Effects of clenbuterol on resting tension and contractile response in vesicourethral smooth muscle of rabbits

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Abstract


The effects of clenbuterol, a selective beta2-agonist, on isolated smooth muscle preparations from the rabbit bladder body, bladder base and proximal urethra have been investigated. The inhibitory effects on resting tension and acetylcholine- and field stimulation-induced contractions in the bladder body were compared with those of flavoxate, atropine, and verapamil.

Clenbuterol (10^{-6}-10^{-7} M) had a strong, concentration-dependent relaxant effect on resting tension of the bladder body, and the relaxant effect was antagonized by propranolol. However, clenbuterol had little effect on the bladder base or proximal urethra. Isoproterenol, a non-selective beta agonist, gave a similar result, but was less potent than clenbuterol. Flavoxate failed to reduce the resting tension, but rather enhanced the spontaneous rhythmic contraction in a concentration-dependent manner. Atropine had little effect. Verapamil produced a concentration-dependent relaxation in the bladder body.

Acetylcholine-induced contraction in the bladder body was completely inhibited by pretreatment with atropine (10^{-7} M). Clenbuterol, flavoxate, and verapamil concentration-dependently inhibited acetylcholine-induced contraction. Field stimulation-induced contraction in the bladder body was not completely inhibited by atropine. However, the residual contraction was completely inhibited by tetrodotoxin. Clenbuterol, flavoxate, and verapamil concentration-dependently inhibited field stimulation-induced contraction. The inhibitory effects of clenbuterol and verapamil were antagonized by an application of propranolol and an increase in external Ca, respectively.

The data suggest that the selective relaxant effect of clenbuterol on the bladder body is due to beta2-antagonistic action, resulting in the inhibition of the contractile response to acetylcholine or field stimulation. Also, its response was different from that of the other drugs used.

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Introduction

It is well known that the nerve supplies to the urinary bladder are mainly parasympathetic, and partly sympathetic. The functional presence of alpha and beta adrenergic receptors for the regulation of the smooth muscle tone and reactivity has been found in both experimental animals (Edvardsen and Setekleiv, 1968; Larsen 1979) and human (Awad et al., 1974; Ek, 1978; Gosling et al., 1977; Larsen, 1979; McGuire and Herlihy, 1979; Nordling, 1980). It is generally accepted that alpha adrenergic receptors predominate in the proximal urethra and bladder base, while beta adrenergic receptors are the predominant adrenergic receptor type in the bladder body (Edvardsen and Setekleiv, 1968; Salimi et al., 1969; Downie et al., 1975; Levin and Wein, 1979). Beta adrenoceptors have been classified into beta_1- and beta_2-subtypes, the latter being the subtype usually involved in smooth muscle relaxation (Barnes et al., 1983).

Clenbuterol, 4-amino-alpha-[([tert-butylamino)methyl]-3, 5-dichlorobenzylalcohol hydrochloride, was introduced by Keck et al. (1972) as a potent bronchodilating drug that was shown to have a selective beta_2-adrenoceptor stimulant activity (Engelhardt, 1972; 1976; O'Donnel, 1976; Miyata et al., 1978; Takayanagi et al., 1981; Okamiya et al., 1984; Hoshina et al., 1984). The contractile activities of several smooth muscles, such as those in guinea pig trachea, ileum, taenia coli, rat uterus, and rabbit aorta have been shown to be inhibited by clenbuterol (Engelhardt, 1976; Takayanagi et al., 1981). It has also been suggested that the order of the tissue-specific difference in the reactivity to clenbuterol is trachea > uterus > taenia coli, ileum > aorta. However, the effect of clenbuterol on the urinary bladder has not yet been studied.

In the present study, we investigated the effect of clenbuterol on resting tension in three segments of the rabbit lower urinary tract, including the bladder body, bladder base, and proximal urethra. The results show a segment-specific difference in the reactivity to clenbuterol, that is, a potent relaxation of the bladder body. Flavoxate or some anti-cholinergic drugs are used for improvement of bladder function, and a drug with Ca antagonistic action has been recently developed. Consequently, the relaxing action of clenbuterol was compared with the effects of flavoxate, atropine, and verapamil.

Materials and methods

Male Japanese White rabbits weighing 2-3 kg were killed by an air embolism after ether anesthesia. Through a vertical midline incision, the bladder and urethra were dissected free of surrounding tissues and immersed in a physiological salt solution (PSS). Smooth muscle strips of approximately equal length (10 mm) were sectioned from three areas (bladder body, bladder base, and proximal urethra) and gently dissected free of mucosal and adipose tissue. Muscle strips from the bladder body were cut longitudinally, but strips from the bladder base and the urethra were cut either longitudinally or transversely.

The normal PSS used was a modified Krebs Henselite solution of the following composition (mM): NaCl (118.4), KCl (4.7), CaCl_2 (1.5), MgSO_4 (1.2), NaHCO_3 (25.0), KH_2PO_4 (1.2) and glucose (10.0). Bathing solutions were kept at 37°C and equilibrated at pH 7.4 with 95% O_2 and
5% CO$_2$.

The contractile tension of muscle strips was recorded isometrically. The strip was suspended in an isolated organ bath (20 ml) and attached to the arm of a strain-gauge transducer (Nihon Kohden), the output of which was displayed on a linear recorder (Yokogawa). The values of isometric tension were reported in grams (g). The strips were allowed to equilibrate for at least 1 hr after the application of an initial tension of 1-2 g. Resting tension at the end of the equilibration period was about 0.5 g.

For field stimulation, muscles were placed between two parallel electrodes which were made of platinum wire. After the equilibration period, muscles were stimulated at 10 Hz or 20 Hz for 20 min using 5 sec trains of 0.2-msec pulses at 90-sec intervals.

The drugs used were clenbuterol hydrochloride (Dr. Karl Thomae GmbH, West Germany), isoproterenol hydrochloride, phenylephrine hydrochloride, propranolol hydrochloride, tetrodotoxin (Sigma, St. Louis, USA) acetylcholine chloride (Daiichi Selyaku, Tokyo, Japan), norepinephrine (Sankyo, Tokyo, Japan), phentolamine mesilate (Ciba Geigy, Basel, Switzerland), flavoxate hydrochloride (Nihonshinyaku, Tokyo, Japan), atropine sulphate (Tokyo Kasei, Tokyo, Japan), and verapamil hydrochloride (Wako-junyaku, Tokyo, Japan).

Results

1. Differences of responses to drugs on the bladder body, bladder base, and proximal urethra isolated from rabbit

Both the bladder body and bladder base exhibited spontaneous rhythmic contractions after 1 hr of equilibration with normal PSS. The contractions were enhanced by an increase in the external Ca concentration from 1.5 mM (normal level) to 3 mM. The muscle strips of the proximal urethra, except some strips, had no spontaneous mechanical activity.

![Fig. 1 Effects of acetylcholine on the body and base of the rabbit bladder. Ordinate: magnitude of the contraction, g. Abscissa: logarithm of the concentration of acetylcholine. Each value is the mean±S.E. for 8-9 muscle strips.](image-url)
Cholinergic agonist

Cumulative addition of acetylcholine \((10^{-7}-10^{-4} \text{ M})\) produced graded contractions in the muscle strips of bladder body and bladder base (Fig. 1). The contractile response to acetylcholine was greater in the bladder body than in the base. In the proximal urethra, the strips without spontaneous mechanical activity did not produce much contraction in response to acetylcholine. This response was small in magnitude.

![Fig. 2](image-url) Effects of phenylephrine on the bladder body, base and proximal urethra in the rabbit. Left: longitudinal muscle strips. Right: circular muscle strips. Ordinate: magnitude of the contraction, g. Abscissa: logarithm of the concentration of phenylephrine. Each value is the mean ± S.E. for 4-8 muscle strips.

![Fig. 3](image-url) Effects of clenbuterol and isoproterenol on the resting tension of the bladder body, base, and proximal urethra in the rabbit. Responses are expressed as percentage of the resting tension before an application of drug, and are plotted on the ordinate as a function of logarithm of the concentration of drug on the abscissa. Each value is the mean ± S.E. for 4-17 muscle strips.
**Alpha adrenergic agonists**

Phenylephrine ($10^{-7}-10^{-4}$ M) had a contractile effect on the bladder base and the proximal urethra. As shown in Fig. 2 (left), both the tissue responses to the drug were concentration dependent and the order of reactivity was bladder base $>$ proximal urethra. In contrast, phenylephrine relaxed the bladder body. The relaxing action was antagonized by propranolol ($2 \times 10^{-6}$ M) but not by phentolamine ($10^{-6}$ M), suggesting a relaxation due to beta adrenergic action. A similar beta-agonist activity for phenylephrine has been demonstrated in the human bladder body (Awad et al., 1974). Norepinephrine ($10^{-7}-10^{-4}$ M) also produced similar relaxation and contraction in the bladder body and base, respectively (data not shown).

It has been reported in the bladder base that circular strips responded better to alpha-agonist than longitudinal strips (Edvardsen and Setekleiv, 1968). Accordingly, some experiments were made with circular strips isolated from the bladder base and proximal urethra. There was a difference in contractile responses to phenylephrine as compared with the above longitudinal strips. As shown in Fig. 2 (right), the order of response was reversed, with proximal urethra $>$ bladder base.

**Beta adrenergic agonists**

Clenbuterol, a selective beta$_2$-agonist, produced a concentration-dependent relaxation of resting tension in the rabbit bladder body (Fig. 3, left). The resting tension of the bladder body was reduced by $66.1 \pm 3.8\%$ at $10^{-7}$ M. However, that of the bladder base and of the proximal urethra was reduced only by $24.4 \pm 3.5\%$ and $12.2 \pm 2.1\%$, respectively, at $10^{-7}$ M. Also, the resting tension of their circular strips was reduced by $14.4 \pm 3.3\%$ and $14.0 \pm 1.8\%$, respectively. This result suggests that the order of the segment-specific difference in the reactivity to clenbuterol is bladder body $>$ bladder base $>$ proximal urethra. Isoproterenol, a non-selective beta agonist, produced a similar result (Fig. 3, right). The resting tension of the bladder body was reduced by $47.3 \pm 2.2\%$ at $10^{-6}$ M, suggesting that its activity is lower than that of clenbuterol.

2. Relaxant action of clenbuterol on resting tension of the bladder body

The effect of clenbuterol on the resting tension of the bladder body was compared with the effects of flavoxate, atropine, and verapamil (Fig. 4). Clenbuterol and verapamil not only relaxed the resting tension but also decreased the magnitude and frequency of spontaneous rhythmic contraction in a concentration-dependent manner. Their inhibitory effects were reversed by an application of propranolol ($2 \times 10^{-5}$ M) in the case of clenbuterol or by an application of 6 mM Ca in the case of verapamil. The concentrations of clenbuterol and verapamil required to relax the resting tension by 50% were $4.7 \times 10^{-9}$ M and $1.1 \times 10^{-5}$ M, respectively (Fig. 5). Flavoxate had no effect on the resting tension, but potentiated the rhythmic contraction in a concentration-dependent manner. Atropine had little effect on either resting tension or rhythmic contraction. Also, the addition of tetrodotoxin ($10^{-6}$ M) had no effect.
3. Effects of clenbuterol on acetylcholine- and field stimulation-induced contractions of the bladder body

Figure 6 shows the effects of clenbuterol on the concentration-response curve for acetylcholine and on the frequency-response curve for field stimulation in the bladder body strips. Clenbuterol (10^{-8} M) reduced the maximum (20 Hz) response to field stimulation by 53.5\pm 6.5\%. Clenbuterol also reduced the acetylcholine (10^{-5} M)-induced contraction by only 27.6\pm 3.1\%. Thus, the data suggest that field stimulation-induced contractions are more sensitive to clenbuterol than acetylcholine-induced ones. This characteristic was compared among flavoxate, atropine, and verapamil in the next two experiments.

After the response to exogenously added acetylcholine (10^{-5} M) was repeated and the size of contraction became stable, drugs were applied. The acetylcholine-induced contraction was inhibited by pretreatment with clenbuterol, flavoxate, atropine, or verapamil in a concentration-dependent manner (Fig. 7). The contraction was reduced by 58.9\pm 6.1\%, 64.3\pm 2.9\%,
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Fig. 5 Effects of clenbuterol, flavoxate, atropine, and verapamil on the resting tension of the bladder body in the rabbit. Responses are expressed as percentage of the resting tension before an application of drug, and are plotted on the ordinate as a function of logarithm of the concentration of drug on the abscissa. Each value is the mean±S.E. for 4-10 muscle strips.

Fig. 6 Effects of clenbuterol on acetylcholine-and field stimulation-induced contractions in rabbit bladder body. Ordinate: magnitude of the contraction, g. Abscissa: logarithm of the concentration of acetylcholine (left) or the frequency of electric stimulation (right). Each value is the mean±S.E. for 5-6 muscle strips.

95.2±0.8%, and 53.9±5.7% at the final concentration of clenbuterol (10^-7 M), flavoxate (10^-4 M), atropine (10^-7 M), and verapamil (10^-8 M), respectively. Also, the concentrations of clenbuterol, flavoxate, atropine, and verapamil required to inhibit acetylcholine-induced contraction by 50% were 1.4×10^-8 M, 5.8×10^-5 M, 6.5×10^-5 M, and 8.5×10^-7 M, respectively. The inhibitory
Fig. 7 Inhibitory effects of clenbuterol, flavoxate, atropine, and verapamil on acetylcholine-induced contraction in rabbit bladder body. Each drug was applied 20 min before the response to acetylcholine (10^-5 M). After application of the final concentration of drug, propranolol (Prop, 2×10^-5 M) was added. Moreover, CaCl2 (Ca, 6 mM) was added hyperosmotically to the preparation in order to bring the final concentration to 7.5 mM. Responses are expressed as percentage of the maximum contraction induced by 10^-5 M acetylcholine before an application of drug. The columns and vertical bars represent means±S.E. for 4 muscle strips.

Clenbuterol, a selective β2-adrenoceptor agonist, caused a concentration-dependent decrease in resting tension of the rabbit bladder body, but only slight relaxation of the bladder base and proximal urethra. The segment-specific difference in the reactivity to cholinergic or adrenergic agonist is discussed as follows. The discussion contains the involvement of β2-adrenoceptors in the relaxation of the bladder body. Moreover, clenbuterol concentration-dependently inhibited acetylcholine- and field stimulation-induced contractions in the rabbit bladder body.
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Fig. 8 Concentration-dependent effects of clenbuterol, flavoxate, atropine, and verapamil on field stimulation-induced contraction in rabbit bladder body. Each drug was cumulatively applied to the muscle strip with steady contraction induced by field stimulation (20 Hz). Prop: $2 \times 10^{-5}$ M propranolol, TTX: $10^{-6}$ M tetrodotoxin, Ca: $6$ mM CaCl₂.

bladder body. This inhibitory effect was compared with that of the other drugs, and the difference from them was found out.

The lower urinary tract receives both cholinergic and adrenergic innervation (Downie and Dean, 1977; Downie et al., 1975). The distribution of adrenergic and cholinergic receptors is considered to parallel closely the pharmacological response to autonomic agonists. In several studies, the urinary tract can be divided into three segments, the bladder body, bladder base, and proximal urethra: the bladder body responds preferentially to cholinergic and beta-adrenergic agonists, whereas the bladder base and proximal urethra respond preferentially to alpha-adrenergic agonists (Khanna et al., 1981; Larsen, 1979; Levin and Wein, 1979; Levin et al., 1980). Results of the present study are partly consistent with the previously cited reports. The contractile response to acetylcholine in the bladder body was greater than in the bladder base, reflecting denser cholinergic innervation in the body. The contractile response to phenylephrine or norepinephrine was most marked in the bladder base and the proximal
urethra and absent in the bladder body. This result confirms a suggestion that alpha adrenergic receptors predominate in the bladder base and the proximal urethra of the rabbit (Edvardsen and Setekleiv, 1968; Salimi et al., 1969; Downie et al., 1975; Levin and Wein, 1979). Similar results have been reported in human (Awad et al., 1974).

On the other hand, clenbuterol and isoproterenol caused a concentration-dependent decrease in resting tension of the rabbit bladder body, but only slight relaxation of the bladder base and proximal urethra. The relaxation was antagonized by propranolol but not significantly altered by phentolamine, suggesting the predominant presence of beta-adrenoceptors in the rabbit bladder body. Awad et al. (1974) and Levin et al. (1980) have also shown that the extent of inhibition induced by isoproterenol was more marked in the bladder body than in the base. Moreover, the relaxant response of the bladder body is considered to be mediated by beta2-adrenoceptor stimulation, since the relaxant effects of isoproterenol and terbutaline, a selective beta2-adrenoceptor agonist, are comparable in magnitude and since dobutamine, a selective beta1 agonist, does not affect the contractile activity of the bladder body (Khanna et al., 1981; Morita et al., 1986). Furthermore, the findings that the relaxant response to isoproterenol is blocked by butoxamine, a selective beta2-antagonist, and atenolol, practolol, or metoprolol, all selective beta1-antagonists, were inactive (Khanna et al., 1981; Larsen, 1979; Morita et al., 1986) support the involvement of beta2-adrenoceptors in the relaxation of the bladder body. Recently, the beta adrenergic receptors in the rabbit bladder body have been characterized further by radioligand studies and found to represent a heterogeneous population of beta1- and beta2-receptors with the latter predominating (Anderson and Marks, 1984). The data from the present investigation show that the relaxant action (IC50: 4.7×10^{-9} M) induced by clenbuterol is greater than that (IC50: 7.1×10^{-8} M) by isoproterenol.
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This finding also confirms that the relaxation is mediated mainly by beta\textsubscript{2}-adrenoceptors.

The inhibitory effects of clenbuterol on resting tension and acetylcholine- and field stimulation-induced contractions in the rabbit bladder body were compared with those of atropine, flavoxate, and verapamil. Clenbuterol at lower concentration reduced a spontaneous rhythmic contraction in rabbit bladder body, and relaxed the resting tension. Moreover, clenbuterol inhibited acetylcholine- and field stimulation-induced contractions. The concentrations required to inhibit resting tension, acetylcholine- and field stimulation-induced contractions by 50\% were $4.7 \times 10^{-9}$ M, $1.4 \times 10^{-8}$ M, and $2.0 \times 10^{-9}$ M, respectively. These inhibitory effects were antagonized by the application of propranolol, suggesting an attribution to beta-agonistic action.

Atropine suppressed the bladder contractions induced by acetylcholine and field stimulation without affecting the spontaneous rhythmic contraction or the resting tension. Acetylcholine-induced contraction was completely inhibited by atropine ($10^{-7}$ M). However, one third of the field stimulation-induced contraction was resistant to atropine. The field stimulation-induced response of the bladder body is mainly mediated by cholinergic and non-cholinergic nerves (Dean and Downie, 1978; Downie and Dean, 1977). The resistance to atropine in this study implies that non-cholinergic component is involved in one third of the response to field stimulation (20 Hz).

Flavoxate caused a concentration-dependent potentiation of spontaneous rhythmic contraction without affecting the resting tension. A similar phenomenon has been previously observed in the bladder body of guinea pigs and rabbits (Miura \textit{et al.}, 1975). However, the mechanism for this potentiation of rhythmic contraction in the presence of flavoxate is not known. While, acetylcholine- and field stimulation-induced contractions were equally inhibited by an application of flavoxate. This action may be attributed to its papaverine-like musculotropic action, as described previously (Miura \textit{et al.}, 1975; Setnikar \textit{et al.}, 1960).

Verapamil produced a concentration-dependent relaxation of the resting tension. The relaxation was accompanied by a reduction of spontaneous rhythmic contraction. The relaxation was antagonized by an increase in the external Ca concentration. Also, the treatment with verapamil inhibited both the acetylcholine- and field stimulation-induced contractions, and the extent of inhibition was approximately equal. The concentrations required to inhibit resting tension, acetylcholine- and field stimulation-induced contractions by 50\% were $1.1 \times 10^{-5}$ M, $8.5 \times 10^{-7}$ M, and $1.4 \times 10^{-6}$ M, respectively.

It is clear from the above that the mechanism of action of clenbuterol differs from that of atropine or flavoxate. It resembles that of verapamil with respect to relaxation of resting tension, but the inhibitory effect of verapamil on resting tension was 10 times less potent than that on acetylcholine- and field stimulation-induced contractions. Moreover, clenbuterol inhibited field stimulation-induced contraction more potently than acetylcholine-induced contraction. Therefore, the inhibitory action of clenbuterol is not mediated by anti-cholinergic nor Ca-antagonistic action but is mainly due to beta-agonistic action.

In summary, clenbuterol produced a strong, concentration-dependent relaxation of resting tension in the bladder body of rabbits, but not in the bladder base or proximal urethra. The bladder contractions induced by acetylcholine and field stimulation were also inhibited by
clenbuterol. The inhibitory effects of clenbuterol were antagonized by propranolol and were
greater than those of isoproterenol, suggesting a beta₂-agonistic action. The data also suggest
that the mechanisms of action of flavoxate, atropine, and verapamil are different from the one
underlying clenbuterol action.

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