Scorpion venoms: taking the sting out of lung disease

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Abstract
Scorpion venoms contain specific toxins which block large conductance calcium-activated potassium (BKCa) channels. Use of these toxins has shown that a significant proportion of the action of bronchodilators such as β-agonists, theophylline, and nitric oxide occurs as a result of the opening of BKCa channels. Similarly, these toxins have shown that inhibitors of airway neurotransmission also operate via BKCa channels. Drugs that open BKCa channels may be alternative bronchodilators (possibly “airway selective” and with fewer side effects) as well as inhibitors of pathophysiological neurogenic influences in asthma, chronic coughing and sneezing, and chronic bronchitis.

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The scorpion’s name is Stingaling, a most repulsive, ugly thing, and I would never recommend that you should treat him as a friend.
The Scorpion (Roald Dahl)

Scorpions may be abhorrent, but their venoms could redefine drug treatment of several airway diseases. Certain toxins in the venom of scorpions selectively occlude large conductance calcium-activated potassium (BKCa) or maxi-K channels in the cell membrane. These channels are involved in the control of muscle tone and neurotransmission, and opening them causes membrane hyperpolarisation with consequent relaxation of muscle and inhibition of neurotransmitter release. Closure of the channels takes off the “brakes” and leads to loss of muscle relaxation (or induction of contraction) and loss of neural inhibition. These processes have relevance to asthma by their involvement in bronchodilation and bronchoconstriction, and to additional airway diseases by involvement in neurogenic inflammation of the airways. The use of scorpion venoms to block BKCa channels has increased our understanding of how bronchodilator drugs work, and has indicated novel approaches to drug treatment of airways disease.

Scientific basis
BKCa CHANNELS AND SCORPION VENOMS
The BKCa channel is characterised electrophysiologically by high conductance (100–300 pS), high selectivity for potassium (K+) over other ions, activation by depolarising voltage and intracellular Ca2+ concentration, and has been visualised as a wide-mouthed whirlpool. Toxins from certain scorpions occlude the mouth of BKCa channels and block K+ flux.1 One such toxin was isolated from the venom of the five-keeled gold scorpion (Leirus quinquenquenstriatus) and was named charybdotoxin after Charybdis, a female sea monster in Greek mythology who created whirlpools by sucking in water. A more selective BKCa channel blocker was derived from the Indian scorpion Mesobuthus tamarus and was named iberiotoxin to acknowledge the contribution of Spanish scientists to its discovery. Charybdotoxin and iberiotoxin have been used to investigate the role of BKCa channels in the control of airway smooth muscle tone and neurotransmission: reversal by the toxin of bronchodilation or neural inhibition indicates the involvement of endogenous BKCa channels in the initial response.

BRONCHODILATION
β2-Adrenoceptor agonist drugs induce bronchodilation. Biochemical and electrophysiological studies have shown that β2-agonists open BKCa channels on airway smooth muscle cells via intracellular signal transduction mechanisms which are dependent and independent of cyclic 3’,5’-adenosine monophosphate (cAMP) (figure).2 Both mechanisms are initiated by β-agonist occupation of β2-adrenoceptors on the airway smooth muscle cells, leading to activation of a stimulatory guanine nucleotide binding protein (Gs). In the cAMP-dependent pathway Gs triggers an enzyme cascade which leads to the phosphorylation of a number of cellular substrates involved with relaxation (figure). Bronchodilation induced by nitric oxide (NO) is the result of a similar enzymatic cascade. One of the phosphorylation events is opening of BKCa channels. In the cAMP-independent pathway the BKCa channel is opened directly by coupling to Gq.
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Use of scorpion venom toxins to investigate bronchodilator drug action and neural inhibition. Left panel: Interaction of β2-agonist drug (for example, salbutamol) with β2-adrenoceptors (β2AR) activates a stimulatory guanine nucleotide binding protein (Gs) which initiates adenylyl cyclase (AC) to convert adenosine triphosphate (ATP) to cAMP. Cyclic AMP activates protein kinase A (PKA) andPKG which phosphorylate cellular substrates involved with relaxation. A similar enzymatic cascade is seen after activation of guanylyl cyclase by, for example, nitric oxide (NO). Phosphorylation processes relevant to relaxation are inhibition of myosin light chain kinase (MLCK), decreased intracellular calcium, inhibition of phosphoinositide hydrolysis (PI), and opening of large conductance calcium-activated potassium (BKCa) channels. BKCa channels may also be coupled directly to Gs. Right panel: Depolarisation of nerves induces neurotransmitter release and neurotransmission. Interaction of agonists which inhibit neurotransmission (A1, e.g. morphine) with specific receptors (Ri, e.g. μ-opioid) inhibits neurotransmitter release via opening of BKCa channels leading to membrane hyperpolarisation. Neuronal BKCa channels appear to be coupled directly to a G protein, as yet not specifically defined (Gα, possibly Go). BKCa channel-selective scorpion venom toxins inhibit β2-adrenoceptor induced relaxation of airway smooth muscle and neurogenic responses, demonstrating the involvement of these channels. Drugs which open BKCa channels may be novel bronchodilators and inhibitors of airway neurotransmission in pathophysiological conditions of the airways.

In functional studies in vitro, iberiotoxin has been shown to inhibit relaxation of guinea pig tracheal smooth muscle induced by the β-stimulatory isoprenaline and salbutamol and by the NO donor sodium nitroprusside. Charybdotoxin reverses relaxation induced by isoprenaline and theophylline in human bronchi. These observations indicate that BKCa channels are involved in smooth muscle relaxation induced by different bronchodilator drugs.

**INHIBITION OF NEUROTRANSMISSION**

Activation of at least eight receptor types - including μ-opioid, α2-adrenergic, and neuropeptide Y - inhibits bronchoconstriction and airway mucus secretion and plasma exudation induced by neural stimulation. Charybdotoxin blocks the inhibitory effects of these receptors on cholinergic nerves and capsaicin-sensitive sensory nerves in guinea pig and human airways in vitro, which indicates that BKCa channels are a common endogenous mechanism for the regulation of nerve activity in the airways.

**Therapeutic potential**

Inhaled β-agonists are effective bronchodilators and are vitally important in the management of asthma. However, because of concerns over the safety of β-agonists and because of the search for new drugs by pharmaceutical companies, novel bronchodilators are being sought.

**Airway nerves**

Airway nerves may contribute to the pathophysiology of a number of bronchial diseases - for example, sensory nerves may be involved in asthma, in chronic coughing and sneezing and, perhaps, in chronic bronchitis. In asthma there is also dysfunction of cholinergic nerves which could exaggerate cholinergic bronchoconstriction and mucus secretion.

Thus, drugs which open BKCa channels may be useful both as novel bronchodilators (possibly with reduced side effects) and as inhibitors of nerve dysfunction in asthma, chronic coughing and sneezing, and chronic bronchitis. To avoid cardiovascular problems associated with non-selective opening of K+ channels - for example, postural hypotension - "airway-selective" K+ channel activators are being sought. A number of drugs which will open BKCa channels are becoming available of which one, NS 1619, is highly selective for this channel in airway smooth muscle and inhibits neurogenic airway mucus secretion.

**Conclusions**

Scorpion venoms have proved invaluable pharmacological research tools in evaluating the role of BKCa channels in the control of airway smooth muscle tone, in the action of bronchodilator drugs, and in regulation of neurotransmission. As a consequence, new BKCa channel activator drugs are being developed.
which may be of value in the treatment of a number of different airway diseases.

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