Characterization of Functional Beta-Adrenoceptor Subtypes in Rabbit Urinary Bladder Smooth Muscle

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MORITA, T., KONDO, S., TSUCHIDA, S. and WEISS, R.M. Characterization of Functional Beta-Adrenoceptor Subtypes in Rabbit Urinary Bladder Smooth Muscle. Tohoku J. exp. Med., 1986, 149(4), 389-395 — Spontaneous contractile force of muscle strips isolated from male rabbit urinary bladder dome (detrusor) and base (trigonal muscle) was significantly inhibited by isoproterenol \((10^{-7} - 10^{-5} \text{M})\), a non-specific beta-adrenoceptor agonist or by terbutaline \((10^{-8} - 10^{-5} \text{M})\), a selective beta2-adrenoceptor agonist. The EC\(_{50}\) values for isoproterenol and terbutaline in detrusor were the same as those in trigonal muscle but the maximum relaxant response to isoproterenol or terbutaline was significantly greater in detrusor than in trigonal muscle. Dobutamine \((10^{-5} - 10^{-4} \text{M})\), a relatively specific beta1-adrenoceptor agonist caused a small but significant relaxant response in trigonal muscle but no change in detrusor. In trigonal muscle the relaxant response to dobutamine was less than that to terbutaline. The relaxant response to \(10^{-6} \text{M}\) isoproterenol in detrusor was completely blocked by butoxamine \((10^{-4} \text{M})\), a selective beta1-antagonist or by propranolol \((10^{-6} \text{M})\), a non-specific beta-antagonist but not by metoprolol \((10^{-6} - 10^{-4} \text{M})\), a selective beta2-antagonist. Relaxation of trigonal muscle induced by \(10^{-6} \text{M}\) isoproterenol was inhibited by \(10^{-5} \text{M}\) metoprolol by 30%, by \(10^{-4} \text{M}\) butoxamine by 70%, or completely by \(10^{-6} \text{M}\) propranolol. These findings are consistent with the view that the density of beta-adrenoceptors is higher in the detrusor than in trigonal muscle, and that the relaxant response to beta-adrenoceptor stimulation is mediated by beta2-subtype in the detrusor and by both of beta1- and beta2-subtypes in trigonal muscle of the male rabbit. ——— beta1- and beta2-adrenoceptor subtypes; rabbit urinary bladder smooth muscle; spontaneous contractile force

The contractile activity of urinary bladder smooth muscle has been shown to be inhibited by beta-adrenoceptor agonists (Todd and Mack 1969; Nergårdh and Boréus 1972; Awad et al. 1974; Downie et al. 1975; Levin et al. 1980, 1983; Rohner and Hannigan 1980). Levin and Wein (1979) have demonstrated the existence of beta-adrenoceptors in dog and rabbit urinary bladder using specific radioligand receptor binding assay. Beta-adrenoceptors have been classified into

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beta1- and beta2- subtypes, the latter being the subtype usually involved in smooth muscle relaxation (Barnes et al. 1983). The present study was designed to study whether or not there exists an inhomogenous distribution of beta-adrenoceptors mediating the inhibitory response in the different regions of urinary bladder. For this purpose the inhibitory responses to selective and non-selective beta-adrenoceptor agonists and the effectiveness of selective beta-adrenoceptor antagonists were compared in the dome (detrusor) and the base (trigonal) muscle of the rabbit urinary bladder.

**Materials and Methods**

Twenty one male New Zealand white rabbits weighing 2.5 kg were stunned and bled. Muscle strips, 2 mm wide and 10 mm long, were dissected from the anterior dome (detrusor) and posterior base (trigonal) muscle of each urinary bladder and were mounted in a 3.0 ml organ bath containing modified Krebs solution of the following composition (mM): NaCl 133.6, KCl 4.7, CaCl2 • 2H2O 1.9, MgCl2 • 6H2O 1.2, glucose 8.3 and were bubbled with 95% O2 and 5% CO2; pH 7.4 at 37°C. One end of the strip was attached to a fixed hook in the bottom of the organ bath with a 4-0 silk thread and other end, to a Statham UC-2 force transducer mounted on a movable slide assembly. A baseline force of 1.0 g was maintained during the experiment. Strips were equilibrated for approximately 90 min prior to drug administration. Drugs were dissolved in distilled water at concentrations to give the following final concentrations in the organ bath when applied in a volume of 0.03 ml: dl-isoproterenol hydrochloride (Sigma), 10^-8-10^-5 M, dobutamine hydrochloride (Eli Lilly), 10^-7-10^-4 M, terbutaline sulfate (Ciba-Geigy), 10^-8-10^-5 M, dl-propranolol hydrochloride (Sigma), 10^-6 M, phentolamine (Ciba-Geigy), 10^-8-10^-4 M, metoprolol tartrate (Ciba-Geigy), 10^-6-10^-4 M and butoxamine hydrochloride (Wellcome), 10^-6-10^-4 M. Agonists were applied in a cumulative manner at intervals of 10 min. Agonists were applied 10 min before the administration of the minimum concentration of agonists. The response was measured as the mean of the changes in peak (baseline + active) force when the response reached a steady level usually one minute after addition of each concentration of drugs.

Statistical analysis of the changes in response to agonists and differences between groups was obtained with a two-way nested analysis of variance (Anova). The Scheffe test (Snedecor and Cochran 1967) was used to determine the concentration of agonists to produce a significant effect compared to control and a non-paired t-test was used to determine the statistical significance between responses in various treatment groups at any given concentration. All statistical analyses were performed based on raw (absolute) data.

**Results**

Both detrusor \((n = 15)\) and trigonal muscle \((n = 15)\) strips exhibited spontaneous rhythmic contractions after 1 hr of equilibration (Fig. 1). The frequency of contractions of detrusor was approximately 11 per min and that of trigonal muscle was approximately 6 per min.

Isoproterenol, 10^-7-10^-5 M, caused a concentration-dependent decrease in the contractile force of both detrusor \((n = 5)\) and trigonal muscle \((n = 5)\) in the presence of phentolamine, 10^-6 M, (Fig. 2). The relaxant responses to isoproterenol at high concentrations, 10^-6-10^-5 M, were significantly greater in detrusor than in trigonal muscle. The EC50 values for isoproterenol \((3 \times 10^-7 M)\) were the same in detrusor and trigonal muscle. Terbutaline, 10^-8-10^-5 M, caused a
concentration-dependent decrease in the contractile force of both detrusor (n=5) and trigonal muscle (n=5) in the presence of phenolamine, 10^{-6} M (Fig. 3). The relaxant responses to terbutaline (10^{-5} M) were greater (p<0.01) in detrusor than in trigonal muscle. The EC_{50} for terbutaline was 3 \times 10^{-7} M in both detrusor and trigonal muscle. Dobutamine, 10^{-7}-10^{-4} M, did not cause an appreciable inhibition in detrusor (n=5), whereas dobutamine, 10^{-5}-10^{-4} M, caused a small but significant decrease in contractile force of trigonal muscle (n=5) in the presence of phentolamine, 10^{-6} M (Fig. 4). The EC_{50} for dobutamine in trigonal muscle was 2 \times 10^{-5} M.

Pretreatment with metoprolol, 10^{-6}-10^{-4} M, (n=3) did not significantly affect responses of detrusor to isoproterenol, 10^{-6} M, whereas pretreatment with butoxamine, 10^{-4} M (n=3) or propranolol, 10^{-6} M (n=3) abolished the relaxant response of detrusor to isoproterenol, 10^{-6} M (Fig. 5). Butoxamine in concentrations of 10^{-5} M and lower did not completely inhibit the response of detrusor induced by isoproterenol, 10^{-6} M (n=2). On the other hand, pretreatment with metoprolol, 10^{-5} M (n=5) inhibited the relaxant response of trigonal muscle to isoproterenol, 10^{-6} M by 30\%, while pretreatment butoxamine, 10^{-4} M, by 70\% (n=5). Propranolol, 10^{-6} M completely abolished the relaxant response of trigonal muscle to isoproterenol, 10^{-6} M (n=3) as shown in Fig. 5. Metoprolol in concentrations of 10^{-6} M and lower (n=3) and butoxamine in concentrations of 10^{-5} M and lower (n=3) decreased slightly but not significantly the relaxant response of trigonal muscle to isoproterenol, 10^{-6} M.

**Discussion**

Smooth muscle strips isolated from the male rabbit urinary bladder dome (detrusor) and base (trigonal muscle) showed spontaneous contractions. The contractile frequency was higher in detrusor than in trigonal muscle. Isoproterenol, a non-specific beta-adrenoceptor agonist, caused a concentration-related decrease in contractile force of rabbit detrusor. This is consistent with previous reports (Todd and Mack 1969; Nergårdh and Borèus 1972; Awad et al. 1974; Downie et al. 1975; Levin et al. 1980, 1983; Rohner and Hannigan 1980). Isoproterenol also inhibited the contractile force of trigonal muscle in a concentration-dependent manner, which is in accord with the findings in several

![Fig. 1. Spontaneous contractions of isolated muscle strips from male rabbit urinary bladder dome (detrusor) and base (trigonal) muscle.](image)
Fig. 2. Responses of detrusor (○) (n = 5) and trigonal muscle (●) (n = 5) to isoproterenol, 10^{-9}-10^{-3} M. Asterisks show significant differences from control (⁎p < 0.05, ⁎⁎p < 0.01). Significance at the bottom of the figure refers to differences between detrusor and trigonal muscle in each concentration. Data are shown as mean ± S.E.

Fig. 3. Responses of detrusor (○) (n = 5) and trigonal muscle (●) (n = 5) to terbutaline, 10^{-8}-10^{-5} M. Asterisks show significant differences from control (⁎⁎p < 0.01). Significance at the bottom of the figure refers to differences between detrusor and trigonal muscle at each concentration. Data are shown as mean ± S.E.

Fig. 4. Responses of detrusor (△) (n = 5) and trigonal muscle (▲) (n = 5) to dobutamine, 10^{-7}-10^{-4} M. Asterisks show significant differences from control (⁎p < 0.05, ⁎⁎p < 0.01). Data are shown as mean ± S.E. Significance at the bottom of the figure refers to differences between detrusor and trigonal muscle at each concentration.
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previous studies (Nergårdh and Boréus 1972; Awad et al. 1974; Downie et al. 1975; Levin et al. 1980). Nergårdh and Boréus (1972), Awad et al. (1974) and Levin et al. (1980) showed that the extent of inhibition caused by isoproterenol was more marked in detrusor than in trigonal muscle. Our results in high concentrations of isoproterenol, $10^{-6}$-$10^{-5}$ M, are in accord with these previous findings. The present results are also consistent with those of Downie et al. (1975) who showed no significant difference in the EC$_{50}$ of isoproterenol in detrusor and trigonal muscle.

The relaxant response to isoproterenol of the detrusor is considered to be mediated by beta$_2$-adrenoceptor stimulation, since (1) the relaxant effects of isoproterenol and terbutaline, a selective beta$_2$-adrenoceptor agonist (Brogden et al. 1973), are comparable in magnitude and (2) the relaxant response to isoproterenol is blocked by butoxamine, a selective beta$_2$-antagonist (Kather and Simon 1980). Furthermore, the finding that dobutamine, a relative beta$_1$-adrenoceptor agonist (Tuttle and Mills 1975), did not affect the contractile activity of the detrusor supports the involvement of beta$_2$-adrenoceptors in the relaxation of the detrusor.

In contrast, the relaxant response to isoproterenol of the trigonal muscle may be mediated not only by beta$_2$- but also by beta$_1$-adrenoceptor stimulation. The relaxant response to isoproterenol of the trigonal muscle was completely blocked by propranolol, a non-specific beta-antagonist, and partially blocked by both metoprolol, a selective beta$_1$-antagonist (Brogden et al. 1977) and butoxamine, a selective beta$_2$-antagonist. The finding that both dobutamine and terbutaline inhibited trigonal muscle activity further supports the view that the relaxation of the trigonal muscle is mediated by both beta$_1$- and beta$_2$-adrenoceptor stimula-
The maximal relaxation produced by terbutaline was greater than that elicited by dobutamine. This suggests that beta$_2$-adrenoceptors predominate beta$_1$-adrenoceptors to relax the trigonal muscle.

Relaxation of most smooth muscles by beta-adrenoceptor agonists involves beta$_2$-adrenoceptors (Barnes et al. 1983; Carswell and Nahorski 1983; Bratveit and Helle 1984; Zaagsma et al. 1984; O'Donnell and Wanstall 1985), although the relaxation of guinea-pig ileum (Grassby and Broadley 1984) and canine left coronary artery (O'Donnell and Wanstall 1985) involves beta$_1$-adrenoceptors and the relaxation of cat and guinea-pig trachea involves both beta$_1$- and beta$_2$-adrenoceptors (Zaagsma et al. 1984; O'Donnell and Wanstall 1983). Previous studies in urinary bladder smooth muscle have not addressed the subtypes of beta-adrenoceptors involved in relaxation elicited via beta-adrenoceptors. The present data suggest that the relaxant response of male rabbit urinary bladder smooth muscle induced by beta-adrenoceptor stimulation is mediated by beta$_2$-subtype in the dome and by both beta$_2$- and beta$_1$-subtypes in the base.

References


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