Different Sensitivities to $\alpha_1$ Adrenoceptor Blockade on Periarterial Sympathetic Nerve-Induced Constriction by Low and High Frequencies in Canine Isolated Splenic Arteries

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YANG, X.-P. and CHIBA, S. Different Sensitivities to $\alpha_1$ Adrenoceptor Blockade on Periarterial Sympathetic Nerve-Induced Constriction by Low and High Frequencies in Canine Isolated Splenic Arteries. Tohoku J. Exp. Med., 2002, 196(3), 151–155 —— The periarterial nerve electrical stimulation at 4 and 10 Hz induced a monophasic vasoconstriction of the canine splenic artery in a pulse number-related manner (1–30 pulses). The responses at 4 Hz were not significantly affected by 0.1 $\mu$M prazosin, but abolished by 1 $\mu$M $\alpha$, $\beta$-methylene ATP. Prazosin (0.1 $\mu$M) partially but significantly inhibited responses at 10 Hz, and the remaining responses were blocked by 1 $\mu$M $\alpha$, $\beta$-methylene ATP. It indicates that the monophasic vasoconstrictor response to short pulses of stimulation at a low frequency is mediated by P2X-receptors, whereas the response at a high frequency may be due to activation of not only P2X-receptors but also $\alpha_1$ adrenoceptors. ——— dog splenic artery; sympathetic nerve electrical stimulation; P2X-purinoceptor; $\alpha_1$-adrenoceptor

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Previously, Yang and Chiba (1998) demonstrated in the isolated, perfused canine splenic arterial preparations that double peaked responses (two phases of the constrictions) were readily induced by periarterial nerve electrical stimulation (PNS) with 30-seconds trains of pulses at 10-V amplitude, 1-millisecond duration in a frequency-related manner. Recently, Yang and Chiba (1999a, 2000a) also found that PNS with trains of 1, 3, and 10 pulses, 1-millisecond pulse duration and 10-V amplitude at 1 Hz, consistently induced monophasic vasoconstriction. The PNS-induced constrictions were not modified by prazosin treatment in doses which completely inhibited the noradrenaline (NA)-induced constriction, but it was suppressed by $\alpha$, $\beta$-methylene ATP ($\alpha\beta$-m ATP), a P2X-purinoceptor desensitizer. Thus, it was concluded that monophasic responses to short pulse trains of PNS at a low frequency might have been mediated by an activation of periarterial purinergic nerves (Yang and Chiba

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1999a, 2000a). However, it has found that the monophasic response to PNS was readily introduced even with higher frequencies if the pulse numbers were small such as 10-30 pulses, although PNS at 30-seconds of pulse trains frequently caused biphasic constrictions as reported before (Yang and Chiba 1998, 1999a, 2000a). Thus, in this study we attempted to investigate whether the monophasic constrictor response to a high frequency with small numbers of pulses is pure purinergic or not.

**MATERIALS AND METHODS**

Mongrel dogs of either sex, weighing 10 to 16 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.). After heparinization (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. Then, the artery (1–2 mm in an outer diameter) was cut into segments (15–20 mm in length), and each segment was cannulated and set up for perfusion as described previously (Hongo and Chiba 1983). 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-type cannula with silk threads. The cannula was 3–4 cm long and 0.8–1.8 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, Tokyo) with Krebs-Henseleit solution gassed with 95% O₂ and 5% CO₂. The solution contained (mM): NaCl 118; KCl 4.7; CaCl₂ 2.5; MgSO₄ 1.2; KH₂PO₄ 1.2; NaHCO₃ 25 and glucose 10. The flow rate was kept at approximately 2 ml/minute. The perfusion pressure was continuously measured with an electric manometer (MPU-0.5A, Nihon Kohden, Tokyo) and recorded with a rectigraph (WT-685G, Nihon Kohden). After a stabilization period of 60 minutes, 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 35–80 mmHg.

For electrical stimulation of the periartrial 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 4 or 10 Hz of stimulation at 10-V amplitude, 1 milliseconds pulse duration, in a train length of 1-30 pulses. The organ bath was sealed with plastic film to maintain the preparation at 37°C. Ten-minutes intervals between electrical stimulation periods were needed to obtain reproducible response. The intervals between frequency-response curves were 60 minutes. The preparations were incubated for 60 minutes with prazosin and αβ-m ATP before the second and third response curves were made for electrical stimulation. Drugs used were dl-noradrenaline hydrochloride (Sankyo, Tokyo), disodium ATP, prazosin hydrochloride (Sigma, St. Louis, MO, USA), α, β-methylene ATP (Research Biochemicals International, Natick, MA, USA). All drugs were dissolved in physiological saline before the start of the experiment. The stock solutions were kept at −20°C until used. Vasoconstrictor responses to electrical stimulation are expressed as the maximal changes in perfusion pressure (mmHg) from their control levels. The data are expressed as mean ± S.E.M. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. A p-value less than 0.05 was considered statistically significant.
RESULTS

PNS at a low frequency of 4 Hz or a high frequency of 10 Hz consistently induced a transient, monophasic vasoconstriction of the canine splenic artery in a pulse-number-related manner. The three reproducible frequency-response curves can be obtained after 60 minutes intervals \( (n=4, \text{data not shown}) \). Typical tracings of vasoconstrictions induced by electrical stimulation at 4 Hz are shown in Fig. 1, and the responses at 10 Hz are shown in Fig. 2. The summarized data are shown in Fig. 3. The vasoconstrictor responses to trains of 1, 3, 10 and 30 pulses at 4 Hz were not modified by the treatment with prazosin \( (0.1 \mu M) \) which abolished constrictor responses to intraluminally administered noradrenaline \( (0.03-3 \text{nmol}) \), but completely inhibited by the P2X-receptor desensitization with \( \alpha\beta\text{-m ATP} (1 \mu M) \) in addition to prazosin \( (0.1 \mu M) \) (Figs. 1 and 3A). On the other hand, the vasoconstrictor responses to trains of 3, 10, and 30 pulses at 10 Hz were partially but significantly inhibited by 0.1 \( \mu M \) prazosin, and remaining responses were abolished by a subsequent treatment with 1 \( \mu M \) \( \alpha\beta\text{-m ATP} \) (Figs. 2 and 3B), in dose which inhibited vasoconstrictor responses to \( \text{ATP} (0.01-1 \mu M) \). As previously reported \( (\text{Ren et al. } 1996; \text{Haniuda et al. } 1997; \text{Yang and Chiba } 1998, 1999a, 2000a) \), ATP-induced vasoconstrictions were strongly inhibited by \( \alpha\beta\text{-m ATP} \) but
that a short train (1 second) electrical stimulation at low, and even at high frequencies (2–32 Hz) evokes a transient purinergic vasoconstriction of the rabbit splenic artery. In the canine splenic artery, it has been demonstrated that short pulse trains of electrical stimulation at a low frequency (1 Hz) can selectively activate a purinergic monophasic vasoconstriction (Yang and Chiba 1999a, 2000a). In the present study, the short trains of 1–30 pulses at 4 Hz apparently induced a purinergic response, since the responses were blocked by αβ-m ATP, but not by prazosin. It has been suggested that the relative contribution of ATP and NA toward the overall constrictive response is variable depending on the preparations, species and stimulation conditions used (Kennedy et al. 1986; Ren and Burnstock 1997; Yang and Chiba 1998; Todorov et al. 1999). In the guinea pig vas deferens, the relative participation of ATP and NA to neurogenic twitch contraction varies with the increasing frequency of nerve stimulation due to the greater amount of NA (Todorov et al. 1999). The present evidences also confirmed that the vasoconstriction to short pulses of trains at a high frequency would be mediated by not only ATP but also NA, since the responses were partially inhibited by blockade of α1-adrenoceptors, and remaining one was abolished by desensitization of P2X-receptors. Previous investigations on this preparation demonstrated that the vasoconstrictor response to pulse trains of up to 10 seconds at 1–10 Hz appeared to be monophasic, whereas it became clearly distinguished into two phases at longer pulse trains of 30-seconds (Yang and Chiba 1998, 1999a, 2000a). At low frequencies (1–3 Hz), the first phase response might contain mainly a purinergic component, whereas at high frequencies (6–10 Hz), each of the two phases seems to be involved in purinergic and adrenergic components (Yang and Chiba 1998). Moreover, in this study it might be recognized that a release of NA by PNS is influenced by either pulse-number- or frequency-dependent mecha-

**DISCUSSION**

Ren and Burnstock (1997) have reported...
nisms. It is hypothesized that ATP and NA are released from two separate populations of exocytotic vesicles within the peripheral sympathetic nerve terminals (Burnstock 1990). This seems to be the case in the canine splenic artery (Yang and Chiba 1999b). Thus, the previous (Ren et al. 1996; Haniuwa et al. 1997; Yang and Chiba 1998, 1999a, 2000a) and present results may reflect the fact that two separate storage vesicles with ATP and NA have a different sensitivity to electrical stimulation.

It is assumed that 1) the monophasic response is purinergic at a low frequency within 30 pulses, and 2) at a high frequency within 30 pulses the monophasic response is mostly purinergic but partially contains the adrenergic component, and 3) the reason for the failure of PNS to cause a double peaked response may be due to a small amount of NA release, since relatively large amounts of NA release usually caused the double peaked response as previously reported (Yang and Chiba 1998, 1999a, 1999b, 2000a, 2000b, 2000c, 2001).

References