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Age-related changes in purinergic and adrenergic components of sympathetic neurotransmission in guinea-pig seminal vesicles

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1 Purinergic and adrenergic components of sympathetic neurotransmission and contractile responses to exogenous α,β-methylene ATP and noradrenaline have been investigated in the seminal vesicles of 1-day (new-born), 2-weeks (young), 12-weeks (adult) and 2-years old (aged) guinea-pigs.

2 In seminal vesicles of new-born guinea-pigs electrical field stimulation (EFS; 80 V, 0.5 ms for 30 s, 2–32 Hz) evoked tonic frequency-related contractions. In 2-weeks old guinea-pigs the tonic contraction masked an initial phasic component of the neurogenic responses, whereas in 12-weeks and 2-years old guinea-pigs, neurogenic responses were biphasic, a phasic response being followed by a tonic contraction. In all experimental groups, prazosin (10^{-6} M) blocked the tonic contraction while desensitization of P2X receptors by α,β-methylene ATP (10^{-4} M) abolished the phasic responses.

3 The phasic purinergic component of the neurogenic response was significantly higher in 12-weeks and 2-years old animals, compared with 2-weeks old guinea-pigs. At 32 Hz phasic contractions were (mN mg^{-1} tissue): 0.047 ± 0.012, 0.018 ± 0.040 and 0.147 ± 0.026 in 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively. In contrast, the tonic adrenergic component of the neurogenic contraction significantly declined at 12-weeks and 2-years compared with 2-weeks old guinea-pigs.

4 Contractile responses (mN mg^{-1} tissue) to the highest concentration of α,β-methylene ATP tested were significantly higher in 2-weeks (0.248 ± 0.022) than in 1-day old animals (0.113 ± 0.012) and decreased in 12-weeks (0.163 ± 0.016) and 2-years old guinea-pigs (0.200 ± 0.008). The pD_2 values for the purinoceptor agonist were also significantly lower in adult (4.74 ± 0.20) and aged guinea-pigs (5.22 ± 0.08) compared with 2-weeks old animals (5.91 ± 0.27). Conversely, responses to the highest concentration of noradrenaline gradually decreased with age, without significant changes in the pD_2 values. Contractile responses to KCl (240 mM) did not differ significantly between the experimental groups.

5 These results demonstrate age-related changes in purinergic and adrenergic components of sympathetic neurotransmission in the guinea-pig seminal vesicles. The purinergic component is absent in new-born animals and it appears fully developed in adult and old guinea-pigs, while the adrenergic component decreases with age. Pre- and postjunctional mechanisms contributing to the age-related changes of sympathetic neurotransmission are discussed.

Keywords: Seminal vesicle; ageing; purinergic neurotransmission; adrenergic neurotransmission

Introduction

The seminal vesicle is one of the accessory male sex glands under control of sympathetic nerves. In adult guinea-pigs, but not in rats, contractions of seminal vesicles in response to hypogastric nerve stimulation are biphasic, with a rapid primary phasic contraction, followed by a secondary tonic contraction (Meldrum & Burnstock, 1985; Chin & Pennefather, 1988; Sadraei *et al*., 1995). It has been shown that the second component is adrenergic, since it is blocked by reserpine pretreatment or by the α-adrenoceptor antagonists, prazosin or phentolamine. The phasic contraction, resistant to α-adrenoceptor or cholinergic blockade, has been shown to be mediated by adenosine 5′-triphosphate (ATP) acting on P2X receptors, as it is blocked by desensitization of P2X receptors by the stable analogue α,β-methylene ATP and it is mimicked by exogenous ATP (Meldrum & Burnstock, 1985).

Plasticity of autonomic nerves during development and ageing has been discovered in the bladder and sexual organs (Burnstock, 1991; Lincoln & Burnstock, 1993). In the rat seminal vesicles both pre- and postjunctional mechanisms appear to contribute to age-related modification of the responsiveness to sympathomimetic agonists, which include loss of function of the neuronal uptake system as well as modifications of the postjunctional adrenoceptors (Docherty & O'Malley, 1983; Hyland & Docherty, 1985). In the guinea-pig vas deferens, the responsiveness to electrical stimulation and exogenous noradrenaline has been shown to be highest at 2-weeks and then gradually to decrease with development (Nagao *et al*., 1994). The influence of sex hormones on the development and function of the sympathetic nervous system in these organs has also been demonstrated with respect to the adrenergic mechanisms of neurotransmission (Bustamante *et al*., 1989; Hamill *et al*., 1984). However, there is little or no information on the purinergic contribution to sympathetic neurotransmission at different stages of neuronal development and sexual maturity. Therefore this study evaluated age-related variations in the relative contribution of noradrenaline and ATP as sympathetic cotransmitters in the seminal vesicles. To this end, neurogenic responses to electrical field stimulation were evaluated in tissue isolated from guinea-pigs 1-day and 2-weeks old, in order to evaluate sympathetic neurotransmission in sexually immature animals at two distinct stages of development. Guinea-pigs of 12-weeks of age were used as sexually mature animals (Soares *et al*., 1993), while 2-years old guinea-pigs were chosen as a model of ageing. In order to discriminate between pre- and postjunctional mechanisms involved in sympathetic neurotransmission, contractile responses to the ATP analogue, α,β-methylene ATP, and to noradrenaline applied exogenously were also assessed in all experimental groups.

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Methods

Tissue preparations and recording of mechanical activity

Albino male guinea-pigs (1-day, 2-weeks, 12-weeks and 2-years old) were killed by a blow to the head and exsanguination. The abdomen was opened and the seminal vesicles were quickly removed and placed in cold, modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.4, $NaHCO_3$ 16.4, $MgSO_4$ 0.6 and glucose 7.7. The seminal vesicles were opened longitudinally and strips proximal to the urethra were cut. Each longitudinal smooth muscle preparation (about 10×2 mm) was threaded through a pair of platinum-ring electrodes (3 mm in diameter, 1 cm apart) connected to a Grass SD9 stimulator; one end was attached to a holder and the other to a Dynamometer UF1 isometric force transducer coupled to a four-channel Grass 79D ink-writing oscillograph. The strips were equilibrated for 1 h in 5 ml organ baths containing modified Krebs solution gassed with 95% O_2 and 5% CO_2 and maintained at $37 \pm 0.5^{\circ}C$. The resting tension for the optimal force development was determined as 1 g (98 mN), and the strips were initially loaded to this resting tension. A slight reduction of this value occurred after the equilibration period.

Experimental procedure

After the equilibration period, each preparation was exposed to noradrenaline (10^{-5} M) until two reproducible contractions were obtained. In order to construct frequency-response curves contractile responses to electrical field stimulation (EFS) were evaluated by applying square wave pulses (80 V, 0.5 ms) delivered for 30 s, at increasing frequencies (2–32 Hz), a 4 min interval being left between two consecutive frequency steps. When repeated, a 30 min interval was left between the frequency-response curves to EFS. In time control experiments there was no significant difference between the first and second frequency-response curves. Tetrodotoxin (10^{-6} M) was then added to the organ bath and EFS repeated. Non-cumulative concentration-response curves to noradrenaline and α,β-methylene ATP were carried out at the resting tone. At the end of the experiment each strip was exposed to KCl (240 mM).

Drugs used

α,β-Methylene ATP lithium salt, noradrenaline (L-arterenol bitartrate), prazosin and tetrodotoxin were all purchased from Sigma. Stock solutions were prepared in distilled water. Subsequent dilutions of the drug were prepared in 0.9% NaCl; dilutions of noradrenaline were made with 0.1 mM ascorbic acid to prevent oxidation.

Analysis of data

All data in the text and figures are expressed as mean ± s.e.mean of at least six experiments. Contractile responses are expressed as % of KCl-induced contraction. Concentration–response curves were compared according to Ludbrook (1994) with analysis of variance (ANOVA) followed by Tukey-Kramer *post hoc* test by use of the computer program Minitab. Maximal contractile responses were compared by ANOVA followed by Bonferroni correction for multiple comparisons (Ludbrook, 1994).

Results

Contractile responses to electrical field stimulation

Figure 1a shows a typical tracing of the neurogenic responses to EFS (4–32 Hz) in seminal vesicle strips of 1-day old guinea-pigs. The sustained tonic contraction was abolished by prazosin 10^{-6} M (Figure 1b). The tracing of the neurogenic responses evoked in the seminal vesicle strip of a 2-weeks old guinea-pig is shown in Figure 1c. In this preparation, blockade of α-adrenoceptors by prazosin 10^{-6} M unmasked a residual frequency-dependent phasic contraction (Figure 1d). Figure 2 shows the biphasic neurogenic responses induced by EFS in

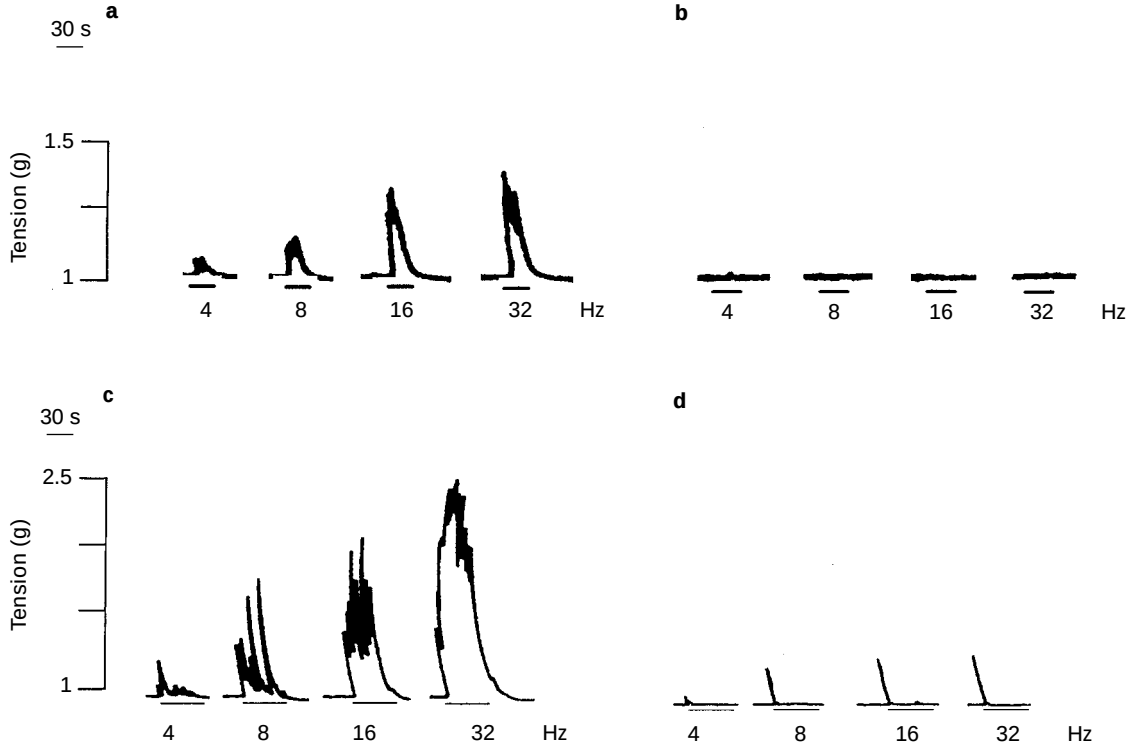

Figure 1 Recordings of contractile responses (g) evoked by EFS (80 V, 0.5 ms for 30 s, 4–32 Hz) in longitudinal smooth muscle strips of seminal vesicle excised from 1-day old (a, b) and 2-weeks old (c, d) guinea-pigs. Prazosin (10^{-6} M) abolished neurogenic responses in new-born tissue (b) and unmasked a phasic neurogenic response in young tissue (d).

seminal vesicle of 12-weeks old guinea-pigs at increasing frequencies of stimulation (4–32 Hz). A phasic response was followed by a long lasting tonic contraction (Figure 2a,c). Desensitization of P2X receptors by α,β-methylene ATP (10^{-4} M) abolished the phasic contraction, without affecting the tonic response (Figure 2b), while incubation with prazosin (10^{-6} M) eliminated the tonic contraction (Figure 2d). About 30% of the preparations excised from adult guinea-pigs exhibited spontaneous activity, as shown in Figure 2a and b. Seminal vesicle strips of 2-years old guinea-pigs also showed biphasic neurogenic responses to EFS. Frequency-response curves of phasic responses to EFS, evaluated in the presence of prazosin (10^{-6} M), demonstrated that neurogenic responses were increased with age, whereas no phasic responses were observed in preparations from new-born guinea-pigs (Figure 3a). Frequency-response curves in 12-weeks and 2-years old animals differed significantly from those obtained from 2-weeks old guinea pigs. At the highest frequency of stimulation (32 Hz), phasic contraction were (mN mg^{-1} tissue): 0.047 ± 0.012, 0.018 ± 0.040 and 0.147 ± 0.026 in 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively. Contractile responses at 32 Hz were significantly higher in 12-weeks and 2-years old animals than in 2-years old guinea-pigs.

Figure 3b shows the frequency-response curves of tonic contractions to EFS in all experimental groups, after desensitization of P2X receptors by α,β-methylene ATP (10^{-4} M). Tonic contraction was significantly larger in 2-weeks than in 1-day old guinea-pigs at all frequencies of stimulation. However, in 12-weeks and 2-years old animals the tonic contractions were significantly reduced compared with 2-weeks old guinea-pigs. Following EFS at 32 Hz, tonic contractions were (mN mg^{-1} tissue): 0.315 ± 0.033, 0.431 ± 0.045, 0.169 ± 0.020 and 0.300 ± 0.037 in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively. At 32 Hz, contractile responses were significantly increased in 2-weeks vs 1-day old animals. However, contractions were significantly lower in 12-weeks compared with 2-years old guinea-pigs and frequency-response curves were significantly different from each other.

Contractile responses to exogenous α,β-methylene ATP and noradrenaline

α,β-methylene ATP elicited concentration-dependent contractile responses significantly larger in strips from 2-weeks than 1-day old guinea-pigs (Figure 4a). However, contractile responses were significantly reduced in 12-weeks and 2-years old animals. The pD_2 values were: 6.31 ± 0.34, 5.91 ± 0.27, 4.74 ± 0.20 and 5.22 ± 0.08 in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively. A significant difference was shown between pD_2 values of the following experimental groups: 1-day vs 12-weeks, 1-day vs 2-years, and 2-weeks vs 12-weeks. Contractile responses to the highest concentration of α,β-methylene ATP tested were (mN mg^{-1} tissue): 0.113 ± 0.012, 0.248 ± 0.022, 0.163 ± 0.016 and 0.200 ± 0.008 in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively. These responses were significantly larger in 2-weeks vs 1-day old animals. A significant difference was also shown between the following experimental groups: 1-day vs 12-weeks, 1-day vs 2-years, and 2-weeks vs 12-weeks.

Figure 4b shows the concentration–response curves to noradrenaline in all groups: strips from new-born guinea-pigs exhibited a larger contraction in response to noradrenaline than strips from 2-weeks, 12-weeks and 2-years old guinea-pigs. Concentration-response curves differed significantly from each other. The pD_2 values were: 4.79 ± 0.05, 4.64 ± 0.20, 4.15 ± 0.13 and 4.60 ± 0.11 in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively. Contractions to the highest concentration of noradrenaline tested were (mN mg^{-1} tissue): 1.017 ± 0.018, 0.734 ± 0.054, 0.621 ± 0.033 and 0.411 ± 0.014 in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs, respec-

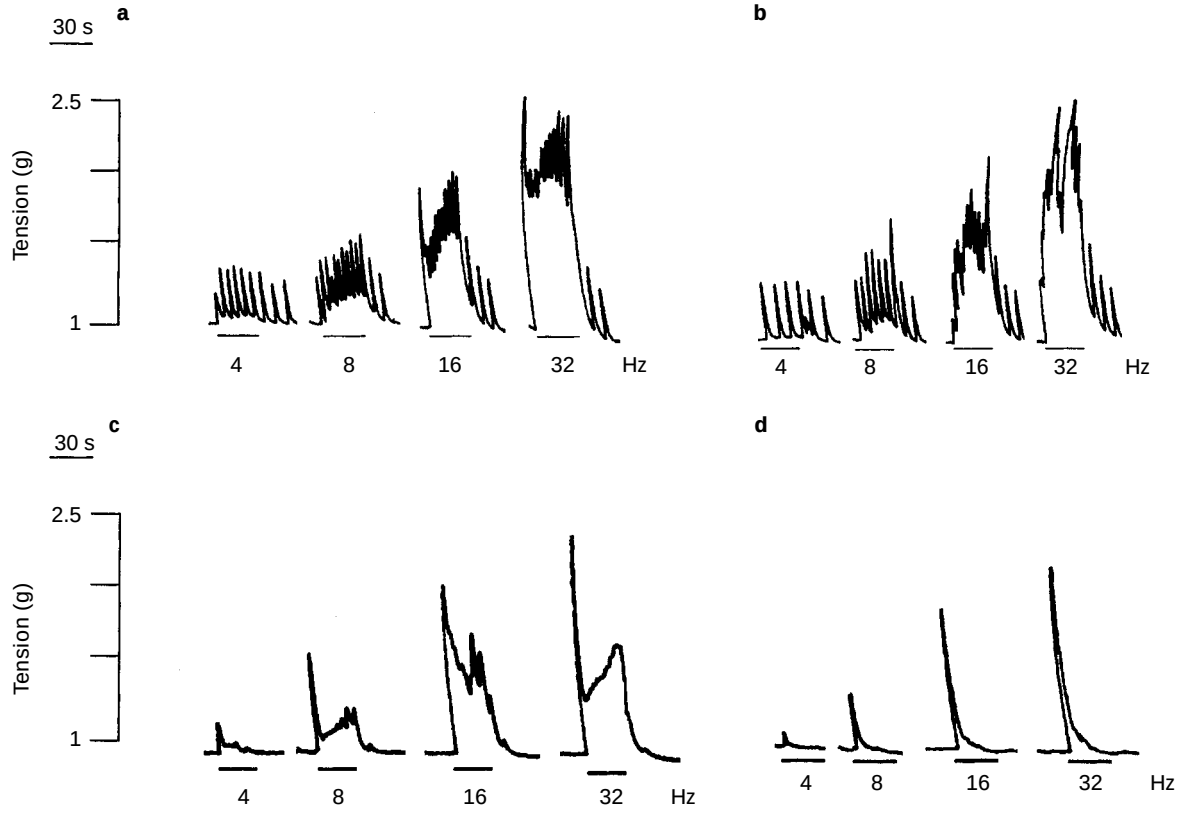

Figure 2 Recordings of contractile responses (g) evoked by EFS (80 V, 0.5 ms for 30 s, 4–32 Hz) in longitudinal smooth muscle strips of seminal vesicle excised from 12-weeks old guinea-pigs before (a) and after (b) desensitization of P2X receptors by α,β-methylene ATP (10^{-4} M) and before (c) and after (d) incubation with prazosin (10^{-6} M). About 30% of the preparations excised from adult guinea-pigs exhibited spontaneous activity.

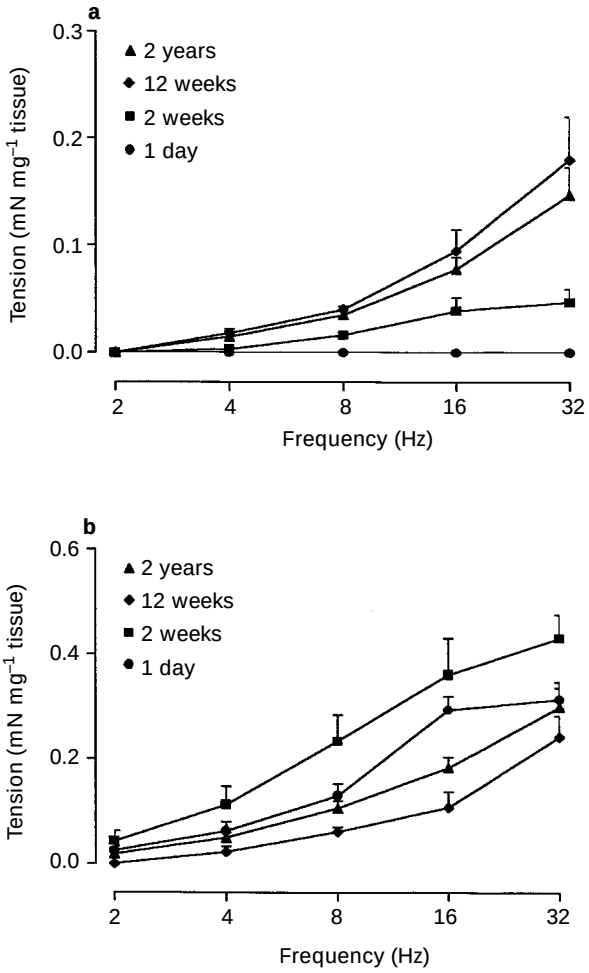

Figure 3 Frequency-response curves to EFS (80 V, 0.5 ms for 30 s, 2–32 Hz) in longitudinal smooth muscle strips of seminal vesicle in the presence of prazosin 10^{-6} M (a) and after desensitization of P2X receptors by α,β-methylene ATP 10^{-4} M (b) in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs. Frequency-dependent curves for the purinergic (a) and adrenergic responses (b) differed significantly between experimental groups. However, no significant difference was shown between adrenergic responses in adult and aged guinea-pigs. Points show mean and vertical lines s.e.mean of 6 experiments, unless occluded by symbol.

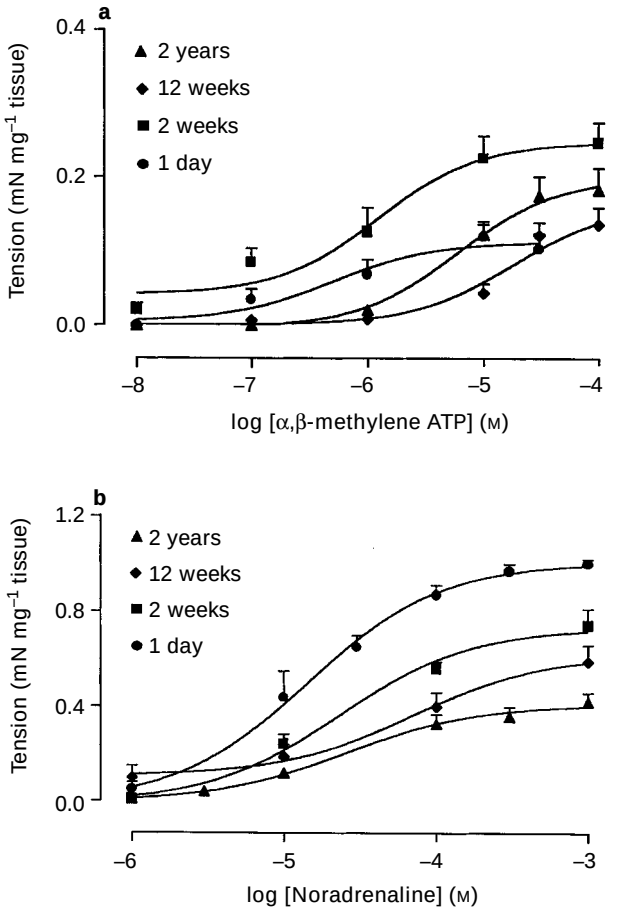

Figure 4 Non-cumulative concentration-response curves to α,β-methylene ATP (a) and noradrenaline (b) in longitudinal smooth muscle strips of seminal vesicle in 1-day old, 2-weeks, 12-weeks old and 2-years old guinea-pigs. Concentration-response curves to α,β-methylene ATP and to noradrenaline differed significantly between experimental groups. However, no significant difference was shown between responses induced by noradrenaline in young and adult guinea-pigs. Points show mean and vertical lines s.e.mean of 6 experiments, unless occluded by symbol.

tively. These responses to noradrenaline were significantly higher in new-born animals, compared with 2-weeks, 12-weeks and 2-years old guinea-pigs. Furthermore, a significant difference was shown between the following groups: 2-weeks vs 2-years and 12-weeks vs 2-years old animals.

Contraction induced by KCl (240 mM) was similar between the experimental groups, being (mN mg^{-1} tissue) 71 ± 12, 72 ± 12, 70 ± 7.5 and 77 ± 8.8 in 1-day, 2-weeks, 12-weeks and 2-years old guinea-pigs, respectively.

Discussion

It is well known that ATP and noradrenaline are cotransmitters in the sympathetic innervation of the sexual organ and lower urinary tract (Hoyle *et al*., 1994; Burnstock, 1995). The plasticity of the autonomic innervation of these tissues has been demonstrated in several pathophysiological conditions, including pregnancy (Alm *et al*., 1979), development (Kiruluta *et al*., 1986; Brauer *et al*., 1994a,b) and ageing (Gilpin *et al*., 1986). The present study provides original evidence for age-related changes in both purinergic and adrenergic components of sympathetic neurotransmission in the guinea-pig seminal vesicles.

In our experimental conditions, neurogenic responses to EFS were biphasic, the first component being blocked by desensitization of P2X receptors by α,β-methylene ATP and the second tonic component being selectively inhibited by prazosin, which confirms that ATP and noradrenaline are cotransmitters in the guinea-pig seminal vesicles (Meldrum & Burnstock, 1985). The phasic response to EFS was not present in 1-day old guinea-pigs, while it was detectable in 2-weeks old animals and was significantly increased in 12-weeks and 2-years old guinea-pigs, thus indicating a lack of purinergic transmission in new-born animals and an increase in the purinergic component with age.

The evaluation of contractile response to α,β-methylene ATP indicated that both pre- and postjunctional mechanisms could account for the observed changes. Lack of purinergic responses to EFS in 1-day old animals was associated with a detectable responsiveness to α,β-methylene ATP, which indicates the presence of postjunctional receptors for ATP and suggests a lack of ATP release in new-born guinea-pigs. Moreover, a significantly higher pD_2 value in the new-born compared with the adult guinea-pig indicates a higher tissue sensitivity to the exogenous agonist, which would occur in the absence on an endogenous purinergic neurotransmitter. These data further support the hypothesis of a lack of purinergic transmission in 1-day old animals. Purinergic transmission in 2-weeks old animals correlated with a higher responsiveness to the ATP analogue, when compared with new-born guinea-

pigs. As larger contractile responses were observed, without changes in the sensitivity (pD_2 values) to the agonist, it can be inferred that together with prejunctional mechanisms, a larger number of receptors for ATP contributes postjunctionally to the increase in purinergic transmission. In adult and aged guinea-pigs the larger purinergic transmission was accompanied by a reduced responsiveness to α,β-methylene ATP, as indicated by reduced contractile responses to the highest concentration of agonist tested and by a reduced receptor sensitivity (pD_2 values), compared with 2-weeks old guinea-pigs. These data suggest that in adult and aged guinea-pigs prerather than postjunctional mechanisms account for the increased purinergic transmission. Prejunctional changes of autonomic neurotransmission might include variation in the neuronal supply to the tissue and/or modification of the neuronal mechanisms of release/uptake, as discussed below.

Tonic contractile responses to EFS were larger in 2-weeks than in 1-day old guinea-pigs, while responsiveness to exogenous noradrenaline was reduced, thus indicating an increased adrenergic transmission in young compared with newborn guinea-pigs via prejunctional mechanisms. However, in contrast to the purinergic component of sympathetic transmission, which increased with age, tonic adrenergic responses to EFS were reduced at 12-weeks and 2-years with respect to 2-weeks old guinea-pigs. Similarly, contractile responses to exogenous noradrenaline gradually decreased with age. A decreased tissue responsiveness to the exogenous transmitter indicates that postjunctional mechanisms are involved in the observed reduction of adrenergic neurotransmission. However, prejunctional events cannot be ruled out by the present results. As the pD_2 values for noradrenaline were not significantly different between the experimental groups, while contractile responses to the highest concentration tested declined with age, a reduced number of postjunctional α-adrenoceptors could contribute to the age-related decline of adrenergic transmission in guinea-pig seminal vesicles. The decline of adrenergic responses with age is in line with an unchanged or reduced adrenergic responsiveness with increased age largely documented in animals and man (Docherty, 1990).

As the depolarizing effect of KCl did not significantly differ between the experimental groups, the observed changes cannot be ascribed to age-related changes in the contractile machinery of the smooth muscle and should be considered as specific alterations in both purinergic and adrenergic responsiveness.

Taken together these data show that, in new-born guinea-pigs, adrenergic mechanisms are mainly responsible for the sympathetic neurotransmission, despite the presence of P2X receptors for ATP and both purinergic and adrenergic components being fully expressed in young animals. In adult sexually mature guinea-pigs (12-weeks old), the purinergic transmission is further increased, while the adrenergic mechanisms of neurotransmission are reduced, leading to speculation on the possible role of purinergic mechanisms in relation to sexual maturity and reproduction. In aged animals purinergic transmission is not significantly affected with respect to adult guinea-pigs, in contrast to a further reduction in the adrenergic responsiveness.

Prejunctional mechanisms responsible for changes in the purinergic and adrenergic components of sympathetic neurotransmission might include a different synthesis and release of neurotransmitter and/or modifications in the mechanisms of neuronal uptake, as well as changes in the neuronal supply of the studied tissue. The pharmacological approach used in the present study does not allow any conclusion to be reached in this respect. However, the parallel evaluation of both components of sympathetic neurotransmission suggests an increased innervation of the seminal vesicles in young compared with new-born guinea-pigs, as both purinergic and adrenergic transmission were enhanced. In contrast, in adult and aged animals, since noradrenaline and ATP are co-stored in and co-released from sympathetic nerves and the increase of the purinergic component paralleled the decrease of the adrenergic neurotransmission, it is unlikely that a different number of neuronal fibres accounts for the observed changes. The inverse relationship between purinergic and adrenergic components of sympathetic neurotransmission suggests rather an age-related variation in the ratio of noradrenaline/ATP release. Previous studies in the rat tail artery have shown a reduction of the purinergic component of sympathetic neurotransmission with age (Bao *et al*., 1989). Furthermore, the ratio of catecholamine : ATP expression in the adrenal medulla varies during development and ageing, being 6 : 1 at 18 days and increasing to more than 10 : 1 in the adult rat (O'Brien *et al*., 1972). The contrasting data obtained in the present study, showing an increase in the purinergic component with age, would support the hypothesis that full development of purinergic transmission can be related to sexual maturity and reproduction.

In summary, the present results demonstrate age-related changes of both purinergic and adrenergic components of sympathetic neurotransmission in the guinea-pig seminal vesicles. While the purinergic transmission is not fully developed in the new-born animal and increases with age, the adrenergic component is present at birth and decreases with age.

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