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Neurogenic double-peaked vasoconstriction of human gastroepiploic artery is mediated by both α_1- and α_2-adrenoceptors

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1 The contribution of postjunctional P2X receptors and subtypes of α-adrenoceptors to vasoconstrictor responses following periarterial electrical nerve stimulation (PNS, 30 s trains of pulses at a frequency of 2, 4 or 8 Hz) was investigated in human gastroepiploic arteries.

2 The vasoconstrictor response to PNS at a stimulation of 4 or 8 Hz was a two-peaked response, whereas at a frequency of 2 Hz it appeared only as a late peak. All vasoconstrictions evoked by PNS were abolished by phentolamine, a nonselective α-adrenoceptor inhibitor, but not by α,β-methylene ATP, a P2X receptor-desensitizing agent.

3 The early peak to PNS at 4 or 8 Hz was abolished by prazosin, an α_1-adrenoceptor antagonist, while the late one still remained, although it was markedly inhibited. The responses remaining after prazosin were blocked by rauwolscine. The vasoconstrictor response to PNS at 2 Hz was not affected by prazosin (0.1 μM), but was abolished by rauwolscine (0.1 μM), an α_2-adrenoceptor antagonist.

4 OPC-28326(10 μM), a newly developed vasodilator, which preferentially exerts its antagonistic actions on the α_{2B}- and α_{2C}-adrenoceptors, significantly reduced the noradrenaline-induced vasoconstriction in the absence or presence of prazosin. OPC-28326 had a greater inhibitory effect on the late peak evoked by PNS than the early one. The neurogenic responses remaining after OPC-28326 were abolished by prazosin.

5 The present results suggest that sympathetic vasoconstriction of the human gastroepiploic artery is mediated by both α_1- and α_2-adrenoceptors postjunctionally, but not by P2X receptors. The α_2-adrenoceptors may be preferentially activated at a low frequency of stimulation, which induces a constriction more slowly than that by α_1-adrenoceptors. The existence of α_2-adrenoceptors may cause an enhancement of α_1-adrenoceptor-induced responses.
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Abbreviations: ATP, adenosine 5′-triphosphate; BHT-933, 2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-(5,4-a)-azepin dihydrochloride; NA, noradrenaline; OPC-28326, 4-(*N*-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionyl-aminobenzoyl) piperidine hydrochloride monohydrate; PNS, periarterial electrical nerve stimulation

Introduction

It is well known that adenosine 5′-triphosphate (ATP) acts as a cotransmitter with noradrenaline (NA) in blood vessels innervated by sympathetic nerves (von Kügelgen & Starke, 1991; Chiba & Yang, 2003). However, the functional evidence obtained in *in vitro* human arterial tissues suggest that neurally evoked vasoconstriction contains an adrenergic component, but has no purinergic component (Parkinson *et al*., 1992; Stephens *et al*., 1992). In the human saphenous vein, the presence of a purinergic component for mediating sympathetic vasomotor responses has been described (Rump & von Kügelgen, 1994; Racchi *et al*., 1999). It seems that the contribution of the purinergic component to the neuroeffector response varies with tissues and species.

From studies on human forearm blood flow, it has been proposed that the postjunctional α_1-adrenoceptors are activated only by neurally released NA, whereas α_2-adrenoceptors are selectively stimulated by circulating catecholamines (Jie *et al*., 1984; 1987). Later results from investigations on human isolated resistance arteries disputed this hypothesis, indicating that both α_1- and α_2-adrenoceptors are activated postjunctionally by neurally released NA (Stevens & Moulds, 1985; Parkinson *et al*., 1992; Stephens *et al*., 1992). Recently, we observed that both α_1- and α_2-adrenoceptors contributed to the vasoconstrictor responses to exogenously applied NA in human isolated, perfused gastroepiploic arteries (Fukui & Chiba, 2003). The purpose of this study was to clarify the possible contributions of purinoceptors or different adrenoceptor subtypes to the neuroeffector response in human gastroepiploic arteries.

Methods

Arterial preparations

A total of 20 patients (age 46–68 years; 12 men, eight women) undergoing gastrectomy for gastric cancer served as vessel

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donors. The patients had no clear cardiovascular risk factors such as ischaemic heart disease, cerebral infarction, hypertension, hyperlipidaemia, diabetes mellitus or renal failure. The experimental protocol was approved by the Medical Ethics Committee of Shinshu University School of Medicine, and informed consent was obtained from all patients. The gastroepiploic arteries were carefully removed just after the omentum was resected. Isolated gastroepiploic arteries, which were 15–20 mm in length and 2–3 mm in outer diameter, were cannulated and set up for perfusion as described previously (Hongo & Chiba, 1983; Tsuji & Chiba, 1984). The cannulated arteries were placed in a bath maintained at 37°C using a thermopump (Haake EF2, Karlsruhe, Germany), and perfused with Krebs-Henseleit solution with a microtube pump (Tokyo Rikakikai MP 3A, Tokyo, Japan). The composition of the Krebs-Henseleit solution was (in mM): 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 solution was bubbled with 95% O_2 and 5% CO_2, and the pH was maintained at 7.2–7.4. The flow rate (approximately 2 ml min^{-1}) was kept constant throughout the experiments, and basal perfusion pressure was 40–60 mmHg in all preparations. The perfusion pressure became stable 60 min after setting up the system.

Periarterial electrical nerve stimulation (PNS)

The preparation was removed from the bath solution and fixed in a horizontal position. Then, two platinum electrodes were placed on the extraluminal side of the arterial wall. PNS 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 2, 4 and 8 Hz, as reported previously (Yang & Chiba, 1998).

Experimental procedure

After a constant perfusion of 1 h, a frequency-dependent double-peaked vasoconstriction was induced by PNS. The preparations were incubated for 10 min with tetrodotoxin, for 30 min with rauwolscine, and for 1 h with other antagonists before the next response curves were made by PNS. Agonists were administered into the rubber tubing close to the cannula in a volume of 0.01–0.03 ml using microinjectors (Terumo, Tokyo, Japan).

Drugs

The drugs used were guanethidine monosulphate; α,β-methylene ATP-lithium salt; DL-NA hydrochloride; phentolamine hydrochloride; prazosin hydrochloride; tetrodotoxin; phenylephrine hydrochloride; suramin sodium salt (Sigma, St Louis, U.S.A.); rauwolscine hydrochloride (Extrasynthese, Genay, France); BHT-933 (Boehringer Ingelheim Pharma KG, Biberach, Germany). OPC-28326 was kindly provided by Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan. All drugs were dissolved in distilled water. The stock solutions were kept at −20°C until used.

Statistical analysis

Vasoconstrictor responses to PNS and exogenously applied agents are expressed as the maximal changes in perfusion pressure (mmHg) from their basal levels. The data are shown as mean ± s.e.m. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons. *P*-values <0.05 were considered statistically significant.

Results

Vasoconstrictor responses to PNS

PNS of the human isolated gastroepiploic artery using a 30 s train of pulses at 4 or 8 Hz caused a biphasic constriction. The early peak started within 10–14 s, and the late one within 30–35 s after the onset of PNS. The early peak of the biphasic response was reached approximately 25 s, and the late peak within 45–50 s after the onset of PNS (Figures 1a and 4a). The vasoconstrictor response to PNS at 2 Hz appeared to be a monophasic response. The time course of the responses to 2 Hz was similar to that of the late peak (Figures 1a and 4a). PNS of human gastroepiploic artery at a low frequency of 2 Hz induced only a late response. The vasoconstriction induced by PNS at the frequencies used was abolished by either tetrodotoxin (1 μM) or guanethidine (10 μM).

The vasoconstrictor responses to PNS were not significantly influenced by the vehicle (distilled water) used in this study ($n=4$, data not shown). Repeating PNS at the frequencies used induced almost the same responses ($n=5$, data not shown).

Effects of α,β-methylene ATP and phentolamine on vasoconstrictor responses to PNS

PNS-induced vasoconstriction of the human gastroepiploic artery was not significantly affected by α,β-methylene ATP (1 μM), but was completely blocked by subsequent treatment with phentolamine (10 μM) ($n=4$, data not shown).

Effects of prazosin, rauwolscine and suramin on vasoconstrictor responses to PNS

Figure 1 is an original tracing of neurogenic vasoconstriction of a human gastroepiploic artery from typical experiments showing the effects of prazosin and rauwolscine. The

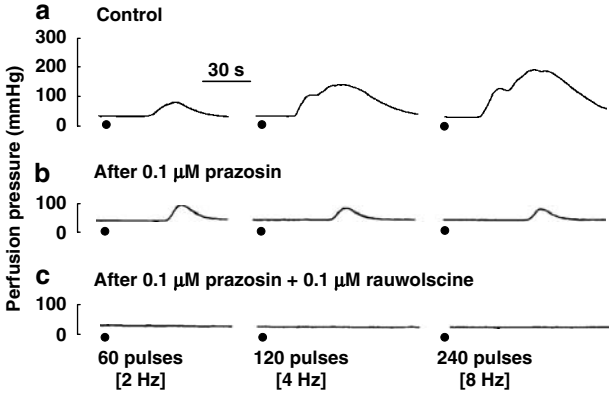

Figure 1 Vasoconstrictor responses to PNS and the effects of prazosin and rauwolscine in a perfused human isolated gastroepiploic artery. The vasoconstriction was induced by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, over a frequency range of 2, 4 and 8 Hz.

summarized data for prazosin and rauwolscine are shown in Figure 2.

The early peaks to PNS at 4 and 8 Hz were abolished by prazosin (0.1 μM), and the late ones were markedly reduced but not completely inhibited (Figures 1b and 2). The remaining late peaks after prazosin were abolished by subsequent application of rauwolscine (Figures 1c and 2). The late peak induced by 2 Hz was unaffected by prazosin (0.1 μM), but abolished by rauwolscine (0.1 μM) (Figures 1 and 2).

Pretreatment with rauwolscine eliminated the late response induced by 2 Hz and markedly inhibited the two peaked responses by 4 or 8 Hz (Figure 3). The remaining response after rauwolscine was abolished by subsequent administration of prazosin (Figure 3). In addition, suramin (30 μM) had no significant effect on the vasoconstriction by PNS after treatment with rauwolscine ($n=3$, data not shown).

Effects of OPC-28326 on vasoconstrictor responses evoked by PNS

Figure 4 is an original tracing of neurogenic vasoconstriction of a human gastroepiploic artery from typical experiments showing the effects of OPC-28326 and prazosin. The summarized data for OPC-28326 and prazosin are shown in Figure 5. The late response to a low frequency of 2 Hz was suppressed by OPC-28326 (10 μM). Both the early and late peaks to PNS at 4 and 8 Hz were significantly reduced by OPC-28326 (Figures 4b and 5). The remaining responses after OPC-28326 were abolished by subsequent application of prazosin (0.1 μM) (Figures 4c and 5).

Effects of OPC-28326 on vasoconstrictor responses induced by exogenously administered NA, phenylephrine and BHT-933

The dose–response curves for NA were shifted to the right by either 10 μM OPC-28326 (Figure 6) or 0.1 μM prazosin (Figure 7). The residual responses after OPC-28326 or prazosin were abolished by a combination of these two drugs (Figures 6 and 7). OPC-28326 (10 μM) suppressed the vasoconstrictor responses induced either by phenylephrine (Figure 8) or by BHT-933 (0.3 μmol) ($n=3$, data not shown).

Discussion

Evidence obtained from animal vascular tissues has suggested that ATP is a cotransmitter with NA in postganglionic sympathetic nerves (von Kügelgen & Starke, 1991; Chiba & Yang, 2003). However, purinergic transmission cannot be confirmed in human arterial blood vessels (Parkinson *et al*., 1992; Stephens *et al*., 1992), although sympathetic vasomotor

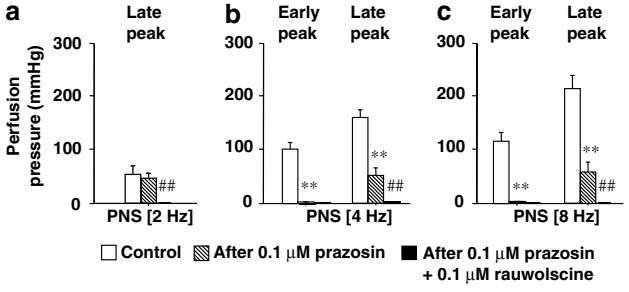

Figure 2 Effects of prazosin and rauwolscine on the vasoconstrictor responses to PNS at 2 Hz (a), 4 Hz (b) and 8 Hz (c) in human gastroepiploic arteries. Data are presented as mean ± s.e.m. ($n=6$). $^{**}P<0.01$ as compared with the control group. $^{\#\#}P<0.01$ as compared with the preceding group.

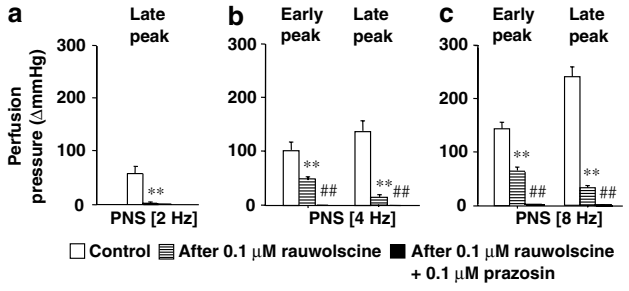

Figure 3 Effects of rauwolscine on the vasoconstrictor responses to PNS at 2 Hz (a), 4 Hz (b) and 8 Hz (c) in human gastroepiploic arteries. Data are presented as mean ± s.e.m. ($n=5$). $^{**}P<0.01$ as compared with the control group. $^{\#\#}P<0.01$ as compared with the preceding group.

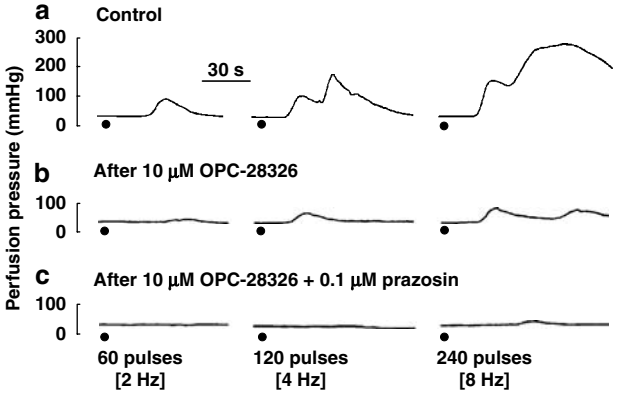

Figure 4 Vasoconstrictor responses to PNS and the effects of OPC-28326 in an isolated, perfused human gastroepiploic artery. The vasoconstriction was induced by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, over a frequency range of 2, 4 and 8 Hz.

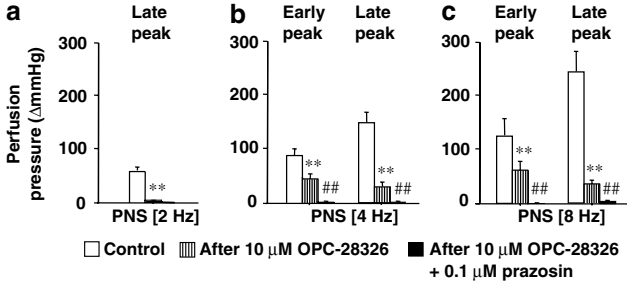

Figure 5 Effects of OPC-28326 on the vasoconstrictor responses to PNS at 2 Hz (a), 4 Hz (b) and 8 Hz (c) in human gastroepiploic arteries. Data are presented as mean ± s.e.m. ($n=6$). $^{**}P<0.01$ as compared with the control group. $^{\#\#}P<0.01$ as compared with the preceding group.

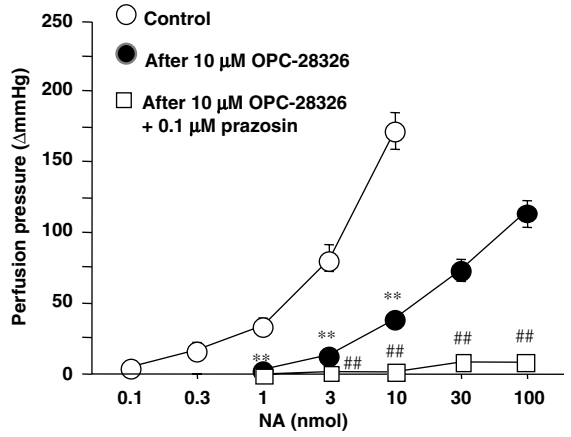

Figure 6 Effects of OPC-28326 on the vasoconstrictor responses to exogenously administered NA in human gastroepiploic arteries. Data are presented as mean ± s.e.m. ($n = 6$). $^{**}P < 0.01$ as compared with the control group. $^{\#\#}P < 0.01$ as compared with the OPC-28326 group.

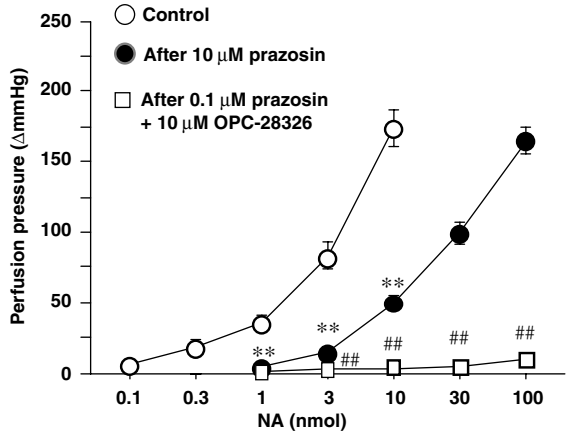

Figure 7 Effects of OPC-28326 on the vasoconstrictor responses of human gastroepiploic arteries to exogenously administered NA in the presence of prazosin. Data are presented as mean ± s.e.m. ($n = 6$). $^{**}P < 0.01$ as compared with the control group. $^{\#\#}P < 0.01$ as compared with the prazosin group.

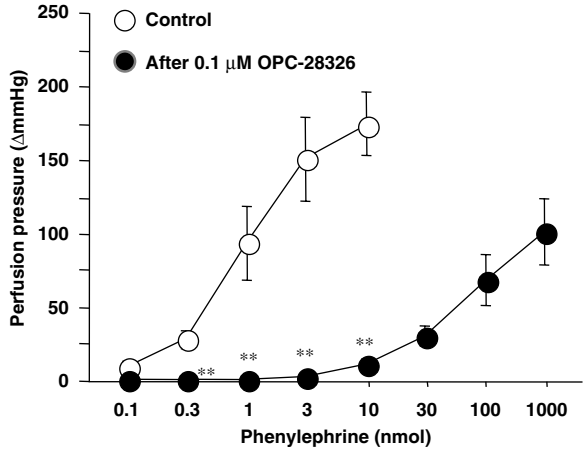

Figure 8 Effects of OPC-28326 on the vasoconstrictor responses of human gastroepiploic arteries to exogenously administered phenylephrine. Data are presented as mean ± s.e.m. ($n = 6$). $^{**}P < 0.01$ as compared with the control group.

constriction in human saphenous vein may contain a purinergic component (Rump & Von Kügelgen, 1994; Racchi *et al*., 1999). The present study provided further evidence that the neurogenic vasoconstriction of the human gastroepiploic artery is adrenergic in origin, since the neurogenic responses were not significantly affected by α,β-methylene ATP, a P2X-receptor desensitizing agent, but were readily blocked by phentolamine, an α-adrenoceptor antagonist. In contrast, a purinergic component was partially involved in mediating neuronal responses in the canine gastroepiploic artery (Tanaka *et al*., 2003). This evidence suggests that the contribution of each component of cotransmission to neuroeffector constriction varies between different species, and there are also tissue differences.

The vasoconstriction of the human gastroepiploic artery by PNS at 4 or 8 Hz appeared to be a double-peaked response, although the early peak was lost at a low frequency of 2 Hz. Using the same experimental conditions and methods, we previously observed that neurogenic vasoconstriction of the canine splenic artery was a double-peaked response consisting of an initial fast, dominantly P2X-receptor-mediated peak, followed by a delayed, mainly α_1-adrenoceptor-mediated peak (Yang & Chiba, 1998; 2000). Unlike the canine splenic artery, which begins to constrict almost immediately (within 0–4 s), the onset of the vasoconstriction in the human gastroepiploic artery developed slowly (within 14–16 s) after PNS. The slower time course of vasoconstriction in the human gastroepiploic artery was similar to that of adrenergic transmission in the canine splenic artery. Previous studies *in vitro* in the rat tail artery (Bao *et al*., 1989; 1993) have shown that the components of neurally evoked contractions can be divided into three phases, that is, initial fast, purinergic phase, intermediate slow, α_1-adrenoceptor-mediated phase, followed by a much delayed α_2-adrenoceptor-mediated phase. In the canine saphenous vein, it was observed that the α_1-adrenergic component develops more rapidly than the α_2-adrenergic component (Hiraoka *et al*., 2000). Both α_1- and α_2-adrenoceptors also contribute to a biphasic constriction in several rabbit isolated blood vessels (MacDonald *et al*., 1992). In the case of the rabbit saphenous vein, the early phase was predominantly mediated by α_1-adrenoceptors, and the late phase was mostly mediated by both α_1- and α_2-adrenoceptors (MacDonald *et al*., 1992). In the present study, we made an attempt to characterize the contributions of α_1- and α_2-adrenoceptors to neurogenic vasoconstriction in the human gastroepiploic artery, using the relevant pharmacological drugs. Recent studies on the same tissue indicated that prazosin at a concentration of 0.1 μM was selective for α_1-adrenoceptors, and rauwolscine at the same concentration was selective for α_2-adrenoceptors (Fukui & Chiba, 2003).

The late response to PNS at 2 Hz was unaffected by prazosin (0.1 μM), but abolished by rauwolscine (0.1 μM), indicating that α_2-adrenoceptors solely contribute to constriction at low frequency. Furthermore, pretreatment with prazosin almost completely antagonized the early peak, and markedly reduced the late one, indicating that the late peak contains an α_1-adrenoceptor-dependent response at the higher frequencies used. The remaining response of late peak was abolished by a subsequent application of rauwolscine. The different sensitivity of two peaked responses of human gastroepiploic arteries to these drugs suggests that α_1-adrenoceptors may be predominantly involved in the early peak, and both α_1- and α_2-adrenoceptors contribute to the late peak.

When analysing the relative contributions of the two adrenoceptor subtypes to the late peak component, it should be noted that the late peak phase is superimposed upon the early one. This overlap of early phase and late phase may be responsible for the fact that not only the early but also the late peak phase was markedly reduced by prazosin.

There is evidence from the feline intestinal microvascular bed that the constrictor response to low-frequency stimulation is inhibited by an α_2- but not an α_1-adrenoceptor antagonist, whereas the response to high frequency is attenuated by both α_1- and α_2-adrenoceptor antagonists (Taylor & Parsons, 1989). Similar results have been obtained in rat skeletal muscle vascular beds (Ohyanagi *et al*., 1991). In this study, we noticed that the α_2-adrenoceptor-mediated component did not increase following an increase in the frequency of stimulation, showing a relatively restricted constrictor response. On the other hand, the α_1-adrenoceptor-mediated component was much more obviously evoked by increasing the frequency of stimulation.

It is well recognized that the α_1-adrenoceptor-mediated response depends mainly on mobilization of Ca^{2+} from intracellular Ca^{2+} stores, whereas both α_1- and α_2-adrenoceptor-mediated responses depend on Ca^{2+} influx from the external medium (Ito *et al*., 1986; Stevens & Moulds, 1986; Sulpizio & Hieble, 1991; Nielsen *et al*., 1992; Bao *et al*., 1993; Docherty, 1998). It was also observed that the contractile response of the rat caudal artery to field stimulation consisted of two phases, that is, an initial phasic contraction and a subsequent tonic contraction (Sulpizio & Hieble, 1991). Furthermore, both contractile components of the rat caudal artery are adrenergic in origin, and the phases can be distinguished by the Ca^{2+} source mobilized. The intracellular stores of Ca^{2+} are mobilized during the phasic component, while extracellular Ca^{2+} is mobilized during the tonic component (Sulpizio & Hieble, 1991). Whether the different sources of Ca^{2+} contribute to the two peaked responses of the human gastroepiploic artery remains to be established.

Pretreatment with rauwolscine did not selectively inhibit the late peak, whereas it markedly reduced two-peaked responses. Since the early peak is almost completely abolished by prazosin, the inhibition of early peak constriction by rauwolscine possibly indicates that there is synergistic interaction between α_1- and α_2-adrenoceptors, as suggested by Docherty (1998). α_2-Adrenoceptors have been reported to enhance the effects of α_1-adrenoceptors in a number of tissues, by activation of different mechanisms to increase the concentration of intracellular free Ca^{2+}, which triggers the contraction (Xiao & Rand, 1989; Bao *et al*., 1993; Docherty, 1998). It remains to be clarified in future studies whether a potentiating interaction between α_1- and α_2-adrenoceptor exists in the human gastroepiploic artery and whether a Ca^{2+} mechanism participates in this process.

When analysing the effects of rauwolscine, it should be also considered that blockade of prejunctional α_2-adrenoceptors would increase the release of NA, resulting in an enhancement of neurally evoked vasoconstriction. However, in this study, rauwolscine did not exert an enhancing action but, rather, it inhibited the response. This is consistent with previous results performed in the human gastroepiploic artery, using another α_2-adrenoceptor antagonist, yohimbine (Toda *et al*., 1988). Our results support the hypothesis that the predominant effect of α_2-adrenoceptors in human blood vessels may be postjunctional (Toda *et al*., 1988; Parkinson *et al*., 1992; Stephens *et al*., 1992).

OPC-23826, a newly developed vasodilator (Orito *et al*., 1999; 2001; Sun *et al*., 2001) was reported to exert its antagonistic actions preferentially on α_{2B}- and α_{2C}-adrenoceptor subtypes. The results showed that, BHT-933 (an α_2-adrenoceptor agonist)-induced vasoconstriction or NA-induced response was markedly attenuated by OPC-23826 in the absence or presence of prazosin, confirming its antagonistic effect on postjunctional α_2-adrenoceptors. However, not only the neuronal late peak but also the early peak was strongly inhibited by OPC-23826. Further observations showed that the phenylephrine-induced response was markedly reduced by OPC-28326, consistent with the result obtained in the canine femoral artery (Orito *et al*., 1999). The results indicate that the inhibition by OPC-23826 of neurogenic two-peaked responses is due to its antagonistic effect on both α_1- and α_2-adrenoceptors. It has been suggested that α_{2B}-adrenoceptors are able to mediate the sympathetic pressor response (Link *et al*., 1996; Dóda, 1997). Thus, the α_{2B}-adrenoceptor subtypes may be involved in mediating the neurogenic response of the human gastroepiploic artery.

In conclusion, our results indicate that both α_1- and α_2-adrenoceptors, but not P2X receptors, receive sympathetic innervation in the human gastroepiploic artery. The neuroeffector response mediated by α_2-adrenoceptors develops more slowly than that mediated by α_1-adrenoceptors. The difference in the onset of α_1- and α_2-adrenoceptor-mediated responses most likely reflects their physiological importance in regulating sympathetic vascular tone.

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