British Journal of Pharmacology (1995) 114, 1710–1716

Prejunctional facilitatory α_1-adrenoceptors in the rat urinary bladder

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1 The effect of activation of α_1-adrenoceptors on acetylcholine (ACh) release and neurally evoked contractile responses induced by electrical field stimulation was investigated in smooth muscle strips from the rat urinary bladder.

2 Neurogenic contractions were facilitated by the α_1-adrenoceptor agonists, phenylephrine (PE) (2–128 μM) and methoxamine (2–128 μM) in a dose-dependent manner. These agents also increased small amplitude spontaneous contractions of bladder strips and in 10% of strips increased basal tone. However, contractions elicited by exogenous ACh (1–10 μM) were not affected by α_1-agonists.

3 The magnitude of the PE facilitation was higher at lower frequencies (1–5 Hz) or at submaximal intensities of stimulation and at lower Ca^{2+} concentrations (0.5–1 mM). The selective α_1-adrenoceptor antagonist, terazosin (TRZ) (0.05–1 μM), competitively inhibited (pA_2 value: 8.6) the PE facilitation of the neurally evoked contractions but not the PE induced increase of spontaneous contractions.

4 [^{3}H]-noradrenaline (NA) and [^{14}C]-ACh release evoked by electrical field stimulation were increased (140% and 173%, respectively) by 2 μM PE. TRZ (0.05–0.1 μM) blocked the PE facilitation of ACh release but not the facilitation of NA release. TRZ alone did not alter the release of ACh or NA nor the amplitude of the neurogenic contractions.

5 PE (2 μM) did not alter the basal release of ACh but did increase (by 180%) the basal release of NA. Desipramine (2 μM) blocked this effect of PE and also the PE-facilitation of evoked ACh and NA release.

6 It is concluded that cholinergic terminals in the rat urinary bladder exhibit α_1-adrenoceptors which can facilitate the release of transmitter. However, under the conditions of our experiments it appears that cholinergic transmission is not modulated by α_1 adrenergic mechanisms. Further studies are necessary to determine whether these receptors can be activated by endogenous noradrenaline released within the bladder.

Keywords: α_1-Adrenoceptors; prejunctional facilitation; urinary bladder

Introduction

Adrenergic modulation of autonomic transmission has been detected at various sites in the peripheral nervous system. For example, activation of α_1-adrenoceptors inhibits acetylcholine (ACh) release from the cholinergic nerve terminals in the ileum (Kapocsi *et al.*, 1986; Starke, 1987) and in autonomic ganglia (Paton & Thompson, 1953; Dawes & Vizi, 1973; de Groat & Booth, 1980) and inhibits noradrenaline (NA) release from adrenergic nerve terminals in the heart (Ledda & Mantelli, 1984), vas deferens (cf. Vizi, 1979) and urinary bladder (Somogyi & de Groat, 1990). α_1-Adrenoceptors which are prominent at postjunctional sites (Langer, 1974), have also been detected at the adrenergic and cholinergic nerve terminals in some peripheral tissues. However, the function of prejunctional α_1-adrenoceptors is more variable. Inhibitory α_1-adrenoceptors were detected on adrenergic nerves in the kidney (Rump & Majewski, 1987) and on adrenergic and cholinergic nerves in the heart (Ledda & Mantelli, 1984; Wetzel *et al.*, 1985). In addition, a facilitatory effect of certain α_1-agonists on ACh release in the heart has been described (Bognar *et al.*, 1990). α_1-Adrenoceptor facilitation of cholinergic transmission has also been reported at nicotinic synapses at the neuromuscular junction (Vizi, 1991) and in parasympathetic ganglia of the cat urinary bladder, where the α_1-adrenoceptors are located on the cell body (Akasu *et al.*, 1985; Keast *et al.*, 1990). In the cat bladder ganglia the facilitation is associated with a slow postsynaptic depolarization (de Groat & Booth, 1980; Akasu *et al.*, 1985). Recent patch clamp studies have revealed that α-adrenoceptor agonists also depolarize bladder parasympathetic neurones isolated from the major pelvic ganglion of the rat (Yoshimura & de Groat, 1992).

The presence of excitatory α_1-adrenoceptors on the soma of bladder parasympathetic neurones raises the possibility that similar receptors might be present at the axon terminals of these neurones in the urinary bladder. This was evaluated in the present experiments by examining the effects of α_1-adrenoceptor agonists on neurally evoked smooth muscle contractions and on ACh and NA release in strips of the rat urinary bladder. The results indicate that activation of α_1-adrenoceptors can facilitate ACh release and excitatory transmission in the bladder. These observations coupled with previous reports of muscarinic receptor (M_2) inhibition (D'Agostino *et al.*, 1986; Somogyi & de Groat, 1992) and muscarinic receptor (M_1) facilitation (Somogyi & de Groat, 1992; Somogyi *et al.*, 1994) of ACh release in the rat bladder indicate that cholinergic transmission in this organ is potentially complex and dependent upon the interaction of a variety of prejunctional modulatory mechanisms.

A preliminary report of some of these results has appeared as an abstract (Somogyi *et al.*, 1992).

Methods

Preparation

The urinary bladder was removed from male Sprague Dawley rats (250–300 g) following decapitation and circular slices were cut from the middle part of the bladder body.

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Contractile experiments

The bladder strips weighing 20–30 mg were mounted in a double jacketed organ bath at 35°C in Krebs solution (mmol l^{-1}: NaCl 113, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 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 1 g and isometric contractions were measured with strain-gauge transducers and recorded on a Grass polygraph. Electrical field stimulation with a Grass 88 stimulator was delivered through platinum electrodes inserted from the top and bottom of the organ bath and separated by 4 cm. Stimulation intensity-response curves were constructed in order to determine the voltage required to elicit a maximal contraction. The preparations were then stimulated at a maximal voltage at 10 Hz with 0.25 ms stimulus duration for 1 s at 1 min intervals. These stimulus parameters yielded contractions at a consistent amplitude over long periods of time (4–6 h). In some preparations the voltage was set to produce 50% of the maximal effect. However, unless otherwise stated, maximal stimulation was applied. When the frequency-response relationships were studied, a frequency-response curve was constructed before and after the addition of drugs by applying stimulus trains consisting of 10 shocks at frequencies ranging from 0.1 to 50 Hz.

The α_1-agonists were added to the bath at 6–8 min intervals in increasing concentrations to construct cumulative dose-response curves. The ED_{50} values for each agonist were calculated. The dose-response relationships were also examined in the presence of different concentrations of the α_1-antagonist, terazosin. Terazosin was added to the bath 20 min before each test.

Experiments on NA and ACh release

A double labelled isotopic technique was used to measure simultaneous release of NA and ACh from the same strip. Strips were incubated consecutively with 10 μCi ml^{-1} (−)-[7-^{3}H]-NA (specific activity: 17 Ci $mmol^{-1}$) for 30 min and after a wash-out, incubated with 0.5 μCi ml^{-1} [methyl-^{14}C]-choline (specific activity: 50 mCi $mmol^{-1}$) for another 30 min at 36°C. After the incubation each strip was suspended in a bath and superfused at a rate of 0.3 ml min^{-1} with oxygenated Krebs solution containing 10 μM hemicholinium-3. After a 60 min washing period, 3 min effluents were collected for 75 min with a fraction collector.

Stimulation and drug administration

Electrical field stimuli were applied through platinum plate electrodes positioned 2.5 cm apart on the top and the bottom of the perfusion bath. Two continuous electrical stimulations (100 shocks, 10 Hz, 100 V and 0.25 ms) were applied consecutively in the 66th (S_1) and 114th min (S_2) of the superfusion. Drugs were added to the perfusion solution 20 min before S_2; and the effects of the drugs were expressed as the S_2/S_1 ratio compared to that of controls.

Calculation of the experimental results

The radioactivity in the effluent was measured by using Optifluor scintillation reagent (Packard Industries) with a Beckman Beta Spectrometer. The measured counts were corrected to absolute activity both for ^{14}C and ^{3}H using the computer programme supplied with the scintillation counter. The acid soluble radioactive content of the tissue slices for both isotopes was determined after the experiments by placing the tissue in 1 ml of 10% trichloro-acetic acid for 16 h before counting. The released amounts of ^{3}H and ^{14}C are expressed as the fraction of the tissue ^{3}H and ^{14}C content, respectively (fractional release). The evoked release of NA and ACh was computed by calculating the area below the increased efflux curve of ^{3}H and ^{14}C, respectively, from the fractional release values as described earlier (Somogyi *et al.*, 1994). For characterization of the effect of treatments on basal release of radioactivity, we calculated the ratio of the basal release (B_2/B_1) of ^{3}H and ^{14}C before starting S_1 and S_2. This value is usually lower than one, unless the basal release of transmitter is increased by the treatment (Somogyi & de Groat, 1993).

Drugs

Atropine sulphate, desipramine hydrochloride, phenylephrine hydrochloride, methoxamine hydrochloride, tetrodotoxin and all constituents of the Krebs solution were purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). Terazosin was provided by Abbott Labs (Chicago, Ill, U.S.A.). (−)-[7-^{3}H]-noradrenaline and [methyl-^{14}C]-choline were purchased from NEN-Du Pont (Boston, MA, U.S.A.).

Statistical analysis

Student's *t* test and the non parametric Rank Sum test, or Kruskall-Wallis one way analysis of variance were used as appropriate. A level of $P<0.05$ was considered as statistically significant. The E_{max} as well as the ED_{50} and the pA_2 values were calculated with a computer programme (Tallarida & Murray, 1987).

Results

Effect of activation of α_1-adrenoceptors on the contractile response of the bladder strips

Phenylephrine (PE) added in increasing concentrations (0.2–128 μM) to the organ bath at 10 min intervals elicited three effects: (1) a concentration-dependent increase in the amplitude of the contractile response induced by electrical stimulation (Figure 1a), (2) increased basal tone in approximately 10% of the bladder strips; the maximal increase usually did not exceed 5–7% of the amplitude of the electrically evoked contractions, but the increase was concentration-dependent (Figure 1b), (3) increased low amplitude spontaneous activity (approximately 20% of the preparations). Similar effects were elicited by methoxamine (0.5–50 μM). Neither the spontaneous contractile activity nor the enhancement of the activity induced by α_1 agonists was inhibited by 1 μM tetrodotoxin or 0.1–1 μM atropine.

Tachyphylaxis did not occur when multiple cumulative dose-response curves were constructed at intervals of 20 min. The maximal effect of each concentration occurred within 4 min after administration of PE or methoxamine. In these experiments the effects of the highest concentration of PE were eliminated within 5–10 min after wash out of the drug.

To measure the direct postjunctional effect of PE on the contractile response of the bladder smooth muscle, dose-response curves for ACh were constructed in the absence and presence of various concentrations of PE (0.5–8 μM). In 3 experiments PE added to the bath 10 min before ACh did not significantly change the ACh dose-response curves (Figure 2). The slight shift of ACh dose-response curves to the right may be due to a weak desensitization of the postsynaptic muscarinic receptors to the high concentrations of ACh (up to 1 mM).

Effect of stimulus intensity and frequency on the PE-induced facilitation

Bladder strips were stimulated (10 Hz, 10 shocks) at an intensity producing 50% of the maximal contractions. As shown in Table 1 the contractions evoked by submaximal stimula-

tion exhibited a greater facilitation (85% ± 10 increase; $n = 13$) than those evoked by maximal stimulation (45% ± 9.2 increase, $n = 27$). Although the magnitudes of these effects were significantly different; $P < 0.01$, (Student's t test) there was no significant difference between the ED_{50} values for PE (1.44 ± 0.3 μM versus 1.1 ± 0.13 μM) during the two types of stimulation. The maximal facilitation produced by methoxamine, another selective α_1-agonist, during submaximal stimulation was in the same range (112 ± 24% increase, $n = 7$; $P > 0.1$ Student's t test) as that of PE; however, the ED_{50} value for methoxamine (Table 1) was significantly higher than that of PE.

The effect of PE was tested at different frequencies (0.1 to 50 Hz) with maximal intensities of stimulation and trains of 10 shocks. The magnitude of the facilitation correlated inversely with the stimulation frequency; the facilitatory effect of PE being more prominent at lower frequencies (0.1–5 Hz) than at higher frequencies (10–50 Hz) (Figure 3).

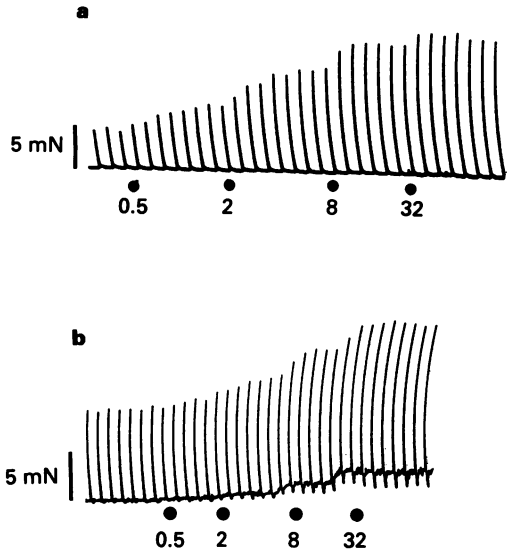

Figure 1 Facilitation by various concentrations of phenylephrine (PE) of the contractile response of a rat urinary bladder strip induced by submaximal electrical field stimulation of 10 Hz, 10 shocks at one minute intervals. (a) A typical tracing showing the facilitatory effect of PE. The concentration of PE was progressively increased at 6–10 min intervals. Note that there is minimal spontaneous activity of the strip, and the baseline is stable even at higher concentrations of PE. (b) In 10% of the bladder strips examined the basal tone and the spontaneous activity were increased by PE in addition to enhancement of the neurogenic contractions. Otherwise, the experiment was done in the same way as (a).

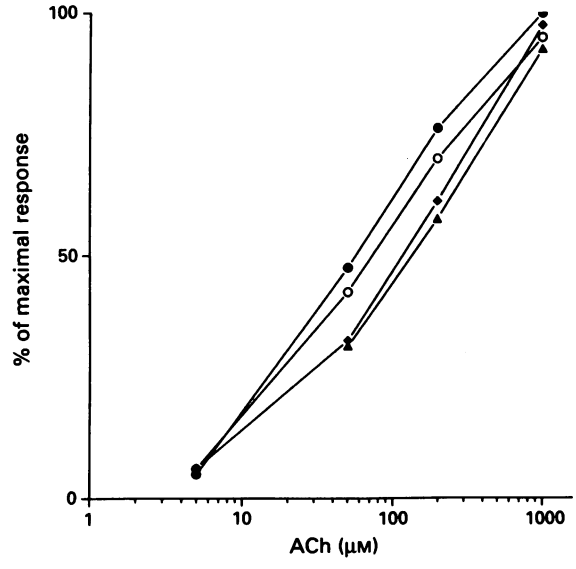

Figure 2 Effect of phenylephrine (PE) on the acetylcholine (ACh)-evoked contractile responses of the circular strips of rat urinary bladder. ACh was washed out after each addition. Increasing concentrations of PE were added 10 min before each ACh dose-response curve and were kept in the washing solution. The concentration of ACh is plotted against the contractile response expressed as the maximal response to ACh. The maximal response was extrapolated from the double reciprocal plot of the dose-response curve (Tallarida & Murray, 1987). The symbols represent (●) control; (○) 0.5 μM PE; (◆) 2 μM PE; (▲) 8 μM PE. Every point is the mean of 3 experiments. The s.e.mean was between 12–17%.

Effect of the extracellular Ca^{2+} concentration on the PE-induced facilitation

Since the M_1 muscarinic receptor mediated facilitation of cholinergic transmission in the bladder was positively correlated with the extracellular Ca^{2+} concentration (Somogyi *et al.*, 1994), the Ca^{2+}-dependence of the α_1 facilitation of the neurally evoked contractions was also examined. In these studies the extracellular Ca^{2+} concentration in the bath was changed in a stepwise fashion from 0 to 2.5 mM while the preparations were electrically stimulated. Then, the Ca^{2+} dose-response curve was repeated in the presence of various PE concentrations (0.5–8 μM). The amplitudes of the contractions at different Ca^{2+} concentrations in the presence and absence of PE are compared in Figure 4. PE was more effective ($P < 0.05$) in facilitating the neurogenic contractions at lower Ca^{2+} concentrations (0.5–1 mM), than at higher concentrations (1.5–2.5 mM).

Effect of terazosin (TRZ) on the PE response

The α_1-adrenoceptor antagonist, terazosin (TRZ), was added to the bath 20 min before constructing cumulative dose-response curves for PE and was present while PE was added to the bath. Experiments were begun with the lowest concentration of TRZ (10 nM) and when the maximal PE facilitation was reached, both PE and TRZ were washed out, and immediately a higher concentration of TRZ was added to the bath. The dose-response curve to PE was shifted to the right by TRZ in a dose-dependent manner (Figure 5). The pA_2 value for TRZ calculated according to Arunlakshana &

Table 1 The facilitatory effect of α_1-agonists on neurogenic contractions of the rat urinary bladder induced by maximal and submaximal intensities of electrical field stimulation (EFS)

	Maximal EFS		*Submaximal EFS*	
Drugs	ED_{50} (μM)	*Max. resp.* (%)	ED_{50} (μM)	*Max. resp.* (%)
PE	1.07 ± 0.13 $n = 27$	145 ± 9.2	1.44 ± 0.3 $n = 13$	185 ± 10
MTX			6.11 ± 1.7 $n = 7$	212 ± 24

The ED_{50} values were determined by calculating the concentrations of α_1-agonists which induce 50% of the maximal increase in electrically evoked contractions. The maximal facilitatory responses (max. resp.) to phenylephrine (PE) and methoxamine (MTX) were calculated as the percentage of the control contractions in strips stimulated with voltages producing a maximal contractile response or a voltage producing 50% of the maximal response (submaximal stimulation).

Schild (1959) was 8.26 and the slope of the Schild plot (1.13 ± 0.18) was not significantly different ($P>0.1$) from unity (Figure 6); indicating that TRZ inhibited the α_1-receptors in a competitive manner. TRZ (20–100 nM) inhibited the PE-induced increase in basal tone; however, it did not change the increased spontaneous activity evoked by PE.

Effect of PE and TRZ on the basal and evoked release of [^{3}H]-NA and [^{14}C]-ACh

The bladder tissues were incubated with [^{3}H]-NA and [^{14}C]-choline, so that the release of NA and ACh could be measured simultaneously. Two successive electrical stimulations using parameters (10 Hz, 100 shocks), which induce facilitation of ACh release via M_1 muscarinic receptors (Somogyi *et al.*, 1994) were applied in the 66th and 105th min of the superfusion (S_1 and S_2). The first stimulation markedly enhanced the efflux of both [^{3}H]-NA and [^{14}C]-ACh above the level of resting efflux. However, the amount of transmitter released was significantly lower during the second stimulation (S_2) producing an S_2/S_1 ratio of 0.49 ± 0.09 for [^{3}H]-NA and 0.3 ± 0.04 for [^{14}C]-ACh. The α_1-agonist, PE applied between S_1 and S_2 at a concentration of 2 μM (which is twice the ED_{50} value for facilitating neurally evoked contractions) significantly ($P<0.05$) facilitated the release of both NA (140.8 ± 47% increase) and ACh (173 ± 37% increase) elicited by the second stimulation. The α_1-antagonist, TRZ (0.1 μM) blocked the PE-induced facilitation of ACh release (53 ± 36% increase; not significantly different from the release in the absence of drugs; $P>0.05$). TRZ did not

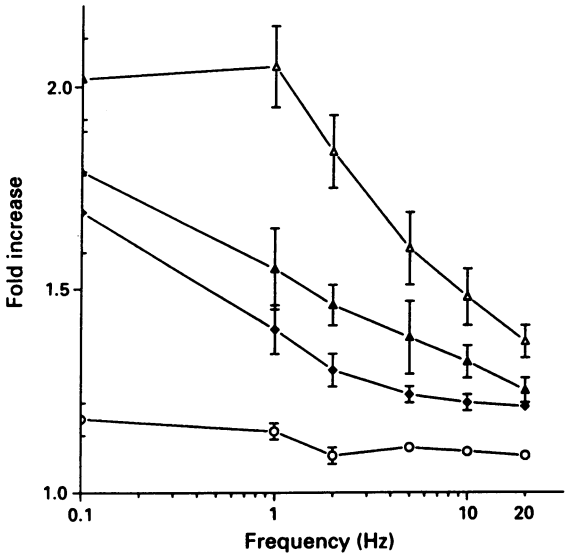

Figure 3 Frequency-dependence of phenylephrine (PE)-induced facilitation of the contractions of rat urinary bladder strips elicited by supramaximal electrical field stimulation. The ratio of the contractions in the presence and absence of various concentrations of PE is plotted against the stimulation frequency. Note that the lower frequencies of stimulation were more sensitive to the PE facilitation than the higher frequency of stimulation. The symbols represent (○) 0.1 μM, (◆) 0.5 μM, (▲) 2 μM and (△) 8 μM PE. Every point is an average of 4 experiments.

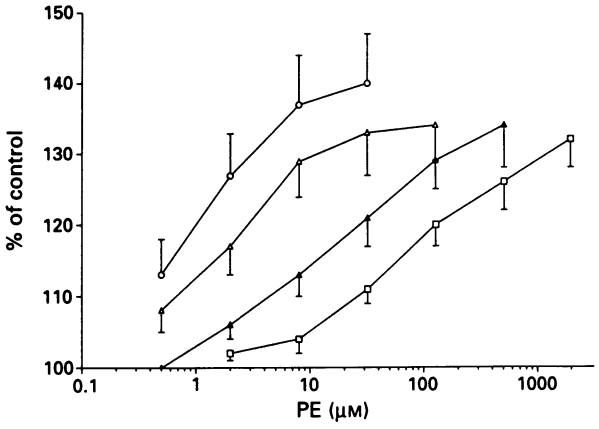

Figure 5 The effect of various concentrations of the α_1-adrenoceptor antagonist, terazosin, on phenylephrine (PE)-induced facilitation of the neurally evoked contractions of rat urinary bladder strips. The facilitatory effect of PE, expressed as a percentage of the control, was plotted against the concentrations of PE in μM. The symbols represent: no terazosin (○), 50 nM (△), 100 nM (▲), and 200 nM (□) terazosin. Every point is the mean ± s.e.mean of 7 experiments.

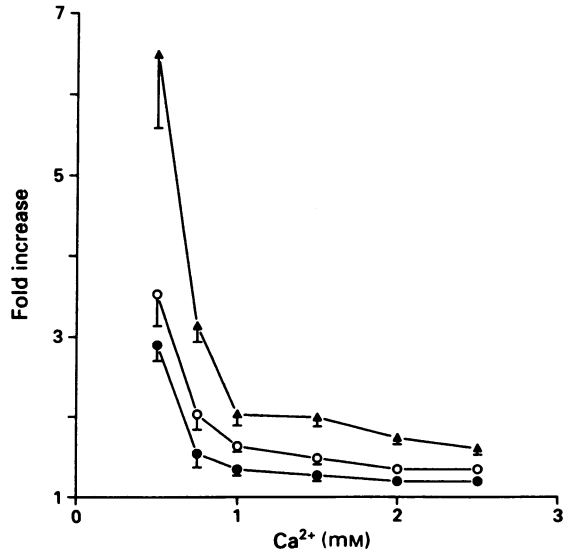

Figure 4 The influence of Ca^{2+} concentrations on the phenylephrine (PE)-facilitation of neurally evoked contractions of rat urinary bladder strips. The ratio of PE facilitated contractile responses to control (fold increase) is plotted against the Ca^{2+} concentration. The symbols represent: (●) 0.5 μM; (○) 2 μM; (▲) 8 μM PE. Every point is the mean (±s.e.mean) of 7 experiments.

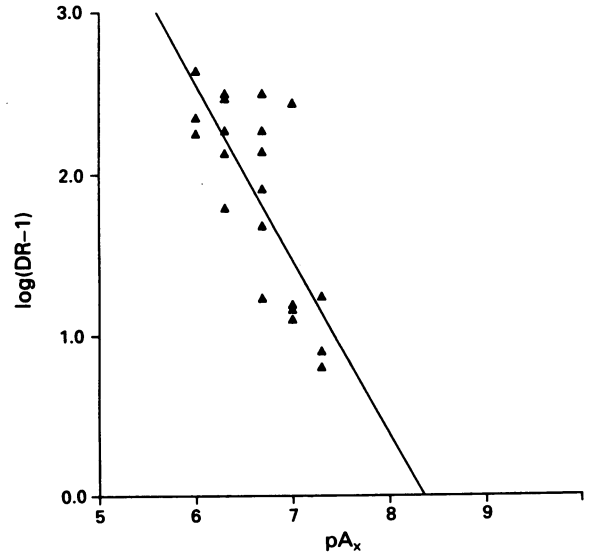

Figure 6 Schild plot showing the effect of the α_1-adrenoceptor antagonist, terazosin, on phenylephrine (PE) facilitation of neurally evoked contractions of rat urinary bladder strips. (pA_2 value: 8.27; slope: 1.13 ± 0.14; r: −0.81, $n=21$) where 'r' is the correlation coefficient. The slope is not significantly different from unity ($P>0.2$, paired t test).

Table 2 Effect of phenylephrine and terazosin on the basal release of [^{14}C]-acetylcholine ([^{14}C]-ACh) and [^{3}H]-noradrenaline ([^{3}H]-NA) in the presence and absence of desipramine

	ACh release		*NA release*	
Treatment	*Normal*	*Desipramine*	*Normal*	*Desipramine*
Control	0.72 ± 0.06 (6)	0.71 ± 0.04 (4)	0.77 ± 0.03 (6)	0.78 ± 0.01 (4)
PE (2 μM)	0.79 ± 0.06 (6)	0.64 ± 0.04 (4)	2.16 ± 0.17 (6)*	0.79 ± 0.02 (4)
TRZ + PE	0.82 ± 0.07 (6)	0.72 ± 0.11 (4)	2.05 ± 0.22 (6)*	0.92 ± 0.11 (4)
TRZ (0.1 μM)	0.79 ± 0.08 (6)	0.79 ± 0.08 (4)	0.91 ± 0.06 (6)	0.79 ± 0.03 (4)

The numbers in the table represent the B_2/B_1 ratio which was computed from the basal release values of [^{3}H]-NA and [^{14}C]-ACh before S_1 and S_2. Desipramine was present in a concentration of 2 μM. Normal values represent the basal release in preparations not treated with desipramine. The drugs (PE = phenylephrine; TRZ = terazosin and TRZ + PE) were added to the perfusion solution 20 min before S_2.

*Indicates values significantly different from the control in the same column ($P<0.05$, Kruskall-Wallis non-parametric one way analysis of variance combined with Rank Sum test). Note that desipramine blocked the effect of PE and PE + TRZ treatment on basal release of [^{3}H]-NA ($P<0.05$; Rank Sum test).

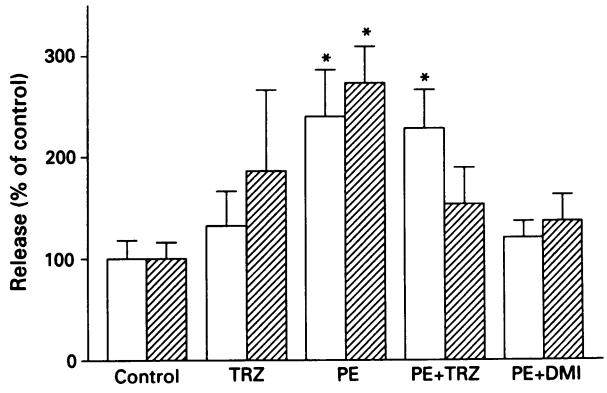

Figure 7 The effect of phenylephrine (PE) and/or terazosin (TRZ) on the stimulation evoked release of [^{14}C]-acetylcholine ([^{14}C]-ACh) and [^{3}H]-noradrenaline ([^{3}H]-NA) in normal and in desipramine (2 μM)-treated rat urinary bladder strips. Two supramaximal electrical field stimulations (100 V, 10 Hz, 100 shocks) were applied in the 66th (S_1) and 114th min (S_2) of superfusion and PE, TRZ or PE + TRZ were administered 20 min before S_2. When desipramine (2 μM) was applied, it was present throughout the experiments. Drug effects are expressed as % of the S_2/S_1 ratios in control experiments. Open columns denote NA release, hatched columns ACh release and the vertical lines denote s.e.mean.

alter the facilitation of [^{3}H]-NA release (128 ± 38% increase; not significantly different from the control PE facilitation of release; $P>0.05$). TRZ alone added to the perfusion solution 20 min before S_2 had no significant effect ($P>0.05$) either on [^{3}H]-NA (33 ± 34% increase) or on [^{14}C]-ACh release (86 ± 80% increase).

PE (2 μM) did not change the basal release of ACh but significantly increased the basal release of [^{3}H]-NA (181 ± 22% increase, $P<0.05$). In the presence of TRZ (0.1 μM) PE produced a similar increase in basal [^{3}H]-NA release (166 ± 28% increase; $P>0.05$) (Table 2), indicating that the effect of PE is not mediated by activation of α_1-receptors. However, desipramine (2 μM) treatment before S_2 completely inhibited (1 ± 2.5% increase; $P>0.05$) the PE-evoked increase of basal release demonstrating that an amine transport mechanism is involved in the basal release of NA (Vizi *et al.*, 1986b). The PE-induced increase in the electrically evoked [^{3}H]-NA and [^{14}C]-ACh release was completely abolished in the presence of desipramine (Figure 7).

Discussion

The present experiments revealed that PE, an α_1-adrenoceptor agonist, increased neurally evoked contractions as well as ACh and NA release in rat urinary bladder strips but did not change ACh-induced bladder contractions. It is concluded that at least part of the facilitatory effect of exogenous α_1-adrenoceptor agents on excitatory transmission in the rat bladder is mediated by prejunctional receptors on the cholinergic nerve terminals.

A review of the literature has not identified other studies which evaluated the effect of α_1-adrenoceptor agonists on neurally evoked bladder contractions; however, there are numerous reports of the direct effects of these agents on the bladder smooth muscle. Experiments in several species revealed that α_1-agonists elicited contractions of the smooth muscle in the bladder base but evoked variable effects on the bladder body (Downie *et al.*, 1975), ranging from no response to prominent excitatory responses (Ordway *et al.*, 1986). Some of this variation could be due to the age of the animals, since Ordway *et al.* (1986) reported that α_1-agonists produced more prominent excitatory responses in older (29 months) than in younger rats (7 months). In the young animals (3–4 months) which were used in the present study only 10% of the preparations exhibited a detectable rise in baseline tone following PE or methoxamine. However, these agents commonly increased the frequency of spontaneous contractions. Since the latter effect of PE or methoxamine was not blocked either by TRZ, tetrodotoxin or atropine, it seems reasonable to conclude that this effect is due to a direct postjunctional action on the smooth muscle. However, several observations indicate that this weak postjunctional action is unlikely to account for the facilitation of neurally evoked bladder contractions. Thus, the α_1-adrenoceptor facilitation of neurogenic contractions is most reasonably attributed to a prejunctional action.

To evaluate the contribution of the putative postjunctional α_1-adrenoceptor activation to the PE-induced increase of neurogenic contractions in the rat bladder, dose-response curves to ACh were constructed in the presence and absence of facilitatory concentrations of PE. Since the contractions evoked by ACh were not enhanced by PE the activation of the postjunctional α_1-adrenoceptors may not contribute significantly to the facilitation of neurogenic contractions. However, it is also possible that α_1-agonists could enhance the postjunctional action of non-cholinergic excitatory transmitters, since it is known that the rat bladder receives a noncholinergic-nonadrenergic excitatory innervation from parasympathetic (Alm & Elmér, 1975; Lincoln & Burnstock, 1993) as well as from afferent nerves (Maggi, 1993). This will be tested in future experiments by examining the effects of PE on the bladder contractions induced by ATP or substance P.

The possibility that a postjunctional action of PE might contribute to the facilitation of neurally evoked bladder contractions is also diminished by two other observations. First, PE elicited a direct effect on bladder smooth muscle baseline tension in only 10% of the preparations; whereas it consistently enhanced the neurally evoked contractions.

Secondly, the effect of PE in enhancing the amplitude of the intrinsic rhythmic activity of the smooth muscle strips was not altered by TRZ in concentrations that blocked the facilitation of neurally evoked contractions.

The present experiments also provided direct evidence that ACh release is facilitated by PE and that this effect of PE is blocked by an α_1-antagonist. This supports the conclusions based on measurements of contractile responses that the facilitatory α_1-adrenoceptors are located prejunctionally in the bladder. Earlier studies revealed facilitatory α_1-adrenoceptors on the cholinergic neurones in the vesical ganglia of the cat (de Groat & Booth, 1980; Akasu *et al.*, 1985; Keast *et al.*, 1990) and on dissociated bladder neurones from the rat major pelvic ganglion (Yoshimura & de Groat, 1992). Thus both the cell body and the nerve terminals of bladder cholinergic neurones exhibit facilitatory α_1-adrenoceptors. In contrast, at the cholinergic nerve terminals in the heart, prejunctional α_1-adrenoceptors are generally thought to inhibit ACh release (Wetzel *et al.*, 1985). One study has indicated that the α_1-agonist, oxymethazone, can facilitate ACh release in the heart; however, other α_1-adrenoceptor agonists did not mimic the effect of oxymethazone (Bognar *et al.*, 1990). Thus, the present demonstration of PE facilitation of neural input to the bladder is the clearest example in the peripheral nervous system of prejunctional α_1-adrenoceptor facilitation.

The α_1-blocker, TRZ, competitively inhibited the PE-induced facilitation of the contractile response of bladder strips to nerve stimulation yielding a pA_2 value of 8.3. Prazosin, another α_1-antagonist, which is generally equiactive with terazosin on α_1-adrenoceptors, has a similar pA_2 value for blocking α_1-receptors in the rat detrusor muscle (8.57; Ordway *et al.*, 1986) and in the rabbit pulmonary artery (8.76; Vizi *et al.*, 1986a). These results indicate that both the postjunctional and prejunctional α_1-receptors bind the non-selective α_1-antagonists with high affinity.

The degree of α_1-facilitation of the contractile response was inversely related to the magnitude of the contractions, being greater at low frequencies or submaximal intensities of stimulation or at low extracellular Ca^{2+}. Previously, we described a marked facilitation of ACh release in the urinary bladder through activation of prejunctional M_1 muscarinic receptors (Somogyi & de Groat, 1992; Somogyi *et al.*, 1994). In contrast to α_1-facilitation, M_1 facilitation was positively correlated with the stimulation strength, stimulation frequency and with the extracellular Ca^{2+} levels. Thus M_1 and α_1-facilitation have very different properties, possibly related to activation of different signal transduction pathways.

PE also enhanced the evoked and basal release of NA. However, the mechanisms involved in the PE-facilitation of ACh and NA release are clearly different. The facilitatory effect on ACh release was blocked by TRZ; whereas, the facilitation of NA release was not blocked. Thus PE could influence the outflow of NA by altering NA reuptake into the nerve terminals. This is consistent with the finding that desipramine, a NA uptake blocker, abolished the PE facilitation of NA release. In addition, PE enhanced basal release of NA but not the basal release of ACh; and this effect of PE was also blocked by desipramine. Thus it seems likely that the effect of PE on NA release is related to an effect on the amine transport system and not mediated by activation of α_1-receptors on the adrenergic terminals. Similar effects of NA uptake blockers on the carrier mediated release of NA have been described in other tissues (Vizi *et al.*, 1986b; Somogyi & Perel, 1991). One unusual observation which was not evaluated in detail was the effect of desipramine in blocking the PE-induced facilitation of ACh release. This might be mediated by a desipramine block of α_1-receptors, an effect that has been noted in other tissues with high concentrations of the drug (Hall & Ögren, 1981).

Although exogenous PE facilitated neurally evoked release of ACh, the physiological significance of this observation is uncertain since we could not detect a similar effect of endogenously released NA. If endogenous NA were exerting a facilitatory effect on the cholinergic nerve terminals, then it would be expected that blockade of α_1-adrenoceptors with TRZ would reduce neurally evoked release of ACh or the neurally evoked bladder contractions. However, neither response occurred.

Thus under the conditions of our experiments it appears that cholinergic transmission is not modulated by α_1-adrenoceptor mechanisms. However, this modulation may be prominent under more physiological conditions *in vivo* or in the presence of pathology. For example in the chronically decentralized bladder of the cat (Sundin *et al.*, 1977) or in human neurogenic bladders (Seiferth, 1976; Swierzewski *et al.*, 1993) it has been reported that excitatory α_1-adrenoceptors are upregulated and that these receptors can contribute to bladder hyperactivity. It is possible that both prejunctional and postjunctional receptor upregulation may occur in this condition. Similarly, the upregulation of α_1-adrenoceptors which occurs postjunctionally in the old rat (Ordway *et al.*, 1986) may occur prejunctionally as well. In future experiments α_1-adrenoceptor modulation of ACh release will be tested in the urinary bladder strips from older animals. Postjunctional α_1-adrenoceptors may also be important in pathological conditions such as benign prostatic hypertrophy (BPH). α_1-Adrenoceptor blocking agents have been used in the treatment of the symptoms of BPH. Although the rationale for the use of these agents in prostate disease is to reduce adrenergic tone in the urethra and thereby reduce urethral resistance and increase urine flow, it has been reported that there is no significant direct correlation between the drug-induced changes of bladder symptoms and urinary flow rates (Lepor *et al.*, 1990). This could occur if the adrenoceptor blocking agents act on neural (prejunctional) receptors as well as on postsynaptic receptors in the smooth muscle of the lower urinary tract. Thus the adrenergic facilitatory mechanism at cholinergic terminals in the urinary bladder may be an important target for drug therapy in the treatment of bladder hyperactivity.

This work was supported by a small grant from Abbott Labs as well as by NSF grant BNS-890-8934 and NIDDK Grants DK-45741 and DK-4930.

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(Received October 3, 1994
Revised December 29, 1994
Accepted January 6, 1995)