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European Journal of Pharmacology 318 (1996) 411–417

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Developmental changes in sympathetic contraction of the circular muscle layer in the guinea-pig vas deferens

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Received 24 September 1996; accepted 1 October 1996

Abstract

Contractile responses of the circular muscle of the isolated vas deferens to electrical stimulation (10–80 Hz) and to noradrenaline significantly decreased with increasing age in 3-week-, 10-week- and 18-month-old guinea pigs, observed by the cannula insertion method. There were no significant differences in the contractile responses induced by α,β-methylene ATP or $BaCl_2$ between 3 and 10 weeks old, but the responses to α,β-methylene ATP or $BaCl_2$ decreased in 18-month-old guinea pigs. The contractile response to electrical stimulation was monophasic in 3-week-old guinea pigs, a small portion of which remained after the treatment with prazosin. Desensitisation of P_{2X}-purinoceptors with α,β-methylene ATP significantly inhibited the contractile responses to stimulation with relatively low frequencies, and the combination of both prazosin and α,β-methylene ATP abolished the stimulation-induced contractions. In 10-week- and 18-month-old guinea pigs electrical stimulation evoked a transient contraction followed by a second contraction at the offset of the stimulation (the after-response). The after-responses were blocked by prazosin. These results show that the dominant component of sympathetic cotransmission is noradrenaline; a purinergic component also exists in the sympathetic contraction in the circular muscle of the vas deferens in young guinea pigs, but is virtually absent in the later stages of development. The sympathetic contractions of the circular muscles significantly decrease with increasing age and this appears to be due to changes in postjunctional, rather than prejunctional, mechanisms.

Keywords: Adrenergic transmission; Purinergic transmission; Contractile response; Circular muscle; Development; Vas deferens; (Guinea pig)

1. Introduction

The vas deferens is one of the most densely innervated organs in the body, and used widely in physiological and pharmacological experiments. However, most information about the vas deferens reflects largely characteristics of the longitudinal muscle layer although the thickness of the circular muscle layer is about 50% of whole muscle wall of the guinea-pig vas deferens (Furness and Iwayama, 1972; Gosling and Dixon, 1972). In the longitudinal muscle layer of the guinea-pig vas deferens, electrical stimulation causes a biphasic contractile response, adenosine 5′-triphosphate (ATP) and noradrenaline respectively contribute to the phasic and tonic components of the biphasic response (Meldrum and Burnstock, 1983; Sneddon and Westfall, 1984). Nagao et al. (1994) reported that some unknown component other than ATP and noradrenaline is involved in the monophasic contraction of the longitudinal muscle layer elicited by electrical stimulation in immature guinea pigs. Additionally, maximal tension of the longitudinal muscle layer of the vas deferens induced by noradrenaline and by electrical stimulation increases with increasing age in guinea pigs and rats (Takayanagi et al., 1987; Chernaeva and Yankova, 1991; Avellar and Markus, 1993), probably due to the increase in the total concentration of postjunctional α_1-adrenoceptors (Higuchi et al., 1982; Takayanagi et al., 1987).

One group has studied the responses of the circular muscle layer of the guinea-pig vas deferens using an isolated perfused preparation, and showed that intramural nerve stimulation causes both a contraction and after-contraction (Anstey and Birmingham, 1978, 1980). Both responses were blocked by α-adrenoceptor antagonists and almost completely abolished by reserpine, indicating that they were due to noradrenaline. As the lumen of the vas deferens increases with age and the increased lumen de-

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PII S0014-2999(96)00791-1

creases the perfusion resistance, it is difficult to analyse developmental changes in pressor responses of the circular muscle of the vas deferens by this method.

In the present study, the changes in responses of the circular muscle of the guinea-pig vas deferens that occur during 3 stages of development, 3 weeks, 10 weeks and 18 months, were examined by using a cannula insertion method which was originally designed for observing pressor responses of different sized large arteries to nerve stimulation and drugs (Hongo and Chiba, 1983; Chiba and Tsukada, 1985; Ren et al., 1996). This method involves inserting a special perforated cannula of suitable diameter into the whole length of the prostatic portion of the vas deferens. α,β-Methylene ATP was used to clarify whether a purinergic component was involved in the contraction elicited by sympathetic nerve stimulation.

2. Materials and methods

2.1. Preparations

Male albino guinea pigs weighing 220–250 g (3 weeks old), 600–650 g (10 weeks old) and 1030–1260 g (18 months old) were killed by stunning and bleeding and the two vasa deferentia were removed. The vas deferens was cleaned of surrounding tissues and the prostatic portion 15–20 mm in length (0.03–0.08 g in wet weight, 1.7–3 mm in outer diameter) was dissected. The preparations were cannulated and set up for perfusion by the cannula insertion method (Hongo and Chiba, 1983; Chiba and Tsukada, 1985; Ren et al., 1996) with a little modification. The cannulae (a gift from Professor S. Chiba) were 3–4 cm long and 0.61, 0.8 and 1.3 mm in outer diameter (for 3, 10 weeks and 18 months old, respectively) and had small side holes 5 mm from the distal sealed end. The prostatic segment was intubated by the stainless steel cannula from the prostatic end towards the epididymal end and the epididymal end was held on the cannula by a ligature 3 mm from the distal end of the cannula. The prostatic end was firmly held on the cannula by a pair of hooked electrodes at the resting length of the vas deferens to prevent it from shortening. The prostatic end was also the outlet for the perfusion solution. The cannulated preparation was placed and fixed in a vertical position in a cup-shaped glass bath, and perfused by a peristaltic pump (type/MC10, Watson-Marlow) with a physiological solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52, pH 7.2. The perfusion solution was aerated with 95% O_2 and 5% CO_2 and the flow rate was kept at 1 ml/min. The organ bath was sealed with plastic film to maintain the preparation at 37°C by a thermostat pump. Perfusion pressure was measured with a Spectramed Statham pressure transducer and recorded on a pen recorder (Grass). After an initial increase in perfusion pressure at the beginning of perfusion, the perfusion pressure decreased and reached a stable baseline (10–20 mmHg). A stabilisation period of 60 min was needed before starting the experiments.

Intramural nerve stimulation was delivered by means of the pair of electrodes hooked to the tissue, using a Grass S11 stimulator. A voltage of 10–24 V and a pulse width of 1 ms were used in experiments and the neurogenic origin of the responses was confirmed by the abolition of all contractions with 0.1 μM tetrodotoxin. The preparation was stimulated over a frequency range of 10–80 Hz for 10 s at 10–15-min intervals. These frequency-dependent response curves were repeated 2 times at 60-min intervals in each preparation.

Agonists and $BaCl_2$ were administered by bolus injection into the rubber tube connecting the cannula in a volume of 0.01–0.03 ml. Dose-response curves for noradrenaline were repeated 2 times in each preparation at 1 h intervals. Only one dose of α,β-methylene ATP (a P_{2X}-purinoceptor agonist) was given per preparation because it rapidly desensitises its own receptors. $BaCl_2$ (30 μmol), which potentiates the contractile responses to many stimuli (Ziganshina et al., 1995), was given at the end of experiments. Antagonists were added to the perfusion solution 60 min before carrying out the next experimental procedure (electrical stimulation, dose-response curve for noradrenaline or administration of α,β-methylene ATP); tetrodotoxin was added 20 min before. The selective α_1-adrenoceptor antagonist prazosin (10 μM) was used to block the adrenergic component of the neurogenic response. This concentration was chosen from preliminary experiments where a concentration of prazosin lower than 10 μM was not able to abolish the responses to exogenous noradrenaline. Desensitisation of P_{2X}-purinoceptors was achieved by perfusion with α,β-methylene ATP (10 μM) for 1 h, in order to block the purinergic component of the neurogenic response (Meldrum and Burnstock, 1983; Sneddon and Burnstock, 1985).

2.2. Drugs

α,β-Methylene adenosine 5′-triphosphate (lithium salt), (−)-noradrenaline bitartrate, prazosin hydrochloride, and tetrodotoxin were all obtained from Sigma. Barium chloride dihydrate was purchased from BDH. All drugs were dissolved in distilled water, except for noradrenaline which was dissolved in 100 μM ascorbic acid.

2.3. Statistical analysis

Contractile responses to drugs and electrical stimulations were expressed as the maximal changes in pressure (mmHg) from their control levels. Values presented here are the mean ± S.E.M. An analysis of variance (general linear model) was used to evaluate any differences be-

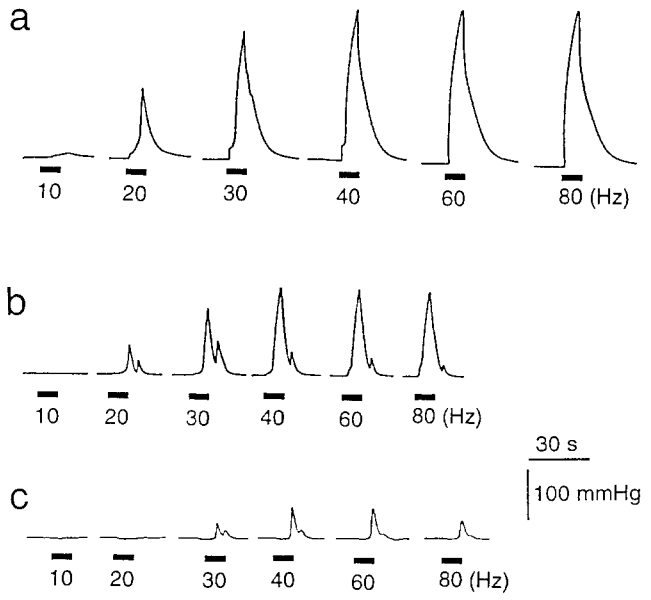

Fig. 1. Traces showing frequency-dependent pressor responses of the response and the after-response of the circular muscle of the guinea-pig vas deferens to electrical stimulation with 10–80 Hz. (a) 3 weeks old, (b) 10 weeks old and (c) 18 months old. Bars show the duration of electrical stimulation.

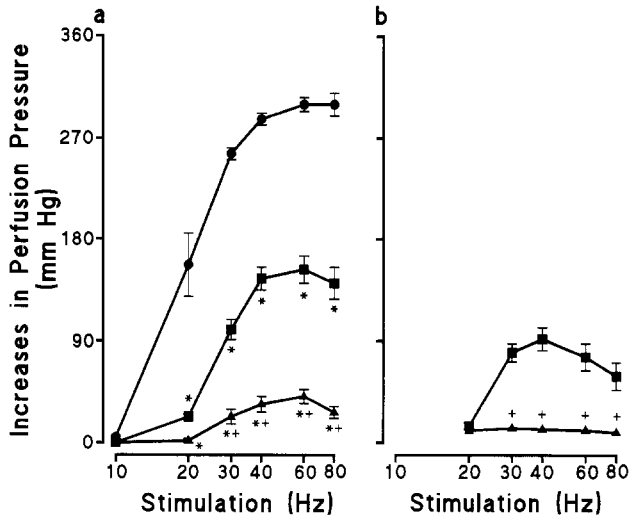

Fig. 2. Comparison of the response (a) and the after-response (b) of circular muscle of the guinea-pig vas deferens to electrical stimulation with 10–80 Hz in 3-week- (●), 10-week- (■) and 18-month-old (▲) guinea pigs. Points represent the mean values with S.E.M. * Represents statistical significance vs. 3 weeks old. + Represents statistical significance vs. 10 weeks old: * $P < 0.05$; + $P < 0.05$, $n = 7$–12.

tween frequency-dependent response curves for electrical stimulation or dose-response curves for drug. If the F statistic was significant, we compared the individual datum with its respective control value by simultaneous multiple comparisons, using Dunnett's method (Wallenstein et al., 1980). Comparisons of the contractile responses to drug between the two groups were analysed by unpaired t-tests. Comparison of a pair of responses to drug before and after treatment was analysed by paired t-tests. P values less than 0.05 were considered statistically significant.

3. Results

3.1. The contractile responses of the circular muscle of the vas deferens to intramural nerve electrical stimulation

In the vas deferens of 3-week-old guinea pigs, electrical stimulation (10–80 Hz) produced monophasic contractile

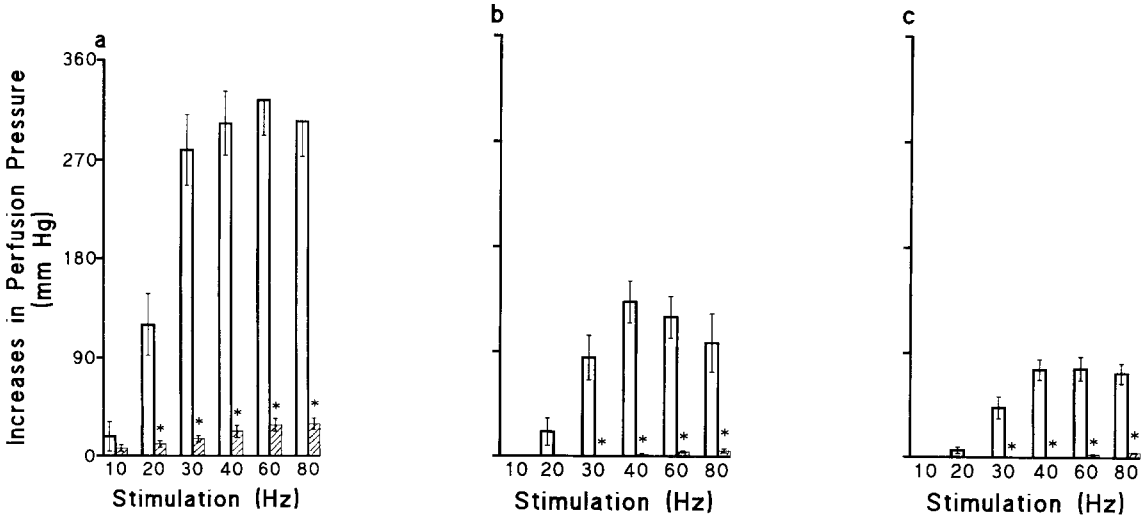

Fig. 3. Effects of 10 μM prazosin (hatched columns) on the response of the circular muscle of the guinea-pig vas deferens to electrical stimulation with 10–80 Hz (open columns). (a) 3 weeks old, (b) 10 weeks old and (c) 18 months old. Columns represent the mean values with S.E.M. Asterisks represent statistical significance vs. the respective control value: * $P < 0.05$, $n = 6$.

responses (Fig. 1a), except for the responses to 20 Hz which consisted of both the response (Fig. 1a) and the after-response (101 ± 49 mmHg) in 4 out of 8 preparations. In 10 weeks and 18 months old, electrical stimulation (20–80 Hz) induced both the response and after-response, the after-response (see the definition by Anstey and Birmingham, 1978; Anstey and Birmingham, 1980) was more obvious in 10 weeks old than 18 months old (Fig. 1b and 1c). Maximal pressor responses of the response to electrical stimulation at 60 Hz significantly decreased with increasing age in the three groups, the values of which were 300 ± 6 (mmHg, 3 weeks old), 153 ± 12 (10 weeks old) and 41 ± 6.1 (18 months old) (Figs. 1 and 2a). The after-response was significantly bigger in 10 weeks old than 18 months old (Figs. 1 and 2b). Frequency-dependent response curves for the response and the after-response were obtained two times without significant differences ($n = 4–6$, in three groups). All the contractile responses, including the after-response, to electrical stimulation were abolished by treatment with tetrodotoxin (0.1 μM, data not shown).

Prazosin (10 μM) significantly inhibited the responses to electrical stimulation at 10–80 Hz in the 3-week-old guinea pigs by 61% at 10 Hz, and 90–94% at 20–80 Hz. In 10-week-old and 18-month-old animals, the responses to stimulation at 20–40 Hz were abolished by prazosin (10 μM) and the maximal pressor responses of the response at 60 Hz were inhibited by 97% in both groups (Fig. 3). The after-response was completely inhibited by treatment with prazosin (10 μM) in both the 10 weeks and 18 months old ($n = 6$, data not shown).

Perfusion with α,β-methylene ATP (10 μM) for desensitisation of P_{2X}-purinoceptors did not cause any changes in the perfusion pressure. α,β-Methylene ATP (10 μM) significantly shifted the response curves elicited by electrical stimulation to the right in 3- and 10-week-old guinea pigs, but it affected neither the after-response curve in 10 weeks old, nor both the response and the after-response curves in 18 months old (Fig. 4, data of the after-response not shown). The pressor responses of the response to electrical stimulation at relatively lower frequencies (20–40 Hz) were significantly inhibited by α,β-methylene ATP (10 μM) by 24–35% in 3 weeks old, and 40–51% in 10 weeks old (Fig. 4a and 4b). Maximal pressor responses of the response at 60 Hz in 3- and 10-week-old guinea pigs were reduced by α,β-methylene ATP (10 μM), but not significantly. The combination of both prazosin (10 μM) and α,β-methylene ATP (10 μM) abolished both the response and after-response to electrical stimulation at 10–80 Hz in the three groups ($n = 4–6$, data not shown).

3.2. The contractile responses of the circular muscle of the vas deferens to noradrenaline, α,β-methylene ATP and $BaCl_2$

Noradrenaline (1–300 nmol) dose-dependently caused contractile responses in the circular muscle of the vas deferens in 3-week-old animals; the maximal response to 300 nmol noradrenaline was 269 ± 21 mmHg. In 10 weeks old, the contractile responses to 10, 30, 100 and 300 nmol noradrenaline significantly decreased by 88%, 72%, 70% and 64% respectively, when compared with the results in 3 weeks old (Fig. 5). The contractile response to noradrenaline in both 3- and 10-week-old guinea pigs consisted of a sustained increase in perfusion pressure with bursts of phasic activity. Noradrenaline (1–300 nmol) did not cause any response in the circular muscle of the vas deferens in 18 months old (Fig. 5), except for 2 out of 10 preparations where 300 nmol noradrenaline induced several transient and smaller pressor responses without consistent increase in pressure. These kinds of transient and smaller responses were observed in all the preparations explored to 3 μmol noradrenaline in the 18-month-old guinea pigs. Dose-response curves for noradrenaline were constructed two times without significant difference (in 3 and 10 weeks old, data not shown). Desensitisation of

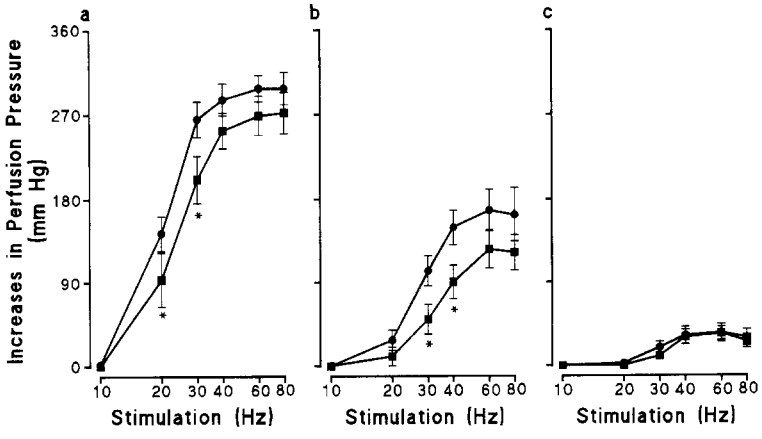

Fig. 4. Effects of 10 μM α,β-methylene ATP on the response of the circular muscle of the guinea pig vas deferens to electrical stimulation with 10–80 Hz. (a) 3 weeks old, (b) 10 weeks old and (c) 18 months old. (●) Control; (■) 10 μM α,β-methylene ATP. Points represent the mean values with S.E.M. Asterisks represent statistical significance vs. the respective control value: * $P < 0.05$, $n = 6–8$.

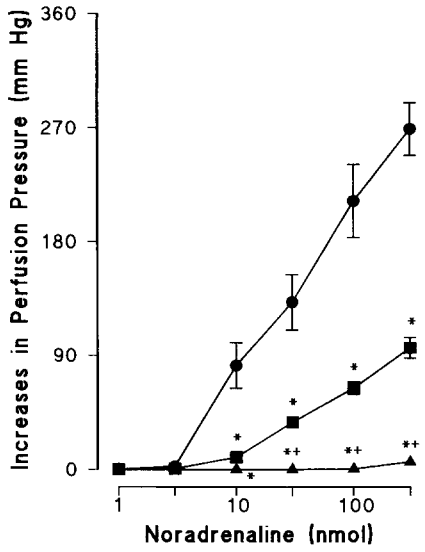

Fig. 5. Dose-response curves for noradrenaline of the circular muscle of the vas deferens in 3-week- (●), 10-week- (■) and 18-month-old (▲) guinea pigs. Points represent the mean values with S.E.M. * Represents statistical significance vs. 3 weeks old. + Represents statistical significance vs. 10 weeks old: * $P < 0.05$; + $P < 0.05$, $n = 8–10$.

P_{2X}-purinoceptors with α,β-methylene ATP (10 μM) and treatment with tetrodotoxin (0.1 μM) did not significantly affect the responses to noradrenaline ($n = 6–8$), while prazosin (10 μM) abolished the contractile responses to noradrenaline (1–300 nmol) in 3- and 10-week-old guinea pigs ($n = 6$), and the transient contractions to noradrenaline (3 μmol) in 18 months old ($n = 4$).

α,β-Methylene ATP (300 nmol, as an agonist) caused a monophasic increase in perfusion pressure by 127 ± 21 (mmHg, $n = 8–10$), 99 ± 17 and 4.9 ± 1.2 in 3-week-, 10-week- and 18-month-old guinea pigs, respectively. There were no differences in the pressor responses to α,β-methylene ATP (300 nmol) between 3 and 10 weeks old, but α,β-methylene ATP-induced responses in 18 months old significantly ($P < 0.05$) decreased by 96%, when compared with that in 3 weeks old. In 3-week-old guinea pigs, the value of the contractile response to 300 nmol α,β-methylene ATP in the absence of prazosin was 127 ± 21 (mmHg, $n = 8$) and in the presence of prazosin (10 μM) was 109 ± 16 ($n = 8$, $P > 0.05$). $BaCl_2$ (30 μmol) evoked a high increase in perfusion pressure of 329 ± 17 (mmHg) and 297 ± 22 in 3- and 10-week-old animals. The responses to $BaCl_2$ (30 μmol) were not significantly different between the 3 and 10 weeks old ($n = 8–10$). The contractile response to $BaCl_2$ (30 μM) in 18 months old, however, decreased from 329 ± 17 (mmHg, 3 weeks old) to 21 ± 4 ($n = 8–10$, $P < 0.05$).

4. Discussion

The results of the present study show that the predominant neurogenic response of the circular muscle layer in the guinea pig vas deferens is due to noradrenaline. However, a small purinergic component contributes to the sympathetic contraction, especially in the young animals. This study further shows that the sympathetic contractile responses of the circular muscle decrease with increasing age due to postjunctional, rather than prejunctional changes, perhaps involving Ca^{2+} mobilisation and/or contractile machinery itself of the circular muscle cells.

It is well-known that the contractile response of the longitudinal muscle of the guinea-pig vas deferens to electrical stimulation consists of both fast (phasic) purinergic and slow (tonic) noradrenergic components (Meldrum and Burnstock, 1983; Sneddon and Westfall, 1984). On the other hand, it was reported that both the contractions and after-contractions of circular muscle layer of the guinea-pig vas deferens to intramural nerve stimulation are largely due to noradrenaline, using a perfusion preparation in the guinea pigs weighing over 600 g (Anstey and Birmingham, 1978, 1980). In the present study, both the response and the after-response to intramural nerve stimulation were observed in the 10-week- and 18-month-old guinea pigs weighing 600–1260 g. The response and the after-response to electrical stimulation were almost abolished by prazosin (10 μM), which blocked the contractile responses to exogenous noradrenaline without effect on that to α,β-methylene ATP, though a small portion (less than 5%) of the response to stimulation at 60 and 80 Hz was insensitive to prazosin. These results were consistent with those reported by Anstey and Birmingham (1978, 1980).

Contractile response of the circular muscle in 3 weeks old, however, was monophasic, and a prazosin-resistant response was observed at each frequency of stimulation. Desensitisation of P_{2X}-purinoceptors with α,β-methylene ATP (Kasakov and Burnstock, 1983; Sneddon and Burnstock, 1985) significantly shifted the frequency-dependent response curve to the right, and inhibited the pressor responses to stimulation at 20–30 Hz by 24–35%. Combination of both prazosin and α,β-methylene ATP completely inhibited the contractile responses to electrical stimulation, indicating a contribution of a purinergic component to the sympathetic contraction of the circular muscle of the vas deferens in immature guinea pigs. It is noteworthy that the contractile response of the longitudinal muscle of the vas deferens is also a monophasic response in the immature guinea pigs (Nagao et al., 1994).

Many studies in the longitudinal muscle of the vas deferens in guinea pigs and rats show either no age-related changes in contraction of the prostatic portion evoked by electrical stimulation or noradrenaline (Docherty and O'Malley, 1983; McAdams and Waterfall, 1986), or increased contraction to electrical stimulation or noradrenaline with increasing age (Takayanagi et al., 1987; Chernaeva and Yankova, 1991; Avellar and Markus, 1993), except for a few reports where the data were calculated by g/g tissue (Higuchi et al., 1982; Nagao et al., 1994). The increase in contractile responses to exogenous noradrena-

line or electrical stimulation is due to age-related decline in neuronal noradrenaline uptake in the prostatic portion (including both longitudinal muscle and circular muscle layers) of the rat vas deferens (Avellar et al., 1990; Markus and Avellar, 1992), and/or age-related increase in total concentration of postjunctional α_1-adrenoceptors (Higuchi et al., 1982; Takayanagi et al., 1987).

On the contrary, the evidence from the present study showed the reduction in response and the after-response of the circular muscle layer to electrical stimulation with increasing age from 3 weeks to 18 months old, and also the exogenous noradrenaline-induced contractions decreased in a similar manner. Receptor binding assays, where both the circular and longitudinal muscle layers were analysed together, show that the total concentration of postjunctional α_1-adrenoceptors increases with increasing age in the vas deferens (Higuchi et al., 1982; Takayanagi et al., 1987). Avellar and Markus (1993) reported that an age-related reduction in noradrenaline release does not occur in the prostatic portion, although noradrenaline release from the sympathetic nerves of the whole vas deferens is higher in immature guinea pigs than that in mature (Chernaeva and Yankova, 1991). Furthermore, histochemical studies show that both the longitudinal and circular muscles are densely innervated by adrenergic fibres in the vas deferens of the guinea pigs weighing 900–950 g (Furness and Iwayama, 1972; Gosling and Dixon, 1972). Therefore, the age-related reduction in sympathetic contraction of the circular muscle layer in the guinea-pig vas deferens appears to be due to changes in postjunctional, rather than prejunctional, sites. The fact that the contractile responses of circular muscle of the vas deferens to $BaCl_2$ and α,β-methylene ATP decreased greatly in 18-month-old guinea pigs is consistent with this suggestion. Barium salts are useful tools for the study of properties of the Ca^{2+} channels at the plasma membrane in the various excitable tissues (Hagiwara and Byerly, 1981) and mechanical properties of the contractile proteins (Saeki et al., 1984). The mechanisms of $BaCl_2$-induced contractile response of smooth muscle have been suggested that it increases the influx of extracellular Ca^{2+} into smooth muscle cells (Antonio et al., 1973), releases Ca^{2+} from an intracellular pool, and directly activates contractile proteins via formation of a Ba^{2+}-calmodulin complex (Kreye et al., 1986; Satoh et al., 1987), although Chao et al. (1984) reported that $BaCl_2$ was unable to activate calmodulin from the bovine brain. Furthermore, the contractile responses to ATP and the purinergic component of biphasic contraction to electrical stimulation of the vas deferens were selectively inhibited by nifedipine, a voltage-operated Ca^{2+} channel blocker (Blakeley et al., 1981; French and Scott, 1981; Stone, 1981; MacKenzie et al., 1988). Recently, it has been suggested that the intracellular calcium pool for circular muscle is of limited size and noradrenaline-induced contraction of circular muscle relies more directly on the influx of extracellular calcium in comparison with longitudinal muscle in the human vas deferens (Amobi and Smith, 1993).

From the results in this study and those reported by others, it is suggested that the changes in the voltage-operated Ca^{2+} channels at the plasma membrane, in intracellular Ca^{2+} mobilisation and/or contractile machinery itself might occur during ageing, which result in the overall reduction in contraction of the circular muscle of the guinea-pig vas deferens. So far, there is no direct evidence to show precise morphological changes in the sympathetic innervation and muscle cells of the circular muscle layer of the guinea-pig vas deferens during development. Further studies should be carried out to clarify the morphological changes which might reflect the basis of the age-related decrease in sympathetic contraction of the circular muscles. The physiological significance of the findings is obscure. Since male guinea pigs of 3-month- to 2-year-old are the best age for breeding, both the decrease in sympathetic contraction of the circular muscle layer and the increase in sympathetic contraction of the longitudinal muscle layer of the vas deferens may act together to produce efficient translocation of sperm from the testes to the seminal vesicles. Certainly, the decreased excitability of the circular muscle does not appear to impair male fertility. Recently, it was reported that a selective blockade of longitudinal, but not circular muscle, contractions to noradrenaline by some α-adrenoceptor antagonists may be the mechanism for its male contraceptive action in the human vas deferens (Amobi and Smith, 1995), indicating an important role of the circular muscle layer of the vas deferens in reproduction.

In conclusion, a purinergic component exists in the sympathetic contraction of the circular muscle of the guinea-pig vas deferens in young animals. Also, the sympathetic contractions of the circular muscle significantly decrease with age, due to changes in postjunctional mechanisms.

Acknowledgements

L.-M.R. was a recipient of an Overseas Research Fellow (FS/94067) of the British Heart Foundation. The financial support of the British Heart Foundation is gratefully acknowledged.

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