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Dissociation of inhibitory effects of guanethidine on adrenergic and on purinergic transmission in isolated canine splenic artery

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Abstract

The aim of this study was both to investigate the effects of progressive inhibition of adrenergic neurons by increasing concentrations of guanethidine (0.1–10 μM) on the double-peaked vasoconstrictor responses to electrical periarterial nerve stimulation in the isolated and perfused canine splenic artery, and to clarify whether release of noradrenaline is presynaptically separate from release of adenosine 5′-triphosphate (ATP). Double-peaked vasoconstrictions (biphases of vasoconstrictions) were consistently observed under the conditions of 30-s trains of pulses at 1–10 Hz frequencies. Guanethidine, at a lower concentration (0.1 μM) did not modify the first (1st) phase vasoconstriction at low frequencies (1–2 Hz), but markedly inhibited the second (2nd) responses. On the other hand, it slightly but significantly inhibited the double-peaked vasoconstrictor responses at high frequencies (6–10 Hz). Furthermore, a 10-fold increase of concentration of guanethidine (1 μM) almost completely inhibited the 2nd phase responses at any frequencies used but did not completely inhibit the 1st phase response. A further increased concentration of guanethidine (10 μM) failed to enhance the 1 μM guanethidine-induced inhibition. The 1 μM guanethidine-resistant 1st phase responses at any frequencies used (1–10 Hz) were sensitive to tetrodotoxin (30 nM). Treatment with 0.1 μM prazosin did not modify the 1st phase response at any frequencies used in the 1 μM guanethidine-treated preparation. The responses remaining after 1 μM guanethidine and 0.1 μM prazosin were completely suppressed by a subsequent application of 1 μM α,β-methylene ATP at any frequencies used. The results indicated that guanethidine, an adrenergic neuron blocker, may exert a dominant inhibitory effect on adrenergic rather than on purinergic components of sympathetic nerve co-transmission, indicating that guanethidine-sensitive mechanisms may mainly contribute to determine noradrenaline secretion from neurosecretory vesicles rather than ATP secretion.

Keywords: Co-transmission; Guanethidine; Sympathetic nerve stimulation; Cannula insertion method; Splenic artery

1. Introduction

Adenosine 5′-triphosphate (ATP) has been proposed as a co-transmitter with noradrenaline in peripheral sympathetic nerves (Burnstock, 1988; Von Kügelgen and Starke, 1991). It has been suggested that the release of noradrenaline and ATP from sympathetic nerve terminals is subject to differential autoinhibition (Von Kügelgen et al., 1994). Several lines of evidence obtained in sympathetically innervated tissues showed that activation of presynaptic α_2-adrenoceptors inhibits the release of noradrenaline to a greater extent than the release of ATP (Bulloch and Starke, 1990; Driessen et al., 1993), whereas activation of presynaptic P1-purinoceptors produces the opposite pattern (Driessen et al., 1994). On the other hand, activation of presynaptic β-adrenoceptors enhances the neuronal release of noradrenaline, but decreases the release of ATP (Driessen et al., 1996; Gonçalves et al., 1996). Moreover, blockade of neuronal uptake usually produces potentiation of noradrenaline release, and a decrease of ATP release, the latter inhibition probably being due to a presynaptic α_2-adrenoceptor-mediated feedback mechanism activated by released noradrenaline (Sneddon and Westfall, 1984; Msghina et al., 1992; Todorov et al., 1996). These findings strongly indicate that there might be some separate modulatory mechanisms for the release of the co-transmitters, ATP and noradrenaline, from postganglionic sympathetic nerves. The evidence obtained with guinea-pig vas deferens showed that guanethidine, at a higher concentration (16 μM) substantially reduced, but did not completely block, the neurogenic release of ATP and subsequent contractile responses (Kirkpatrick and Burnstock, 1987).

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Recently, Yang and Chiba (1998) reported that purinergic and adrenergic components contributed differently to double-peaked vasoconstrictor responses to periarterial electric nerve stimulation in the isolated canine splenic artery. They demonstrated that the first (1st) phase constriction might have mainly a purinergic component, and the second (2nd) response, mostly an adrenergic component. In preliminary experiments, we also observed that nerve stimulation-induced vasoconstrictions in canine splenic artery were partly resistant to a higher concentration of guanethidine (10 μM), and that guanethidine-resistant responses were consistently abolished by P2X-receptor desensitization with α,β-methylene ATP, suggesting that the purinergic component of co-transmission might be resistant to adrenergic neuron blocking agents. Thus, it seems that purinergic and adrenergic components of co-transmission are subject to different inhibition by adrenergic neuron blockade. The purpose of this study was to determine whether progressive inhibition exerted on adrenergic neurons, by increasing concentrations of guanethidine produces different effects on co-transmission of purinergic and of adrenergic components of the double-peaked vasoconstrictor responses to periarterial nerve stimulation in isolated canine splenic artery, and thereby to clarify whether a guanethidine-sensitive mechanism determines differently the release of co-transmitters, ATP and noradrenaline.

2. Materials and methods

2.1. Arterial preparations

Mongrel dogs of either sex, weighing 7–16 kg, were anaesthetized with sodium pentobarbitone (30 mg/kg i.v.). After treatment with sodium heparin (200 units/kg i.v.), the dogs were killed by rapid exsanguination from the right femoral artery. The arterial main branches of the splenic artery were isolated, and side branches of the artery were tied with silk threads. The artery (1–1.2 mm in outer diameter) was cut into segments (15–20 mm in length), and only four segments were obtained from each splenic artery. In our experiments, *n* values represent the numbers of blood vessel preparations, but in the same treated group, only 1–2 preparations obtained in each splenic artery were used. Each segment was cannulated and set up for perfusion as described previously (Hongo and Chiba, 1983; Chiba and Tsukada, 1985). Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. A proximal portion of the segment was fixed to the distal portion of a needle with silk threads. The cannula was 3–4 cm long and 0.8–1.0 mm in an outer diameter and had small side holes 5 mm from the distal sealed end. The cannulated arterial segment was placed in a cup-shaped glass bath and was perfused by a roller pump (Tokyo Rikakikai) with Krebs–Henseleit solution gassed with 95% O_2 and 5% CO_2. The solution contained 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 10 mM glucose. The flow rate was kept at approximately 2 ml/min. The perfusion pressure was continuously measured with an electric manometer (Nihon Kohden, MPU-0.5A) and recorded with a rectigraph (Nihon Kohden, WT-685G). After a stabilization period of 60 min, the preparation was removed from the bath solution and fixed in a horizontal position. The preparation was perfused at a constant flow rate during the experiment. The basal perfusion pressure was within 40–80 mm Hg.

For electrical stimulation of the periarterial sympathetic nerve terminals, two platinum electrodes were placed on the extraluminal side of the arterial wall. Electrical stimulation was delivered by an electric stimulator (SEN-7203, Nihon Kohden) using 30-s trains of pulses at 10-V amplitude, 1-ms pulse duration, and a frequency range of 1–10 Hz. The organ bath was sealed with plastic film to maintain the preparation at 37°C. Ten-minute intervals between electrical stimulation periods were needed to obtain reproducible responses. The intervals between frequency–response curves were 60 min. The preparations were incubated for 60 min with prazosin, α,β-methylene ATP or

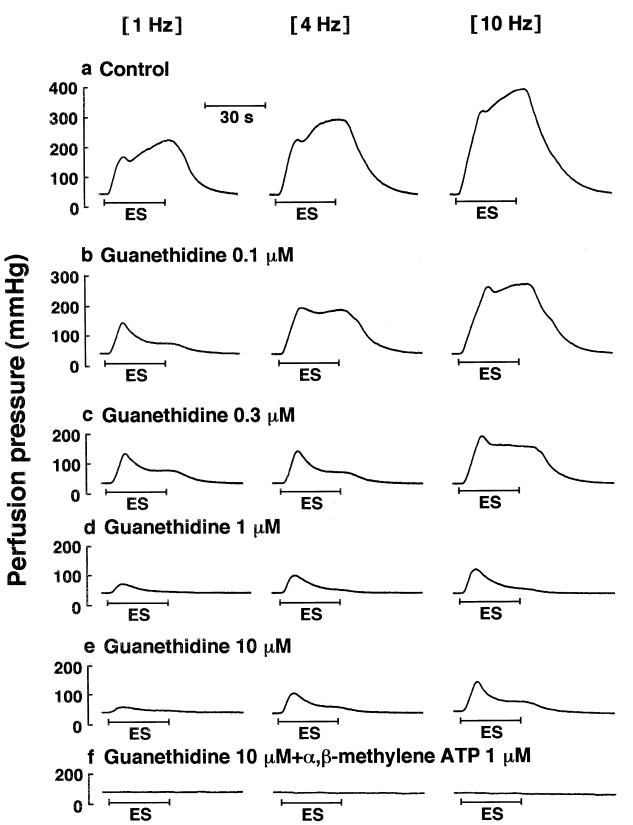

Fig. 1. Double-peaked vasoconstrictor responses and the effects of increasing concentrations of guanethidine as well as effects of α,β-methylene ATP on the 10 μM guanethidine-resistant responses in an isolated, perfused canine splenic artery. The vessel was electrically stimulated by 30-s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz. ES: Electrical nerve stimulation.

guanethidine before the next response curves were made for electrical stimulation. α,β-Methylene ATP produced a transient increase of perfusion pressure with return to the original level after approximately 60 min. The drug solution was administered into the rubber tubing close to the cannula in a volume of 0.01–0.03 ml by means of microinjectors.

2.2. Drugs

Drugs used were α,β-methylene ATP (Research Biochemicals International, Natick, MA, USA), disodium ATP (Sigma, USA), DL-noradrenaline hydrochloride (Sankyo, Tokyo, Japan), prazosin hydrochloride (Sigma), tetrodotoxin (Sigma) and guanethidine sulfate (Sigma). All drugs

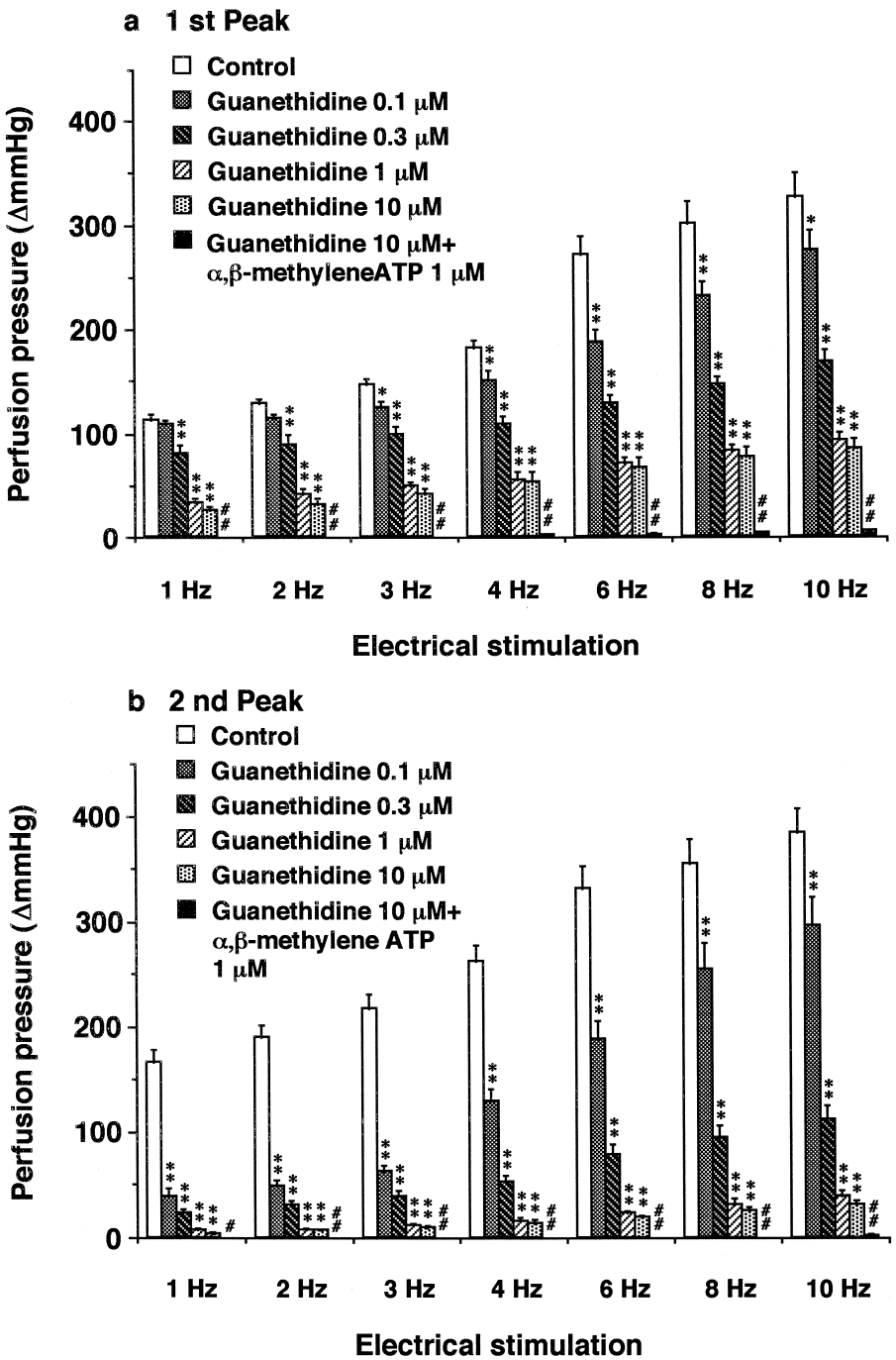

Fig. 2. Effects of increasing concentrations of guanethidine (0.1–10 μM) on the first peak (a) and the second peak (b) of the biphasic vasoconstrictor responses to electrical nerve stimulation (10 V amplitude, 1 ms pulse duration and 30-s trains of pulses at stated frequencies) as well as effects of α,β-methylene ATP (1 μM) on the 10 μM guanethidine-resistant responses in the canine splenic artery. Data are presented as means ± S.E.M., $n = 8$. $^{*}P < 0.05$; $^{**}P < 0.01$ as compared with the control group. $\#P < 0.05$; $\#\#P < 0.01$ as compared with the preceding treatment.

were dissolved in physiological saline before the start of the experiment. The stock solutions were kept at −20°C until used.

2.3. Statistical analysis

Vasoconstrictor responses to electrical stimulation are expressed as the maximal changes in perfusion pressure (mm Hg) from their control levels. The data are shown as means ± S.E.M. An analysis of variance with Bonferroni's test was used for statistical analysis of multiple comparisons of data. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Vascular responses to periarterial electrical nerve stimulation

The periarterial electrical nerve stimulation (30-s trains of pulses) induced a double-peaked vasoconstriction (two phases of the vasoconstriction) in the isolated and perfused canine splenic artery in a frequency-related manner and usually separated by an intervening dip in the increasing perfusion pressure (Fig. 1a and Fig. 3a) as reported previously (Yang and Chiba, 1998). The 1st peak of vasoconstriction was reached within 8–12 s, and the 2nd peak, within 30–35 s after the start of electrical stimulation as shown in Fig. 1a and Fig. 3a. In only some cases were monophasic vasoconstrictor responses observed. Any unclear double-peaked responses were omitted. As reported previously (Ren et al., 1994), the vasoconstrictor responses to electrical stimulation at 6–12 V, 0.1–10 Hz and 0.2–1 ms pulse duration were completely inhibited by tetrodotoxin (30 nM). In addition, the reproducibility of vasoconstrictor responses to electrical nerve stimulation or exogenous ATP and noradrenaline had been verified. The results of a corresponding time control showed that there were no significant influences on responsiveness of vasoconstrictions induced by electrical nerve stimulation or exogenous ATP and noradrenaline under the present experimental conditions (data not shown, $n = 8–12$).

3.2. Effects of increasing concentrations of guanethidine on vasoconstrictor responses to electrical nerve stimulation

Intraluminal application of various doses of guanethidine (0.1–10 μM) did not produce any significant vascular response by itself. As shown in Fig. 1, a lower concentration of guanethidine (0.1 μM) did not modify the 1st phase vasoconstriction at a low frequency (1 Hz), but markedly inhibited the 2nd phase responses. On the other hand, it slightly but significantly inhibited the biphasic responses at a high frequency (10 Hz) (Fig. 1b). A three-fold increase of concentration of guanethidine (0.3 μM) slightly but clearly inhibited the 1st phase constriction even at a low frequency, and also markedly reduced the biphasic responses at a high frequency (Fig. 1c). Furthermore, a 10-fold increase of concentration of guanethidine (1 μM) almost completely inhibited the 2nd phase responses at any frequencies used, but did not completely inhibit the 1st phase response (Fig. 1d). A further increase of concentration of guanethidine (10 μM) failed to enhance the 1 μM guanethidine-induced inhibition (Fig. 1e), and the remaining responses after 10 μM guanethidine were completely suppressed by subsequent application of 1 μM α,β-methylene ATP (Fig. 1f). Fig. 2 shows the summarized data for the effects of increasing concentrations of guanethidine (0.1–10 μM) on the 1st phase vasoconstrictor responses (a), and the 2nd responses (b) to electric nerve stimulation. The results show that 1 μM guanethidine had a more pronounced inhibitory effect on the 2nd phase responses at 1–10 Hz than on the 1st responses, i.e., the 1st phase response at any of the frequencies used was inhibited by about 70% (Fig. 2a), and 2nd responses by 95% (Fig. 2b). The effects of 10 μM guanethidine show almost the same results on both phases.

3.3. Effects of an α_1-adrenoceptor antagonist, prazosin, and a P2X-purinoceptor desensitizer, α,β-methylene ATP, on 1 μM guanethidine-treated preparations

The treatment with 0.1 μM prazosin abolished the vasoconstrictor response to exogenous noradrenaline (0.1–10 nmol), but did not influence the contractile responses to

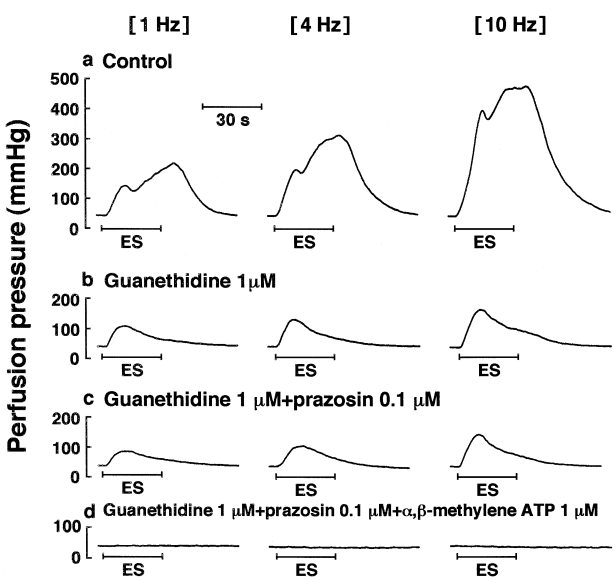

Fig. 3. Double-peaked vasoconstrictor responses and the effects of prazosin (0.1 μM) and α,β-methylene ATP (1 μM) on the 1 μM guanethidine-resistant responses in an isolated, perfused canine splenic artery. The vessel was electrically stimulated by 30-s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz. ES: Electrical nerve stimulation.

ATP (0.01–1 μmol). On the other hand, the perfusion with α,β-methylene ATP (1 μM) blocked the contractile responses to ATP, but not those to noradrenaline as reported previously (Yang and Chiba, 1998). The vasoconstrictions remaining after 1 μM guanethidine were blocked by subsequent administration of 30 nM tetrodotoxin (data not shown, $n = 4$). Fig. 3b shows the effects of 1 μM guanethidine on double peaked vasoconstrictor responses to nerve stimulation. As shown in Fig. 3c, the treatment with 0.1 μM prazosin did not modify the 1st phase response at any frequencies used in the 1 μM guanethidine-treated preparation. The remaining responses after 1 μM guanethidine and 0.1 μM prazosin were completely suppressed by a subsequent application of 1 μM α,

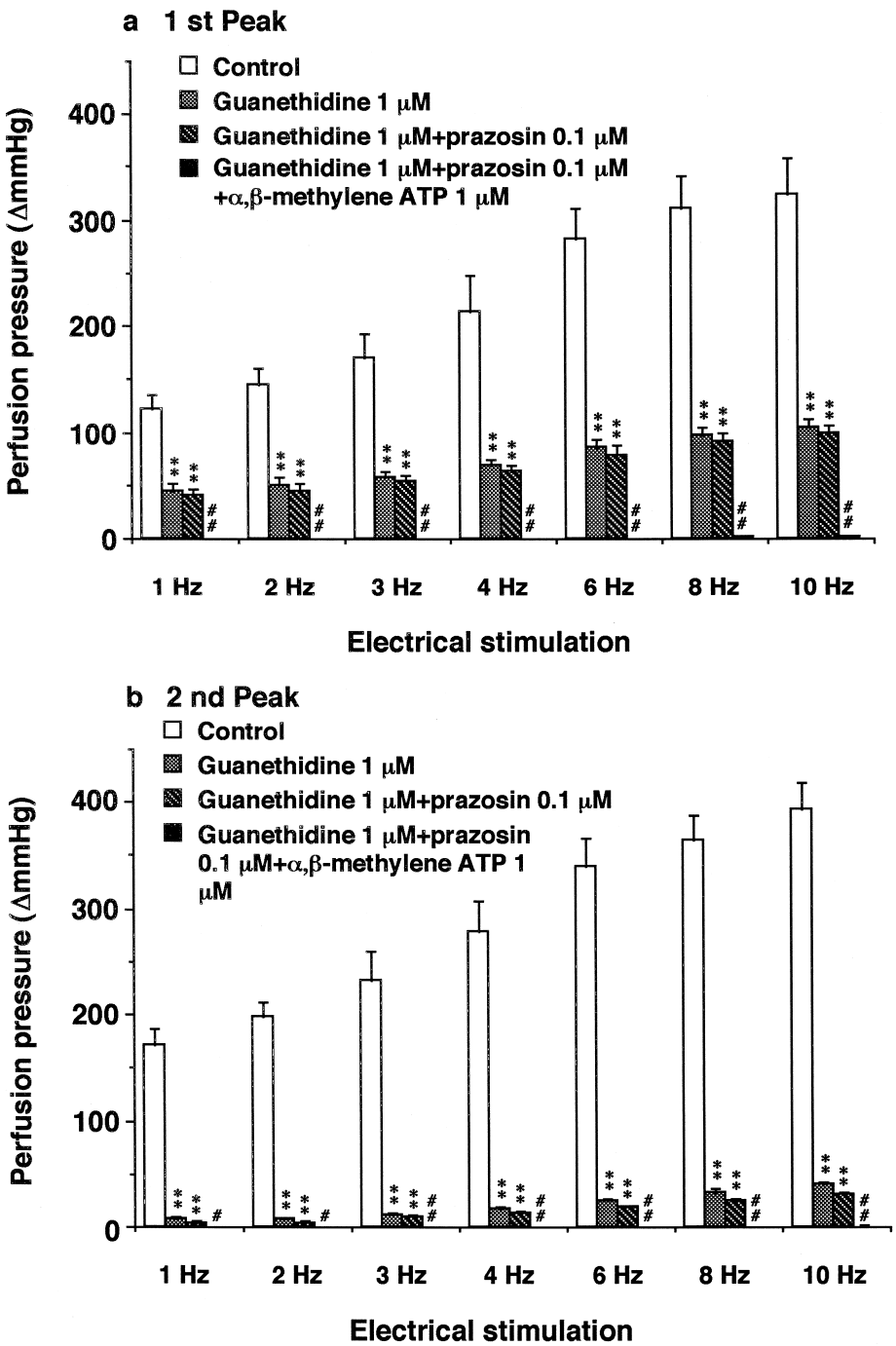

Fig. 4. Effects of guanethidine (1 μM) on the first phase (a) and the second phase (b) vasoconstrictor responses to electrical nerve stimulation (10 V amplitude, 1 ms pulse duration and 30-s trains of pulses at stated frequencies) as well as effects of prazosin (0.1 μM) and α,β-methylene ATP (1 μM) on guanethidine-resistant responses in the isolated canine splenic artery. Data are presented as means ± S.E.M., $n = 6$. $^{**}P < 0.01$ as compared with the control group. $\#P < 0.05$; $\#\#P < 0.01$ as compared with the preceding treatment.

β-methylene ATP at any frequencies used as shown in Fig. 3d. Summarized data are shown in Fig. 4. Fig. 4a shows control, effects of 1 μM guanethidine, prazosin and α,β-methylene ATP on the 1st phase responses, and Fig. 4b shows effects of these on the 2nd phase responses.

4. Discussion

Observations made with a variety of blood vessel preparations have demonstrated that an adrenergic neuron blocking agent, guanethidine, at a μmolar concentration can abolish the biphasic vasoconstrictor responses to electric nerve stimulation, in which ATP acts as a co-transmitter with noradrenaline (Von Kügelgen and Starke, 1985; Kennedy et al., 1986; Burnstock and Warland, 1987; Machaly et al., 1988; Sjöblom-Widfeldt et al., 1990). The present results showed that guanethidine at lower concentrations exerts a preferential inhibition on the 2nd peaked vasoconstrictions, but that the 1st phase response is rather resistant even with a higher concentration. On the other hand, 1 μM guanethidine appeared to produce a maximal inhibition of vasoconstrictor responses to nerve stimulation, but only the 1st phase responses still remained in part. As reported previously by Yang and Chiba (1998, 1999a), the nerve-stimulated 1st phase constriction might be mainly mediated by the release of ATP, and the 2nd one might be mostly mediated by the release of noradrenaline. Therefore, it is reasonable that the low concentrations of guanethidine may preferentially inhibit the adrenergic component and not the purinergic component, since the 2nd phase responses were inhibited more markedly, whereas the 1st phase responses were only slightly decreased. Furthermore, the results also showed that 1 μM guanethidine-resistant responses, which were sensitive to tetrodotoxin, were not affected by the treatment with prazosin, but were abolished by subsequent P2X-receptor desensitization with α,β-methylene ATP, indicating that 1 μM guanethidine-resistant responses might involve mainly a neurogenic purinergic component of co-transmission. However, this result contrasts with earlier results from our laboratory (Ren et al., 1996), in which the 100 μM guanethedine-resistant response to nerve stimulation in the same preparation was abolished by an α-adrenoceptor antagonist, phentolamine (10 μM). It is also reported that phentolamine, at a low perineuronal concentration of noradrenaline may show some characteristics of a partial α_2-agonist, and even inhibit transmitter release (Limberger and Starke, 1984). In addition, there is good evidence that activation of presynaptic α_2-adrenoceptors inhibits not only the release of noradrenaline co-localized with ATP in sympathetic nerve terminal but also the release of ATP (Brock et al., 1990; Driessen et al., 1993). Thus, it is possible that phentolamine inhibits the guanethidine-resistant responses mediated by the release of ATP via activation of presynaptic α_2-adrenoceptors. From the present results, it is reasonable to assume that guanethidine may exert a preferential inhibitory effect on the adrenergic and not on the purinergic component of co-transmission, and that the purinergic component might be in part resistant to adrenergic neuron blockade.

The mechanism of the inhibition of neurotransmission by adrenergic neuron blocking agents such as guanethidine and bretylium has been widely investigated. Previous observations suggested that these agents may alter impulse propagation in sympathetic nerve terminals (Haeusler et al., 1969). Furthermore, using focal extracellular recording techniques, it was definitely demonstrated that guanethidine and bretylium inhibit impulse propagation and thus block the release of transmitters (Brock and Cunnane, 1988; Åstrand and Stjärne, 1989). Moreover, it was also demonstrated that guanethidine may have an additional inhibitory effect on depolarization–secretion coupling processes in addition to its effects on the nerve impulse (Brock and Cunnane, 1988). Thus, our results may be explained by considering that noradrenaline secretion from neurosecretory vesicles might be more dependent on the guanethidine-sensitive neural impulse and depolarization–secretion coupling mechanisms than is ATP secretion. However, the question raised is that of which guanethidine-sensitive mechanisms accounts for its preferential inhibition of adrenergic and not of purinergic transmission. This remains to be established in future studies.

It is hypothesized that ATP and noradrenaline are released as co-transmitters from the same axons (Burnstock, 1988; Von Kügelgen and Starke, 1991). Interestingly, more recently it was suggested that prolonged cold storage, which causes irreversible degeneration of adrenergic nerve fibres, may preferentially depress the co-transmission of the purinergic component, whereas the adrenergic component is largely unaffected (Yang and Chiba, 1999b). Thus, we considered that ATP and noradrenaline are possibly released from two separate populations of exocytotic vesicles within the periarterial sympathetic nerve in the canine splenic artery, as suggested by Burnstock (1990).

Progressive inhibition of adrenergic neurons by guanethidine may produce a preferential inhibitory effect on adrenergic rather than on purinergic components of the biphasic vasoconstrictor responses to nerve stimulation in the isolated canine splenic artery and, in turn, the purinergic component of co-transmission might be in part resistant to adrenergic neuron blockade. These results suggest that guanethidine-sensitive mechanisms contribute dominantly to determine noradrenaline rather than ATP secretion from neurosecretory vesicles.

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