Perivascular Purinergic Nerve-Induced Vasoconstrictions in Canine Isolated Splenic Arteries

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ABSTRACT—We tried to induce selective perivascular purinergic nerve stimulation in isolated canine splenic arterial preparations, using the cannula insertion method. Under the conditions of periarterial electrical stimulation (ES), i.e., trains of 1, 3 and 10 pulses, 1-ms pulse duration and 10-V amplitude at 1 Hz, monophasic vasoconstriction was consistently induced. The ES-induced vasoconstriction was not influenced by prazosin in doses that completely inhibited noradrenaline-induced vasoconstrictions, but it was suppressed by α,β-methylene ATP, a P2X purinoceptor desensitizer. Thus, it is indicated that a selective purinergic transmitter release is readily obtained in the isolated splenic arterial preparation.

Keywords: Dog splenic artery, Perivascular electric stimulation, P2X Purinoceptor

It has been hypothesized that ATP and noradrenaline are released from two separate populations of exocytotic vesicles within the peripheral sympathetic nerve terminals (1). Recently, Yang and Chiba (2) demonstrated in canine isolated, perfused arterial preparations that double-peaked responses (two phases of the constriction) were readily induced with the condition of 30-s trains of pulses at 10-V amplitude, 1-ms duration in a frequency-related manner. The first phase might contain mainly a purinergic component, and the second one mainly an adrenergic component, because the first one was suppressed by treatment with α,β-methylene ATP, a desensitizer of P2X purinoceptor, and the second one by prazosin, an α1-adrenoceptor antagonist (2). Moreover, the first phase was not modified by a small dose of tetrodotoxin, but the second one was readily inhibited, although a large dose of tetrodotoxin blocked the double-peaked constrictions (3). The second phase was also suppressed by a small dose of guanethidine, but the first one was not (4). However, previous reports indicated (2 – 4) that the selective purinergic vasoconstriction was not produced without a release of noradrenaline in the perivascular electrical nerve stimulation of splenic arterial preparations (2 – 4).

Thus, in the present study, we tried to produce a selective release of purinergic transmitter but no adrenergic one, because we wanted to investigate pharmacologically the mechanisms of purinergic transmitter release.

Mongrel dogs of either sex, weighing 8 to 14 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The heparinized dogs (200 units/kg, i.v.) 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 (5, 6). Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. The proximal portion of the segment was fixed to the distal portion of a needle-type cannula with silk threads. The cannula was 3- to 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 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3 and 10 mM glucose. The flow rate was kept at approximately 2 ml/min. 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 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 35 – 80 mmHg.

For electrical stimulation of the periarterial sympathetic

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nerve terminals, two platinum electrodes were placed on the extraluminal side of the arterial wall (7). Electrical stimulation was delivered by an electric stimulator (SEN-7203, Nihon Kohden), using 1 Hz of stimulation at 10-V amplitude, 1-ms 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-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 and α,β-methylene ATP before the second response curves were made for electrical stimulation or ATP or noradrenaline was administered. α,β-Methylene ATP produced a transient increase of perfusion pressure, which returned to its original level by 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 use of microinjectors. Drugs used were disodium adenosine 5'-triphosphate (ATP), prazosin hydrochloride, tetrodotoxin (Sigma, St. Louis, MO, USA), α,β-methylene ATP (Research Biochemicals International, Natick, MA, USA) and dl-noradrenaline hydrochloride (Sankyo, Tokyo). 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 or an agonist are expressed as the maximal changes in perfusion pressure (mmHg) from their control levels. The data are expressed as the mean ± S.E.M. An analysis of variance with Bonferroni’s test was used for the statistical analysis of multiple comparisons of data. P values <0.05 were considered statistically significant.

Periarterial electrical nerve stimulation at 1 Hz readily induced a vasoconstrictor response of the canine splenic artery in a pulse-number-related manner. The vasoconstrictor response to trains of up to 10 pulses appeared to be monophasic, whereas it became distinguished into two phases at over 15 pulse trains. A typical tracing of vasoconstrictions induced by electrical stimulation is shown in Fig. 1.

Fig. 1. Vasoconstrictor responses of an isolated, perfused canine splenic arterial preparation. The vessel was electrically stimulated by 1 Hz at 10-V amplitude and 1-ms pulse duration, with trains of 1 – 30 pulses.

Fig. 2. Effects of prazosin on the monophasic vasoconstrictor responses to trains of 1, 3 and 10 pulses at 1 Hz of stimulation (A) and the vasoconstrictor responses induced by exogenous noradrenaline (B) in the canine splenic arteries. □: Control, ■: after 0.1 μM prazosin. *P<0.01, as compared with the control group. Symbols represent the mean values ± S.E.M., n=6.

Fig. 3. Effects of α,β-methylene ATP on the monophasic vasoconstrictor responses to trains of 1, 3 and 10 pulses at 1 Hz of stimulation (A) and the vasoconstrictor responses induced by exogenous noradrenaline (B) in the canine splenic arteries. □: Control, ■: after 1 μM α,β-methylene ATP. *P<0.01, as compared with the control group. Symbols represent the mean values ± S.E.M., n=6.
The vasoconstrictor responses under the used experimental conditions were completely abolished by the treatment with tetrodotoxin (30 nM) (n = 4, data not shown).

The vasoconstrictor responses to 1 – 10 pulses of electrical stimulation were consistently monophasic as shown in Fig. 1. These monophasic vasoconstrictions were not influenced by treatment with 0.1 μM prazosin, which significantly blocked the noradrenaline-induced vasoconstrictions as shown in Fig. 2. Electrical stimulation-induced vasoconstrictions were abolished by 1 μM α,β-methylene ATP as shown in Fig. 3. However, α,β-methylene ATP did not modify the noradrenaline-induced vasoconstrictions (Fig. 3). The present results demonstrated that the vasoconstrictor responses to pulse trains of 1 – 10 s at 1 Hz only caused the release of purinergic transmitters from sympathetic nerve terminals. Although ATP is contained together with noradrenaline in sympathetic nerve terminal vesicles (8–10), it is enough to consider that ATP may be released without noradrenaline. In the rabbit jejunal arteries, ATP has been claimed to be the sole mediator of the contractile response to sympathetic nerve stimulation (11). As reviewed by Lundberg (12), in general, the rapid and short-lasting ATP-mediated control of vascular tone may be most appropriate in vascular beds demanding very rapid and precise adjustments as a pathophysiological factor. Using the present demonstrated conditions of electrical stimulation on vessel preparations, the mechanisms of purinergic transmitter release will be precisely investigated in the near future.

REFERENCES


