POSSIBLE ROLE OF THE α_1 -ADRENOCEPTOR IN THE MODULATION OF ADRENERGIC CO-TRANSMISSION IN THE GUINEA-PIG VAS DEFERENS

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ABSTRACT

We observed the effects of prazosin (10^{-8} – 10^{-5} M) on noradrenaline (NA) and dihydroxyphenylethylene glycol (DOPEG) overflow, and on excitatory junction potentials (e.j.p.s) before and after pretreatment with yohimbine, using high pressure liquid chromatography (HPLC), double sucrose-gap and microelectrode methods. Prazosin and yohimbine (10^{-8} – 10^{-5} M) dose-dependently increased the resting and stimulated overflow of DOPEG, or the stimulated overflow of NA. After pretreatment with yohimbine (10^{-7} M) or prazosin (10^{-7} M), additional application of prazosin or yohimbine, respectively, further increased the stimulated NA overflow. Yohimbine (10^{-7} M), but not prazosin (10^{-7} M), significantly increased the amplitude of the e.j.p. without changing the resting membrane potential or input membrane resistance of the smooth muscle cells. Both prazosin (10^{-7} M) and yohimbine (10^{-7} M) significantly enhanced the growth curve (facilitation phenomenon) of e.j.p.s evoked by repetitive stimulation at 0.2 or 0.5 Hz. Each of the drugs produced an additional facilitatory effect on e.j.p.s which had been facilitated by pretreatment of the tissue with the other drug. During prolonged treatment of the vasa with α, β -methylene adenosine 5'-triphosphate ($\alpha\beta$ mATP), single or repetitive field stimulation evoked small and slow e.j.p.s (s.s.e.j.p.s) which were sensitive to tetrodotoxin (10^{-7} M) or guanethidine (10^{-6} M), but not to the P_{2y} -purinoceptor blocking agent, reactive blue 2 (10^{-5} M). E.j.p.s recorded from reserpinized vasa showed enhanced facilitation at stimulus frequencies of 0.2 and 0.5 Hz. Neither prazosin nor yohimbine (up to 10^{-6} M) had any effect on the e.j.p. amplitude or facilitation after reserpinization. The present results may indicate that prejunctional α_1 - and α_2 -adrenoceptors both contribute to the negative feedback control of NA and ATP release from sympathetic nerve terminals in the guinea-pig vas deferens.

The prejunctional negative feedback control of noradrenaline (NA) release in adrenergic neuro-effector transmission in visceral smooth muscle tissues has been considered to be exclusively (28), or at least predominantly (9, 27) due to the α_2 prejunctional-adrenoceptor. However, a possible involvement of prejunctional α_1 -adrenoceptors in this control has been reported. For example, in cardiac tissue, the α_1 -selective agonist methoxamine inhibited the tachycardia evoked by electrical stimulation in the rat (7, 8, 17) and this inhibition was more susceptible to antagonism by the α_1 -adrenoceptor

antagonist prazosin than by the α_2 -adrenoceptor antagonist yohimbine. The measurement of NA output from cardiac tissue indicated a direct inhibition by methoxamine of NA release in the rat atria (30). However, the later reviews did not interpret these results as evidence for a distribution of prejunctional α_1 -adrenoceptors, since the concentrations of agonists and antagonists used in these studies were much higher than necessary for actions at prejunctional α_1 -adrenoceptors, (8, 26, 27). Furthermore, in the guinea-pig vas deferens, prazosin has been reported to increase the ampli-

tude of excitatory junction potentials (e.j.p.s) (24), which are most likely due to release of ATP from adrenergic nerve terminals (24, 25). However, this effect was attributed to an interaction of prazosin with α_2 -prejunctional adrenoceptors.

To shed more light on a possible contribution by the prejunctional α_1 -adrenoceptor to the negative feedback control of neurotransmitter release from the guinea-pig vas deferens, we re-examined the actions of prazosin, before and after pretreatment with yohimbine, on the release of endogenous NA and its metabolite dihydroxyphenylethylene glycol (DOPEG) using high pressure liquid chromatography (HPLC), as well as on e.j.p.s recorded by means of double sucrose-gap and microelectrode methods. Vasa deferentia from reserpinized guinea pigs were also used to investigate the prejunctional action of endogenous NA on the modulation of e.j.p. amplitude.

MATERIALS AND METHODS

Male guinea pigs (200–300 g) were killed by a blow to the head and bled. Vasa deferentia were removed and dissected free of connective tissue and cut open along the longitudinal axis. The prostatic part of the vasa was trimmed to a width of 2.0–3.0 mm and a length of 15–20 mm for the microelectrode, double sucrose-gap and HPLC methods. The epithelial layer was carefully removed using fine forceps under microscopic observation.

NA and DOPEG Assay

The resting and stimulated overflow of NA and its deaminated metabolite DOPEG were assayed using HPLC following the modified alumina extraction method of Oishi *et al.* (21). After incubation for 30 min in aerated (5% CO₂ in O₂) Krebs's solution at room temperature, the prostatic parts of the vasa were fixed vertically between two platinum electrodes (4 mm I.D. and 1.5 cm apart) attached by threads to force displacement transducers, placed under an initial load of 0.5 g and superfused at 1 ml/min with Krebs solution (36°C) containing 3×10^{-7} M desipramine to inhibit NA re-uptake, by means of a peristaltic pump. Two initial stimulations (0.5 msec duration, 10 Hz, 50 V with trains of 200 and 600 pulses) were given to the vasa during a 1 h equilibration period. Subsequently, the vasa were stimulated for periods of 1 min (600 pulses in a train) at 40 min intervals and 5 ml of the super-

fusate collected in ice-cold, 10 ml test tubes. Two experimental protocols were used in this study. In the first, five samples (5 ml) were collected at 40 min intervals from each of the two vasa deferentia prepared from each animal. One of the preparations was stimulated for 1 min at the beginning of each collection and the amounts of NA and DOPEG were measured. The other preparation received no stimulation in order to estimate the resting NA and DOPEG overflow. The resting efflux was subtracted from the total efflux measured upon stimulation to obtain the stimulated efflux. The drugs (when tested) were given to both preparations simultaneously. In the second protocol, both vasa deferentia were stimulated 3 times at 40 min intervals and different drugs in various concentrations were given at the beginning of the second and third collections and the effects examined on the stimulated NA and DOPEG overflows. The collected samples were acidified by adding 50 μ l of perchloric acid (60%) and stored frozen. The vasa were carefully blotted on filter paper and weighed. For the measurement of NA and DOPEG content, the samples after addition of dihydroxybenzylamine (DHBA) were subjected to alumina extraction and, following elution in 100 μ l 0.1 N HCl (19), NA, DOPEG and DHBA levels were measured using HPLC (Yanagimoto MFC, L-200L, Tokyo, Japan) with electrochemical detection (layer voltammetric detector BMD-101) in which 50 μ l of the eluates were injected. Separation was achieved on a Yanapak ODS-T reversed-phase column (250 mm $\times$ 4 mm I.D., 5 μ m average particle size) using a mobile phase of 0.1 mM sodium phosphate buffer (pH 2.6) containing 0.4 mM sodium heptane sulphonate and 10 μ M Na₂ EDTA at a constant flow rate of 0.7 ml/min. NA and DOPEG levels were corrected for recovery using the DHBA as an internal standard. Tissue content of NA and DOPEG was calculated per g wet weight tissue and expressed as relative values against the first control stimulation, which was accepted as 1.0.

Recording of E.j.p.s

The double sucrose-gap method was used to record the membrane potential and tension development in the tissue. The chamber used has been described elsewhere (13). To produce neurogenic responses, electrical field stimulation was applied, via a ring electrode placed in the centre pool of the apparatus, using an electronic stimulator (Nihon Kohden,

SEN-7103). Single or repetitive stimulation was applied with current pulses of 50 μ sec duration and about 10–20 V intensity to stimulate the nervous elements in the muscle tissue. The voltage of the current pulse was adjusted so that an e.j.p. of a given amplitude (10 mV) was evoked by a single pulse. Drugs were dissolved in Krebs solution and applied to the tissue through the centre pool of the double sucrose-gap apparatus using a multi-way tap (dead-time approximately 30 sec).

For intracellular recording of the membrane potential from a single cell, thin strips of tissue 10–15 mm long and 2–3 mm wide were used. A conventional microelectrode filled with 3M KCl (30–50 M Ω) was inserted from the outer surface of the preparation. Field stimulation was applied to the nerves through a pair of Ag-AgCl wires 3–5 mm apart and placed so that a current pulse would pass transversely across the tissue. Single or repetitive stimuli at 20 Hz were applied, with a pulse of 30–100 μ sec duration and 30–50 V intensity, using an electronic stimulator (Nihon Kohden, SEN-7103). The chamber in which the strips were mounted had a volume of 2 ml, and was superfused at a rate of 3 ml/min at a temperature of 35–36°C. To avoid recording artifacts due to twitch-like contractions of the muscle tissue, the preparation was firmly fixed to the rubber plate in the chamber using insect pins of 100 μ m diameter.

Reserpinization of Guinea Pigs

Pretreatment with reserpine consisted of two injections (5 mg/kg i.p.) 24 h apart. The guinea pigs were killed 30 min after the second injection (15). Subsequently, one of the preparations from each animal was used for measurement of NA and DOPEG overflows and the other for recording e.j.p.s by means of the double sucrose-gap method.

Solutions and Drugs

The Krebs solution used had the following composition (mM): Na⁺ 137.4; K⁺ 5.9; Ca²⁺ 2.5; Mg²⁺ 1.2; HCO₃⁻ 15.5; H₂PO₄⁻ 1.2; Cl⁻ 134; glucose 11.5. The solution was continuously bubbled with 5% CO₂ in O₂ and kept at 36°C. The following drugs were used: α , β -methylene adenosine 5'-triphosphate (AMP-CPP; $\alpha\beta$ mATP); DHBA; DOPEG; NA; phentolamine; prazosin; reserpine; yohimbine, reactive blue 2, desipramine (SIGMA).

Statistics

The results (amount of NA and DOPEG release, amplitude of e.j.p.s) are expressed as mean $\pm$ SD, or 2 $\times$ SD and were analyzed for statistical significance using a Student's *t*-test.

RESULTS

Effects of Prazosin on NA and DOPEG Overflows

The resting NA and DOPEG overflows were measured from samples collected according to the first experimental protocol using preparations which were not stimulated (see Materials and Methods). The resting NA overflow was not detectable, however the amount of DOPEG released gave a mean value of 11 ± 4 ng/g tissue/min in the first sample, though this gradually decreased to 8.5 ± 3 ng/g tissue/min in the fifth sample collected. Electrical field stimulation (0.5 msec duration, 10 Hz, 50 V intensity for 60 sec) induced a measurable overflow of NA (185 ± 12 ng/g tissue/min) and more than doubled that of DOPEG to 25 ± 2 ng/g tissue/min. The amounts of NA and DOPEG were highest in the first collection and decreased with each of the following stimulations.

Prazosin (10^{-8} – 10^{-5} M) did not apparently influence the resting NA overflow, which remained undetectable (not shown), but it increased the resting DOPEG overflow, in a dose-dependent manner (Fig. 1a). In the vasa pretreated with prazosin, the stimulated NA overflow was considerably increased compared to the control. In fact, prazosin increased NA overflow in a dose-dependent manner in the range 10^{-8} to 10^{-5} M (Fig. 1d). Further increase in the prazosin concentration (to 10^{-4} M), however, led to a decrease of the stimulated NA and DOPEG overflows. The prazosin-induced increase in the stimulated DOPEG overflow did not differ significantly from its effects on the resting DOPEG overflow (Fig. 1, b vs a). Fig. 1c shows effects of yohimbine (10^{-8} – 10^{-5} M) on the stimulated NA overflow, exhibiting a bell-shaped concentration-response curve producing the peak effect at 10^{-7} M.

Yohimbine-Prazosin Interaction on NA and DOPEG Overflows and on E.j.p.s

After the first control stimulation, one preparation of the vas deferens was treated for 30 min with

Fig. 1 Effects of prazosin (10^{-8} – 10^{-5} M) and yohimbine (10^{-8} – 10^{-5} M) on the resting and stimulated overflow of DOPEG and NA. Each column with bar indicates the mean value of 4–7 experiments with SD. The resting efflux was subtracted from the total efflux measured upon stimulation to obtain the stimulated overflows of DOPEG or NA.

10^{-7} M prazosin, the other with 10^{-7} M yohimbine and then both preparations were stimulated according to the second experimental protocol. Yohimbine and prazosin increased the stimulated NA release to 1.38 ± 0.08 ($P < 0.01$) and 1.27 ± 0.02 ($P < 0.01$) times control, respectively, in the second collection (Fig. 2). After an additional application of yohimbine or prazosin to the tissues pre-treated with prazosin or yohimbine respectively, an additional increase of the stimulated NA release was observed. Thus, after pretreatment with yohimbine, prazosin increased the NA overflow to 1.66 ± 0.09 times control and after pretreatment with prazosin, yohimbine increased the release of NA to 1.75 ± 0.12 times control (Fig. 2). The stimulated DOPEG overflow was not significantly influenced by yohimbine but was markedly increased in the presence of prazosin (Fig. 2).

Fig. 2 Effects of pretreatment of tissues with yohimbine (10^{-7} M) or prazosin (10^{-7} M) on the enhancing action of prazosin (10^{-7} M) and yohimbine (10^{-7} M), respectively, on the stimulated overflow of NA and DOPEG. Each column with bar indicates the mean values of 4–8 experiments with SD.

Fig. 3 Effects of yohimbine (10^{-7} M), prazosin (10^{-7} M) and combined application of both agents on changes in amplitude of e.j.p.s (facilitation phenomenon) during short trains of impulses at 0.2 (a and c) and 0.5 Hz (b and d). (○), control; (●), yohimbine or prazosin; (□), combined application of both agents. Each point indicates the mean value of 5–7 experiments and bar $2 \times \text{SD}$. SP shows the generation of action potential by e.j.p.s.

Effects of Prazosin, Yohimbine or Combined Application of the Two Agents on E.j.p. Amplitude

Effects of prazosin (10^{-7} M), yohimbine (10^{-7} M) or the combined application of the two agents on e.j.p. amplitude were observed using the double sucrose-gap method. Prazosin (10^{-7} M) slightly increased the amplitude of the e.j.p. evoked by a single stimulus (50 μsec duration, and 10–20 V intensity) to 1.03 ± 0.027 times the control value ($n=6$), but this effect was not statistically significant. Additional application of yohimbine (10^{-7} M), significantly increased the e.j.p. amplitude to 1.20 ± 0.03 times control ($n=6$, $\pm \text{SD}$, $P < 0.01$). Yohimbine (10^{-7} M), by itself, on the other hand, significantly increased the e.j.p. amplitude to 1.09 ± 0.04 times control ($n=4$, $\pm \text{SD}$, $P < 0.01$), and additional application of prazosin (10^{-7} M) further increased the amplitude to 1.18 ± 0.05 ($n=6$) times control. Prazosin (10^{-7} M), yohimbine (10^{-7} M) or combined application of both agents did not affect the resting membrane potential measured by the

microelectrode method [control: -62.1 ± 1.1 mV ($n=50$, $\pm \text{SD}$); in prazosin (10^{-7} M): -62.5 ± 1.7 mV ($n=50$, $\pm \text{SD}$); in yohimbine (10^{-7} M): -63.2 ± 1.1 mV ($n=50$, $\pm \text{SD}$); in both drugs together: -63.0 ± 1.5 mV ($n=35$, $\pm \text{SD}$)]. The drugs also had no significant effect on the input membrane resistance measured from the amplitude of electrotonic potentials recorded by means of the double sucrose-gap method.

The e.j.p.s evoked by repetitive stimulation showed facilitation at any stimulus frequency (>0.2 Hz), and at the stimulus frequency of 3–5 Hz, facilitation appeared rapidly and the enhanced amplitude e.j.p.s triggered action potentials with contraction (4, 12). Therefore, to observe the effects of prazosin or yohimbine on the facilitation of e.j.p.s, low stimulus frequencies (0.2 and 0.5 Hz) were used. When the amplitude of e.j.p.s was expressed relative to that evoked by the first pulse (expressed as 1.0), the growth of the e.j.p. during short trains of impulses appeared as an exponential curve with a time constant of about 6 sec (Fig. 3). After the application of yohimbine (10^{-7} M), the amplitude of the first e.j.p. was

Fig. 4 Changes in amplitude of e.j.p.s (facilitation phenomenon) during short trains of impulses at 0.2 Hz (a) and 0.5 Hz (b) recorded from the reserpinized vasa deferentia before (○) and after application of yohimbine (10^{-7} M) (●). Each point indicates the mean value of 5–7 experiments and bar $2 \times$ SD.

increased to 1.10 ± 0.03 times the control value, and the growth curve of the e.j.p.s was markedly enhanced, as shown in Fig. 3, c and d at either stimulus frequency (0.2 or 0.5 Hz). Additional application of prazosin further enhanced the growth curve at either 0.2 or 0.5 Hz (Fig. 3c).

Similar experiments were done with a reversed sequence of drug application. As shown in Fig. 3, a and b, prazosin markedly enhanced the facilitation of e.j.p.s at frequencies of 0.2 and 0.5 Hz, and additional application of yohimbine (10^{-7} M) further enhanced the facilitation phenomenon.

Effects of Prazosin, Yohimbine and NA on the Reserpinized Vasa

To investigate the possible role of endogenously

released NA in the negative feedback control of transmitter release, we reserpinized the guinea pigs. After the pretreatment with reserpine, e.j.p.s in response to a single or repetitive field stimulation were recorded by microelectrode or double sucrose-gap methods. The most striking features of the e.j.p.s recorded from the reserpinized vasa were the enhanced facilitation of e.j.p.s and the shift of the growth curve from an exponential curve to a linear line within a limited stimulus number (with slopes of 0.2 ± 0.01 mV/stimuli and 0.6 ± 0.02 mV/stimuli at frequencies of 0.2 and 0.5 Hz, respectively). Furthermore, in the reserpinized vasa, yohimbine (10^{-7} M) produced no effect on the e.j.p. amplitude or on the growth curve of the e.j.p.s (Fig. 4).

To investigate the properties of the e.j.p. recorded after pretreatment with reserpine, we used $\alpha\beta$ mATP. As shown in Fig. 5, application of $\alpha\beta$ mATP depolarized the membrane, and evoked repetitive action potentials accompanied by a phasic contraction. The effect on the membrane potential of this drug consisted of a transient depolarization followed by a gradual repolarization over several minutes, but without a return to the original level. The resting membrane potentials measured by the microelectrode before, and 30 min after application of $\alpha\beta$ mATP (10^{-5} M) were -62.6 ± 1.2 mV ($n=50$, $\pm$ SD) and -54.2 ± 1.0 mV ($n=25$, $\pm$ SD), respectively. Single electrical field stimulation applied 20–30 min after the pretreatment with $\alpha\beta$ mATP did not evoke any membrane response. However, during repetitive field stimulation at 0.2 or 0.5 Hz, there were small (1–2 mV) but discrete membrane depolarizations with a facilitation phenomenon (Fig. 5). These potential changes and the facilitation of the potential changes at 0.2 and 0.5 Hz were insensitive to yohimbine (10^{-6} M) and to prazosin (10^{-6} M) (Fig. 5, d and e). However, the potential changes were completely inhibited by guanethidine (10^{-6} M) (Fig. 5f) or by tetrodotoxin (Fig. 5i), indicating that the generation of these small e.j.p.s (small and slow e.j.p.) is generated due to excitation of adrenergic nerve fibers.

To confirm the above observations, we applied repetitive stimulations at high frequency (20 Hz) after the desensitization of the reserpinized vasa to $\alpha\beta$ mATP (10^{-5} M). As shown in Fig. 5, g–i, a single stimulus did not evoke any membrane response, but more than two stimuli at 20 Hz evoked small and slow e.j.p., and their amplitude increased in proportion to the increase in the

Fig. 5 Effects of $\alpha\beta$ mATP (10^{-5} M) on e.j.p.s recorded from reserpinized vasa deferentia. a and a': Control e.j.p.s during short trains of impulses at 0.2 Hz (a) and 0.5 Hz (a'). b and b': Effects of $\alpha\beta$ mATP (10^{-5} M) on the membrane potential (b) and muscle tone (b') of the reserpinized vasa deferentia. c and c': E.j.p.s recorded 30 min after the application of $\alpha\beta$ mATP (10^{-5} M) at 0.2 Hz (c) and 0.5 Hz (c'). d and d', e and e' & f and f': Effects of yohimbine (10^{-6} M), yohimbine with prazosin (10^{-6} M) and guanethidine (10^{-6} M) on e.j.p.s in the presence of $\alpha\beta$ mATP. g-i: E.j.p.s evoked by repetitive stimulation at 20 Hz in the presence of $\alpha\beta$ mATP (g), $\alpha\beta$ mATP with reactive blue 2 (10^{-5} M), or tetrodotoxin (10^{-7} M). Note: a-f and g-i shows e.j.p.s recorded from the same preparation. g.p.; guinea pig. The double-sucrose gap method was used to record the changes in membrane potential and tension.

stimulus number.

The P_2 -purinoceptor is classified into two subtypes (P_{2x} and P_{2y} -purinoceptors), mainly on the basis of the rank order of agonist potency of the structural analogues of ATP. For the P_{2x} - and P_{2y} -purinoceptor agonists, the order of potency has been reported to be: $\alpha\beta$ mATP, $\beta\gamma$ mATP > ATP = 2-methylthioATP, and 2-methylthioATP > ATP > $\alpha\beta$ mATP, $\beta\gamma$ mATP for the P_{2y} receptor (5). On this basis, it might be expected that the P_{2y} -purinoceptor would be less sensitive to $\alpha\beta$ mATP and, as a consequence, the desensitization to $\alpha\beta$ mATP would not involve complete inhibition of the e.j.p.s. To investigate the above possibility the effects of reactive blue 2, a P_{2y} -purinoceptor antagonist (5) were studied on the amplitude of small and slow e.j.p. As shown in Fig. 5h, however, reactive blue 2 (10^{-5} M) had no effect on the amplitude of small and slow e.j.p. evoked by repetitive stimuli at 20 Hz. On the other hand, the e.j.p.s

were blocked by 10^{-6} M guanethidine (data not shown) or tetrodotoxin (Fig. 5i).

The effects of exogenously applied NA on e.j.p. amplitude was examined after reserpinization. NA ($>10^{-9}$ M) dose-dependently suppressed the amplitude of e.j.p. evoked by a single stimulus (Fig. 6), without affecting resting membrane potential, or input membrane resistance of the smooth muscle cells (Fig. 6f). This observation indicates that exogenously applied NA acts mainly at presynaptic sites to reduce ATP release. NA (10^{-9} – 10^{-6} M), however, did not affect the growth curve of e.j.p.s at stimulus frequencies of 0.2 and 0.5 Hz (Fig. 6, g and h).

Effects of $\alpha\beta$ mATP on E.j.p. Amplitude of Control Vasa

After the desensitization to $\alpha\beta$ mATP of the reserpinized vasa, repetitive field stimulation evoked

Fig. 6 Effects of NA (10^{-9} – 10^{-6} M) on the amplitude of e.j.p.s (a–e and f), resting membrane potential (R.P.) and input membrane resistance (Rin) (f), and growth curve of e.j.p.s at 0.2 Hz (g) and 0.5 Hz (h) of smooth muscle cells of reserpinized guinea-pig vas deferens in the presence or absence of NA (10^{-7} M). Each point with bar indicates the mean value of 5–25 experiments with $2 \times$ SD.

small and slow e.j.p. These observations led us to re-examine the effects of $\alpha\beta$ mATP on the e.j.p. amplitude of control animals. As shown in Fig. 7, $\alpha\beta$ mATP (10^{-5} M) evoked transient depolarization as it did in the reserpinized vasa, again with repetitive generations of action potentials followed by a phasic contraction. The resting membrane potentials measured by microelectrode before, and 30 min after application $\alpha\beta$ mATP (10^{-5} M) were -63.0 ± 2.0 mV ($n=50$, $\pm$ SD) and -55.2 ± 1.2 mV ($n=25$, $\pm$ SD), respectively, indicating that the membrane was still depolarized to some extent even after 30 min. Single electric field stimulation evoked small but discrete e.j.p.s (small and slow e.j.p.) which showed facilitation when evoked at stimulus frequencies of 0.2 or 0.5 Hz. Neither the amplitude of the small and slow e.j.p. nor their facilitation was affected by reactive blue 2 (10^{-5} M), or the com-

bined application of yohimbine (10^{-6} M) and prazosin (10^{-6} M). However, the small and slow e.j.p. were sensitive to TTX (10^{-7} M) and to guanethidine (10^{-6} M).

To confirm the above observations, we recorded e.j.p.s with the microelectrode. Fig. 7, g and h shows typical recordings of e.j.p.s before and after treatment with $\alpha\beta$ mATP. The mean amplitude of small and slow e.j.p. in response to a single field stimulation (0.5 msec duration, 100 V intensity) after $\alpha\beta$ mATP was 0.9 ± 0.34 mV ($n=5$). The corresponding value under control conditions was 5–10 mV. The time course of the control e.j.p.s could be expressed as two components with time constants of 140.4 ± 9 msec ($n=5$, $\pm$ SD) and 307.3 ± 24 msec ($n=4$, $\pm$ SD), respectively. The time constant of small and slow e.j.p. after the treatment with $\alpha\beta$ mATP had only one component which had

Fig. 7 Effects of $\alpha\beta$ mATP(10^{-5} M) on e.j.p.s recorded from control guinea-pig vas deferens using double sucrose-gap (a-f) and microelectrode (g and h) methods. a and a': Changes in amplitude of e.j.p.s during short trains of impulses at 0.2 Hz (a) and 0.5 Hz (a'). b and b': Effects of $\alpha\beta$ mATP(10^{-5} M) on the membrane (b) and muscle tone (b') of the vas deferens. c and c': Small e.j.p.s evoked in the presence of $\alpha\beta$ mATP(10^{-5} M) at 0.2 Hz (c) and 0.5 Hz (c'). d and d', e and e' & f and f': Effects of reactive blue 2 (10^{-5} M), yohimbine (10^{-6} M) with prazosin (10^{-6} M), and tetrodotoxin (10^{-7} M). g and h: E.j.p.s recorded with a microelectrode before (g) and after (h) the application of $\alpha\beta$ mATP. g.p.; guinea pig

a much slower time course, giving a mean value of 357.2 ± 35 msec ($n=5$, $\pm$ SD).

DISCUSSION

Two essential features of sympathetic neuro-effector transmission are: first, the negative feedback control of NA release via α_2 -adrenergic receptors, and second, the co-existence and co-release of several transmitter substances together with NA from the sympathetic nerves. The evidence concerning the local modulation of sympathetic neurotransmission has been frequently reviewed over the last two decades (for a recent review, see 27). In most of the reviews, however, NA is assumed to be the only

transmitter which undergoes prejunctional modulation and the mechanism involved is assumed to be prejunctional autoinhibition via α_2 -adrenoceptors. As the hypothesis of co-transmission became more widely recognized (3) more attention was paid to the modulatory mechanisms affecting co-transmitter release. Indeed recent studies on the guinea-pig vas deferens have shown that the release of ATP may also be subject to presynaptic inhibition via α_2 -adrenergic receptors (24, 28).

In the present experiments, α_1 -adrenoceptor antagonist prazosin (10^{-7} M) markedly enhanced the release of NA and DOPEG, and augmented the facilitation of e.j.p.s. Recently, it was reported that the α_2 -adrenoceptor can be subdivided into α_{2A} and α_{2B} subtypes, and that oxymetazoline and prazosin are selective antagonists for the α_{2A} and α_{2B} adrenoceptors, respectively (6, 20). On this basis, the enhancing action of prazosin on NA output and on the facilitation of e.j.p.s observed in the present experiments would be due to a blockade of α_{2B} -adrenoceptors by prazosin. However, the following observation ruled out this possibility, namely that after pretreatment of the tissue with yohimbine (10^{-7} M), which blocks both α_{2A} and α_{2B} adrenoceptors, prazosin (10^{-7} M) further augmented both the NA and DOPEG overflows and the facilitation of e.j.p.s. Thus, the present observations on the action of prazosin may indicate a role for the prejunctional α_1 -adrenoceptor in the negative feedback control of the NA and ATP release from the guinea-pig vas deferens.

In the perfused dog heart, it has been reported that intra-arterial injections of prazosin (1–30 μ g) and yohimbine (0.3–10 μ g) during cardiac sympathetic nerve stimulation increased the evoked tachycardia, whereas continuous infusions of methoxamine or clonidine caused a sustained reduction of the tachycardia. These observations also suggest that there are prejunctional α_1 -adrenoceptors regulating NA release, in addition to the α_2 -adrenoceptor distribution on the cardiac sympathetic nerve terminals of the dog (32). Further, in perfused SHR-tail arteries prelabelled with [3 H]NA, both α_2 - and α_1 -adrenoceptor antagonists increased the stimulation-evoked release of tritium. This result confirms the presence of prejunctional α_2 -adrenoceptors and the presence of prejunctional α_1 -adrenoceptors which are not present in the tail artery of normotensive WKY rats (11). Thus, it seems probable that the prejunctional α_1 -adrenoceptor plays a role in the modulation of transmit-

ters release in some animal tissues including the guinea-pig vas deferens.

To estimate the role of endogenous NA involved in the prejunctional effects, guinea pigs were pretreated with reserpine. The scheme used for reserpization seems to totally remove the NA from the tissue (15), and in our experiments, neither NA nor DOPEG overflow could be detected by the HPLC procedure (see Materials and Methods). The e.j.p.s recorded from vasa dissected from animals pretreated with reserpine however, showed a marked facilitation phenomenon, and the facilitation curve shifted from an exponential to a linear one (Fig. 4). On this background, yohimbine as well as prazosin failed to affect the e.j.p.s amplitude or the facilitation phenomenon, while exogenous NA depressed the e.j.p.s dose-dependently. Thus, the actions of prazosin and yohimbine could be attributed to interruption of a tonic presynaptic inhibition exerted by endogenous NA via activation of the α_1 and α_2 -adrenoceptors on release of whichever transmitter generates the e.j.p.s in this tissue. The transmitter generating the e.j.p.s in the guinea-pig vas deferens, previously considered to be NA (4), now appears to be ATP (2, 24, 27). Several pieces of evidence support this view: firstly, local application of ATP but not of NA mimicks the e.j.p.s (24, 25); secondly, the generation of e.j.p.s is blocked, (i) after desensitization of the P_{2x} -purinoceptor with $\alpha\beta$ mATP (18) or (ii) by the P_{2x} antagonist ANAPP₃ but (iii) not by adrenergic antagonists (10, 24). Our data are further evidence of the possible role of ATP as co-transmitter in the guinea-pig vas deferens and additionally suggest the existence of a third as yet unknown transmitter which generates small and slow e.j.p. During continuous treatment with $\alpha\beta$ mATP, e.j.p.s were strongly suppressed in amplitude probably because of the desensitization of the P_{2x} -purinoceptor, but small, non-adrenergic, and $\alpha\beta$ mATP-insensitive e.j.p.s (small and slow e.j.p.) were still recorded (Figs. 5 and 6). The treatment of the vasa from normal or reserpized animals with reactive blue 2 failed to affect the small and slow e.j.p., suggesting that they are not due to an action of ATP on the P_{2y} -purinoceptor subtype (5). The small and slow e.j.p. may correspond to the slow e.j.p.s described by Stjärne and Astrand (29) which were also not sensitive to $\alpha\beta$ mATP or reserpine. The time course of small and slow e.j.p. in the present experiments was also slow compared to that of control e.j.p.s. It is relevant to this argument that there are at least three classes of vesicle in the sym-

pathetic nerve terminals, viz., larger dense-cored vesicles (LDVs), small dense-cored vesicles (SDVs) and small clear vesicles (SCVs). Both LDVs and SDVs have been shown to store NA and ATP, and some LDVs also store a neuropeptide. No types of vesicle in the adrenergic terminals have been shown to contain only ATP (28).

Prazosin and yohimbine dose-dependently increased the resting DOPEG overflow in all concentrations used (Fig. 2). It is well known that DOPEG originates from nerve terminals where the cytosolic NA is converted to DOPEG after deamination by monoamine oxidase, an enzyme located on the outer leaflet of the inner mitochondrial membrane (1, 22). The concentration of NA in the cytosol is a function firstly, of the balance between inward transport into the vesicles (16, 34) and 'back leakage' from vesicles into cytosol (33), and secondly, of the balance between outward leakage from the cytosol to the extraneuronal medium and inward transport, via a Na^+ -dependent carrier in the plasma membrane (23, 31). In our experiments, however, neuronal NA uptake was blocked by desipramine which was always present in the Krebs solution and therefore the possibility that prazosin acted by stimulating NA uptake could be ruled out. Under our experimental conditions, prazosin increased the resting DOPEG release though it seems unlikely that this effect is related to an action on the presynaptic adrenoceptor, since the stimulated DOPEG release was not increased. Therefore, it is probable that prazosin blocks NA transport into vesicles and/or stimulates 'back leakage' from the vesicles resulting in an increase in cytosolic NA. However, no resting NA overflow was detectable even after the administration of 50 μ M prazosin (not shown), which suggests that at rest, all the NA is deaminated to DOPEG. Prazosin has been reported to increase the resting tritium overflow from the [³H]NA preloaded guinea-pig vas deferens, and this has been interpreted as evidence that this chemical increases the resting NA overflow (14). However, the present results indicate that prazosin increases the overflow of DOPEG but not NA. Prazosin did not significantly increase the stimulated DOPEG overflow in comparison with that in the resting state, but markedly increased the stimulated NA overflow (Figs. 1 and 2). The enhancing effect of prazosin on the stimulated NA overflow cannot be explained by its increasing the cytosolic NA in the manner described above, but is most likely due to blockade of the negative feed-

back control of transmitter release through pre-junctional α_1 -adrenoceptors. The present observations on the actions of prazosin may indicate a role of prejunctional α_1 -adrenoceptors in the negative feedback control of NA and ATP release from the guinea-pig vas deferens.

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