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α- AND β-ADRENOCEPTORS IN THE DETRUSOR MUSCLE AND BLADDER BASE OF THE PIG AND β-ADRENOCEPTORS IN THE DETRUSOR MUSCLE OF MAN

JENS-JØRGEN LARSEN

Department of Pharmacology and Toxicology, Royal Veterinary and Agricultural University, DK-1870 Copenhagen V, Denmark

1 The presence and type of adrenoceptors in the smooth muscle of the pig and human urinary bladder was assessed on the basis of the relative potency of α- and β-adrenoceptor agonists and antagonists.

2 In isolated, carbachol-contracted bladder strips from the pig detrusor muscle the relaxing potency of isoprenaline was four times that of salbutamol and ritodrine and thirty times that of noradrenaline.

3 Propranolol caused a parallel shift to the right of the noradrenaline dose-response curve which was not changed by phentolamine.

4 Propranolol and butoxamine showed, in contrast to practolol, a dose-dependent antagonism of the response to isoprenaline. A pA_2 value of 9.2 ± 0.2 and 6.8 ± 0.2 (mean ± s.e. mean) for the first two antagonists was calculated.

5 In the bladder base of the pig, propranolol caused a parallel shift to the right and phentolamine a shift to the left of the dose-response curve to noradrenaline.

6 In the human detrusor muscle the potency and maximum effect of isoprenaline and salbutamol were less than those in the pig detrusor muscle. The potency of isoprenaline was sixty times that of salbutamol.

7 Whereas a parallel shift to the right of the dose–response curve to isoprenaline was obtained with propranolol, no antagonism was obtained with butoxamine or practolol.

8 The results are interpreted as indicating the presence of β_2-adrenoceptors in the detrusor muscle of the pig and β-adrenoceptors with neither β_1- nor β_2-characteristics in the detrusor muscle of man. An indication of the presence of α-adrenoceptors in the bladder base but not in the detrusor muscle of the pig was obtained.

Introduction

The mammalian urinary bladder muscle is both parasympathetically and sympathetically innervated via the pelvic and the hypogastric nerves respectively (Learmonth, 1931; Edvardsen, 1967). The parasympathetic nervous system is of considerable importance during the expulsion phase and causes a contraction of the bladder (Langworthy, Reeves & Tauber, 1934; Carpenter & Root, 1951; Hald, Agrawal & Kantrowitz, 1966; Holmquist, Staubitz & Greatbatch, 1967). Electrical stimulation of parasympathetic nerves or administration of cholinomimetic substances give rise to an increase in intravesical pressure. This effect is only partially inhibited by antimuscarinic compounds (Ursillo & Clark, 1956; Carpenter 1963; Vanov, 1965; Ambache & Zar, 1970). Therefore, it has been proposed that the motor nerve terminals of the bladder are entirely or chiefly non-cholinergic (Ambache & Zar, 1970; Dumsday, 1971; Burnstock, Dumsday & Smythe, 1972).

The sympathetic nervous system, while not essential for the expulsion phase is thought to exert an inhibitory effect during the collecting phase (Edvardsen, 1967; Boyarsky, Labay, Gregg & Levie, 1968; De Sy, Lacroix & Leusen, 1974). Electrical stimulation of the hypogastric nerves produces a quick rise of the intravesical pressure followed by a secondary pressure decrease below the control value (Edvardsen & Setekleiv, 1968; Taira, 1972; De Sy *et al.*, 1974). The contraction phase is thought to be mediated through a direct α-adrenoceptor stimulation in the muscle cells (Edvardsen & Setekleiv, 1968) or through a synaptic link between postganglionic adrenergic elements of the hypogastric nerves and parasympathetic ganglionic cells in the bladder wall

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(De Sy *et al.*, 1974). The relaxation phase is thought to be mediated through a direct stimulation of β-adrenoceptors in the detrusor muscle (Edvardsen & Setekleiv, 1968; De Sy *et al.*, 1974). Demonstration of β-adrenoceptors in the detrusor muscle confirms this assumption (Edvardsen & Setekleiv, 1968; Nergårdh & Boréus, 1972; Elmér, 1974). The type of β-adrenoceptors involved in the adrenergically induced relaxation of the detrusor muscle vary from species to species. The adrenoceptors are of the β_1-type in the cat bladder (Nergårdh, Boréus & Naglo, 1977) and the β_2-type in the rat bladder, (Elmér, 1974). In the human bladder the β-adrenoceptors seem to belong neither to the β_1- nor the β_2-group (Nergårdh *et al.*, 1977). An indication of the presence of β-adrenoceptors in the bladder of the pig has been given by Young & Macht (1923) who observed a relaxation of detrusor muscle strips from this species with adrenaline. The present study was carried out to investigate the distribution of α- and β-adrenoceptors in the bladder of the pig and to compare the type of β-adrenoceptors in the detrusor muscle with that in man.

Methods

Specimens from pigs

Thirty-six female and three male (boars) pigs weighing 20 to 90 kg were used. The female pigs were killed by a blow on the neck and bled out or pretreated with azaperonum (Sedaperone vet., Janssen) and anaesthetized with pentobarbitone sodium (mebumalum, NFN). The boars were obtained at a slaughter house and were anaesthetized by electrical stimulation and bled out. In the females the urinary bladder was removed *in toto* within a few minutes and transferred to Krebs solution kept at room temperature. A period of 25 min elapsed between killing of the three boars and removal of the bladders. The muscle strips from these bladders were kept at 4°C in Krebs solution during transport (about 45 min) to the laboratory.

From each bladder four to eight pieces of muscle tissue, 20 mm long and 3 mm wide, were dissected from different parts of the detrusor muscle and bladder base. The pieces were removed by an incision either parallel or transverse to the longitudinal axis of the bladder.

Specimens from man

Specimens from fifteen men aged 39 to 77 years and from one woman aged 30 years were used. The patients were premedicated with morphine–scopolamine and atropine or diazepam (Valium, Roche) and atropine and anaesthetized with halothane, nitrous oxide and oxygen. Gallamine triethiodide (Relaxan, GEA) or succinylcholine hydrochloride (Suxamethonium, DAK) was used as a muscle relaxant. The specimens were removed during retropubic prostatectomy ($n = 14$) or ureteral neoimplantation ($n = 2$) by a longitudinal incision from the anterior detrusor muscle and placed in Krebs solution at 4°C for transport (about 30 min) to the laboratory. One to eight muscle pieces, 20 mm long and 3 mm wide, were dissected from each specimen. Some of the bladders from patients undergoing prostatectomy showed considerable muscular hypertrophy.

Isometric measurement of muscle tension

The muscle pieces were suspended in 70 ml organ baths filled with Krebs solution at 37°C and connected to isometric Grass FT 03 force-displacement transducers. Two to four muscle pieces were investigated simultaneously in each experiment. The Krebs solution had the following composition (g/l): NaCl 7.0, KCl 0.4, $CaCl_2.2H_2O$ 0.4, KH_2PO_4 0.2, $MgSO_4.7H_2O$ 0.3, $NaHCO_3$ 2.1 and glucose 2.0. It was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was 7.4. Recordings were made by means of a Beckman type R dynograph.

Carbachol tension

After a period of equilibration (60 min) without tension during which the bathing solution was changed several times, the muscle pieces were stretched gradually until a stable tension of 0.5 g was reached. Then carbachol was added in cumulative doses, increasing the tension up to 2.0 g. The concentration of carbachol in the bath varied from 10^{-7} to 10^{-6} M. The tension thus obtained was stable for at least 60 min.

Relaxation with β-adrenoceptor stimulants

The relaxing potency on the carbachol-induced tension in the muscle strips was examined for the non-selective, β-adrenoceptor stimulant isoprenaline in the pig and in man, and for the selective β_2-adrenoceptor stimulants salbutamol in the pig and in man, and ritodrine in the pig. The α- and β-adrenoceptor stimulant noradrenaline was tested in the pig. The effect of the agonists which were given in cumulative doses was expressed as percentage relaxation from the carbachol-induced tension level. The potency of isoprenaline was evaluated in the same strips as salbutamol or ritodrine.

Inhibition of relaxation with adrenoceptor blocking agents

The receptor blocking potency of the non-selective agent propranolol, the selective β_2-adrenoceptor blocking agent butoxamine and the selective β_1-adrenoceptor blocking agent practolol was determined in the pig and in man and that of the α-adrenoceptor blocking agent phentolamine only in the pig. The dose-response curves for isoprenaline and noradrenaline before and 30 min after the addition of the blocking agent were compared and the pA_2 values of the β-blocking agents were determined (Arunlakshana & Schild, 1959).

The necessity for inclusion of a neuronal uptake inhibitor drug and an extraneuronal uptake inhibitor drug was investigated by means of protriptyline and metanephrine in the pig and human detrusor muscle and in the pig bladder base muscle. The detrusor muscle relaxing potency of isoprenaline, salbutamol and noradrenaline and the antagonistic effect of propranolol on the isoprenaline-induced relaxation were evaluated in parallel experiments with and without the uptake inhibiting agents protriptyline (10^{-6} M) and metanephrine (5×10^{-5} M). Similar experiments were carried out with noradrenaline, propranolol and phentolamine in the pig bladder base muscle.

The position of the receptors stimulated by the β-adrenoceptor agonists was examined by means of tetrodotoxin. The muscle relaxing effect of isoprenaline was evaluated in the pig detrusor muscle before and 2 min after addition of the neurone blocking toxin.

Drugs

The following drugs were dissolved in Krebs solution containing ascorbic acid (100 µg/ml): carbachol (carbamylcholine hydrochloride); isoprenaline sulphate; salbutamol base; ritodrine hydrochloride (Utopar, Philips-Duphar); noradrenaline base; propranolol hydrochloride; practolol base; butoxamine hydrochloride; phentolamine (Regitin, Ciba-Geigy); tetrodotoxin (Boehringer Mannheim); protriptyline hydrochloride; metanephrine hydrochloride (Sigma). A volume of 1 ml of the solutions was added directly to the bath. All doses in the text refer to the final concentration of the drug in the bath, calculated as base.

Values of P were calculated according to Student's t test. The number of muscle strips examined with each drug is given in parentheses.

Results

The muscle relaxing potency of isoprenaline ($n = 8$), salbutamol ($n = 8$) and noradrenaline ($n = 8$) and the antagonistic effect of propranolol ($n = 8$) on the response to isoprenaline were not influenced by inclusion of protriptyline (10^{-6} M) and metanephrine (5×10^{-5} M) in the pig or human detrusor muscle. In the pig bladder base muscle, pretreatment with the uptake blocking substances resulted in a minor but non-significant increase ($P > 0.05$) of the muscle relaxing potency of noradrenaline ($n = 8$) and in no change in the antagonistic effect of propranolol ($n = 8$) or phentolamine ($n = 8$) on the response to noradrenaline. The muscle relaxing effect of isoprenaline on the pig detrusor muscle was not influenced by pretreatment with tetrodotoxin 0.2 µg/ml ($n = 8$).

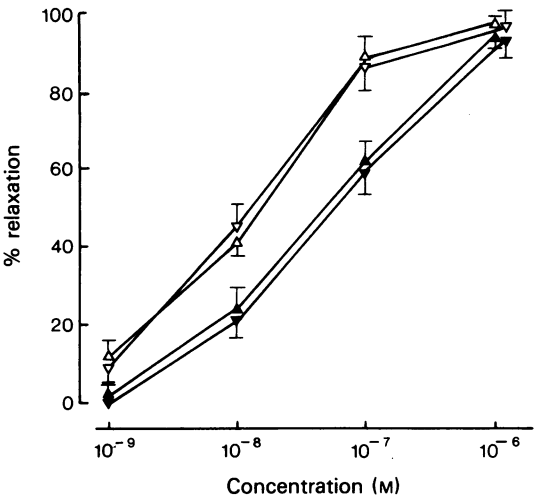

Figure 1 Effects of isoprenaline (△ and ▽), salbutamol (▲) and ritodrine (▼) on the pig detrusor muscle ($n = 16$ and 10 respectively). Mean values are shown; vertical lines indicate s.e. means.

Adrenoceptors in the detrusor muscle of the pig

Addition of isoprenaline, salbutamol, ritodrine and noradrenaline to the bath caused, in all cases, a dose-dependent relaxation of the muscle strips kept under tension by means of carbachol (Figures 1 and 2). In a dose range from 10^{-9} to 10^{-5} M, a relaxing effect from 0 to 100% was obtained with each agonist. Parallel dose–response curves were obtained with the four agonists. The potency of isoprenaline was about 4 times that of salbutamol and ritodrine and 30 times that of noradrenaline.

The blocking effect of propranolol and phentolamine on the noradrenaline-induced, relaxation of the muscle is shown in Figure 2. Whereas propranolol in a dose of 10^{-7} M caused a parallel shift to the right of the noradrenaline dose-response curve with a significant reduction ($P < 0.05$) of the noradrenaline

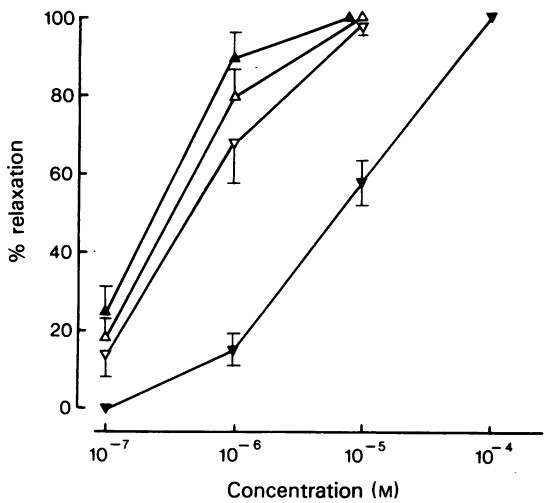

Figure 2 Effects of propranolol and phentolamine on the dose-response curve of noradrenaline in the pig detrusor muscle. Controls (▽ and △); propranolol 10^{-7} M (▼); phentolamine 10^{-5} M (▲) ($n = 8$). Mean values are shown; vertical lines indicate s.e. means.

response at all dose levels, phentolamine in a dose of 10^{-5} M did not influence the noradrenaline response to a significant extent ($P > 0.05$) at any dose level. Pretreatment with propranolol (10^{-9}, 10^{-8} and 10^{-7} M) or butoxamine (10^{-7}, 10^{-6} and 10^{-5} M) resulted in a dose-dependent parallel shift to the right of the isoprenaline dose-response curve. Addition of the lowest dose resulted for both antagonists in a minor but non-significant inhibition ($P > 0.05$) of the agonist responses. Addition of the next to lowest dose of propranolol and butoxamine resulted in a further and significant inhibition ($P < 0.05$) of the agonist responses, and pretreatment with the highest dose of the two antagonists gave rise to a marked and significant reduction ($P < 0.02$) of the isoprenaline effects. The calculated pA_2 value for propranolol and butoxamine was 9.2 ± 0.2 (mean $\pm$ s.e. mean, $n = 8$) and 6.8 ± 0.2 ($n = 8$) respectively. Pretreatment with practolol (10^{-6} and 10^{-5} M) did not on the other hand result in a dose-dependent shift to the right of the isoprenaline dose-response curve. No significant reduction ($P > 0.05$) of the agonist responses was obtained except at a single dose level (practolol 10^{-6} M and isoprenaline 10^{-8} M, $P < 0.05$).

Adrenoceptors in the bladder base of the pig

The muscle relaxing potency of noradrenaline on strips from the bladder base was not significantly different ($P > 0.05$) from that obtained on the detrusor strips (Figure 3). After pretreatment of the muscle pieces with propanolol (10^{-7} M) noradrenaline in a low dose (10^{-7} M) induced a contraction but after pretreatment with phentolamine (10^{-5} M), noradrenaline (10^{-7} M) induced a higher degree of relaxation in comparison with the results on the detrusor muscle. In the higher doses (10^{-6} to 10^{-4} M) the noradrenaline responses in the presence of propranolol (10^{-7} M) and phentolamine (10^{-5} M) were not significantly different ($P > 0.05$) from those obtained on the detrusor strips.

Adrenoceptors in the detrusor muscle of man

Addition of isoprenaline and salbutamol to the organ bath caused in both cases a dose-dependent relaxation of the muscle strips (Figure 4). The dose-response curves being broadly speaking parallel, were less steep than the similar ones obtained in the pig detrusor muscle and the maximum effect obtained was only 80% and 63% reduction of the carbachol-induced tension with isoprenaline and salbutamol respectively. The relaxing potency of isoprenaline in man was about 60 times that of salbutamol and about 40 times less than that of isoprenaline in the pig detrusor muscle.

Figures 5 and 6 show the antagonistic effect of propranolol, butoxamine and practolol on the isoprenaline-induced relaxation of the human detrusor muscle. Whereas a parallel shift to the right of the isoprenaline dose-response curve was obtained with propranolol in a dose of 10^{-7} M with significant reduction ($P < 0.05$) of the isoprenaline response at all dose levels (Figure 5), no antagonistic effect was obtained with butoxamine or practolol at a concentration of 10^{-5} M (Figure 6).

Discussion

Evidence suggesting the existence of different types of β-adrenoceptors was presented by Lands and co-workers (Lands & Brown, 1964; Lands, Arnold, McAuliff, Luduena & Brown, 1967a; Lands, Luduena & Buzzo, 1967b; Furchgott, 1967). A subdivision of the receptors into a β_1- and a β_2-group was based upon differences in the relative potency of a number of adrenoceptor agonists. The excitatory effect of β-adrenoceptor stimulating agents such as isoprenaline and noradrenaline on the heart is mediated through the β_1-adrenoceptors and the relaxation of uterine, bronchial and vascular smooth muscle of agents such as isoprenaline and salbutamol is mediated through the β_2-adrenoceptors (Lands *et al.*, 1967a, b; Farmer, Kennedy, Levy & Marshall, 1970a; Farmer, Levy & Marshall, 1970b). Similarly a selective blockade of the β_1-adrenoceptors can be obtained

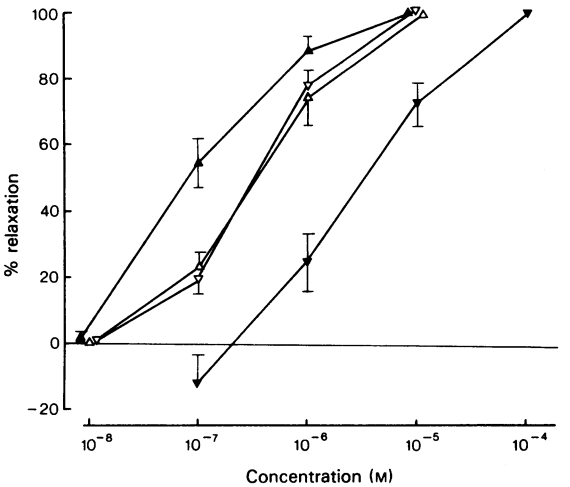

Figure 3 Effects of propranolol and phentolamine on the dose-response curve of noradrenaline in the pig bladder base. Controls (▽ and △); propranolol 10^{-7} M (▼); phentolamine 10^{-5} M (▲) ($n = 8$). Mean values are shown; vertical lines indicate s.e. means.

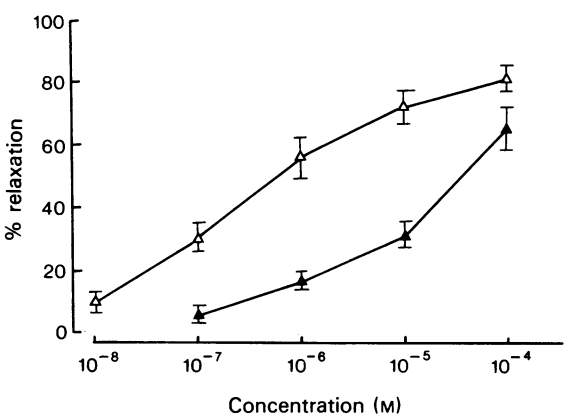

Figure 4 Effects of isoprenaline (△) and salbutamol (▲) on the human detrusor muscle ($n = 10$). Mean values are shown; vertical lines indicate s.e. means.

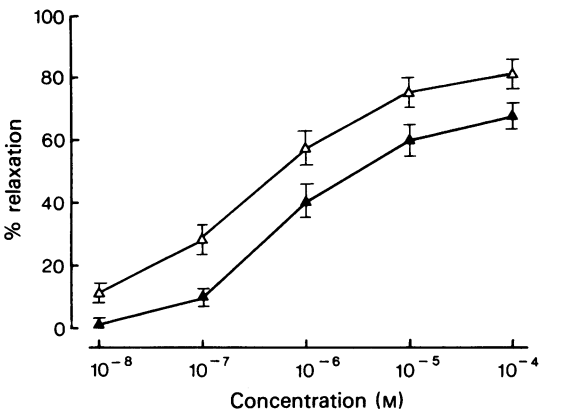

Figure 5 Effect of propranolol on the dose-response curve of isoprenaline in the human detrusor muscle. Controls (△); propranolol 10^{-7} M (▲) ($n = 9$). Mean values are shown; vertical lines indicate s.e. means.

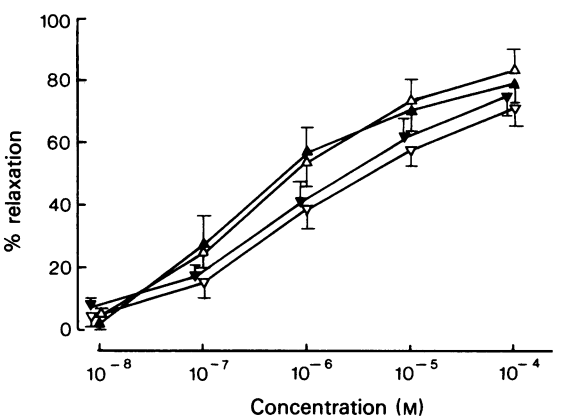

Figure 6 Effects of butoxamine and practolol on the dose-response curve of isoprenaline in the human detrusor muscle. Controls (△ and ▽) butoxamine 10^{-5} M (▲); practolol 10^{-5} M (▼) ($n = 9$). Mean values are shown; vertical lines indicate s.e. mean.

with practolol (Dunlop & Shanks, 1968) and of the β_2-adrenoceptors with butoxamine (Levy, 1966), whereas a non-selective blockade of the two receptor populations is achieved with propranolol (Black, Duncan & Shanks, 1965).

The combination of selective and non-selective agonists and antagonists has been used in the present study in order to characterize the adrenoceptors in the bladder of pig and man.

The neuronal uptake blocking agent protriptyline and the extraneuronal uptake blocking agent metanephrine were used in concentrations which in other *in vitro* muscle preparations have resulted in a high and specific uptake blockade (Burgen & Iversen, 1965; Foster, 1968, 1969; Almgren & Jonason, 1971; Maxwell, Ferris, Burcsu, Chaplin Woodward, Tang & Williard, 1974). Since neuronal uptake and extraneuronal uptake did not appear to affect responses of the pig or human detrusor muscle or pig bladder base muscle to adrenoceptor stimulating or blocking drugs, uptake$_1$ and uptake$_2$ inhibiting agents have not been used in all the experiments. An

explanation for the lack of effect of uptake blocking agents on the responses to noradrenaline in the detrusor and bladder base muscle might lie in the sparse sympathetic innervation of the muscle tissue.

An indication that the β-adrenoceptors stimulated by isoprenaline in the muscle tissue were postsynaptic was given by the lack of effect of tetrodotoxin on the isoprenaline response.

As shown in Figure 1, salbutamol is about four times less potent than isoprenaline in relaxing the detrusor muscle of the pig. This value corresponds to those between 3 and 13 obtained in uterine, bronchial and vascular smooth muscle of the rat, guinea-pig and dog but is in contrast to those between 100 and 2500 obtained in cardiac muscle of the same three species (Farmer *et al.*, 1970a, b; Daly, Farmer & Levy, 1971). The results thus indicate the presence of β_2-adrenoceptors in the pig detrusor muscle. This indication was confirmed by the high muscle relaxing potency of ritodrine, which is a selective β_2-adrenoceptor stimulating agent (Coutinho, Bomfim de Sousa, Wilson & Landesman, 1969; Baumgarten, Frölich, Seidl, Sokol, Lim-Rachmat & Hager, 1971) and by the moderate muscle relaxing potency of the predominant β_1-adrenoceptor stimulating agent noradrenaline. The dose-dependent β-adrenoceptor blocking effect of propranolol and butoxamine and the lack of antagonism of practolol for the isoprenaline-induced muscle relaxation further indicate that the β-adrenoceptors in the pig detrusor muscle belong to the β_2-group. Thus, the calculated pA_2 values for propranolol and butoxamine of 9.2 ± 0.2 and 6.8 ± 0.2 respectively correspond to the equivalent ones of 9.56 and 6.91 in the rat uterine smooth muscle and are in contrast to the results obtained in the rat cardiac muscle in which butoxamine (10^{-6} and 10^{-5} M) was without effect, and a pA_2 value for propranolol and practolol of 10.12 and 7.37 was obtained (Wasserman & Levy, 1972).

In the present study of the pig detrusor muscle the α-adrenoceptor blocking agent phentolamine failed to cause a significant shift to the left of the noradrenaline dose-response curve and after pretreatment with the β-adrenoceptor blocking agent propranolol, noradrenaline in no case gave rise to a contractor response indicating that no α-adrenoceptors are present in the detrusor muscle of the pig.

In the muscle strips from the bladder base of the pig, phentolamine, however, gave rise to a significant increase in the noradrenaline response in a dose of 10^{-7} M and after pretreatment with propranolol the same dose of noradrenaline resulted in a marked contraction of the muscle. These results thus indicate that both α- and β-adrenoceptors are present in the smooth muscle of the bladder base in the pig.

The experiments on human detrusor muscle revealed that the muscle relaxing potency of isoprenaline is about 40 times less in man than in the pig which might be an expression of a smaller number of β-adrenoceptors in the detrusor muscle of man than in the pig. Further, the far less steep dose-response curve for isoprenaline in man than in the pig indicates that the β-adrenoceptor populations in the detrusor muscle in the two species are different from each other. The muscle relaxing potency of salbutamol in man was found to be about 60 times less than that of isoprenaline and a maximum muscle relaxing effect of 63% was obtained with salbutamol in doses up to 10^{-4} M. When compared to the earlier described potency ratios between isoprenaline and salbutamol in 'classical' β_1- and β_2-adrenoceptor containing organs, the present ratio between the two agonists indicate that the β-adrenoceptors in the human detrusor muscle can be classified neither as β_1- nor as β_2-adrenoceptors. Whereas the isoprenaline-induced relaxation of the detrusor muscle from man could be antagonised with propranolol, no inhibition of the isoprenaline response was obtained with practolol or butoxamine, confirming that the β-adrenoceptors in question belong neither to the β_1- nor to the β_2-group.

The results of the present study of the β-adrenoceptors in the detrusor muscle of pig and man are comparable to those obtained in corresponding investigations carried out in rat (Elmér, 1974), cat and man (Nergårdh *et al.*, 1977). The β-adrenoceptors demonstrated in the pig are very much like the β_2-adrenoceptors found in the rat and are in marked contrast to the β_1-adrenoceptors found in the cat detrusor muscle. The present finding of β-adrenoceptors in the human detrusor muscle, not belonging to the β_1- or the β_2-group, is in agreement with the results in man obtained by Nergårdh *et al.* (1977) who have suggested the existence of a third type of β-adrenoceptor in the human detrusor muscle.

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