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European Journal of Pharmacology 337 (1997) 251–258

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Characterisation of excitatory and inhibitory transmitter systems in prostate glands of rats, guinea pigs, rabbits and pigs

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Received 7 April 1997; revised 5 August 1997; accepted 12 August 1997

Abstract

Excitatory and inhibitory transmitter systems were investigated in strips of prostate glands from rats, guinea pigs, pigs and rabbits. In strips from all species, electrical field stimulation (1 ms pulses at 1–30 Hz for 10 s) produced frequency-dependent contractions which were abolished by tetrodotoxin (1 μM). In strips from rats, guinea pigs and rabbits, contractions were reduced by prazosin (1 μM), guanethidine (10 μM) and atropine (2 μM), indicating the presence of noradrenergic and cholinergic mechanisms. However, the smooth muscle in the pig prostate appears to have a non-(nor)adrenergic non-cholinergic (NANC) excitatory innervation for which the transmitter was not identified. When noradrenergic and cholinergic mechanisms were blocked by guanethidine and atropine, respectively, and tone was raised with noradrenaline or methoxamine, field stimulation produced relaxations only in strips of rabbit prostate, and these were greatly reduced by N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), providing functional evidence for a nitrergic relaxant innervation. In accord with this, nitric oxide (NO) synthase activity was considerably higher in rabbit than in rat or pig prostates. © 1997 Elsevier Science B.V.

Keywords: NANC (non-adrenergic non-cholinergic) innervation; Noradrenergic innervation; Nitrergic innervation; Prostate gland; (Rat); (Guinea pig); (Pig); (Rabbit)

1. Introduction

Benign prostatic hypertrophy is increasingly recognised as a major health problem in middle-aged and older males (Garraway et al., 1991). The main untoward consequences are due to obstruction of the urethra caused not only by the increased mass of tissue around the urethra but also to active noradrenergically mediated constriction of smooth muscle in the capsule and trabeculae of the gland since it can be relieved by α_1-adrenoceptor antagonists (Caine, 1986; Chapple et al., 1990; Oesterling, 1995). If the smooth muscle in the prostate gland is also controlled by a relaxant mechanism, the possibility exists for exploiting this in the treatment of prostatic hypertrophy. To this end, we thought it worthwhile to search for a suitable animal model for investigating the neuronal control of smooth muscle in the prostate gland.

Recently, nitric oxide (NO) has been proposed to have a functional role in the prostate gland (Burnett, 1995; Hedlund et al., 1997). NO synthase activity has been detected by biochemical assay in extracts of prostate glands of the rat (Burnett et al., 1992), human (Ehrén et al., 1994, 1996; Burnett et al., 1995), rabbit and pig (Di Iulio et al., 1996b), and histochemical staining techniques have demonstrated the presence of NO synthase in nerves adjacent to the prostate gland in rat (Burnett et al., 1992) and within the nerves of the human prostate (Burnett et al., 1993; Addicks et al., 1996; Hedlund et al., 1997), indicating the likelihood of nitrergic transmission. In fact, it has been shown that nerve stimulation-induced relaxations of isolated preparations of rabbit, canine and human prostates are blocked by NO synthase inhibitors (Aikawa et al., 1994; Takeda et al., 1995; Hedlund et al., 1997).

The primary aim of this study is to determine the extent to which nitrergic transmission is involved in relaxant responses of rat, guinea pig, rabbit and pig prostates, and in the course of this to characterise the contractile transmitter systems, using functional pharmacodynamic analyses in isolated preparations of the gland. A preliminary communication of this study has been presented (Najbar-Kaszkiel et al., 1996).

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2. Materials and methods

2.1. Animals

Sexually mature male animals were killed in the following manners: rats (Sprague–Dawley, 250–350 g body weight) were killed by cervical dislocation; guinea pigs (Dunkin–Hartley, 300–550 g body weight) were killed with an overdose of pentobarbitone and treatment with heparin, followed by cervical dislocation; rabbits (New Zealand White, 1.5–2 kg body weight) were killed with an overdose of pentobarbitone, followed by bleeding; pigs were slaughtered in an abattoir. The prostate glands were isolated from these animals.

2.2. Functional studies

For each species and experiment, strips (approximately 3 mm in width, 10 mm in length and 2–4 mm in thickness) were obtained by cutting transversally through the lobes of the prostate gland parallel to the urethra. Consistent responses were obtained in functional studies when strips were taken from approximately the same location. The strips were mounted in organ baths in physiological salt solution (PSS) for isometric recording of tension under a resting tension of 2 g. The composition of the PSS (mM) was: NaCl (118), KCl (4.7), $NaHCO_3$ (25), $MgSO_4$ (0.45), KH_2PO_4 (1.03), $CaCl_2$ (2.5), D-(+)-glucose (11.1) and disodium edetate (0.067). The PSS was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The tissues were equilibrated for 1 h before experimental procedures were commenced. Electrical field stimulation (1 ms pulses at 1–50 Hz for 10 s, supramaximal voltage) was delivered through platinum wire electrodes placed at either side of the strip.

2.3. Determination of NO synthase activity

NO synthase activity in extracts of pig, rabbit and rat prostate glands were assessed by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline, as described by Di Iulio et al. (1996a), with slight modifications.

2.4. Drugs, chemicals and solutions

The following drugs were used: acetylcholine perchlorate (BDH Chemicals, Australia); atropine sulphate, calcitonin gene related peptide (CGRP), methoxamine hydrochloride, α,β-methyleneATP (α,β-MeATP), N^G-nitro-L-arginine (NOLA), N^G-nitro-L-arginine methyl ester (L-NAME), neurokinin A, noradrenaline bitartrate ((−)-arterenol), L-phenylephrine hydrochloride, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), serotonin creatinine sulphate, sodium nitroprusside, substance P and tetrodotoxin (Sigma, USA); prazosin hydrochloride (Pfizer, USA); neuropeptide Y (Auspep, Australia). A saturated NO solution (2 mM) was prepared on the day of the experiment, using the method described by Feelish (1991), with slight modifications, that is, distilled water was deoxygenated by gassing with argon for 1 h, followed by bubbling with NO for 30 min. Argon and NO were obtained from Commonwealth Industrial Gases (Australia).

All drugs, except prazosin were dissolved in distilled water and diluted in PSS. Prazosin was dissolved in a mixture of 10% glycerol and 5% dextrose.

2.5. Statistical analysis of results

Data are expressed as means ± standard error of the means (S.E.M.), and n indicates the number of experiments. The significance of differences between means was determined by analysis of variance, followed by Student's t-test. Probability levels less than 0.05 were considered significant.

2.6. Ethics

The study was approved by the Animal Experimentation Ethics Committee of the Royal Melbourne Institute of Technology and conformed to the guide lines laid down by the National Health and Medical Research Council of Australia.

3. Results

3.1. Contractile responses to electrical field stimulation

Electrical field stimulation (1–50 Hz for 10 s) produced frequency-dependent contractile responses in strips of prostate gland from all four species (Fig. 1). The contrac-

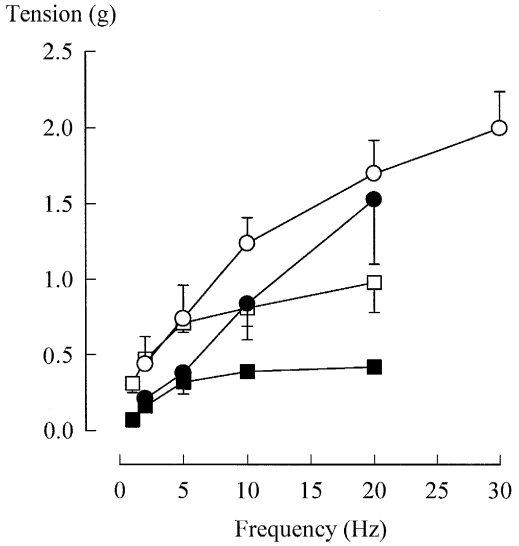

Fig. 1. Contractile responses produced by electrical field stimulation with 1 ms pulses at various frequencies for 10 s periods in strips of prostate glands isolated from guinea pigs (●, $n = 5$), pigs (□, $n = 6$), rabbits (○, $n = 4$) and rats (■, $n = 4$–6). Symbols represent means and vertical bars indicate S.E.M., which in some cases are smaller than the size of the symbol.

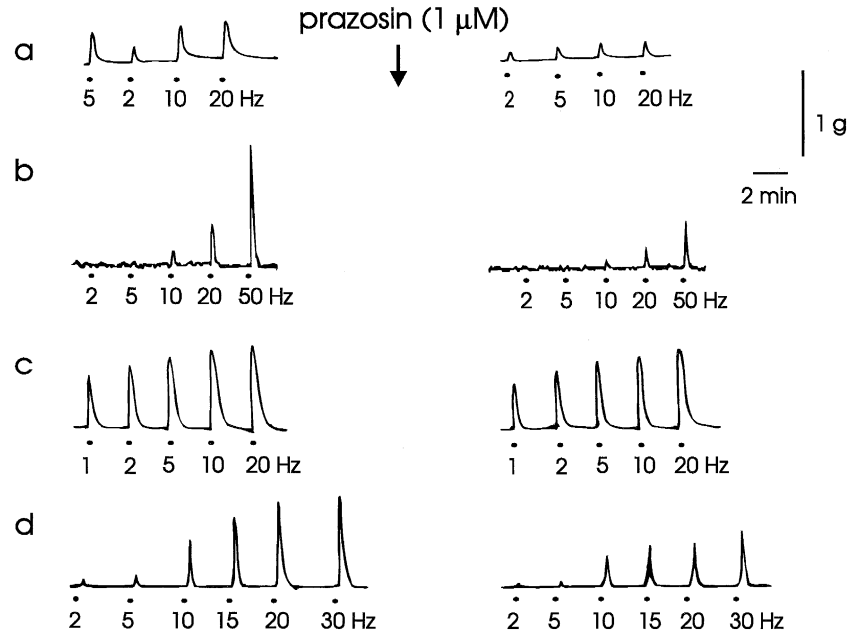

Fig. 2. Records of the effect of prazosin (1 μM) on contractile responses to electrical field stimulation (1–50 Hz for 10 s periods) in strips of prostate glands isolated from (a) rat, (b) guinea pig, (c) pig and (d) rabbit. Note, that prazosin reduced contractile responses to field stimulation in strips from all species except the pig.

tions were abolished by the neuronal sodium channel blocker tetrodotoxin (1 μM) (data not shown).

In strips of rat, guinea-pig and rabbit prostates, field stimulation-induced contractions were reduced by the α_1-adrenoceptor antagonist prazosin (1 μM; $n = 4$ for each species; examples in Fig. 2) or the noradrenergic neurone blocking drug guanethidine (10 μM; $n = 5$ for each species; data not shown). In contrast, contractions of strips of pig prostate elicited by field stimulation were not affected by prazosin (1 μM, $n = 3$; example in Fig. 2) or guanethidine (10 and 20 μM, $n = 3$, data not shown).

The muscarinic cholinoceptor blocker atropine (2 μM) reduced field stimulation-induced contractions (1–10 Hz) of strips of rabbit ($n = 4$) and guinea-pig ($n = 4$) prostates,

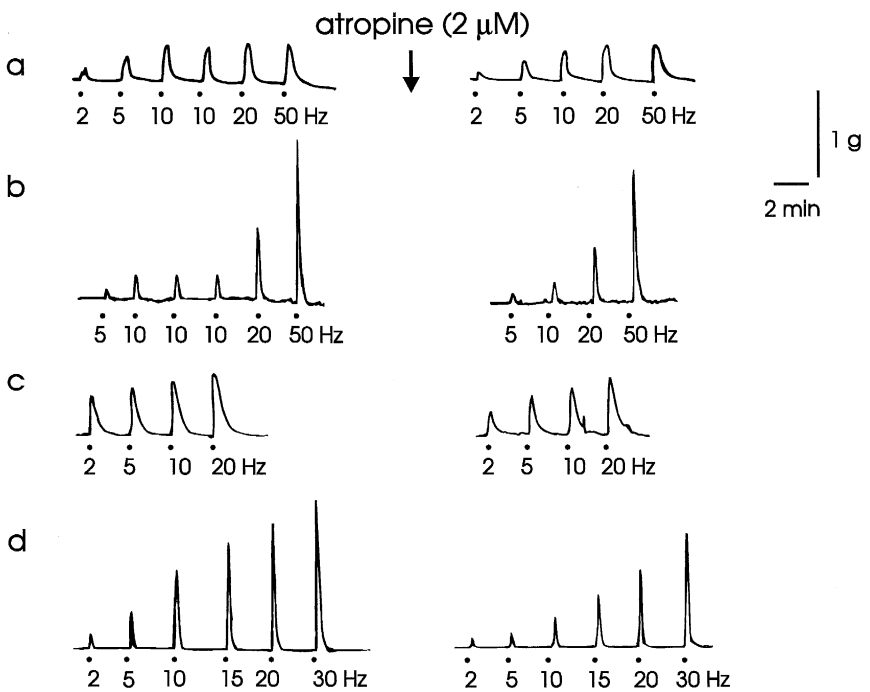

Fig. 3. Records of the effect of atropine (2 μM) on contractile responses to electrical field stimulation (2–50 Hz for 10 s periods) in strips of prostate glands isolated from (a) rat, (b) guinea pig, (c) pig and (d) rabbit.

Table 1
Effects of various agonist drugs with contractile activity on smooth muscle on strips of rat, guinea-pig, pig and rabbit prostate gland

Agonist drug	Species			
	Rat	Guinea pig	Pig	Rabbit
Noradrenaline (1–100 μM)	↗	↗ or ↗P	↗	↗
Methoxamine (10–20 μM)	↗	↗P	↗	—
Acetylcholine (1–10 μM)	↗P	↗	↗	↗
Serotonin (10 μM)	↗P	↗P	X	—
$PGF_{2\alpha}$ (1–10 μM)	↗P	↗P	↗	—
Phenylephrine (1–10 μM)	↗P	↗P	↗P	—
KCl (50 mM)	—	—	X	—
α,β-MethyleneATP (10 μM)	—	—	X	—
Neuropeptide Y (1–3 μM)	—	—	↗	—
Substance P (100 nM)	—	—	X	—
Neurokinin A (1–100 nM)	—	—	X	—
CGRP (0.1 μM)	—	—	X	—

In each case, $n = 3–8$.
↗ = the respective agonist drug(s) produced simple sustained contractions (refer to text).
↗P = the respective agonist drug(s) produced contractions which were immediately followed by phasic contractions and relaxations (refer to text).
X = the respective agonist drug(s) produced no contraction.
— = the effect of the respective agonist drug(s) was not tested.

but produced only slight reductions of those of strips of rat ($n = 4$) and pig prostates ($n = 3$). Representative traces are shown in Fig. 3.

A combination of guanethidine (10 μM) and atropine (2 μM) abolished the field stimulation-induced contractions in strips of rabbit prostate, reduced them greatly in rat and guinea-pig prostate and had no effect on pig prostate ($n = 5$ for each species, data not shown). Thus, noradrenergic and cholinergic mechanisms accounted almost entirely for the field stimulation-induced contractions of strips of rabbit prostate and largely for those of strips of rat and guinea-pig prostates, but made little or no contribution to those of strips of pig prostate.

L-NAME (100 μM) had no effect on contractile responses to electrical field stimulation in strips of prostate gland from any of the species used ($n = 4$ for each species, data not shown).

3.2. Contractile responses to agonist drugs

The effects of various agonist drugs with a contractile potential on various smooth muscle-containing tissues on the resting tension of strips of prostate gland are summarised in the Table 1.

Noradrenaline (1–100 μM) produced contractions in strips from all species. In strips of rat, rabbit and pig prostates, the peak increases produced by 10 μM noradrenaline were 0.5 ± 0.1 g ($n = 4$), 1.3 ± 0.2 g ($n = 8$) and 0.5 ± 0.1 g ($n = 4$), respectively, and thereafter the tension often fell slightly but remained elevated at a constant level. In strips from guinea-pig prostate, the initial contractions produced by noradrenaline (10 μM) were 0.4 ± 0.1 g ($n = 4$), and these were sometimes followed by phasic contractions and relaxations. Methoxamine produced simple sustained contractions in strips of rat and pig prostates, the initial peaks to 10 μM being 0.5 ± 0.1 g

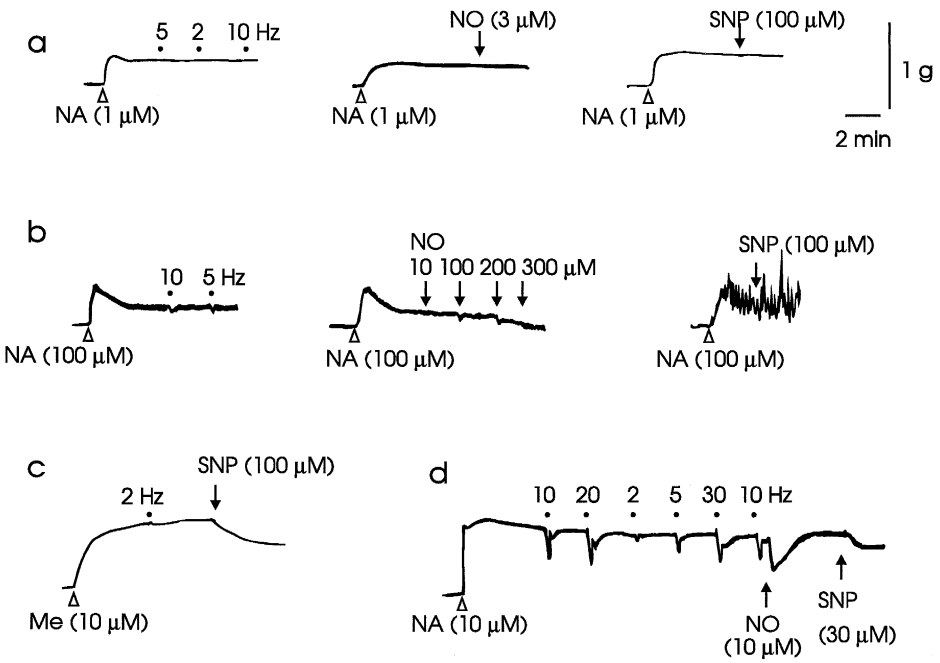

Fig. 4. Records of attempts to obtain relaxations in response to electrical field stimulation, NO and sodium nitroprusside in strips of prostate glands isolated from (a) rat, (b) guinea pig, (c) pig and (d) rabbit. The strips were initially treated with guanethidine (10 μM) and atropine (2 μM) and then precontracted with noradrenaline (NA) or methoxamine (Me) in the concentrations indicated. Field stimulation was at the frequencies shown for 10 s periods.

($n = 3$) and 0.7 ± 0.3 g ($n = 3$), respectively, but produced contraction followed by phasic activity in strips of guinea-pig prostate (and was not tested on strips of rabbit prostate since the noradrenaline-induced contractions were suitable for further experiments). Examples of contractile responses to noradrenaline and methoxamine are illustrated in Fig. 4.

Acetylcholine (1–10 μM) produced concentration-dependent contractions, which were of the simple type in strips of rabbit, guinea-pig and pig prostates but were followed by phasic activity in strips of the rat prostate.

In strips of the rat, guinea-pig and pig prostates, in addition to the above agents the effects of serotonin (10 μM), $PGF_{2\alpha}$ (1–10 μM) and phenylephrine were tested. These agonists produced no contraction or small contractions (≤ 0.2 g) which were either of the simple sustained type or were followed by increased phasic activity (see Table 1).

In strips of the pig prostate, in an attempt to find an agonist that mimicked the response to field stimulation, the other drugs listed in the relevant column of Table 1 were tested: they produced either no contraction at all, or slight increases in tension (≤ 0.2 g) (see Table 1).

3.3. Relaxant responses to electrical field stimulation, NO and sodium nitroprusside

These experiments were carried out in the presence of guanethidine (10 μM) and atropine (2 μM) to block, respectively, noradrenergic and cholinergic components of contractile responses, and noradrenaline (1–100 μM) or methoxamine (10 μM) was used to raise the tone.

Rat prostate strips did not relax in response to electrical field stimulation (2–10 Hz for 10 s), sodium nitroprusside (100 μM) or NO (3 μM) (Fig. 4a).

Guinea-pig prostate strips relaxed slightly (less than 10% of the raised tone) in response to high (5 and 10 Hz for 10 s) but not low (1–2 Hz for 10 s) frequencies of field stimulation, and in response to high concentrations of NO (≥ 100 μM) (Fig. 4b).

Since the mediator of the field stimulation-induced contractile response in the pig prostate was not identified, it was not possible to block it in an attempt to observe whether stimulation-induced relaxation could be revealed. When the tone was raised by methoxamine, sodium nitroprusside (100 μM) produced relaxation, but field stimulation had no effect (Fig. 4c).

Strips of rabbit prostate relaxed in response to field stimulation (2–30 Hz for 10 s), NO (1–30 μM) and sodium nitroprusside (30 and 100 μM) (Fig. 4d and Fig. 5). The field stimulation-induced relaxant responses were abolished by tetrodotoxin (1 μM), and were markedly reduced or abolished by 100 μM L-NAME (Fig. 5a), and in 3 out of 4 experiments the relaxations to higher frequencies of stimulations (10–30 Hz for 10 s) were converted to contractions in the presence of L-NAME. In contrast, L-NAME (100 μM) had no significant effect on relaxant responses produced by NO (1–30 μM) (Fig. 5b).

3.4. NO synthase activity

The NO synthase activities in terms of fmol of [^{3}H]L-citrulline formed per hour per mg protein from [^{3}H]L-arginine in the supernatant of homogenates of rat, pig and rabbit prostate glands were 3.62 ± 0.9 ($n = 5$), 24.2 ± 6.4

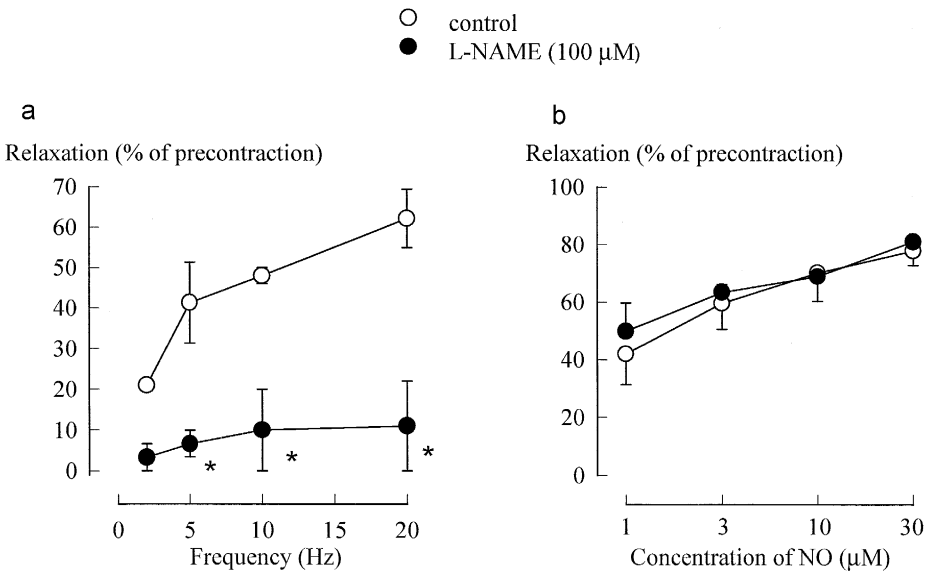

Fig. 5. Relaxations of strips of rabbit prostate glands elicited by (a) electrical field stimulation (2–20 Hz for 10 s, $n = 3$) or (b) NO (1–30 μM, $n = 3$) in the absence (○) and presence of 100 μM L-NAME (●). The symbols represent means and vertical bars indicate S.E.M., which in some cases are smaller than the size of the symbol. In (a), the asterisks (*) indicate a significant difference from control ($P < 0.05$).

($n = 4$) and 388.3 ± 47.9 ($n = 4$), respectively. The activity in the guinea-pig prostate was not tested.

4. Discussion

It has been previously reported that the motor transmitter to smooth muscle in prostate glands is predominantly noradrenergic in nature, since these tissues contracted to α_1-adrenoceptor agonists, such as noradrenaline, and to nerve stimulation, and these responses were reduced by α_1-adrenoceptor antagonists, as shown for the rat and guinea pig (Cohen and Drey, 1989), dog (Hieble et al., 1986; Normandin and Lodge, 1996), human (Raz et al., 1973; Caine et al., 1975; Hieble et al., 1985, 1986; Kunisawa et al., 1985; Marshall et al., 1995) and rabbit (Hiraoka et al., 1996). In addition, the presence and, in some cases, the subtype(s) of α_1-adrenoceptor in prostates of various species have been investigated by radioligand binding, cloning and/or in situ hybridisation studies (rat, human, rabbit and dog: Testa et al., 1993; Hiraoka et al., 1996; human: Price et al., 1993; Marshall et al., 1995; Tseng-Crank et al., 1995; Nasu et al., 1996; Chess-Williams et al., 1996; Michel et al., 1996; Chueh et al., 1996).

Our findings confirm the presence of noradrenergic contractile responses in rat, guinea-pig and rat prostates in that field stimulation-induced contractions were greatly reduced by prazosin or guanethidine in rat, guinea-pig and rabbit prostates. In contrast, electrical field stimulation-induced contractions in pig prostates were not reduced by either prazosin or guanethidine, suggesting that there was no noradrenergic innervation of its smooth muscle.

The nature of the motor neurotransmitter in the pig prostate could not be identified in the present study, but the transmitter is unlikely to be acetylcholine since atropine did not affect electrical field stimulation-induced contractions. Our results also exclude neuropeptide Y, substance P, neurokinin A or CGRP since these substances produced little or no contraction. The P_2 purinoceptor desensitising agent α,β-MeATP (10 μM) or the cyclooxygenase inhibitor indomethacin (10 μM) had no effect on field stimulation-induced contractions in strips of pig prostate, thus excluding the involvement of prostaglandins or ATP. Further experiments beyond the scope of the present paper would be required to identify the nature of the NANC excitatory transmitter in the pig prostate.

The production of [^{3}H]L-citrulline from [^{3}H]L-arginine has been previously reported to occur in rat prostate gland homogenates prepared in a similar fashion to those in the present study, but it was not demonstrated unequivocally that this was due to NO synthase activity (Burnett et al., 1992). More recently, it has been shown that only 20% of the conversion was due to NO synthase in that it was blocked by the NO synthase inhibitor NOLA (100 μM), and the remainder was due to activity of other enzymes; furthermore, the total NO synthase activity was considerably less than in similar extracts of the rabbit prostate (Di Iulio et al., 1996b). In the rat prostate, the slight NO synthase activity was of the calcium-dependent constitutive type (Di Iulio et al., 1996b), suggesting that it was probably due to endothelial type NO synthase.

The NO synthase activity was greatest in extracts of the rabbit prostate, followed by the pig and the rat prostates, with about 6% and 1%, respectively, of that in the rabbit prostate. Stimulation-induced relaxations that were blocked by the NO synthase inhibitor L-NAME and attributable to a nitrergic innervation were readily demonstrable in strips of rabbit prostate, and relaxations were also produced by exogenous NO or the NO donor sodium nitroprusside. It is possible that the amount of NO produced by NO synthase in rat and pig prostate glands is not sufficient to elicit relaxation of the smooth muscle. Although NO synthase activity in the guinea-pig prostate was not measured in the present study, no evidence for a nitrergic innervation was found in functional studies since the prostate did not relax in response to nerve stimulation, NO or sodium nitroprusside. On the other hand, although the amount of NO synthase activity in the pig prostate was almost seven times that of the rat prostate, the excitatory innervation could not be blocked; so conditions for demonstration of an inhibitory innervation were not obtainable.

The results from the present study suggest that, of the species studied, the prostate gland in rabbit most closely resembles the human prostate in terms of NO synthase content. NO synthase inhibitors reduced [^{3}H]L-citrulline production by more than 90% in extracts of human (Burnett et al., 1995) and rabbit (Di Iulio et al., 1996b) prostates. Moreover, the measurement of NO synthase activity in the rabbit prostate (6.5 fmol citrulline per min per mg protein) was of a similar order of magnitude to that reported by Burnett et al. (1995) for the human prostate (6.4 ± 1.7 fmol citrulline per min per mg protein in the transition zone and 15.7 ± 2.0 fmol citrulline per min per mg protein in the peripheral zone).

In tissues such as the rat anococcygeus muscle, which is endowed with a noradrenergic innervation (Gillespie, 1972; Gibson and Gillespie, 1973) as well as a nitrergic innervation (Li and Rand, 1989), NO synthase inhibitors enhance the stimulation-induced noradrenergically mediated contractions as a consequence of blockade of the counteracting nitrergic relaxation (for example, see Li and Rand, 1989; Brave et al., 1993). Such an effect has been observed in preparations of human and canine prostates in which L-NAME enhanced electrical field stimulation-induced contractions (Takeda et al., 1995; Hedlund et al., 1997). However, in the present study, L-NAME did not enhance electrical field stimulation-induced contractions in strips of rabbit prostate, even though it has a demonstrable nitrergic relaxant innervation. The reason may be that the relaxing component is so much weaker than the contractile that eliminating it does not make an appreciable difference to the contractions.

In conclusion, in the present study, functional evidence was obtained for a nitrergic inhibitory innervation in the rabbit prostate, but not in rat, guinea-pig or pig prostates. The motor innervation is predominantly noradrenergic in rat, guinea-pig and rabbit prostates, whereas the pig prostate had a NANC excitatory innervation, utilising a transmitter that was not identified.

Acknowledgements

This work was supported by grants from the National Health and Medical Research Council of Australia, the MSD Research Foundation and the Smoking and Health Research Foundation of Australia.

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