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Research report

Plasticity of non-adrenergic non-cholinergic bladder contractions in rats after chronic spinal cord injury

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ABSTRACT

The purpose of this study was to examine the pharmacologic plasticity of cholinergic, non-adrenergic non-cholinergic (NANC), and purinergic contractions in neurogenic bladder strips from spinal cord injured (SCI) rats. Bladder strips were harvested from female rats three to four weeks after T_9–T_{10} spinal cord transection. The strips were electrically stimulated using two experimental protocols to compare the contribution of muscarinic and NANC/purinergic contractions in the presence and the absence of carbachol or muscarine. The endpoints of the study were: (1) percent NANC contraction that was unmasked by the muscarinic antagonist 4-DAMP, and (2) P2X purinergic contraction that was evoked by α,β-methylene ATP. NANC contraction accounted for 78.5% of the neurally evoked contraction in SCI bladders. When SCI bladder strips were treated with carbachol (10 μM) prior to 4-DAMP (500 nM), the percent NANC contraction decreased dramatically to only 13.1% of the neurally evoked contraction ($P = 0.041$). This was accompanied by a substantial decrease in α,β-methylene ATP evoked P2X contraction, and desensitization of purinergic receptors (the ratio of subsequent over initial P2X contraction decreased from 97.2% to 42.1%, $P = 0.0017$). Sequential activation of the cholinergic receptors with carbachol (or with muscarine in neurally intact bladders) and unmasking of the NANC response with 4-DAMP switched the neurally evoked bladder contraction from predominantly NANC to predominantly cholinergic. We conclude that activation of muscarinic receptors (with carbachol or muscarine) blocks NANC and purinergic contractions in neurally intact or in SCI rat bladders. The carbachol-induced inhibition of the NANC contraction is expressed more in SCI bladders compared to neurally intact bladders. Along with receptor plasticity, this change in bladder function may involve P2X-independent mechanisms.

1. Introduction

The muscarinic and purinergic pathways play important roles in urinary bladder contraction. During neurally evoked contractions of the bladder, acetylcholine (ACh) and ATP are co-released from parasympathetic nerve terminals and activate post-junctional muscarinic (M) and purinergic (P2X1) receptors, respectively, to elicit a bladder contraction in the rat [8]. Thus, muscarinic antagonists such as atropine or 4-DAMP are not fully effective in inhibiting neurally evoked bladder contraction due to a significant non-adrenergic, non-cholinergic (NANC) contractile component [1,26,30]. The P2X purinergic pathway contributes significantly to this NANC contraction since P2X antagonists abolish most of the remaining NANC bladder contraction after atropine treatment [15,17,28].

The relative contribution of cholinergic and NANC/purinergic transmission to neurally evoked bladder contraction is influenced by a number of factors, such as animal age [13,22,31], frequency of electrical stimulation, and pathology. For example, at higher frequency of nerve stimulation (10–40 Hz), more ACh is released and the bladder contraction becomes more cholinergic [23,29]. After spinal cord injury (SCI), the rat bladder becomes more responsive to the cholinergic transmitter ACh and less responsive to the purinergic transmitter ATP [24,26]. Thus, muscarinic antagonists, like atropine or 4-DAMP, exhibit greater inhibition on neurally evoked contractions subsequently following SCI [26].

We have recently shown that activation of muscarinic receptors by the cholinergic agonist carbachol, or by endogenous ACh induces a cascade of events that leads to reduced purinergic con-

Abbreviations: 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine methiodide; α,β-mATP, α,β-methylene ATP; CCh, carbachol; NI, neurally intact; SCI, spinal cord injury.

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tractile response, and consequently inhibition of the bladder NANC response [16]. Correspondingly, in the presence of the cholinergic agonist carbachol, the muscarinic antagonist 4-DAMP becomes more effective in inhibiting neurally evoked bladder contractions, whereas the P2X receptor agonist α,β-methylene ATP (α,β-mATP) produced a diminished contractile response. This pharmacologic plasticity, i.e. an abrupt shift from NANC/purinergic contraction to cholinergic/muscarinic contraction, occurs rapidly after carbachol administration, and may be caused by the desensitization of P2X receptors [16].

The pharmacologic plasticity of bladder cholinergic and NANC response was first demonstrated in normal rats [16]. In this paper we investigated the cholinergic-NANC plasticity in SCI rats where the micturition circuitry is reorganized due to permanent disruption of ascending and descending pathways to the brain. Specifically, we compared the effectiveness of 4-DAMP (a muscarinic receptor antagonist) to inhibit neurally evoked bladder contraction, and the effectiveness of α,β-mATP (a P2X purinergic receptor agonist) to evoke bladder contraction in the presence and absence of carbachol or muscarine. Our findings have significant importance because the plasticity of cholinergic, NANC, and purinergic transmission is poorly understood in the rat model of neurogenic bladder. Although treatment with antimuscarinic agents has been widely used in the management of neurogenic detrusor overactivity in humans, the efficacy is modest while the side effects may be substantial. Understanding the pharmacologic modulation of cholinergic and purinergic contractions in the rat model of SCI bladders, and the mechanisms underlying this modulation, may have potential impact on the management of neurogenic bladder conditions as long as the results are translatable to humans.

2. Materials and methods

All the experimental procedures were approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine and were conducted in accordance with NIH guidelines for the care and use of laboratory animals.

2.1. Spinal cord injury (SCI)

Experiments were performed on 31 neurally intact (NI) and 17 spinal cord transected (SCI) female Sprague-Dawley rats weighing 250–300 g as previously described [20]. Rats were anesthetized with isoflurane (1.5%). A midline dorsal incision and laminectomy were performed to expose the spinal segment T_9–T_{10}. The dura mater was opened and the spinal cord was completely transected. The overlying muscle and skin were closed and the rats were given 2 mL of saline solution subcutaneously. All rats received ampicillin (100 mg/kg) intramuscularly once a day for 3 days. Rat bladders were manually expressed twice a day until spinal reflex micturition developed.

2.2. Bladder strips preparation and experimental paradigms

Three to four weeks after spinal cord transection rats were euthanized, the urinary bladder was removed above the trigone, and 4 longitudinal bladder strips were prepared taking special care to preserve the integrity of the urothelial layer during preparation. The strips were mounted in 5 mL 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, D-glucose 11.5 mM) at 37 °C. Pretension of 10 mN was applied to all strips, isometric contractions were measured with a force transducer (FT-2, World Precision Instruments, Sarasota, FL, USA), and normalized based on strip cross-sectional area. Neurally evoked contractions were induced using electrical field stimulation via platinum wire electrodes. The data were collected in real-time using the WINDAQ data acquisition program (DataQ Instruments, Akron, OH, USA) at a sampling rate of 20 Hz. Trains of square wave impulses (0.25 ms, 20 Hz, 200 shocks every 100 s) were applied at a voltage (100 V) that produced maximal contractions. Drugs were added to the organ bath according to the experimental protocols A or B as outlined in Fig. 1. 10 μM α,β-mATP was applied at the beginning and at the conclusion of the experiment to evoke P2X purinergic bladder contractions. The two α,β-mATP applications (A1, A2) were approximately 35 min apart. After A1 and wash-out, neurally evoked contractions were applied until a stable baseline (expressed as B) was established. Bladder NANC contractions were unmasked by applying the muscarinic antagonist 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP). In paradigm A (Fig. 1A), 500 nM 4-DAMP was added between the α,β-mATP contractions while in paradigm B (Fig. 1B), 10 μM carbachol (or 10 μM muscarine) and 500 mM 4-DAMP were added sequentially. That portion of the neurally evoked contraction that was inhibited by 4-DAMP was considered cholinergic while the residual contraction (R) that remained after 4-DAMP administration was considered as the NANC response. There was no wash-out between carbachol (or muscarine), 4-DAMP, and the second application of α,β-mATP (A2). At the end of all experiments, the viability of bladder strips was confirmed by contracting the bladder strips with 100 mM K^+. At 500 nM concentration, 4-DAMP loses its selectivity towards M_3 and functions as a non-specific muscarinic antagonist [9,10,25]. We evaluated: (1) the percent NANC contraction that was unmasked by the muscarinic antagonist 4-DAMP (expressed as R/B × 100%), and (2) P2X purinergic contraction that was evoked by α,β-mATP. The A2/A1 ratio was used as a measure of the purinergic receptor desensitization.

2.3. Drugs

All pharmacologic agents and the constituents of Krebs solution were obtained from Sigma–Aldrich (St Louis, MO, USA).

2.4. Statistics

The data are expressed as mean ± S.E.M. For statistical analysis, one way ANOVA followed by a Bonferoni's post-test was used for analyzing the results obtained with carbachol and 4-DAMP in NI and SCI bladders. Paired two-tailed *t*-tests were performed for data obtained with muscarine and 4-DAMP in neurally intact bladders. Statistical analysis and figure preparation were performed with PRISM 4 (GraphPad Prism Software, San Diego, CA, USA). Statistical significance was considered at a level of $P \leq 0.05$.

3. Results

3.1. Effect of activation of muscarinic receptors on the magnitude of NANC contraction in NI and SCI rat bladders

In SCI rats, the muscarinic antagonist 4-DAMP (500 nM) decreased the amplitude of neurally evoked bladder contractions by approximately 21% (Fig. 2, $n = 10$). Thus, the NANC contraction accounted for 78.5 ± 21.9% of the neurally evoked contraction in SCI rat bladder strips. The experimental paradigm is shown in Fig. 1A. When SCI bladder strips were treated with the mixed cholinergic agonist carbachol (10 μM) prior to 4-DAMP application (500 nM) (paradigm B in Fig. 1), 4-DAMP decreased the amplitude of neurally evoked bladder contraction by 87% (Fig. 2, $n = 6$). Therefore, the NANC contraction only accounted for 13.1 ± 5.6% of the neurally evoked bladder strip contraction. This percent NANC contraction was significantly smaller than that without carbachol pre-treatment (13.1 ± 5.6% versus 78.5 ± 21.9%, $P = 0.041$). In other words, in the presence of cholinergic receptor activation (with 10 μM carbachol), the percent NANC contraction became smaller while the percent cholinergic contraction became more prominent.

When the experiments were performed in neurally intact (NI) normal rat bladders (Fig. 2), a similar reduction of the NANC response and an enhancement of the cholinergic contractile component were also observed when carbachol (10 μM) was applied prior to 4-DAMP (500 nM) [16]. In the presence of carbachol, the NANC response in SCI rat bladders (13.1 ± 5.6%) was significantly smaller than the NANC response in NI rat bladder (32.0 ± 3.2%) ($P = 0.0059$). In other words, the negative effect of carbachol on bladder NANC contractions is more prominent in SCI rats than in NI rats.

In another series of experiments the selective muscarinic agonist, muscarine was applied instead of carbachol in NI rat bladder strips. As shown in Fig. 2, application of muscarine (10 μM) before 4-DAMP produced a significant inhibition of NANC contraction by 42% ($P < 0.05$, $n = 5$) as compared to 4-DAMP only treated strips. The significantly reduced contractions to electrical stimulation obtained after the application of muscarine and 4 DAMP confirms that muscarinic receptors mediate the enhanced inhibition of the NANC response.