Effects of Clenbuterol on Rabbit Vesicourethral Muscle Contractility

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Abstract

Clenbuterol (10^-10-10^-7 M), a selective β2-adrenoceptor agonist, reduced spontaneous contractile force of isolated rabbit bladder dome, bladder base and proximal urethra. Clenbuterol inhibited both acetylcholine (Ach)- and electrical field stimulation (EFS)-induced contractions of rabbit bladder dome, but was more potent in inhibiting EFS-than Ach-induced contractions. Acetylcholine-but not EFS-induced contractions in the bladder dome were completely inhibited by pretreatment with 10^-6 M atropine. The atropine resistant component of the EFS-induced contractions was completely inhibited by tetrodotoxin, 10^-6 M. Clenbuterol and a non-selective β-adrenoceptor agonist, isoproterenol, potentiated the EFS-induced contractions of isolated striated muscle preparations from the external urethral sphincter and from the extensor digitorum longus in the rabbit. Clenbuterol was more potent than isoproterenol in increasing EFS-induced contractile force in the external urethral sphincter, whereas isoproterenol was more potent than clenbuterol in increasing EFS-induced contractile force in the extensor digitorum longus. These data suggest that clenbuterol may have a role in the treatment of urinary incontinence by inhibiting the detrusor contraction and facilitating the external urethral sphincter selectively.

Introduction

Functional α- and β-adrenergic receptors are present in the bladder and urethra of humans and other animals (Gosling and Dixon, 1997; Larsen, 1979; McGuire and Herlihy, 1979; Nording et al., 1980). Alpha-adrenoceptors predominate in the proximal urethra and in the bladder base, while β-adrenoceptors predominate in the bladder dome (Edvardsen and Setkleiv, 1968; Salimi et al., 1969; Downie et al., 1975; Levin and Wein, 1979). Although the roles of the sympathetic nerves in the function of the bladder and the proximal urethra has been extensively studied, the role of the sympathetic nerves in the function of the external urethral sphincter remains controversial (Steers, 1992). Urinary continence is maintained by both the relaxation of the urinary bladder and the contraction of the urethra. It is well known that β2-adrenoceptors relax the urinary bladder (Morita et al., 1986; Levin et al., 1988; Morita et al., 1990) and it has long been known that adrenaline contracts striated muscles (Goffart and Ritchie, 1952).
Clenbuterol, 4-amino-alpha-[tert-butylamino) methyl]-3, 5-dichlorobenzylalcohol hydrochloride, a selective $\beta_2$-adrenoceptor agonist, was introduced by Keck et al. (1972) as a potent bronchodilator. In the present study, we investigated the effects of clenbuterol on contractile activities in rabbit lower urinary tract smooth and striated muscles.

**Methods**

Twenty female Japanese white rabbits weighing 2-3 kg were anesthetized with 45 mg/kg of sodium pentobarbital and then bled to kill. Urinary bladder and urethra were removed, trimmed and dissected free from the vaginal wall. The bladder dome was dissected from the bladder base at the level of the ureteral orifices, and the bladder base was separated from the urethra at the level of the bladder neck. Muscle strips, 2 mm wide and 10 mm long, were dissected longitudinally from bladder dome and base, and were also dissected circularly from the proximal urethra and from the external urethral sphincter which was confirmed histologically. The extensor digitorum longus muscle was also dissected into 2 mm wide and 10 mm long segments. Muscle strips were mounted in a 3.0 ml chamber containing modified Krebs solution of the following composition (mM): NaCl 118.6, KCl, 4.7; CaCl$_2$ 2H$_2$O, 1.9; MgCl$_2$ 6H$_2$O, 1.2; NaHCO$_3$, 25.0; and glucose, 8.3; the strips were gassed with 95% O$_2$ and 5% CO$_2$ (pH 7.4) at 37°C. One end of the strip was attached with a 4-0 silk thread to a hook fixed at the bottom of the chamber and the other end was similarly attached to a Statham UC-2 force transducer mounted on a movable slide assembly. An initial force of 1 g was applied and the strips were allowed to equilibrate for at least 1 hr. Following a period of stress relaxation force was approximately 0.3 g in bladder and urethral smooth muscles. Spontaneous contractions of approximately 0.7 g in magnitude were superimposed on the basal force in bladder dome and bladder base. Spontaneous contractions were not observed in the proximal urethra, external urethral sphincter or extensor digitorum longus muscle. Electrical field stimulation (EFS) was applied at 90 sec intervals (voltage, supramaximal: pulse duration, 0.2 msec; frequency, 10-20 Hz; train duration, 5 sec) to bladder dome smooth muscle segments via two parallel wire platinum electrodes. Striated muscle strips, external urethral sphincter and extensor digitorum longus, were stimulated at 40 Hz using 0.5 sec trains of 0.5 msec duration pulses delivered at 15 sec intervals. EFS induced contractions of approximately 2 g in bladder muscles and of approximately 1.5 g in external sphincter and extensor digitorum longus muscles. Peak contractile force (basal force + active contractile force) was measured after drug administration.

Drugs used were clenbuterol hydrochloride (Teijin Institute, Tokyo), isoproterenol hydrochloride, tetrodotoxin, acetylcholine chloride, atropine sulfate, propranolol hydrochloride (Sigma, St. Louis) and phentolamine mesilate (Ciba Geigy, Basel). Drugs were made up as solutions in distilled water and were added directly in a volume of 30 μl to the chamber.

Data are expressed as mean ± S.E.M. Statistical analysis of drug effect and differences between treatment groups was determined by using analysis of variance (ANOVA). The Scheffe test was used to determine what concentration of drug produced a significant effect. A non-paired t-test was used to determine significance between responses at any given drug
Concentration. \( p < 0.05 \) was regarded as the level of significance.

**Results**

Clenbuterol, a selective \( \beta_2 \)-adrenoceptor agonist, produced a concentration-dependent decrease in spontaneous contractile force of rabbit bladder dome, bladder base and proximal urethra (Fig. 1). Clenbuterol, at \( 10^{-7} \) M reduced peak contractile force by 67% in the bladder dome, by 25% in the bladder base and by 12% in the proximal urethra. Isoproterenol, a non-selective \( \beta \)-adrenoceptor agonist, reduced peak contractile force in bladder dome, bladder base and proximal, urethra by 47%, 18% and 7%, respectively. The EC\(_{50}\) for clenbuterol induced relaxation in the bladder dome was \( 0.5 \times 10^{-9} \) M, whereas the EC\(_{50}\) for isoproterenol induced relaxation...
relaxation in the bladder dome was significantly greater at $3.0 \times 10^{-9}$ M. There was no significant difference in the EC$_{50}$ values for clenbuterol and isoproterenol in the bladder base and proximal urethra. Clenbuterol and isoproterenol had little effect on spontaneous contractile force of the external urethral sphincter (Fig. 1).

Acetylcholine ($10^{-6}$-$10^{-4}$ M) increased peak contractile force of bladder dome and bladder base but had no effect on muscle strips from either the proximal urethra or external urethral sphincter (Fig. 2).

Figures 3A, and B show the inhibitory effects of clenbuterol and atropine on peak contractile force and on acetylcholine-induced increases in peak contractile force of the bladder dome. Clenbuterol inhibited both the spontaneous and the acetylcholine-induced forces, whereas atropine inhibited only the acetylcholine-induced force. The inhibitory effect of clenbuterol,
but not that of atropine, was blocked by propranolol, $10^{-6}$ M.

Figure 4 shows EFS frequency–response curves of the bladder dome and external urethral sphincter strips. Atropine ($10^{-6}$ M), clenbuterol ($10^{-7}$ M) and tetrodotoxin ($10^{-6}$ M) inhibited EFS (20 Hz)–induced contractions of bladder dome by 58%, by 73% and 100%, respectively (Fig. 5). The inhibitory effects of clenbuterol were significantly greater than that of atropine on EFS–induced contractions of bladder dome.

EFS (40 Hz)–induced contractions of the external urethral sphincter were not inhibited by atropine, $10^{-5}$ M, phentolamine, $10^{-5}$ M or propranolol, $3 \times 10^{-8}$ M, but were completely inhibited by tetrodotoxin, $3 \times 10^{-8}$ M (Fig. 6). Clenbuterol, $10^{-8}$ M produced a significant potentiation of
EFS-induced contractions. The contractile force increased by approximately 40% within 10-20 min after the application of clenbuterol. Propranolol, $3 \times 10^{-6}$ M which had little effect on EFS-induced external urethral sphincter contractions on its own, antagonized the potentiation induced by clenbuterol (Fig. 6). Phenolamine, a $\alpha$-antagonist and atropine, a cholinergic antagonist did not antagonize the potentiation induced by clenbuterol.

The effects of clenbuterol and isoproterenol on EFS-induced contractions in rabbit external urethral sphincter and extensor digitorum longus are shown in Fig. 7. The magnitude of potentiation of EFS-induced contractions by clenbuterol was significantly greater in the external urethral sphincter than in the extensor digitorum longus, whereas the potentiation of EFS-induced contractions by isoproterenol was similar in the external urethral sphincter and the extensor digitorum longus. The magnitude of potentiation of EFS-induced contractions in the external urethral sphincter by clenbuterol was significantly greater than that by isoproterenol, whereas the magnitude of potentiation of EFS-induced contractions in the extensor digitorum longus by isoproterenol was significantly greater than that by clenbuterol.

**Discussion**

Clenbuterol, a selective $\beta_2$-adrenoceptor agonist and isoproterenol, a non-selective $\beta$-adrenoceptor agonist, both caused a marked concentration-dependent relaxation of spontaneous contractile force of rabbit urinary bladder dome, but only a slight relaxation of spontaneous contractile force of bladder base and proximal urethra. Clenbuterol neither significantly affected the contractile activity of the external urethral sphincter. The relaxant effects of clenbuterol on rabbit bladder dome were more marked than that of isoproterenol. Acetyl-
choline caused a marked increase in peak contractile force of the rabbit bladder dome and bladder base but did not affect the proximal urethra or the external urethral sphincter.

The lower urinary tract receives both cholinergic and adrenergic innervation and the distribution of cholinergic and adrenergic receptors closely parallels the pharmacological response to the respective agonists (Downie and Dean, 1997; Levin et al., 1980). The contractile response to acetylcholine in the bladder dome was greater than that in the bladder base, reflecting the denser cholinergic innervation of the bladder dome than of the bladder base (Latifpour et al., 1990). The relaxant responses to isoproterenol and clenbuterol were greater in the bladder dome than in the bladder base or proximal urethra, which are in accord with the higher density of β-adrenoceptors in the bladder dome than in the other tissues (Latifpour, 1990). The observation that the relaxant response in the bladder dome to clenbuterol was more marked than that to isoproterenol is consistent with previous reports (Morita et al., 1986; Levin et al., 1988; Morita et al., 1990) that support the involvement of β2-adrenoceptors in the relaxation of bladder dome.

Atropine suppressed acetylcholine-and EFS-induced increases in peak contractile force of rabbit bladder dome strips but did not affect the spontaneous contractions of the rabbit bladder dome. Acetylcholine-induced forces were completely inhibited by atropine, whereas one third of the magnitude of the EFS-induced contractions was resistant to atropine. As it is known that EFS-induced contractions in the urinary bladder are mediated by both cholinergic and non-cholinergic nerves (Dean and Downie, 1978), the resistance to atropine implies that a non-cholinergic component is involved in one third of the contractile response to electrical field stimulation (20 Hz).

Clenbuterol inhibited spontaneous contractions and EFS-induced and Ach–induced increases in peak contractile force of the bladder dome. Clenbuterol, 10⁻⁷ M, inhibited the EFS–induced force by 75% and the acetylcholine–induced increases in peak contractile force by 55%. EFS–induced force were more strongly inhibited by clenbuterol, 10⁻⁷ M, than by atropine, 10⁻⁶ M, whereas acetylcholine–induced increase in peak contractile force were more strongly inhibited by atropine, 10⁻⁷ M, than by clenbuterol, 10⁻⁷ M. These data show that β2-adrenoceptor agonists inhibit both nerve–induced and acetylcholine–induced contractions in rabbit urinary bladder dome smooth muscle with the degree of inhibition being greater with respect to EFS–induced contractions than to contractions evoked by acetylcholine. The data suggest the existence of modulation of cholinergic neurotransmission by prejunctional β2-receptors in rabbit urinary bladder smooth muscles. Inhibition of cholinergic neurotransmission in human airways by β2-adrenoceptors has been demonstrated (Rhoden et al., 1988). Otherwise, the data show the possibility that β2-adrenoceptor modulates the non-cholinergic (purinergic) components in the urinary bladder contraction.

It has been shown that the tension of tetanic contractions in fast contracting skeletal muscles is increased by β-adrenoceptor stimulation (Bowman and Zaimis, 1958; Bowman and Nott., 1969; Tashiro, 1973; Homberg and Waldeck, 1977; Waldeck, 1977). Al–Jeboory and Marshall (1977) and Fellenius et al. (1980) demonstrated that the β2-adrenoceptor agonists, terbutaline or sulbutamol, increase the force of subtetanic contractions of guinea pig extensor digitorum longus muscle and that this was associated with significant elevations in cAMP
levels. In the present study, clenbuterol was significantly more potent than isoproterenol in increasing the force of EFS-induced contractions in rabbit external urethral sphincter, which is a fast contracting skeletal muscle (1987). There has not been reported that the contraction of external urethral sphincter is effectively induced by $\beta_2$-adrenoceptor agonists. So the data presented in this study that clenbuterol, a selective $\beta_2$-adrenoceptor agonist, relaxes the urinary bladder smooth muscle and also contracts the external urethral sphincter may suggest the usefulness of the effects of clenbuterol on the contractility of the lower urinary tract smooth and striated muscles which have an important role on urinary continence mechanism.

References


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