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Inhibition of contractions by tricyclic antidepressants and xylamine in rat vas deferens

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Abstract

The effects of noradrenaline uptake inhibitors on contractions evoked by electric field stimulation, noradrenaline, clonidine, 5-hydroxytryptamine, ATP, high K^+, and $BaCl_2$ in the epididymal half of rat isolated vas deferens were examined. Protriptyline, amitriptyline and xylamine concentration-dependently inhibited monophasic contractions induced by low frequency electrical stimulation (0.3 Hz, 1 ms duration, 60 V). Protriptyline and xylamine inhibited in a noncompetitive manner the contractile response induced by noradrenaline (3×10^{-8}–3×10^{-5} M) and the inhibitory effect of protriptyline was reversible, while xylamine produced long-lasting inhibition. All three noradrenaline uptake blockers inhibited the clonidine (3×10^{-6} M) or 5-hydroxytryptamine (10^{-5} M)-induced contraction. Protriptyline and amitriptyline at concentrations of 3×10^{-6}–3×10^{-5} M reversibly inhibited the ATP (10^{-4} M)-induced monophasic contraction. In contrast, xylamine ($(1–3) \times 10^{-5}$ M) had no effect. Protriptyline and amitriptyline but not xylamine concentration-dependently reduced the high K^+ (6×10^{-2} M)-induced sustained contraction with respective IC_{50} values of 1.81×10^{-6} M and 8.6×10^{-7} M. Protriptyline and amitriptyline at 10^{-5} M reversibly inhibited $BaCl_2$ (3×10^{-3} M)-induced phasic contractions and xylamine (10^{-5} M) had no effect. These findings demonstrate that tricyclic antidepressants might exert direct inhibitory action on mechanical contraction pathway, whilst xylamine, a structurally different inhibitor of noradrenaline uptake, may act mainly at α-adrenoceptors and other amine receptors on the smooth muscle of the rat vas deferens as a long-lasting nonselective antagonist, and it at least in part blocks sympathetic transmission.

Keywords: Noradrenaline uptake inhibitor; Protriptyline; Amitriptyline; Xylamine; Electric field stimulation; Contraction; Vas deferens, rat

1. Introduction

The antidepressant effect of tricyclic agents such as protriptyline and amitriptyline is thought to be caused by inhibition of noradrenaline uptake in the central nervous system (Iversen, 1965), so that a greater concentration of noradrenaline is made available at the postsynaptic surface, resulting in potentiation of adrenergic transmission (Bassett et al., 1969; Sulser et al., 1969). However, the adverse effects are generally attributed to their antagonistic effects at muscarinic receptors and adrenoceptors on the postsynaptic membrane in peripheral tissues (Klerman and Cole, 1965; Hrdina and Ling, 1970; Doggrell and Vincent, 1981; Rehavi et al., 1987). For example, tricyclic antidepressants relax smooth muscle of rat blood vessels (Hrdina and Ling, 1970), rat anococcygeus (Doggrell and Vincent, 1981), and human and guinea-pig bladder (Rehavi et al., 1987). Recently, more detailed study has demonstrated that tricyclic drugs can have direct inhibitory effects on the mechanical contraction of arterial smooth muscle (Huang, 1996). On the other hand, xylamine, a nontricyclic inhibitor of noradrenaline uptake (Cho et al., 1980; Ransom et al., 1985) was found to exert inhibitory action on both presynaptic and postsynaptic sites in sympathetic transmission (Ransom et al., 1984; Nedergaard et al., 1992).

Antidepressant-related sexual dysfunction including impaired ejaculation and impotence has been reported in some patients undergoing tricyclic therapy (Harrison et al., 1986); on the other hand, imipramine has been used to treat retrograde ejaculation (Brooks et al., 1980). Many noradrenaline uptake blockers were reported to influence neurotransmission in vas deferens (Ursillo and Jacobson, 1965; Kasuya and Goto, 1968; Leedham et al., 1985). Desipramine and nisoxetine at low concentrations caused potentiation of the postjunctional response to noradrenaline probably through inhibition of noradrenaline uptake into

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nerve terminals (Leedham et al., 1985). However, these agents could reduce contractile response in different smooth muscles at high concentrations (Hrdina and Ling, 1970; Doggrell and Vincent, 1981; Leedham et al., 1985; Rehavi et al., 1987; Huang, 1996). This paper describes the results of studies investigating the inhibitory effects of noradrenaline uptake inhibitors on contractile responses induced by different stimuli in isolated rat vas deferens. It was found that protriptyline and amitriptyline appear to act at multiple sites on smooth muscle to cause relaxation, e.g. noncompetitive antagonism of α-adrenoceptors and inhibition of Ca^{2+} influx. Xylamine, a structurally distinct inhibitor, seems to nonselectively antagonize the effect of noradrenaline, clonidine and 5-hydroxytryptamine, and at least partially to block sympathetic transmission.

2. Materials and methods

2.1. Preparation

Male Sprague-Dawley rats weighing about 400 g were anaesthetized with pentobarbital sodium (60 mg/kg body weight) and a pair of vas deferens were dissected out. The epididymal segment was cut and cleared of surrounding connective tissue. The tissue was then mounted between two platinum electrodes placed 1 cm apart in a 20 ml organ bath containing Krebs-Henseleit solution of the following compositions (mM): 119 NaCl, 4.2 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaCO_3$, 0.03 Na_2EDTA, 11.1 *d*-glucose, 0.2 ascorbic acid. The segment was positioned vertically with one end attached to a glass support and another end to the force transducer (Grass Instruments). The bath solution was maintained at 37°C and constantly gassed with 95% O_2 plus 5% CO_2. Tissues were allowed to equilibrate under 1 g resting tension for 1.5 h during which time the bath medium was changed every 30 min.

2.2. Electrical field stimulation

The electrical stimulation was repetitively delivered to vas deferens with use of a Grass SD9 Stimulator via two platinum electrodes (0.3 Hz, 1 ms pulse duration, 60 V). The electrically evoked monophasic contractions were abolished by tetrodotoxin (3×10^{-6} M). Noradrenaline uptake inhibitors were added cumulatively to the bath to induce concentration-dependent reduction of electrically evoked contractions.

2.3. The noradrenaline-, clonidine-, 5-hydroxytryptamine- and ATP-induced contraction

Tissues were contracted with noradrenaline at concentrations ranging from 3×10^{-7} to 3×10^{-5} M to construct the first concentration–response curves. Once the maximum response to noradrenaline has been obtained, preparations were rinsed with Krebs-Henseleit solution every 20 min until the tension fell to the basal level. Tissues were then equilibrated with different concentrations of noradrenaline uptake inhibitors for 30 min and another cumulative concentration–response curve to noradrenaline was repeated. In another set of experiments, a single concentration of clonidine (3×10^{-6} M) or 5-hydroxytryptamine (10^{-5} M) or adenosine 5′-triphosphate (ATP, 10^{-4} M) was used to evoke the transient contractile response. Similar peak amplitude of three consecutive contractions by each agonist with intervals of about 45 min were determined as control. When each of the noradrenaline uptake inhibitors was tested, it was allowed to equilibrate for 30 min prior to the second application of the agonist. The magnitude of the second evoked peak contraction was measured as an index of the effect for each drug on the agonist-induced response in vas deferens. The reversibility of the effect of each drug was tested by measuring the third contractile response induced by the agonist after washout of the drug for four times (between 45 min and 3 h) with normal Krebs-Henseleit solution.

2.4. The high K^+- and $BaCl_2$-induced contraction

Responses to a single concentration of K^+ (6×10^{-2} M) were obtained in the epididymal half of vas deferens. Cumulative application of noradrenaline uptake inhibitors caused concentration-dependent inhibition of the high K^+-induced sustained contraction. The possible inhibitory effect of noradrenaline uptake inhibitors on the Ba^{2+}-induced response was examined with the same protocol as used in effects of these drugs on ATP-induced contraction.

2.5. Drugs

The following chemicals were used: (−)-noradrenaline bitartrate, clonidine hydrochloride, 5-hydroxytryptamine hydrochloride, prazosin hydrochloride, disodium adenosine 5′-triphosphate, α,β-methylene ATP, tetrodotoxin, glibenclamide (Sigma, St. Louis, MO, USA), protriptyline hydrochloride, amitriptyline hydrochloride, xylamine hydrochloride, charybdotoxin, yohimbine hydrochloride (Research Biochemicals, Natick, MA, USA). All agents were dissolved in Krebs solution except for prazosin and glibenclamide in dimethyl sulfoxide (0.2%). Dimethyl sulfoxide (0.2%) in organ baths did not affect electrically evoked contractions.

2.6. Statistical analysis

Results were expressed as mean ± S.E.M. from *n* experiments. The effects of noradrenaline uptake inhibitors on contractions induced by field stimulation or high K^+ were expressed as a percentage of the control value. Cumulative concentration–response relations were analyzed with a nonlinear curve fitting by a logistic equation

(Graft, Erithacuc Software) and IC_{50} values were calculated as the drug concentration causing a half-maximal inhibition. Student's *t*-test was used to evaluate the significance between mean values.

3. Results

3.1. Effect of tricyclic antidepressants and xylamine on neurotransmission

Monophasic contractions (6.25 ± 0.04 mN, $n = 34$) of the epididymal half of rat vas deferens were evoked by repetitive electric field stimulation. Prazosin (3×10^{-6} M) and α,β-methylene ATP (3×10^{-6} M) reduced the amplitude of electrically evoked contraction to $42.5 \pm 9.8\%$ and $67.3 \pm 8.5\%$ of control, respectively ($n = 5$ in each case), indicating that the contraction was caused by noradrenaline and ATP probably co-released from sympathetic nerve endings. Protriptyline and xylamine induced concentration-dependent inhibition of electrically evoked contractions with similar potency. IC_{50} values for protriptyline and xylamine were $2.19 \times 10^{-6} \pm 0.18 \times 10^{-6}$ M ($n = 8$) and $2.03 \times 10^{-6} \pm 0.25 \times 10^{-6}$ M ($n = 8$), respectively (Fig. 1). In contrast to a complete inhibition by protriptyline and xylamine, amitriptyline, an analogue of protriptyline, only caused a maximal 68% reduction of electrically evoked contraction (Fig. 1B). Blockers of K^+ channels such as charybdotoxin (10^{-7} M, $n = 4$), and glibenclamide (10^{-5} M, $n = 6$) did not reverse the relaxant effects of noradrenaline uptake blockers (data not shown). Protriptyline, amitriptyline and xylamine at concentrations between 10^{-8} and 3×10^{-5} M did not alter the resting tension in the preparations.

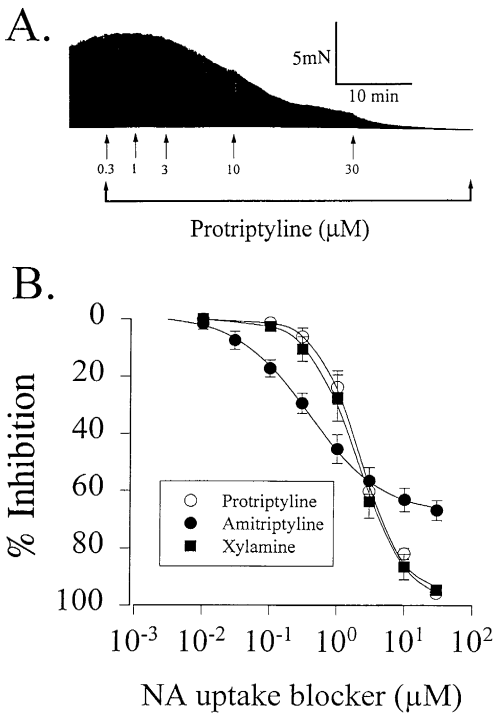

Fig. 1. (A) A typical trace of the concentration-dependent inhibition by protriptyline of electrically evoked contractions in rat vas deferens. (B) Concentration–response curves for the inhibitory effects of protriptyline (○), amitriptyline (●) and xylamine (■). Curves were drawn by fitting the data points to the logistic equation. Values are mean ± S.E.M. from eight experiments.

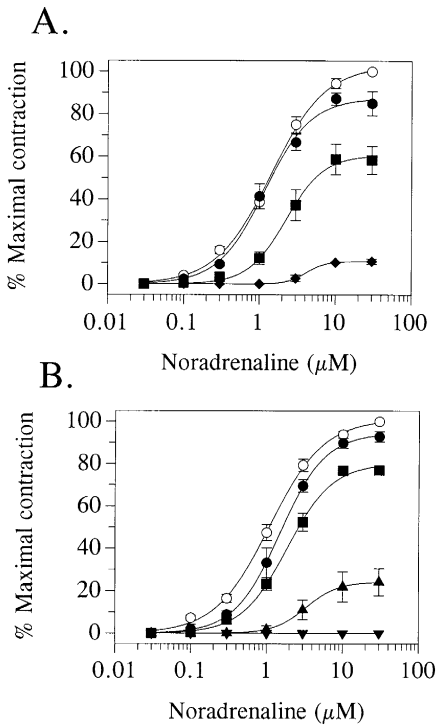

Fig. 2. (A) Logarithmic concentration–response curves of contractions of rat vas deferens for noradrenaline in the absence (○, control, $n = 18$) and presence of protriptyline (●, 10^{-6} M, $n = 6$; ■, 3×10^{-6} M, $n = 6$; ◆, 10^{-5} M, $n = 6$). (B) Concentration–contraction curves for noradrenaline in the absence (○, control, $n = 22$) and presence of xylamine (●, 10^{-7} M, $n = 6$; ■, 3×10^{-7} M, $n = 6$; ▲, 10^{-6} M, $n = 6$; ▼, 3×10^{-6} M, $n = 4$). Drugs were incubated for 30 min before repeating the second concentration-response curve. Data are expressed as a percentage of the maximum response obtained in the first (control) concentration-response curve. Curves are drawn by fitting the data points to the logistic equation. Values are mean ± S.E.M. from *n* experiments.

3.2. Effect of tricyclic antidepressants and xylamine on the noradrenaline-, clonidine-, 5-hydroxytryptamine and ATP-induced contractile response

Noradrenaline produced contractions consisting of intermittent spikes superimposed upon a tonic component with an EC_{50} value of $1.26 \times 10^{-6} \pm 0.04 \times 10^{-6}$ M ($n = 40$) and a maximum contraction (measured as the mean height of intermittent spikes) of 9.56 ± 0.32 mN ($n = 40$). Both protriptyline (Fig. 2A) and xylamine (Fig. 2B) noncompetitively antagonized contractions induced by noradrenaline (3×10^{-7}–3×10^{-5} M) after 30 min incubation in the

bath. The inhibitory effect of protriptyline was reversible, but xylamine at a concentration greater than 3×10^{-6} M appeared to induce long-lasting inhibition since noradrenaline failed to contract the preparation after repeated washout of the drug over 3 h. Similarly, protriptyline (10^{-5} M), amitriptyline (10^{-5} M) and xylamine (10^{-6}–10^{-5} M) reduced the clonidine (3×10^{-6} M)- or 5-hydroxytryptamine (10^{-5} M)-induced contractile responses (Table 1). Besides, protriptyline (3×10^{-6}–3×10^{-5} M) and amitriptyline (3×10^{-6}–3×10^{-5} M) reversibly inhibited the ATP (10^{-4} M)-induced monophasic contraction. In contrast, xylamine (10^{-5}–3×10^{-5} M) had no effect (Table 1). However, xylamine at 10^{-5} M reduced electrically evoked contractions by about 94% (Fig. 1B). In order to test a possibility that the increased synaptic noradrenaline levels due to blockade by xylamine of noradrenaline uptake could activate the prejunctional α_2-adrenoceptors to inhibit electrically stimulated release of contractile transmitters, yohimbine was used to block the α_2-adrenoceptors. Yohimbine alone at 3×10^{-6} M reduced electrically evoked contractions by $12.6 \pm 2.8\%$ ($n = 4$) of control. However, xylamine was still able to inhibit electrically evoked contractions in the presence of yohimbine ($IC_{50} = 2.48 \times 10^{-6} \pm 0.28 \times 10^{-6}$ M, 91% maximum inhibition, $n = 4$, in yohimbine-treated tissue compared to $IC_{50} = 2.03 \times 10^{-6} \pm 0.25 \times 10^{-6}$ M, 94% maximum inhibition, $n = 8$, in untreated tissue, Fig. 1B, $P > 0.05$). In addition, xylamine at 3×10^{-5} M reduced by $91.4 \pm 6.8\%$ ($n = 5$, data not shown) electrically stimulated contractions in the presence of both prazosin (3×10^{-6} M) and yohimbine (3×10^{-6} M).

3.3. Effect of tricyclic antidepressants and xylamine on the high K^+-induced contraction

Results obtained from above experiments suggest that three agents used in the present study nonselectively antagonize effects of noradrenaline and 5-hydroxytryptamine as previously reported in smooth muscle of rat arteries and anococcygeus (Hrdina and Ling, 1970; Doggrell and Vin-

Table 1
Effects of tricyclic antidepressants and xylamine on the clonidine-, 5-hydroxytryptamine-, ATP- and $BaCl_2$-induced contraction (in mN) in rat vas deferens

		Contractile response			*n*
		First	Second	Third	
Clonidine					
Control		7.40 ± 1.07	8.02 ± 1.07	7.96 ± 1.04	5
Protriptyline	10^{-5} M	6.33 ± 0.88	0.03 ± 0.03 [a]	3.13 ± 0.82 [b]	4
Amitriptyline	10^{-5} M	6.90 ± 1.36	0.05 ± 0.03 [a]	3.23 ± 1.40 [b]	4
Xylamine	10^{-6} M	7.75 ± 0.49	4.02 ± 0.80 [a]	5.30 ± 0.60	4
	3×10^{-6} M	7.28 ± 0.87	0 [a]	0	4
5-Hydroxytryptamine					
Control		3.33 ± 0.56	3.60 ± 0.48	4.15 ± 0.59	5
Protriptyline	10^{-5} M	3.75 ± 0.30	0.03 ± 0.03 [a]	3.13 ± 0.47 [b]	4
Amitriptyline	10^{-5} M	3.33 ± 0.68	0 [a]	0.20 ± 0.14 [b]	4
Xylamine	3×10^{-6} M	3.17 ± 0.45	1.23 ± 0.25 [a]	1.57 ± 0.22	4
	10^{-5} M	3.23 ± 0.11	0 [a]	0	4
ATP					
Control		4.13 ± 0.20	4.11 ± 0.21	4.07 ± 0.16	6
Protriptyline	3×10^{-6} M	4.00 ± 0.59	3.20 ± 0.27	4.03 ± 0.39	4
	10^{-5} M	3.88 ± 0.37	2.07 ± 0.29 [a]	3.92 ± 0.51 [b]	5
	3×10^{-5} M	4.23 ± 0.46	1.03 ± 0.15 [a]	4.13 ± 0.41 [b]	5
Amitriptyline	3×10^{-6} M	4.38 ± 0.59	3.93 ± 0.48	4.10 ± 0.48	4
	10^{-5} M	3.82 ± 0.37	2.52 ± 0.38 [a]	3.78 ± 0.64 [b]	5
	3×10^{-5} M	4.48 ± 0.58	1.45 ± 0.23 [a]	3.70 ± 0.58 [b]	5
Xylamine	10^{-5} M	4.05 ± 0.23	3.83 ± 0.49	4.37 ± 0.34	6
	3×10^{-5} M	4.37 ± 0.27	4.32 ± 0.42	4.10 ± 0.18	4
$BaCl_2$					
Protriptyline	10^{-5} M	6.68 ± 0.47	1.65 ± 0.23 [a]	4.96 ± 0.59 [b]	4
Amitriptyline	10^{-5} M	6.43 ± 0.59	1.14 ± 0.33 [a]	5.49 ± 0.51 [b]	4
Xylamine	10^{-5} M	6.85 ± 0.41	6.94 ± 0.53	6.78 ± 0.34	4

The mean peak amplitude of three consecutive contractions induced by clonidine (3×10^{-6} M), 5-hydroxytryptamine (10^{-5} M), ATP (10^{-4} M) or $BaCl_2$ (3×10^{-3} M) was measured and muscle tension was presented in mN. The effect of each drug was tested by comparing the amplitude of the second evoked contraction in the presence of drugs to the first evoked contraction in the absence of drugs. Drugs were added to the bath 30 min prior to the second application of each agonist. The interval between second and third application of the agonist was 3 h. Significant difference between the first and second evoked contractions ([a] $P < 0.01$) and between the second and third evoked contractions ([b] $P < 0.05$) in paired data. Values are mean ± S.E.M. from *n* experiments indicated.

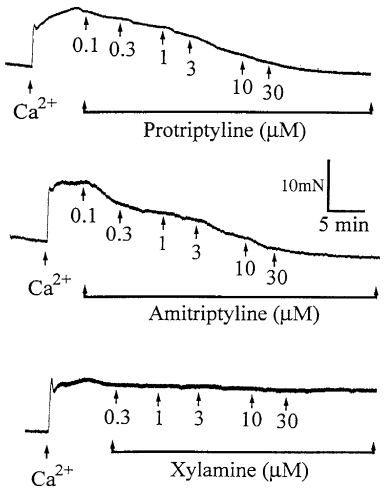

Fig. 3. Traces are representative records for effects of protriptyline, amitriptyline and xylamine on the high K^+ (6×10^{-2} M)-induced sustained contraction of rat vas deferens. Calibration bars apply to all traces.

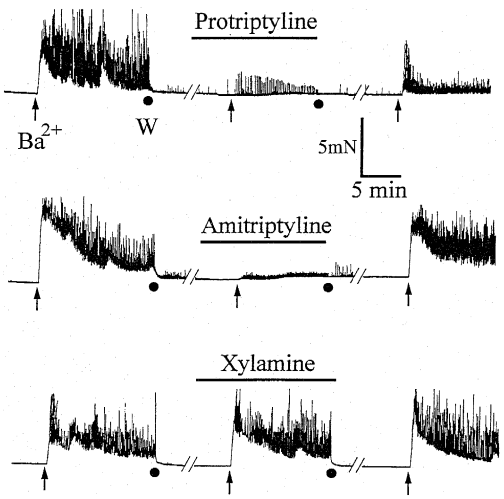

Fig. 5. Traces are representative records for effects of protriptyline, amitriptyline and xylamine on the $BaCl_2$-induced contraction in rat vas deferens. Ba^{2+} (3×10^{-3} M) evoked a reproducible contractile response. Calibration bars apply to all traces.

cent, 1981). On the other hand, noradrenaline uptake inhibitors have been shown to reduce the depolarization-induced contraction in arterial smooth muscle (Hrdina and Garattini, 1967; Huang, 1996). In order to further investigate whether these drugs directly inhibited muscle contraction other than antagonism of α-adrenoceptors or other monoaminergic receptors, 6×10^{-2} M K^+ was used to produce a steady tension (10.57 ± 0.89 mN, $n=26$). Subsequent application of protriptyline and amitriptyline caused concentration-dependent inhibition (Figs. 3 and 4). IC_{50} values for inhibition of the high K^+-induced contraction were $8.6\times10^{-7}\pm1.2\times10^{-7}$ M ($n=6$) for amitriptyline and $1.81\times10^{-6}\pm0.11\times10^{-6}$ M ($n=6$) for protriptyline, respectively. In contrast, xylamine (up to 3×10^{-5} M, $n=6$) had no effect (Figs. 3 and 4). In the time-matched control, the high K^+-induced contractile response was not affected by prazosin at 10^{-6} M ($n=4$), suggesting that the effect of noradrenaline released from the depolarized sympathetic nerves is negligible. Furthermore, nifedipine (3×10^{-7} M) completely reversed the high K^+-induced contraction ($n=4$, data not shown).

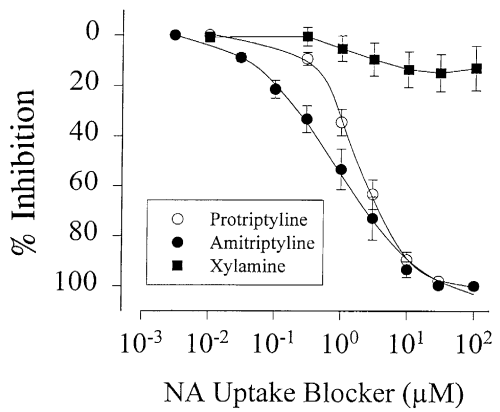

Fig. 4. Concentration-dependent effects for protriptyline (○, $n=6$), amitriptyline (●, $n=6$) and xylamine (■, $n=6$) of the high K^+-induced sustained contraction in rat vas deferens. Curves for two tricyclic agents were drawn by fitting the data points to the logistic equation. Values are mean ± S.E.M. from six experiments.

3.4. Effect of tricyclic antidepressants and xylamine on the $BaCl_2$-induced contractile response

Fig. 5 and Table 1 show that protriptyline and amitriptyline at 10^{-5} M ($n=4$ in each case) markedly reduced the contractile response to $BaCl_2$ (3×10^{-3} M) and the effects of these drugs were partially reversible within the 45–70 min washing period. Similar to the lack of effect on the high K^+-induced contraction, xylamine (10^{-5} M, $n=4$) did not affect the $BaCl_2$-induced phasic activity.

4. Discussion

The present study shows that three noradrenaline uptake blockers inhibited the contractile responses induced by electrical stimulation and various agonists in rat isolated vas deferens. Protriptyline and amitriptyline reduced electrically evoked contractions at high concentrations (3×10^{-7}–3×10^{-5} M). Desipramine was shown to potentiate the response to sympathetic nerve stimulation in rabbit ear artery at low concentrations (2×10^{-8} M) while it produced a transient potentiation followed by inhibition of electrically stimulated response at high concentrations (2×10^{-6} M) (Bassett et al., 1969). In addition, the concentrations of desipramine required to cause maximum potentiation of the postjunctional α-adrenoceptor-mediated response in the epididymal vas deferens (3×10^{-8}–1×10^{-7}

M) inhibited accumulation of [^{3}H]noradrenaline in the prostatic segment by over 50%, while a high concentration of desipramine ($> 3 \times 10^{-7}$ M) was found to inhibit noradrenaline-induced contraction (Leedham et al., 1985). These findings may suggest that tricyclic antidepressants primarily act on the prejunctional site to inhibit noradrenaline uptake at low concentrations, but they could possess α_1-adrenoceptor blocking action at high concentrations. Direct evidence for antagonism by antidepressants of α-adrenoceptor comes from the radioligand binding studies at the human brain (Richelson and Nelson, 1984).

Protriptyline and amitriptyline at high concentrations ($> 10^{-6}$ M) suppressed the contractile responses to exogenous noradrenaline, clonidine, 5-hydroxytryptamine, ATP in rat vas deferens. Inhibitory effects of tricyclic antidepressants on smooth muscle have been suggested via nonspecific antagonism of neurotransmitter receptors or direct inhibitory mechanisms (Klerman and Cole, 1965; Hrdina and Ling, 1970; Doggrell and Vincent, 1981, Rehavi et al., 1987, Huang, 1996). It is probable that tricyclic agents act at sites other than monoamine receptors on vas deferens smooth muscle. In fact, both drugs reduced the high K^+-induced sustained contraction in a concentration-dependent manner. It is known that Ca^{2+} influx through the depolarized plasma membrane is the major driving force for steady tension development by high extracellular K^+ in smooth muscle (Van Breemen and McNaughton, 1970). Furthermore, both drugs inhibited the Ba^{2+}-induced phasic contractions in vas deferens. Ba^{2+} was demonstrated to promote Ca^{2+} entry in smooth muscle by blocking K^+ channels and subsequent Ca^{2+}-induced Ca^{2+} release in the same preparation (Huang, 1995). These results indicate that tricyclic antidepressants could inhibit Ca^{2+} influx through depolarized cell membrane in smooth muscle of rat vas deferens. Interference with Ca^{2+} influx has been proposed for the inhibitory effects on arterial smooth muscle of tricyclic antidepressants such as desipramine and protriptyline (Hrdina and Garattini, 1967; Huang, 1996). In addition, imipramine was also found to inhibit Ca^{2+} current in isolated bovine ventricular myocytes (Isenberg and Tamargo, 1985). These findings indicate that inhibition of Ca^{2+} influx through plasma membrane Ca^{2+} channels might at least in part underlie the inhibitory actions of tricyclic agents on contractile responses to noradrenaline, clonidine, 5-hydroxytryptamine, ATP, high K^+ and $BaCl_2$, since the larger portion of the contractions induced by these agonists depends on the presence of extracellular Ca^{2+} and are sensitive to blockade of nifedipine. The exact site of action in excitation–contraction coupling for tricyclic antidepressants is at present unclear; however, it cannot be ruled out that tricyclic antidepressants might act at other steps in excitation–contraction coupling.

Xylamine, a β-halobenzylamine agent previously found to block noradrenaline uptake into both peripheral and central adrenergic nerve endings probably through alkylation of the noradrenaline carrier (Cho et al., 1980; Ransom et al., 1984; Ransom et al., 1985), is structurally different from tricyclic antidepressants. Similar to tricyclic drugs, this agent inhibited contractions induced by electrical stimulation, noradrenaline, clonidine and 5-hydroxytryptamine, but its action appeared to be long-lasting because the effect of xylamine ($> 3 \times 10^{-6}$ M) cannot be removed after repeated washout over 3 h. Xylamine was reported as an alkylating-type irreversible inhibitor of noradrenaline uptake in both peripheral and central noradrenergic neurons (Cho et al., 1980; Dudley et al., 1981). Different from inhibitory effects of tricyclic drugs, xylamine did not affect contractions evoked by exogenous ATP, high K^+ or Ba^{2+} at concentrations that abolished the agonist-induced and electrically stimulated responses. Xylamine did not alter the K^+-evoked contractions of rabbit and rat aorta (Nedergaard et al., 1992; Huang, 1996) and the endothelin-induced tension of rat aorta (Huang, 1996). Xylamine was also shown to reduce the contractile responses to serotonin and histamine in rabbit aorta (Nedergaard et al., 1992). It is apparent that xylamine can act on different monoaminergic receptors. Lack of effect on depolarization-, $BaCl_2$- and ATP-induced contractile responses in vas deferens indicates that xylamine might act primarily at α-adrenoceptors and other monoaminergic receptors without affecting Ca^{2+} entry in smooth muscle. The noncompetitive nature of the antagonism has been suggested to be due to an alkylation of the α-adrenoceptors or other monoaminergic receptors by xylamine (Nedergaard et al., 1992). In addition, radiolabelling with [^{3}H]xylamine shows that a large number of proteins were alkylated in tissues including rat vas deferens (Ransom et al., 1985; Koide et al., 1986). It is interesting to note that xylamine did not affect the ATP-induced contraction at a concentration of up to 3×10^{-5} M but this concentration inhibited by approximately 94% the electrically evoked contraction which consisted of adrenergic and purinergic components. The present results discount the possibility that xylamine might increase synaptic noradrenaline concentration which activates the prejunctional α_2-adrenoceptors to inhibit electrically stimulated release of contractile transmitters in vas deferens, since yohimbine (3×10^{-6} M), an antagonist of α_2-adrenoceptors, did not alter the inhibitory effect of xylamine. This concentration of yohimbine was sufficient to block the clonidine-mediated activation of presynaptic α_2-adrenoceptors in vas deferens (Demichel et al., 1981). Besides, xylamine also inhibited contractions induced by field stimulation in the presence of α_1- and α_2-adrenoceptor antagonists; this raises the possibility that xylamine might have a blocking effect on sympathetic neurons innervating rat vas deferens. It was suggested that xylamine, like bretylium, in part blocks neuronal activity upon being taken up inside the noradrenergic nerve endings in the peripheral tissues (Nedergaard et al., 1992).

In summary, these experiments have shown that pro-

triptyline and amitriptyline are nonspecific relaxants in rat vas deferens, they nonselectively blocked the effect of noradrenaline, clonidine, 5-hydroxytryptamine, ATP and $BaCl_2$, and possibly inhibited Ca^{2+} influx to induce relaxation. In contrast, xylamine, a structurally unrelated inhibitor, appeared to act mainly at α-adrenoceptors and other monoaminergic receptors as a nonselective antagonist in smooth muscle of rat vas deferens. The concentrations of tricyclic drugs in plasma that have been suggested to correlate best with satisfactory antidepressant effects range between 50 and 300 ng/ml after normal dosing conditions (Baldessarini, 1991), equivalent to 1.7×10^{-7}–10^{-6} M for protriptyline and $(1.6–9.6) \times 10^{-7}$ M for amitriptyline. These values are within or close to the concentration range used in the present study. The present results might indicate the possible clinical implications of male reproductive function in patients undergoing tricyclic antidepressant medication.

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