Abstract The main objective of this study has been to analyse the electrophysiological differences in the prostatic portion of vas deferens between spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY). Resting membrane potentials (RMP) recorded in SHR (–63.8±0.3 mV) and WKY (–68.1±0.3 mV) were significantly different. Bath applications of suramin (30 µM), α,β-methylene adenosine 5’-triphosphate (α,β-meATP; 30 µM) or prazosin (0.1 µM) did not modify the control values of RMP. In control conditions, spontaneous excitatory junction potentials (SEJPs) were recorded in preparations from both groups of animals. SEJPs registered in SHR were greater than those in WKY in amplitude (7.0±0.4 mV vs. 2.6±0.1 mV) and frequency (0.25±0.02 Hz vs. 0.14±0.01 Hz). SEJP amplitude was abolished by bath applications of suramin (30 µM) or α,β-meATP (30 µM). However, tetrodotoxin (TTX; 1 µM) and prazosin (0.1 µM) had no effect on this spontaneous activity. Electrical-field stimulation (EFS; 0.1 ms, 20 V, 0.2 Hz) induced an enhanced excitatory junction potential (EJP) in SHR but not in WKY (16.0±0.6 mV vs. 12.2±0.5 mV) which was abolished by TTX (1 µM), suramin (30 µM) and α,β-meATP (30 µM). The degree of inhibition of both SEJP and EJP produced by α,β-meATP (0.3–30 µM) was greater in SHR than in WKY. This study demonstrates an altered purinergic contribution to the co-transmission process in the prostatic portion of vas deferens from SHR.

Keywords Vas deferens · Prostatic half · SHR · WKY · Co-transmission · Purinergic neurotransmission

Introduction

It is well accepted that the neurogenic mechanical response induced by electrical-field stimulation (EFS) of rodent vas deferens is biphasic, consisting of an initial short-lasting twitch, followed by a slower, maintained contraction (Sneddon and Westfall 1984; von Kügelgen and Starke 1991; Mallard et al. 1992). Considerable evidence supports the proposal that in the rodent vas deferens, ATP underlies the initial twitch response of the biphasic contraction to EFS acting on P2x-purinoceptors to elicit excitatory junction potentials (EJPs) leading to action potentials and the predominantly purinergic component of the muscular contraction (Sneddon 1995; Lundberg 1996). Noradrenaline, however, does not contribute significantly to the EJPs and induces the slow and predominantly noradrenergic component of the contractile response (van Helden and Woolridge 1990; von Kügelgen and Starke 1991; Sneddon 1995).

It has long been recognised that vascular smooth muscles of spontaneously hypertensive rats (SHR) show an altered responsiveness to α₁-adrenoceptor agonists (Vila et al. 1993; Tabernero et al. 1996) and EFS (Muir and Wardle 1989) when compared to normotensive Wistar Kyoto rats (WKY). This phenomenon has also been reported in non-vascular smooth muscle such as the stomach (Altman et al. 1977) or vas deferens (Katsuragi et al. 1991; Vivas et al. 1997; Guitart et al. 1999). Previous studies performed in vas deferens of SHR confirmed an altered postjunctional functionality of α₁-adrenoceptors (Caufield et al. 1977; Caricati-Neto 1992; Vivas et al. 1997) although the endogenous content and the electrically evoked release of noradrenaline from this tissue were similar in both SHR and WKY (Katsuragi et al. 1991). These facts might indicate a general alteration in SHR smooth muscle responsiveness involving modifications in α₁-adrenoceptors. Likewise, an increased purinergic contribution to the co-
transmission process has been described in the enhanced nerve-mediated contractions to EFS recorded in isolated caudal (Vidal et al. 1986) and mesenteric arteries of SHR (van Helden and Woolridge 1990). However, other authors have reported that the increased muscular contraction to EFS observed in whole vas deferens (Katsuragi et al. 1991), tail (Dalziell et al. 1989; Muir and Wardle 1989) and mesenteric arteries (Muir and Wardle 1989) of SHR was not induced by a greater contribution of ATP to the contractile response. Thus, the aforementioned results do not provide clear information about the extent to which ATP contributes to the enhanced responsiveness to EFS in the sympathetic co-transmission in SHR as compared to WKY.

A widely described feature when studying the vas deferens is the existence of a regional variation in the electrical and contractile responses to α-adrenoceptor agonists along the length of the tissue (Anton et al. 1977; Sneddon and Machaly 1992; Granà et al. 1997). Although ATP and noradrenaline are co-released from sympathetic nerve terminals in the whole vas deferens, segments from prostatic and epididymal halves of the tissue show a different post-junctional sensitivity to noradrenaline and ATP (Anton et al. 1977; Sallés and Badia 1991; Sneddon and Machaly 1992). Accordingly, a number of differences in the electrical properties of the cells between both halves have also been described. Thus, the resting membrane potential (RMP) in the prostatic end of guinea-pig vas deferens is greater than in the epididymal segment, although EJPs are similar in amplitude and electrical features (Sneddon and Machaly 1992).

It is generally accepted that SHR smooth muscle shows an increased post-synaptic responsiveness to sympathetic stimulation. However, most of the studies about changes in the electrical properties in hypertensive animals have been performed in vascular smooth muscle and no data are available about electrical features in vas deferens from SHR. As ATP is the transmitter underlying the electrical activity in vas deferens and in view of the greater sensitivity of the prostatic end of the organ to ATP, this portion of the vas deferens has been used in our experiments. The aims of the present study were: (1) to characterise the electrical properties of the prostatic end in SHR and WKY, and (2) to assess the contribution of the purinergic component in the increased sympathetic response observed in SHR vas deferens.

**Materials and methods**

**General experimental procedures.** The experiments were performed on 16- to 18-week-old male SHR (350–375 g) and age-matched WKY (375–395 g) supplied by Ifa-Credo (France). Animals were killed by decapitation and vasa deferentia were quickly removed and placed in modified Krebs-Henseleit physiological salt solution (PSS) of the following composition (mM): NaCl 112.0, KCl 4.7, CaCl2 2.5, KH2PO4 1.1, MgSO4 1.2, NaHCO3 25.0 and glucose 11.1. The preparation was cleaned of connective tissue and transversally bisected yielding the epididymal and the prostatic ends. The prostatic portion, about 1 cm in length, was opened longitudinally showing the lumen, and the epithelium was carefully removed.

**Recordings from smooth muscle cells.** Intracellular recordings of the smooth muscle were made using glass microelectrodes (resistance of 40–60 MΩ) filled with 3 M KCl. Membrane potential was measured using a standard electrometer Duo 773 (WPI) and displayed on a digital storage oscilloscope 4026 (Racal-Dana). Simultaneously, records were digitised (500 Hz) and collected on a PC-based acquisition system (EG&G software). Impalements were accepted only if the following criteria were both satisfied (Brock and Cunnane 1996): (1) the cell penetration was abrupt and the membrane potential increased to a value more negative than the initial potential, and (2) the membrane potential was stable.

**Electrophysiological response to EFS.** EFS stimulation was performed using a pair of silver chloride wires placed perpendicularly to the longitudinal axis of the preparation and located at the same distance from the recording electrode. Repeated electrical pulses (pulse width 0.1 ms, amplitude strength 20 V, frequency 0.2 Hz) were delivered from a two-channel stimulator (Grass S44). In order to abolish stable impalements (3 µM) was performed to abolish mechanical activity. The tested parameters were measured under control conditions and after incubation (30 min) of the different drugs.

**Drugs.** Prazosin hydrochloride, α,β-methylene adenosine 5’-triphosphate (α,β-mATP; lithium salt), nifedipine and tetrodotoxin (TTX) were obtained from Sigma Chemical; suramin hexadecylamine and TTX were a gift from Bayer (Spain). Stock solutions were made by dissolving drugs in distilled water and serially diluted in PSS to the required final concentration. Prazosin and nifedipine were made as a stock solution in 30% ethanol and 100% ethanol, respectively. All other chemicals used were of analytical grade and supplied by Merck Farma y Química.

**Statistics.** Data are expressed as the means ± SEM. The number of animals used (n) is stated in the legends of figures and tables. Where appropriate, the dependency of electrical activity on strain and treatment was analysed by a two-way analysis of variance (ANOVA) within the framework of the general linear model approach (Littell et al. 1991). Two-way ANOVA is precluded if a significant value for the interaction term (strain × treatment) is present because a misleading interpretation of main effects can be made. When this happened in our study, strain and treatment were analysed separately. Differences between strains (SHR and WKY) at each treatment level were tested by Student’s t-test with P-values adjusted according to the Šidák (1967) procedure to correct for multiple testing. Differences among treatment levels for each strain were assessed by a one-way ANOVA followed by Dunnett’s test for comparisons to a control. 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 1996).

**Results**

**Determination of RMP**

The mean RMP values for the smooth muscle cells from the prostatic end of vas deferens were significantly lower (P<0.001) in the normotensive than in the hypertensive animals (Table 1). Application of TTX (1 µM) had no significant effect on the RMP recorded in either WKY or SHR. The RMPs recorded ranged from −61.9 mV to −65.1 mV in SHR and from −68.0 mV to −70.0 mV in WKY. Bath