Br. J. Pharmac. (1984), **82**, 025–034

Post-natal development of functional neurotransmission in rat vas deferens

A. MacDonald & J.C. McGrath*

Department of Biological Sciences, Glasgow College of Technology, Cowcaddens Road, Glasgow G4 0BA and Institute of Physiology*, University of Glasgow, Glasgow G12 8QQ

1 Responses of the rat vas deferens to drugs and to field stimulation were examined in sexually immature rats.

2 The vasa from immature rats often exhibited spontaneous contractions and displayed greater sensitivity to the contractile effects of α-adrenoceptor agonists.

3 The responses of the vasa from immature rats to single pulse field stimulation lacked the adrenergic component of the response although the non-adrenergic component was present. The responses were antagonized by α_2-adrenoceptor agonists. In the presence of cocaine, an adrenergic component of the response did appear.

4 During trains of pulses the pre- and postjunctional effects of adrenergic transmission which are found in adult rats were absent in vasa from immature rats.

5 Electron microscopic studies showed no qualitative differences in adrenergic innervation in vasa from immature and adult rats.

6 It is concluded that a state of 'pre-innervation supersensitivity' associated with a lack of functional adrenergic transmission exists in the vas deferens of immature rats. The supersensitivity disappears and functional transmission develops during the period in which testosterone secretion increases in the rat. The reason for the lack of functional transmission at a time when the innervation appears to be morphologically mature is not clear but may be due to the noradrenaline release mechanism not being fully operative.

Introduction

Development of the autonomic nervous system is affected by various hormones although their role is not well understood (Burnstock & Costa, 1975; Gibson, 1981). Previous work showed that testosterone affected adrenergic neurotransmission in the rat vas deferens (MacDonald & McGrath, 1980a) and the present study was undertaken to investigate neurotransmission in the vas deferens from sexually immature rats before and during the development of testosterone secretion which increases at 5–6 weeks of age (Knorr *et al.*, 1970).

In the adult rat, the response of the vas deferens to single pulse field stimulation consists of two phases: an initial non-adrenergic response and a secondary adrenergic response (McGrath, 1978). These can be partially separated anatomically, with the non-adrenergic response dominant in the prostatic portion and the adrenergic response dominant in the epididymal portion (McGrath, 1978; Brown *et al.*, 1979; MacDonald & McGrath, 1980a, b). Further separation of the phases can be achieved pharmacologically as the adrenergic component can be selectively removed by postjunctional α_1-adrenoceptor antagonists (McGrath, 1978; MacDonald & McGrath, 1980a, b) while the non-adrenergic component is selectively removed by nifedipine (French & Scott, 1981).

After castration, several changes occurred in the rat vas deferens: the vasa exhibited spontaneous contractions; noradrenaline no longer produced a tonic contraction but increased the phasic spontaneous activity; the adrenergic component of the contractile response to field stimulation was lost whereas the non-adrenergic component remained; prejunctional inhibition of field stimulation-induced contractions by either endogenous or exogenous activation could not be demonstrated (MacDonald & McGrath, 1980a). In spite of the loss of adrenergic neurotransmission, microscopy revealed a similar density of adrenergic innervation in the vasa from

castrate rats and controls (MacDonald & McGrath, 1980a). As testosterone treatment partially reversed the effects of castration, it appeared that the function of the adrenergic nerves depended on testosterone levels. Hence in the present work similar studies of neuroeffector sensitivity and adrenergic innervation of the vas deferens in immature rats were carried out.

Preliminary accounts of some of these results have previously been published (MacDonald & McGrath, 1981;1982b).

Methods

The minimum age at which vasa could be examined individually and in a manner comparable with adult tissues was found to be 3 weeks. Tissues from rats aged 3 weeks and over were therefore tested. The rats were killed by a blow on the head and exsanguination. Vasa deferentia were isolated and set up bisected transversely into two portions of equal length in a 30 ml organ bath containing Krebs-bicarbonate solution of the following composition: (m M) NaCl 119, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1, and gassed with 95% O_2 and 5% CO_2.

Vasa were connected by thread to Grass FT03 transducers and isometric tension recorded on a Grass Model 7 polygraph. Field stimulation was applied via Ag:AgCl 'ring and hook' electrodes with supramaximal pulses of 0.5 ms duration applied either individually or in a train (8 Hz, 5s).

Dose-response curves to noradrenaline were constructed by exposing the tissues to each concentration for a period of 5 min, washing between each addition. Dose-response curves to the inhibitory effects of oxymetazoline and xylazine on nerve-induced responses were cumulative.

Tissues from rats of various ages were prepared for light and electron microscopy. For light microscopy, tissues were stained with haematoxylin and eosin (H & E) or Masson. For examination in the electron microscope tissues were fixed and prepared by the method of Tranzer & Richards (1976) as modified by Jones (1979). This method uses a modification of the chromaffin reaction to produce dense-cored vesicles in adrenergic terminals only when noradrenaline is present.

The following drugs were used: atropine sulphate (Sigma) cocaine hydrochloride (Cockburn's), corynanthine tartrate (Aldrich), nifedipine (Bayer), noradrenaline bitartrate (Koch-Light), oxymetazoline hydrochloride (Merck), prazos in hydrochloride (Pfizer), rauwolscine (α-yohimbine) base (Inverni della Beffa), tetrodotoxin (Sigma), yohimbine hydrochloride (Sigma) WB 4101 (N-[-(2,6 dimethoxyphenoxy) ethyl]-1,4 benzodioxane-2-methylamine), Ward Blenkinsop), xylazine hydrochloride (Bayer).

Results

Spontaneous activity

The vasa from immature rats sometimes showed spontaneous activity (Figure 1), not normally seen in the adult rat (MacDonald & McGrath, 1980a). The spontaneous activity was more likely to occur in younger rats with its presence disappearing at 6–7 weeks (Figure 1). The spontaneous contractions were not reduced by prazosin (6 μM), corynanthine (6 μM), WB 4101 (0.1 μM), atropine (1 μM) or tetrodotoxin (1 μg ml^{-1}). The spontaneous activity occurred more often in the prostatic end at all ages tested (figure 1) and the size and frequency of contractions also tended to be greater in the prostatic portion (figure 1).

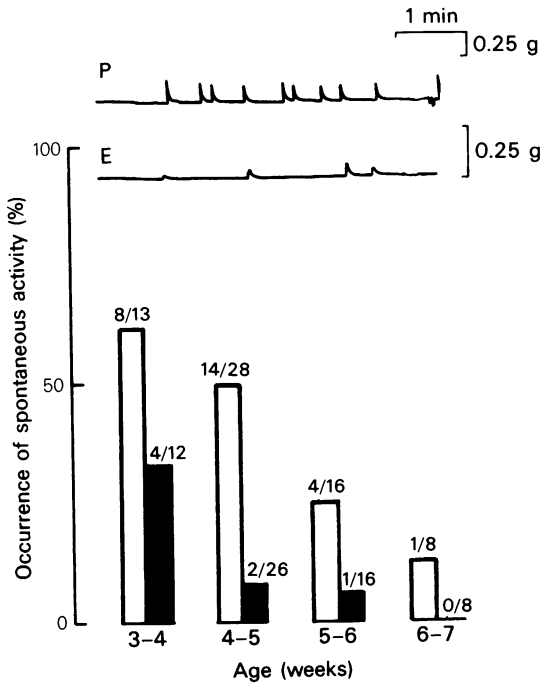

Figure 1 Upper trace; isometric tension recording of vasa (P, prostatic portion, E, epididymal portion) from a 4 week old rat. Lower histogram; frequency of occurrence of spontaneous activity in vasa, prostatic portion (open columns) and epididymal portion (solid columns), from rats of different ages.

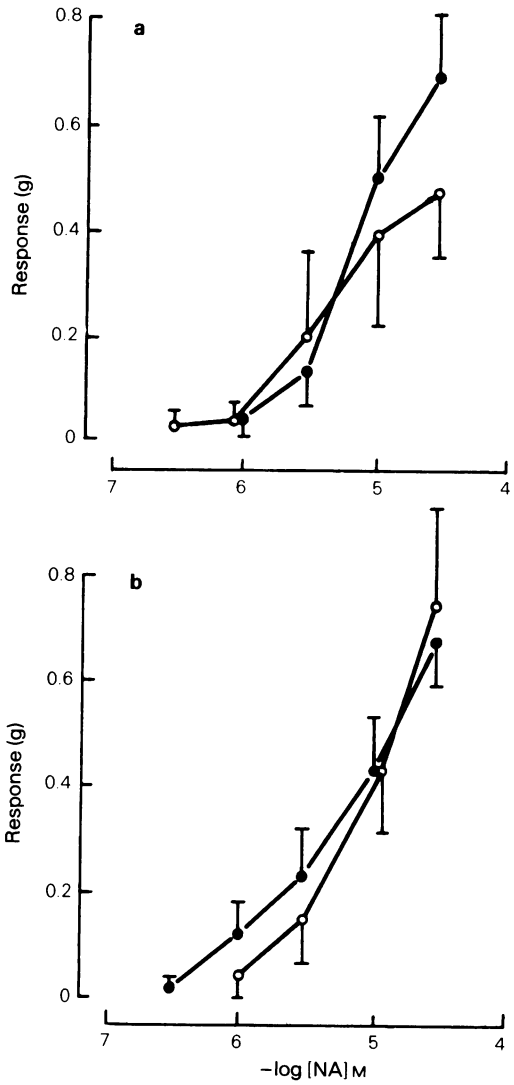

Figure 2 The effects of noradrenaline (NA) on isometric tension in vasa deferentia from rats aged 3–4 weeks (immature) (●) and over 12 weeks (adult) (○). (a) prostatic portions (b) epididymal portions. Values shown are means ± s.e.mean (vertical lines) 5 determinations.

Postjunctional α-adrenoceptor agonists

The response to exogenous noradrenaline of vasa from immature rats was qualitatively similar to that in adult rats, with both phasic and tonic components as described previously (MacDonald & McGrath, 1980a). The sensitivity to noradrenaline appeared similar in immature and adult rats (Figure 2) but this is without taking the tissue weights into consideration. Since vasa from adult rats were three to four times as heavy as vasa from immature rats, when the magnitude of response is corrected for the difference in tissue weight then the response of the immature rat is greater. As in adult rats (MacDonald & McGrath, 1982a), nifedipine (10 μM) blocked the contractile effects of exogenous noradrenaline in the immature rats.

The vasa of immature rats also appeared to be more sensitive to the contractile effects of oxymetazoline, an α-adrenoceptor agonist not subject to neuronal uptake, with a lower threshold for contraction of 1 nM compared with 10–100 nM in adult rats. It was not possible to construct dose-response curves for oxymetazoline due to the nature of the contractile activity, being mainly intermittent phasic twitches which were difficult to quantify.

Nerve-stimulation – single pulse

Unlike the biphasic responses of adults, the responses to single pulse field stimulation of prostatic and epididymal portions from the vasa of immature rats were monophasic and were often prolonged (Figures 3 and 4). The responses were sensitive to tetrodotoxin (1 μg ml^{-1}). Corynanthine (3 μM), which reduced the second component of the response in adult rats, had either no effect or caused only a slight reduction in the responses (Figure 3). Nifedipine (10 μM), which blocked only the first component in adult rats, almost completely abolished the responses (Figure 4). The responses were potentiated by cocaine (3 μM) and in the presence of cocaine both nifedipine-sensitive and corynanthine-sensitive components of the nerve-induced responses were present, as in adult rats (Figure 5).

Nerve-stimulation – train of pulses

There was no qualitative difference in the shape of the responses to stimulation with a train of pulses at 8 Hz for 5 s between the vasa from immature and adult rats. In adult rats, α_1-adrenoceptor blockade reduced the response to a train of pulses and subsequent addition of yohimbine (0.6 μM) or rauwolscine (0.3 μM) produced a potentiation to beyond the initial level (MacDonald & McGrath, 1980a; Brown *et al.*, 1983): this demonstrates the pre- and postjunctional effects of endogenous noradrenaline. However, both the pre- and postjunctional effects of adrenergic transmission were absent at around 4 weeks of age, after which time they began to develop (Table 1).

Prejunctional α_2-adrenoceptor agonists

Oxymetazoline inhibited the prostatic peak response to a single pulse in the vasa from immature rats

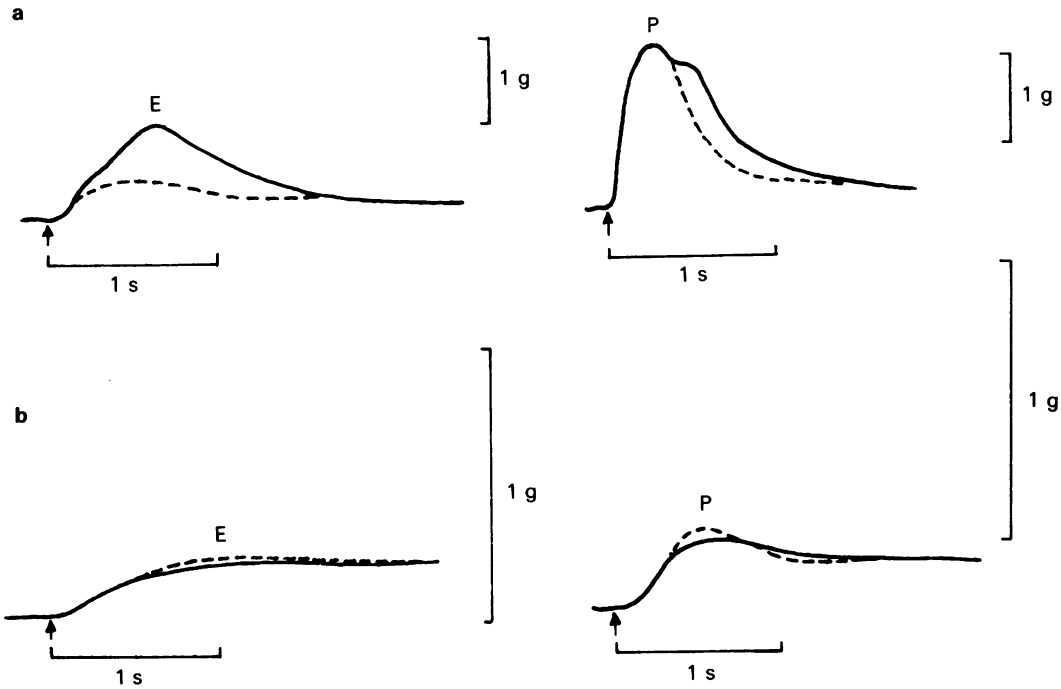

Figure 3 Effects of corynanthine on responses of isolated portions of rat vas deferens to single pulse (0.5 ms) field stimulation. (a) Adult rat (> 12 weeks old), (b) immature rat (4 weeks old). Control, solid line; corynanthine 3 μM, broken line. E, epididymal portion; P, prostatic portion.

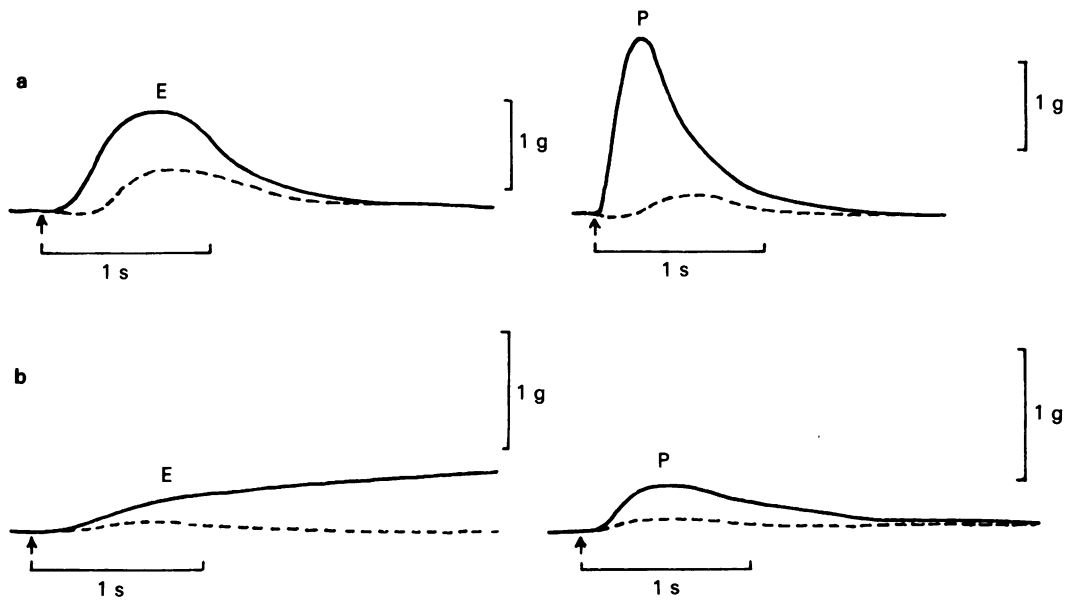

Figure 4 Effects of nifedipine on responses of isolated portions of rat vas deferens to single pulse (0.5 ms) field stimulation. (a) Adult rat (> 12 weeks old), (b) immature rat (4 weeks old). Control, solid line; nifedipine 10 μM, broken line. E, epididymal portion; P, prostatic portion.

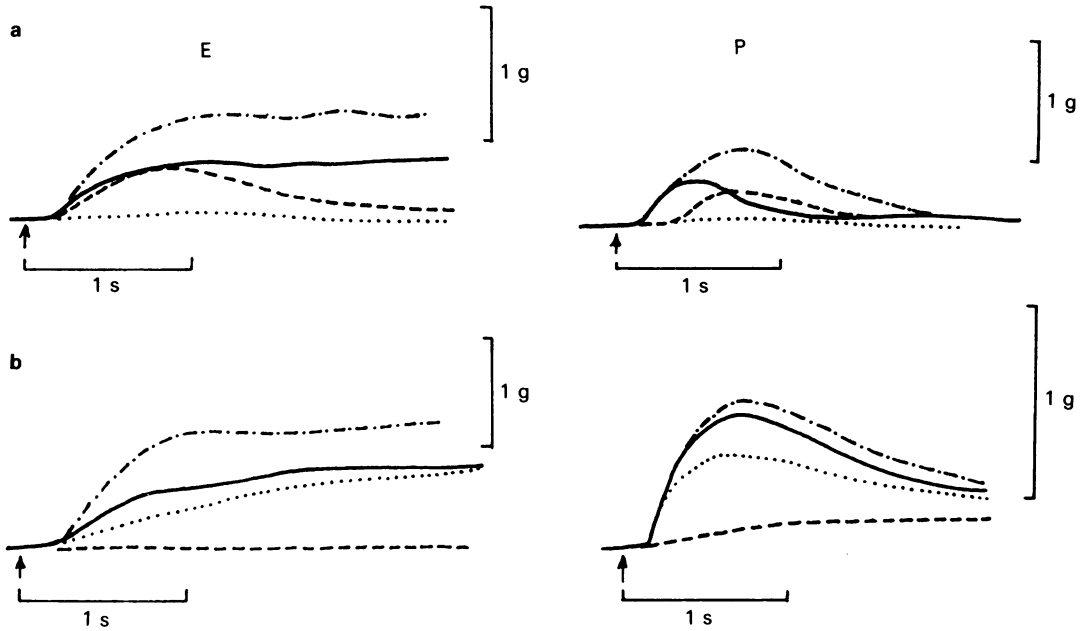

Figure 5 Responses of isolated vasa deferentia from immature (4 week-old rats. E, epididymal portions. P, prostatic portions. (a) Upper traces: control, solid line; cocaine 3 μM (—·—·—·); cocaine + nifedipine 10 μM, (- - - - - -); cocaine + nifedipine + corynanthine 3 μM, (. . . . .) (b) Lower traces: control, solid line; cocaine 3 μM, (—·—·—·—.); cocaine + corynanthine 3 μM, (. . . . .); cocaine + corynanthine + nifedipine 10 μM, (- - - - -).

although the inhibitory effect was not so marked in higher doses as in adult rats (Figure 6). In the epididymal portion, oxymetazoline had a biphasic effect in adult vasa: the initial inhibition is due to activation of prejunctional inhibitory α_2-adrenoceptors and the secondary increase to activation of postjunctional excitatory α_1-adrenoceptors (MacDonald & McGrath, 1980b). In immature rats the postjunctional effects tended to obscure the prejunctional inhibitory effects, probably due to the increased sensitivity of the vasa from immature rats to oxymetazoline. Although the mean results show no clear inhibitory effects, clear prejunctional inhibition was seen in individual experiments.

The postjunctional excitatory effects of xylazine were less marked than those of oxymetazoline and clear inhibitory effects were observed in both prostatic and epididymal portions of vasa from immature rats (Figure 6). Again the excitatory effects of xylazine were more apparent in vasa from immature rats, especially in the epididymal end, indicating a greater sensitivity to postjunctional excitatory effects (Figure 6). Mediation by α_2-adrenoceptors of the inhibitory effects of xylazine in the vasa of immature

Table 1 Development of pre- and postjunctional effects of adrenergic transmission

		Single pulse		*Train of pulses (8 Hz, 5s)*	
		a	b	c	d
Age of rat (days)	*Number of vasa tested* (n)	*Biphasic response* (*% of* n)	*α_1-Blockade* (*% of* n)	*α_1-Blockade* (*% of* n)	*α_2-Mediated feedback* (*% of* n)
20–30	7	0	0	0	0
30–40	18	44	44	6	22
40–50	9	56	56	56	78
>84	>50	100	100	100	100

Postjunctional effects of adrenergic transmission were taken as: (a), a biphasic response to a single pulse; (b), reduction of the single pulse response by prazosin (6 μM) or corynanthine (3 μM); (c) reduction of the response to a train of pulses by prazosin or corynanthine. Prejunctional effects of adrenergic transmission were taken as (d), potentiation of the response to a train of impulses by yohimbine (0.6 μM) or rauwolscine (0.3 μM) after α_1-blockade.

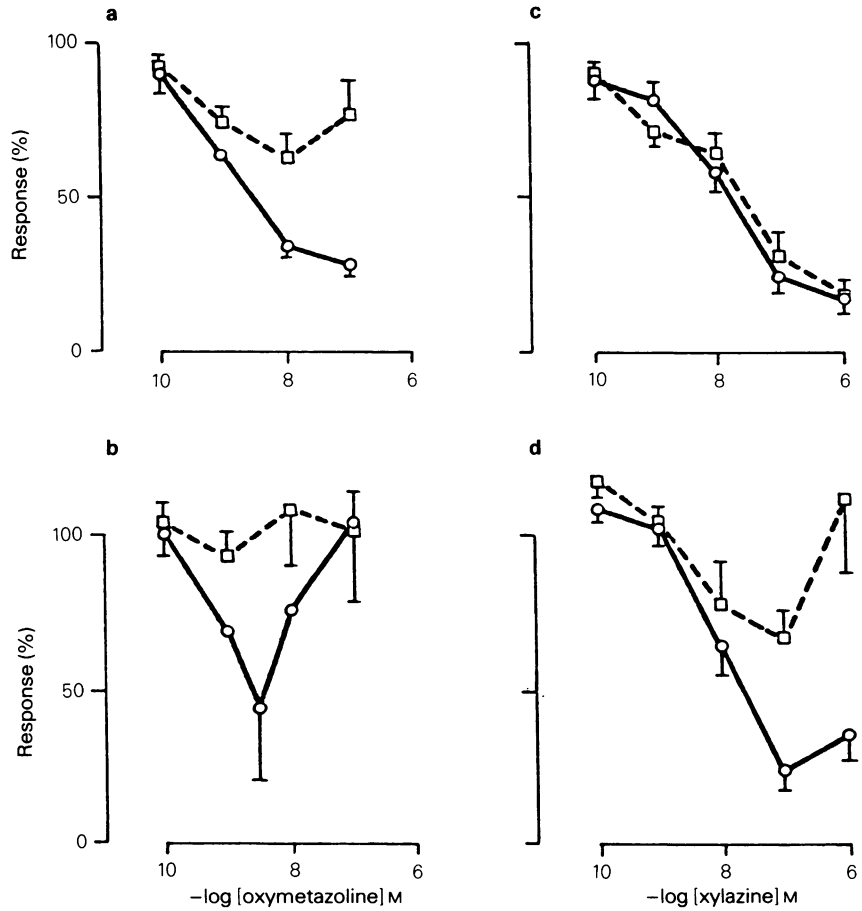

Figure 6 Effects of oxymetazoline (a and b) and xylazine (c and d) on the responses of portions of isolated rat vas deferens to single pulse (0.5 ms) field stimulation. Responses are expressed as % of pre-drug control values. (a and c) Prostatic peak response, (b and d) epididymal peak response. (○—○), Adult rats (> 12 weeks old), (□- - -□), immature rats (4 weeks old). Values shown are means ± s.e.mean (vertical lines of at least 6 determinations).

rats was confirmed by the use of rauwolscine: mean prostatic response, (% of control, mean ± s.e.mean, $n=7$); xylazine (1 μM) 28 ± 5, xylazine (1 μM) + rauwolscine (0.3 μM) 53 ± 8, $P<0.05$.

Microscopy

Histological staining with H & E and Masson showed that at 4 weeks of age, the arrangement of smooth muscle in the vas appeared similar to that in adult rats except that the cells were smaller and more closely packed together. Electron microscopy of vasa from 4 week-old rats revealed varicosities containing a mixture of small granular vesicles and large granular vesicles, as was observed in adult rats. Occasional profiles with only small clear vesicles were found in both adult and immature vasa. No qualitative differences between mature and immature rats as regards distribution or number of vesicles or in nerve-muscle distances could be distinguished (Figure 7). The density of innervation was not studied quantitatively but did not appear to be substantially different between immature and mature animals.

Discussion

The results confirm an old observation by Perutz & Taigner (1921) that vasa deferentia of young rats are more inclined to show spontaneous activity than those of adult rats, which are not normally spontaneously active. The spontaneous activity appears to be myogenic in origin as it is unaffected by muscarinic or α-adrenoceptor antagonists or tetrodotoxin. Spon-

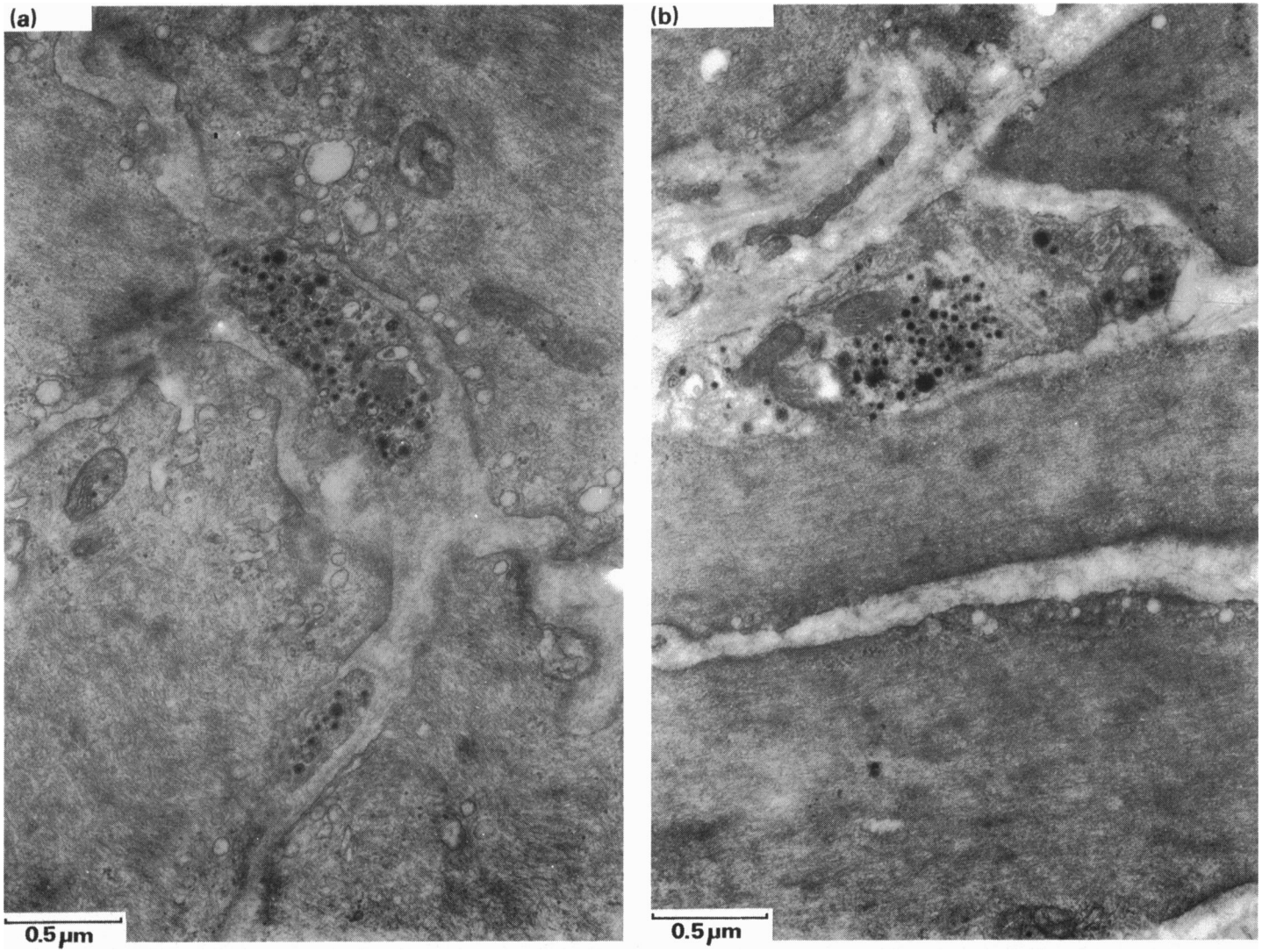

Figure 7 Electron micrographs of rat deferens. Fixation procedure (Tranzer & Richards, 1976; Jones, 1979) shows dense-cored vesicles in adrenergic terminals. (a) Left-hand micrograph, adult rat; (b) right-hand micrograph, immature rat, 4 weeks old.

taneous activity of the vas deferens *in vitro* has been previously observed after castration (Martins & Valle, 1939; MacDonald & McGrath, 1980a), reserpine treatment (Pollock *et al.*, 1972; Lee *et al.*, 1975), during morphine withdrawal (Pollock *et al.*, 1972), after surgical denervation (Lee *et al.*, 1975), after transplantation of the vas to a site adjacent to the wall of the colon (Jurkiewicz *et al.*, 1977) and after a chemical sympathectomy with 6-hydroxydopamine or guanethidine (MacDonald & McGrath, 1980a; MacDonald & McGrath, unpublished results). A common factor, which was postulated for each of these situations in which spontaneous activity was found, is a functional adrenergic denervation which has been present for at least five days Thus the spontaneous activity may be an index of denervation-induced postjunctional supersensitivity, as suggested by Lee *et al.*, (1975). This would correlate well with the present results showing a lack of functional adrenergic transmission in the vasa deferentia of young rats. As the spontaneous activity in vasa from young rats disappears with the appearance of functional adrenergic transmission this suggests that a state of 'pre-innervation postjunctional supersensitivity' exists in young rats due to a lack of the restraining influence of the adrenergic nerves on the postjunctional sensitivity of the smooth muscle of the vas deferens. It is not known whether this adaptive mechanism is due to a trophic substance released by the nerves, perhaps noradrenaline itself, or to the activity of the effector cells themselves, but from the present study it would seem that the mere presence of the nerves is not sufficient to govern the postjunctional sensitivity; they have to be functional as well.

Since the non-adrenergic response is present at an age when spontaneous activity occurs, then this suggests that the functioning of the nerves which are responsible for this response is not sufficient to prevent the 'pre-innervation supersensitivity'.

The greater propensity of the prostatic portion of the vasa from immature rats to display spontaneous activity has not been noted in the other situations in which spontaneous activity occurs. The reason for this increase in spontaneous activity in one half of the vas is not clear, although it may be related to the greater density of adrenergic innervation in the prostatic end of the tissue (Owman & Sjöstrand, 1965).

The disappearance of the spontaneous activity at 6–7 weeks of age coincides with the age at which testosterone secretion increases in the rat (Knorr *et al.*, 1970) and it is tempting to speculate that the function of the adrenergic nerves and hence the spontaneous activity depends on the testosterone levels as was postulated for castrate rats (MacDonald & McGrath, 1980a).

The postjunctional sensitivity to agonists of the vasa from immature and adult rats was difficult to compare because of the difference in tissue weights. However the results with noradrenaline, oxymetazoline and xylazine do suggest an increased sensitivity in the vasa from immature rats, supporting the hypothesis that a 'pre-innervation postjunctional supersensitivity' exists in the vasa deferentia from immature rats. The contractile responses of the vasa from immature rats showed both phasic and tonic components unlike other situations in which postjunctional supersensitivity is found, i.e. after reserpine, during morphine withdrawal (MacDonald, 1970), after castration (MacDonald & McGrath, 1980a or after chemical sympathectomy (MacDonald & McGrath, unpublished observations) where the tonic component was lost. The reason for this difference is not known.

The responses of the vasa from immature rats to single pulse field stimulation of the intramural nerves were monophasic, nifedipine-sensitive and corynanthine-resistant, confirming that they consisted mainly of the non-adrenergic component seen in adult rats, with the adrenergic component lacking. However after cocaine a clear adrenergic component does emerge, suggesting that the reason for the lack of functional adrenergic transmission may be insufficient noradrenaline release.

The responses of the rat vas deferens to trains of impulses are complex and difficult to interpret (Brown *et al.*, 1983) but evidence of pre- and postjunctional effects of endogenous noradrenaline could be demonstrated in adult rats using the appropriate blockers. The absence of postjunctional adrenergic effects at four weeks of age agrees with the results obtained using single pulse stimulation.

The effects of the relatively-selective α_2-adrenoceptor agonists on the responses of the vasa to single pulse field stimulation showed that the prejunctional α_2-adrenoceptrs can be activated in vasa from immature rats. The lack of prejunctional effects of endogenous noradrenaline during trains of stimuli would therefore appear to be due to insufficient release of noradrenaline.

Several possibilities regarding the reason for the lack of functional adrenergic transmission have been considered:

(1) *Ability of tissue to contract*

The vasa from immature rats give good contractions to exogenous agonists including noradrenaline. In addition, there is a nerve-induced contraction which corresponds to the non-adrenergic response of adults.

(2) *Presence of adrenergic nerves*

The fact that adrenergic nerves are present in the rat vas deferens from an earlier age than four weeks has

been shown previously by others using catecholamine fluorescence techniques (Owman *et al.*, 1971) and confirmed in the present study by electron microscopy.

(3) *Formation of close neuromuscular junctions*

The electron microscopic studies showed that the close neuromuscular distances seen in adult rat vas deferens (Richardson, 1962) are present at four weeks of age.

(4) *Density of innervation*

The density of innervation in immature rats may be less than in adult rats since Furness *et al.*, (1970) showed that the density increases up to six months in mouse vas deferens. However, although no quantitative estimation of density was carried out in the present study, no impressions of a substantial difference were gained from the electron microscopy. In addition, adult levels of noradrenaline in the rat vas deferens are reached three to five weeks after birth (Owman *et al.*, 1971).

(5) *Operative prejunctional receptors*

As these could be activated by exogenous agonists the lack of prejunctional effects on adrenergic transmission would appear to be due to insufficient release of noradrenaline.

(6) *Operative postjunctional receptors*

Exogenous noradrenaline produced good contractions but this may be a poor guide to the functional state of the receptors for neuronally-released noradrenaline. For example, in adult rats nifedipine blocks the effects of exogenous noradrenaline but has no effect on the response to neuronally-released noradrenaline, suggesting either different receptors or a different activation mechanism via the same receptor (MacDonald & McGrath, 1982a). Nevertheless, exogenous agonists did induce contraction.

(7) *An operative noradrenaline release mechanism*

There is no direct evidence here as we have not measured release. However the results with the α_2-adrenoceptor agonists provide indirect evidence of a lack of neuronally-released noradrenaline (see (5)).

A delay in the onset of adrenergic neurotransmission has also been observed in mouse vas deferens (Furness *et al.*, 1970) and in the carotid artery of the foetal lamb (Su *et al.*, 1977). The results of the latter study suggested that the mechanism for neurotransmitter release developed after the mechanisms for transmitter inactivation and action on the muscle cells.

Development of neurotransmission in the rat vas deferens has also previously been examined by Swedin (1972). However this study examined responses from birth to around 3 weeks and is not comparable with the present work. It is interesting, however, that Swedin (1972) found a nerve-mediated contraction in newborn rats which was susceptible to α-adrenoceptor antagonists up to 10 days but resistant thereafter. Presumably this resistant response corresponds to the non-adrenergic response found in immature rats in the present study.

In conclusion, there is a lack of functional adrenergic transmission in the vas deferens of immature rats which is probably due to insufficient release of noradrenaline because the release mechanism is not fully operative. Sub-sensitivity of the postjunctional apparatus for neuronally-released noradrenaline cannot be discounted as an additional factor. As with the disappearance of spontaneous activity, the appearance of functional adrenergic transmission coincides with the increase in testosterone secretion in the rat, supporting the suggestion that the function of the adrenergic nerves in the rat vas deferens depends on the testosterone levels (MacDonald & McGrath, 1980a). The effects of testosterone on the postjunctional sensitivity of smooth muscle in the male reproductive tract may therefore be mediated via changes in adrenergic transmission.

The authors are grateful to the Medical Research Council, the Medical Research Funds of the University of Glasgow and the Small Grants Fund of Glasgow College of Technology for financial support and Mr I. Montgomery for the electron microscopy.

References

BROWN, C.M., McGRATH, J.C. & SUMMERS, R.J. (1979). The effects of α-adrenoceptor agonists and antagonists on responses of transmurally stimulated prostatic and epididymal portions of the isolated vas deferens of the rat. *Br. J. Pharmac.*, **66**, 553–564.

BROWN, D.A., DOCHERTY, J.R., FRENCH, A.M., MacDONALD, A., McGRATH, J.C. & SCOTT, N.C. (1983). Separation of adrenergic and non-adrenergic contractions to field stimulation in the rat vas deferens. *Br. J. Pharmac.*, **79**, 379–393.

BURNSTOCK, G. & COSTA, M. (1975). *Adrenergic neurons*, pp. 114–117 London: Chapman & Hall.

FRENCH, A.M. & SCOTT, N.C. (1981). A comparison of the effects of nifedipine and verapamil on rat vas deferens. *Br. J. Pharmac.*, **73**, 321–323.

FURNESS, J.B., MCLEAN, J.R. & BURNSTOCK, G. (1970). Distribution of adrenergic nerves and changes in neuromuscular transmission in the mouse vas deferens during postnatal development. *Developmental Biology*, **21**, 491–505.

GIBSON, A. (1981). The influence of endocrine hormones on the autonomic nervous system. *J. Auton. Pharmac.*, **1**, 331–358.

JONES, C.J. (1979). Perivascular nerves in the rat submandibular salivary gland. *Neurosci. Lett.*, **13**, 19–23.

JURKIEWICZ, N.H., JURKIEWICZ, A. GOMES, C.B. & AUCELIO, J.G. (1977). Pharmacodynamic analysis of the rat isolated vas deferens after transplantation to intestinal wall. *J. Pharmac. exp. Ther.*, **203**, 112–119.

KNORR, D.W., VANHA-PERTTULA, T. & LIPSETT, M.B. (1970). Structure and function of rat testes through pubescence. *Endocrinology*, **86**, 1298–1304.

LEE, T.J.-F., WESTFALL, D.P. & FLEMING, W.W. (1975). The correlation between spontaneous contractions and postjunctional supersensitivity of the smooth muscle of the rat vas deferens. *J. Pharmac. exp. Ther.*, **192**, 136–148.

MACDONALD, A. (1970). The effects of agonists on the isolated vas deferens of the rat after chronic treatment with morphine and reserpine. *B.Sc. (Hons) Thesis*, University of Glasgow.

MACDONALD, A. & MCGRATH, J.C. (1980a). The effects of castration on neuro-transmission in the rat vas deferens. *Br. J. Pharmac.*, **69**, 49–58.

MACDONALD, A. & MCGRATH, J.C. (1980b). The distribution of adrenoceptors and other drug receptors between the two ends of the rat vas deferens as revealed by selective agonists and antagonists. *Br. J. Pharmac.*, **71**, 445–448.

MACDONALD, A. & MCGRATH, J.C. (1981). Effects of nerve stimulation and agonist drugs on the vas deferens of immature rats *Br. J. Pharmac.*, **72**, 527–528P.

MACDONALD, A. & MCGRATH, J.C. (1982a). Characterization and location of α-adrenoceptors in the rat vas deferens. *Br. J. Pharmac.*, **76**, 211P.

MACDONALD, A. & MCGRATH, J.C. (1982b). Lack of functional adrenergic transmission in the vas deferens of immature rats. *Br. J. Pharmac.*, **77**, 475P.

MCGRATH, J.C. (1978). Adrenergic and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol.*, **283**, 23–39.

MARTINS, T. & VALLE, J.R. (1939). Endocrine control of the motility of the male accessory organs. *Endocrinology*, **25**, 80–90.

OWMAN, CH., SJÖBERG, N.-O. & SWEDIN, G. (1971). Histochemical and chemical studies on pre- and postnatal development of the different systems of 'short' and 'long' adrenergic neurons in peripheral organs of the rat. *Z. Zellforsch. microsk. Anat.*, **116**, 319–341.

OWMAN, CH. & SJÖSTRAND, N.-O. (1965). Short adrenergic neurons and catecholamine-containing cells in vas deferens and accessory male genital glands of different mammals. *Z. Zellforsch. microsk. Anat.*, **66**, 300–320.

PERUTZ, A. & TAIGNER, E. (1921). Beiträge zur experimentellen Pharmakologie des männlichen Genitales II. Experimentellen pharmacologische Untersuchungen der peripher auf den Samenstränge wirkenden Mittel. *Archs. Derm. Syph.*, **131**, 316–328.

POLLOCK, D., MUIR, T.C., MACDONALD, A. & HENDERSON, G. (1972). Morphine-induced changes in the sensitivity of the isolated colon and vas deferens of the rat. *Eur. J. Pharmac.*, **20**, 321–328.

RICHARDSON, K.C. (1962). The fine structure of autonomic nerve endings in smooth muscle of the rat vas deferens. *J. Anat.*, **96**, 427–442.

SU, C., BEVAN, J.A., ASSALI, N.S. & BRINKMAN III, C.R. (1977). Development of neuroeffector mechanisms in the carotid artery of the foetal lamb. *Blood vessels*, **14**, 12–24.

SWEDIN, G. (1972). Postnatal development of the mechanical response of the isolated vas deferens to nerve stimulation. *Acta physiol. scand.*, **84**, 217–223.

TRANZER, J.-P. & RICHARDS, J.G. (1976). Ultrastructural cytochemistry of biogenic amines in nervous tissue: Methodologic improvements. *J. Histochem. Cytochem.*, **24**, 1178–1193.

(Received November 2, 1983.)