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EFFECT OF DENERVATION ON MUSCARINIC RECEPTOR CONTRACTILE SIGNAL TRANSDUCTION MEDIATED BY PHOSPHOLIPASES AND PROTEIN KINASES IN THE RAT BLADDER

Hypothesis / aims of study

Whereas the M_3 muscarinic receptor subtype mediates normal bladder contraction, the M_2 receptor subtype mediates contraction of the denervated bladder. We used inhibitors of enzymes thought to be involved in muscarinic receptor mediated contractile signal transduction to determine denervation induced changes in muscarinic receptor subtype excitation contraction coupling mechanisms in the rat bladder.

Study design, materials and methods

In-vitro muscle bath contractility assays were performed on normal rat bladders and bladders from rats denervated for 3 days by bilateral major pelvic ganglion electrocautery. After determining maximal contractile responses to 120 mM KCl, strips were exposed to phospholipase inhibitors (ET-18-OCH3, D-609 and neomycin), protein kinase inhibitors (H7, H89 and chelerythrine), and Rho kinase inhibitors (HA-1077 and Y-27632) at 1, 3 and 10 times their reported concentrations that inhibit 50% of enzyme activity. Initial experiments in control tissues revealed that doses of D609 greater than the published K_i suppressed the carbachol response to less than 20% thus doses approximately 0.1, 0.3 and 1 times the published K_i were used. These enzyme inhibitors were added to separate groups of 6-8 separate muscle strips in the presence and absence of 10 nM darifenacin. After 30 minutes, a cumulative concentration response curve (CRC) to carbachol was determined. Thus each muscle strip was only exposed to a single concentration of a single enzyme inhibitor (with and without 10 nM darifenacin) and a single cumulative agonist CRC so that any desensitizing effects of one CRC on subsequent agonist responses were avoided. We found that exposure of normal rat bladder (n=16 strips) to 5 successive carbachol CRCs caused the affinity of the M_3 selective antagonist p-F-HHSiD to be statistically significantly decreased from 7.6 in the first CRC to 7.1 in the fifth CRC suggesting that the repeated exposure to cumulative carbachol CRCs alters the mechanism of contraction possibly by suppression of M_3 receptor mediated signaling. Results of the carbachol induced contractions are expressed as the percent of the initial KCl response . Since many drugs, particularly at the higher doses, prevented the strips from reaching 50% of the maximal KCl response, the carbachol affinity was defined as the dose producing 25% of the initial KCl response (EC_{25}). The affinity of darifenacin was based on the shift in carbachol EC_{25} induced by 10 nM darifenacin. Higher darifenacin doses significantly suppressed maximal responses and lower doses had little effect on carbachol EC_{25}.

Results

Data from normal and sham operated controls was not statistically different and these results were pooled. Results are shown in the Table below.

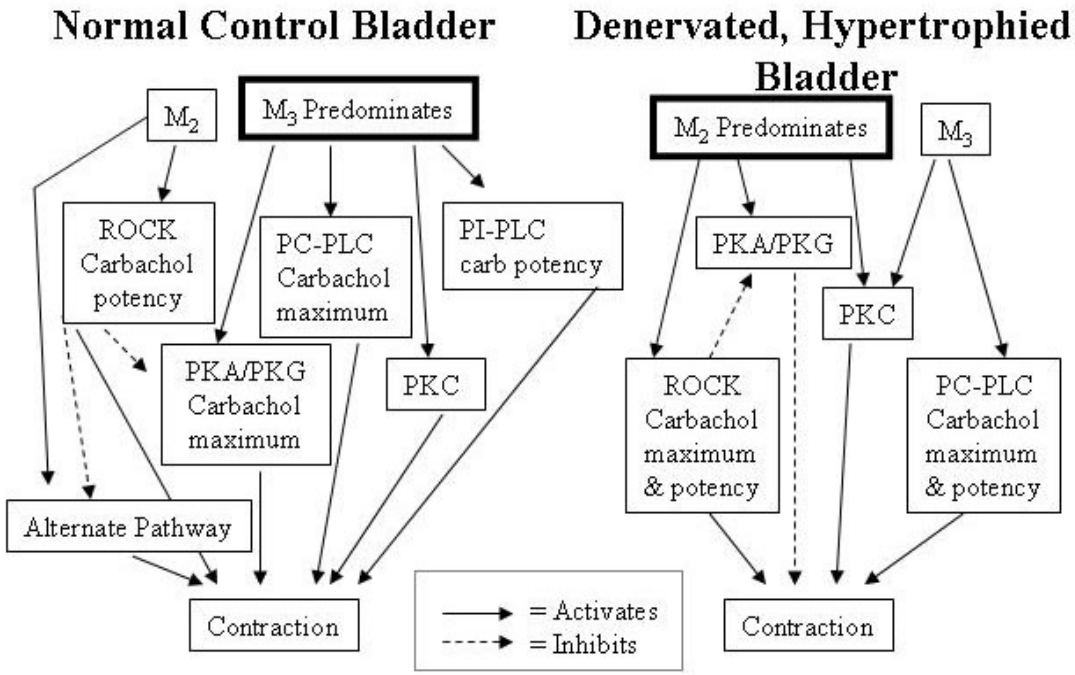
Interpretation of results

These results demonstrate the complex interactions of M_2 and M_3 receptors, and different signal transduction pathways for contraction induced by bladder pathology. For example, Y27632 which inhibits ROCK, reduces the maximal contraction in denervated bladder strips with no effect in normal bladder, and reduces carbachol potency in both control and denervated strips. Moreover, the higher doses of Y-27632 result in a significant increase in darifenacin affinity in the denervated tissue from values consistent with M_2 mediated contractions to values consistent with M_3 mediated contractions. Darifenacin affinity in control tissue is also increased by ROCK inhibition with Y27632. This suggests that ROCK is activated by the M_2 receptor and explains why ROCK inhibition has greater effects in the predominantly M_2 mediated contraction in denervated bladders.

		Control			Denervated		
Drug	Dose (μM)	Max (%KCl)	EC_{25} (μM)	DAR Aff. (nM)	Max (%KCl)	EC_{25} (μM)	DAR Aff (nM)
None	None	117±4	2.5±0.2	5.3±1.0	181±15	0.55±0.1†	13.3±2.5
ET-18-OCH_3	10	111±5	4.5±1.8	8.7±3.3	158±20	0.42±0.05	9.8±2.3
(PI-PLC)	30	102±3	12.7±3.2**	3.7±1.0	152±32	0.35±0.11	9.9±5.9
	100	103±5	32.7±16.9**	15.4±4.9*	200±27	0.46±0.13	22.9±3.5
D609	10	114±7	2.8±0.8	4.2±0.9	184±26	1.0±0.3	6.4±2.1
(PC-PLC)	30	91±6	3.4±0.3	3.8±1.1	166±18	1.3±0.3*	18.9±12
	100	50±10*	10.8±5.9	2.1±1.1	106±22*	3.6±1.0**	17.9±10
Neomycin	10	90±5**	1.3±0.2	3.1±1.2	245±63	0.3±0.05	9.9±5
(PLC)	30	115±10	2.1±0.6	5.3±1.9	147±10	0.3±0.02	19±8.5
	100	125±15	1.6±0.5	1.1±0.5	198±38	0.2±0.06	5.9±2.4
Y-27632	1	101±7	3.0±0.7	0.2±0.03**	203±20	3.7±1.8**	5.0±4.0
(ROCK)	3	85±12	6.7±1.8**	0.7±0.3	189±43	3.9±1.2**	0.4±0.1**
	10	108±17	12.8±3.4**	0.3±0.1*	111±10*	9.1±2.0**	1.6±.08*
HA-1077	10	73±5**	22±6.3**	9.8±3.2	249±67	0.3±0.1	8.6±4.9
(ROCK> PKA=PKG)	30	71±6**	16.4±9.7**	6.4±4.8	227±35	0.3±0.1	1.2±0.2*
	100	47±10**	25.8±10.3**	20.4±3.8**	151±18	1.0±0.3	10.8±5.1
Chelerytherine	1	102±10	5.4±3.2	2.6±0.6	138±19	0.5±0.3	1.9±.06*
(PKC)	3	115±11	2.0±0.5	0.3±0.04**	178±13	0.7±0.4	16.6±5.4
	10	129±18	2.4±0.3	0.3±0.1**	201±26	1.5±0.8	29.2±13
H7	10	75±5**	17.3±5.4**	10.7±4.9	155±24	1.6±0.3*	11.7±5.6
(ROCK>PKA>	30	82±7**	8±2.1**	0.3±0.1**	145±29	2.5±0.9**	2.8±2.7**
PKG=PKC)	100	58±10**	19.6±6.8**	0.5±0.2**	170±21	3.4±1.2**	6.9±5.8
H89	1	143±12	5.6±1.4**	6.6±5.2	185±25	0.5±0.1	16.8±9
(PKA>ROCK> PKG)	3	100±11*	3.8±0.8*	3.1±1.4	181±15	0.7±0.3	10.8±9.2
	10	53±7**	13.5±4.6**	6.2±3.2	160±34	1.4±0.3	1.3±0.1**

Concluding message

Combining the results in Table 4 for all of the inhibitors used leads us to propose the following model of receptor signal transduction interaction:



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