Catecholamines and Neuropeptide Y in the Prostate Gland Of the Streptozotocin-treated Diabetic Rat.

John F.B. Morrison [1*], S.Dhanasekaran [2] and E.P.K. Mensah-Brown[3]

Departments of Physiology and Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University, PO Box 17666, Al Ain, United Arab Emirates*

Abstract: The concentrations of noradrenaline (NA), adrenaline (ADR), dopamine (DOP), serotonin (5-HT), and the distribution and intensity of staining of nerves containing tyrosine hydroxylase (TH) and neuropeptide Y (NPY) have been studied in the prostate gland of control and streptozotocin (STZ) -diabetic rats. The weight of the prostate of diabetic animals was uniformly less than that of age-matched controls. The immunohistochemical study of the axons in the prostate showed an increase in the density of TH and NPY axons after 12 weeks of STZ diabetes. It is suggested that this is an indication of the presence of diabetic autonomic neuropathy, and that it may correspond to the retraction and regrowth of sympathetic nerve terminals. We conclude that diabetic autonomic neuropathy can affect the prostate gland, and the presence of increased levels of amines and peptides may be of interest in relation to the pathogenesis of benign prostatic hyperplasia and the spread of prostatic carcinoma.

Keywords: Male Reproductive Dysfunction, Prostate, Noradrenaline, NPY

Introduction

The primary objective of these experiments was to study the late changes in sympathetic nerves innervating the prostate gland following prolonged periods of hyperglycaemia in STZ-diabetic rats. A previous study after 8 weeks of diabetes has shown significant changes in the weight of the prostate gland and of levels of noradrenaline and NPY[1]. This paper describes changes in noradrenaline, adrenaline, dopamine and serotonin concentration in the prostate of the streptozotocin (STZ) -diabetic rat over a time-course of 10 months of hyperglycaemia; the results are significant and may be relevant to the development of diabetic autonomic neuropathy in this organ, and the role of autonomic nerves in the normal and diseased prostate. There is a positive relationship between diabetes and benign prostatic hyperplasia [2-5], and the catecholamines as well as neuropeptide Y (NPY), found in autonomic nerves in this tissue, may influence prostatic tone or urethral pressure [6-7]. angiogenesis [8-11] and the development or spread of prostatic carcinoma [12-14]. The prostate gland of humans and animals receive a substantial sympathetic innervation [15] in addition to afferents and some parasympathetic efferents [16]. The sympathetic efferents travel through three main pathways involving neurones in the sympathetic chain, inferior mesenteric ganglia and the pelvic ganglia [16]. The sympathetic nerves are known to contract prostatic smooth muscle using alpha-1 adrenergic receptors [17-19]. The central control of these structures is mediated by spinal and supraspinal pathways similar to and

overlapping with those that regulate the activity of other pelvic viscera and male reproductive organs [20-21], but when looked at in detail, it is clear that the pathways are distinct and separate [22].

The integrity of the peripheral sympathetic innervation of the prostate gland is believed to be maintained by the presence of nerve growth factor (NGF) in the organ [23], and there is a relationship between the level of beta-NGF mRNA and the density of the sympathetic innervation [24-25]. In STZ-diabetes, the concentration of NGF in the prostate gland are decreased and it was of interest to see whether the level of noradrenaline and the density of NPY- and TH-containing axonal terminals [26], change as they do in some other tissues of diabetic animals [27-28]. It is believed that some of the late changes are associated with a neuropathy in which remodelling (nerve retraction and regrowth) may be present, and this may influence the efficacy of neurotransmission.

Materials and Methods

*Measurement of amines and glucose :*Experiments were performed on adult male Wistar rats which had been injected with STZ (60 mg/Kg) at ten weeks of age. The tissues of these animals were compared with those from age-matched controls. The prostate gland was removed up to 42 weeks after the administration of STZ under pentobarbitone anaesthesia (60 mg/Kg) and blood glucose was estimated on samples of inferior vena caval blood at that time using the glucose oxidase method. Measurements of catecholamines were performed as described previously [27, 29-30]. All experiments were performed within the guidelines of the Animal Ethics Committee of the Faculty of Medicine and Health Sciences, United Arab Emirates University.

Immunohistochemistry : Six rats that had had STZ-diabetes for 12 weeks and 6 age-matched control rats were anaesthetised with ether and perfused with 4% paraldehyde. The prostate gland was removed sectioned and immunostained for TH and NPY as described previously [29-30].

Results

Blood Glucose: The control blood glucose concentrations were in the range 53 to 70 mg/dl (Mean 62 mg/dl) and the diabetic levels were 189 to 600 mg/dl (Mean 302 mg/dl). Age had no effect on blood glucose in the control group, but in the diabetic group there was a negative correlation between blood glucose and duration of diabetes that was statistically significant [30].

Effects of Age on amine concentrations in the prostate gland of control animals : We have previously reported that in some tissues the concentrations of noradrenaline and other amines changed with age. In the present experiments noradrenaline concentration in the prostate fell slightly and significantly with age (P=0.036), as in the penis [30], but there were no significant trends with age for any of the other amines. 5-HIAA was not detected in any sample of the prostate gland, whether from control or from diabetic rats.

EFFECTS of STZ-DIABETES

Tissue Weight. : The weight of the diabetic prostate became significantly less than that of the control animals, soon after the animals became diabetic (Fig. 1), which, in these experiments, was at the age of 10 weeks. The weight of the prostate gland of

diabetic rats also remained significantly less than the controls throughout the period of the study,

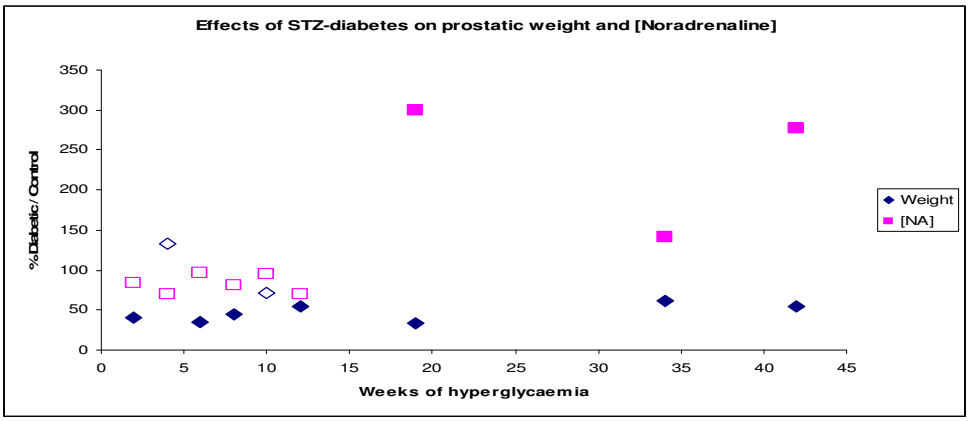

Figure 1. The graph shows the changes in the weight and noradrenaline concentration in the prostate gland as a percentage of control values during the time course of STZ-diabetes. Filled symbols indicate a statistical significance of $p<0.05$.

Amine concentrations: Figure 1 also shows that the concentration of noradrenaline in the prostate gland is not statistically different from control tissues during the first 12 weeks of hyperglycaemia, but increases markedly by 19 weeks and remains significantly higher than the controls, despite the fact that there was no further change in the weight of the prostate. The raised noradrenaline levels at this late stage of the diabetes are therefore not due to a sudden atrophy of the gland. Other amines also showed significant changes compared with the control tissues of the same age (see Table 1). During the period 19-42 weeks following the onset of hyperglycaemia, dopamine and serotonin concentrations increased significantly, while adrenaline concentrations decreased significantly.

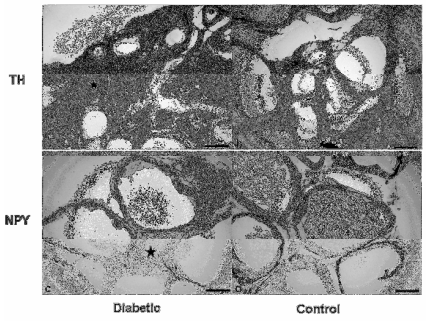

Figure 2.: Light micrograph of prostate gland of STZ-treated diabetic (a and c), and age-matched normal (b, d) rats immunostained with antisera to tyrosine hydroxylase (TH) (a, b) and neuropeptide Y (NPY) (c, d). Note the greater density of nerve fibres and varicose terminals immunoreactive to both TH and NPY especially in the expanded interstitium (star) in the diabetic prostate (a, c) compared to that of normal (b, d) rats. Bar =100 µm

Immunohistochemical observations on tyrosine hydroxylase ands NPY and in the prostate gland: Immunohistochemistry revealed a dense layer of TH -immunoreactive thick and fine nerve fibres as well as varicose terminals around glandular elements of the prostate of both diabetic and normal rats. This distribution was more extensive and denser in the 12-week STZ-diabetic rats. In addition, there appeared to be an increase in the interstitial tissue space of the diabetic gland with a dense layer of immunoreactive TH fibres. This was absent in the control gland (Figure 2 a, b). NPY-immunoreactive fibres also appeared to be

more dense and extensive in the diabetic than in the control rats (Figure 2 c, d)

	Duration of Diabetes		
	19 weeks	34 weeks	42 weeks
	Noradrenaline pg/mg		
Control	511 ± 25.2 (4)	504 ± 11.5 (5)	449 ± 36.5 (4)
Diabetic	1535 ± 48.1 (4)	713 ± 35.7 (5)	1243 ± 164.9 (6)
P	5.66E-05	0.027	0.0053
% Diabetic/Control	300	141	277
	Adrenaline pg/mg		
Control	529 ± 42.4 (4)	498 ± 14.6 (5)	247 ± 35.4 (4)
Diabetic	74 ± 3.0 (4)	71 ± 5.1 (5)	66 ± 5.8 (6)
P	0.005	2.07E-05	0.015
% Diabetic/Control	14	14	27
	Dopamine pg/mg		
Control	465 ± 42.1 (4)	403 ± 12.5 (5)	315 ± 19.4 (4)
Diabetic	1416 ± 169.0 (4)	603 ± 40.3 (5)	541 ± 21.8 (6)
P	0.035	0.046	0.00005
% Diabetic/Control	305	150	172
	Serotonin pg/mg		
Control	280 ± 56.1 (4)	337 ± 33.6 (5)	313 ± 34.7 (4)
Diabetic	825 ± 154.1 (4)	518 ± 38.6 (5)	654 ± 40.0 (6)
P	0.03	0.011	0.0002
% Diabetic/Control	295	154	209

Table 1. Concentrations of amines in the prostate of control and diabetic rats. Data in pg/mg wet weight (mean± SEM, Number of animals); P= statistical significance.
The table shows that in the prostate gland, [NA] increases markedly and to highly significant levels statistically, particularly after 20 weeks of hyperglycaemia. [ADR] falls and the changes are consistently statistically significant. [SER] and [DOP] both increase and the P values are significant.

Discussion

The present experiments indicate that STZ- diabetes has a profound effect on the weight of the rat prostate, and that there are late changes in the levels of increases in noradrenaline and NPY that may reflect neuronal retraction and remodelling as has been suggested in other urogenital organs [29-30]. It has been suggested that the reduction in organ weight may be related to changes in the secretion of gonadotrophins or gonadal hormones [31]; gonadal hormones are known to influence the development of noradrenergic and other autonomic nerves in the prostate [26, 32-33]. The development and remodelling of these nerves appears to depend on nerve growth factor (NGF) [24], and the level of NGF in the prostate is known to decrease in STZ-diabetes [25, 34]. It is clear therefore that the neuropathic changes observed in this paper may be influenced by a number of different mechanisms, each of which may in turn be relevant to the development of prostatic tone, growth and angiogenesis. The neuropathic changes, together with the altered release of transmitters and neurotrophins could possibly influence the development of the prostate, the development of prostatic and urethral tone, the hyperplasia of the gland itself and the spread of prostatic carcinoma [35-36]; however the effects of diabetes on the incidence on prostatic carcinoma are conflicting [37-39]. Human and experimental diabetes is accompanied by changes in the autonomic innervation [40] of many organs [41-42], and the changes that have been observed in both include the presence of dilated axons, engorged nerve endings and other changes that are often described as degenerative in nature [43]. However there remains the possibility that the sympathetic nerves respond to axonal retraction by remodelling and regrowth, and some authors suggest that regeneration of unmyelinated axons occurs in diabetes [44]. It may be that the fine distribution of the innervation does not return to normal, but a change in the number and size of axons may be accompanied by a different distribution of terminals[45], which may have implications for the function of the target organs. Such changes have been described recently in various organs subjected to STZ-induced prolonged hyperglycaemia [28, 29, 46]. In humans, the prostate gland receives a rich sympathetic innervation [15] which contains noradrenaline [7] and NPY [47]; in the cat and dog, these autonomic neurons arise from the inferior mesenteric ganglia, sympathetic chain ganglia, and pelvic plexus ganglia [16, 48]. Dopamine beta-hydroxylase was found in most of the neurons of the sympathetic chain and inferior mesenteric ganglion, mainly the former, but only in about 65-75% of pelvic ganglia neurons [16, 48]. In the mouse virtually all non-cholinergic neurons in the pelvic ganglion are noradrenergic and contain NPY, and most innervate the smooth muscle of reproductive organs including the prostate gland [33]; in the monkey they also innervate the acini [49]. In the rat however, there is evidence that an additional non-adrenergic (probably cholinergic) sympathetic supply targets the glandular tissue and enhances secretory outflow which is normally stimulated by the hypogastric sympathetic nerves [50-51]. Stimulation of the sympathetic nerves to the prostate gland causes its smooth muscle to contract [18-19] and the sympathetic tone to the gland is said to be the source of approximately half the resting urethral tone [6] in cases of human benign prostatic

hypertrophy . The results presented here indicate that the concentration of noradrenaline increases in the prostate gland during the course of prolonged STZ-induced hyperglycaemia. This is in keeping with observations on other tissues, including the heart, tail artery and penis [27-29, 46, 52], and the increased level of noradrenaline is often associated with an increased presence of TH- and NPY-containing nerve terminals. TH is present in nerve fibres that are generally larger than normal and may have bulbous nerve endings; their density is also greater than in the controls (see Figure 2). It is known that diabetes can reduce the levels of gonadotrophins [31] and therefore of testosterone, which influences the weight of reproductive organs and function of some autonomic neurones. Certain neurones in the pelvic ganglia are sensitive to testosterone [26, 32-33] and show morphological changes associated with the presence of this hormone. It seems likely that some of the large rise in noradrenaline concentration observed in these tissues could be related to the failure of the organs to grow at the same rate as the controls. However, there was still a substantial and statistically significant increase in the concentration of noradrenaline after corrections for the weight of the organs (of around 250%). We cannot comment on the direct effects of changes in testosterone levels on the parameters measured in these experiments, but we do acknowledge a probable role for this mechanism in some of the neurones.

Conclusions

Changes in the concentration of catecholamines and the distribution of TH- and NPY- containing axons occur in the prostate gland of STZ-diabetic rats. These changes are of interest because of the incidence of diabetes and prostatic disease in the general population, and evidence that amines and NPY have an influence on influence prostatic tone, urethral pressure, angiogenesis and the development or spread of prostatic carcinoma.

Acknowledgements.

We would like to thank the Faculty of Medicine and Health Sciences and the Sheikh Hamdan Medical Research Foundation for their support of this study.

Reference

1. Crowe R, Milner P, Lincoln J, Burnstock G (1987) Histochemical and biochemical investigation of adrenergic, cholinergic and peptidergic innervation of the rat ventral prostate 8 weeks after streptozotocin-induced diabetes. J Auton Nerv Syst 20: 103-112
2. Joseph MA, Harlow SD, Wei JT, et al. (2003) Risk factors for lower urinary tract symptoms in a population-based sample of African-American men. Am J Epidemiol 157: 906-914
3. Berger AP, Bartsch G, Deibl M, et al. (2006) Atherosclerosis as a risk factor for benign prostatic hyperplasia. BJU Int 98: 1038-1042
4. Berger AP, Deibl M, Halpern EJ, et al. (2005) Vascular damage induced by type 2 diabetes mellitus as a risk factor for benign prostatic hyperplasia. Diabetologia 48: 784-789
5. Berger AP, Deibl M, Leonhartsberger N, et al. (2005) Vascular damage as a risk factor for benign prostatic hyperplasia and erectile dysfunction. BJU Int 96: 1073-1078
6. Furuya S, Kumamoto Y, Yokoyama E, Tsukamoto T, Izumi T, Abiko Y (1982) Alpha-adrenergic activity and urethral pressure in prostatic zone in benign prostatic hypertrophy. J Urol 128: 836-839
7. Ishigooka M, Hashimoto T, Suzuki Y, et al. (1997) Functional property, norepinephrine content and morphometric findings in human hyperplastic prostate. Prostate 33: 183-187

8. Lee EW, Grant DS, Movafagh S, Zukowska Z (2003) Impaired angiogenesis in neuropeptide Y (NPY)-Y2 receptor knockout mice. Peptides 24: 99-106
9. Kitlinska J, Lee EW, Movafagh S, Pons J, Zukowska Z (2002) Neuropeptide Y-induced angiogenesis in aging. Peptides 23: 71-77
10. Ekstrand AJ, Cao R, Bjorndahl M, et al. (2003) Deletion of neuropeptide Y (NPY) 2 receptor in mice results in blockage of NPY-induced angiogenesis and delayed wound healing. Proc Natl Acad Sci U S A 100: 6033-6038
11. Kitlinska J (2007) Neuropeptide Y in neural crest-derived tumors: effect on growth and vascularization. Cancer Lett 245: 293-302
12. Magni P, Motta M (2001) Expression of neuropeptide Y receptors in human prostate cancer cells. AnnOncol 12 Suppl 2: S27-S29
13. Ruscica M, Dozio E, Boghossian S, et al. (2006) Activation of the Y1 receptor by neuropeptide Y regulates the growth of prostate cancer cells. Endocrinology 147: 1466-1473
14. Ruscica M, Dozio E, Motta M, Magni P (2007) Role of neuropeptide Y and its receptors in the progression of endocrine-related cancer. Peptides 28: 426-434
15. Gosling JA (1986) The distribution of noradrenergic nerves in the human lower urinary tract. ClinSci(Lond) 70 Suppl 14: 3s-6s
16. Danuser H, Springer JP, Katofiasc MA, Thor KB (1997) Extrinsic innervation of the cat prostate gland: a combined tracing and immunohistochemical study. J Urol 157: 1018-1024
17. deGroat WC, Booth AM (1980) Physiology of male sexual function. AnnInternMed 92: 329-331
18. Ohkawa H (1983) Sympathetic neuromuscular transmission in the smooth muscle of guinea-pig prostate gland. IntJ Fertil 28: 68-77
19. Yonese J, Kihara K, Sato K, Fukuda H, Kamata S, Oshima H (2000) Sympathetic efferent pathways projecting to the prostate in the dog. Prostate 44: 225-232
20. Marson L, rd CC (1999) Central Nervous System Innervation of the Penis, Prostate, and Perineal Muscles: A Transneuronal Tracing Study. MolUrol 3: 43-50
21. Orr R, Marson L (1998) Identification of CNS neurons innervating the rat prostate: a transneuronal tracing study using pseudorabies virus. J AutonNervSyst 72: 4-15
22. Nadelhaft I, Miranda-Sousa AJ, Vera PL (2002) Separate urinary bladder and prostate neurons in the central nervous system of the rat: simultaneous labeling with two immunohistochemically distinguishable pseudorabies viruses. BMCNeurosci 3: 8
23. Paul A, Habib F (1998) Low-affinity nerve growth factor receptors (p75LNGFR) in human prostate tissue: stromal localisation. UrolRes 26: 111-116
24. MacGrogan D, Despres G, Romand R, Dicou E (1991) Expression of the beta-nerve growth factor gene in male sex organs of the mouse, rat, and guinea pig. J Neurosci Res 28: 567-573
25. Hellweg R, Hartung HD (1990) Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: a possible role for NGF in the pathogenesis of diabetic neuropathy. J Neurosci Res 26: 258-267
26. Kepper M, Keast J (1995) Immunohistochemical properties and spinal connections of pelvic autonomic neurons that innervate the rat prostate gland. Cell Tissue Res 281: 533-542
27. Morrison JFB, Howarth FC, Sheen R (2001) Catecholamines in the Heart and Adrenal gland of Streptozotocin-treated (STZ) Diabetic Rats. ArchPhysiol Biochem 109: 206-208
28. Morrison JF, Dhanasekaran S, Sheen R (2004) Effects of age and streptozotocin-induced diabetes on biogenic amines in rat tail artery. MolCell Biochem 261: 77-82
29. Morrison JFB, Dhanasekaran S, Sheen R, Frampton CM, Mensah-Brown E (2006) The effect of streptozotocin-induced diabetes on the rat seminal vesicle: a possible pathophysiological basis for disorders of ejaculation. . AnnNYAcadSci 1084: 267-279
30. Morrison JFB, Sheen R, Dhanasekaran S, Mensah-Brown EPK (2007) Long term changes in sympathetic innervation in the corpus cavernosum of the STZ-diabetic rat. IntJImpotRes 19: 509-516
31. Dong Q, Lazarus RM, Wong LS, Vellios M, Handelsman DJ (1991) Pulsatile LH secretion in streptozotocin-induced diabetes in the rat. J Endocrinol 131: 49-55

32. Keast JR, Saunders RJ (1998) Testosterone has potent, selective effects on the morphology of pelvic autonomic neurons which control the bladder, lower bowel and internal reproductive organs of the male rat. Neuroscience 85: 543-556
33. Wanigasekara Y, Kepper ME, Keast JR (2003) Immunohistochemical characterisation of pelvic autonomic ganglia in male mice. Cell Tissue Res 311: 175-185
34. Hellweg R, Wohrle M, Hartung HD, Stracke H, Hock C, Federlin K (1991) Diabetes mellitus-associated decrease in nerve growth factor levels is reversed by allogeneic pancreatic islet transplantation. NeurosciLett 125: 1-4
35. De Jong M, Kwekkeboom D, Valkema R, Krenning EP (2003) Radiolabelled peptides for tumour therapy: current status and future directions. Plenary lecture at the EANM 2002. EurJ NuclMedMolImaging 30: 463-469
36. Krenning EP, Kwekkeboom DJ, Valkema R, Pauwels S, Kvols LK, De Jong M (2004) Peptide receptor radionuclide therapy. AnnNYAcadSci 1014: 234-245
37. Gong Z, Neuhouser ML, Goodman PJ, et al. (2006) Obesity, diabetes, and risk of prostate cancer: results from the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev 15: 1977-1983
38. Velicer CM, Dublin S, White E (2007) Diabetes and the risk of prostate cancer: the role of diabetes treatment and complications. Prostate Cancer ProstaticDis 10: 46-51
39. Schiel R, Beltschikow W, Steiner T, Stein G (2006) Diabetes, insulin, and risk of cancer. Methods Find Exp Clin Pharmacol 28: 169-175
40. Schmidt RE, Plurad SB, Parvin CA, Roth KA (1993) Effect of diabetes and aging on human sympathetic autonomic ganglia. AmJ Pathol 143: 143-153
41. Schroer JA, Plurad SB, Schmidt RE (1992) Fine structure of presynaptic axonal terminals in sympathetic autonomic ganglia of aging and diabetic human subjects. Synapse 12: 1-13
42. Schmidt RE (2002) Neuropathology and pathogenesis of diabetic autonomic neuropathy. Int Rev Neurobiol 50: 257-292
43. Tomlinson DR, Yusof AP (1983) Autonomic neuropathy in the alloxan-diabetic rat. J AutonPharmacol 3: 257-263
44. Britland ST, Young RJ, Sharma AK, Clarke BF (1992) Acute and remitting painful diabetic polyneuropathy: a comparison of peripheral nerve fibre pathology. Pain 48: 361-370
45. Beggs J, Johnson PC, Olafsen A, Watkins CJ (1992) Innervation of the vasa nervorum: changes in human diabetics. J NeuropatholExpNeurol 51: 612-629
46. Morrison JF, Pallot DJ, Sheen R, Dhanasekaran S, Mensah-Brown EP (2007) The effects of age and streptozotocin diabetes on the sympathetic innervation in the rat penis. MolCell Biochem 295: 53-58
47. Lange W, Unger J (1990) Peptidergic innervation within the prostate gland and seminal vesicle. UrolRes 18: 337-340
48. Zacharko A, Arciszewski MB, Wasowicz K (2004) Origin of the primary efferent neurons projecting to the prostate of the dog. AnnAnat 186: 349-356
49. Yokoyama R, Inokuchi T, Satoh H, Kusaba T, Yamamoto K, Ando K (1990) Distribution of tyrosine hydroxylase (TH)-like, neuropeptide Y (NPY)-like immunoreactive and acetylcholinesterase (AChE)-positive nerve fibers in the prostate gland of the monkey (Macacus fuscatus). Kurume MedJ 37: 1-8
50. Eckard C (1896) Untersuchungen □ber die erction des penis beim hunde. Beite Anat Physiol 3: 123-150
51. Farrell JI, Lyman Y (1936) A study of the secretory nerves of, and the action of certain drugs on the prostate gland. Am J Physiol 118: 64-70
52. Mensah-Brown E, Adem A, Conlon JM, Morrison JFB (2005) Changes in Neuropeptide Y (NPY) - nerves and in NPY binding in the seminal vesicles and prostate gland of the streptozotocin-treated diabetic rat. Emirates Medical Journal: 221-228

*All correspondences to: Professor John Morrison, Department of Physiology, Faculty of Medicine and Health Sciences, United Arab Emirates University, PO Box 17666 Al Ain UAE. Email:jfbmorrison@gmail.com