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Alpha- and Beta-Adrenergic Receptors in the Horse Ureter

A. Labadia*, L. Rivera, G. Costa and A. García-Sacristán

Cátedra de Fisiología
Facultad de Veterinaria
Universidad Complutense de Madrid
28040 Madrid (Spain)

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The presence of both alpha and beta adrenergic receptors in the caudal third ureter of the horse were studied *in vitro* under isometric conditions using adrenergic agonist and antagonist drugs. Isoprenaline and the β_2- stimulating agent, salbutamol, elicited relaxation of the ureter smooth muscle strips. The responses were not affected by the β_1- blocking agent, practolol, but were totally abolished by propranolol and the β_2- blocking agent, butoxamine. The stimulation of α-adrenergic receptors with noradrenaline and phenylephrine evoked a contractile effect which was totally inhibited by phenoxybenzamine and the α_1- blocking agent, prazosin. It is concluded that in the horse ureter the alpha receptors are dominant and belong to α_1 subtype while the β receptors are recessive and belong to β_2- adrenoceptor subtype.

Key words: Adrenergic receptors, Caudal ureter, Horse.

The control of ureteral peristaltism by the autonomic nervous system has been dealt with in numerous reports. Some investigations (10) have noted that sympathomimetic and cholinergic agents have no effect on ureteral peristalsis, whereas others (8) have suggested that any ureteral response to neurohumoral agents is secondary to direct local action of these agents on the muscle cells and not via reflex mechanisms of the autonomic nervous system. However, the demonstration of high levels of catecholamines in the ureter (1), as well as the histological evidence of an adrenergic innervation of mammal ureter (6) provides a solid basis to the suggested role for the adrenergic receptor mechanisms in the control of the ureteral functions. Since certain differences in relation to the presence of adrenergic receptors between different animal species (2, 16) as well as the participation of different receptor subtypes(α_1, α_2, β_1, β_2), in dog ureter have been shown, in the present study the presence and action of the different types and subtypes of adrenoceptors in the ureteral smooth muscle of horse have been studied.

Materials and Methods

The horses studied were obtained from the Madrid slaughterhouse. The ureters extracted *in situ* were immediately sub-

* To whom all correspondence should be addressed.

merged in a Krebs solution and transported to the laboratory. After cleaning the ureters for fat and adherences they were stored at 4° C, since there is no reduction in responses when kept in this condition for up to 72 h (7, 11, 15).

From the caudal third of each ureter a helicoidal strip of smooth muscle tissue, 25-30 mm long and 2-5 mm wide, was dissected. The strips were suspended in 40 ml organ baths with Krebs solution at 37° C and bubbled with a mixture of 95% O_2 and 5% CO_2. The distal end of the muscle strip was fixed to the base of the bath and the proximal end to a force transducer (Grass FT 03 C) with a tension of 2 g. The recording was made on a Grass polygraph model 79 B. Before starting the recording a time lapse of 60 minutes was allowed to stabilize the activity. Eight experiments per dose were carried out.

The results are expressed in terms of means ± standard error of the mean (SEM) and the statistical evaluation was calculated using analysis of variance and the multiple comparison test.

Substances: butoxamine hydrochloride (Wellcome); isoprenaline sulphate (Boehringer Sohn Ingelheim); L-noradrenaline bitartrate, phenylephrine hydrochloride and yohimbine hydrochloride (Sigma); phenoxybenzamine hydrochloride (Smith Kline & French); practolol and propranolol hydrochloride (ICI); prazosin hydrochloride (Pfizer), and salbutamol sulphate (Glaxo).

Results

Once the period of time allowed for the adaptation of the strips had passed, the different adrenergic agonist and antagonist substances were tested on preparations with spontaneous contractions as

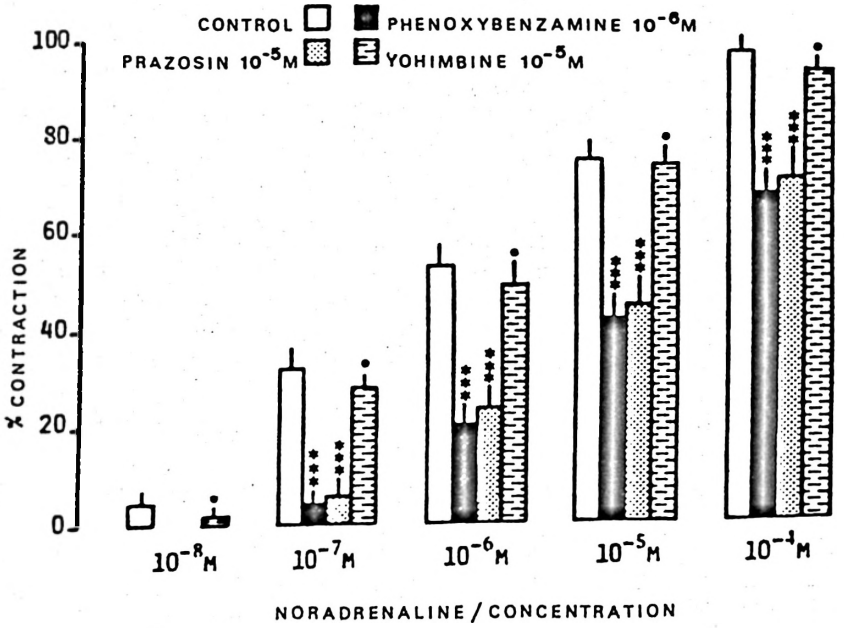

Fig. 1. *Phenoxybenzamine, prazosin and yohimbine blocking action on the noradrenaline contractile activity in ureter of horse.*

Mean values are shown, vertical lines indicate SEM of 8 experiments per dose (significantly different from control at *** $p < 0.001$, ●: n.s.).

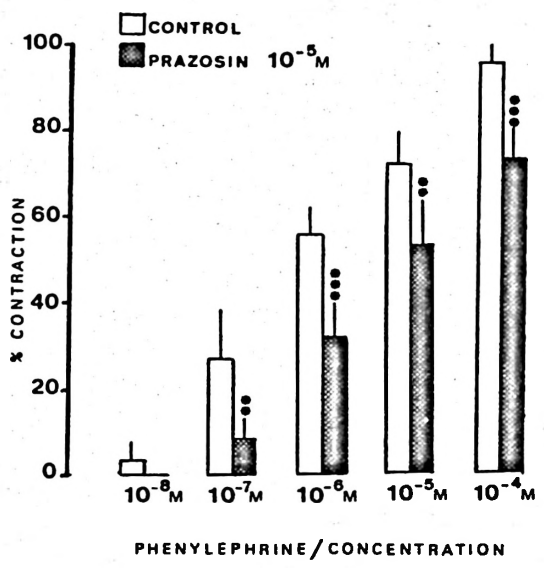

Fig. 2. *Influence of prazosin on phenylephrine dose-response curve in horse ureter.*
Mean values are shown, vertical lines indicate SEM of 8 experiments per dose (significantly different from control at ** $p < 0.01$, *** $p < 0.001$).

well as on inert ones. Different noradrenaline concentrations (10^{-8} M to 10^{-4} M), on stimulating the α-adrenergic receptors in the absence of antagonist substances, caused a dose-dependent contraction of the muscle strips. Prior administration of phenoxybenzamine (10^{-6} M) or prazosin (10^{-5} M) induced a shift of the noradrenaline dose-response curve to the right causing a total blockage at 10^{-8} M and significant inhibition of the contractile activity at doses from 10^{-7} M to 10^{-4} M. Yohimbine did not inhibit the noradrenaline contractile action (fig. 1).

Phenylephrine provoked 3.16% to 94% contractile response at 10^{-8} M to 10^{-4} M concentrations respectively (figure 2). But this contractile activity was totally inhibited at 10^{-8} M when prazosin (10^{-5} M) was previously added, and significantly reduced for the other phenylephrine doses.

Stimulation of β-adrenergic receptors with 10^{-8} M to 10^{-4} M isoprenaline provoked a 1.62% to 62.50% dose-de-

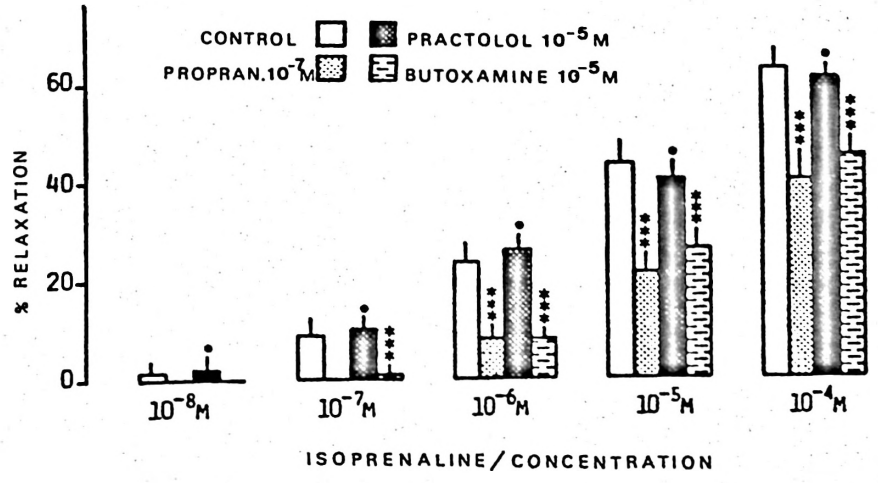

Fig. 3. *Blocking action of propranolol, practolol and butoxamine on the isoprenaline dose-response curve in the ureter of horse.*
Mean values are shown, vertical lines indicate SEM of 8 experiments per dose (significantly different from control at *** $p < 0.001$, •: n.s.).

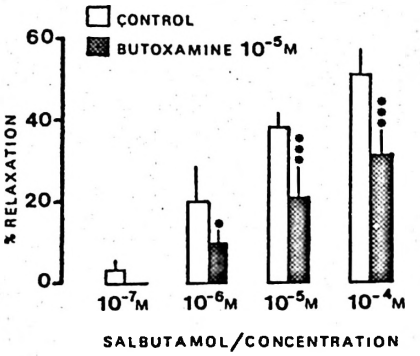

Fig. 4. *Butoxamine inhibitory effect on salbutamol dose-response curve in horse ureter.*
Mean values are shown, vertical lines indicate SEM of 8 experiments per dose (significantly different from control at * $p < 0.05$, *** $p < 0.001$).

pendent relaxation respectively (fig. 3). The addition of propranolol (10^{-7} M) shifted the isoprenaline dose-dependent curve to the right causing total inhibition at 10^{-8} M and 10^{-7} M and a significant reduction between 10^{-6} M and 10^{-4} M concentrations. Practolol (10^{-5} M) did not inhibit the relaxation activity of isoprenaline at any of the concentrations assayed (fig. 3), while butoxamine (10^{-5} M) caused the total blockage in the range 10^{-7} M to 10^{-4} M.

Salbutamol caused a relaxation activity of 3.66% and 50.33% at 10^{-7} M and 10^{-4} M respectively (fig. 4), but butoxamine (10^{-5} M) significantly reduced this relaxation.

Discussion

The results obtained in this study with the stimulation of α-adrenoceptors show the presence of this type of receptors in the ureteral smooth muscle of horse and are in agreement with those found in dog (13), pig (4) and rabbit (16). Thus, and with the aim of differentiating more specifically to which type of α-adrenoceptors the contractile action of noradrenaline was due, selective antagonists such as prazosine and yohimbine were used.

The fact that the stimulation of α-adrenergic receptors in the presence of prazosin has an inhibitory effect on ureteral contractile activity, which is not observed with yohimbine, and that the contractile ureteral activity was present after the administration of phenylephrine, leads us to believe that the ureteral contractions in horse are mediated by means of α_1-adrenergic receptor. These results agree with those obtained in dog (3) by *in vivo* and *in vitro* experiments.

The results obtained in relation to the β-adrenergic receptors are a clear indication of the presence of this type of receptor, since isoprenaline evoked the relaxation of the ureteral smooth muscle and the prior addition of propranolol inhibited this effect. Thus, these results prove the existence of β-adrenoceptors in horse ureter as has already been reported in pig, cow, man (2) and dog (5, 11). In rat, however, BOYARSKY and LABAY (2) could not observe any response to isoprenaline, and the strong contractions induced by noradrenaline were not inhibited by any of the α-adrenergic antagonist. This shows the differences among the animal species in relation to the adrenergic receptors.

Since the existence of different β-adrenergic receptor subtypes has been demonstrated (9) various reports, using the combination of specific and non specific agonist and antagonist substances, have shown the presence of β- and β_2-adrenoceptors in different muscle tissues. In our study the relaxation induced by isoprenaline was inhibited by previous addition of butoxamine, a β_2-adrenoceptor antagonist while prior administration of practolol did not abolished the isoprenaline relaxation action.

In general, the results found in this study reveal a predominance of α-adrenergic receptors over β-adrenergic receptors, since the responses obtained at equal

concentrations were much stronger when stimulating α-adrenoceptors, as MALIN *et al.* (12) found in different animal species, but the presence of both types of adrenergic receptors was always evident. Thus our results contrast with the concept that the ureteral peristaltism control is exclusively a miogenic process (14) and that the sympathomimetic substances did not induce any effect on the ureteral peristalsis (10). The fact of having observed a clear response both with the stimulation of alpha and beta-adrenoceptors proves the important role that the autonomic nervous system plays on the ureteral activity in horse, the α_1-adrenoceptor being responsible for the contractile activity and the β_2-adrenoceptors for the relaxation.

Resumen

Se estudia la presencia de receptores α- y β-adrenérgicos en la porción caudal del uréter equino utilizando para ello agonistas y antagonistas adrenérgicos en condiciones isométricas *in vitro*. Tanto el isoproterenol como el salbutamol provocan la relajación de todas las tiras de músculo liso ureteral, efecto que no es inhibido por el practolol y sí, en cambio, cuando previamente se administraban propranolol y butoxamina observándose un bloqueo total del efecto relajante inducido por los agonistas α-adrenérgicos. La estimulación de los receptores α-adrenérgicos con noradrenalina y fenilefrina causa un efecto contráctil que es totalmente inhibido por la fenoxibenzamina y el prazosín, bloqueante específico de los receptores α_1. De los resultados obtenidos se puede concluir que, en el uréter caudal de los équidos, los receptores α-adrenérgicos son predominantes y pertenecen al subtipo α_1 mientras que los receptores β-adrenérgicos son recesivos y pertenecen al subtipo β_2.

Palabras clave: Receptores adrenérgicos, Ureter caudal, Equidos.

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