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Protein kinase C and the sub-sensitivity and sub-reactivity of the diabetic rat prostate gland to noradrenaline

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Abstract

Concentration–response curves to noradrenaline (1 nM–100 μM) were obtained in prostates from 6-week streptozotocin diabetic, insulin-treated diabetic or control rats. Compared to the curve obtained in controls, those obtained in prostates from diabetic and insulin-treated diabetic rats were shifted rightward. The α_1-adrenoceptor antagonist, prazosin (100 nM), caused a rightward shift of the curves in prostates from all groups. In contrast, the uptake 1 inhibitor, nisoxetine (300 nM), only produced a leftward shift of the curves in prostates from control and insulin-treated diabetic rats. However, frequency–response curves obtained in prostates from both control and diabetic rats were shifted leftward by nisoxetine (300 nM). The concentration–response curve to the α_1-adrenoceptor agonist, methoxamine (10 nM–100 μM), obtained in prostates from diabetic rats was shifted rightward compared with controls. Calphostin C (500 nM), a protein kinase C inhibitor, caused a leftward shift of the curve in prostates from diabetic, but not control, rats. The protein kinase C inhibitor, bisindolylmaleimide I (500 nM), β-adrenoceptor antagonist, propranolol (500 nM) and muscarinic cholinoceptor antagonist, atropine (300 nM), had no effect on the noradrenaline concentration–response curves of prostates from control or diabetic rats. Our results suggest that diabetes reduces the sensitivity and reactivity of the prostate to noradrenaline-induced stimulation, and this reduction may be due to changes in protein kinase C activity.

Keywords: Diabetes mellitus, type I; Prostate gland; Benign prostatic hyperplasia; Noradrenaline; Protein kinase C

1. Introduction

The prostate gland is a major accessory gland of the male reproductive system and plays an important role in maintaining the viability of sperm and assisting its passage through the female reproductive tract. It is a tubuloalveolar gland consisting of two lobes contained within a capsule. The gland is composed of secretory alveoli surrounded by fibro-elastic connective tissue interspersed with smooth muscle cells (Crowe et al., 1987). The rat prostate gland, like that of the human, is located around the urethra, in close proximity to where the urethra joins the bladder. Both the sympathetic and parasympathetic nervous systems innervate the rat prostate gland (McVary et al., 1998), but contractile responses to electrical nerve stimulation are mediated by α_1-adrenoceptors (Lau et al., 1998; Nishi et al., 1998).

Benign prostatic hyperplasia is the nonmalignant growth of the prostate gland and, due to the strategic position of the prostate, frequently produces problematic lower urinary tract symptoms in older men (Madsen and Bruskewitz, 1995). It is produced by an age- and androgen-dependent increase in the physical size of the gland, as well as an increase in the sympathetic tone of the prostatic smooth muscle, which consequently puts added pressure on the urethra (Cooper et al., 1999). The predominant pharmacological treatment of benign prostatic hyperplasia involves α_1-adrenoceptor antagonists, such as prazosin (Hedlund and Andersson, 1988) and terazosin (Lepor et al., 1996), which act by decreasing the sympathetic stimulation of the prostatic smooth muscle. These drugs are used to reduce problematic symptoms and to improve urinary flow (Cooper et al., 1999).

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Recent epidemiological studies have shown a correlation between diabetes and the symptoms of benign prostatic hyperplasia in patients without an enlarged prostate (Klein et al., 1999). This suggests that diabetes is related to the increased sympathetic tone component of benign prostatic hyperplasia. Another study found a correlation between the development of benign prostatic hyperplasia and men with Type II, non-insulin-dependent diabetes mellitus, obesity, and/or hyperinsulinaemia indicating that these may all be risk factors (Hammarsten and Hogstedt, 1999). Based on evidence that both obesity (Troisi et al., 1991) and hyperinsulinaemia (Rowe et al., 1981) are conditions associated with an increase in sympathetic outflow, it was also suggested that men with fast-growing benign prostatic hyperplasia might have increased sympathetic nerve activity. Furthermore, this effect may be due to the sympatho-excitatory effect of insulin (Rowe et al., 1981).

Previous studies have shown that streptozotocin-diabetic rats display a significant reduction in body weight (James and Hodgson, 1997) and serum insulin level, as well as high serum glucose levels (James and Hodgson, 1997), and decreases in fertility and spermatogenesis (Frenkel et al., 1978) when compared to nondiabetic rats. They also exhibit parallel reductions in reproductive organ weights, including the prostate gland (Crowe et al., 1987; Nishi et al., 1998). This reduction in body and prostatic weight can be prevented or reversed by early chronic insulin administration (Crowe et al., 1987; Nishi et al., 1998).

Induction of diabetes mellitus causes a significant decrease in catecholamine-containing nerve fibres of the rat prostate (Crowe et al., 1987), as well as large reductions in the densities of prostatic α_1- (Crowe et al., 1987) and β-adrenoceptors (Gousse et al., 1991). Streptozotocin-diabetic rats also display decreased densities of prostatic muscarinic receptors (Latifpour et al., 1991), an effect which is prevented or reversed with insulin treatment (Fukumoto et al., 1993).

Protein kinase C has been shown to play a role in α_1-adrenoceptor-mediated contraction of guinea-pig vas deferens (Kamimura et al., 2000). Protein kinase C in the rat ventral prostate is of the Ca^{2+}-dependent form, and is thought to play an important role in the physiological activities and mechanisms of cell proliferation and differentiation in the prostate gland (Garcia-Paramio et al., 1993). Prostate glands from streptozotocin-diabetic rats have also been shown to exhibit an increased protein kinase C activity in the membrane fraction, and a decreased protein kinase C activity in the cytosolic fraction, when compared with control rats (Garcia-Paramio et al., 1995). This effect was found to be restored toward control conditions by insulin treatment (Garcia-Paramio et al., 1993).

Although it is widely accepted that enlargement of the prostate gland is inevitable as men age, the mechanisms involved in the development of benign prostatic hyperplasia are largely unknown. To date, investigations into the diabetes-induced alterations in prostatic receptors and prostate size have only been preliminary. This study investigated whether changes occur in the sensitivity and reactivity to noradrenaline of prostate glands from diabetic rats in addition to changes in size.

2. Materials and methods

2.1. Animals

Male Wistar rats were weighed and lightly anaesthetised (4% halothane, 2:1 O_2/N_2O) to enable measurement of blood glucose levels via a tail vein sample. Blood glucose was measured using an Ames Glucometer II. Diabetes was induced by a single tail vein injection of streptozotocin (60 mg/kg), dissolved immediately prior to use in citrate buffer (50 mM citric acid and 50 mM trisodium citrate; pH 4.5). An equivalent volume of citrate buffer was injected into age-matched control rats. Rats were housed in treatment pairs (one diabetic and one control) for a 6-week period.

A subgroup of rats was treated with a single daily dose of Lente insulin (4 units for the first 3 days, and 6 units every day thereafter, s.c.; Monotard human insulin zinc suspension) commencing 2 days after streptozotocin administration. Ethical approval for all experiments was obtained from the Monash University Pharmacology Animal Ethics Committee.

2.2. Isolated organ bath experiments

After a 6-week period, rats were weighed, killed and blood glucose was measured. A lower abdominal incision was made and prostate glands were dissected out and vertically mounted onto wire tissue holders in 5-ml organ baths. Experiments were paired with one prostate lobe placed in Krebs solution ((mM): NaCl 118.4, KCl 4.7, $NaHCO_3$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.1) and the other placed in Krebs solution containing an antagonist or inhibitor, as indicated. Following a 1-h equilibration period, discrete dose–response curves to noradrenaline (1 nM–100 μM) or methoxamine (10 nM–100 μM) were performed. Agonists were kept in contact with the tissue until the response plateaued (approximately 10–15 s), with a 10-min period between each dose.

In a subset of experiments, isolated prostate preparations were electrically field stimulated. Tissues were mounted onto perspex tissue holders incorporating platinum electrodes connected to a Grass S88 stimulator. Parameters for stimulation applied to tissues were: 10 pulse trains of 0.5-ms duration, at 80 V. The frequencies tested were 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 Hz. Stimulation at frequencies greater than 1 Hz were given as 10-s trains. A resting period of 10 min was allowed between stimulations.

2.3. Histochemical studies

Rats from all three treatment groups (see Section 2.1) were killed and prostate glands removed. Tissues were placed in

Tissue TEK and frozen at −21 °C to be stained for noradrenaline. At least 12 sections (12 μM) were cut from each prostate specimen and thawed onto gelatin-coated slides. Prostates were exposed to sucrose–potassium phosphate–glyoxylic acid solution (SPG) for 3 s (De la torre and Surgeon, 1976). Sections were dried for approximately 20 min and heated at 80 °C for an additional 5 min. Slices were mounted in paraffin oil and examined with an Olympus photomicroscope fitted with an Olympus mercury burner fluorescent light source attachment and DM55 dichroic mirror, BP400-410 exciter filter and BA 455 barrier filter.

For staining with haematoxylin and eosin, tissues were fixed for 2 h in a solution containing 4% paraformaldehyde in phosphate buffered saline (PBS; (mol/l) NaCl 0.137, KH_2PO_4 0.002 and Na_2HPO_4 0.008). Tissues were washed four times, each for 10 min, in a solution containing 7% sucrose and 0.01% sodium azide in PBS. Prostates were stored in this solution for 48 h at 4 °C. Tissues were placed in Tissue TEK, snap frozen in liquid nitrogen and stored at −80 °C. At least 12 sections (12 μM) were cut from each prostate specimen and thawed onto gelatin-coated slides. Sections were stained routinely in Mayer's Haemalum for 10 min, rinsed in distilled water for 2 min, and placed in Scott's solution for a further 2 min. After rinsing in gentle running tap water for 2 min, prostate slices were stained in Eosin for 30 s and carefully rinsed again in tap water for 3 min. Sections were dehydrated once in 70% and 90% ethanol for 4 min, and three times in 100% ethanol for 4 min each. Histoclear was used, three times for 4 min each, to clear sections, and slides were mounted in DPX and coverslipped before viewing under an Olympus BX60 microscope. Micrographs were taken using an Olympus PM 30 photographic system.

2.4. Drugs and solutions

Drugs used included: the muscarinic receptor antagonist, atropine sulfate (Sigma); the protein kinase C inhibitor, bisindolylmaleimide I (Calbiochem); protein kinase C inhibitor, calphostin C (Calbiochem); α_1-adrenoceptor agonist, methoxamine hydrochloride (Wellcome); uptake 1 inhibitor, nisoxetine hydrochloride (Eli Lilly); α-adrenoceptor agonist, noradrenaline bitartrate (Sigma); α-adrenoceptor antagonist, prazosin hydrochloride (Sigma); β-adrenoceptor antagonist, propranolol hydrochloride (ICI).

Atropine, nisoxetine, prazosin, and propranolol were all dissolved in distilled water. Calphostin C and bisindolylmaleimide I were dissolved in 1% dimethyl sulphoxide. Noradrenaline and methoxamine were dissolved in a catecholamine diluent (0.9% NaCl, 0.0156% $NaH_2PO_4 \cdot 2H_2O$, 0.004% ascorbic acid). All subsequent dilutions were made in distilled water.

Table 1
Body weights, blood glucose levels and prostate weights of control ($n=41$), diabetic ($n=36$) and insulin-treated diabetic ($n=14$) rats

	Body weight (g)		Blood glucose (mM)		Prostate weight (mg)
	Initial	Final	Initial	Final	
Control	329 ± 4	451 ± 5[a]	7.1 ± 0.2	7.6 ± 0.2	93.3 ± 5.2
Diabetic	329 ± 3	282 ± 4[a]	7.1 ± 0.2	21.4 ± 0.5[b]	41.8 ± 6.5[c]
Insulin	328 ± 4	390 ± 5[a]	7.2 ± 0.4	6.3 ± 1.5	38.3 ± 3.0[c]

Initial measurements were made at the time of streptozotocin or vehicle injection, and final measurements made 6 weeks later.

[a] Significantly different from initial value for corresponding treatment group, $P<0.01$.

[b] Significantly different from initial value for corresponding treatment group, $P<0.001$.

[c] Significantly different from corresponding values from control group, $P<0.001$.

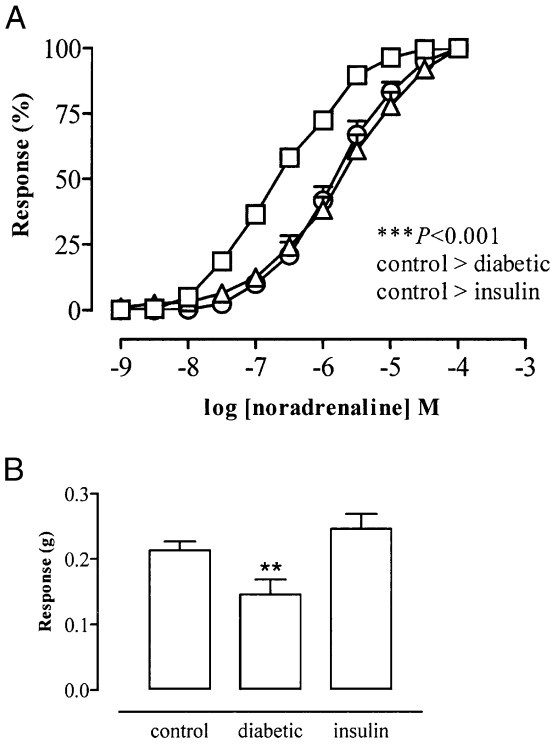

Fig. 1. (A) Discrete dose–response curves of prostates from control (□, $n=41$), diabetic (△, $n=36$) and insulin-treated diabetic (○, $n=14$) rats to noradrenaline. Data are expressed as means ± S.E.M. (B) The maximum response of prostate glands from control ($n=41$), diabetic ($n=36$) and insulin-treated ($n=14$) diabetic rats to noradrenaline. Data are expressed as means ± S.E.M. ** $P<0.01$, when compared with the control group.

2.5. Statistical analysis

The data are expressed as a percentage of the maximum response achieved by each individual tissue, and are presented as means ± S.E.M. Differences in mean concentration–response curves of two or more treatment groups were compared at all concentrations on the log concentration–response curve using a two-way repeated measure analysis of variance (ANOVA). Differences in maximum response, blood glucose levels and body and prostate weights between treatment groups were compared using a one-way ANOVA. Bonferroni correction for multiple comparisons was per-

formed when required. In all cases, $P \leq 0.05$ was taken as statistically significant.

EC_{50} values were determined using Graph Pad Prism (version 2.0), and were used to calculate 95% confidence intervals and the shift of the noradrenaline concentration–response curves in the presence of an antagonist or inhibitor from its corresponding control.

3. Results

3.1. Laboratory animals

The mean body weights of 6-week control (vehicle-treated) and insulin-treated diabetic rats were significantly increased compared to their corresponding mean pre-injection

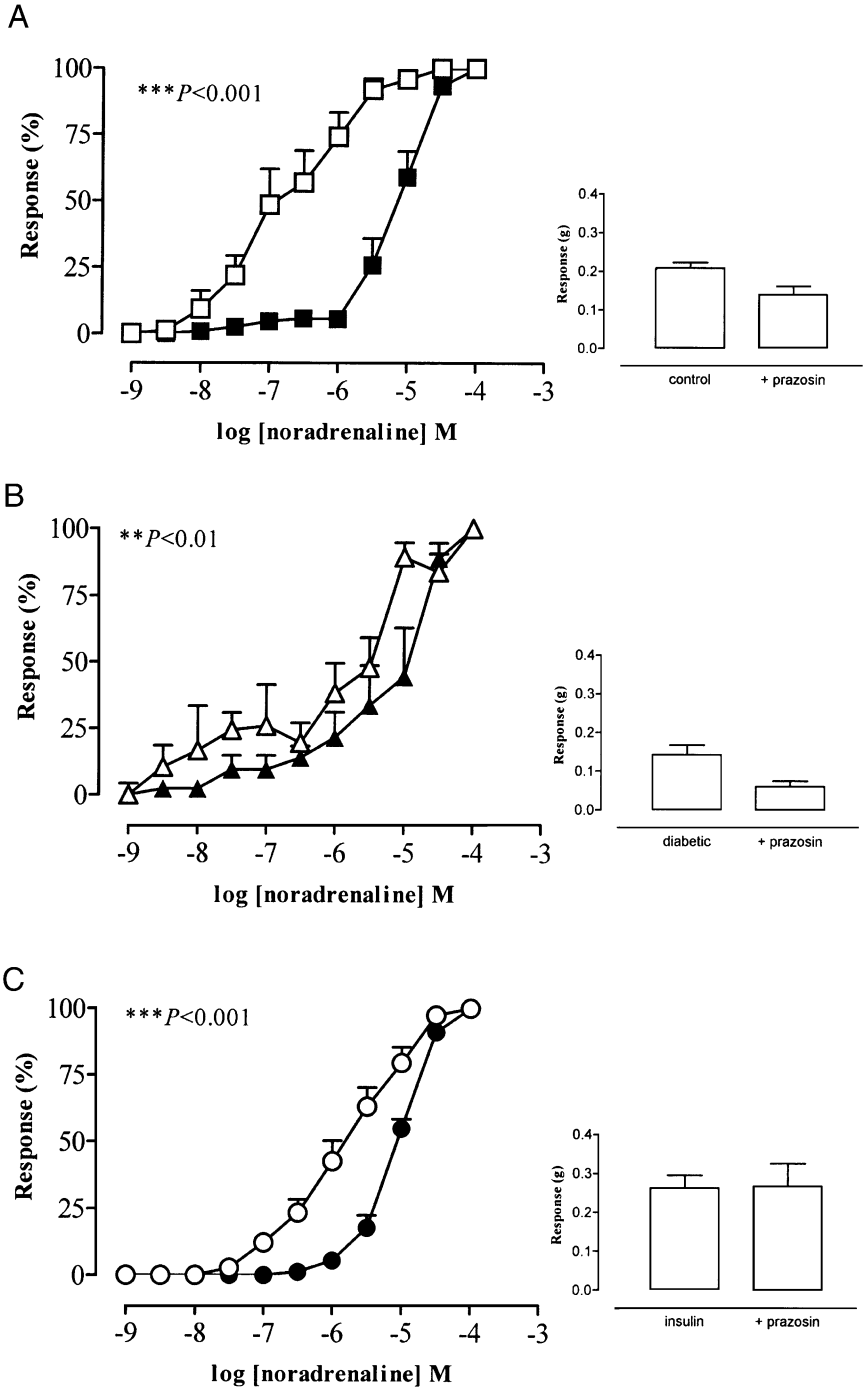

Fig. 2. The effect of prazosin (100 nM) on the noradrenaline concentration–response curve of prostate glands from (A) control (□, $n=7$), (B) diabetic (△, $n=6$) and (C) insulin-treated diabetic (○, $n=6$) rats. Solid symbols represent response in the presence of prazosin. Histogram: maximum response of tissues in the absence and presence of prazosin. Data are expressed as means ± S.E.M. ** $P<0.01$ and *** $P<0.001$, compared with response in the absence of prazosin.

weights ($P<0.01$). In contrast, diabetic rats displayed significantly reduced mean body weights when compared with their mean pre-injection weights ($P<0.01$) (Table 1).

Mean blood glucose levels of control and insulin-treated diabetic rats remained normoglycaemic. However, diabetic rats exhibited significantly increased mean blood glucose levels compared to their corresponding pre-injection levels ($P<0.001$; Table 1).

Mean wet weights of prostate tissue from diabetic and insulin-treated diabetic rats were significantly reduced when compared to prostate glands from age-matched control rats ($P<0.001$) (Table 1).

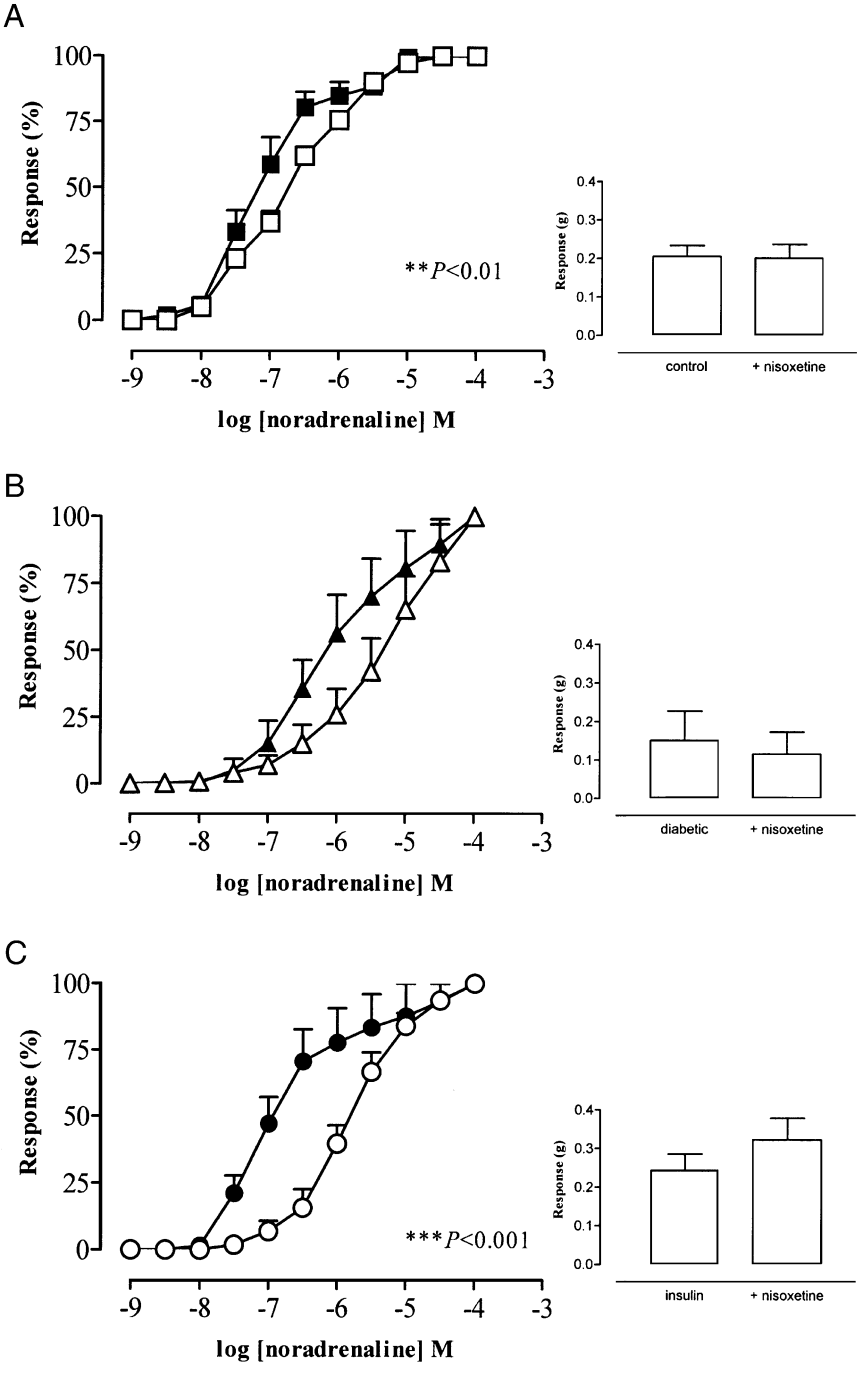

Fig. 3. Effect of nisoxetine (300 nM) on the noradrenaline concentration–response curve of prostate glands from (A) control (□, $n=9$), (B) diabetic (△, $n=7$) and (C) insulin-treated diabetic (○, $n=6$) rats. Solid symbols represent response in the presence of nisoxetine. Histogram: maximum response of tissues in the absence and presence of nisoxetine. Data are expressed as means ± S.E.M. ** $P<0.01$ and *** $P<0.001$, compared with corresponding curve in the absence of nisoxetine.

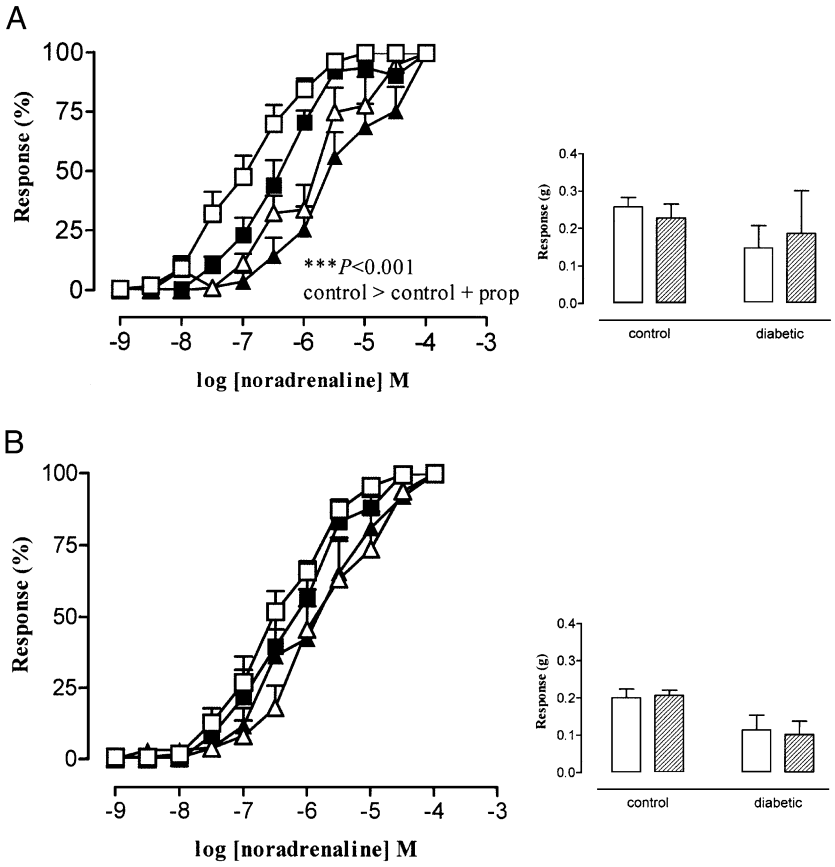

Fig. 4. (A) Effect of propranolol (1 μM) on the noradrenaline concentration–response curve of prostate glands from control (□, $n=6$) and diabetic (△, $n=6$) rats. Solid symbols represent response in the presence of propranolol. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of propranolol. Data are expressed as means ± S.E.M. (B) Effect of atropine (300 nM) on the noradrenaline concentration–response curve of prostate glands from control (□, $n=7$) and diabetic (△, $n=6$) rats. Solid symbols represent response in the presence of atropine. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of atropine. Data are expressed as means ± S.E.M.

3.2. Effects of noradrenaline

Discrete addition of noradrenaline (1 nM–100 μM) produced dose-dependent contractions in prostates from all rats. The mean noradrenaline concentration–response curve obtained in tissues from diabetic and insulin-treated diabetic rats were shifted rightward 8.5-fold (95% confidence limit = 4.1–17.4) and 6.7-fold (95% confidence limit = 3.9–11.5), respectively, compared to the mean noradrenaline concentration–response curve obtained in tissues from con-

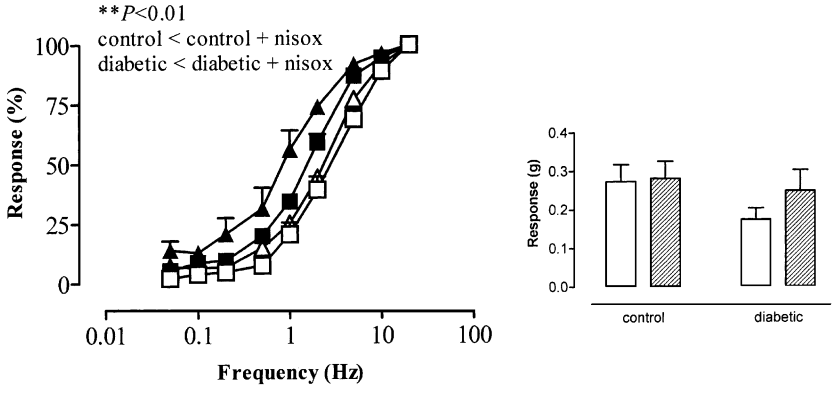

Fig. 5. The effect of nisoxetine (300 nM) on the frequency response curve of electrically field stimulated prostates from control (□, $n=6$) and diabetic (△, $n=5$) rats. Solid symbols represent response in the presence of nisoxetine. EFS parameters: 10 trains of 0.5-ms duration; 80 V; frequency 0.05–20 Hz every 10 min. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of nisoxetine. Data are expressed as means ± S.E.M.

trol rats ($P<0.001$) (Fig. 1A). The noradrenaline concentration–response curve obtained in preparations from insulin-treated diabetic rats did not differ from that seen in tissues from diabetic rats (Fig. 1A). The mean maximum response to noradrenaline in prostate glands from diabetic rats was significantly decreased when compared with the maximum responses observed in tissues from control and insulin-treated diabetic rats ($P<0.01$) (Fig. 1B).

3.3. Effects of the α-adrenoceptor antagonist, prazosin

Prazosin (100 nM) produced significant 60.1-fold (95% confidence limit = 24.4–147.9; $P<0.001$) and 3.8-fold (95% confidence limit = 0.7–19.7; $P<0.01$) rightward shifts of the mean noradrenaline concentration–response curves in tissues from control and diabetic rats, respectively (Fig. 2A and B, respectively). In the presence of prazosin, there was also a 7.7-fold (95% confidence limit = 3.8–15.8) shift of the mean noradrenaline concentration–response curve in prostates from insulin-treated diabetic rats ($P<0.001$) (Fig. 2C). There was no difference between the maximum responses of tissues from control, diabetic or insulin-treated diabetic rats when they were compared with their corresponding groups in the absence of prazosin.

3.4. Effects of the uptake 1 inhibitor, nisoxetine

Nisoxetine (300 nM) produced a significant leftward shift of the mean noradrenaline concentration–response curve in prostates from control (2.8-fold (95% confidence limit = 1.4–6.1); $P<0.01$) (Fig. 3A), but not diabetic (Fig. 3B), rats. Nisoxetine produced a significant 16.4-fold leftward shift of the mean noradrenaline concentration–response curve of prostates from insulin-treated diabetic rats (95% confidence limit = 9.7–27.7; $P<0.001$) (Fig. 3C). Nisoxetine had no effect on the maximum responses obtained for tissues from control, diabetic or insulin-treated diabetic rats.

3.5. Effects of the β-adrenoceptor antagonist, propranolol

Propranolol (1 μM) had no significant effect on the noradrenaline concentration–response curve of prostate glands from diabetic rats ($P>0.05$). However, in the presence of propranolol, there was a small rightward shift of the nor-

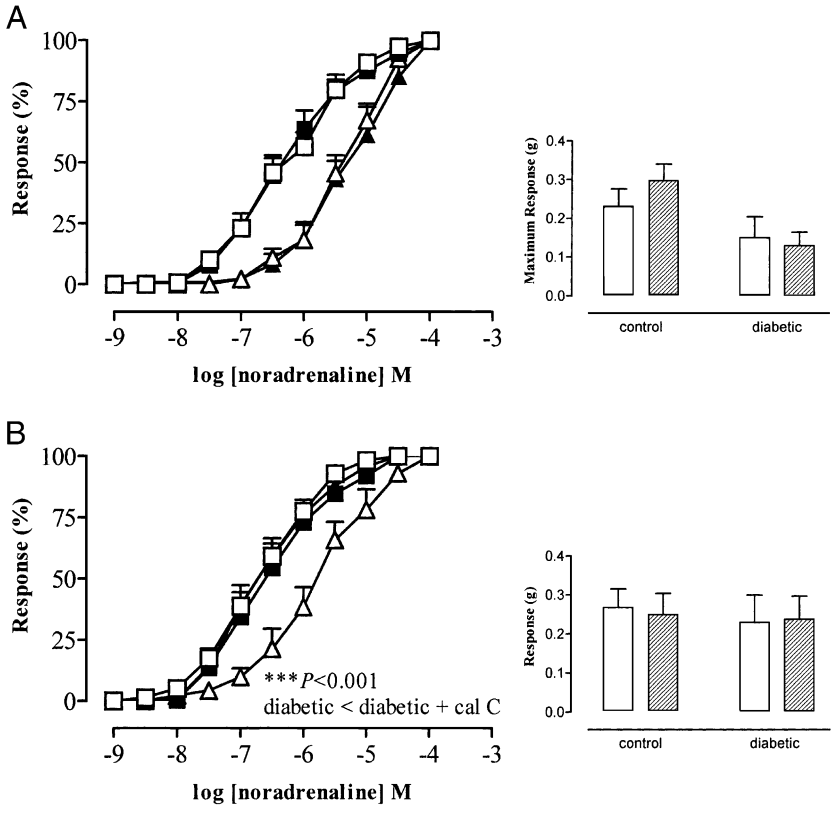

Fig. 6. (A) The effect of bisindolylmaleimide I (500 nM) on the noradrenaline concentration–response curve of prostate glands from control (□, $n=6$) and diabetic (△, $n=6$) rats. Solid symbols represent response in the presence of bisindolylmaleimide I. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of bisindolylmaleimide I. Data are expressed as means ± S.E.M. (B) The effect of calphostin C (500 nM) on the noradrenaline concentration–response curve of prostate glands from control (□, $n=6$) and diabetic (△, $n=6$) rats. Solid symbols represent response in the presence of calphostin C. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of calphostin C. Data are expressed as means ± S.E.M. ** $P<0.001$, when compared with corresponding curve in the absence of calphostin C.

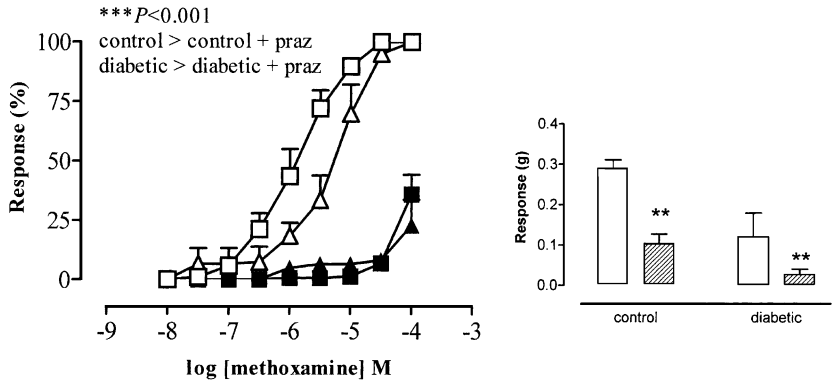

Fig. 7. The effect of prazosin (100 nM) on the methoxamine concentration–response curve of prostate glands from control (□, $n=5$) and diabetic (△, $n=5$) rats. Solid symbols represent response in the presence of prazosin. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of prazosin. Data are expressed as means ± S.E.M.

adrenaline concentration–response curve of prostate glands from control rats ($P<0.001$) (Fig. 4A). Propranolol had no effect on the maximum responses obtained for tissues from control or diabetic rats.

3.6. Effects of the muscarinic receptor antagonist, atropine

Lau et al. (2000) have shown that stimulation of muscarinic receptors of the guinea-pig prostate gland are able to enhance noradrenaline mediated contractions. In the present study, atropine (300 nM) had no significant effect on the noradrenaline concentration–response curves of prostate glands from control or diabetic rats ($P>0.05$) (Fig. 4B). Atropine had no effect on the maximum responses obtained for tissues from control or diabetic rats.

3.7. Effects of electrical field stimulation

In electrical field stimulated preparations, frequency response curves obtained from control and diabetic rat prostate glands did not differ significantly. Nisoxetine (300 nM) caused a significant 3.6-fold (95% confidence limit = 1.7–16.7) and 4.0-fold (95% confidence limit = 1.5–9.8) leftward shift of the mean frequency–response curve in prostates from control and diabetic rats, respectively ($P<0.01$) (Fig. 5). Nisoxetine did not effect the maximum responses of tissues to stimulation at 20 Hz.

3.8. Effects of the protein kinase C inhibitors, bisindolylmaleimide I and calphostin C

Bisindolylmaleimide I (500 nM) did not affect the mean noradrenaline concentration–response curves in prostates from control or diabetic rats (Fig. 6A). Mean maximum responses of control and diabetic tissues were not affected by the addition of bisindolylmaleimide I.

Calphostin C (500 nM) produced a significant 8.6-fold (95% confidence limit = 4.7–15.9) leftward shift of the mean noradrenaline concentration–response curve in tissues

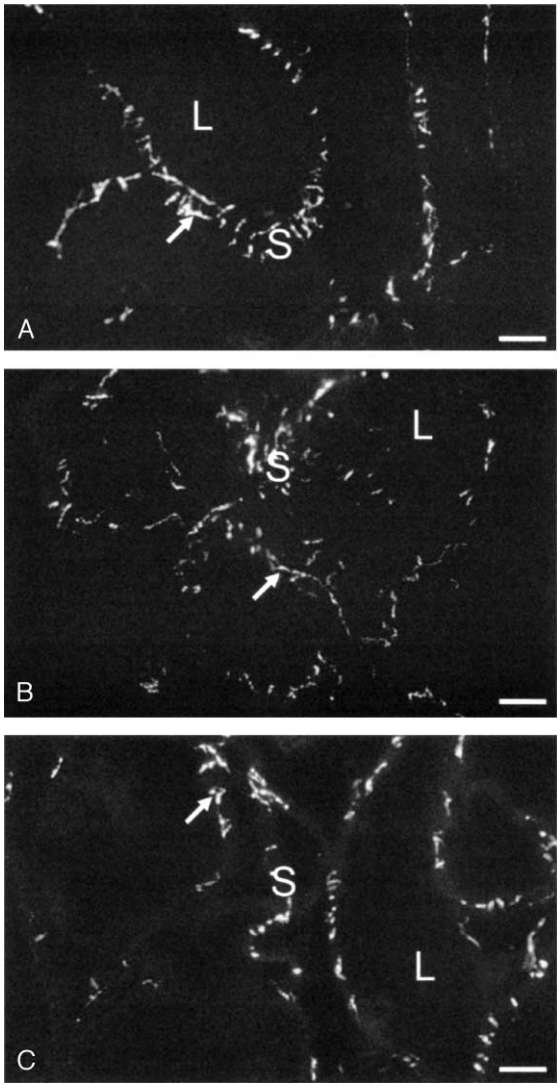

Fig. 8. Photomicrographs showing cross-sections stained for catecholamine fluorescence in prostate glands from (A) control, (B) diabetic and (C) insulin-treated diabetic rats. Arrows indicate fluorescent catecholamine staining neurons.

from diabetic rats ($P<0.001$) (Fig. 6B). Mean maximum responses of control and diabetic tissues to noradrenaline were not affected by the addition of calphostin C.

3.9. Effects of the α_1-agonist, methoxamine

Discrete additions of methoxamine (10 nM–100 μM) produced dose-dependent contractions in prostates from control and diabetic rats. The mean methoxamine concentration–response curve obtained in prostates from diabetic rats was shifted 5.0-fold (95% confidence limit = 3.1–8.1) rightward compared with the mean methoxamine concentration–response curve obtained in tissues from control rats ($P<0.001$) (Fig. 7). Prazosin (100 nM) produced a rightward shift of at least 100-fold in the mean methoxamine concentration–response curves in preparations from both control and diabetic rats ($P<0.001$) (Fig. 7). Responses to methoxamine in both control and diabetic tissues treated with prazosin did not reach maximum.

3.10. Histochemical studies

Catecholamine fluorescence stained dense populations of nerves in the prostatic smooth muscle stroma in between the secretory acini, and was similar in cross sections of prostates taken from control, diabetic and insulin-treated diabetic rats (Fig. 8A–C).

Staining with haematoxylin and eosin displayed similar muscle profiles of prostates taken from 6-week control, diabetic and insulin-treated diabetic rats (results not shown).

4. Discussion

In the present study, streptozotocin-diabetic rats exhibited symptoms similar to those observed in humans with uncontrolled type I, insulin-dependent diabetes mellitus, e.g. hyperglycaemia and weight loss. These observations are consistent with the findings of other studies (Crowe et al., 1987; Gousse et al., 1991; Latifpour et al., 1991; Fukumoto et al., 1993; Nishi et al., 1998).

The mean noradrenaline concentration–response curves of prostate glands from diabetic and insulin-treated diabetic rats were significantly shifted rightward when compared to the curve obtained in tissues from control rats. This effect clearly demonstrates that prostate glands from diabetic rats were less sensitive to noradrenaline when compared to the control, and this sub-sensitivity to noradrenaline was not reversible by chronic insulin treatment. Furthermore, the mean maximum response of prostates from diabetic rats to noradrenaline was significantly reduced when compared with the maximum responses of prostates from control and insulin-treated diabetic rats to noradrenaline. Therefore, diabetes also appears to affect the reactivity of the prostate gland to noradrenaline. In contrast to the diabetes-induced sub-sensitivity, this decrease in reactivity was reversible with insulin. However, it must also be noted that the reversal of reactivity of prostates from diabetic rats to control levels was not associated with a parallel reversal in prostate weights, despite the chronic administration of insulin reversing some of the effects of streptozotocin treatment, i.e. hyperglycaemia and reduced body weight. Crowe et al. (1987) have suggested that the reduction in prostate size may be due to a decrease in the thickness of the smooth muscle of this tissue. However, in our study, the morphology of prostates taken from 6-week control, diabetic and insulin-treated diabetic rats did not display any obvious differences as seen with histological haematoxylin and eosin staining (results not shown). The α_1-adrenoceptors, responsible for the contraction of the prostate (Garcia-Paramio et al., 1995; Nishi et al., 1998), and the increase in sympathetic tone seen in benign prostatic hyperplasia (Cooper et al., 1999), are predominantly found in the muscle stroma. Therefore, any alterations in prostatic morphology may not only be related to changes in the size of this gland, but may also affect its ability to contract in response to the addition of exogenous noradrenaline.

In contrast to Crowe et al. (1987), no obvious differences in catecholamine fluorescence were seen between prostates from control, diabetic or insulin-treated diabetic rats. This variance may be due to the fact that the present study used a 6-week streptozotocin-diabetic model whilst that of Crowe et al. (1987) employed an 8-week streptozotocin-diabetic model. This result is consistent with the other findings of the present study. If the diabetic prostate had displayed a decreased catecholamine fluorescence, then we would expect to see an increased sensitivity to noradrenaline due to the loss of uptake 1, a decreased sensitivity of the diabetic prostate gland to electrical field stimulation, and an equal sensitivity to methoxamine of the diabetic prostate. An increase in the innervation of prostatic smooth muscle could explain the sub-sensitivity of the prostate gland seen in this study, but such an increase in innervation was not observed.

Methoxamine is an α-adrenoceptor agonist with a greater selectivity for the α_1-subtype and decreased susceptibility to neuronal uptake, compared to noradrenaline (Wikberg, 1978). Prostate glands from diabetic rats were less sensitive to methoxamine than prostates from control rats. This result further supports the notion that subsensitivity is not due to changes in neuronal uptake.

Haynes and Hill (1997) have shown that β-adrenoceptors are involved in the contraction of prostatic smooth muscle in the guinea pig. In addition, previous radioligand receptor binding studies have shown that streptozotocin-diabetic rat prostates exhibit a 45–50% reduction in β-adrenoceptor density (Fukumoto et al., 1993). Similarly, prostate glands from streptozotocin-diabetic rats exhibit decreased densities of muscarinic-receptors of up to 30% (Latifpour et al., 1991; Fukumoto et al., 1993). Previous studies have also shown that cholinergic drugs are able to modify noradrenergic contraction in the isolated guinea-pig prostate gland (Lau et al.,

2000). Such down-regulation in receptors could lead to changes in sympathetic tone. However, in the present study, propranolol and atropine did not shift the noradrenaline concentration–response curve of the diabetic prostate gland towards that of the control, suggesting that changes in β-adrenoceptors or muscarinic receptors play no role in the diabetes-induced changes in the sensitivity of the rat prostate glands to noradrenaline.

Many of the physiological activities and mechanisms of cell proliferation and differentiation in the prostate gland involve protein kinase C activity, which is known to exist in both Ca^{2+}-dependent and Ca^{2+}-independent forms. In addition to playing a role in the noradrenaline-induced contraction of smooth muscle (Kamimura et al., 2000), protein kinase C is involved in the signal transduction of neurotransmitters, hormones and growth factors that control cell function and proliferation (Garcia-Paramio et al., 1993). In the present study, the protein kinase C inhibitor, bisindolylmaleimide I (500 nM), had no effect on the noradrenaline concentration–response curves of prostates from either control or diabetic rats. In contrast, another protein kinase C inhibitor, calphostin C (500 nM), produced a leftward shift of the noradrenaline concentration–response curve of prostates from diabetic rats, resulting in its return to a position similar to that seen in prostates from control rats. These findings strongly suggest that the sub-sensitivity of the diabetic prostate gland to noradrenaline is due to an alteration in the activity of a protein kinase C isoform other than those blocked by bisindolylmaleimide I. One reason why calphostin C, and not bisindolylmaleimide I, has an effect on the noradrenaline concentration–response curve of prostates from diabetic rats may be explained by their differing modes of action. Bisindolylmaleimide I has a competitive inhibitory action at the ATP binding site of protein kinase C and shows high selectivity for protein kinase Cα, β_I, β_{II}, γ, δ and ε isozymes (Toullec et al., 1991; Gekeler et al., 1996). Alternatively, calphostin C exerts its inhibitory effect by competing at the binding site of diacylglycerol and phorbol esters, thereby inhibiting the activation of protein kinase C (Kobayashi et al., 1989; Bruns et al., 1991; Gopalakrishna et al., 1992). The concentrations of bisindolylmaleimide I and calphostin C used in the present study have been shown to be selective despite their differing modes of action (Bruns et al., 1991; Gopalakrishna et al., 1992; Kobayashi et al., 1989; Tamaoki et al., 1990; Toullec et al., 1991).

The findings of the present study suggest that the induction of diabetes by streptozotocin results in a reduction in the sensitivity and reactivity of the rat isolated prostate gland to noradrenaline. Interestingly, despite the diabetes-induced decreases in rat body and prostate weight, these reductions do not appear to be due to alterations in the morphology of the gland. Our results suggest that these changes may involve diabetes-induced changes in protein kinase C activity. Similar effects have been seen in other sympathetically innervated tissues following the induction of diabetes (James and Hodgson, 1997).

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