Br. J. Pharmac. (1978), **63**, 25–34.

SPECIES DIFFERENCES IN POSTGANGLIONIC MOTOR TRANSMISSION TO THE RETRACTOR PENIS MUSCLE

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1 Graded motor responses were elicited in isolated, desheathed, thin strips of dog, horse, pig and sheep retractor penis (RP) muscles by field stimulation with trains of 0.2 ms pulses at 10 hertz. These twitches were shown to be neurogenic in all four species, by their prompt extinction in tetrodotoxin.

2 α-Adrenoceptor blocking drugs abolished the contractile response to noradrenaline and to tyramine in all four species.

3 Motor transmission was wholly adrenergic in the horse as in the dog RP because phentolamine rapidly abolished the electrically induced twitches in both these species; but in the pig and in the sheep RP a large proportion of the motor transmission was unaffected by phentolamine given in many times the concentration required to abolish matching noradrenaline-induced contractions.

4 Because of the occurrence of periodic spasms in sheep preparations, further investigation of the phentolamine-resistant transmission was confined to the pig RP. Its responses were shown to be entirely postganglionic in origin because they were unaffected by pentolinium.

5 In the pig RP a considerable proportion of the phentolamine-resistant motor transmission persisted after combined blockade of α- and β-adrenoceptors by phenoxybenzamine plus propranolol and was more resistant to guanethidine and bretylium than the motor transmission to the dog RP; it was not extinguished after reserpine treatment.

6 The pig RP is contracted by histamine but is rather insensitive to acetylcholine, 5-hydroxytryptamine and adenosine-5′-triphosphate. The motor transmission remained unaffected after responses to these substances were blocked by the following antagonists, given alone or in combination: mepyramine, burimamide, atropine, (+)-tubocurarine, methysergide and 2-2′-pyridylisatogen tosylate.

Introduction

The retractor penis, a paired muscle which is present on the under surface of the penis in some mammalian species, receives a dual innervation. Langley & Anderson (1895) found that the hypogastric nerves were motor and the sacral nerves, inhibitory. In the dog retractor penis the sympathetic postganglionic motor transmission is wholly adrenergic because the whole of it is easily paralysed by α-adrenoceptor blocking agents such as chrysotoxin (Dale, 1906), yohimbine, ergotoxin or piperoxan (Luduena, 1939) and piperoxan or phentolamine (Luduena & Grigas, 1966; 1972). This is not so in other species and the present investigation has shown that the nature of the postganglionic motor transmission is subject to species variation because it is paralysed by α-adrenoceptor blockade with phentolamine or with phenoxybenzamine in the retractor penis of the horse, as in the dog, but not in that of the pig or of the sheep.

A preliminary account of these results has been given (Ambache & Killick, 1976).

Methods

Most of the experiments were performed on slaughterhouse material, with the exception of the dog retractor penis muscles, which were taken from 4 uncastrated animals of various breeds, anaesthetized in the laboratory with pentobarbitone sodium, 40 mg/kg (Veterinary Nembutal, Abbott Laboratories) and killed by air embolism. For the rest, the retractor penis muscles were excised, immediately after death at various abattoirs, from 5 horses (two of which were castrated), 36 castrate pigs and 5 castrate sheep. The tissues were then transported to the laboratory in Krebs–Henseleit solution of the composition given below, kept ice-cold in a Dewar flask. The muscle preparations were used within 3 h of death; occasionally they were stored overnight at 4°C for use next day.

The parallel halves of the retractor penis muscle may either be well separated by a distinct gap as in the pig (and ox) or more adherent, as in the horse, but could, in all four species, be easily separated from

each other by midline dissection between the pair. The preparations were taken from the middle third of one of the pairs, after careful desheathing. Thin strips, 30 × 2 × 2 mm, were cut along the longitudinal axis and suspended at an initial load of 0.5 g in 2 ml organ-baths (with built-in, parallel, vertical platinum–iridium electrodes) containing Krebs–Henseleit solution at 35°C (±0.1°), bubbled with a 95% O_2 and 5% CO_2 mixture. The composition of the Krebs–Henseleit solution was as follows (mM): NaCl 112.9, KCl 4.69, $CaCl_2 \cdot 2H_2O$ 2.52, $MgSO_4 \cdot 7H_2O$ 1.5, KH_2PO_4 1.18, $NaHCO_3$ 25.0 and glucose 22.2. Contractions or relaxations were recorded isometrically by means of transducers coupled to potentiometric recorders (Servocorder, model SR 652, Watanabe Instrument Corp., Tokyo, Japan). Intermittent field stimulation, usually at 1 min intervals with single pulses or with trains of pulses, was provided by a stimulator of low output impedance (Bell & Stein, 1971). The stimulation voltage was kept constant throughout each experiment; pulse width was fixed at 0.2 ms and the frequency at 10 Hz for all the experiments.

Reserpine treatment

A male, uncastrated, 19 kg pig was injected intraperitoneally with reserpine, 10 mg/kg per day for three consecutive days, and was killed 24 h after the last injection. The reserpine solution was prepared according to Leydon *et al.* (1956), i.e. by dissolving 600 mg of reserpine (Ciba) in a mixture of 4.8 ml benzyl alcohol, 24 ml Tween '80', 600 mg anhydrous citric acid and 240 ml distilled water. The animal was killed under intraperitoneal Nembutal anaesthesia.

Drugs

Except for prostaglandins E_2 and $F_{2\alpha}$ (Upjohn), tetrodotoxin (Sankyo) and burimamide (Smith, Kline & French), all doses refer to the respective salts, namely: acetylcholine chloride (Hopkin & Williams), adenosine 5'-triphosphate disodium salt (ATP; Sigma), atropine sulphate (Hopkin & Williams), bradykinin triacetate (Sigma), bretylium tosylate (Burroughs Wellcome), guanethidine sulphate (Ciba), histamine dihydrochloride (Koch-Light), 5-hydroxytryptamine creatinine sulphate (Sigma), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), (−)-noradrenaline hydrogen tartrate (Koch-Light), pentolinium tartrate (May & Baker), phenoxybenzamine hydrochloride (Smith, Kline & French), phentolamine mesylate (Ciba), propranolol hydrochloride (ICI), (+)-tubocurarine chloride (Sigma) and tyramine hydrochloride (Koch-Light).

We wish to thank Dr M. Hooper of the School of Pharmacy, Sunderland Polytechnic, for the gift of a sample of 2-2'-pyridylisatogen tosylate.

Results

Species difference in motor transmission revealed by α-adrenoceptor blocking agents

Phentolamine. That the motor transmission to the retractor penis is wholly adrenergic in the dog has been confirmed repeatedly in the present experiments by the fact that the α-adrenoceptor blocking agent, phentolamine (2.6 μM), never failed to produce rapid and total paralysis. Similar results were obtained on preparations from another species, the horse.

On the other hand, with pig and sheep preparations the results were quite different. In both these species a substantial proportion of the motor transmission to the retractor penis was spared after blockade of noradrenaline or tyramine contractions by low concentrations of phentolamine, and there was no further decline in motor transmission when the concentration of phentolamine was raised tenfold. It should be emphasized that in all four species the responses to transmural stimulation with narrow pulses of 0.2 ms were wholly neurogenic and postganglionic in origin because they were unaffected by pentolinium, 0.19 mM, but unfailingly abolished by tetrodotoxin, 1.6 μM (0.5 μg/ml). Figures 1 and 2 illustrate these species differences.

Figure 1 contrasts the classical susceptibility of the motor transmission to paralysis by α-adrenoceptor blocking drugs in the dog with its considerable insusceptibility in the pig. In the dog retractor penis not only did phentolamine, 5.3 μM, abolish the motor responses to exogenous noradrenaline, 3 μM, or to tyramine, 29 μM (which releases endogenous noradrenaline), but it also very rapidly extinguished the electrically induced twitches; this process was complete within 10 min for the responses elicited by 4 pulses and within 15 and 18 min for those elicited by the longer trains of 8 and 16 pulses, respectively.

On the other hand, in the pig retractor penis the contractile responses to noradrenaline, 6 μM, and to tyramine, 115 μM, were easily abolished by an even lower concentration of phentolamine, i.e. 2.6 μM, but there was no extinction of the motor transmission either at this low concentration or after the tenfold increase in concentration to 26 μM. The presence, initially, of a small adrenergic component in the electrically induced twitches was shown by their slight reduction after phentolamine, 2.6 μM. This reduction was more marked at greater train lengths. Thus, the diminution in twitch height brought about by 2.6 μM was 4 ± 1% (s.e. mean; $n = 5$) for the responses to the single pulses and 32 ± 4% (s.e. mean; $n = 5$) for the responses elicited by the 16-pulse trains. It will be noted that there was no further decrease in response height when the phentolamine concentration was raised to 26 μM.

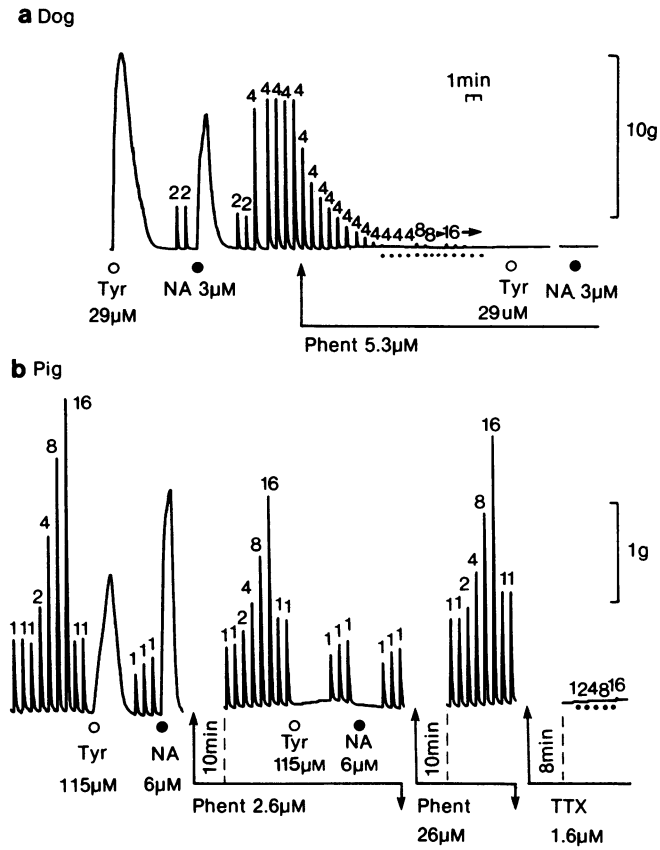

Figure 1 Species difference between the dog and the pig in phentolamine-resistance of the motor transmission to the retractor penis (RP) muscle. In this and all subsequent figures the twitches were elicited at 1 min intervals by transmural stimulation with 1–32 pulses, as indicated by superscripts (pulse width, 0.2 ms; frequency, 10 Hz; constant voltage throughout); and the small dots show the application of electrical stimuli after motor paralysis. At Tyr, tyramine and at NA, noradrenaline given for 60 seconds. (a) Dog RP: Initial motor responses to tyramine, 29 μM, and to noradrenaline, 3 μM, and their extinction by phentolamine, 5.3 μM (2 μg/ml), associated with a rapid and total paralysis of motor transmission. (b) Pig RP: Responses to transmural stimulation with 1–16 pulses, to tyramine, 115 μM, and to noradrenaline, 6 μM, before and after phentolamine (2.6 μM for 0.5 h followed by 26 μM for 17 minutes). Although the lower concentration of phentolamine abolishes the response to tyramine and to noradrenaline, it fails to extinguish the motor transmission and reduces only the response to long trains. The tenfold increase in phentolamine concentration to 26 μM does not produce any further decline in motor transmission at all train lengths. The phentolamine-resistant motor responses to 1–16 pulses are abolished by tetrodotoxin, 1.6 μM (last panel), and are therefore entirely neurogenic.

Mention should be made of the fact that in experiments on pig retractor penis preparations, the twitch responses showed a natural tendency to increase in height with time, even in the absence of any drugs, so that the increases in response height seen in the penultimate panel of Figure 1 and in Figures 3 and 4 could not be attributed to the drug treatment.

Figure 2 contrasts the motor transmission in the horse and in the sheep. The preparations taken from both these species were for different reasons less satisfactory than those from the dog and the pig. In the horse retractor penis, motor transmission was often poorly maintained even without drug treatment and in order to demonstrate the rapid effect of phentolamine it was necessary to select preparations in which the motor transmission was well maintained and to use a higher concentration of phentolamine (13 μM) in order to increase the rapidity of onset of the paralysis, which in Figure 2a occurred within 3 minutes.

With some sheep preparations difficulties of a different kind were created by a tendency to go into spasm periodically; stimulation accelerates the

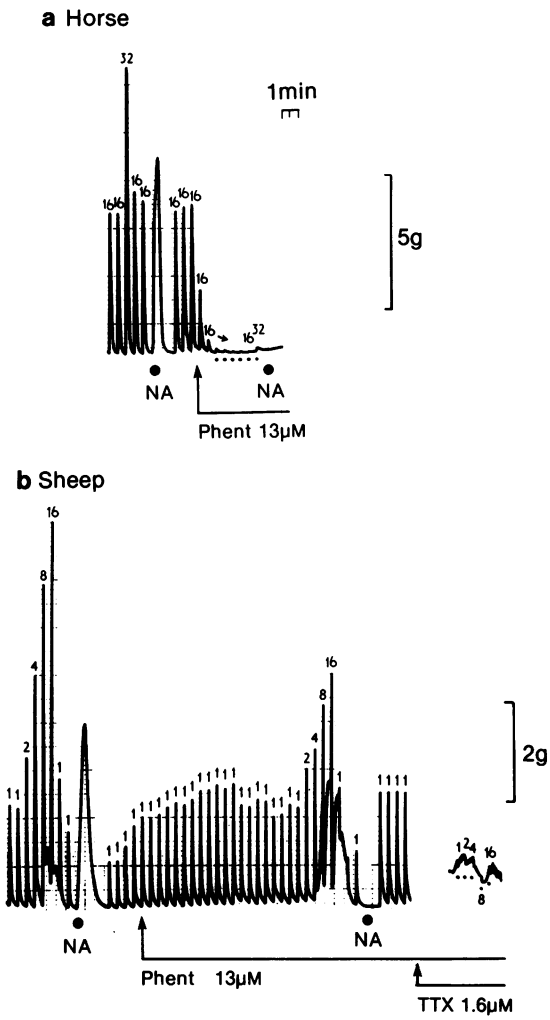

Figure 2 Species difference between the horse and the sheep in motor transmission to the retractor penis. At NA, noradrenaline, 3 or 9 μM, given for 60 seconds. (a) Horse RP: Rapid and total paralysis of motor transmission, and of response to noradrenaline (3 μM) by phentolamine (Phent) 13 μM. (b) Sheep RP: Even after 32 min exposure to phentolamine, 13 μM, and extinction of contraction to noradrenaline (9 μM) the twitch height remains virtually unaltered with 1 or 2 pulses but is reduced with 4–16 pulses. Note the appearance, as a result of the development of tone, of an inhibitory component in the response to 8–16 pulses. The phentolamine-resistant motor responses to 1–16 pulses were abolished by tetrodotoxin, 1.6 μM (last panel), and were therefore wholly neurogenic.

appearance of such spasms. It was nevertheless clear, even with these difficulties, that a considerable proportion of the motor transmission to the retractor penis is substantially spared by phentolamine in the sheep, as in the pig. Thus, after phentolamine (13 μM) in the experiment of Figure 2b, the height of the response to single pulses was initially unaltered; but with the long trains of 8 or 16 pulses some reduction was apparent, as in the pig; however, it was difficult to quantify this reduction because of the development of tone and the appearance of an inhibitory component in the response to each train. Because of these difficulties the further investigation of the phentolamine-resistant motor transmission was confined to preparations taken from pigs.

Phenoxybenzamine. The species difference in motor transmission revealed by phentolamine was confirmed with the irreversible α-adrenoceptor blocking agent, phenoxybenzamine, the paralytic effect of which was total and prompt in the dog but small in the pig. Thus, the experiment on the pig retractor penis illustrated in Figure 3 shows that, concurrently with extinction of noradrenaline-induced contractions, phenoxybenzamine produced a slight initial decrease in twitch-height which was more pronounced with longer trains and was due to the removal of the adrenergic component in the motor responses which was noticed in the experiments with phentolamine. But no further reduction in twitch-height occurred even after prolonged soaking in this drug for nearly 3 hours. At the end of the experiment the phenoxybenzamine-resistant twitches were rapidly extinguished, as usual, by tetrodotoxin, 1.6 μM.

Further analysis of the phentolamine-resistant motor transmission in the pig retractor penis

As can be seen from Figure 4b, the phentolamine-resistant component in the motor transmission persisted after combined blockade of the β- as well as the α-adrenoceptors, by administration of phentolamine and propranolol sequentially; it will be noted there was no further reduction in the non-adrenergic component of the motor transmission by the superimposition of the β-blockade.

Figure 4a also illustrates an inhibition of motor transmission by noradrenaline, which was noted in several phentolamine-treated pig retractor penis preparations. This inhibition was due to β-adrenoceptor stimulation because it was obtained also with isoprenaline, 3.6 μM, in other experiments and was abolished by propranolol (Figure 4b).

Lastly, Figure 4 also excludes cholinergic motor transmission of the phentolamine-resistant motor component, because it persisted after atropine, 2.9 μM, and, in other experiments, after (+)-tubocurarine, 113 μM. The pig retractor penis is, anyway, rather insensitive to acetylcholine, as can be seen in Figure 4b.

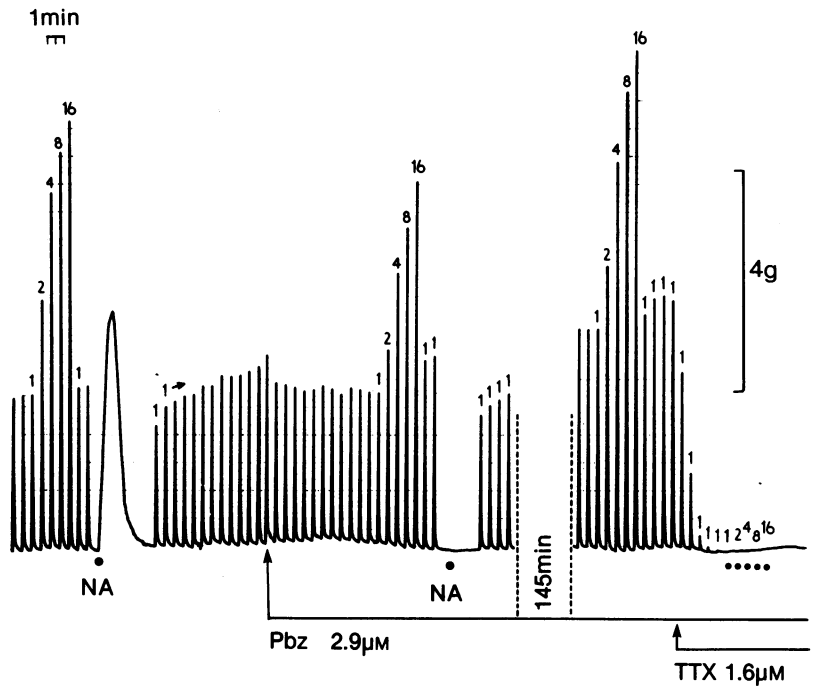

Figure 3 Persistence of motor transmission in pig retractor penis after α-adrenoceptor blockade with phenoxybenzamine (Pbz) 2.9 μM. Although the response to noradrenaline, 3 μM (at NA), is soon abolished by phenoxybenzamine, the motor transmission persists even after exposure to phenoxybenzamine for nearly 3 h, but is promptly paralysed by tetrodotoxin, 1.6 μM, at the end of the experiment.

Reserpine-treatment. The results obtained on the retractor penis preparation taken from the reserpine-treated pig (Figure 5) illustrate several points. The preparation was supersensitive to noradrenaline, responding by contraction to as little as 0.03 μM. This ability of the preparation to detect much smaller amounts of noradrenaline than usual increases the significance of the fact that there was no motor response to the noradrenaline-releasing agent tyramine, 115 μM, a dose which had never failed to cause large contractions in preparations taken from untreated pigs. Despite the noradrenaline-depletion which can be inferred from these results, tetrodotoxin-susceptible motor responses could still be elicited by electrical stimulation with trains of 4–16 pulses, though not with 1–3 pulses, thus differing from normal preparations. Another difference from the normal muscles, which is illustrated in Figure 5c, was that the electrically induced twitches underwent no reduction whatsoever in phentolamine (5.3 μM), showing again that the adrenergic portion of the motor transmission had been fully removed.

Adrenergic neurone blockers. A further species difference was noted when adrenergic neurone blocking drugs were used. It is known that such drugs induce spasm as a side effect.

Luduena & Grigas (1966; 1972) were the first to report that in the dog retractor penis, guanethidine or bretylium produce a reversal of the response to transmural stimulation, due to a selective paralysis of the motor and a sparing of the inhibitory fibres, the effect of which becomes manifest only when sufficient tone is present. This has been repeatedly confirmed for guanethidine in the present experiments. But when the effect of guanethidine was studied on the pig retractor penis, a further species difference emerged, which is illustrated in Figure 6.

With the dog retractor penis the motor paralysis by guanethidine was total even with 20 μM (Figure 6a). In the contracted state obtained when guanethidine (20 μM) was present in the organ-bath the transmural stimulation with the narrow 0.2 ms pulses (which earlier had produced pure contractions overshadowing the underlying inhibitory responses) now produced pure relaxations. Even after the subsidence of tone when the guanethidine was washed out, the total motor paralysis persisted for some time, with little or no response to 2–16 pulses.

On the other hand, in the pig retractor penis the

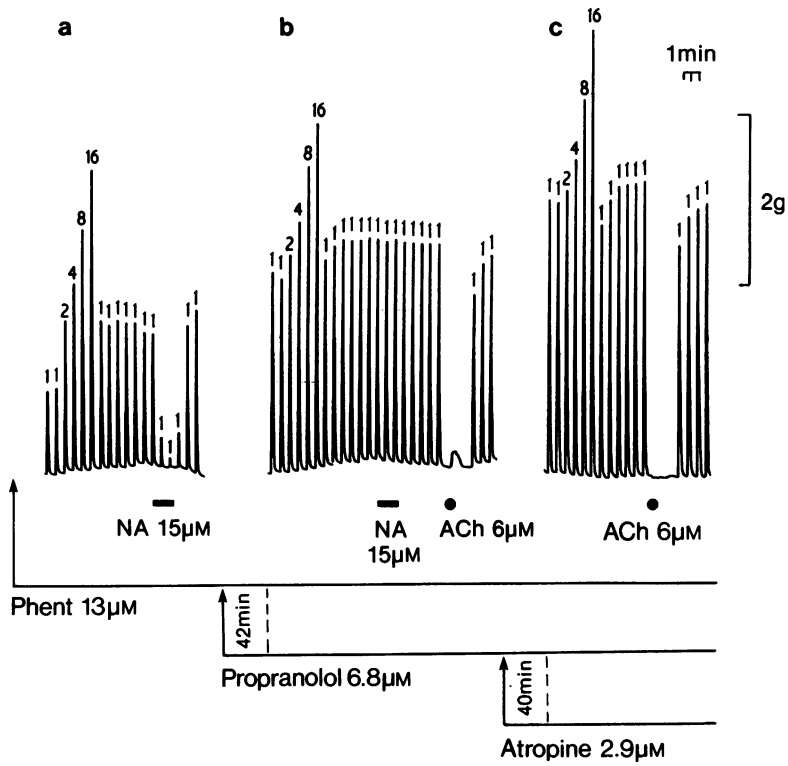

Figure 4 The phentolamine-resistant motor transmission of the pig retractor penis persists after β-adrenoceptor blockade with propranolol, 6.8 μM, and after the administration of atropine, 2.9 μM. At NA, noradrenaline, 15 μM, for 2 min and at ACh, acetylcholine, 6 μM, for 60 seconds. In the presence of phentolamine (a) noradrenaline produces an inhibition of motor transmission which is due to a β-effect because it is blocked by propranolol (b).

motor transmission was considerably more guanethidine-resistant than in the dog. Thus, Figure 6b shows that although the concentration of guanethidine was doubled (40 μM) and the exposure was more prolonged, the inhibitory responses elicited by transmural stimulation with 2–16 pulses were still preceded by a distinct motor component. And in the relaxed state obtained after the guanethidine-spasm was abolished by the administration of phentolamine (5.3 μM) electrical stimulation with 2–16 pulses still elicited contractions; with 4–16 pulses, these twitches represented a sizeable proportion of the initial motor responses at the same train lengths. These residual guanethidine-resistant responses were shown to be neurogenic by their extinction with tetrodotoxin.

Likewise, bretylium (48 μM) failed to extinguish the motor responses in pig retractor penis preparations, even when the exposure was prolonged for 40 minutes.

It is evident, then, that the motor transmission in the pig retractor penis is considerably more resistant to adrenergic neurone blocking drugs than in the dog.

Exclusion of some putative transmitters. The foregoing results show that the phentolamine-resistant motor transmission in the pig retractor penis is not mediated either by catecholamines or by acetylcholine. Mediation by 5-hydroxytryptamine (5-HT) could also be excluded because the retractor penis muscle is relatively insensitive to this substance and because the motor transmission persisted after 5-HT blockade with methysergide (1.1 μM) or phentolamine (2.6–26 μM).

Autonomically innervated smooth muscles usually display a high sensitivity to their motor transmitter, whether this is acetylcholine or noradrenaline. But the pig retractor penis was found to be relatively insensitive to ATP; for instance in the two experiments of Figure 7, concentrations of 18 and 90 μM were required to induce a motor response.

There is as yet no satisfactory antagonist for ATP. Thus, with 2-2'-pyridylisatogen tosylate (PIT) Hooper, Spedding, Sweetman & Weetman (1974) found it necessary, in order to achieve blockade of ATP, to use such high concentrations that non-specific side

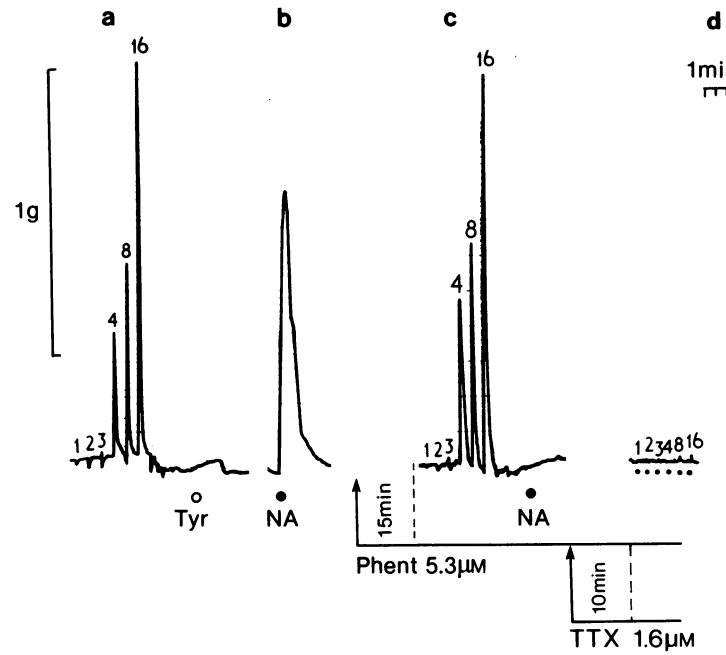

Figure 5 Retractor penis preparation taken from a reserpine-treated pig (details in Methods). Survival of the motor transmission despite the noradrenaline-depletion shown by the fact that, although the muscle was supersensitive to noradrenaline (b), there was a virtual absence of motor response to the noradrenaline-releasing drug, tyramine (a). At Tyr, tyramine, 115 μM, for 2 min and at NA, noradrenaline, 0.03 μM, for 30 seconds. With 1–3 pulses small inhibitory responses were recorded; with 4–16 pulses, motor responses were elicted which were wholly neurogenic because they were paralysed by tetrodotoxin (d). Note that the motor transmission in the reserpinized preparation was completely unaffected by phentolamine, 5.3 μM, indicating the absence of the adrenergic component which is detectable with longer trains in normal preparations.

effects were always exerted. However, in the present experiments on the pig retractor penis PIT did always suppress ATP-contractions to a much greater extent than the phentolamine-resistant motor transmission. For example, in the two experiments illustrated in Figure 7, there was little or no reduction of the motor transmission after ATP-blockade with PIT, 20–50 μM. And in a third experiment (not shown) 20 μM of PIT depressed 2-pulse twitches by 33%, whereas the initially larger responses to ATP, 18 μM (10 μg/ml) were depressed by 95%.

The pig retractor penis is fairly sensitive to histamine, which elicited contractions in concentrations of 0.54–54 μM. But histaminergic transmission was excluded by the fact that the electrically induced twitches remained completely unaffected after block of H_1- and H_2-receptors with, respectively, mepyramine (2.5 μM) and burimamide (4.7 μM), given alone or in combination.

The pig retractor penis is also contracted by bradykinin, 0.08–0.8 μM, and prostaglandin $F_{2\alpha}$, 0.88 μM, for which no satisfactory antagonist is as yet available.

Discussion

The results show that after α-adrenoceptors are fully blocked a clear-cut species difference is revealed in the postganglionic motor transmission to the retractor penis muscle between, on the one hand, the dog and the horse and, on the other, the pig and the sheep. The total paralysis of motor responses to single pulses and to trains of up to 32 pulses, which is obtained with adrenergic neurone or α-adrenoceptor blocking agents in the dog and in the horse, has shown that the whole of the motor transmission is adrenergic in these two species. On the other hand, in the pig and in the sheep the use of these drugs has revealed the presence of only a small adrenergic motor component and shown that a substantial proportion of the motor transmission at these train lengths persists after guanethidine or after α-adrenoceptor blockade with phentolamine or phenoxybenzamine.

The unidentified non-adrenergic postganglionic motor transmission in the retractor penis of the pig

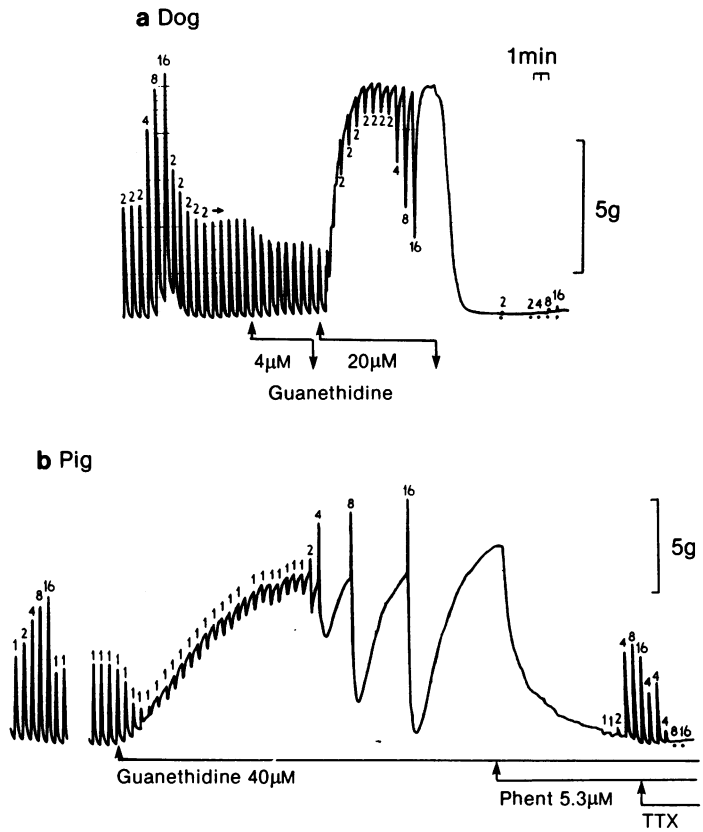

Figure 6 Species difference between the dog (a) and the pig (b) retractor penis in susceptibility to guanethidine. The contractions or relaxations were elicited at 1 min intervals by transmural stimulation with 1–16 pulses, as indicated by superscripts or subscripts (pulse width, 0.2 ms; frequency, 10 Hz; constant voltage throughout); the small dots at the end of each experiment show the application of electrical stimuli after motor paralysis. (a) Dog RP: Slow rate of motor paralysis and absence of contracture with 4 μM of guanethidine; 20 μM produced contracture and a reversal of responses to stimulation with 2–16 pulses, which elicited pure inhibitions during the guanethidine-spasm and little or no motor responses for at least 15 min after the guanethidine was washed out and the preparation had relaxed to its original baseline. (b) Pig RP: Persistence of a proportion of the motor transmission in guanethidine, 40 μM. Note that at the height of the guanethidine-spasm, 2–16 pulses still elicited motor responses which preceded each inhibition. These motor responses were also present after the spasm was abolished by phentolamine, 5.3 μM; their susceptibility to tetrodotoxin shows that they were neurogenic.

and sheep may resemble the non-adrenergic sympathetic motor transmission found in the vas deferens of many species and studied by Ambache & Zar (1971), Ambache, Dunk, Verney & Zar (1972), Euler & Hedqvist (1975), Hedqvist & Euler (1976) and Henderson & Zar (1976). The retractor penis muscle receives its postganglionic motor innervation from the hypogastric nerve, which is also the motor nerve to the vas deferens. It is interesting to note that a similar species difference in sympathetic postganglionic motor transmission has recently been detected in the vasa of three of the four species that have been examined in this paper (sheep were not available). Adebanjo and Ambache (unpublished observations) have found that in the vas deferens of the dog and of the horse the motor transmission is wholly adrenergic and can be easily paralysed by phentolamine or phenoxybenzamine (as in human vasa; Thompson & Zar, personal communication 1975). But in the vas deferens of the pig and of seven other species a considerable proportion of the motor transmission persists after α-adrenoceptor blockade.

In the present experiments another species difference was observed, namely the spontaneous appear-

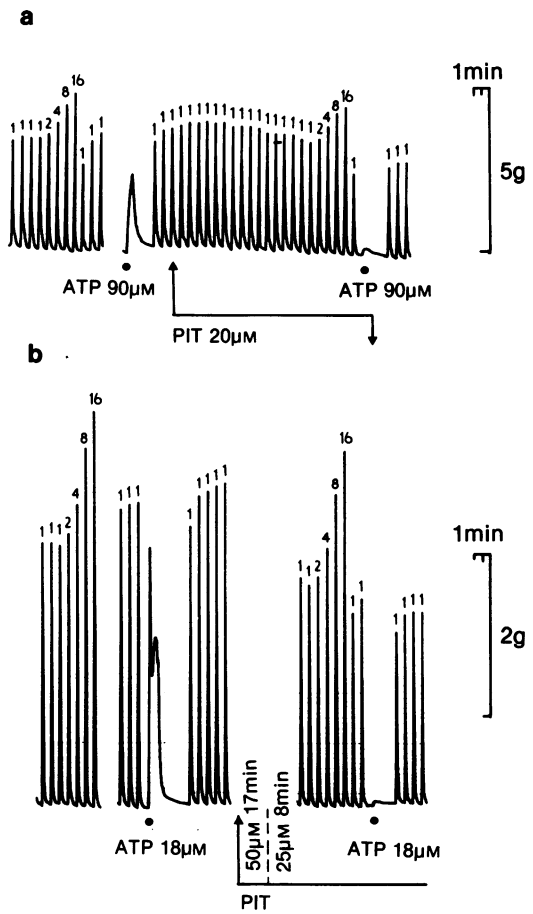

Figure 7 Persistence of the phentolamine-resistant motor transmission in pig retractor penis after ATP-blockade with pyridylisatogen tosylate (PIT). The two preparations were taken from different animals and were stimulated transmurally at 1 min intervals with 1–16 pulses of 0.2 ms; phentolamine, 13 μM, propranolol, 6.8 μM, and atropine, 1.4 μM, were present throughout. (a) After 22 min exposure to PIT, 20 μM, the contractile response to ATP, 90 μM (50 μg/ml) given for 30 s, was virtually abolished without significant paralysis of the motor transmission. (b) The response to single pulses was matched by ATP, 18 μM, given for 60 seconds. After longer exposure to high concentrations of PIT (50 μM for 17 min, reduced to 25 μM for another 8 min preceding the last panel and then left in the organ-bath for a further 14 min to the end of the experiment) the response to the twitch-matching dose of ATP was virtually abolished without corresponding motor paralysis. These higher concentrations of PIT (known to exert other non-specific effects) produced some slight depression of the twitch.

ance of periodic spasms in the retractor penis of sheep, but not of the other species. It was thought that this spontaneous tone could be useful for physiological investigations of neurogenic inhibition; for, whenever spasm was present, it unmasked an inhibitory component in the response to electrical stimulation, as found previously in the contracted ox retractor penis (Ambache, Killick & Zar, 1975); this relaxation is due to concurrent stimulation of the sacral postganglionic fibres whose inhibitory effect was shown by Langley & Anderson (1895). However, the sheep muscle is much less useful than the ox muscle for studying the inhibitory transmission because the spontaneous tone is not maintained, whereas the ox muscle remains steadily contracted for many hours in Krebs–Henseleit solution at 35°C, a natural property which obviates completely the need to create tone artificially with drugs such as bretylium or guanethidine, as for instance in the dog retractor penis (Luduena & Grigas, 1966; 1972).

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(Received March 23, 1977.
Revised September 29, 1977.)