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Interaction between neuropeptide Y Y_1 receptors and α_{1B}-adrenoceptors in the neurovascular junction of canine splenic arteries

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Abstract

Previous study has demonstrated that periarterial electrical nerve stimulation (30-s trains of pulses at a frequency of 1 or 4 Hz) induces a double-peaked vasoconstriction consisting of an initial transient, predominantly P2X-receptor-mediated constriction followed by a prolonged, mainly α_1-adrenoceptor-mediated response in the isolated canine splenic artery. Treatment with 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY 7378, 0.1 μmol/l), a selective α_{1D}-adrenoceptor antagonist, produced a slight but significant inhibition of the second peaked responses. A marked inhibition of second peaked responses was obtained by exposure of the tissues to chloroethylclonidine (60 μmol/l), an α_{1B}- and α_{1D}-adrenoeptor antagonist. Neither BMY 7378 nor chloroethylclonidine affected the first peaked vasoconstrictor responses. [Leu^{31},Pro^{34}]Neuropeptide Y (10–30 nmol/l), a selective neuropeptide Y Y_1 receptor agonist, enhanced the second peaked responses in the presence of BMY 7378 but failed to enhance the responses in the presence of chloroethylclonidine. The results indicate that the postjunctional α_{1B}-adrenoceptor subtype is likely coupled to neuropeptide Y Y_1 receptors responsible for the cooperation of the sympathetic adrenergic and neuropeptide Yergic transmission in the canine splenic artery.

Keywords: Neurojunctional transmission; α_{1B}-Adrenoceptor; Neuropeptide Y Y_1 receptor; Splenic artery, canine

1. Introduction

Neuropeptide Y Y_1 receptors are suggested to be a postjunctional facilitatory subtype of neuropeptide Y receptors in sympathetically innervated vascular preparations (Malmström and Lundberg, 1995; Donoso et al., 1997; Han et al., 1998; Phillip et al., 1998; Cortés et al., 1999; Racchi et al., 1999; Yang and Chiba, 2000a). Neuropeptide Y released from periarterial sympathetic nerve terminals exerts its direct and indirect synergistic vasoconstriction, primarily through the activation of the neuropeptide Y Y_1 receptor subtype (Donoso et al., 1997; Cortés et al., 1999; Yang and Chiba, 2000a). Activation of neuropeptide Y Y_1 receptors by neuronal or exogenous neuropeptide Y can enhance the α_1-adrenoceptor-mediated sympathetic vasoconstriction, whereas it is unable to affect an exogenous noradrenaline-induced response in the canine splenic artery (Yang and Chiba, 2000a,b). The explanation for this difference is thought to be that there is an α_1-adrenoceptor subtype-specific role involved in these responses, i.e., the postjunctional α_{1B}- and α_{1D}-adrenoceptor subtypes were demonstrated to receive a sympathetic adrenergic innervation in this preparation, whereas the α_{1A}-adrenoceptor subtype may be extrajunctionally activated by exogenous noradrenaline (Yang and Chiba, 2000c, 2001). Thus, it is hypothesized that the postjunctional α_{1B}- or α_{1D}-adrenoceptor subtype is a target that interacts with the neuropeptide Y Y_1 receptor. The purpose of this study was to clarify which of the α_1-adrenoceptor subtypes is responsible for this interactive process of the canine splenic artery, using 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro-[4.5]decane-7,9-dione (BMY 7378), a selective α_{1D}-adrenoceptor antagonist (Goetz et al., 1995; Hieble et al., 1995), and chloroethylclonidine, an α_{1B}- and α_{1D}-adrenoceptor antagonist (Han et al., 1987; Hieble et al., 1995; Schwinn et al., 1995).

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2. Materials and methods

2.1. Arterial preparations

Mongrel dogs of either sex, weighing 9–13 kg, were anaesthetized with sodium pentobarbitone (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 (1–1.2 mm in an outer diameter) was cut into segments (15–20 mm in length). Four segments were obtained from each splenic artery. Each segment was cannulated and set up for perfusion as described previously (Hongo and Chiba, 1983; Tsuji and Chiba, 1984). 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, Japan) with Krebs–Henseleit solution gassed with 95% O_2 and 5% CO_2. The solution contained (in mmol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25 and glucose, 10. The flow rate was kept at approximately 2 ml/min. The perfusion pressure was measured continuously with an electric manometer (MPU-0.5A, Nihon Kohden, Tokyo, Japan) 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 40–80 mm Hg.

For electrical stimulation of the periarterial sympathetic nerve terminals, two platinum electrodes were placed on the extraluminal side of the arterial wall. Periarterial electrical nerve 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, over a frequency range of 1 and 4 Hz. The organ bath was sealed with plastic film to maintain the preparation at 37 °C. It required 10-min intervals between electrical stimulation periods to obtain a reproducible response. The intervals between frequency–response curves were over 1-h. The preparations were incubated for 30 min with BMY 7378 or with [Leu^{31},Pro^{34}]neuropeptide Y before the next response curves were made for nerve stimulation. Pretreatment of preparations with chloroethylclonidine was achieved by perfusion of tissue segments with drug-containing physiological solution for 30 min, followed by perfusion with drug-free solution for 30 min before frequency–response curves to nerve stimulation were made.

2.2. Drugs

Drugs used were [Leu^{31},Pro^{34}]neuropeptide Y (human) (Sigma, St. Louis, USA); chloroethylclonidine dihydrochloride; BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) (Research Biochemicals, Natick, MA, USA). [Leu^{31},Pro^{34}]Neuropeptide Y was dissolved in 0.5% (w/v) bovine serum albumin in distilled water. Other drugs were dissolved in distilled water. The stock solutions were kept at −20 °C until used.

2.3. Statistical analysis

Vasoconstrictor responses to nerve stimulation are expressed as the maximal changes in perfusion pressure (mm Hg) from their basal levels. The data are shown as means ± S.E.M. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Vasoconstrictor responses to nerve stimulation

Periarterial electrical nerve stimulation at a frequency of 1 or 4 Hz induced a double-peaked (two-phase) vasoconstriction consisting of an initial transient constriction followed by a prolonged contractile response in the isolated, perfused canine splenic artery (Figs. 1A and 3A), as reported previously (Yang and Chiba, 1998).

3.2. Effects of [Leu^{31},Pro^{34}]neuropeptide Y on nerve stimulation-induced vasoconstrictions in the presence of BMY 7378

Fig. 1 shows an original tracing of nerve stimulation-induced double-peaked responses from typical experiments showing the effects of [Leu^{31},Pro^{34}]neuropeptide Y after treatment with BMY 7378. The perfusion with 0.1 μmol/l BMY 7378 produced a slight, but significant inhibition on the second peaked responses induced by nerve stimulation (1 and 4 Hz), but did not affect the first peaked responses (Fig. 1B). The subsequent application of [Leu^{31},Pro^{34}]neuropeptide Y (10–30 nmol/l) potentiated only the second peaked constrictions dose dependently, but failed to influence the first peaked responses (Fig. 1C and D). The summarized data show that BMY 7378 treatment significantly decreased the second peaked responses, and that additional treatment with [Leu^{31},Pro^{34}]neuropeptide Y (10–30 nmol/l) reversed the BMY 7378-induced effects. The potentiating effect by 30 nmol/l [Leu^{31},Pro^{34}]neuropeptide Y was greater than the response of the control in the absence of BMY 7378 (Fig. 2).

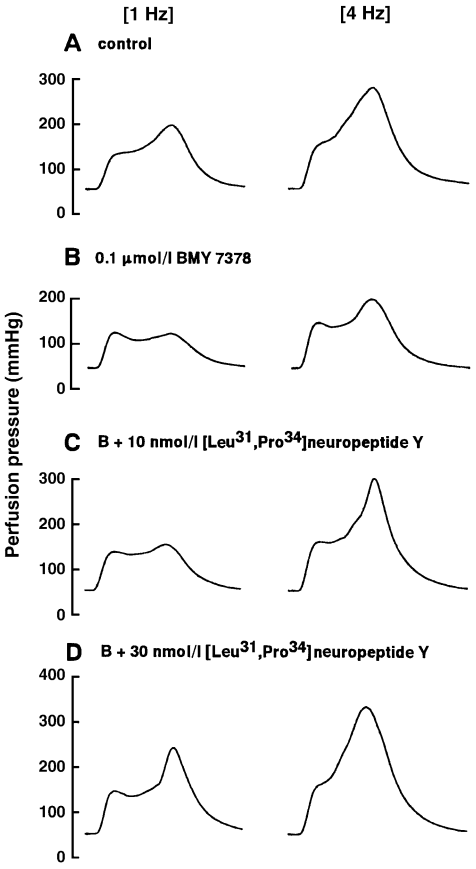

Fig. 1. Double-peaked vasoconstrictor responses to periarterial electrical nerve stimulation and the effects of [Leu^{31},Pro^{34}]neuropeptide Y on BMY 7378-resistant responses in an isolated, perfused canine splenic artery. The double-peaked vasoconstriction was induced by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 or 4 Hz.

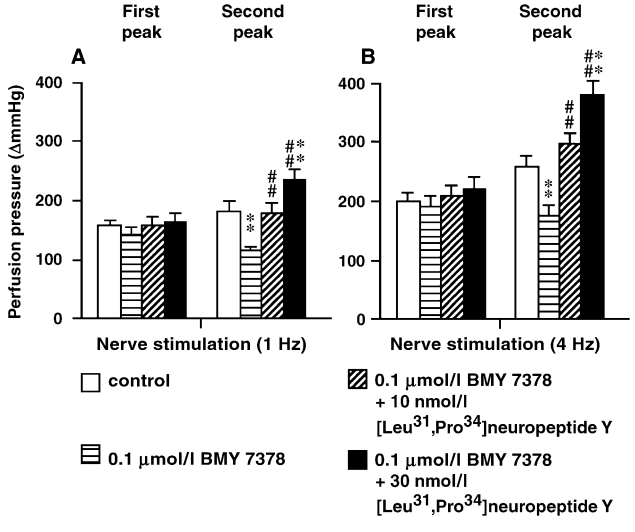

Fig. 2. Effects of [Leu^{31},Pro^{34}]neuropeptide Y on BMY 7378-resistant vasoconstrictor responses to periarterial electrical nerve stimulation at 1 Hz (A) and 4 Hz (B) in canine splenic arteries. The vessels were electrically stimulated by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with frequencies of 1 and 4 Hz. Data are presented as means ± S.E.M., $n=6$. $^{**}P<0.01$ as compared with the control group. $^{\#\#}P<0.01$ as compared with the preceding group.

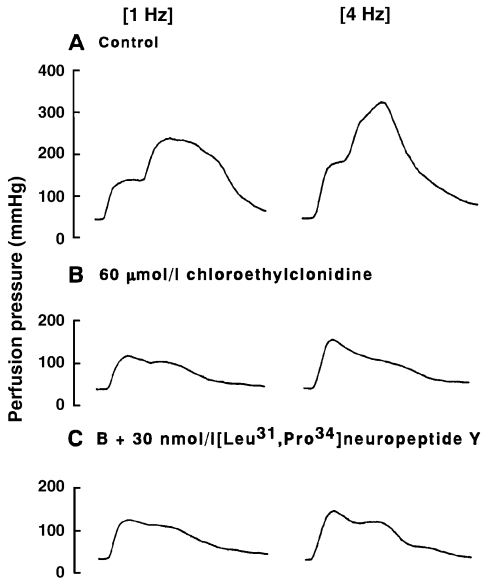

Fig. 3. Double-peaked vasoconstrictor responses to periarterial electrical nerve stimulation and the effects of [Leu^{31},Pro^{34}]neuropeptide Y on chloroethylclonidine-resistant responses in an isolated, perfused canine splenic artery. The double-peaked vasoconstriction was induced by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 or 4 Hz.

3.3. Effects of [Leu^{31},Pro^{34}]neuropeptide Y on nerve stimulation-induced vasoconstriction in the presence of chloroethylclonidine

Fig. 3 shows an original tracing of double-peaked responses to nerve stimulation from typical experiments showing the effects of [Leu^{31},Pro^{34}]neuropeptide Y after treatment with chloroethylclonidine. As shown in Fig. 3B,

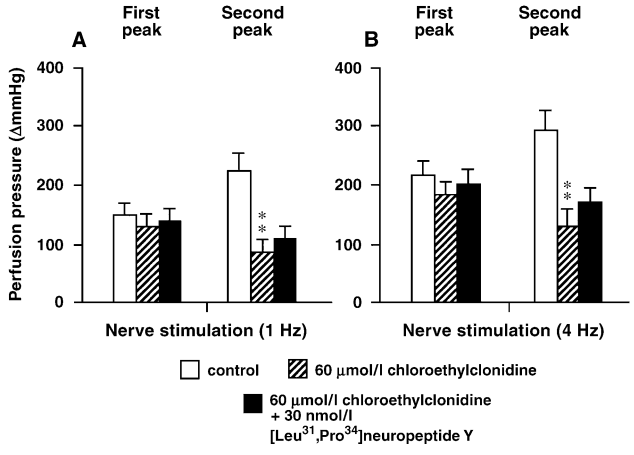

Fig. 4. Effects of [Leu^{31},Pro^{34}]neuropeptide Y on chloroethylclonidine-resistant vasoconstrictor responses to periarterial electrical nerve stimulation at 1 Hz (A) and 4 Hz (B) in the canine splenic arteries. The vessels were electrically stimulated by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with frequencies of 1 and 4 Hz. Data are presented as means ± S.E.M., $n=10$. $^{**}P<0.01$ as compared with the control group.

chloroethylclonidine at a concentration of 60 μmol/l markedly suppressed the second peaked vasoconstrictions, but did not affect the first peaked responses. Since a small dose of 1 μmol/l chloroethylclonidine has no blocking effect on nerve stimulation-induced responses as reported previously (Yang and Chiba, 2001), we used 60 μmol/l chloroethylclonidine, the concentration which strongly inhibited the nerve stimulation-induced second peaked vasoconstrictions. Subsequent treatment with [Leu^{31},Pro^{34}]neuropeptide Y (30 nmol/l) failed to enhance the second peaked vasoconstrictions (Fig. 3C). The summarized data are shown in Fig. 4.

4. Discussion

Results of previous studies on the canine splenic artery have suggested that the neuropeptide Y Y_1 receptor is coupled to the specific subtype of α_1-adrenoceptor responsible for the neuropeptide Y-mediated cooperation of sympathetic adrenergic vasoconstriction (Yang and Chiba, 2000a,b). The present results provide evidence that the specific subtype of α_1-adrenoceptors seems to be an α_{1B}-subtype.

It was initially observed that periarterial electrical nerve stimulation induced a double-peaked vasoconstriction consisting of an initial transient, predominantly P2X-receptor-mediated constriction followed by a prolonged, mainly α_1-adrenoceptor-mediated response in the canine splenic artery (Yang and Chiba, 1998). Further observations showed that neuropeptide Y, via activation of neuropeptide Y Y_1 receptors, acted as a neuromodulator in the cooperation of the sympathetic adrenergic response (Yang and Chiba, 2000a). However, activation of neuropeptide Y Y_1 receptors by [Leu^{31},Pro^{34}]neuropeptide Y potentiated the nerve-stimulated, α_1-adrenoceptor-mediated second peaked constriction, whereas it did not affect the response induced by exogenous noradrenaline (Yang and Chiba, 2000b). Furthermore, the potentiating effect of [Leu^{31},Pro^{34}]neuropeptide Y was not only abolished by BIBP 3226, a selective neuropeptide Y Y_1 receptor antagonist, but it was also prevented by prazosin, a nonselective α_1-adrenoceptor antagonist (Yang and Chiba, 2000b). These findings led us to consider that neuropeptide Y may produce its potentiation of sympathetic adrenergic vasoconstriction through a synergistic effect between neuropeptide Y Y_1 receptors and the specific subtype of α_1-adrenoceptors. Interestingly, we recently found that the sympathetic-innervated subtypes of α_1-adrenoceptors in the canine splenic artery were mainly α_{1B}-, and in part, α_{1D}-subtype, rather than α_{1A}-subtype, since the nerve-stimulated second peaked response was much reduced by chloroethylclonidine, an α_{1B}- and α_{1D}-adrenoceptor antagonist, and was partially inhibited by BMY 7378, a selective α_{1D}-adrenoceptor antagonist, but was not inhibited by 2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane (WB 4101), an α_{1A}-adrenoceptor antagonist (Yang and Chiba, 2000c, 2001). On the other hand, the dose–response curves for noradrenaline were shifted in parallel to the right by treatment with WB 4101. However, they were mostly unaffected by either chloroethylclonidine or BMY 7378 (Yang and Chiba, 2001). Pharmacological evidence has demonstrated that chloroethylclonidine can inactivate both α_{1B}- and α_{1D}-adrenoceptors, although the former subtype is relatively more sensitive (Schwinn et al., 1995). The finding that activation of neuropeptide Y Y_1 receptors by [Leu^{31}, Pro^{34}]neuropeptide Y failed to potentiate the α_1-adrenoceptor-mediated second peaked vasoconstriction after treatment with chloroethylclonidine possibly indicates that the subtype of α_1-adrenoceptors potentiated by neuropeptide Y Y_1 receptors is of the α_{1B}- or α_{1D}-subtype. However, we observed that [Leu^{31}, Pro^{34}]neuropeptide Y enhanced the second peaked response even after blockade of α_{1D}-adrenoceptors with BMY 7378. Thus, the specific subtype of α_1-adrenoceptors in cooperation with neuropeptide Y Y_1 receptors is postulated to be mostly α_{1B}-subtype.

It has been suggested that the synergism between neuropeptide Y Y_1 receptors and α_1-adrenoceptors plays an important role in the regulation of the sympathetic vasomotor effect in the rat arterial mesenteric bed (Donoso et al., 1997; Cortés et al., 1999). The present results provided supporting evidence for this suggestion applied to the canine splenic artery, and further clarified that neuropeptide Y enhanced sympathetic adrenergic vasoconstriction possibly through the synergistic effects between neuropeptide Y Y_1 receptor and the α_{1B}-adrenoceptor subtypes. The question now raised is how do we explain the findings concerning the specific synergistic effect in our experiments? Functional studies on the rat arterial mesenteric bed have shown that neuropeptide Y requires precontraction with noradrenaline or other receptor agonists linked to a phospholipase C signaling pathway to elicit its vasomotor effect (Cortés et al., 1999). Phospholipase C has been demonstrated to be activated by noradrenaline via stimulation of α_{1B}-adrenoceptors in rat medullary thyroid carcinoma 6–23 cells (Esbenshade et al., 1994). Furthermore, activation of neuropeptide Y Y_1 receptors in the porcine aortic smooth muscle cells stimulates mobilization of Ca^{2+} from intracellular store sites via a phospholipase C-dependent pathway (Shigeri et al., 1995). Thus, it is likely that a phospholipase C-dependent pathway is a biochemical basis for the synergism of neuropeptide Y Y_1 receptors and α_{1B}-adrenoceptors. Whether this mechanism represents what actually occurs in the canine splenic artery still remains to be studied.

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