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Alpha- and Beta-Adrenoceptors in the Female Dog Urethra

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The existence and subtypes of α- and β-adrenoceptors in the female dog urethra were studied *in vivo* and *in vitro* by means of agonist and antagonist drugs. Noradrenaline, phenylephrine and B-HT 920, stimulants of α, α-1 and α-2 receptors respectively, caused an increase in the urethra tonicity. Thus indicating that the contractile activity is mediated by α-1 and α-2 adrenoceptors subtypes. On the other hand, the inhibitory urethral activity is under control of β-adrenoceptors of β-2 subtype, since the isoprenaline relaxing action is inhibited when β-2 receptors are blocked, whilst this effect was not observed when β-1 receptors were blocked. This fact was proved when β-2 receptors were stimulated with salbutamol.

Key words: Adrenoceptors, Urethra, Dog.

The presence of an abundant sympathetic innervation in the urethra of the dog has been demonstrated (17). The relaxation of this organ follows stimulation of β-adrenoceptors (12) whilst stimulation of α-adrenoceptors increases the urethral muscle tone (3). The blocking of these latter receptors results in a decrease in urethral pressure. These effects can be used in the therapy of functional disorders of the lower urinary tract (2).

Different subtypes of α and β-adrenoceptors occur (13, 14) and the aim of this study was to determine the effect of these subtypes on urethral function in the dog.

Materials and Methods

In vitro. The urethras of 36 female dogs, obtained immediately after euthanasia, were used.

From each urethra a longitudinal strip of smooth muscle was dissected and suspended at a tension of 2 g in a 20 ml organ bath containing a Krebs solution, bubbled with a mixture of 95 % O_2 and 5 % CO_2. The recordings were made using force transducers and displayed on a four-channel Multicorde (Hugo Sachs Elektronik).

Before starting the recordings a time lapse of 60 to 90 min was allowed to

stabilize the activity. Five minutes were allowed for each drug to act and one minute for the blocking agent.

All the results were expressed as mean ± standard error of the mean, an the statistical evaluation was made using analysis of variance and the multiple comparison test.

In vivo. Twelve mongrel bitches varying from 18 to 25 kg of body weight were used. They were anesthetized with sodium thiopentane (25 mg/kg, i.v.) and maintained with halothane (0.5 to 2.5 %) as an inhalation anesthetic.

A bad catheter was introduced in the urethra which after having been filled with 3 to 5 ml of water, depending on the urethra size, fitted perfectly to this structure wall so that the mechanic variations which would take place in the urethra could be transmitted through a circuit full of water in a Statham pressure transducer P23AC which was connected to a Grass Polygraph with a paper speed of 1 cm/min.

Substances used were: B-HT 920 (6-Allyl-2-amino-5,6,7,8-tetrahydro-4-H-thiazolo-4,5-d azepin-dihydrochloride) (Lab. Dr. K. Thomae); butoxamine hydrochloride (Wellcome); isoprenaline sulphate (Boehringer); (—)-noradrenaline bitartrate (Serva), phenylephrine hydrochloride, yohimbine hydrochloride (Sigma); phenoxybenzamine hydrochloride (Smith Kline & French); practolol, propranolol hydrochloride (ICI); prazosin hydrochloride (Pfizer) and salbutamol sulphate (Glaxo).

Results

In vitro. In all cases the stimulation of α-receptors with noradrenaline at concentrations of 10^{-8} M to 10^{-4} M resulted in a concentration-dependent contraction of the muscular strips varying from 17.00 ± 2.36 to 94.62 ± 1.17 % (fig. 1 A).

Phenoxybenzamine (10^{-6} M), an α-adrenergic blocking drug, caused a shift to the right of the noradrenaline concentration-response curve with a total blockade of the muscle contraction induced by noradrenaline at 10^{-8} M, while

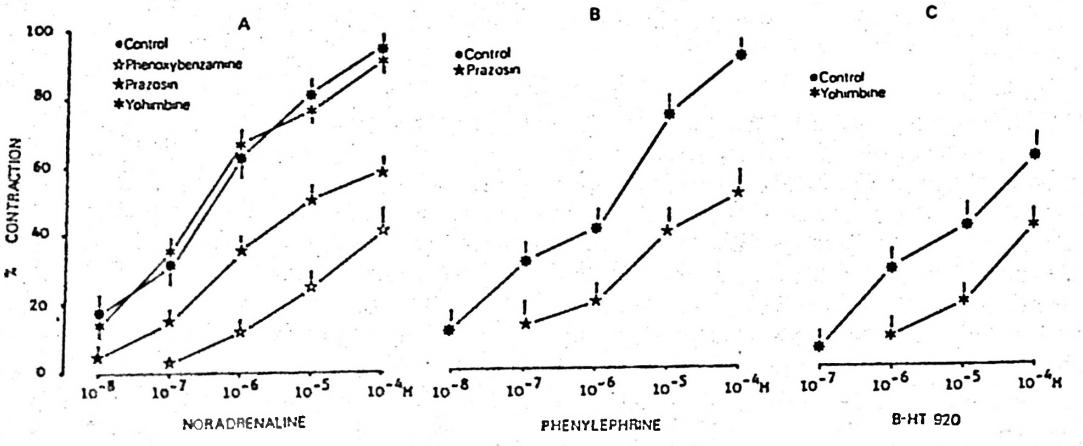

Fig. 1. *Studies in vitro on α- and β-adrenoceptors in the isolated female dog urethra.* A) Effects of phenoxybenzamine, prazosin and yohimbine on the concentration-response curve to noradrenaline. B) Effect of prazosin on the concentration-response curve to phenylephrine. C) Effect of yohimbine on the concentration-response curve to B-HT 920. Mean values are shown; vertical lines indicate S.E.M. (n = 8).

the contractile response to noradrenaline at 10^{-7} M to 10^{-4} M was significantly inhibited ($p < 0.02$). The prior addition of prazosin, a selective antagonist of α-1-receptors, at a concentration of 10^{-5} M blocked, partially, the response to noradrenaline at 10^{-8} M ($p < 0.05$) and from 10^{-7} M to 10^{-4} M ($p < 0.02$), although the blocking effect was less than that obtained with phenoxybenzamine (fig. 1 A).

Yohimbine, a selective antagonist of α-2-receptors, at a concentration of 10^{-5} M did not inhibit the contractile action of noradrenaline (fig. 1 A).

With the administration of selective α-adrenergic agonists it was observed that the urethra tonicity was increased by stimulation of α-1-receptors with phenylephrine from 10^{-8} M to 10^{-4} M (11.30 ± 1.76 to 91.66 ± 2.27 %) (figure 1 B) and α-2-receptors with B-HT 920 from 10^{-7} M to 10^{-4} M (5.00 ± 1.52 to 61.00 ± 3.21 %) (fig. 1 C). Phenylephrine was more potent than B-TH 920.

The urethral muscle responses to phenylephrine and B-HT 920 were significantly inhibited ($p < 0.02$) by previous addition of prazosin (10^{-5} M) and yohimbine (10^{-5} M), respectively (fig. 1 B and 1 C).

Adding isoprenaline (10^{-7} M to 10^{-4} M), an agonist of β-adrenergic receptors, to the urethral muscle, evoked a concentration-dependent relaxation, varying from 11.25 ± 2.14 to 85.75 ± 2.86 % (fig. 2 A). Adding propranolol (10^{-7} M), a non selective blocking agent of β-adrenergic receptors, and butoxamine (10^{-5} M), a selective blocking agent of β-2-receptors, to the bath, resulted in a shift to the right of the concentration-response curve to isoprenaline at all the concentration levels used, with a significant reduction in activity ($p < 0.05$ for 10^{-7} M and $p < 0.02$ for 10^{-6} 10^{-5} and 10^{-4} M). On the other hand, no antagonistic effect was observed after adding

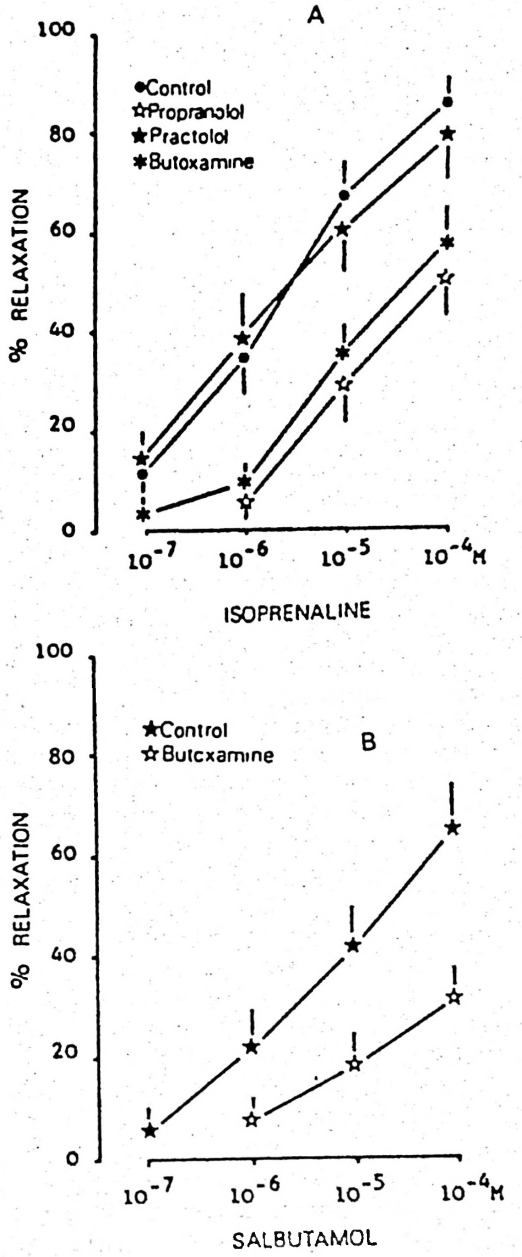

Fig. 2. *Studies in vitro on α- and β-adrenoceptors in the isolated female dog urethra.* A: Effects of propranolol, practolol and butoxamine on the concentration-response to isoprenaline. B: Effect of butoxamine on the concentration-response to salbutamol. Mean values are shown; vertical lines indicate SEM ($n = 8$).

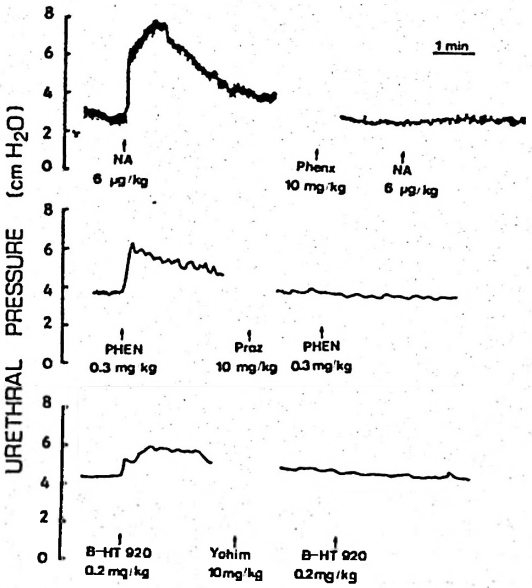

Fig. 3. *Studies in vivo on α- and β-adrenoceptors in the female dog urethra.* *Left:* Effects of noradrenaline (NA), phenylephrine (PHEN) and B-HT 920 on urethral pressure of female dogs, *in vivo*. *Right:* The addition of phenoxybenzamine (Phenx), prazosin (Praz) and yohimbine (Yohim) blocked the effects of noradrenaline, phenylephrine and B-HT 920.

practolol (10^{-5} M), a selective blocking agent of β-1-receptors.

When salbutamol, a selective β-2-adrenergic receptors agonist, was added at concentration from 10^{-7} M to 10^{-4} M, a relaxing effect occurred, varying from 6.38±1.20 to 65.00±5.90 % (fig. 2 B). This relaxing action was significantly reduced when butoxamine (10^{-5} M) had been previously added ($p < 0.05$ for the salbutamol concentration of 10^{-6} M and $p < 0.02$ for the concentrations of 10^{-5} M and 10^{-4} M).

In vivo. The administration of noradrenaline (2 to 6 µ/kg, i.v.), phenylephrine (0.1 to 0.5 mg/kg, i.v.) or B-HT 920 (0.1 to 0.3 mg/kg, i.v.) (figure 3) resulted in a concentration-dependent increase in the urethral tonicity, but showing a greater response when α-1-receptors were stimulated, either by the administration of noradrenaline (an α-receptor agonist) or, specifically, by phenylephrine, than when α-2-receptors were stimulated with B-HT 920.

Previous administration of phenoxybenzamine, prazosin or yohimbine, at a dose of 10 mg/kg i.v., inhibited the contractile action of noradrenaline, phenylephrine or B-HT 920, respectively (figure 3).

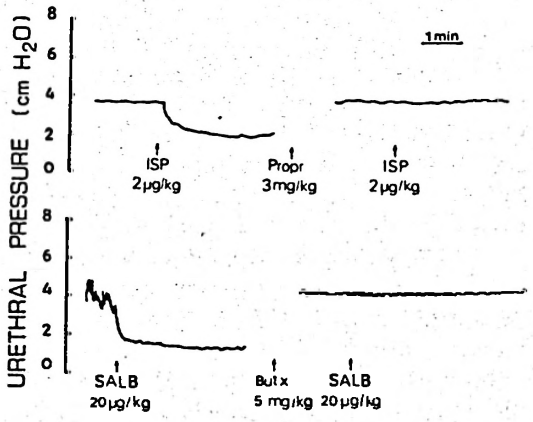

Fig. 4. *Studies in vivo on α- and β-adrenoceptors in the female dog urethra.* *Left:* Effects of isoprenaline (ISP) and salbutamol (SALB) on urethral pressure of female dog. *Right:* The addition of propranolol (Prop) and butoxamine (Butx) blocked the effect of isoprenaline, and salbutamol.

The administration of isoprenaline (1 to 5 µg/kg, i.v.) and salbutamol (15 to 25 µg/kg, i.v.) (fig. 4) induced a concentration-dependent relaxation of the urethral pressure. Prior addition of propranolol (3 g/kg, i.v.) or butoxamine (5 mg/kg, i.v.) inhibited the relaxing action of isoprenaline or salbutamol, respectively (fig. 4).

Discussion

In spite of the amount of published works, quantitative differences in relation to the sympathetic innervation of the lower urinary tract have been found in several studies. Thus, EK *et al.* (6) found little sympathetic innervation in the human urethra, TANAGHO *et al.* (18) concluded that the sympathetic autonomic nervous system only controls the rich vascular bed of the urethra, and not the proper urethral smooth muscle, whereas other authors have observed an abundant sympathetic innervation in the rabbit (1), cat (16), dog (12, 17), sheep (8), pig (15), cattle (10) and horse (9).

Since LANGER (14) suggested the existence of different α-adrenergic receptor subtypes, several studies have shown the presence of α-1 and α-2-receptor in different muscular tissues, e.g. HOFFMAN *et al.* (11). In the present study, noradrenaline caused an increase in the urethral tonicity in the dog and this effect was inhibited by phenoxybenzamine and prazosin; it indicated that the muscle contractile activity is mediated by α-adrenergic receptors, including the α-1 subtype. This effect has already been observed in the urethra of cattle (10) and horse (9) but the fact that the inhibiting action of prazosin is lower than the one obtained with phenoxybenzamine on the noradrenaline induced contractions suggests the existence of another subtype of α-adrenergic receptor in the mediation of the contractile activity of the urethra in the dog. Yohimbine had no effect on the response to noradrenaline (fig. 1 A) which shows that this receptor subtype was not α-2. The use of α-1 as well as α-2 selective agonist proved that the tonicity of the urethra increased when α-1-receptors were stimulated with phenylephrine and when α-2-receptors were stimulated with B-HT 920, both in *in vitro* and *in vivo* preparations.

These results are clear evidence of the presence of both subtypes of α-adrenergic receptors and their role in the contractile activity in the urethra of the dog. Similar results were obtained by ANDERSSON *et al.* (1) who detected the presence of both subtypes of α-adrenergic receptors in the urethra of the rabbit.

Following confirmation that relaxation of the lower urinary tract was controlled by direct stimulation of β-adrenergic receptors (5) and since LANDS *et al.* (13) showed the existence of different types of β-adrenergic receptors, various studies have shown the presence of β-1 and β-2-receptors in different muscle tissues (4, 7).

In the present work, smooth muscle relaxation of the canine urethra was shown to be controlled by adrenergic receptors of β-2-subtype, since salbutamol, a selective β-2-receptor agonist, evoked this effect (fig. 2 B). Likewise, the relaxing activity of isoprenaline, which is a powerful sympathomimetic substance and acts almost exclusively on β-receptors, is inhibited when butoxamine, a selective β-2-receptor antagonist, was previously added (fig. 2 A). This relaxing action of isoprenaline did not disappear with prior addition of practolol, a selective β-1-receptor antagonist, results which agree with those obtained by GARCÍA-SACRISTÁN *et al.* in the urethra of the sheep (8), horse (9) and cattle (10).

It may be concluded that in the female dog urethra there exists clear evidence for the presence of both α and β-adrenergic receptors, and that the contractile activity is mediated by α-1 as well as by α-2-receptors, whereas the relaxation is controlled by adrenergic receptors of β-2 subtype.

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Resumen

Se estudia la presencia y subtipos de adrenoceptores α y β en la uretra de perra, tanto en *in vitro* como *in vivo*, mediante la utilización de substancias agonistas y antagonistas de esos receptores. El hecho de que la noradrenalina, fenilefrina y el compuesto B-HT 920, estimulantes de los receptores α, α-1 y α-2 respectivamente, produzcan un incremento de la tonicidad de la uretra indica claramente que la actividad contráctil está mediada por receptores de los subtipos α-1 y α-2. Por el contrario, la actividad inhibitoria de la uretra está gobernada por acción de los β-adrenoceptores del subtipo β-2, ya que la acción relajante del isoproterenol queda inhibida cuando se bloquean los receptores β-2, pero no cuando se antagonizan los β-1, circunstancia que se corrobora cuando se estimulan los receptores β-2, especialmente con salbutamol.

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