Brain Research Bulletin 77 (2008) 420–426

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Activation of cholinergic receptors blocks non-adrenergic non-cholinergic contractions in the rat urinary bladder

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ARTICLE INFO

Article history:
Received 28 July 2008
Accepted 29 July 2008
Available online 26 August 2008

Keywords:
Nicotinic acetylcholine receptors
Muscarinic acetylcholine receptors
P2X purinergic receptors
Non-adrenergic
Non-cholinergic (NANC) contractions
Receptor interaction
Bladder smooth muscle
Bladder contractility

ABSTRACT

In the present study, the plasticity of the non-adrenergic non-cholinergic (NANC) response was investigated. Isolated rat bladder strips were electrically stimulated and the evoked contractions were isometrically recorded. The NANC part of the contractions were unmasked by applying 500 nM 4-DAMP, a potent muscarinic antagonist. Treatment of the bladder strips with 10 μM carbachol (a cholinergic agonist) increased the muscle tone but did not alter the neurally evoked contractions. However, carbachol decreased: (1) the NANC response from 74.6% to 33.3% of control and (2) the purinergic contractile response to α,β-methylene ATP (α,β-mATP) (10 μM) from 97.0% to 43.4% ($p < 0.05$). Treatment with the cholinesterase inhibitor eserine (10 μM) also significantly decreased the NANC response to 21.1% ($p < 0.0001$). The purinergic receptor antagonist suramin (100 μM) did not affect the neurally evoked contractions, however; subsequent addition of 4-DAMP decreased the contractions to 31%. Activation of the smooth muscle cholinergic receptors (with carbachol or eserine) and purinergic receptors (with α,β-mATP) decreased the NANC contractions and the direct contractile response to α,β-mATP. When the electrically evoked contractions were facilitated by the L-type Ca^{2+} channel activator, Bay-K 8644 the subsequent application of 4-DAMP did not unmask inhibited NANC contractions. We conclude that activation of muscarinic receptors by cholinergic agonist, carbachol or by endogenous acetylcholine (ACh) induce a cascade of events that leads to diminished purinergic response and consequently an inhibition of the bladder NANC response.

1. Introduction

During electrical stimulation of the rat bladder, acetylcholine (ACh) and ATP are released from parasympathetic nerve terminals and activate M_3 muscarinic and $P2X_1$ purinergic receptors, respectively, to elicit a bladder contraction. Consequently, muscarinic antagonists such as atropine or 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine methiodide) are not fully effective in inhibiting neurally evoked bladder contractions [1,18,24] due to a non-adrenergic, non-cholinergic (NANC) contractile component. In addition, P2X purinergic antagonists inhibit most of the remaining electrically evoked contraction after atropine treatment indicating that the NANC contraction in rat bladders is regulated, in part, by purinergic pathways [8,9,20]. The relationship between the cholinergic and the NANC response is influenced by the frequency of nerve stimulation since at higher frequencies (10–40 Hz) more ACh was released and the bladder contractions became more cholinergic [14,18]. Although the participation of the NANC response is fairly constant at a given frequency of stimulation in normal bladders, it can be altered during pathology. For instance, spinal cord injury or bladder injury in rats induces bladder contractions which become more responsive to the cholinergic transmitter ACh, and less responsive to the purinergic transmitter ATP. In concert with these findings, the muscarinic blocker atropine has a greater inhibitory effect in these pathological models [18,16].

Several bodies of evidence demonstrate that ATP can be released from postjunctional sites (i.e. smooth muscle) unlike traditional nerve transmitters such as ACh or norepinephrine (NE). For example, ACh and NE binding to smooth muscle induce postjunctional release of ATP that may overcome its neuronal release. This kind

Abbreviations: 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine methiodide; ATP, adenosine triphosphate; α,β-mATP, alpha,beta-methylene ATP; CCh, carbachol; NANC, non-adrenergic, non-cholinergic.

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doi:10.1016/j.brainresbull.2008.07.011

of ATP release in the adrenergic system of the vas deferens was described as cascade transmission [22,23] Similar postjunctional release of ATP has been also demonstrated in the cholinergic system of the guinea pig ileum [10] where released ACh or exogenously applied cholinergic agonist induced the release of considerable amounts of ATP.

In this paper, we investigated the NANC response in the rat bladder in the presence of the cholinergic agonist carbachol, or in the presence of the cholinesterase inhibitor eserine, conditions associated with high extracellular levels of ACh. We also measured changes in the purinergic response to the P2X agonist α,β-methylene ATP (α,β-mATP) or the P2X antagonist suramin following muscarinic receptor activation. Finally, we determined the change in NANC response to carbachol induced by pretreatment of the preparation with a muscarinic antagonist.

2. Materials and methods

2.1. Surgical procedures, electrical stimulations, and pharmacologic agents

Experiments were performed on female Sprague–Dawley rats weighing 250–300 g. After euthanization of the rats the urinary bladder was removed and 4 longitudinal strips were prepared. The strips were mounted in 5 mL double jacketed organ baths containing oxygenated Krebs (NaCl 113, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, and D-glucose 11.5 mM) at 37 °C. Electrical field stimulation was applied with a Grass 88 stimulator (Astro-Med, W. Warwick, RI) via platinum wire electrodes. A pretension of 10 mN was applied to all strips prior to starting the experiments and isometric contractions were measured with a force transducer (World Precision Instruments, Sarasota, FL), connected to a bridge amplifier (Transbridge 4M, World Precision Instruments, Sarasota, FL). The data were collected in real-time using the WINDAQ data acquisition program (DataQ Instruments, Akron, OH) at a sampling rate of 20 Hz. Trains of square wave impulses (0.25 ms, 20 Hz, 200 shocks) were applied at a voltage that produced maximal contractions every 100 s. Drugs were added to the organ bath following the experimental paradigms described in Fig. 1. All animal experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the institutional review board.

2.1.1. Experimental protocol

The experimental protocol is summarized in Fig. 1. The α,β-mATP was added at the start and the end of the experiments as shown in Fig. 1a–c. The time difference between the two applications was approximately 60 min. The amplitudes of the electrically evoked contractions were measured and the average of three contractions before the first drug addition was considered as 100% (A1). All the changes were expressed as percent of control. In Fig. 1a–c the change in the amplitude of α,β-mATP induced contractions to treatment was expressed as percent of the contraction amplitude taken at the beginning of the experiment (A1). At the end of all experiments the viability of tissue contractions was tested by adding 100 mM K^+ to the tissue bath.

2.2. Statistics

The experimental results were expressed as mean ± S.E.M. Paired or unpaired *t*-tests were performed as appropriate using the statistical function of PRISM graphical program (GraphPad Software Inc., San Diego, CA). Statistical significance was considered at the level of $p \leq 0.05$.

2.3. Drugs

The pharmacologic agents carbachol, eserine, atropine, 4-DAMP, α,β-mATP, suramin and BAY-K 8644 and all constituents of Krebs solution were obtained from Sigma–Aldrich, Inc. (St. Louis, MO).

3. Results

3.1. Effect of carbachol on the amplitude of NANC contractions

In rat bladder strips, 4-DAMP decreased the amplitude of neurally evoked stimulations by 25.4% ($n = 14$, Fig. 3). In other words, the NANC response accounted for 74.6 ± 5.0% of the neurally evoked contractions in the controls. Treatment with 10 μM carbachol prior to 4-DAMP application (paradigm 'b', Fig. 1) caused a robust contraction of the bladder strips that persisted until 4-DAMP was applied subsequently (Fig. 2A). In the presence of carbachol the neurally evoked contractions were superposed on the increased baseline but the contraction amplitude did not change significantly as compared to the control. The contraction amplitude was significantly decreased after 0.5 μM 4-DAMP was added to the solution to 33.3 ± 3.5% of controls ($n = 13$, $p < 0.0001$, Figs. 2A and 3). However, when bladder strips were treated with a lower concentration of carbachol (1.0 μM) that also caused contractions of the bladder strips, the subsequent application of 4-DAMP did not significantly decrease the contraction amplitude over the inhibitory effect of 4-DAMP alone (69.1 ± 14.0% of controls, $n = 4$, $p > 0.1$). Adding 4-DAMP before 10 μM carbachol to preemptively block the muscarinic receptors (paradigm 'c') did not significantly alter the NANC response (72.0 ± 12.7% of controls, $n = 5$, $p = 0.82$) as compared to the inhibition produced by 4-DAMP alone (Fig. 1, paradigm "a").

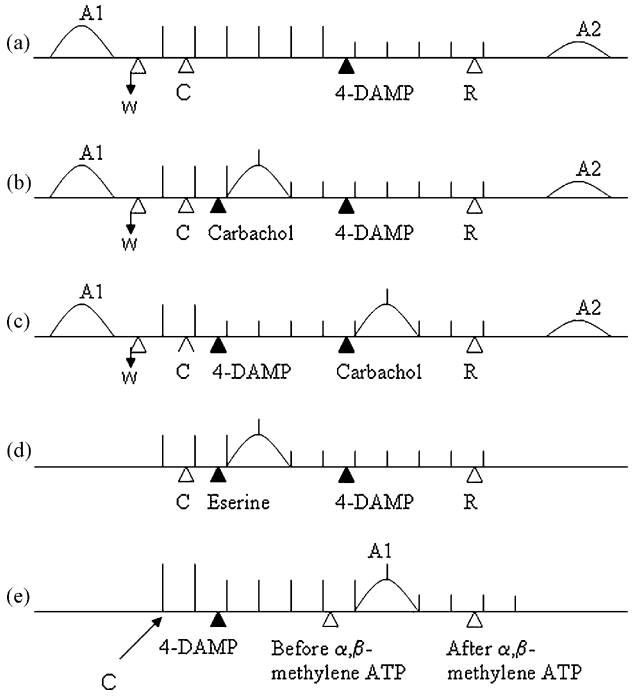

Fig. 1. Experimental paradigms of bladder strips using various sequences of agonists and antagonists to study the plasticity of the NANC response. Paradigm 'a' served as control experiment where only the muscarinic antagonist 4-DAMP was applied to unmask the NANC response; while paradigm 'b' examined the effects of cholinergic stimulation (carbachol) on the NANC response. In paradigm 'c', 500 nM 4-DAMP was administrated before carbachol to preemptively block the muscarinic receptors. Paradigm 'd' investigated the effects of increasing synaptic acetylcholine concentration by applying the cholinesterase inhibitor eserine. Paradigm 'e' studied the effects of $P2X_1$ purinergic receptor activation by α,β-mATP. For the experiments with BAY-K 8644 and suramin basically the paradigm 'b' was applied but the drugs were added to the bath in lieu of carbachol. Symbols (Δ + W) washout; (Δ + C) control for the calculations; (Δ + R) reading the drug effect (result); (▲) addition of drug; A1 α,β-mATP at the beginning and A2 at the end of the experiments.

3.2. Effect of the cholinesterase inhibitor eserine on the amplitude of NANC contractions

In this series of experiments, 10 μM of eserine was added to the organ bath. After 30 min incubation time, the amplitude of the electrically evoked contractions was initially increased then slightly decreased while the baseline tone became elevated. When 4-DAMP (0.5 μM) was added to the bath, the elevated baseline tone returned to the control level and at the same time the amplitude of the electrically evoked contractions was abruptly reduced (21.1 ± 8.0% of controls; $p < 0.0001$; $n = 4$, Figs. 2B and 3).