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Facilitation of transmitter release in the urinary bladders of neonatal and adult rats via α_1-adrenoceptors

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Abstract

Age-dependent changes in the effects of the α_1-adrenoceptor agonist, phenylephrine were investigated on neurally evoked contractile responses and basal tone in smooth muscle strips from rat urinary bladder. Phenylephrine facilitated the neurogenic contractions in both neonatal and 7-month-old adult rats. However, phenylephrine increased the basal tone in adult but not neonatal rats. In adult rats, phenylephrine-induced facilitation of neurally evoked contractions occurred before and after the block of cholinergic contractions with 1 μM atropine. In adult rats, the phenylephrine facilitation was reduced at stimulation parameters (20 Hz, 80 shocks and maximal voltage) which activated muscarinic receptor mediated facilitation of acetylcholine release. The results indicate that pre-synaptic α_1-adrenoceptors facilitate the release of both acetylcholine and the non-cholinergic non-adrenergic transmitter. In summary, α_1-adrenoceptor-mediated facilitation is less expressed when muscarinic M_1 receptor mediated facilitation is functioning; pre-junctional α_1-adrenoceptors are present in the bladder of both neonatal and adult rats, whereas post-junctional α_1-adrenoceptors are expressed only in older adult rats.

Keywords: α_1-Adrenoceptor; Muscarinic receptor; Bladder, neonate; Bladder, adult; Facilitation

1. Introduction

In peripheral cholinergic and adrenergic post-ganglionic nerves, the release of transmitters is primarily regulated by negative feedback mechanisms mediated via pre-synaptic muscarinic M_2 receptors or α_2-adrenoceptors (for reviews see Starke, 1987; Starke et al., 1989). However, at the parasympathetic nerve terminals in the rat urinary bladder, facilitatory mechanisms mediated by α_1-adrenoceptors and muscarinic M_1 receptors can increase acetylcholine and/or noradrenaline release (Somogyi et al., 1994, 1995, 1996; Somogyi and De Groat, 1999). Activation of pre-synaptic α_1-adrenoceptors by the α_1-agonists, phenylephrine or methoxamine increased acetylcholine release and enhanced neurogenic contractions in a dose-dependent manner (Somogyi et al., 1995; De Groat et al., 1999).

In mature rats (4 months or older) but not in young rats, phenylephrine also activated post-junctional α_1-adrenoceptors and increased basal tone in bladder strips (Somogyi et al., 1995, 2000) supporting the report of Ordway et al. (1986) that α_1-adrenoceptor agonists evoked an increase in bladder tone in older rats but not in young rats. Our data suggest that the α_{1A}-adrenoceptor subtype mediates the pre-junctional facilitation, whereas an α_{1B} or α_{1D} subtype mediates post-junctional facilitation (Széll et al., 2000).

In addition to the α_1-adrenoceptor-mediated facilitation, muscarinic M_1 receptor-mediated facilitation of acetylcholine release was also described for the cholinergic nerve terminals. The muscarinic receptor mediated facilitation had characteristics different from α_1-adrenergic-receptor mediated facilitation, e.g. it was more expressed at high extracellular calcium concentrations and during stimulation with high stimulus intensities (Somogyi et al., 1994), whereas α_1-adrenoceptor mediated facilitation was more expressed at lower calcium concentrations and submaximal stimulation (Somogyi et al., 1995).

We now address the following questions relating to the rat urinary bladder. (1) Is pre-synaptic α_1-adrenoceptor-mediated facilitation age dependent? (2) Is there any negative or positive interaction between α_1-adrenoceptor-mediated facilitation and muscarinic M_1 receptor mediated

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facilitation? (3) Does activation of α_1-adrenoceptors facilitate non-cholinergic non-adrenergic (NANC) transmission?

Some of the findings were presented as an abstract (Somogyi et al., 1999).

2. Materials and methods

2.1. General

The urinary bladder was removed following decapitation. Two to four circular slices were cut from the bladder body of adult female rats (7 months old; 380–450 g) and two longitudinal strips were prepared from neonatal rat bladder (14–21 days old). Bladder strips weighing 15–25 mg were mounted in a double-jacketed organ bath at 35°C in Krebs solution (mmol/l; NaCl 113, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.5) and constantly bubbled with a mixture of 95% O_2 and 5% CO_2. The initial tension was set at 10 mN and isometric contractions were measured with strain-gauge transducers and recorded with a computerized data acquisition program (Windaq, DATAQ Instruments, Akron, OH, USA). The amplitude of the stimulation evoked contractions was computed with the WindaqEx program (DATAQ Instruments). Electrical field stimulation using maximal or submaximal voltage at 20 Hz frequency and 0.25 ms stimulus duration was delivered with a Grass S88 stimulator through platinum electrodes inserted from the top and bottom of the organ bath and separated by 4 cm. The stimulus protocol applied in a particular series of experiments is described in Section 3.

2.2. Statistical analysis

The data were analyzed by one way analysis of variance or Student's *t*-test using the Prism Statistical Program (Graphpad, San Diego, CA). A level of $P < 0.05$ was considered statistically significant. The data are expressed as means ± S.E.

2.3. Drugs

Phenylephrine hydrochloride, 5-methyl-urapidil, atropine sulfate, and all constituents of the Krebs solution were purchased from Sigma (St. Louis, MO, USA).

3. Results

3.1. Effect of activation of α_1-adrenoceptors on the contractile response of bladder strips from neonatal and adult rats

The facilitatory effect of phenylephrine on neurally evoked contractions of strips from neonatal and adult rat bladders occurred in a similar range of phenylephrine concentrations. Facilitation was detected at a concentration of 0.5 μM and was maximal between 8 and 16 μM (Somogyi et al., 1995). In adult bladders, 8 μM phenylephrine also elicited a near maximal increase in baseline tension (Széll et al., 2000). Thus, 8 μM phenylephrine was selected as the test concentration to evaluate the pre-junctional and post-junctional effects of phenylephrine on bladder strips prepared from neonatal (14–21 days old), or 7-month-old rats. Electrical stimulation (20 Hz, 10 shocks) of neonatal and adult bladder strips with submaximal voltages evoked large contractions (amplitudes: 8.98 and 10.62 mN/mm^2, respectively) (Table 1). As shown in Fig. 1, phenylephrine (8 μM) induced a similar increase in the amplitude of the neurogenic contractions in neonatal and adult bladder strips. However, the phenylephrine-evoked elevation of baseline tension which was prominent (3.2 mN/mm^2) in the adult strips, did not occur in the neonatal bladder strips (Table 1, Fig. 1).

3.2. Interaction between muscarinic and α_1 facilitation in adult bladders

As noted earlier (Somogyi et al., 1994, 1998), when the stimulation frequency was 10 Hz or higher, muscarinic receptor mediated facilitation of acetylcholine release could be evoked with trains of 80–100 shocks at supramaximal voltage, whereas shorter duration trains (10 shocks) or submaximal voltages did not facilitate acetylcholine release. In the present experiments, the interaction between muscarinic and α_1-adrenoceptor mediated facilitation was tested in bladder strips from 7-month-old rats using two stimulation protocols: one which did not produce muscarinic facilitation (20 Hz, 10 shocks at a submaximal voltage which produced 50% of the maximal contraction) (Somogyi et al., 1995) and the other which produced muscarinic facilitation (20 Hz, 80 shocks with supramaxi-

Table 1
Amplitude of the neurally evoked and phenylephrine-induced contractions in adult and neonatal rat bladders

	20 Hz/10 shocks (mN/mm^2)[a]	20 Hz/100 shocks (mN/mm^2)[a]	PE 8 μM (mN/mm^2)
Neonatal	8.98 ± 0.68 (8)	14.96 ± 1.18 (8)	0 (4)
Adult	10.62 ± 0.8 (7)	22.3 ± 1.58 (7)[b]	3.21 ± 0.4 (7)

The peak contractile force was standardized to the resting cross-sectional area of the bladder strips. The cross-sectional area (CA) was calculated with the equation $CA = weight/1.05 \times L$, where weight is the wet weight of the bladder strips measured after the experiments, and L is the length of the strips measured after the experiments. The constant 1.05 is the specific density of the bladder tissue.

The numbers in brackets represent the number of experiments.

[a] Neurally evoked contractions were elicited by a submaximal voltage, which produced 50% of the maximal responses.

[b] Significantly different from the neonatal contraction amplitude ($P < 0.05$, Student's *t*-test).

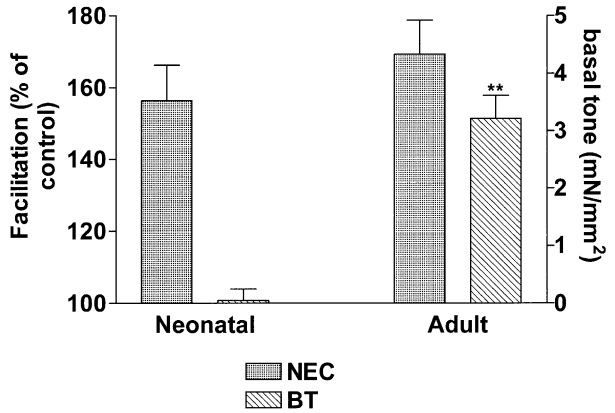

Fig. 1. Pre- and post-junctional effect of phenylephrine (8 μM) in neonatal and adult bladder strips. Phenylephrine-induced increase of the neurally evoked contractions (NEC) is expressed as percent of the control amplitude. Note that there is no statistically significant difference between the facilitatory effect of phenylephrine on neurally evoked contractions in neonatal and in adult rat ($P > 0.05$). The basal tone increase in adult rats is prominent, whereas, in neonatal rats it is not significantly different from zero ($P > 0.05$). Data obtained from four neonatal and seven adult rats.

mal voltage). Phenylephrine was tested in a concentration of 8 μM which produced close to maximal facilitation of the electrically evoked contractions. As shown in Fig. 2, the facilitatory effect of phenylephrine was significantly greater during non-facilitating stimulation than during facilitating stimulation.

3.3. Effects of atropine on phenylephrine-evoked facilitation of neurogenic contractions in adult bladders

The cholinergic contractions of bladder strips from adult rats were inhibited by 1 μM atropine, which produces a maximal block of muscarinic receptors in the bladder (Zoubek et al., 1993). Under these circumstances the contractions are mediated only by a NANC transmitter,

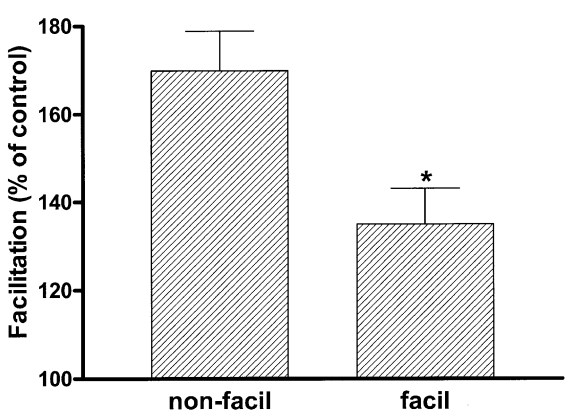

Fig. 2. The effect of 8 μM phenylephrine on the neurally evoked contractions of bladder strips from adult rats (7 months old) using non-facilitatory (non-facil; train of 10 shocks at 20 Hz with submaximal voltage; $n = 7$) or facilitatory (facil; train of 100 shocks, maximal voltage $n = 6$) stimulation protocols. $^{*}P < 0.05$.

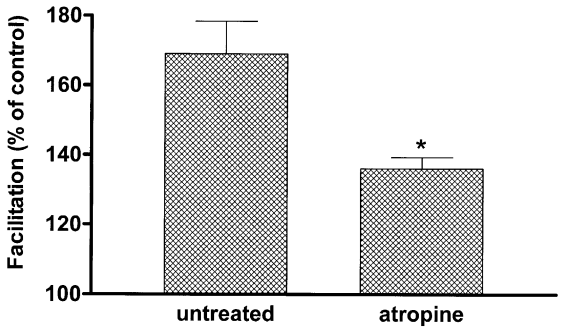

Fig. 3. The effect of 8 μM phenylephrine on the neurally evoked contractions of bladder strips in adult rats (7 months old). Contractions were elicited by non-facilitatory stimulation (train of 10 shocks at 20 Hz with submaximal voltage) in atropine (1 μM)-treated ($n = 6$) or untreated ($n = 7$) preparations. $^{*}P < 0.05$.

presumably ATP. Atropine reduced the submaximal contractions of the stimulated bladder strips by approximately 30%. As shown in Fig. 3, phenylephrine facilitated the neurally evoked contractions even when the post-junctional effect of acetylcholine was completely blocked by atropine; however, this facilitation was significantly less than that in the absence of atropine. The selective α_{1A}-adrenoceptor inhibitor, 5-methyl-urapidil (50 nM), completely blocked the phenylephrine evoked facilitation in both normal and atropine-treated strips indicating that α_{1A}-adrenoceptors mediate the facilitation of neurally evoked NANC contractions.

4. Discussion

Previous experiments in our laboratory (Somogyi et al., 1995; Széll et al., 2000) revealed that, in strips from the bladder body, phenylephrine, an α_1-adrenoceptor agonist, has both pre- and post-junctional actions to enhance neurally evoked contractions (pre-junctional action) and to increase basal tone (post-junctional action). Our present results showed that phenylephrine increased basal tone in strips from adult rats, but not in strips from neonatal rats. On the other hand, phenylephrine enhanced the neurally evoked contractions of bladder strips from both adult and neonatal rats. Thus, pre-junctional and post-junctional facilitatory α_1-adrenoceptors seem to be expressed at different times during maturation of the bladder.

Experiments with several species revealed that α_1-adrenoceptor agonists elicit contractions of the smooth muscle in the bladder base but evoke variable effects on the bladder body (Downie et al., 1975). Some of this variation in the rat could be due to the age of the animals, since Ordway et al. (1986) reported that α_1-adrenoceptor agonists consistently increase the basal tone of bladder strips from older rats. Accordingly, in the present study the bladder strips from adult rats (7 months old) had a detectable rise in baseline tone following phenylephrine ad-

ministration, while, in neonatal bladders, the increase in baseline was minimal. On the other hand, the neurally evoked contractions were facilitated via α_1-adrenoceptors and the degree of facilitation was not significantly different between strips from neonatal and from adult rat bladders. These findings suggest that the α_1-adrenoceptors are expressed from an early age in the pre-synaptic post-ganglionic nerve terminals in the bladder, whereas, the post-junctional α_1-adrenoceptors appear only in adult bladders, mostly after 7 months of age.

The α_1-adrenoceptors at pre-junctional and post-junctional sites are different. Pre-junctional receptors can be inhibited by α_{1A}-adrenoceptor blockers (5-methyl-uradipil, and REC 15237) but not by chloroethylclonidine, whereas the post-junctional receptors can be inhibited by chloroethylclonidine but not by α_{1A}-adenoceptor blockers (Széll et al., 2000). Thus, it seems reasonable to conclude that α_{1A}-adrenoceptors are expressed in the cholinergic nerve terminals in neonatal bladders, whereas, the chloroethylclonidine sensitive post-junctional α_{1B} or α_{1D}-adrenoceptors appear at a later age in the bladder smooth muscle.

When muscarinic receptors on the smooth muscle are blocked by atropine, phenylephrine still enhances the amplitude of the non-cholinergic neurally evoked contractions, which are mediated by ATP (MacKenzie and Burnstock, 1984; Brading and Mostwin, 1989). However, phenylephrine elicited a smaller facilitatory effect in the presence of atropine (35% increase) than in untreated preparations (70% increase) suggesting that enhancement of both acetylcholine and ATP release contributes to the phenylephrine-induced facilitation of neurally evoked contractions. Facilitation of non-cholinergic transmission was inhibited by α_{1A} selective concentrations of urapidil (Gross et al., 1988), indicating that the α_{1A}-adrenoreceptor subtype is involved in the facilitation of ATP release.

Previous studies (Somogyi et al., 1994) showed that when the bladder strips are stimulated at a frequency of 10 or 20 Hz the release of acetylcholine is positively correlated with the number of shocks in the train between 5 and 100 shocks. When a higher number of shocks (80–100) was used the released acetylcholine activated pre-synaptic muscarinic M_1 facilitatory receptors and the amount of transmitter released was greatly enhanced. In the present experiments, we evaluated whether the pre-junctional phenylephrine-evoked facilitation was altered by stimulation parameters that induced pre-junctional muscarinic facilitation. It might be expected that, when acetylcholine release is already facilitated by activation of pre-junctional muscarinic receptors, further enhancement via stimulation of α_{1A}-adrenoceptors would be blunted. Although phenylephrine-induced facilitation still occurred during long trains of stimulation, the magnitude of the facilitation was reduced by 50%. This suggests that α-adrenoceptor mediated pre-junctional facilitation has a limited role in the voiding function in normal bladders because, after activation of the muscarinic facilitatory mechanisms, there is sufficient acetylcholine and ATP released to evoke a maximal detrusor contraction. However, α_1-adrenoceptor mediated pre-junctional facilitation may have an important pathophysiological function to amplify small-amplitude non-voiding contractions of the bladder, when parasympathetic nerve activity is low and muscarinic receptor mediated facilitation is weak. This might occur in the presence of outlet obstruction or in neurogenic bladders both of which exhibit non-voiding contractions during bladder filling (Brading, 1997).

Acknowledgements

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