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The Prejunctional Inhibitory Effect of α-Methylnoradrenaline in the Rat Vas Deferens is Not Mediated via α_2-Adrenoceptors

MABAYOJE A. ORIOWO

Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat, 13110, Kuwait

Abstract

In field-stimulated rat vas deferens, clonidine (10^{-9}–10^{-7} M) and α-methylnoradrenaline (10^{-7}–3×10^{-5} M) concentration-dependently inhibited twitch response to electrical field stimulation. The inhibitory effects of clonidine and α-methylnoradrenaline were similarly reduced by phenoxybenzamine (10^{-6} M). However, idazoxan (3×10^{-7} M) antagonized clonidine but not α-methylnoradrenaline-induced responses. The inhibitory effect of α-methylnoradrenaline was also not antagonized by phentolamine (3×10^{-7} M), SK&F 104856 (2-vinyl-7-chloro-3,4,5,6-tetrahydro-4-methylthienol[4,3,2 ef][3]benzazepine) 3×10^{-6} M) or yohimbine 3×10^{-7} M). These antagonists however, attenuated the twitch-inhibiting effect of clonidine. The $-\log K_B$ values were $8{\cdot}02 \pm 0{\cdot}05$, $6{\cdot}91 \pm 0{\cdot}10$, and $7{\cdot}58 \pm 0{\cdot}07$ for phentolamine, SK&F 104856 and yohimbine respectively. In-vivo treatment of rats with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (6 mg kg^{-1}), to inactivate prejunctional α_2-adrenoceptors, attenuated clonidine, but not α-methylnoradrenaline-induced responses.

It was concluded that adrenoceptors are not involved in the prejunctional inhibitory effects of α-methylnoradrenaline in the rat vas deferens.

The rat vas deferens is a preparation commonly used in functional studies of prejunctional α_2-adrenoceptor mechanisms. Stimulation of prejunctional α_2-adrenoceptors in this preparation inhibits the twitch response evoked by electrical stimulation. There are two main classes of α-adrenergic agonists, the phenylethylamine and imidazoline derivatives. It has been suggested that the phenylethylamine and imidazoline derivatives bind to different recognition sites on the α_2-adrenoceptor (Langer & Shepperson 1982; Mottram 1982b; Vizi et al 1983). α-Methylnoradrenaline is a phenylethylamine derivative which potently stimulates prejunctional α_2-adrenoceptors centrally and peripherally. In the rat vas deferens, α-methylnoradrenaline is not only less potent in inhibiting the twitch response to electrical stimulation, it is also less susceptible to antagonism by yohimbine (Mottram 1982a) or idazoxan (Langer & Shepperson 1982). This was interpreted to suggest that clonidine binds to both high- and low-affinity sites of the α_2-adrenoceptor, while α-methylnoradrenaline binds only to the low-affinity site (Mottram 1982a). It has also been suggested that the phenylethylamines and imidazolines interact with non-overlapping discrete sites (Mottram 1982b). Results are presented in this paper which suggest that conventional α_2-adrenoceptors are not involved in the twitch-inhibiting effect of α-methylnoradrenaline in the rat vas deferens.

Materials and Methods

Drugs

α-Methylnoradrenaline hydrochloride, prazosin hydrochloride, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and idazoxan hydrochloride were obtained from RBI (Natick, MA) while clonidine hydrochloride, SK&F 104856 (2-vinyl-7-chloro-3,4,5,6-tetrahydro-4-methylthienol[4,3,2ef][3]benzazepine) and phenoxybenzamine hydrochloride were gifts from SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Phentolamine mesylate and yohimbine hydrochloride were obtained from Sigma (St Louis, MO). For in-vivo administration, EEDQ was dissolved in absolute ethanol and diluted with dimethylsulphoxide (DMSO). Stock solution of prazosin was made in methanol while phenoxybenzamine was dissolved in absolute ethanol. All other compounds were dissolved in distilled water.

Methods

Adult male Wistar or Sprague-Dawley rats, 200–300 g, were killed by a blow to the head followed by exsanguination. Both vasa deferentia were isolated and carefully stripped of any connective tissue. Each tissue was then set up in a 25-mL organ bath containing Krebs solution at 37°C and aerated with a 5% CO_2-95% O_2 mixture. The Krebs solution was of the following composition (mM): NaCl, 119; KCl, 4·7; $NaHCO_3$, 25·0; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 2·5; and glucose, 11·0. Each tissue was passed through two ring platinum electrodes (approximately 2 cm apart) for electrical stimulation. The tissues were allowed to equilibrate under a resting tension of 1·0 g for 60 min, during which the bathing fluid was changed twice. Square-wave pulses (Grass stimulator model S 88) of 1 ms duration were delivered at a frequency of 10 Hz in 0·5 s trains at 90-s intervals and supramaximal voltage. Isometric contractions were recorded through FT 03 transducers on a Grass polygraph model 7D. Prazosin (5×10^{-7} M) was included

in the Krebs solution to block any possible influence of postjunctional α_1-adrenoceptor effects. In preliminary experiments, β-adrenoceptor blockade with propranolol (10^{-6} M) did not modify the response to electrical stimulation or agonists. Propranolol was therefore not routinely used in this study. When four consecutive contractions to electrical field stimulation were reproducible, agonists were added to the bath in a cumulative manner. In all experiments involving α-methylnoradrenaline, ascorbic acid (0·07 mM) was added to the Krebs solution to prevent its oxidation. At the end of the agonist concentration–response curve, the preparations were washed at 20-min intervals during a 60-min rest period. Only one agonist was tested per tissue. When antagonists were used, the appropriate concentrations were added to the bath and left in contact with the tissue for 30 min before adding the agonists. Antagonist potency was expressed as $-\log K_B$ values. In one series of experiments, the vasa deferentia were treated with phenoxybenzamine (10^{-6} M) for 30 min, followed by wash-out and 30-min equilibration in normal Krebs solution. Thereafter, the inhibitory effects of the agonists on electrically-induced twitches were examined. In another series of experiments, rats were treated with EEDQ (6 mg kg^{-1}) overnight before the experiment and the vasa deferentia isolated and treated as above.

Statistical analysis

Results are presented as arithmetic mean ± s.e. of n experiments. Statistical analysis was performed using Student's *t*-test (paired or unpaired as appropriate); differences between mean values were considered statistically significant when $P < 0.05$.

Results

Clonidine ($10^{-9} - 10^{-7}$ M) and α-methylnoradrenaline ($10^{-7} - 3 \times 10^{-5}$ M) concentration-dependently inhibited the twitch response of the vas deferens to electrical field stimulation (Fig. 1). −logEC50 values were 8·39 ± 0·09 and 5·65 ± 0·12 for clonidine and α-methylnoradrenaline respectively. The concentration–response curves had slopes (calculated using the Grafit, nonlinear regression, computer programme (Leatherbarrow 1992) of 1·10 ± 0·04 and 0·72 ± 0·03 for clonidine and α-methylnoradrenaline, respectively. These values were significantly different ($P < 0.05$) from each other. Phenoxybenzamine (10^{-6} M) shifted both α-methylnoradrenaline and clonidine curves to the right and significantly reduced the maximum response (Fig. 2). As shown in Fig. 3, idazoxan (3×10^{-7} M) had little or no effect on the inhibitory effect of α-methylnoradrenaline, but produced a concentration ratio of approximately 30 against clonidine ($-\log K_B = 7.81 \pm 0.11$ n = 4). Phentolamine (3×10^{-7} M), SKF 104856 (3×10^{-6} M) and yohimbine (3×10^{-7} M) did not antagonize the twitch inhibiting effect of α-methylnoradrenaline in the vas deferens; however, at these concentrations, they antagonized the twitch-inhibiting effect of clonidine with $-\log K_B$ values of 8·02 ± 0·05, 6·91 ± 0·10 and 7·58 ± 0·07 respectively. Treatment of rats with EEDQ (6 mg kg^{-1}) overnight did not modify the inhibitory potency of α-methylnoradrenaline on the vas deferens (Fig. 4). In contrast, clonidine-induced

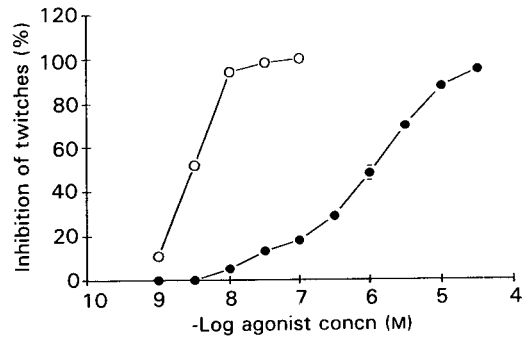

FIG. 1. Inhibitory effects of clonidine (○) and α-methylnoradrenaline (●) on field stimulation-induced twitch response of the rat vas deferens. Each point is the mean ± s.e. of 4–6 experiments.

inhibition of the twitch response was attenuated by treatment with EEDQ (Fig. 4). The −logEC50 value was reduced from 8·39 ± 0·09 to 6·77 ± 0·37 and the maximum response was reduced by 36%. In addition, the slope of the concentration–response curve was also significantly reduced from 1·10 ± 0·04 to 0·85 ± 0·03.

Discussion

The results described above confirm previous reports that α_2-adrenergic agonists of the imidazoline and phenylethylamine groups interact differently with the receptors (Pelayo et al 1980; Langer & Shepperson 1982; Mottram 1982b; Ruffolo et al 1983; Hicks et al 1985). In the present study,

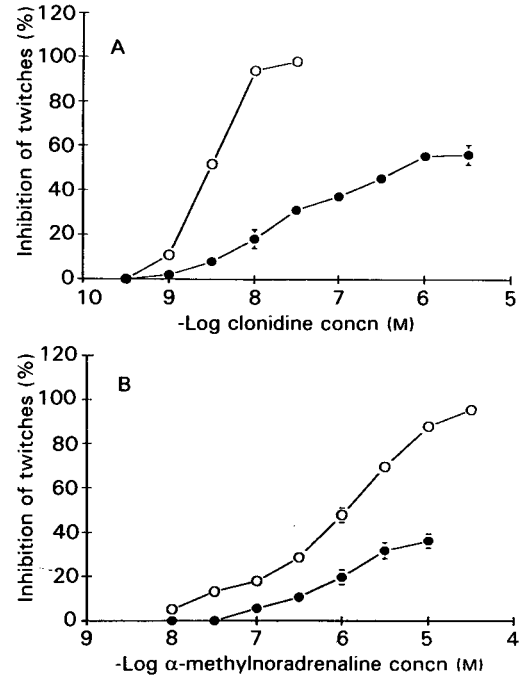

FIG. 2. Effect of phenoxybenzamine (10^{-6} M) on the prejunctional effects of clonidine (A) and α-methylnoradrenaline (B) in the field-stimulated rat vas deferens. ○, Agonist effect before; ●, agonist effect after phenoxybenzamine. Each point is the mean ± s.e. of four experiments.

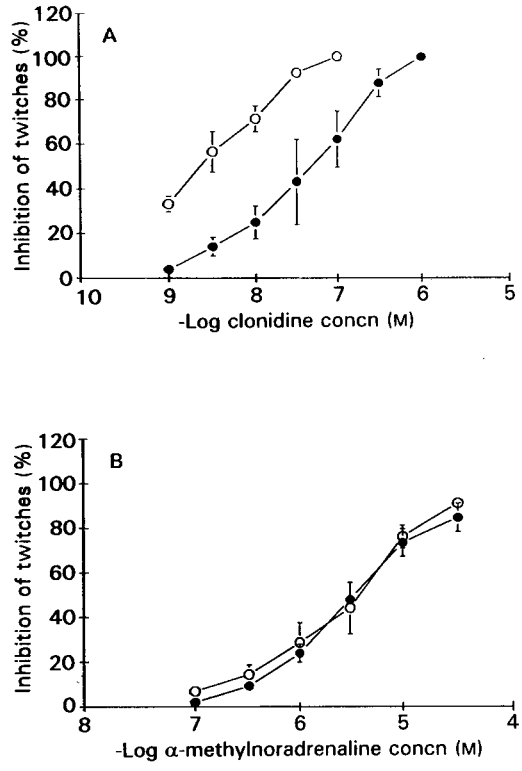

FIG. 3. Effect of idazoxan (3×10^{-7} M) on the inhibitory effect of clonidine (A) and α-methylnoradrenaline (B) in the field-stimulated rat vas deferens. Response to the agonists in the absence (○) and in the presence (●) of idazoxan. Each point is the mean ± s.e. of four experiments.

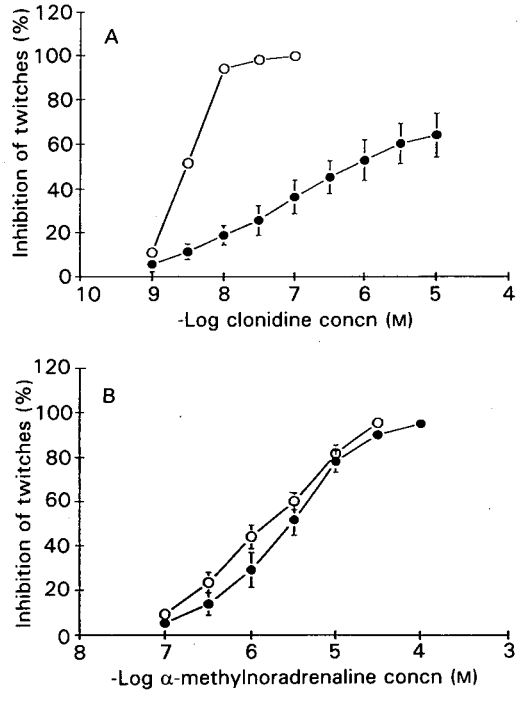

FIG. 4. Effect of EEDQ treatment (6 mg kg^{-1}, 16 h overnight) on the inhibitory effects of clonidine (A) and α-methylnoradrenaline (B) in the field-stimulated rat vas deferens. ●, Agonist effect in control; ○, agonist effect in EEDQ-treated rats. Each point is the mean ± s.e. of 4–6 experiments.

clonidine was more potent than α-methylnoradrenaline in inhibiting the twitch response, the slopes of the concentration–response curves were significantly different from each other, and the inhibitory effect of clonidine but not α-methylnoradrenaline was antagonized by idazoxan. The inhibitory effects of clonidine and α-methylnoradrenaline were similarly reduced by the same concentration of phenoxybenzamine suggesting that the inhibitory effect of α-methylnoradrenaline was receptor mediated.

Previous studies have shown that SK&F 104078, a 3-benzazepine derivative (Ruffolo et al 1987), could differentiate between two types of prejunctional α_2-adrenoceptors in the rat vas deferens (Ruffolo et al 1991). This was based on the differential interaction between SK&F 104078 and a series of α_2-adrenoceptor agonists. In the study of Ruffolo et al (1991), SK&F 104078 antagonized clonidine-induced inhibition of twitch responses while having little or no effect on twitch inhibition induced by UK 14304, BHT 920 or BHT 933. In the same study, rauwolscine equipotently antagonized all agonists indicating that they all activated prejunctional α_2-adrenoceptors in this tissue. Thus it was concluded that there are SK&F 104078-sensitive and insensitive prejunctional α_2-adrenoceptor subtypes in the rat vas deferens. In the present study, SK&F 104856, an analogue of SK&F 104078 (Hieble et al 1991), attenuated the twitch-inhibiting effect of clonidine but not that of α-methylnoradrenaline in the rat vas deferens, confirming our previous report (Ruffolo et al 1991) that there are two subtypes of prejunctional α_2-adrenoceptors in the rat vas deferens. Therefore, failure of idazoxan to antagonize the inhibitory effect of α-methylnoradrenaline would suggest that the SK&F 104078-insensitive prejunctional α-adrenoceptors in the rat vas deferens do not constitute a homogeneous population.

Idazoxan is a potent and selective α_2-adrenoceptor antagonist (Doxey et al 1983). It is also a potent antagonist of the imidazoline receptor (Michel & Insel 1989; Atlas 1991). The differential antagonism of the inhibitory effects of clonidine and α-methylnoradrenaline by idazoxan could, therefore, not be due to interactions at the prejunctional α_2-adrenoceptors and imidazoline receptors. This is supported by the observation that yohimbine, which has very weak affinity for the imidazoline receptors, also inhibited clonidine- but not α-methylnoradrenaline-mediated effects (Mottram 1982a; this study). The inability of phentolamine to antagonize the twitch-inhibiting effect of α-methylnoradrenaline would further make it unlikely that prejunctional α_2-adrenoceptors are involved in this response.

EEDQ is an irreversible antagonist of dopamine, α-adrenergic (α_1 and α_2) and 5-HT receptors (Meller et al 1988; Pilc et al 1989; Engberg & Eriksson 1991). Treatment with EEDQ (6 mg kg^{-1}) has been shown to produce more than 90% reduction in α_2-adrenoceptor density, accompanied by a significant attenuation of agonist responses (Agneter et al 1991). Therefore, the observation that EEDQ treatment attenuated clonidine- and not α-methylnoradrenaline-mediated effects would confirm that conventional prejunctional α_2-adrenoceptors are not involved in the prejunctional effects of α-methylnoradrenaline in the rat vas deferens. Imidazoline receptors are also not involved in this action of α-methylnoradrenaline since the inhibitory

effects were not antagonized by idazoxan. Since EEDQ also binds to and inactivates dopamine and 5-HT receptors, it is doubtful that these receptors could mediate the prejunctional inhibitory effect of α-methylnoradrenaline in the rat vas deferens. The significance of these findings is not immediately obvious. However, since the phenomenon is not restricted to the vas deferens, it could have some physiological significance, especially since the natural transmitter is a phenylethylamine derivative.

Acknowledgement

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