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SECOND MESSENGER PATHWAYS INVOLVED IN THE NKA POTENTIATION OF DETRUSOR RESPONSES TO ATP

Hypothesis / aims of study

Neurokinin A (NKA) potentiates the contraction of bladder detrusor smooth muscle to adenosine triphosphate (ATP) [1]. The mechanism linking NKA and ATP responses is unknown, but NKA acts primarily through G-protein coupled NK2 receptors, which are linked to the $G_{q/11}$ signal transduction pathway producing inositol triphosphate (IP_3) and diacylglycerol (DAG) as second messengers [2]. The main action of IP_3 is the release of intracellular calcium stores while DAG is believed to stimulate the influx of extracellular calcium. The aim of the present study was to determine the contribution of each of these second messenger pathways to NKA-mediated potentiation of purinergic signalling in detrusor smooth muscle.

Study design, materials and methods

Tissue strips were prepared from the dome of porcine bladders and the urothelial layer carefully dissected away. The remaining strips of detrusor muscle were mounted under 2g tension in organ baths containing Krebs bicarbonate solution, gassed with 5% CO_2 in oxygen at 37^{O}C. Isometric developed tension was recorded as tissues were exposed to ATP (1mM). After wash-out, tissues were incubated with NKA (100nM) in the absence or presence of drugs to inhibit calcium influx (R59022, 300nM; calphostin C, 1µM) or interfere with the storage/release of intracellular calcium (2-aminoethoxydiphenylborane (2APB), 30µM; cyclopiazonic acid (CPA), 100µM; ryanodine, 30µM). Responses to ATP (1mM) were then re-examined. Control experiments were also performed without the addition of NKA. Responses are expressed as the percentage potentiation of responses to ATP (1mM) by NKA following the incubation period. Statistical differences between responses were examined using the Student's paired or unpaired t-test as appropriate.

Results

ATP (1mM) induced contractions of detrusor strips (0.95±0.11g, n=50), which were significantly enhanced in the presence of NKA (100nM) in each group under study (Table 1).

In the presence of 2APB (30µM) (an IP_3 receptor antagonist), NKA (100nM) still significantly potentiated responses to ATP (1mM). Similarly in the presence of R59022 (300nM) and calphostin C (1µM), a DAG-kinase and protein kinase C (PKC) inhibitor respectively, detrusor responses to ATP (1mM) were again potentiated in the presence of NKA (100nM).

Only ryanodine (30µM), an inhibitor of calcium release from the sarcoplasmic reticulum (SR), and CPA (100µM), an inhibitor of calcium reuptake into the SR, significantly reduced the NKA-mediated potentiation of detrusor responses to ATP (1mM).

		Potentiation of responses to ATP (1mM) in the presence of NKA (100nM) alone		**Potentiation of responses to ATP (1mM) in the presence of NKA (100nM) and during second messenger pathway modulation**	
Drug	**Drug action**	**Potentiation (%)**	**n**	**Potentiation (%)**	**n**
2APB (30µM)	IP_3 receptor antagonist	265.94 ± 43.78$^{###}$	13	253.14 ± 64.75	14
R59022 (300nM)	DAG-kinase inhibitor	248.78 ± 57.05$^{###}$	11	297.93 ± 57.51	11
Calphostin C (1µM)	PKC inhibitor	235.05 ± 66.09$^{###}$	8	119.46 ± 15.03	8
Ryanodine (30µM)	Inhibitor of SR calcium release	175.00 ± 30.71$^{##}$	8	86.43 ± 21.74**	8
CPA (100µM)	Calcium ATPase pump inhibitor	273.10 ± 61.61$^{#}$	10	104.61 ± 27.99*	9

Table 1. The influence of second messenger pathway modulation on the NKA-mediated potentiation of detrusor smooth muscle responses to ATP (1mM)

$^{#}$P<0.05, $^{##}$P<0.01, $^{###}$P<0.001 compared with control tissues in the absence of NKA.
*P<0.05, **P<0.01 compared to potentiation of ATP responses in the presence of NKA alone

Interpretation of results

These data suggest that calcium stores in the SR are an important component of NKA-mediated potentiation of detrusor smooth muscle responses to ATP. Modulation of the IP_3/DAG pathway at the level of IP_3 receptors, DAG and PKC had no significant influence on responses to ATP in the presence of NKA.

Concluding message

Purinergic signalling in the bladder detrusor smooth muscle is modified by the presence of NKA, and this appears to be related to activation of SR calcium stores.

References

1. Hausman N, Burcher E, Moore K, Mansfield K, Grundy D, Chess-Williams R (2010). The influence of neurokinin A on purinergic and muscarinic receptor signalling in the bladder. Abstract 1041, online at https://www.icsoffice.org/ASPNET_Membership/Membership/Abstracts/Publish/105/001041.pdf
2. Alexander S.P.H, Mathie, A, Peters, J.A (2009). 7TM receptors, British Journal of Pharmacology, 158: S5-S101

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
Statement that no ethical approval was needed	**Ethical approval was not required as tissue samples were obtained from an abattoir (animals not killed for research).**