a major role in transmission at the level of the first synapse in the nociceptive pathway. Tachykinin-containing neurons in sensory ganglia have small cell bodies with unmyelinated C-fibers or thinly myelinated Aδ-fibers and slow conduction velocities. These neurons mediate nociceptive responses to physical

(thermal, mechanical) and chemical stimuli. In addition, by releasing neuropeptides from peripheral endings, they generate "neurogenic inflammation," which includes arteriolar dilatation and plasma extravasation and granulocyte infiltration from postcapillary venules **(FIGURE 8)**. SP- and NKA-expressing neurons, representing 30–50% of neurons of the rat DRG, coexpress multiple neuropeptides that have been detected by immunochemical techniques. However, convincing evidence for neuropeptide release from the central or peripheral nerve terminals, a prerequisite for physiological function, is available for only a limited number of neuropeptides, notably SP and CGRP (44).

Peptidergic sensory neurons express TRP ion channels, including the thermosensors TRPV1, TRPV2, TRPV3, and TRPV4, the menthol sensor TRPM8, and the irritant sensor TRPA1. Once activated, TRP channels induce release of neuropeptides, including tachykinins. The observation that chronic treatment with the TRPV1 agonist capsaicin depletes neuropeptide from sensory nerve terminals implies that all peptidergic neurons express TRPV1. Moreover, TRPA1-positive neurons are a component of the TRPV1 neuronal population (324), with TRPA1 localized to peptidergic neurons (34). However, a proportion of nonpeptidergic neurons express TRPA1 (150). Mature sensory neuropeptides are produced from pre-pro-hormones synthesized in the neuronal cell body to be transported by active mechanisms to both central and peripheral nerve endings, where they are stored in dense-core vesicles. Neurotrophins, including NGF, regulate the expression of neuropeptides by sensory neurons, as well as the development of the neurons themselves (314).

Two modes of neuropeptide release from peripheral terminals of peptidergic sensory neurons have been identified. The first, induced in vitro by EFS and putatively in vivo by antidromic invasion of a propagated action potential to the terminal region of nerve fibers, results in a tetrodotoxin-sensitive and neuropeptide-mediated response. This pathway offers a neurochemical and ionic basis for the seminal hypothesis of Bayliss (27) and Lewis (182), whereby injury induces a neurogenic flare response, which we now know is mediated by CGRP released from cutaneous perivascular sensory nerves (309). The TRPV1 agonist capsaicin activates the second tetrodotoxin-insensitive pathway of neuropeptide release, providing insights into the function of peptidergic sensory neurons (326). An identical differentiation exists in the activation of neuropeptide release from central endings of primary sensory neurons in response to peripheral noxious stimuli (328). However, while there is no doubt that the release of neuropeptides from peripheral endings of sensory neurons causes neurogenic inflammation, there is uncertainty of the pathophysiological importance of centrally released tachykinins to nociceptive transmission.

1. NKR activation in DRG and spinal cord

NKRs are expressed by neurons of the DRG and dorsal horn of the spinal cord. Primary sensory neurons express both NK_1R and NK_2R that may serve as autoreceptors for SP and NKA released from the same neurons. Thus Ca^{2+}-dependent release of SP enhances TRPV1 activity of DRG neurons in an autocrine manner by activating neuronal NK_1R and NK_2R (298, 360). PKC-ϵ mediates NK_1R-dependent sensitization of TRPV1, probably by phosphorylating TRPV1 and altering channel gating (360). The NK_1R and NK_2R are also expressed by neurons of the dorsal horn, where SP/NKA released from the central projections of primary sensory neurons can activate NKRs on spinal neurons and induce endocytosis (see sect. III*D*). Additionally, mediators released locally within the spinal cord may stimulate tachykinin release and thereby activate spinal NKRs. Thus spinal cytochrome *P*-450 activity can generate *5′,6′*-epoxyeicosatrienoic acid, which activates TRPV4 on DRG neurons to release sensory neuropeptides (134, 344). One consequence of NK_1R activation is the formation of ROS (104, 319). In DRG neurons, the intracellular ROS-mediated pathway augments M-type K^+ channels and thereby counterbalances the ability of SP to sensitize TRPV1 and induce thermal hypersensitivity (190). This mechanism may explain the finding that SP causes hyperalgesia but not acute nociception. However, the sensitizing effect of SP on neurons is not confined to TRPV1, since NK_1R activation also sensitizes P2X3 channels of trigeminal nociceptive, nonpeptidergic neurons (254).

2. Tachykinin-induced transmission in the dorsal spinal cord

A large body of evidence supports the view that SP and the NK_1R contribute to nociception and hyperalgesia in experimental animals **(FIGURE 8)**. Thus *Tac1* deletion attenuates moderate to intense pain and prevents neurogenic inflammation (50), and NK_1R deletion suppresses stress-induced pain (73). However, NK_1R antagonists have failed as analgesics in clinical trials (126), and it is now recognized that of all the neuropeptides released by C-fibers, CGRP is a more likely contributor to pain typical of migraine headaches (31). However, despite the negative results of clinical trials, preclinical studies still support a role for SP and NKRs in the modulation of pain and hyperalgesia, although their role may be more subtle than that previously proposed.

Normally, Aδ and C fibers transmit pain while Aβ fibers signal touch. However, after nerve injury, Aβ fibers can transmit pain. In models of inflammatory and neuropathic pain, SP is upregulated in large-diameter neurons (237). Intraplantar injection of carrageenan leads to spinal production of 12-lipoxygenase-derived hepoxilin A3, which enhances release of SP and contributes to inflammatory hyperalgesia via TRPV1 and TRPA1 (113). In a chronic constriction model of nerve injury, the NK_1R is upregulated in

spinal neurons, which could also amplify pain (71). Studies of *Tac1*-deficient mice reveal a contribution of SP to pain-related behavior induced by intraplantar Formalin and capsaicin, although the contribution of SP is small and transient (29). In a mouse model of peripheral diabetic neuropathy, increased SP expression in the presynaptic sensory fibers innervating lamina I-III has been found in association with enhanced ERK1/2 phosphorylation and induction of other markers of activation of the nociceptive pathway (72).

Intraplantar and intrathecal pretreatment with the NK_1R antagonist CP96345 inhibits mustard oil-induced thermal hyperalgesia and nocifensive behaviors (229). Peripheral inflammation induced by intraplantar Formalin activates a cascade of intracellular events in NK_1R-positive spinal neurons that can lead to hyperalgesia, including PI3K, Akt, and the mammalian target of rapamycin (mTOR) (353), which have previously been observed in DRG and spinal cord neurons (352). Intraplantar carrageenan also induces NK_1R-dependent activation of PI3K and Akt in dorsal horn neurons (64).

Several analgesics have been proposed to act by modulating peptidergic sensory neurons. Among a series of Ca^{2+} channel antagonists, only ziconotide reduced NK_1R internalization evoked by noxious stimulation (328). The vesicular glutamate transporters in $Nav1.8^{Cre}$-positive neurons and SP are required for Formalin-evoked nociception (171). Intrathecal botulinum neurotoxin B inhibits SP release from spinal primary afferent sensory fibers and attenuates the ensuing nociceptive response in models of inflammatory and neuropathic pain (130).

Finally, SP has been linked to intrinsic spinal cord pathways that modulate pain as indicated by the observation that descending facilitation from rostral ventral medial medulla contributes to facilitation of nociceptive transmission and hyperalgesia via NK_1Rs (46). An opposing function has been reported for SP in the locus coeruleus, where NK_1R activation facilitates spinal noradrenergic transmission and attenuates mechanical allodynia after chronic constriction injury (226).

G. Immune System

Tachykinins and their receptors are expressed by multiple immune cells, where they participate in inflammatory responses and immunity.

1. Neutrophils

SP regulates the activity and function of neutrophils and can control their production and infiltration into inflamed tissues. Thus SP stimulates the generation and release of cytokines, chemokines, matrix metalloproteases, and ROS from neutrophils and promotes bacterial phagocytosis (16, 244). Treatment of neonatal rats with capsaicin depletes the levels of SP in bone marrow, yet enhances neutrophil generation and release, which may be related to enhanced expression of *Tac1* and *Tacr1* by bone marrow cells (99). While some reports fail to demonstrate a role for the NK_1R in cutaneous neutrophil recruitment (262), others have shown that NK_1R antagonism attenuates neutrophil influx (295), suggesting selective involvement of the NK_1R under different experimental conditions.

2. Monocytes and macrophages

There is convincing evidence for a role of tachykinins in regulating macrophage function during wound healing, inflammation, autoimmunity, and infection (266, 354). Moreover, NKR expression by macrophages is affected in certain disorders. For example, hypoxia in rats upregulates NK_1R expression by pulmonary macrophages (359), and the NK_1R is upregulated by threefold in macrophages of smokers compared with nonsmokers (26). In a patient with myelofibrosis, which is often associated with inflammatory and neoplastic conditions, increased levels of SP and TGF-β were observed (121). Finally, *Tac4* mRNA, which encodes HK-1, is present in $CD11b^+$ macrophages and $CD11c^+$ dendritic cells in mice, suggesting a role of macrophage- or DC-derived HK-1 in immunoregulation (235).

3. Dendritic cells

Dendritic cells (DC) coordinate the activation of antigen-specific effector and memory lymphocytes, and thereby play a major role in acquired immunity. Both murine and human DCs express functional NK_1R (238) and NK_2R (153), where activation engages the NF-κB pathway and regulates T-cell function. SP and the NK_1R are critically involved in controlling the immunoregulatory role of Langerhans DCs of the mouse skin (205). Human Langerhans cells also express SP (321), which may control T-cell proliferation (176) and regulate other dermal cells, such as keratinocytes. Tachykinins have been implicated in stress-evoked alterations in immunity. Thus acute immobilization stress of mice exacerbated cutaneous infection with Leishmania, which correlated with decreased numbers of epidermal Langerhans cells and greater SP immunoreactivity in skin (289). In the lung, SP regulates pulmonary responses to inhaled antigens by promoting the recruitment of DCs (166).

4. Mast cells

Mast cells are closely associated with SP-positive sensory nerves in many tissues, and there is a bidirectional communication between mast cells and primary sensory neurons, whereby sensory neuropeptides stimulate mast cells, and mast cell products control neuropeptide release. SP induces the release of TNF-α (78) and vascular endothelial growth factor (301) from mast cells, thereby contributing to inflam-

mation, immunity, and angiogenesis. SP also induces Ca^{2+} signals and leukotriene B_4 release from mast cells, but only when co-cultured with fibroblasts (243). However, other studies suggest that SP stimulates the release of preformed mediators, such as histamine, from skin mast cells, but does not affect the transcription of cytokine genes (115). The NK_1R also mediates stress-evoked mast cell degranulation in rat skin (90).

5. Eosinophils

Like mast cells, eosinophils are also found in close proximity to SP-positive neurons in inflammatory diseases (313). However, the pathways that lead to eosinophil activation by tachykinins or the production of tachykinins by eosinophils are poorly understood and may involve epithelial cells (139). In the skin, SP-induced eosinophil infiltration is under the control of mast cell stimulation (239). In a model of allergic asthma in mice, NK_1R activation promotes the release of leukotriene B_4 and eosinophil recruitment (4), and in a guinea pig model of asthma, SP and NKA concentrations correlate positively with higher numbers of eosinophils (267).

6. Lymphocytes

T cells synthesize SP and express the NK_1R during inflammation and infection, and SP regulates T-cell proliferation, cytokine and chemokine release, and killer activity (343). This regulation may be important in the development of autoimmune conditions such as type 1 diabetes, where T cell-mediated death of pancreatic beta cells causes insulin deficiency. For example, pancreatic sensory nerves that express TRPV1 are required for the development of islet inflammation and insulin resistance in diabetes-prone mice, whereas SP delivery to the pancreas reverses insulin resistance, inflammation, and diabetes (277). SP and the NK_1R also regulate release of cytokines in autoimmune encephalomyelitis in mice (280). Both HK-1 and the NK_1R are found in murine B cells and can regulate B-cell development and differentiation, probably in an autocrine fashion (111, 362).

7. Natural killer cells

Natural killer cells of rodents and humans express NK_1R and respond to SP (92). SP enhances cytotoxicity of natural killer cells (177), promoting release of cytokines (186). The protective actions of SP against certain tumors and viruses may depend on activation of natural killer cells and T cells (197).

H. Cancer

Many types of tumor cells express NKRs, and tachykinins from tumor cells or infiltrating nerves or immune cells can influence proliferation, apoptosis, and metastasis of tumor cells in an autocrine, paracrine, or neurocrine manner.

1. Neural cancer

Neural tumors and cell lines often express tachykinins and the NK_1R (119, 179, 219). The NK_1R activates signaling pathways in U373MG cells that are related to growth and survival (see sect. IIIC). Notably, NK_1R blockade reduces basal Akt phosphorylation, which suggests expression of a constitutively active receptor in glioblastoma cell lines (3). HK-1 also causes NK_1R-mediated expression of the matrix metalloproteases by glioma cells, which promotes migration (217). NK_1R antagonists may be a therapy for neural tumors since blockade reduces growth of U373MG cells xenografts (252).

Neuroblastoma is a sympathetic nervous system-derived childhood tumor with a poor prognosis. SY5Y and CHP212 neuroblastoma cell lines express full-length and truncated NK_1R as well *Tac1*, and NK_1R silencing blocks mitogenesis (218). Interestingly, stimulation of P2X7 nucleotide receptor triggers proliferation of neuroblastoma cell lines in part due to SP release, suggesting the existence of an autocrine pathway (272). Thus NK_1R antagonists could be a therapy for neuroblastoma.

2. Colon cancer

Colon cancer is the leading cause of death among gastrointestinal diseases. Multiple observations support the involvement of SP and the NK_1R in proliferation and tumorigenesis in the colonic epithelium. In human NCM460 colonic epithelial cells, SP activates multiple signaling pathways that are linked to proliferation (see sect. IIIC). The NK_1R is expressed in SW-403 colorectal cancer cells, and the NK_1R antagonist L-733,060 blocks proliferation in these cells with and without exogenous SP, suggesting existence of an autocrine mechanism (288). The truncated NK_1R is preferentially upregulated in colonic epithelial cells of patients with ulcerative colitis who develop colonic carcinoma, suggesting a functional role for the truncated NK_1R in transformation (108). The diminished desensitization and endocytosis of the truncated NK_1R could amplify its tumorigenic potential.

The inactivation of tumor suppressor genes, often associated with promoter hypermethylation, can be exploited to identify novel tumor suppressor genes. Importantly, *Tac1* was found to be a frequent target of methylation in primary colon cancers (47%) (216). The intensity of *Tac1* methylation was higher in Dukes A/B than in Dukes C/D cancers and was associated with a small but significant downregulation of *Tac1* mRNA in MSI-high colon tumors, indicating that *Tac1* expression is reduced in colon carcinogenesis through hypermethylation.

3. Pancreatic cancer

Pancreatic cancer has the lowest survival rates of any gastrointestinal malignancy. The NK_1R is upregulated in human pancreatic tumors, especially in advanced tumors with a poor prognosis (101). Several pancreatic cancer cell lines also express the NK_1R, and SP exposure stimulates growth of these cells by an NK_1R-dependent process (101).

4. Breast cancer

Cell lines and malignant breast biopsies have increased levels of *Tac1*, NK_1R, and NK_2R mRNA and produce high levels of SP (35, 310). Studies of human breast carcinoma cells in culture and after xenograft implantation into mice indicate that NK_1R and NK_2R antagonists inhibit growth by a cytostatic mechanism, in both the presence and absence of exogenous SP and NKA (35). The RE-1 silencer of transcription (REST) is a transcriptional regulator that, together with NF-κB, suppresses *Tac1* gene expression in non-neuronal cells (112). REST inhibits *Tac1* in breast cancer cell lines and in primary breast cancer cells, suggesting an important role for REST in the oncogenic function of SP (278). Immunoneutralization of SP strongly inhibits MAPK activation, cell cycle progression, and growth of breast cancer cell lines, while promoting apoptosis (208). Overexpression of the short form but not the full-length NK_1R induces transformation of breast cancer cells, suggesting that targeting the truncated version of this receptor could be a therapeutic strategy, if achievable (257).

5. Lung cancer

Small cell lung cancers express a variety of neuropeptides that can act as autocrine growth factors. NKA has been detected in small cell lung cancers lines (32), and *Tac1* and its products are present in some lung cancers (37). NK_1R antagonism or deletion predisposes the development of bleomycin-associated lung adenocarcinoma (196). Blockade of the NK_1R in mice treated with bleomycin also affects cytoplasmic translocation of the death receptor Nur77 and alters expression of Bcl2 and Bak, which are associated with apoptosis. Consistent with these observations, treatment of mice with aerosolized SP impedes cigarette smoke-induced lung damage and tumor development, and reverses cellular and genetic precursors of emphysema and malignancy (120).

6. Skin cancer

SP and the NK_1R are expressed by melanomas and melanoma cell lines (148, 220). NK_1R blockade inhibits growth and apoptosis of melanoma cells, in the presence or absence of SP, indicating an autocrine SP/NK_1R system (220). While SP and the NK_1R can directly promote melanoma cell growth, NK_1R signaling may indirectly suppress melanoma development in vivo. SP infusion protects mice against melanoma cell growth, and this protection is lost in mice depleted of T and natural killer cells (197). The adoptive transfer of these cells from SP-treated mice also suppressed tumor growth, suggesting that SP can prime the immune system to defend against melanoma tumorigenesis.

V. THERAPEUTIC POTENTIAL OF NEUROKININ RECEPTOR ANTAGONISTS

A. Overview

Preclinical studies implicating tachykinins in diverse pathological processes (see sect. IV) spurred intense efforts by the pharmaceutical industry to develop NKR antagonists. These efforts were highly successful. Over 500 patents for NKR antagonists have been filed during the last 20 years, and some compounds have been evaluated in clinical trials. Despite intensive efforts, there is but one success: approval of the NK_1R antagonist aprepitant (EMEND^T) and its prodrug fosaprepitant (IVEMEND^T) to treat chemotherapy-induced nausea and vomiting (CINV) and postoperative nausea and vomiting **(FIGURE 3*D*)** (118). Other large-scale trials of NKR antagonists for diverse conditions have either failed to demonstrate a benefit compared with existing therapies or placebo, or the results have been equivocal. In other areas, such as pruritus and viral infection, small-scale trials show promise, and large studies are necessary to determine the clinical utility. Finally, there are some indications, such as postsurgical formation of fibrous adhesions and cancer, where studies in animals show that NKR antagonists are effective treatments, but data from humans are lacking.

The challenges of developing NKR antagonists to treat human diseases are formidable, and there are multiple possible reasons for the failure. Many of the disease targets are poorly understood, with unknown etiology and complex pathology. There are obvious caveats in extrapolating the results from experimental animals to humans. Some animal models faithfully recapitulate human disorders, including models of nausea and emesis, where NK_1R antagonists are effective in multiple species, including humans. However, this is not usually the case, and the difficulties of developing an animal model of a human disorder of unknown cause are daunting. The challenge of extrapolating from animal to human studies is complicated by differences in the potency with which certain antagonists interact with NKRs of different species (98). The molecular nature of the interactions between antagonists and receptors can markedly influence efficacy. Considerations include whether antagonists interact with the same sites as the endogenous agonists (orthosteric), or whether they bind to different sites and thereby alter receptor interactions with endogenous tachykinins (allosteric). Whereas orthosteric antagonists shut down signaling, allosteric antagonists fine-tune signaling by

endogenous tachykinins and thereby exert more subtle and perhaps beneficial effects. Whether drugs are neutral antagonists (no effects in the absence of agonist), inverse agonists (decrease basal activity of constitutively active receptors), or insurmountable antagonists (sustained interactions with receptors) can also affect the outcome. The redundancy inherent in the tachykinin system also complicates therapeutic strategies. When one receptor is antagonized, the other two may compensate, and antagonists that target two or more NKRs, or even NKRs and other GPCRs, could be necessary. Interactions between NKRs and other GPCRs adds complexity since receptor heterodimers exhibit distinct signaling properties and likely pharmacology (261). Finally, there are challenges of targeting antagonists, not only to appropriate tissues but also to appropriate subcellular domains. PET imaging with labeled antagonists enables verification of central penetrance, allowing optimization of dosing regimens for effective drug occupancy of brain NK_1Rs. However, the subcellular targeting of antagonists to block NKR signaling from specific subcellular domains has not been given similar attention. NKRs traffic to different subcellular compartments from which they can transmit unique signals with distinct outcomes (223). Thus targeting antagonists to defined subcellular compartments, possibly by lipidation of antagonists, could allow more selective antagonism, with fewer side effects than global antagonism of all signaling events.

Despite these disappointments, the pharmaceutical interest has not waned, as reflected by the numerous and continued efforts to develop antagonists of all three NKRs, and the large number of ongoing clinical trials. This section highlights the successes and failures of clinical trials of NKR antagonists. The development of NKR antagonists has been reviewed (6).

B. CINV

Cytotoxic chemotherapy is the mainstay treatment for cancer, with the debilitating and feared side effects of nausea and vomiting. Although steroids and antagonists of serotonin 5-HT_3 and dopamine D_2 receptors are effective treatments for the initial nausea, they are ineffective against the later phases. The realization that SP is concentrated in brain areas involved in vomiting (9), coupled with the findings that NK_1R antagonists block emesis induced by peripherally and centrally acting emetics (345), provided the impetus for development of NK_1R antagonists as treatments for CINV. Studies in humans substantiated these findings in animals, confirming the predictability of animal models (125). The development of PET tracers for brain imaging in humans verified central penetrance of peripherally administered NK_1R antagonists and facilitated the design of dosing regimens for aprepitant that allowed blockade of >90% of the targeted receptors (118). In patients receiving cisplatin-based chemotherapy, aprepitant combined with the standard treatment of ondansetron (5-HT_3 antagonist) and dexamethasone provided better antiemetic protection than the standard regimen alone (265). Aprepitant was approved in 2003 for oral administration in combination with the standard regimen. Fosaprepitant, a prodrug of aprepitant, was approved for intravenous use to treat CINV in 2008, and aprepitant was approved to treat postoperative nausea and vomiting in 2006. A recent review of multiple trials of NK_1R antagonists for treatment of emesis confirmed their effectiveness but also revealed that use may be associated with increased rates of infection, suggesting that ongoing safety assessment is required (82).

C. Affective and Addictive Disorders

The development of NKR antagonists to treat the stress-related disorders has been reviewed (86). The presence of SP in brain areas involved in emotion and affective functions, and its colocalization with serotoninergic and noradrenergic transmitters that regulate emotions, suggest involvement of tachykinins in affective disorders. Thus immobilization stress in rats induces SP release in the medial nucleus of the amygdala, and central administration of an NK_1R antagonist blocks stress-evoked anxiogenic-like behavior (85). A role for tachykinins in depression was provided by the report that NK_1R blockade suppressed depressive behavior in guinea pigs and that the NK_1R antagonist MK-869 had antidepressant effects in patients with moderate to severe major depression (167). Phase II trials confirmed effectiveness of NK_1R antagonists to treat depression (86). However, a large placebo-controlled multisite phase III trial of patients with major depressive disorder did not confirm the efficacy of MK869, despite a dosing regimen that achieved near-maximum receptor occupancy in the brain (147). Although subsequent trials confirmed these results, the findings are difficult to interpret since the drugs selected as a positive control were also no more effective than the placebo. Recent trials also failed to show beneficial effects of NK_1R antagonists in posttraumatic stress disorder and generalized anxiety (206, 214).

The lack of efficacy of antagonists in depression may relate to patient heterogeneity, assessment of end-points, and pharmacokinetic properties of the antagonists. A comparison of the NK_1R antagonists aprepitant, CP-99994, and ZD6021 suggests that slow functional reversibility is associated with long-lasting in vivo efficacy of NK_1R antagonists, and may be an important determinant of therapeutic potential (189). A recent clinical trial of the efficacy of the NK_1R antagonist orvepitant to treat major depressive disorder supports this proposal (276).

Antagonists of other NKRs may be of value for stress-related disorders. NK_3R antagonists may be effective treatments for schizophrenia and schizoaffective disorder (211), panic attacks, and Parkinson's disease (308). Antagonists of

the NK_2R have been shown to be effective in clinical trials for the treatment of major depression (86).

The NK_1R has been implicated in addiction. NK_1R deletion suppressed alcohol consumption in mice, and the NK_1R antagonist LY686017 blunted the urge of detoxified alcoholic patients to consume alcohol (105). The NK_1R antagonist CJ-11,974 decreases sucrose consumption in rats (322). NK_1R deletion also attenuates the rewarding properties of opioids (225), and the NK_1R antagonist L822429 reduces the acute reinforcing actions of heroin in rats (25). Thus NK_1R antagonists could control several addictive behaviors.

D. Gastrointestinal Disorders

Preclinical studies and small-scale trials in IBS patients support the proposal that antagonists of all three NKRs may be useful therapies for IBS and related disorders. Analysis of two phase II studies of women with diarrhea-predominant IBS treated with the dual NK_1R/NK_2R antagonist DNK333 revealed relief of IBS symptoms compared with placebo (358). A small-scale study of female IBS patients evaluated the effectiveness of the NK_1R antagonist AV608 on anxiety and visceral pain during painful and nonpainful visceral stimulation, with functional magnetic resonance imaging to evaluate brain activity (334). AV608 reduced anxiety and pain ratings, and suppressed activation of the amygdala, hippocampus, and anterior cingulate gyrus during visceral distension. IBS and IBD are associated with decreased binding of an NK_1R PET agonist in the brain, which may reflect release of endogenous tachykinins and NK_1R endocytosis, with a consequent reduction in binding of a PET ligand (135). Considered together, these studies support further exploration of NKR antagonists in IBS.

Although IBD is another potential target for NK_1R antagonists, NK_1R disruption delays healing of the inflamed colon, and antagonists could have the side effect of delayed resolution of colitis (see sect. IV*B*). Preclinical studies indicate that NK_1R antagonists prevent the formation of fibrous adhesions after invasive abdominal surgery (see sect. IV*B*), which remains to be examined in clinical trials.

E. Respiratory Disorders

The contributions of tachykinins to neurogenic inflammation and hyperreactivity of the airways led to the suggestion that NKR antagonists could be treatments for asthma, bronchitis, and COPD (see sect. IV*C*). Although FK888, a peptidic NK_1R antagonist, improved exercise-induced asthma (133), the small molecule NK_1R antagonist CP99,994 had no protective effect on hypertonic saline-induced bronchial constriction or cough in patients with mild asthma (91). Moreover, the dual NK_1R/NK_2R antagonist AVE5883 did not protect asthmatics challenged with allergen, despite its antagonistic activity against inhaled NKA (39). A review of clinical trials of NKR antagonists as a treatment for asthma concluded that while there is a potential of NKR antagonist to decrease airway hyperresponsiveness and improve lung function, their effects on airway inflammation and asthma symptoms have not been studied in sufficient detail to warrant firm conclusions, suggesting the need for large-scale trials (274).

F. Urogenital Disorders

Preclinical studies indicate that tachykinins regulate contractility of smooth muscle in the urinary tract, suggesting that NKR antagonists may be treatments for bladder disorders (see sect. IV*D*). Overactive bladder, which is defined as urgency with or without incontinence, is a common disorder that severely compromises quality of life, and existing treatments with anticholinergic drugs have side effects such as constipation, dry mouth, and dry eyes. A large trial of patients with overactive bladder revealed that although the NK_1R antagonist serlopitant decreased the daily number of urination episodes compared with placebo, it was no more effective than tolterodine (M_2/M_3 muscarinic receptor antagonist) and, unlike tolterodine, serlopitant did not reduce urgency or incontinence relative to placebo (100). Whether the NK_2R, which is expressed by bladder detrusor muscle of all mammalian species studied, is a target for overactive bladder remains to be clinically evaluated.

G. Sensory Disorders of Pain and Pruritus

Extensive preclinical studies implicate SP and the NK_1R in somatic and visceral pain (see sect. IV, *B* and *F*). Although the NK_1R antagonist CP-99,994 reduced postsurgical pain of patients undergoing tooth extraction compared with placebo (81), NK_1R antagonists were subsequently shown to be ineffective in other pain states in humans (126).

A novel strategy to develop improved analgesics has been to generate chimeric molecules composed of an NK_1R antagonist and an opioid agonist, with the rationale that blockade of the algesic NK_1R and activation of the analgesic opioid receptor would be a more effective therapy than targeting the individual receptors. A bifunctional chimeric peptide, [Dmt-D-Arg-Aba-Gly-NMe-30,50-(CF3)2-Bn], is a NK_1R antagonist, a balanced agonist of μ- and δ-opioid receptors, and is algesic in mice (20, 116). The usefulness of such compounds in humans awaits evaluation.

Chronic pruritus accompanies diverse diseases that affect the skin (atopic dermatitis) and other organs (kidney, liver). Preclinical studies indicate that NK_1R antagonism attenuates scratching behavior in a picrylchloride-induced dermatitis model in mice (241). A small study of patients with

untreatable chronic pruritus revealed that the NK_1R antagonist aprepitant caused a marked reduction of itch intensity (320). These studies justify a randomized, controlled clinical trial to fully evaluation the effectiveness of NK_1R antagonists to treat chronic pruritus.

H. Viral and Bacterial Infections and Sepsis

In view of the major role of SP and the NK_1R in regulation of immune functions (see sect. IV*G*), there has been considerable interest in use of NK_1R antagonists to treat viral and bacterial infections. SP levels are elevated in the circulation of HIV-infected individuals (83), and SP enhances HIV replication in mononuclear phagocytes and macrophages by an NK_1R-mediated mechanism (174). This effect of SP may depend on NK_1R-induced expression of the chemokine receptor CCR5, a coreceptor for HIV. A small-scale trial of HIV patients revealed that aprepitant was safe but at the doses used did not show significant antiviral or immunologic improvement (333). Deletion of *Tac1* or antagonism of the NK_1R protects mice from polymicrobial sepsis and airway inflammation induced by cecal ligation and puncture (122, 268). Thus NK_1R antagonist may be effective therapies for bacterial sepsis, which remains to be studied in humans.

I. Cancer

Multiple studies suggest that tachykinins induce proliferation and migration of tumor cells and that NKR blockade has antiproliferative and proapoptotic actions (see sect. IV*H*). Although these studies point to therapeutic utility of NKR antagonists in cancer, this possibility has yet to evaluated in clinical trials.

VI. CONCLUSIONS AND FUTURE PERSPECTIVES

Tachykinins are one of the most intensively studied families of neuropeptides. The early realization that tachykinins regulate important pathophysiological processes, such as inflammation, immunity, and nociception, provided the impetus for these studies. Knowledge of the structure, function, and regulation of tachykinins undoubtedly guided studies of other neuropeptides, confirming the importance of tachykinins as "pioneering neuropeptides." The NKRs are also one of the most studied families of GPCRs. Information about the signaling, trafficking, and properties of NKRs certainly influenced studies of other GPCRs, establishing their position as "pioneering receptors." Preclinical studies implicating involvement of NKRs in diverse disorders, including inflammatory diseases, pain, affective disorders, and vomiting, stimulated the pharmaceutical industry to develop antagonists of all three NKRs. On one hand, these efforts were successful: NKR antagonists are selective, potent, safe, and show good bioavailability. On the other hand, they were a major disappointment: apart from the usefulness of NK_1R antagonists to treat nausea and vomiting after chemotherapy and surgery, large-scale trials failed to support further development of antagonists for the treatment of pain, depression, or bladder disorders.

Has the lack of widespread clinical utility dampened interest in tachykinins, and are NKRs still a viable therapeutic target? Judging from the ongoing reports of involvement of tachykinins in important physiological processes and diseases, including cancer and infection, the tachykinin field remains vibrant. There are also multiple ongoing clinical trials of NKR antagonists, suggesting that interest in industry is sustained. Moreover, recent preclinical studies of the involvement of tachykinins in fibrosis and pruritus support clinical studies of NKR antagonists in other disorders.

The reason why NKR antagonists failed in many clinical trials remains a mystery. Plausible explanations for failure include the use of inappropriate experimental models for preclinical development, the inappropriate selection of patients and end-points for efficacy in clinical trials, and a lack of understanding of the structural basis of antagonist interaction with NKRs. A deeper understanding of the molecular mechanisms of human disease is required for the development of suitable experimental models for evaluation of effectiveness of therapies, including NKR antagonists. This knowledge will inform trials of more homogeneous patient groups and permit the identification of more sensitive and specific end-points. Knowledge of the three-dimensional structure of NKRs in combination with agonists, antagonists, and signaling partners, including G proteins and β-arrestins, will facilitate drug design. The next generation of NKR antagonists could include allosteric modulators, which would permit finer control of signaling by endogenous tachykinins. Since tachykinins often act in concert with CGRP, drugs that simultaneously antagonize tachykinin and CGRP receptors may be more effective, and NKR antagonists in combination with opioid receptor agonists are already being studied for pain. Finally, the subcellular targeting of NKR antagonists to disrupt disease-relevant compartmentalized signaling may lead to increased specificity and fewer side-effects. Clearly, there is much still to learn from the study of tachykinins and NKRs.

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