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Nicotinic acetylcholine receptors in the autonomic control of bladder function

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Abstract

Micturition is achieved through complex neurological mechanisms involving somatic, autonomic and central components. This article briefly reviews recent findings on the autonomic control of urinary bladder function. Neuronal nicotinic acetylcholine receptors mediate fast synaptic transmission in autonomic ganglia, and activation of nicotinic receptors in parasympathetic bladder neurons produces contraction of the destrusor muscle. Autonomic ganglia contain transcripts for the α_3, α_4, α_5, α_7, β_2 and β_4 nicotinic subunits, which can assemble to form multiple nicotinic receptor subtypes, but the exact nicotinic receptor subunit composition in bladder ganglia is unknown. Mutant mice lacking the α_3 or the β_2 and the β_4 nicotinic subunits have enlarged bladders with dribbling urination and develop urinary infection and bladder stones. Bladder strips from α_3 null mice do not respond to nicotine but contract when stimulated with a muscarinic agonist or electric field stimulation. Mice lacking the β_2 subunit have no overt bladder phenotype, and their bladders contract in response to nicotine. Surprisingly, bladder strips from β_4 mutant mice do not respond to nicotine despite the absence of major bladder dysfunction in vivo. These findings suggest that nicotinic receptors containing the α_3 and the β_4 subunits are necessary for normal bladder function.

Keywords: Nicotinic receptor; Nicotinic receptor subunit; Bladder; Autonomic dysfunction; Knockout mice

1. Introduction

The autonomic nervous system has a prominent influence on both the filling and voiding phases of micturition. The bladder acts like a reservoir during the filling phase, and as a pump during the voiding phase (De Groat, 1998; De Groat and Booth, 1980). The urethra is closed during filling to prevent involuntary leakage of urine, but opens during voiding. Micturition is achieved by a complex neuromuscular apparatus where the bladder smooth muscle is influenced by ganglionic, spinal and supraspinal mechanisms (De Groat, 1998; De Groat and Booth, 1980; De Groat et al., 1996, 1999). Bladder filling is primarily controlled by sympathetic inputs, whereas voiding is under parasympathetic influence. During filling, distension of the destrusor muscle in the body of the bladder is achieved by suppression of the excitatory efferent parasympathetic influence (through neural reflexes) and the concomitant closure of the bladder outlet through sympathetic-mediated tonic contractions of the base of the bladder and the urethra. Afferent sensory inputs from the bladder and a sustained excitatory parasympathetic discharge underlie the voiding process (Applebaum et al., 1980; Maggi et al., 1987, 1988; Elbadawi, 1988).

The autonomic ganglia that provide innervation to the urinary tract receive inputs from preganglionic fibers located in the lumbar and sacral regions of the spinal cord. Postganglionic sympathetic neurons innervating the bladder release norepinephrine, which binds to both α and β adrenoreceptors (De Groat and Booth, 1980; Maggi and Meli, 1982). Norepinephrine binding to β_2 adrenoreceptors promotes relaxation of the destrusor muscle whereas binding to α_1 and α_2 adrenoreceptors produces contraction of the base of the bladder and the urethra. Postganglionic parasympathetic neurons innervating the bladder release acetylcholine and ATP, producing contraction via M_3 and M_2 muscarinic receptors and via P_{2x} and possibly P_{2Y1} purinoreceptors (Burnstock et al., 1978; Kasakov and Burnstock, 1982; Acevedo and Contreras, 1985; Maggi et al., 1985; Hoyle and Burnstock, 1993; Obara et al., 1998;

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Hegde and Eglen, 1999). In the urinary tract of rats and mice, the majority of the postganglionic projections originate from the major pelvic ganglion and some small adjacent ganglia (Purinton et al., 1973; Keast et al., 1989). The bladder wall also contains a small number of intramural ganglia (Chesher, 1967; Ekstrom and Elmer, 1980; Elbadawi, 1982; Elbadawi and Schenk, 1966; Xu et al., 1999a,b) that are not easily detected and whose presence might be age-dependent (Carpenter and Rubin, 1967; Alm and Elmer, 1975; Alian and Gabella, 1996).

Neuronal nicotinic acetylcholine receptors play a key role in the control of bladder function mediating fast synaptic transmission between preganglionic and postganglionic bladder neurons. Wherever it has been examined, autonomic ganglia express more than one nicotinic receptor subtype (Listerud et al., 1991; Mandelzys et al., 1994; Rust et al., 1994; Poth et al., 1997), and bladder ganglia likely express multiple nicotinic receptor subtypes. Transcripts for the α_3, α_4, α_5, α_7, β_2 and β_4 subunits are found in peripheral neuronal cells, with α_3 and β_4 having higher expression levels (Listerud et al., 1991; Mandelzys et al., 1994; Rust et al., 1994; Poth et al., 1997; Zhang et al., 1998; Xu et al., 1999a). Because the subunit composition of native nicotinic receptors mediating synaptic transmission in the peripheral nervous system is unknown, the relative contribution of each nicotinic subunit remains elusive. In the past year, the analysis of mutant mice for nicotinic receptor subunits has provided evidence that nicotinic receptors containing the α_3 and the β_4 subunits are important for normal bladder function (Xu et al., 1999a,b). This article briefly reviews the results obtained in mice deficient in the α_3 ($\alpha_3-/-$) and the β_2 and β_4 ($\beta_2-/-\beta_4-/-$) nicotinic subunits.

2. Results and discussion

Absence of the α_3 nicotinic receptor subunit caused autonomic dysfunction at different organ system levels with unexplained lethality in the first week of life. The $\alpha_3-/-$ mice displayed impaired growth, mydriasis, and bladder enlargement as their most relevant phenotypic traits (Xu et la., 1999a). Severe bladder distension was visible within 2 days after birth, and as time progressed, the mice developed overflow incontinence. In the animals that survived more than 1 week, urine was often cloudy and infected with bacteria, and sometimes bladder stones completely filled the bladder. Similarly, $\beta_2-/-\beta_4-/-$ mice had severe autonomic dysfunction, growth retardation, premature death, bladder distension and dribbling urination often accompanied by urinary bacterial infection (Xu et al., 1999b). Conversely, the $\beta_2-/-\beta_4+/+$ and $\beta_2+/+\beta_4-/-$ single mutants grew to adult age and had no overt bladder dysfunction.

Bladder dysfunction in the $\alpha_3-/-$ and $\beta_2-/-\beta_4-/-$ mice was consistent with severe reduction or lack of contractility at the level of the bladder. Impaired smooth muscle contractility would prevent voiding and cause the bladder to progressively enlarge. After reaching maximal bladder distensibility, urine would start to dribble, possibly due to a simultaneous increase in intravescical pressure and reduced sympathetic tone of the sphincters. Infection with urea metabolizing bacteria would finally lead to the formation of stones in older animals.

Several studies indicate that in newborn animals, micturition occurs in response to a short-latency somatovescical spinal reflex activated when the mother licks the perineal area (De Groat and Booth, 1980). In particular, such a mechanism is essential for survival of pups less than 15 days old (De Groat and Booth, 1980; Henning, 1981; Sato et al., 1983). Thereafter, a supraspinal reflex develops that subserves micturition in normal adult animals (De Groat and Booth, 1980; Sato et al., 1983). Based on this evidence, the bladder phenotype of the $\alpha_3-/-$ and $\beta_2-/-\beta_4-/-$ mice between postnatal days 2 and 5 is likely to reflect anomalies of the spinal portion of the micturition reflex. Because activation of postganglionic nicotinic receptors leads to neurotransmitter release onto the target organ, bladder ganglia are candidate sites for the bladder dysfunction of $\alpha_3-/-$ and the $\beta_2-/-\beta_4-/-$ mice. To test our hypothesis, we measured contractile responses to nicotine in bladder strips from 2- to 5-day-old mutant mice. That experimental paradigm provides information about the function of intramural ganglia, because postganglionic fiber tracts originating in the pelvic ganglion are severed during isolation of the bladder.

Fig. 1 summarizes the contractile responses to nicotine obtained in bladder strips from $\alpha_3-/-$, $\beta_2-/-\beta_4+/+$, $\beta_2+/+\beta_4-/-$, and $\beta_2-/-\beta_4-/-$ mice,

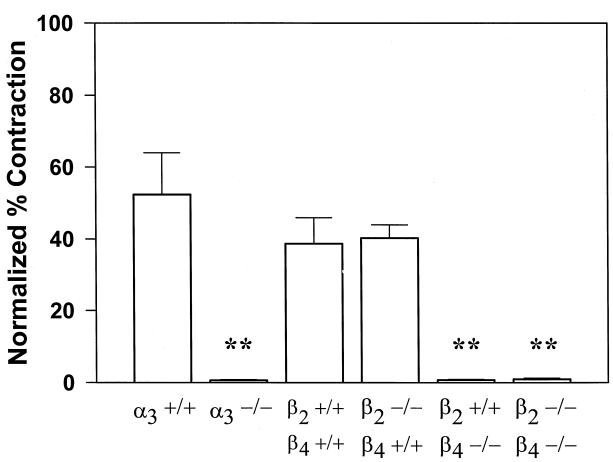

Fig. 1. Reduced bladder contraction in response to nicotine in $\alpha_3-/-$, $\beta_2-/-\beta_4-/-$, $\beta_2+/+\beta_4-/-$ and $\beta_2-/-\beta_4-/-$ mice. Response to nicotine in bladder strips from $\alpha_3+/+$ ($n=10$), $\alpha_3-/-$ ($n=8$), $\beta_2+/+\beta_4+/+$ ($n=4$), $\beta_2-/-\beta_4+/+$ ($n=18$), $\beta_2+/+\beta_4-/-$ ($n=17$) and $\beta_2-/-\beta_4-/-$ ($n=12$) mice, respectively. Data represent the contractile response to nicotine as a percentage of that to carbamylcholine ($\pm$S.E.M.). (**) indicates P values <0.01. Adapted from Xu et al. (1999a,b).

respectively (Xu et al., 1999a,b). Nicotine was added as a single dose (0.1 mM) directly to the organ bath and was washed out after a maximum contraction was recorded (see Xu et al., 1999a). Normally, bath-applied nicotine activates nicotinic receptors that then excite the intramural neurons to release acetylcholine onto muscarinic receptors on the bladder smooth muscle, causing contraction. To compare nicotine responses from different animals, we normalized the nicotine data to the contraction induced by a single dose (0.1 mM) of the mixed muscarinic and nicotinic agonist, carbamylcholine. The contractile response to nicotine in wild-type animals usually corresponds to 40–55% of the maximal contraction induced by carbamylcholine. Fig. 1 illustrates how nicotine failed to produce a strong smooth muscle contraction in both the $\alpha_3 -/-$ and the $\beta_2 -/- \beta_4 -/-$ mice. The contractile response for both genotypes corresponded to 2% of the carbamylcholine-induced response, suggesting that autonomic transmission is impaired in the bladder of these mutant mice. The nicotine responses of single mutant $\beta_2 -/- \beta_4 +/+$ mice were superimposable with those of their wild-type littermate controls. Surprisingly, bladder responses were significantly reduced in the $\beta_2 +/+ \beta_4 -/-$ mice, despite the absence of bladder distension and urine retention. Nicotine responses in the $\beta_2 +/+ \beta_4 -/-$ mice were similar to those observed in the $\alpha_3 -/-$ and the $\beta_2 -/- \beta_4 -/-$ mice, and corresponded to 3% of the contraction elicited by 0.1 mM carbamylcholine.

Despite a poor response to nicotine, the ability of bladder smooth muscles to respond to direct muscarinic stimulation was preserved in the $\alpha_3 -/-$, the $\beta_2 +/+ \beta_4 -/-$, and the $\beta_2 -/- \beta_4 -/-$ mice. Furthermore, when intramural nerve terminals were depolarized via electric field stimulation, neurotransmitter release onto the smooth muscle produced a contraction that was equal to or even stronger than that observed in the wild-type controls (Xu et al., 1999a,b). In all the experiments that were performed, the contractile responses of bladder strips from $\beta_2 -/- \beta_4 +/+$ mice were comparable to those obtained in their littermate controls. These results, together with histochemistry results showing normal intramural innervation, indicate that bladder dysfunction is produced by a defect in ganglionic nicotinic receptors. The α_3 and the β_4 subunits seem to be the most important contributors to these nicotinic receptors.

It is interesting that bladder strips from $\beta_2 +/+ \beta_4 -/-$ mice do not respond to nicotine, but the animals do not develop bladder distension. The following explanation seems most likely. Our data are consistent with intramural parasympathetic ganglion cells predominantly expressing $\alpha_3\beta_4$-containing nicotinic receptors. However, the bladder also receives substantial innervation from the pelvic ganglion, which is located outside the bladder and was not studied in our experiments. Neuronal nicotinic receptors expressed in pelvic neurons may contain combinations of $\alpha_3\beta_4$, $\alpha_3\beta_2$ and $\alpha_3\beta_2\beta_4$ subunits, possibly with additional subunits. When only one of the two β subunits is absent, nicotinic receptors containing the remaining β subunit would be sufficient to ensure enough acetylcholine release onto the smooth muscle to sustain micturition. Compensatory mechanisms involving nicotinic receptor upregulation or assembly of receptors with different subunit compositions may also explain the results obtained in the $\beta_2 +/+ \beta_4 -/-$ mice.

The results obtained with the $\alpha_3 -/-$ and the $\beta_2 -/- \beta_4 -/-$ mice provide information that will help delineate the complex mechanisms governing bladder function. A detailed investigation of the structure and activity of bladder ganglia is far from complete. As a consequence, bladder disorders as common as urinary incontinence are not fully understood, and the therapeutic approaches are not always successful. The phenotype produced by the absence of α_3 or β_2 and β_4 also offers new perspectives for the study of human disorders characterized by multiorgan autonomic dysfunction. An example is the autosomal recessive megacystis microcolon intestinal hypoperistalsis syndrome (MMHIS) (Anneren et al., 1991), which is reminiscent of the in vivo phenotype of the $\alpha_3 -/-$ and the $\beta_2 -/- \beta_4 -/-$ mice.

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