Effects of L-765,314, a Selective and Potent $\alpha_{1B}$-Adrenoceptor Antagonist, on Periarterial Nerve Electrical Stimulation-Induced Double-Peaked Constrictor Responses in Isolated Dog Splenic Arteries

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ABSTRACT—The periarterial nerve electrical stimulation (PNS) at a frequency of 1 or 4 Hz (30-s trains of pulses) readily caused a double peaked vasoconstriction in the canine splenic artery. The treatment with $1 \mu M$ L-765,314, a selective and potent $\alpha_{1B}$-adrenoceptor antagonist, markedly inhibited the second peaked constriction, whereas it did not modify the vasoconstrictor responses to exogenous noradrenaline (0.03 – 1 nmol) and A61603 (1 – 30 pmol), a selective $\alpha_{1A}$-agonist. A large dose of $10 \mu M$ L-765,314 significantly blocked exogenous noradrenaline- and A61603-induced responses. It is concluded that PNS-induced responses are mediated via the postjunctional $\alpha_{1B}$-adrenoceptor subtype.

Keywords: L-765,314, Perivascular nerve electrical stimulation, $\alpha_{1}$-Adrenoceptor subtype

It has been suggested that neuronal and exogenous noradrenaline (NA)-induced contraction of the rat vas deferens is mediated by different subtypes of $\alpha_{1}$-adrenoceptors (1, 2). Recent observations further confirm this fact in the canine splenic artery and indicated that NA released from sympathetic nerves may junctionally exert its vasoconstrictor effect via activation of postjunctional $\alpha_{1B}$- and in part $\alpha_{1D}$-adrenoceptors, whereas exogenous NA extrajunctionally activates $\alpha_{1A}$-adrenoceptors to produce its response (3, 4). In the canine splenic artery, periarterial nerve electrical stimulation (PNS) induced a double-peaked vasoconstriction consisting of an initial transient, predominantly P2X-purinoceptor-mediated constriction followed by a prolonged, mainly $\alpha_{1A}$-adrenoceptor-mediated response (5). Furthermore, BMY 7378, an $\alpha_{1D}$-adrenoceptor antagonist (6), reduced the PNS-induced second-peaked, adrenergic response by approximately 30%. Exposure of tissues to chloroethylclonidine (CEC), an $\alpha_{1B}$-adrenoceptor antagonist, attenuated the second-peaked response by approximately 60%, even in the presence of BMY 7378. However, blockade of $\alpha_{1A}$-adrenoceptors with WB4101 failed to affect the neuronal adrenergic response, although exogenous NA-induced responses were inhibited (3, 4). It has been reported that both $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes are effectively inactivated by CEC, although the former subtype is relatively more sensitive (7). Therefore, a much more selective competitive antagonist is needed for the precise, quantitative characterization of $\alpha_{1B}$-adrenoceptors in the junctional area.

In 1996, a selective $\alpha_{1B}$-adrenoceptor antagonist, cyclazosin which displays a 90- to 130-fold selectivity for binding to rat $\alpha_{1B}$-adrenoceptors compared to $\alpha_{1A}$ and $\alpha_{1D}$ subtypes was reported (8). However, in functional pharmacological experiments using the rat spleen, cyclazosin did not behave as a selective $\alpha_{1B}$-adrenoceptor antagonist (9). More recently, L-765,314 was reported as a potent and selective $\alpha_{1B}$-adrenoceptor antagonist (10). Thus, in this study, we tried to examine effects of L-765,314 on PNS-induced adrenergic vasoconstrictor responses.

Mongrel dogs of either sex, weighing 10 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 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 (11). 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 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 recti-

![Graph A](image1)

**Fig. 1.** Effects of L-765,314 on the vasoconstrictor responses to exogenously given noradrenaline (NA) (A) and A61603 (B) in the canine splenic arteries. Data are presented as the mean ± S.E.M., n = 6. **P<0.01, as compared with the control group.

![Graph B](image2)

**Fig. 2.** Effects of L-765,314 on double-peaked vasoconstrictor responses to periarterial nerve electrical stimulation (PNS) (1 and 4 Hz) of the isolated, perfused canine splenic artery. Control (A); after treatment with 0.1 μM (B), 1 μM (C) and 10 μM (D) L-765,314.
the extraluminal side of the arterial wall. Electrical stimulation was delivered by an electric stimulator (SEN-7203, Nihon Kohden), using 1 and 4 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 L-765,314 before the second response curves were made for electrical stimulation.

Drugs used were dl-noradrenaline hydrochloride (Sigma, St. Louis, MO, USA), A61603 hydrobromide (N-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]methylene sulphonamide) (Tocris Cookson, Bristol, UK), L-765,314 hydrochloride (4-amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[1,1-dimethylethyl]amino]carboxyl]-piperazinyl]-6,7-dimethoxyquinazoline) (Merck & Co., Inc. Rahway, NJ, USA). All drugs used were dissolved in distilled water. The stock solutions were kept at 20°C until used.

Vasoconstrictor responses to PNS and agonists are expressed as the maximal changes in perfusion pressure (mmHg). 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.

When L-765,314 was intraluminally given in a dose range of 1 – 10 μM, no significant vascular changes were observed. The vasoconstrictor responses to NA (0.03 – 1 nmol) were not significantly inhibited by 0.1 (n = 6, data not shown) and 1 μM L-765,314. However, 10 μM L-765,314 significantly inhibited NA-induced vasoconstrictions as shown in Fig. 1A, causing a parallel shift of the dose-response curve for NA to the right. As shown in Fig. 1B, A61603 (a selective α1A-adrenoceptor agonist) at concentrations of 1 – 30 pmol induced a strong dose-dependent vasoconstriction. The dose-response curve for A61603 was not significantly inhibited by a smaller dose of L-765,314 (1 μM), but was shifted to the right in parallel manner by a larger dose of L-765,314 (10 μM) (Fig. 1B). Figure 2 shows a typical tracing of effects of L-765,314 (0.1 – 10 μM) on PNS-induced responses to stimulation at 1 and 4 Hz. The PNS-induced adrenergic responses (second peaked responses) were significantly inhibited by 0.1 and 1 μM L-765,314 (Fig. 2: B and C). After treatment with 10 μM L-765,314, PNS-induced second peaked responses were mostly completely inhibited, although the first peaked responses were not influenced (Fig. 2D). Summarized data are shown in Fig. 3.

Previously, we reported that the PNS-induced adrenergic responses were significantly blocked by treatment with 60 μM CEC, an α1B-adrenoceptor antagonist, but were not inhibited by WB4101, an α1A-adrenoceptor antagonist (3, 4). On the other hand, the vasoconstrictor responses to administered NA were dose-dependently antagonized by WB4101 (10 – 100 nM), but were not significantly by CEC (60 μM) (3, 4). These results indicated that the sympathetic adrenergic vasoconstriction of the canine splenic artery is likely mediated via an activation of the postjunctional α1B-adrenoceptors, whereas the exogenous NA-induced response is possibly induced by α1A-adrenoceptors (3, 4). The present results showed that L-765,314 at relatively small doses (0.1 and 1 μM) did not significantly affect
vасoсonstrictor responses to exogenous NA or A61603, a selective $\alpha_{1A}$-adrenoceptor agonist (12), but markedly inhibited PNS-induced adrenergic responses. A tenfold increase in the dose of L-765,314 (10 $\mu$M) not only produced a further inhibition on PNS-induced adrenergic responses but also significantly inhibited the vasoconstrictor responses to these agonists. Thus, it is considered that L-765,314 at a smaller dose range (0.1 and 1 $\mu$M) might cause selective $\alpha_{1D}$-adrenoceptor blocking activity, whereas it might also have $\alpha_{1A}$-adrenoceptor blocking activity at a large dose. It has recently been reported that the phenylephrine-induced responses in the canine external carotid circulation were antagonized by 5-methylurapidil, an $\alpha_{1A}$-adrenoceptor antagonist, but unaffected by L-765,314 (13). It is therefore considered that a suitable dose of L-765,314 has a selective $\alpha_{1B}$-adrenoceptor blocking properties, although it is still necessary to obtain a much more selective $\alpha_{1B}$-adrenoceptor antagonist than L-765,314.

From these results, it is confirmed that PNS-induced adrenergic responses are mostly mediated via the $\alpha_{1B}$-adrenoceptor subtype.

REFERENCES


