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Effects of ONO-1078, a leukotriene antagonist, on cardiovascular responses induced by vagal stimulation, capsaicin, and substance P in guinea pigs[1]

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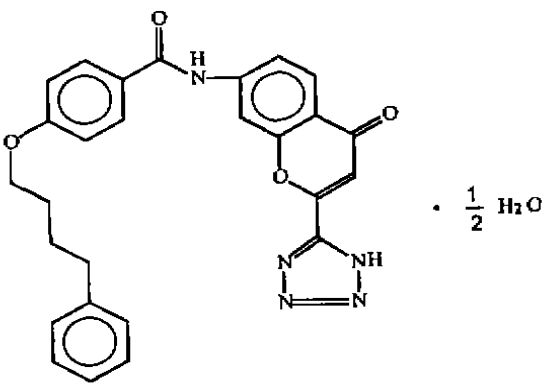
AIM: To determine the role of ONO-1078, 4-oxo-8-[*p*-(4-phenylbutyloxy) benzoyl-amino]- 2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate, in cardiovascular responses induced by vagal stimulation, capsaicin, and substance P. **METHODS**: Evans blue extravasation in the atrium and ventricle, and mean arterial pressure (MAP) were observed. **RESULTS**: Electric stimulation of vagus (ESV, 10 Hz, 5 ms, 2 or 10 V, for 90 s) increased Evans blue extravasation in the hearts of atropine (1 mg·kg^{-1}, iv)-pretreated guinea pigs. Capsaicin (0.05 mg·kg^{-1}, iv) and substance P (1 μg·kg^{-1}, iv) enhanced the dye extravasation and elicited a drop in MAP. ONO-1078 (0.03 and 0.1 mg·kg^{-1}, iv) inhibited ESV-induced response, especially at stimulation of 2 V. ONO-1078 (0.03 mg·kg^{-1}) attenuated capsaicin-induced cardiac microvascular leakage and hypotensive response, but failed to inhibit substance P-induced responses. **CONCLUSION**: ONO-1078 can modulate the cardiovascular responses in neurogenic inflammation, possibly mediated by inhibiting sensory neuropeptide release.

KEY WORDS ONO-1078; benzopyrans; heart; capsaicin; substance P; electric stimulation; vagus nerve; capillary permeability; Evans blue

The heart is a target organ of neurogenic inflammation induced by stimuli of sensory C fibers, such as electric stimulation of vagus (ESV) and capsaicin, in which sensory neuropeptides are released[1,2], and microvascular leakage occurred[3]. Peptido-leukotrienes (LT), potent inflammatory mediators, cause coronary vasoconstriction and myocardial depression[4-6], but their effects on heart neurogenic inflammation is unknown. ONO-1078, 4-oxo-8-[*p*-(4-phenylbutyloxy) benzoyl-amino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate, is a specific antagonist for LTD_4, C_4 and E_4, and 1000 times more potent than FPL-55 712, a typical leukotriene antagonist[7,8]. In the airways, endogenous LT play a role in the microvascular leakage and smooth muscle contractile responses to stimulation of sensory C fibers, and ONO-1078 or other LT antagonists partly inhibit these responses[9,10]. In this study, we utilized ONO-1078 to determine whether endogenous



ONO-1078

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LT were also involved in the heart microvascular leakage and functional responses to sensory nerve stimulation (ESV and capsaicin), and a sensory neuropeptide, substance P.

MATERIALS AND METHODS

Drugs ONO-1078 (ONO, Ono Pharmaceutical Co, Osaka, Japan); Evans blue and capsaicin (Sigma, USA); substance P (SP, Peptide Institute, Osaka, Japan); and atropine sulfate (Minsheng Pharmaceutical Factory, Hangzhou, China).

ESV-induced Evans blue extravasation in heart Hartley guinea pigs of either sex ($n=121$) weighing 334±s 54 g were anesthetized with sodium pentobarbital (30 mg·kg^{-1}, ip) and ventilated with a rodent ventilator (DH-140, Medical Instrumental Factory of Zhejiang Medical University, tidal volume 5 mL, 80 breaths·min^{-1}) via a tracheal cannula. Bilateral cervical vagi were cut, and the distal ends were stimulated (10 Hz, 5 ms, 2 or 10 V, for 90 s) after iv pretreatments: atropine (1 mg·kg^{-1}, 10 min); Evans blue (30 mg·kg^{-1}, 2 min); and ONO (0.03 and 0.1 mg·kg^{-1}, 2 min) or solvent (5 % ethanol in saline, 2 min). Five min after ESV, the animal was perfused with 50 mL saline within 1 min. The extravasated Evans blue in atrium and ventricle was extracted and measured[31].

Effects of capsaicin and SP on Evans blue extravasation and mean arterial pressure (MAP) MAP was measured via a left carotid arterial cannula by a transverse piezoresistive pressure transducer (MPX 50DP, Motorola, USA). The animals were injected iv with capsaicin (0.05 mg·kg^{-1}), SP (1 μg·kg^{-1}), or saline 10 min after pretreatment. Ten min later, the extravasated Evans blue in the heart was measured.

Statistical analysis Significance of difference between groups was determined by t test, ANOVA, or Mann-Whitney u-test.

RESULTS

ESV enhanced Evans blue extravasation in the atrium and ventricle of atropine-pretreated guinea pigs. ONO 0.03 and 0.1 mg·kg^{-1} iv inhibited ESV-induced increase of Evans blue extravasation in the atrium and ventricle when stimulated at 2 V, and 0.1 mg·kg^{-1}, iv inhibited that in atrium at 10 V (Tab 1).

Tab 1. Effects of ONO-1078 (ONO, 0.03, 0.1 mg·kg^{-1}, iv) on Evans blue extravasation increased by electric stimulation of vagus (ESV, 10 Hz, 5 ms, 2 or 10 V, for 90 s) in the atrium and ventricle of guinea pig. $\bar{x}\pm s$; $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **control**; $^dP<0.05$, $^eP<0.01$ *vs* **ESV alone**; $^fP<0.01$ *vs* **ESV (2 V).**

	n	Evans blue extravasation (μg/g wet tissue) Atrium	Ventricle
Control	8	8.4±3.8	3.4±1.8
ONO 0.03 mg·kg^{-1}	6	10.4±3.9^a	3.5±2.1^f
0.1 mg·kg^{-1}	5	12.6±2.8^a	4.3±0.8^a
ESV (2 V)	10	19.2±4.4^c	6.2±2.3^b
+ONO 0.03 mg·kg^{-1}	7	13.0±6.7^e	4.3±1.9^a
+ONO 0.1 mg·kg^{-1}	7	11.4±4.2^f	3.8±1.5^{ef}
ESV (10 V)	13	32.2±8.8^{ef}	10.1±4.4^{ef}
+ONO 0.03 mg·kg^{-1}	9	35.8±12.4^e	10.1±4.3^f
+ONO 0.1 mg·kg^{-1}	7	17.7±2.8^{ef}	6.3±1.6^e

ONO elicited a transient drop in MAP by 5 %—15 % which rapidly returned to normal levels within 2—5 min. Capsaicin (0.05 mg·kg^{-1}, iv) and SP (1 μg·kg^{-1}, iv) increased Evans blue extravasation and reduced MAP. ONO 0.03 mg·kg^{-1} iv attenuated the dye extravasation induced by capsaicin, not that by SP (Tab 2). Capsaicin-evoked reduction of MAP lasted within 5 min in ONO group, and over 10 min in solvent group, however, ONO did not alter the hypotensive responses to SP (Fig 1).

DISCUSSION

In the atropine-pretreated guinea pigs, ONO-1078 inhibited microvascular leakage in the atrium and ventricle elicited by ESV. We further observed the effects of ONO-1078 on the cardiovascular responses to capsaicin

Tab 2. Effects of ONO-1078 (ONO, 0.03 mg·kg^{-1}, iv) on Evans blue extravasation induced by capsaicin (Cap, 0.05 mg·kg^{-1}, iv) and substance P (SP, 1 μg·kg^{-1}, iv) in the atrium and ventricle of guinea pig. $\bar{x}\pm s$: $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; $^dP<0.05$, $^eP<0.01$ *vs* Cap.

	n	Evans blue extravasation (μg/g wet tissue) Atrium	Ventricle
Control	7	7.5±3.9	2.7±1.9
ONO 0.03 mg·kg^{-1}	7	8.7±4.4^a	3.2±1.8^a
Cap 0.05 mg·kg^{-1}	10	34.9±8.8^c	13.1±5.5^c
ONO−Cap	9	22.4±7.1^{cf}	7.7±2.1^{ce}
SP 1 μg·kg^{-1}	8	38.0±9.6^c	11.3±4.4^c
ONO+SP	8	36.8±7.0^c	10.6±4.1^c

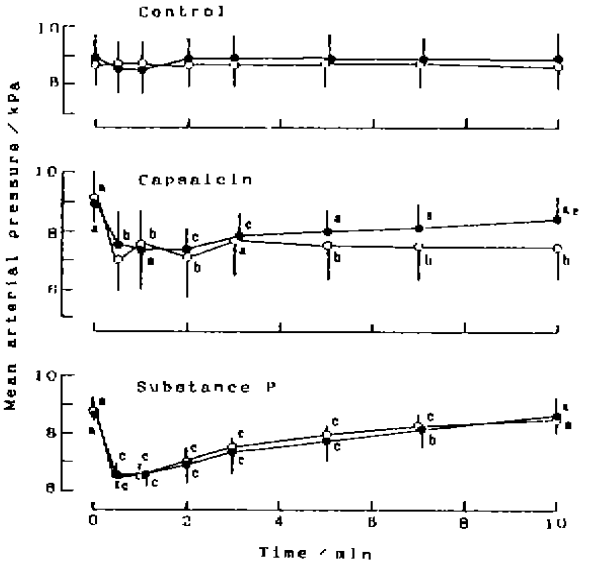

Fig 1. Effects of ONO-1078 (0.03 mg·kg^{-1}, iv) on the hypotensive response to capsaicin (0.05 mg·kg^{-1}, iv) and substance P (1 μg·kg^{-1}, iv) in guinea pigs. (○) solvent; (●) ONO-1078. $n=7-10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$. $^cP<0.01$ *vs* control; $^dP<0.05$ *vs* solvent.

and SP to clarify the mechanisms of its effects. Capsaicin promotes sensory neuropeptide release in many mammalian tissues including the heart[1,2], while SP is an agonist for NK_1 receptors that have been proven to be involved in microvascular leakage[11]. This study showed that both capsaicin and SP enhanced Evans blue extravasation in the heart, and elicited a hypotensive response. The responses of capsaicin, not those of SP, were attenuated by ONO-1078 (0.03 mg·kg^{-1}). It is likely that the effects of ONO-1078 on the cardiovascular responses to capsaicin or ESV may be mediated by inhibiting release of sensory neuropeptides, not by directly blocking the postsynaptic effects of SP and related neuropeptides.

In the airways and skin microvasculatures, ONO-1078 selectively blocks the effects of LTC_4, D_4, and E_4[7,8], not those of LTB_4, histamine, arachidonic acid and $PGF_{2\alpha}$ (Ono Pharmaceutical Co, personal communication, 1991). However, it remains to be investigated whether the inhibition by ONO-1078 of heart microvascular leakage and hypotensive response in this study are mediated by specific antagonism of LT or by other actions. On the other hand, why the agent had a mild transient hypotensive action when given intravenously is unclear.

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白三烯拮抗剂 ONO-1078对电刺激迷走神经、辣椒素和 P 物质引起的豚鼠心血管反应的作用

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目的：阐明 ONO-1078(ONO，4-氧-8-[对-(4-苯丁氧基)苯甲酰氨基]-2-(5-四唑基)-4*H*-1-苯并吡喃)对神经原性刺激诱导心血管反应的作用。**方法**：观察豚鼠心房和心室伊文思蓝渗出以及平均主动脉压(MAP)变化。**结果**：在阿托品(1 mg·kg^{-1}，iv)预先处理后，电刺激迷走神经(ESV，10 Hz，5 ms，2或10 V，90 s)显著增高伊文思蓝渗出；辣椒素和 P 物质也增加染料渗出并降低平均动脉压(MAP)。ONO (0.03，0.1 mg·kg^{-1}，iv)抑制 ESV 的反应，在刺激强度低(2 V) 时更明显；ONO 0.03 mg·kg^{-1}减弱辣椒素引起的微血管渗漏和低血压，但对 P 物质无影响。**结论**：ONO-1078可能通过抑制感觉神经肽释放而调节神经原性炎症时的心血管反应。

关键词 ONO-1078；苯并吡喃类；心脏；辣椒素；P 物质；电刺激；迷走神经；毛细血管渗透性；伊文思蓝

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