Periarterial Electrical Nerve Stimulation-Induced Adrenergic Vasoconstriction Inhibited by Adrenergic α_{1B} -Receptor Blockade but Not by α_{1A} -Blockade

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ABSTRACT—The periarterial electrical nerve stimulation at a frequency of 4 Hz (30-s trains of pulses) induced a double-peaked vasoconstriction in the canine splenic artery. The treatment with chloroethylclonidine (CEC, 60 μ M) markedly inhibited the second-peaked constriction, whereas it produced an insignificant effect on the first-peaked response. The vasoconstriction to noradrenaline (NA, 1 nmol) was not significantly influenced by 60 μ M CEC. On the other hand, WB 4101 (1 μ M) consistently abolished the vascular response induced by NA (1 nmol), but rather potentiated the double-peaked constriction. The results indicate that neuronal NA may junctionally exert its vasoconstrictor effect via an activation of postjunctional α_{1B} -receptors, whereas exogenous NA may extrajunctionally activate α_{1A} -receptors for its vascular action in the canine splenic artery.

Keywords: α_1 -Adrenoceptor subtype, Adrenergic transmission, Chloroethylclonidine

The evidences obtained in the rat vas deferens support the hypothesis that neuronal and hormonal noradrenaline (NA) may activate a different subtype of α_1 -receptors (1, 2). The previous report by Mallart et al. (1) suggested that the α_{1B} -receptor subtype mediates the sympathetic nerve-induced adrenergic contraction to a single impulse, whereas the α_{1A} -receptor subtype is involved in the response to exogenous NA. A recent observation further confirmed these differences in the same preparation and indicated that the α_1 -receptor subtype involved in the nerve-stimulated response resembles the α_{1D} -receptor (2). However, in canine vessels, NA released from sympathetic nerves and exogenous NA may couple to the same subtype of α_1 -receptors, although it varied with regions (3, 4). An α_1 -receptor has been identified as a functional subtype for the vasoconstrictions to neuronal and exogenous NA in the canine splenic artery (5). The present study was attempted to determine whether α_1 -receptor subtypes differently mediate the nerve-stimulated and administered NA-evoked vascular responses in the canine splenic artery.

Mongrel dogs of either sex, weighing 9 to 16 kg, were anesthetized with sodium pentobarbital (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. Then the artery (1–2 mm in outer diameter) was cut into segments (15–20 mm in length), and each segment was cannulated and set up for perfusion. 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 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.

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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, with a frequency of 4 Hz. The organ bath was sealed with the plastic film to maintain the preparation at 37°C. Ten-min intervals between electrical stimulation periods were needed to obtain a reproducible response. The preparations were incubated for 60 min with chloroethylclonidine (CEC) and WB 4101 before the second stimulation was made or NA was administered, respectively. The drug solution of NA 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 *dl*-noradrenaline hydrochloride (Sigma, St. Louis, MO, USA), chloroethylclonidine dihydrochloride and WB 4101 (2-(2,6-dimethoxyphenoxyethyl)-amino-methyl-1,4-benzodioxane) (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 or an agonist are expressed as the maximal changes in perfusion pressure (mmHg) from their control levels. The data are expressed as the mean $\pm$ 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.

The periarterial electrical nerve stimulation (PNS) at 4 Hz (30-s trains of pulses) induced a double-peaked vasoconstriction (2 phases of vasoconstriction) in the isolated and perfused canine splenic artery and usually separated by an intervening dip in increasing perfusion pressure (Fig. 1) as reported previously (5).

Figure 1 shows an original tracing of vascular contractile force from typical experiments showing the effects of CEC (A) and WB 4101 (B) on the vasoconstrictor responses to

Fig. 1. Double-peaked vasoconstrictor responses of the isolated, perfused canine splenic arteries and the effects of chloroethylclonidine (CEC) (A) and WB 4101 (B). The vessels were electrically stimulated by 30-s trains of pulses at a frequency of 4 Hz (10-V amplitude and 1-ms pulse duration). PNS: Periarterial electrical nerve stimulation.

nerve stimulation at 4 Hz. The summarized data are shown in Fig. 2. As shown in Fig. 2A, treatment with 60 μ M CEC markedly inhibited the second-peaked constriction, whereas it failed to affect the first peak. On the other hand, 1 μ M WB 4101 did not inhibit the double-peaked vasoconstriction, but rather consistently potentiated the vascular constriction. Furthermore, the vasoconstriction induced by exogenously-applied NA (1 nmol) was not significantly influenced by 60 μ M CEC, but markedly depressed by an application of 1 μ M WB 4101 (Fig. 3)

The vascular responses to PNS in the canine splenic artery are shown to be a double-peaked vasoconstrictions consisting of an initial, transient constriction (first peak) followed by a prolonged response (second peak) (5). The

Fig. 2. Effects of chloroethylclonidine (CEC) (60 μ M, A) and WB 4101 (1 μ M, B) on the double-peaked vasoconstrictor responses to PNS (10-V amplitude, 1-ms pulse duration and 30-s trains of pulses at a frequency of 4 Hz) in the canine splenic arteries. $\square$: control, ▨ : after treatment. Each point represents the mean $\pm$ S.E.M., *n* = 6; **P* < 0.05, ***P* < 0.01, as compared with the control group.

Fig. 3. Effects of chloroethylclonidine (CEC) (60 μ M, A) and WB 4101 (1 μ M, B) on the vasoconstrictor responses to administered NA (1 nmol) in the isolated canine splenic arteries. $\square$: control, ▨ : after treatment. Each point represents the mean $\pm$ S.E.M, $n = 6$; ** $P < 0.01$, as compared with the control group.

first peak is induced mainly via an activation of P2X-purinoreceptors, and the second peak involves mostly α_1 -receptors (5). The present results showed that the second peak induced by PNS was selectively inhibited by CEC, an α_{1B} -receptor antagonist (6), but was rather enhanced by WB 4101, an α_{1A} -receptor blocker (7). On the other hand, the exogenous NA-induced vasoconstriction was inhibited by WB 4101, but not significantly affected by CEC. The results suggest that the vasoconstriction of canine splenic artery induced by exogenous NA is likely mediated by α_{1A} -receptors, whereas nerve-evoked response is possibly mediated by α_{1B} -receptors. Thus, it is reasonable to hypothesize that α_{1B} -receptors may locate in the junctional region, whereas α_{1A} -receptors may reside in the extrajunctional region of the sympathetic neurovascular junction in the canine splenic artery.

In the rat vas deferens, α_{1B} -receptor was initially assumed to mediate nerve-evoked contraction, since CEC selectively inhibited that response without the interference of an exogenous NA-induced response (1). The recent evidence obtained in the same preparation revealed an involvement of α_{1D} -receptors for the nerve stimulation-evoked contraction because BMY 7378, a selective α_{1D} -receptor antagonist (8), exhibited a relatively high potency against the nerve stimulation-induced response (2). The question raised is whether the α_{1D} -receptor subtype is functionally responsible for the α_1 -receptor-mediated constriction in the canine splenic artery. It has been suggested that both α_{1B} - and α_{1D} -receptor subtypes are effectively inactivated by CEC, although the former subtype is relatively more sensitive (9). On the other hand, WB 4101 also has a higher affinity for α_{1A} - and α_{1D} -receptor subtypes (10). Since the PNS-induced response was not inhibited by WB 4101,

there may not functionally exist α_{1D} -receptors in the junctional region. However, it will be necessary to use a selective α_{1D} -receptor antagonist in the future.

In the rat kidney, an activation of prejunctional α_{1A} -receptors may inhibit the release of transmitters (11). Since WB 4101 inhibited the vasoconstriction induced by exogenous NA, the potentiation of PNS-induced constriction by WB 4101 is probably explained by prejunctional effect, presumably via a blockade of prejunctional α_{1A} -receptors.

In conclusion, the present results indicate that there may be a heterogeneity of α_1 -receptor subtypes in the sympathetic neurovascular junctional and extrajunctional regions of the canine splenic artery. The junctional α_{1B} -receptors may receive a sympathetic adrenergic innervation, whereas α_{1A} -receptors may be extrajunctionally activated by an exogenous agonist.

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