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Adrenergic and purinergic components in bisected vas deferens from spontaneously hypertensive rats

[1]Mònica Guitart, [2]Jesús Giraldo, [3]Eduard Goñalons, [1]Elisabet Vila & *,[1]Albert Badia

[1]Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; [2]Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain and [3]Departament de Biologia Cel.lular i Fisiologia, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

1 Purinergic and adrenergic components of the contractile response to electrical field stimulation (EFS) have been investigated in epididymal and prostatic portions of Wystar Kyoto (WKY) and spontaneously hypertensive rat (SHR) vas deferens.

2 In both halves of SHR and WKY vas deferens, EFS (40 V, 0.5 ms for 30 s, 0.5–32 Hz) evoked frequency-related contractions. The neurogenic responses were biphasic, consisting of a rapid non-adrenergic response, dominant in the prostatic portion, followed by a slow tonic adrenergic component, dominant in the epididymal half.

3 Phasic and tonic components of the frequency-response curves evoked by EFS were significantly higher in the epididymal but not in the prostatic portion of vas deferens from SHR compared to WKY rats.

4 The α_1-adrenoceptor antagonist prazosin (0.1 μM) was more effective against both components of the contractile response in the epididymal end of SHR than in WKY rats.

5 Inhibition by α,β-methylene adenosine 5′-triphosphate (α,β-meATP 3 and 30 μM) was higher in both components of the contractile responses in WKY preparations than in SHR.

6 Combined α_1-adrenoceptor and P_{2x}-purinoceptor antagonism virtually abolished the EFS-evoked contractile response in both strains. The degree of inhibition by prazosin (0.1 μM) after P_{2x}-purinoceptor blockade was higher in SHR than in WKY rats.

7 These results demonstrate a modification in the purinergic and noradrenergic contribution to neurogenic responses in SHR and WKY animals besides a co-participation of ATP and noradrenaline in both contractile components of the response to EFS.

Keywords: Epididymal half; prostatic half; vas deferens; SHR; purinergic neurotransmission; noradrenergic neurotransmission

Abbreviations: α,β-meATP, α,β-methylene adenosine 5′-triphosphate; $ANAPP_3$, arylazidoaminopropionyl-adenosine 5′-triphosphate; ANOVA, two-way analysis of variance; ATP, adenosin 5′-triphosphate; EFS, electrical field stimulation; SHR, spontaneously hypertensive rats; WKY, Wystar Kyoto rats

Introduction

One of the most extensively investigated examples of co-transmission in the peripheral nervous system has been the rodent vas deferens, where adenosine 5′-triphosphate (ATP) is co-released with noradrenaline from sympathetic innervation (Westfall *et al*., 1978; Fedan *et al*., 1981; Sneddon & Westfall, 1984; Burnstock, 1990; Von Kügelgen & Starke, 1991). In the vas deferens of rat, mouse, rabbit and guinea-pig, the electrical field stimulation (EFS) produces a biphasic contraction of the smooth muscle (McGrath, 1978; Sneddon *et al*., 1984; Von Kügelgen & Starke, 1991; Ralevic & Burnstock, 1996). The initial peak (phasic component or twitch), resistant to α-adrenoceptor or cholinergic blockade, has been shown to be mediated by ATP acting on P_{2x}-purinoceptors. This response is blocked by arylazidoaminopropionyl-ATP ($ANAPP_3$) (Fedan *et al*., 1981), by selective desensitization of P_{2x}-purinoceptors using α, β-methylene ATP (α,β-meATP) (Meldrum & Burnstock, 1983; Allcorn *et al*., 1986) and by suramin (Dunn & Blakely, 1988; Mallard *et al*., 1992). The second, slower contraction (tonic component), is adrenergic, since it is blocked by reserpine pretreatment or by α-adrenoceptor antagonists (Sneddon & Westfall, 1984; Sneddon *et al*., 1984; Von Kügelgen & Starke, 1991). In mouse, rabbit and guinea-pig vas deferens, each component of the response to EFS is easily isolated using selective α_1-adrenoceptor or P_{2x}-purinoceptor antagonists to inhibit one component of the biphasic contraction, indicating that ATP and noradrenaline are the main neurotransmitters of phasic and tonic responses respectively (Meldrum & Burnstock, 1983; Kasakov *et al*., 1988; Burnstock, 1990; Von Kügelgen & Starke, 1991). The underlying electrical responses to nerve stimulation, the excitatory junction potentials, are also thought to be mediated by ATP (Sneddon *et al*., 1982; Sneddon & Burnstock, 1984; Brock *et al*., 1990; Stjärne & Stjärne, 1997).

An important feature relevant to the function of the rodent vas deferens is the existence of a regional variation in the contractile response to nerve stimulation along the length of the tissue (Anton *et al*., 1977; Sneddon & Machaly, 1992). Moreover, one of each component of the contractile response is dominant in one end of the organ. Segments from the prostatic end of the muscle exhibit primarily a rapid, phasic, non-adrenergic response whilst segments from the epididymal end show mainly a slower, tonic, adrenergic contraction (Brown *et al*., 1983; Sneddon & Machaly, 1992). The regional variation observed in rodent vas deferens is due to a different postjunctional sensitivity of the smooth muscle cells to ATP

*Author for correspondence.

and noradrenaline (Anton *et al*., 1977; Sallés & Badia, 1991; Sneddon & Machaly, 1992). Thus, the prostatic end is approximately ten times more sensitive to exogenous ATP and ten times less sensitive to adrenoceptor agonists than the epididymal end (French & Scott, 1983; Sallés & Badia, 1991; Sneddon & Machaly, 1992).

The spontaneously hypertensive rats (SHR) have been widely used as a pathophysiological animal model for human essential hypertension (Okamoto & Aoki, 1963). The vascular smooth muscle of SHR exhibits an altered responsiveness to α_1-adrenoceptor agonists (Vila *et al*., 1993; Tabernero *et al*., 1996) and EFS (Muir & Wardle, 1989) when compared to tissues from normotensive Wistar Kyoto (WKY) rats. This phenomenon has also been demonstrated in non-vascular smooth muscle from SHR, such as stomach (Altman *et al*., 1977) and vas deferens (Katsuragi *et al*., 1991; Vivas *et al*., 1997). The contractions induced by EFS of sympathetic nerve endings (Katsuragi *et al*., 1991) and by exogenous noradrenaline (Vivas *et al*., 1997) are increased in vas deferens from SHR when compared to WKY rats. However, the endogenous content and the electrically evoked release of noradrenaline from whole vas deferens are almost identical in SHR and WKY (Katsuragi *et al*., 1991). These facts could indicate a general alteration of the smooth muscle function in SHR possibly related to modifications in α_1-adrenoceptors (Mulvany, 1988). An increase (Caufield *et al*., 1977; Caricati-Neto *et al*., 1992; Vivas *et al*., 1997), no modification (Corbett *et al*., 1980; Sakai *et al*., 1984) and even a decrease (Docherty & Warnock, 1986) in postjunctional α_1-adrenoceptor mediated responses are reported in vas deferens from SHR. On the other hand, an enhanced purinergic contribution to the co-transmission process has been described in the SHR isolated tail artery (Vidal *et al*., 1986) as compared to WKY. This enlarged purinergic component in the EFS-induced contraction could not be confined to this tissue and exists in others. In contrast, other authors have reported that the enhancement of sympathetic nerve-mediated contractions observed in tail artery (Dalziell *et al*., 1989; Muir & Wardle, 1989), mesenteric artery (Muir & Wardle, 1989) and whole vas deferens (Katsuragi *et al*., 1991) from SHR animals do not seem to be related to a greater contribution of ATP to the contractile response.

The present study was designed to elucidate the relative contribution of the adrenergic and purinergic components of the EFS-induced contraction in the epididymal and prostatic portions of vas deferens from SHR and WKY animals. Our experimental strategy was to use selective antagonists to determine the involvement of noradrenaline, ATP or both, in the phasic and tonic components of the contraction induced by EFS in SHR and WKY bisected vas deferens.

Methods

Tissue preparations and recording of mechanical activity

The experiments were performed on 16–18-week-old male SHR (365.31 ± 6.23 g) and age-matched normotensive WKY rats (385 ± 5.48 g) supplied by Iffa-Credo, France. The animals were killed by decapitation and exsanguination. Vasa deferentia were quickly removed and placed in modified Krebs-Henseleit physiological salt solution of the following composition (mM): NaCl 112.0; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.1; $MgSO_4$ 1.2; $NaHCO_3$ 25.0 and glucose 11.1. The preparation was cleaned from connective tissue and transversally bisected giving the epididymal and the prostatic ends.

Epididymal and prostatic portions, about 1 cm in length, were set up in a 20 ml organ bath containing PSS maintained at $32 \pm 0.5^{\circ}$C and continuously gassed (95% O_2, 5% CO_2). The preparations were left to equilibrate for 45 min at a resting tension of 4.9 mN with bath fluid changes every 15 min. Tension was readjusted if necessary. Responses were recorded isometrically by a Harvard UF1 force displacement transducer and displayed on an Omniscribe two channel pen recorder.

Experimental procedure

Electrical field stimulation was applied using a Grass S88 stimulator through two platinum-wire rings, one on either side

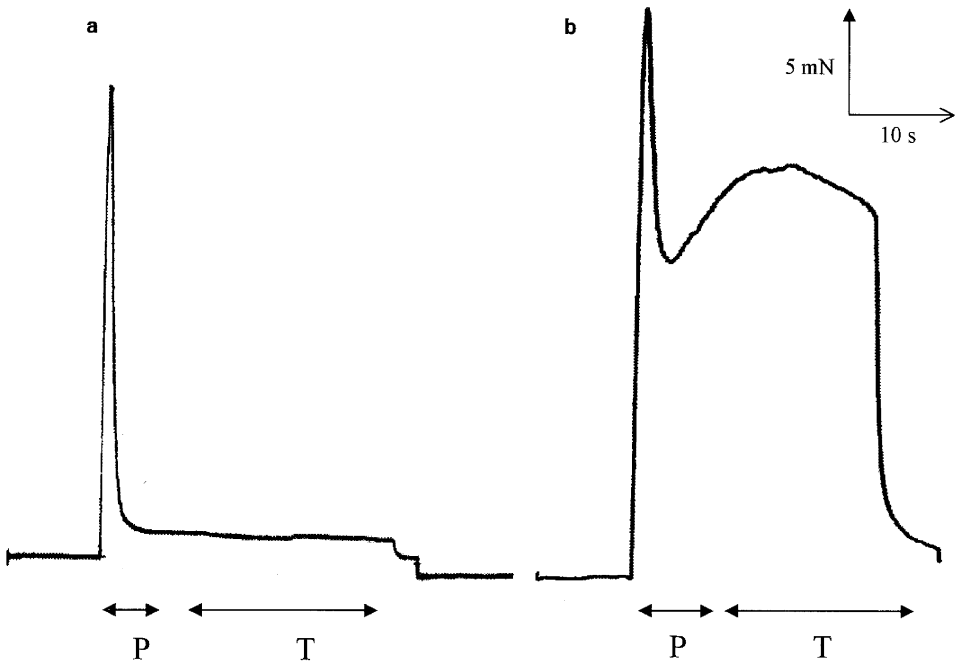

Figure 1 Recordings of contractile responses (mN) evoked by EFS (40 V, 0.5 ms for 30 s, 4 Hz) in the prostatic (a) and epididymal (b) portions of rat vas deferens. Phasic (P) and tonic (T) components of the EFS contractile response are shown.

of the tissue. Responses to EFS were obtained at 0.5–16 Hz using 30 s train pulses at 40 V and 0.5 ms pulse duration. These responses were abolished by tetrodotoxin (1 μM) and guanethidine (10 μM), indicating that they were mediated entirely by sympathetic nerves. From pilot experiments, it was found that three frequency-response curves separated by a 30 min period could be obtained with each tissue, so a multiple curve design was used. This methodology allows a comparison of the frequency-response curves in three situations without involving tissue differences. In a first set of experiments, two frequency-response curves were obtained in the absence and in the presence of either prazosin (0.1 μM) or α,β-meATP (3, 30 or 100 μM) for 30 min. Each preparation was used to test a single concentration of each drug. In the second set of experiments, three frequency-response curves were obtained in each tissue. The first and second curves were carried out before and after the incubation of α,β-meATP (100 μM) for 30 min, and the third one with the further addition of prazosin (0.1 μM) for 30 min. Results were expressed either in absolute values (mN) or as a percentage of the maximal contractioin (at 16 Hz stimulation) reached in the first curve.

Statistics

All data in the text and figures are expressed as mean ± s.e.mean. The number of experiments performed (n) are indicated in the figures. The dependency of contractile response on treatment and frequency was studied by a two-way analysis of variance (ANOVA) within the framework of the general linear model approach (Littell *et al*., 1991). Planned contrasts were used to test for differences among the levels of treatment factor at selected frequencies (vertical pairwise comparisons). P values were adjusted according to Šidák (1967) procedure. For specific two means comparisons Student's t-test was used. Statistical significance was set as a P value of less than 0.05. Statistical analyses were carried out with the SAS/STAT® statistical package (SAS Institute Inc., 1989).

Drugs

(−)-Noradrenaline bitartrate, prazosin hydrochloride, α,β-meATP (lithium salt) and tetrodotoxin were obtained from Sigma Chemical Co. Noradrenaline was made up as a stock solution of 100 μM in 0.1 mM ascorbic acid and diluted in physiological salt solution. Prazosin was made up as a stock solution of 10 μM in 30% ethanol and diluted in physiological salt solution. All other chemicals used were of analytical grade and supplied by Merck.

Results

Contractile responses to electrical field stimulation

Figure 1 shows a typical tracing of the neurogenic response to EFS at 4 Hz in the prostatic (Figure 1a) and epididymal (Figure 1b) portions of rat vas deferens. It consisted of two clearly discernible phases, an initial short-lasting twitch (phasic) component that declines and is replaced by a second slower (tonic) component. There was a regional variation in the contractile response to nerve stimulation along the length of the vas deferens: segments from the prostatic end (Figure 1a) exhibited primarily a phasic response, while the epididymal end showed mainly a tonic response (Figure 1b).

Phasic and tonic components of EFS-induced contractions of the prostatic end are shown in Figure 2a (phasic) and Figure 2b (tonic). No significant differences were observed between strains neither in the phasic nor the tonic components of the frequency-response curve. In the epididymal portion, however, the phasic (Figure 3a) and the tonic (Figure 3b) phases of the response were significantly greater in SHR than in WKY rats ($P<0.0001$). Responses obtained at high (4–16 Hz) but not those obtained at low (0.5–2 Hz) frequencies of stimulation were significantly increased ($P<0.05$) in SHR.

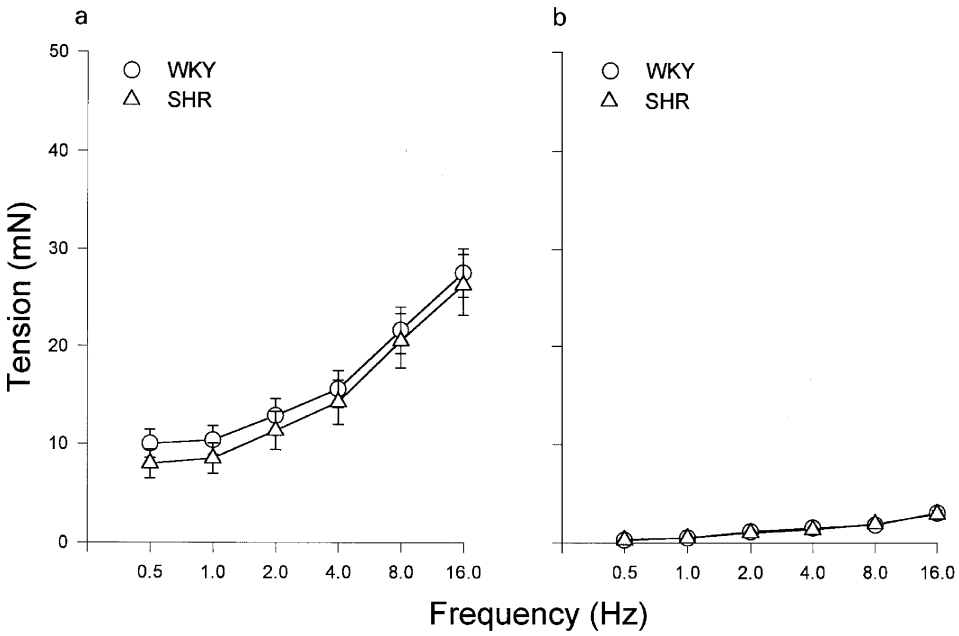

Figure 2 Frequency-response curves to EFS (40 V, 0.5 ms for 30 s, 0.5–16 Hz) in the prostatic portion of vas deferens from SHR and WKY rats. (a) Phasic and (b) tonic components of the contractile response. Results are expressed as absolute values (mN). Points are mean ± s.e.mean of at least 23 experiments. SHR and WKY curves are not significantly different; two-way (strain, frequency) ANOVA with repeated measures on frequency factor.

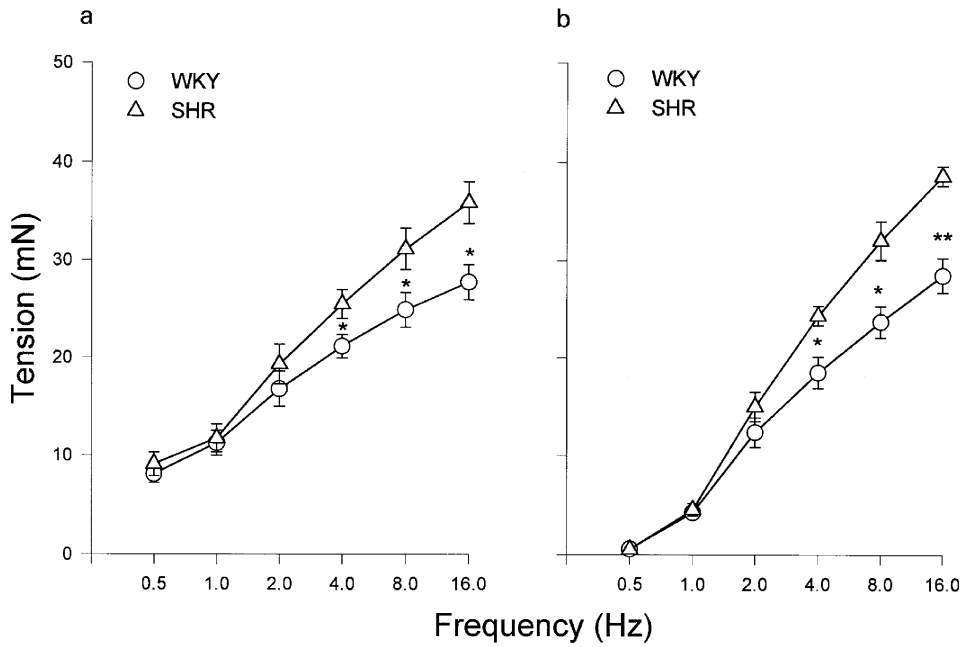

Figure 3 Frequency-response curves to EFS (40 V, 0.5 ms for 30 s, 0.5–16 Hz) in the epididymal portion of vas deferens from SHR and WKY rats. (a) Phasic and (b) tonic components of the contractile response. Results are expressed as absolute values (mN). Points are mean ± s.e.mean of 26 experiments. SHR and WKY curves diverge significantly ($P<0.0001$); two-way (strain, frequency) ANOVA with repeated measures on frequency factor. Vertical pairwise contrasts: $*P<0.05$, $**P<0.01$.

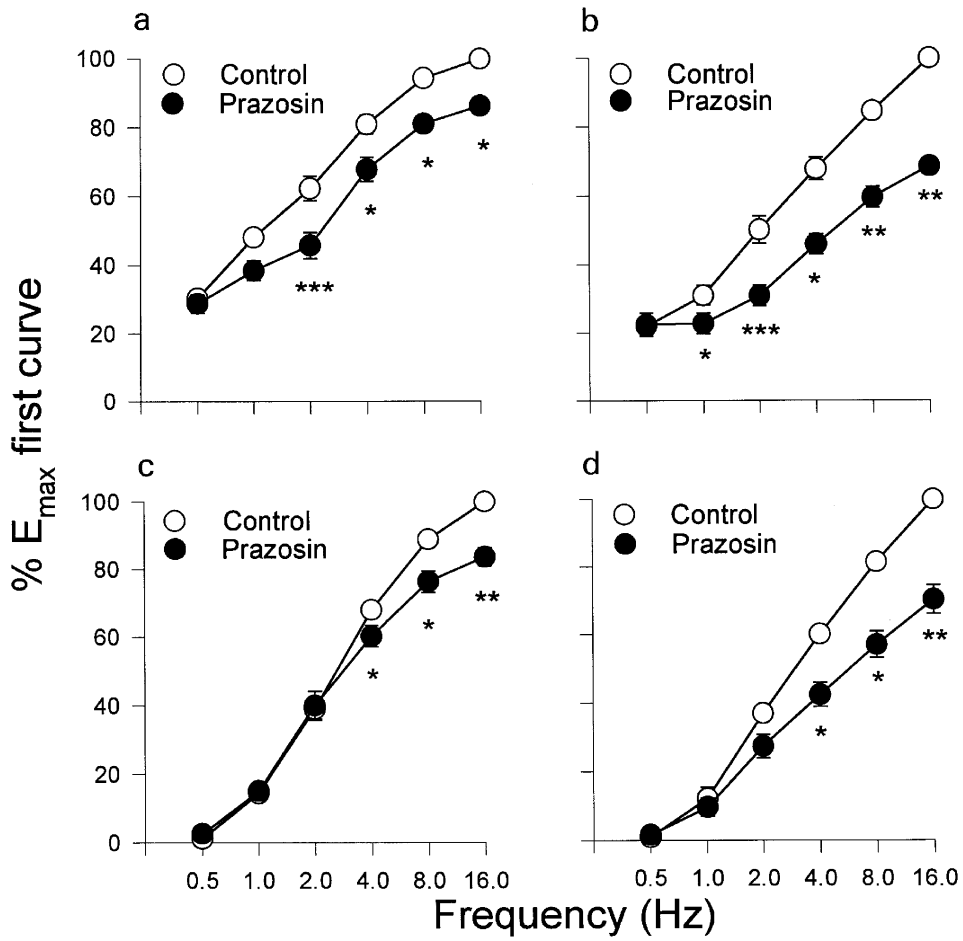

Figure 4 Frequency-response curves to EFS (40 V, 0.5 ms for 30 s, 0.5–16 Hz) in the epididymal portion of vas deferens in the absence (control) and presence of prazosin (0.1 μM). Phasic component in (a) WKY and (b) SHR. Tonic component in (c) WKY and (d) SHR. Points are mean ± s.e.mean of six experiments expressed as a percentage of the maximal contraction (E_{max}) obtained in the first curve. Control and prazosin curves diverge significantly in all figures ($P<0.0001$); two-way (treatment, frequency) ANOVA with repeated measures on both factors. Vertical pairwise contrasts: $*P<0.05$, $**P<0.01$, $***P<0.001$.

Effects of α_1-adrenoceptor blockade

Phasic component of the neurogenic response was reduced ($P<0.0001$) by prazosin (0.1 μM) in WKY (Figure 4a) and SHR (Figure 4b). The degree of inhibition of the maximal contraction by the α_1-adrenoceptor antagonist was greater ($P<0.05$) in hypertensive ($33.0\pm3.3\%$) than in normotensive ($13.7\pm1.1\%$) rats. However, when maximum responses were expressed as absolute values, no differences after prazosin incubation were observed between SHR (22.6 ± 1.6 mN) and WKY (21.4 ± 1.3 mN) animals. The tonic component of the response was also reduced ($P<0.0001$) by prazosin in WKY (Figure 4c) and SHR (Figure 4d). Prazosin inhibited to a greater extent ($P<0.05$) the tonic adrenergic component in SHR ($29.7\pm2.6\%$) than in WKY ($15.4\pm2.1\%$) at 16 Hz stimulation. When this maximal response was represented as absolute values, the remaining fraction after α_1-adrenoceptor blockade did not differ between normotensive (23.8 ± 1.1 mN) and hypertensive (25.2 ± 2.2 mN) animals.

Effects of P_{2x}-purinoceptor blockade

Application of α,β-meATP (3, 30 or 100 μM) for 30 min to desensitize P_{2x}-purinoceptors produced a quick and short contraction which returned to basal line in 15 s. α,β-meATP (3 μM) reduced ($P<0.0001$) the phasic frequency-response curve in normotensive animals (Figure 5a), but not in hypertensive rats (Figure 5b). Application of α,β-meATP (30 μM) further decreased ($P<0.0001$) the phasic component of the contractile response in WKY rats and significantly reduced the maximal response in SHR. The tonic component of the response was reduced ($P<0.0001$) by α,β-meATP (3 μM) in both strains (Figure 5c and d, respectively). The degree of reduction of the maximal response produced by the purinoceptor antagonist was greater ($P<0.05$) in the normotensive ($37.3\pm6.3\%$) than in the hypertensive ($26.5\pm3.0\%$) animals. α,β-meATP (30 μM) further reduced the maximal tonic response to EFS in SHR ($P<0.0001$) but not in WKY rats. A higher concentration of α,β-meATP (100 μM) did not increase the inhibitory effects of the antagonist (30 μM) in any of the contractile response components neither in SHR nor WKY rats.

Combined α_1-adrenoceptor and P_{2x}-purinoceptor blockade

To determine the contribution of noradrenaline and ATP on both components of the contractile response, the influence of prazosin (0.1 μM) was studied after blockade of P_{2x}-purinoceptors with α,β-meATP (100 μM). The addition of prazosin in the presence of α,β-meATP almost abolished the phasic and the tonic component of the contraction to EFS in

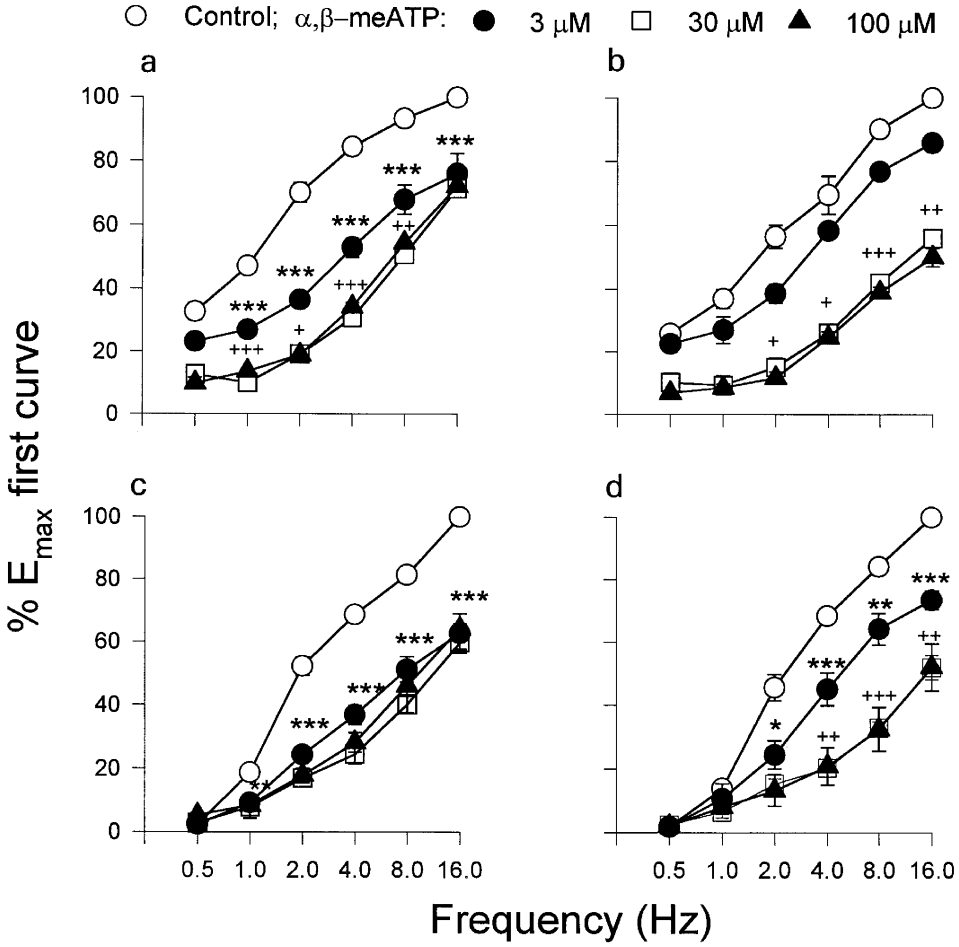

Figure 5 Frequency-response curves to EFS (40 V, 0.5 ms for 30 s, 0.5–16 Hz) in the epididymal portion of vas deferens in the absence (control) and presence of α,β-meATP (3, 30 or 100 μM). Phasic component in (a) WKY rats and (b) SHR. Tonic component in (c) WKY rats and (d) SHR. Points are mean$\pm$s.e.mean of six experiments expressed as a percentage of the maximal contraction (E_{max}) obtained in the first curve. Minimal concentration values at which α,β-meATP and control curves diverge significantly: 3 μM (a,c,d), 30 μM (b). Minimal concentration values from which a concentration increase of α,β-meATP does not reduce EFS response: 3 μM (c), 30 μM (a,b,d); two-way ANOVA with repeated measures on frequency factor. Vertical pairwise contrasts: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ control *vs* 3 μM and $+P<0.05$, $++P<0.01$, $+++P<0.001$ 3 μM *vs* 30 μM.

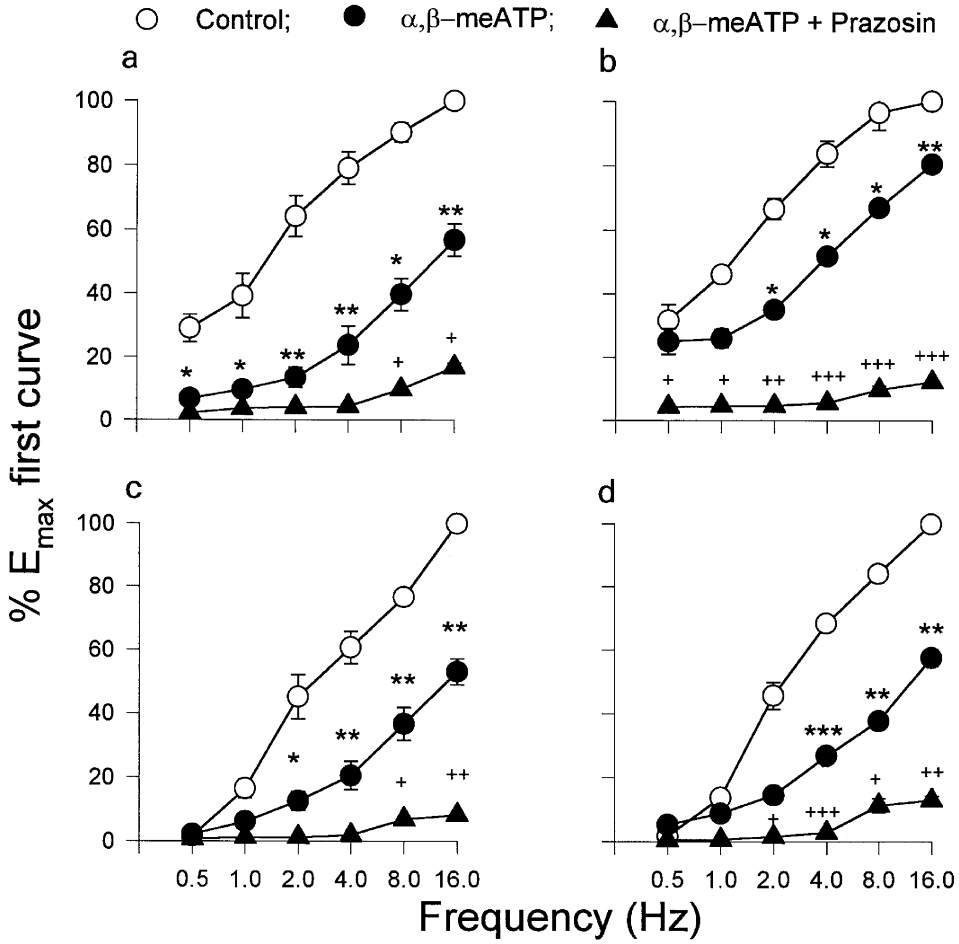

Figure 6 Frequency-response curves to EFS (40 V, 0.5 ms for 30 s, 0.5–16 Hz) in the epididymal portion of vas deferens in the absence (control) and presence of α,β-meATP (100 μM) and α,β-meATP (100 μM) plus prazosin (0.1 μM). Phasic component in (a) WKY and (b) SHR and tonic phase in (c) WKY and (d) SHR. Points are mean±s.e.mean of four experiments expressed as a percentage of the maximal contraction (E_{max}) obtained in the first curve. In all figures α,β-meATP *vs* control and α,β-meATP plus prazosin *vs* α,β-meATP curves diverge significantly ($P<0.0001$); two-way (treatment, frequency) ANOVA with repeated measures on both factors. Vertical pairwise contrasts: $*P<0.05$, $**P<0.01$, $***P<0.001$ α,β-meATP *vs* control and $+P<0.05$, $++P<0.01$, $+++P<0.001$ α,β-meATP plus prazosin *vs* α,β-meATP.

both strains (Figure 6). All that remained was a small, slow, monophasic response. The degree of reduction of the phasic component by prazosin in the presence of α,β-meATP was greater ($P<0.005$) in SHR (68.2±0.9%) than in WKY (42.6±2.0%) rats. Nevertheless, no differences were observed in the degree of reduction after prazosin in the tonic phase between SHR (44.5±2.9%) and WKY (44.9±4.2%) animals.

Discussion

Most of the early studies establishing sympathetic co-transmission of noradrenaline and ATP were performed on the whole rodent vas deferens (Westfall *et al*., 1978; Fedan *et al*., 1981; Meldrum & Burnstock, 1983; Allcorn *et al*., 1986). It was shown that the biphasic contractile response to single pulse EFS consisted of an initial rapid twitch response blocked by desensitization of P_{2x}-purinoceptors with α,β-meATP and predominantly mediated by ATP, and a secondary tonic contraction blocked by the α_1-adrenoceptor antagonist prazosin and mediated predominantly by noradrenaline (Burnstock, 1990; Von Kügelgen & Starke, 1991). The employment of the whole vas deferens now seems inexpedient for two reasons: (i) Interpretation of the response in the whole vas is complex, whether this is with respect to nerve stimulation or to agonists and (ii) it is clearly demonstrated that both ends of the organ show a different relative sensitivity to noradrenaline and ATP (Brown *et al*., 1983; Sneddon & Machaly, 1992). Similarly, previous studies on SHR and WKY rat vas deferens were also carried out in whole vas (Caufield *et al*., 1977; Katsuragi *et al*., 1991; Caricati-Neto *et al*., 1992). However, comparison of the two ends of the organ has been performed to further study modifications due to hypertension (Sakai *et al*., 1984; Docherty & Warnock, 1986; Vivas *et al*., 1997). The existence of an adrenergic and a non-adrenergic component in the biphasic contractile response in both halves of the rat vas deferens, therefore, needs further studies to set their relative contribution to the modified contractile response in hypertensive animals.

In our experimental conditions, neurogenic responses to EFS in the bisected vas deferens were biphasic either in SHR or WKY rats. In the epididymal, but not in the prostatic half of the vas deferens, phasic and tonic components of the contractile response to EFS were higher in hypertensive than in normotensive animals. These results agree with previous observations in the whole rat vas deferens where the contractile response to sympathetic nerve stimulation was greatly enhanced in the SHR compared to WKY (Katsuragi *et al*., 1991). It could also be related to the reported increase in density of α_1-adrenoceptors in the epididymal portion of SHR

vas deferens (Caufield *et al*., 1977; Caricati-Neto *et al*., 1992; Vivas *et al*., 1997). To clarify the role of the two putative neurotransmitters in each component of the contractile response, the influence of selective antagonists was studied. In contrast to previous results obtained in whole vas deferens from guinea-pig or rabbit (Meldrum & Burnstock, 1983; Sneddon & Westfall, 1984; Sneddon *et al*., 1984), where the selective antagonists abolished one component of the response leaving intact the other, prazosin and α,β-meATP affected both phases of the contractile response in the epididymal end of rat vas deferens. Similar conflicting results emerging from experimentation with rat vas deferens have been previously reported (Ralevic & Burnstock, 1996) when the effects of hypophysectomy in both components of the EFS-induced contraction in vas deferens were studied.

The relative resistance of the phasic and tonic components of the contractile response to inhibition by the antagonists used differed between strains. Thus, prazosin diminished the tonic adrenergic component and the phasic non-adrenergic response in both groups of animals, but a greater inhibition of both components of the contractile response was obtained in SHR. This is in contrast with results obtained in the guinea-pig vas deferens, where the fast component of the biphasic contractile response was only affected by P_{2x}-purinoceptors antagonists (Meldrum & Burnstock, 1983; Kasakov *et al*., 1988). The maximal response of the phasic and tonic components was more resistant to α_1-adrenoceptor blockade in WKY than in SHR, although when expressed as absolute values, the remaining fractions of the response after prazosin were similar in both groups of animals. A similar phenomenon has been previously reported (Vivas *et al*., 1997) when concentration-response curves to noradrenaline were analysed in the epididymal end of hypertensive and normotensive animals: the maximal response achieved by the agonist was higher with vas deferens from SHR than with that from WKY rats. After exposure of the epididymal half of vas deferens to the alkylating agent phenoxybenzamine, the maximal response elicited by noradrenaline was decreased to a similar extent in the tissues from both strains, the reduction being greater in the SHR than in WKY animals. Our results seem to suggest that the epididymal end of SHR vas deferens may have a greater noradrenergic component than the WKY animals. On the other hand, a similar content of endogenous and electrically evoked release of noradrenaline in SHR and WKY whole vas deferens has been reported (Katsuragi *et al*., 1991). Thus, it is likely that the enhanced electrical evoked contraction of vas deferens in SHR could be due to alterations in postjunctional α_1-adrenoceptors as reported before (Caufield *et al*., 1977; Caricati-Neto *et al*., 1992; Vivas *et al*., 1997). However, other factors such as modifications in the functionality of G proteins, could account for the altered EFS-induced contraction observed in the epididymal end of vas deferens from SHR animals (Vivas *et al*., 1997).

To study the purinergic component of the contractile response to EFS in the epididymal portion of vas deferens, the action of ATP was blocked by α,β-meATP. As observed with prazosin, this compound was able to reduce both phases of the contractile responses. The maximal phasic non-adrenergic and the tonic adrenergic components of the contractile response were more resistant to α,β-meATP (3 μM) in SHR than in WKY, although a similar reduction was obtained after incubation with α,β-meATP (30 μM). These facts could indicate a proportionally greater participation of purines in the contractile response to EFS in WKY when compared to SHR. After P_{2x}-purinoceptor blockade, the remaining contraction was completely adrenergic, and greater in SHR than in WKY. The further addition of an α_1-adrenoceptor antagonist almost abolished the contractile response, indicating that a major fraction is susceptible to α_1-adrenoceptor antagonism in SHR than in WKY. When the tonic contractile response was examined, the fractions resistant to α,β-meATP and prazosin were similar in both groups of animals.

Our results seem to suggest that in the epididymal portion of SHR vas deferens, noradrenaline has a proportionally more important co-transmitter role than in the corresponding WKY preparations, where noradrenaline and ATP seem to have a similar participation in co-transmission. On the other hand, a decrease in the purinergic component of the EFS contractile response in SHR cannot be discarded and electrophysiological studies have to be done. In addition, it is well established that responses to noradrenaline are potentiated in the presence of ATP in guinea-pig (Holck & Marks, 1978; Kazic & Milosavljevic, 1980), rat (Huidobro-Toro & Parada, 1988) and mouse (Witt *et al*., 1991) vas deferens. It remains to be examined if this phenomenon of synergism could play a role in hypertension in this tissue.

In summary, a co-participation of noradrenaline and ATP can be demonstrated in both phases of the neurogenic contractile response in the epididymal end of vas deferens from SHR and WKY rats. Moreover, the noradrenergic contribution to the contractile responses is enhanced in the biphasic contraction of SHR animals. However, postjunctional modifications in P_{2x}-purinoceptor sensitivity, which could account for the altered contraction in response to EFS observed in the epididymal portion of vas deferens from SHR, cannot be excluded.

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