Antagonistic Interaction Between BIIE 0246, a Neuropeptide Y Y2-Receptor Antagonist, and ω-Conotoxin GVIA, a Ca2+ Channel Antagonist, in Presynaptic Transmitter Releases in Dog Splenic Arteries

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ABSTRACT—Isolated dog splenic arteries were perfused with Krebs-Henseleit solution at 37°C, using the cannula inserting method. Periarterial nerve electrical stimulation (10-V amplitude; 1-ms duration; 30-s trains of pulses; 1, 4 and 10 Hz) readily caused double peaked vasoconstrictions, i.e., 1st peaked response was mostly inhibited by α,β-methylene ATP and the 2nd one, by prazosin. These responses were consistently inhibited by ω-conotoxin GVIA (ω-CTX), whereas they were facilitated by BIIE 0246, a neuropeptide Y (NPY) Y2-receptor antagonist. The ω-CTX-induced blocking effects of transmitter release were significantly antagonized by BIIE 0246. It is possible that the NPY Y2 receptor activity may partially be linked to presynaptic Ca2+ channels.

Keywords: Dog splenic artery, Perivascular nerve electrical stimulation, α-Adrenoceptor

It has been well recognized that neuropeptide Y (NPY) presynaptically inhibits a release of neurotransmitters via activation of NPY Y2 receptors, whereas NPY postsynaptically facilitates transmitter-induced effect via Y1 receptors in the rat vas deferens and in the dog splenic artery (1 – 6). Previously, we demonstrated in the isolated canine splenic artery that double peaked responses (two phases of the vasoconstriction) were consistently induced by periarterial nerve electrical stimulation (PNS) with the condition of 30-s trains of pulses at 10-V amplitude, 1-ms duration in a frequency-related manner; i.e., the 1st phase response might mainly contain a purinergic component and the 2nd response, mostly an adrenergic one (7, 8). The inhibition of the double-peaked responses by NPY Y2-receptor activation seems to be different from that by tetrodotoxin (TTX), a potent sodium inward current inhibitor, because the double-peaked vasoconstrictions were inhibited in parallel by Y2 receptor activation, but TTX blocked only the 2nd peaked one in a small dose (4, 9). On the other hand, an N-type Ca2+ channel antagonist, ω-conotoxin GVIA (ω-CTX), which specifically blocks N-type Ca2+ channels, caused a parallel inhibition of the double-peaked vasoconstrictions (10). The Ca2+ entry through neuronal Ca2+ channels is a key step in neurotransmitter release, and the cellular mechanisms that regulate the activities of presynaptic Ca2+ channels are still widely investigated (11). Thus, we considered that Y2 receptor-activated inhibitory effects may be related to presynaptic regulating mechanisms of Ca2+ channels. In the present study, we made an attempt to investigate the effects of a selective NPY Y2-receptor antagonist, BIIE 0246, on Ca2+ channel-dependent release of neurotransmitters.

Mongrel dogs of either sex, weighing 12 to 15 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 main branches of the splenic artery were isolated, and side branches of the artery were tied with silk threads. Then, the artery (1 – 1.2 mm in outer diameter) was cut into segments (15 – 20 mm in length). Each segment was cannulated and set up for perfusion as described previously (12, 13). 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 – 1.0 mm in outer diameter with 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 CaCl$_2$, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 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 (MPU-0.5A; Nihon Kohden, Tokyo) and recorded with a rectigraph (WT-685G, Nihon Kohden). After a stabilization period of 1 h, 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 30–80 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 1, 4 and 10 Hz of stimulation at 10-V amplitude, 1-ms pulse duration, in a train length of 30-s pulses. The organ bath was sealed with plastic film to maintain the preparation at 37°C. The reproducible responses to PNS were obtained at 10-min intervals. The intervals between frequency-response curves were 60 min. The preparations were incubated for 60 min with ω-CTX and/or BIIE 0246 before the second response curves were made for electrical stimulation.

Drugs used were ω-CTX (Sigma, St. Louis, MO, USA) and BIIE 0246, (S)-N$^2$-[1-[2-[4-[(R,S)-5,11-dihydro-6(6H)-oxodibenzo[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]acetyl]-N-[2-[1,2-dihydro-3,5(4H)-dioxo-1,2-diphenyl-3H-1,2,4-triazol-4-yl]ethyl]-argininamide (Boehringer Ingelheim Pharma KG, Biberach, Germany). BIIE 0246 was dissolved in 15% dimethyl sulfoxide. The concentrations of dimethyl sulfoxide (vehicle) used did not modify the PNS-induced and exogenous noradrenaline (NA)-induced vasconstrictions. ω-CTX was dissolved in 0.5% (w/v) bovine serum albumin in distilled water before the start of the experiment. The stock solutions were kept at −20°C until used.

The effects of ω-CTX and BIIE 0246 on PNS-induced vasocostrictions are expressed as a percentage of the control response. 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.

PNS at frequencies of 1, 4 or 10 Hz induced a double-peaked vasoconstriction of the canine splenic artery as reported previously (5, 7). Figure 1 shows a typical tracing of vasoconstrictions induced by electrical stimulation at 4 Hz and effects of ω-CTX and BIIE 0246. Perfusion with 10 nM ω-CTX almost completely inhibited the first and second peaked responses (Fig. 1A), as previously reported (10). Perfusion with 1 μM BIIE 0246 did not change basal perfusion pressure (n = 8, data not shown), whereas it slightly enhanced PNS-induced double peaked constric-

![Fig. 1. Double-peaked vasoconstrictor responses to PNS and the effects of ω-conotoxin GVIA (ω-CTX) and BIIE 0246 in the isolated and perfused canine splenic arteries. The vessel was electrically stimulated by 4 Hz at 10-V amplitude and 1-ms pulse duration, with a train of 30-s pulses. PNS: periartrial nerve electrical stimulation.](image)
receptor inhibition antagonizes \( \omega \)-CTX-induced effects. However, the inhibition by \( \omega \)-CTX was antagonized significantly by a pretreatment with BIIE 0246. It seems that NPY \( Y_2 \)-receptor activation causes a modification of neuronal N-type Ca\(^{2+} \) channels which in turn disturbs Ca\(^{2+} \) channel function, and finally causes a decrease in the transmitter release. On the other hand, presynaptic Ca\(^{2+} \) channels, which were functionally blocked by \( \omega \)-CTX, were partially reserved by NPY \( Y_2 \)-receptor inhibition. Recently, it has been reported that the dihydropyridine compound H394/84 is a highly selective NPY \( Y_1 \)-receptor antagonist (14), although the dihydropyridine compound has in general Ca\(^{2+} \) inward current inhibitory properties (15). Since it is well recognized that a dihydropyridine compound modifies the reactivity to Ca\(^{2+} \) channels, NPY receptors may partially participate in the Ca\(^{2+} \) channel reactivity.

Thus, it is considered that NPY \( Y_2 \)-receptor inhibition by BIIE 0246 may partially contribute to antagonize the \( \omega \)-CTX-induced Ca\(^{2+} \) channel inhibiting effect, suggesting the interaction between NPY \( Y_2 \) receptor and presynaptic Ca\(^{2+} \) channels in sympathetic nerve terminals. Therefore, the possibility that presynaptic NPY \( Y_2 \) receptors may in part regulate the neurotransmitter release via neuronal Ca\(^{2+} \) channels is estimated, although it is still not ruled out that NPY \( Y_2 \)-receptor-activation causes a disturbance of transmitter releasing process that follows the Ca\(^{2+} \) channel activation.

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