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SIGNAL TRANSDUCTION MECHANISMS OF M2 AND M3 MEDIATED BLADDER CONTRACTILE RESPONSE TO CHOLINERGIC STIMULATION

Hypothesis / aims of study

We have previously shown that contraction of hypertrophied bladders is mediated at least partially by the M_2 receptor subtype. Additionally we have shown that in these hypertrophied bladders, the M_2 and M_3 receptor signal transduction mechanisms synergize to mediate the contractile response. This same synergy is seen in thapsigargin treated normal bladders. In normal bladders no synergistic response is seen. This led to our hypothesis that either the M_2 or the M_3 receptors can activate signal transduction pathways resulting in contraction. However, in normal bladders, the M_2 mediated pathway is inhibited by some component of the M_3 pathway. Inhibition of a signal transduction pathway involved in mediating the contractile signal could result in:

1. a decrease in the maximal contractile response to carbachol,
2. a decrease in the potency of carbachol, or
3. a change in the potency of subtype selective antagonists to inhibit carbachol contractions.

This is based on the assumption that either the M_2 mediated component or the M_3 mediated component of contraction could be dominant. For instance, the M_2 receptor may dominate in mediating contraction when a second messenger system activated by the M_3 receptor is inhibited. The M_3 receptor dominates in mediating the contractile response in normal bladders and the M_2 receptor appears to dominate in mediating contraction in hypertrophied bladders.

Study design, materials and methods

We determined the effects of protein kinase, rho kinase (ROCK) and phospholipase inhibitors on the above parameters in an effort to delineate the signal transduction mechanism activated by the M_2 and M_3 receptor subtypes. Since the available phospholipase and protein kinase inhibitors are not completely selective for only one isoform of these enzymes, several different chemical agents were used at 3 different concentrations. For these experiments, after equilibrating the strips to the bath, the enzyme inhibitors were exposed to the tissues for 30 minutes before performing cumulative carbachol concentration response curve. Each strip was only exposed to one enzyme inhibitor concentration and thus one concentration response curve. The isoform specificity of these inhibitors in shown in Table 1.

Table 1.

INHIBITOR	TARGETS (conc used)
ET-18-OCH3 (ET)	PI-PLC (10, 30, 100 µM)
D-609	PC-PLC (100, 300, 1000 µM)
HA-1077 (HA)	ROCK, PKA, PKG (10, 30, 100 µM)
H7	PKA, PKG, PKC (10, 30, 100 µM)
H89	PKA (1, 3, 10 µM)
Chelerythrine (CHEL)	PKC (1, 3, 10 µM)

Results

The results of all the enzyme inhibitors on carbachol potency and efficacy and darifenacin affinity is summarized in table 2. Asterisks indicate statistically significant differences.

Table 2									
Inhibitor	µM	DAR Affinity	%Carb/KCL	EC50 shift	Inhibitor	µM	DAR Affinity	%Carb/KCL	EC50 shift
ET	0	8.5	107		H7	0	8.3	120	
	10	8.1	78	2		1	7.4	76*	0
	30	8.3	78	2		3	8.3	79*	0
	100	7.7	76	10*		10	8.7	63*	0
D609	0	8.7			H89	0	8.5	120	
	10	8.7	50*	0		3	8.7	79	0
HA	0	8.5	120			10	8.5	65	0
	10	8.8	76*	2	CHEL	0	9.7	120	
	30	8.3	80*	3		1	8.9	76	0
	100	7.6	71*	10*		3	9.5	73	0
						10	9.3	83	0

Interpretation of results

Inhibition of PI-PLC by ET reduces the carbachol potency. The resulting contraction is mediated by the M_2 receptor subtype as the affinity of darifenacin for inhibition the carbachol induced contraction in the presence of 100 µM ET is 7.7. Inhibition of PC-PLC by D609, reduced the maximal contraction with no effect on carbachol potency. PC-PLC is not likely involved in the M_3 mediated signal transduction pathway because the darifenacin affinity remains high (9.1) consistent with M_3 receptors following D609 treatment.

Inhibition of either PKC with CHEL up to 10 µM or PKA with H89 up to 10 µM had no effect on the carbachol potency, the carbachol maximal contraction or the affinity of darifenacin. This suggests that neither PKC nor PKA are involved in transducing the M_3 mediated bladder contraction. However, this conclusion assumes that there are no redundant signal transduction pathways. Inhibition of PKA, PKG, and PKC with H7 reduces the maximal carbachol induced contraction with no effect on the carbachol potency. The darifenacin affinity indicates that the M_2 receptor mediates contraction at low concentrations of H7 (1 µM), while at higher concentrations the M_3 receptor mediates contraction. This result suggests that either PKG is involved in mediating the M_3 signal transduction mechanism or that either of these kinases can substitute for one other, but when all three are inhibited, maximal contraction is inhibited.

Inhibition of rho Kinase with HA inhibits the maximal contraction and reduces the carbachol potency. Rho Kinase is likely involved in mediating the M_3 signal because the affinity of darifenacin is reduced to 7.6 in the presence of 100 µM HA which is consistent with M_2 receptors mediating the response. ROCK phosphorylates myosin light chain phosphatase thus preventing myosin light chain dephosphorylation induced by myosin light chain kinase. Inhibition of ROCK with HA prevents the removal of the myosin light chain phosphatase brake on contraction. Thus the M_2 contractions that we observed with high concentrations of HA may be mediated by mechanisms unrelated to myosin light chain phosphorylation such as thin filament regulated contractile events.

Concluding message

Multiple, redundant, overlapping and interacting contractile signal transduction pathways exist in the bladder that mediate both M_2 and M_3 receptor activated contraction. These pathways and their interaction with each other are altered by bladder hypertrophy and aging. Understanding these alterations will pave the way for the development new classes of highly selective drugs to restore normal bladder function.

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