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Mode of Inhibitory Action of Amitriptyline on Carbachol-Induced Contraction of Isolated Rabbit Detrusor Muscle

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=ABSTRACT=

The present study was aimed at elucidating the mode of inhibitory action of tricyclic antidepressants on the smooth muscle. Effects of amitriptyline on the isolated detrusor muscle strips of the urinary bladder of the rabbit were examined. The spontaneous rhythmic movement of the muscle preparation was frequently observed, which was decreased or abolished by addition of amitriptyline (10^{-5}~10^{-3} M). The muscle preparation responded with contraction dose-dependently to carbachol, of which dose-response curve shifted to the right in the presence of either amitriptyline or atropine. However, amitriptyline produced a nonparallel shift, whereas atropine caused a parallel one. In calcium-free medium, the contraction response to carbachol was markedly decreased, which was resumed by the addition of $CaCl_2$ (2.5mM), but not in the presence of either amitriptyline or nifedipine. KCl (60 mM) produced a potent contraction, which was abolished in the presence of amitriptyline or nifedipine. These results suggest that amitriptyline, unlike atropine, not only acts as a noncompetitive antagonist at cholinergic muscarine receptors but also inhibits Ca-influx through the muscle cell membrane.

Key Words: Tricyclic antidepressants; Amitriptyline; Ca-antagonist.

INTRODUCTION

Tricyclic antidepressants, such as amitriptyline and imipramine, have been used in the treatment of depression. However, it has been recognized that long-term administration of these drugs results in various side effects such as urinary retention, constipation, blurred vision and dry mouth. These suggest that these drugs affect the activity of the peripheral autonomic nerve function.

It is indeed well known that tricyclic antidepressants have potent parasympatholytic effects (Sigg, 1959; Hollister, 1964) on both the central nervous system (Loew & Taeschler, 1965; Ho et al, 1966; Cupta et al, 1967; El-Fakahny & Richelson, 1983) and peripheral organs (Theobald et al, 1965; Baimbleocombe & Green, 1967; Atkison & Ladinsky, 1972; Snyder & Yamamura, 1977; Peroutka & Snyder, 1981). Particularly, atropine-like effect in the peripheral organs was emphasized (Snyder & Yamamura, 1977). In addition, many authors have observed that the release of acetylcholine from cholinergic nerve terminals is influenced by presynaptic muscarinic autoreceptor (Sawynok & Thamandas, 1977; Szerb, 1979; Kolbinger & Wessler, 1980) and that the stimulated release of acetylcholine is increased in the presence of scopalamine (Kilbinger & Kruel, 1981). Furthermore, Richardson et al (1984) suggested that the anticholinergic effect of these drugs are also associated with the reduced release of acetylcholine from the parasympathetic nerve terminals.

On the other hand, calcium ions are im-

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plicated in the smooth muscle contraction. Therefore, the inhibitory effects of tricyclic antidepressants on smooth muscles may also be associated with an altered calcium metabolism in these cells.

The present study was undertaken to elucidate the mode of anticholinergic action of amitriptyline in the detrusor muscle. Effects of the drug on carbachol-induced contraction of the isolated detrusor muscle were investigated with comparison to the effect of atropine. In addition, effects of the drug were also compared to those of nifedipine, a calcium antagonist, on the contraction responses induced by carbachol or KCl.

MATERIALS AND METHODS

Rabbits weighing 2.0-2.5 Kg of either sex were anesthetized with pentobarbital (50 ng/Kg, IP) and whole urinary bladder was removed. The detrusor muscle strips, 5 mm long with 2 mm width, were then prepared in cold physiological salt solution (1-4°C).

Each preparation was hooked with stainless steel wires and suspended in a 20 ml bath of physiological salt solution at 37 ± 0.05°C and continuously bubbled with 95% O_2 and 5% CO_2 for the saturation of bath fluid. The lower end of the preparation was fixed to the bottom of the bath and other end was attached to a force displacement transducer connected to a Grass Polygraph. Base-line load placed on the muscle strip was 2g. Spontaneous rhythmic movement of the muscle preparation was recorded. The muscle strip was subjected to the exposure to the drugs after two hours of equilibrium. The composition of physiological salt solution used was as follows: NaCl 8.0g, KCl 0.2g, $CaCl_2$ 0.2g, $MgCl_2$ 0.01g, $NaHPO_4$ 0.05g, glucose 1.0g, $NaHCO_3$ 1.09g and distilled water 1000 ml.

Concentration-response was obtained by a cumulative administration of the drug. IC_{50} values were obtained by linear regression using log transformation of the concentration. For statistical analysis, Student's t-test was employed. Drugs used were carbachol chloride, amitriptyline hydrochloride, atropine sulfate and nifedipine. All were obtained from Sigma Co.

RESULTS

Spontaneous rhythmic movement of the detrusor muscle strip was frequently observed, which was not affected by low concentrations of amitriptyline (less than 10^{-5} M) but was decreased or abolished by higher concentrations of the drug ($10^{-5} \sim 10^{-3}$ M) (Fig. 1).

Carbachol induced a contraction of the muscle strip in a dose-dependent manner. In the presence of amitriptyline (10^{-3} M), the carbachol dose-response curve shifted to the right and the maximum contraction attained was markedly decreased. EC_{50} (effective concentra-

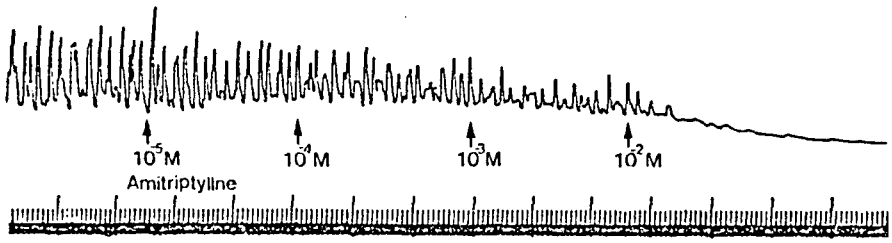

Fig. 1. Effect of amitriptyline on the spontaneous rhythmic movement of isolated detrusor muscle strip. Time marker in one minute intervals.

Table 1. EC_{50} and slope of the curves representing dose-contraction of isolated detrusor muscle strips to carbachol in the absence and presence of amitriptyline (10^{-3} M) or atropine (10^{-3} M)

		EC_{50}	Slope	Max. Cont. (%)
Control	(n=7)	3.7 ± 0.6 ($\times 10^{-5}$)	23.46 ± 5.00	100
+ Ami	(n=9)	3.9 ± 2.3 ($\times 10^{-2}$)	17.82 ± 1.83	46.98 ± 6.07*
+ Atr	(n=8)	1.5 ± 0.2 ($\times 10^{-3}$)	28.02 ± 2.27	80.65 ± 5.98*

* $p<0.01$ compared with control. The maximum contraction induced by carbachol prior to the addition of amitriptyline or atropine was regarded 100%. Values are means ± SE.

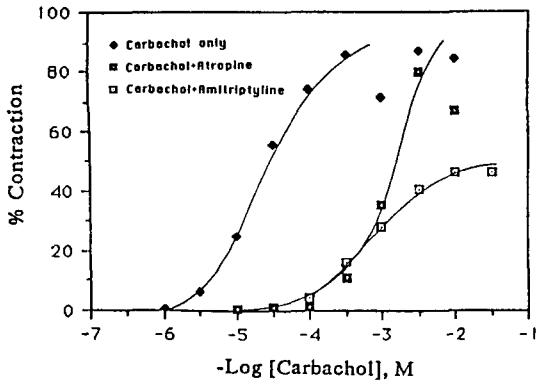

Fig. 2. Effects of amitriptyline and atropine on the dose-contraction responses of isolated detrusor muscle strips to carbachol. Control curve represents the dose-response in the absence of amitriptyline or atropine. Amitriptyline (10^{-3} M) or atropine (10^{-3} M) was added 15 minutes prior to the administration of the first dose of carbachol.

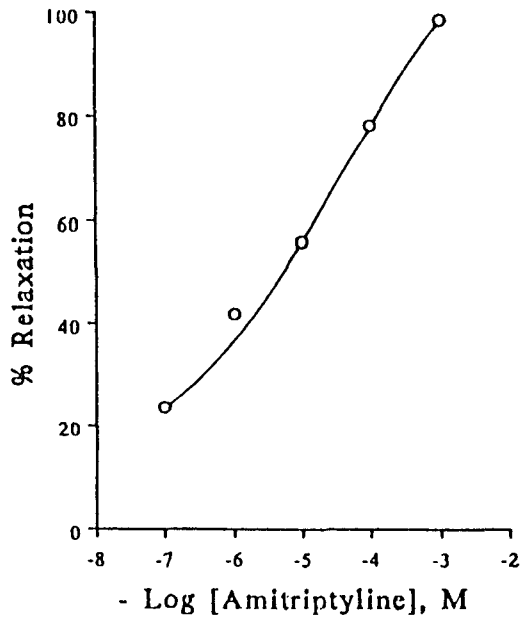

Fig. 3. Effect of cumulative dose of amitriptyline on the contraction of isolated detrusor muscle strip elicited by carbachol. Maximal contraction (100%) was produced and maintained by carbachol (10^{-3} M).

tion producing 50% of maximum contraction) was significantly increased and slope of the dose-response curve was decreased. In contrast, while the dose-response curve was also shifted to the right in the presence of atropine, the maximum contraction height was not affected (Table 1 and Fig. 2).

The contraction of the detrusor preparation by carbachol (10^{-3} M) was dose-dependently inhibited by amitriptyline, in which IC_{50} (effective concentration inhibiting 50% of maximum contraction) was 2.0 ± 1.1 ($\times 10^{-6}$ M) and slope of dose-relaxation curve was 0.52 ± 0.08 (Fig. 3).

Carbachol (10^{-3} M)-induced contraction was markedly decreased in calcium-free medium. The response to carbachol was resumed by the addition of $CaCl_2$, however, which was decreas-

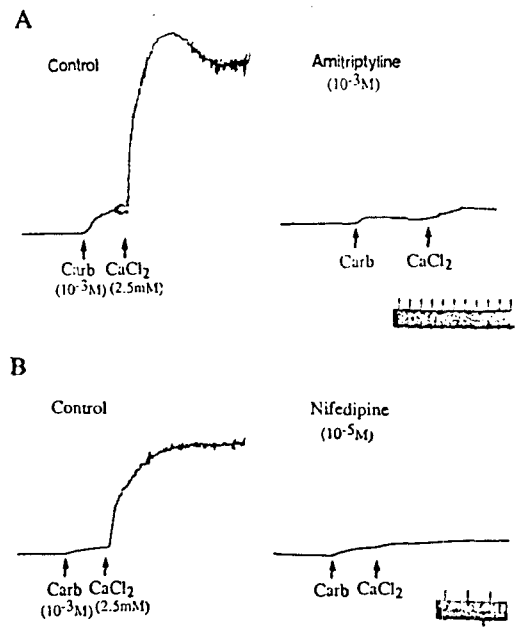

Fig. 4. Effect of calcium chloride on the carbachol (Carb) response of the isolated detrusor muscle strip suspended in a calcium-free Tyrode solution in the absence and presence of amitriptyline (A) or nifedipine (B). Other legend as in Fig. 1.

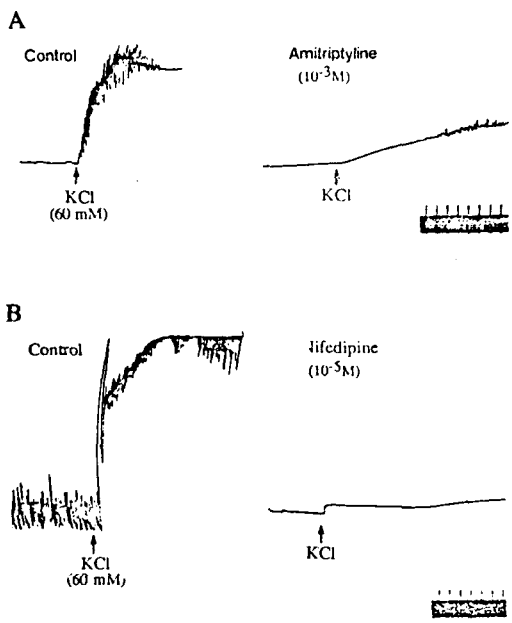

Fig. 5. Effects of amitriptyline or nifedipine on the response of the isolated detrusor muscle strips to potassium chloride. Other legend as in Fig. 1.

ed or abolished by the pretreatment with either amitriptyline (10^{-3} M) or nifedipine (10^{-5} M) (Fig. 4).

KCl (60 mM) produced a potent contraction of the detrusor preparation, which was decreased or abolished by the pretreatment with either amitriptyline or nifedipine (Fig. 5).

DISCUSSION

It has been reported that amitriptyline and imipramine have an atropine-like antimuscarinic effect due to their direct blockade of muscarinic receptors (Snyder & Yamamura, 1977; Sheim & Smith, 1978; Peroutka & Snyder, 1981). Furthermore, Richardson et al (1984) suggested that amitriptyline and imipramine, inhibit the release of acetylcholine from parasympathetic nerve terminals in addition to their postsynaptic muscarinic receptor blocking action. However, the mechanisms for the presynaptic autoregulation of acetylcholine as a neurotransmitter are so complicated that the detailed effects of these drugs on the autoregulation remains to be invetigated.

In this study, antimuscarinic effect of amitriptyline on the detrusor preparation was also recognized. In addition, amitriptyline produced a non-parallel right shift of the dose-response curve and the maximum contraction attained was decreased, whereas atropine caused a parallel one and the maximum contraction was not affected. These results indicate that amitriptyline, unlike atropine, is a non-competitive antagonist at cholinergic muscarinic receptors of the detrusor smooth muscle. In our previous study (Gill et al, 1990), similar results were obtained in the isolated rat ileum.

It is well known that calcium ions are essential in the smooth muscle contraction. The cellular calcium may be increased in the smooth

muscle either by activation of the receptor or alterations in membrane potential. The depolarization of the cell membrane due to high concentration of KCl may open the potential-operated calcium channels. In this study, the muscle preparation responded with a potent contraction to high concentration of KCl solution and the response was abolished in the presence of either amitriptyline or nifedipine. In addition, carbachol-induced contraction of the detrusor muscle was markedly decreased in calcium-free salt solution, which was resumed by the addition of $CaCl_2$, and was abolished in the presence of amitriptyline and nifedipine. These results suggest that amitriptyline, apart from its antimuscarinic antagonistic action interferes with calcium-influx through the cell membrane possibly by blocking the potential-operated calcium channels.

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