Do β3-adrenoceptor agonists cause urinary bladder smooth muscle relaxation by inhibiting acetylcholine release?

Focus on “Inhibition of cholinergic neurotransmission by β3-adrenoceptors depends on adenosine release and A1 receptors activation in human and rat urinary bladder”

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β3-Adrenoceptor (β3-AR) agonists such as mirabegron are a novel drug class to treat the overactive bladder syndrome (OAB) (4). Classic concepts have assumed that β3-AR agonists work by directly acting on a β3-AR located in the plasma membrane of smooth muscle cells in the detrusor of the urinary bladder (15). Recently, this concept has been critiqued increasingly (7) because mirabegron, the only β3-AR agonist currently in clinical use, has an EC\textsubscript{50} for
relaxation of isolated detrusor strips of 588-776 nM in humans and 288-5113 nM in rats with comparable values in mice and monkeys (12) but maximum plasma levels upon therapeutic dosing reach only 83-167 nM (9). On the other hand, several cell types potentially involved in the control of detrusor smooth muscle tone have been reported to express β3-AR and/or be responsive to β3-AR agonists. These include the urothelium, afferent nerves, interstitial cells of Cajal, blood vessels supplying the urinary bladder and the major pelvic ganglion (7, 12, 14). However, for none of these it has been shown that mirabegron works at concentrations of about 100 nM. Therefore, uncertainty remains which cell type or combination of cell types mediates detrusor smooth muscle relaxation in response to systemic administration of β3-AR agonists.

A related receptor, the β2-AR facilitates release of noradrenaline from sympathetic nerve endings but its effects on acetylcholine release from parasympathetic nerve endings have remained controversial as both enhancing and inhibiting effects have been reported. Recently, it has been shown that β3-AR agonists can inhibit not only electrical field stimulation-induced contraction of isolated human detrusor strips but also acetylcholine release in this preparation (6). Sources of acetylcholine in the urinary bladder include not only parasympathetic nerves but also non-neuronal release from the urothelium (16). In the preparation studied by D’Agostino, acetylcholine release occurred primarily from nerves as it was concentration-dependently inhibited by tetrodotoxin. Importantly, mirabegron had high potency for inhibition of contraction (EC50 123 nM) and of acetylcholine release (EC50 129 nM) in that study. While the high potency of mirabegron for relaxation may refute the non-smooth-muscle hypothesis (7), the observation that inhibition of contraction and of acetylcholine release exhibited similar potency raised the possibility that the latter may be the cause of former.
A paper in the present issue of the Journal confirms that β3-AR agonists can substantially inhibit electrical field stimulation-induced acetylcholine release from an isolated human detrusor preparation, and that such inhibition is prevented by a selective β3-AR antagonist (13). Notably, mirabegron was effective in this model already at a concentration of 100 nM. As β3-AR, similar to other β-AR subtypes, couple to stimulation of cAMP formation and cAMP is degraded to adenosine, Silva et al. tested whether adenosine may mediate inhibition of acetylcholine release observed in the presence of β3-AR agonists. Indeed, isoprenaline and mirabegron increased extracellular adenosine concentrations in the detrusor strips.

Antagonism of A₁ adenosine receptors by 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) or blockade of the equilibrative nucleoside transporters with dipyridamole or S-(4-nitrobenzyl)-6-thioinosine (NBTI) prevented inhibition of acetylcholine release, suggesting that it did not necessarily occur via a β3-AR located in the nerve ending but rather indirectly by intermediate formation of adenosine subsequent activation of A₁ inhibitory receptors. The physiological relevance of this observation was demonstrated by in vivo experiments in anesthetized rats, in which DPCPX, dipyridamole and NBTI reversed the decrease in voiding frequency caused by isoprenaline. To further substantiate the mechanistic basis of an indirect inhibition of acetylcholine release, the authors also performed immunohistochemical staining of β3-AR and vesicular acetylcholine transporter, a marker of cholinergic neurons. Given the limited target selectivity of many β3-AR antibodies, they used a previously proposed approach (2) to concomitantly stain by multiple antibodies targeted against different epitopes of the β3-AR. However, little colocalization of β3-AR and acetylcholine transporter was observed, suggesting that the receptor is primarily expressed by cells other than parasympathetic nerve endings.

The findings of D’Agostino et al. (6) and of Silva et al. (13) in combination make a convincing point that β3-AR agonists, including therapeutically achieved concentrations of
mirabegron can inhibit neuronal acetylcholine release in human detrusor. They support a
hypothesis that (indirect) inhibition of acetylcholine release may be the mechanism for
detrusor smooth muscle relaxation. However, it is not fully clear yet whether such inhibition
of acetylcholine release indeed occurs exclusively indirectly via adenosine formation and A₁
adenosine receptor activation or may also involve a neuronally expressed β₃-AR. Thus,
another group of investigators has recently also reported immunohistochemical studies of β₃-
AR and vesicular acetylcholine transporter in the human bladder (5). These authors have used
the same approach of concomitant labeling with multiple antibodies targeting different
epitopes in the β₃-AR, actually even the same antibodies. Despite using the same antibodies,
Coelho et al. report very different findings. In their hands, β₃-AR colocalized not only with
the acetylcholine transporter but also with β3-tubulin, another marker of neurons; such
colocalization would allow for a direct effect of β₃-AR agonists on cholinergic neurons. The
two studies also differ in other ways: Silva et al. have detected β₃-AR in human bladder in
smooth muscle fibers and, to a lesser extent, in urothelium and sub-urothelium (13). In
contrast, Coelho et al. (5) detected β₃-AR primarily in nerve fibers in the mucosa and
muscular layers of the bladder but not in urothelium or smooth muscle. The cholinergic fibers
expressing β₃-AR were mostly found in the sub-urothelium, where they mingled adrenergic
fibers (staining positive for tyrosine hydroxylase) and peptidergic fibers (staining positive for
calcitonin gene-related peptide). Earlier studies based on validated antibodies have reported
β₃-AR expression to a greater extent in urothelium than smooth muscle of the human bladder,
and also in sub-urothelial myofibroblast-like cells, intramural ganglions, Schwann cells and
intramural nerves (10). However, the latter study did not explore whether the nerves
expressing β₃-AR were sympathetic, parasympathetic and/or peptidergic. Thus, various
investigators using similar approaches and antibodies have obtained at least in part different
results with regard to the localization of β₃-AR in the human urinary bladder. The reasons for
these divergent results are not fully clear. However, it should be noted that sensitivity and
specificity of immunohistological staining depend not only on the antibody being used but
also on other factors including thickness of slices, fixation and denaturalization protocols (8)
and type of microscopy. Therefore, it is possible that rather minor differences in experimental
protocol may have led to major differences in staining pattern, making it difficult to determine
in which cell types within the urinary bladder β3-AR are expressed at the protein level. As the
Coelho and the Silva groups work within the same institution, a collaborative study between
them appears an obvious approach to settle this issue.

The physiological question is whether and to which extent parasympathetic nerves contribute
to OAB symptoms and are a target for its treatment. The original Eastham argument (7) is that
mirabegron effects on smooth muscle occur at concentrations considerably exceeding those
achieved in patients after administration of therapeutic doses. Inhibition of neuronal
acetylcholine release is the only cellular response to mirabegron in the human urinary bladder
that has been consistently reported to occur at concentrations that are found in plasma of
mirabegron-treated subjects (6, 13). On the other hand, D’Agostino et al. also report such low
mirabegron concentrations to cause smooth muscle relaxation (6); why they found much
higher potency of mirabegron in isolated human detrusor than several previous studies (12)
remains to be determined. Of note, the potency of β-AR agonists to cause relaxation of
detrusor smooth muscle can markedly differ depending on the stimulus used for inducing tone
in the preparation (3).

The conundrum with the prejunctional parasympathetic nerve hypothesis is that
parasympathetic nerves are physiologically active in the voiding phase of the micturition
cycle whereas OAB symptoms occur in the storage phase; acetylcholine released during the
storage phase, e.g. by bladder distension is believed to largely come from non-neuronal
sources such as the urothelium (11). To settle such questions additional studies are required
that determine the potency of β3-AR agonists not only for relaxation of smooth muscle and
inhibition of acetylcholine release but also for functional responses of other cell types
implicated in the regulation of detrusor tone. It is not an improbable hypothesis that
improvement of OAB symptoms after administration of β3-AR agonists is the net effect of
concomitant action on multiple cell types, including urothelium, afferent nerves and
interstitial cells. Of note, as highlighted by the prejunctional findings of Silva et al., release of
ATP and/or adenosine could act as intermediate player in all of these cell types; after all, the
original concept of ATP as a co-transmitter was developed by Burnstock largely based on
findings in the urinary bladder (1).

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REFERENCES


