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RESEARCH PAPER

A ketolide antibiotic, telithromycin, inhibits vascular adrenergic neurotransmission in the rat mesenteric vascular bed

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Background and purpose: A ketolide antibiotic, telithromycin, has side effects including temporary loss of consciousness in clinical use, but the underlying mechanisms remain unclear. This study investigated the effects of telithromycin on perivascular nerve function in rat mesenteric arteries, in comparison with those of macrolide (erythromycin and clarithromycin) and new quinolone antibiotics (levofloxacin and gatifloxacin).
Experimental approach: *In vitro*, vascular responses and release of noradrenaline induced by periarterial nerve stimulation (PNS) of rat perfused mesenteric vascular beds were measured in the presence of each antibiotic. *In vivo* blood pressure measurement was performed in Wistar rats.
Key results: In mesenteric preparations with resting tone, telithromycin (10 nM–10 μM) markedly inhibited PNS (4–12 Hz)-induced adrenergic nerve- and exogenous noradrenaline-mediated vasoconstriction, whereas the other antibiotics slightly inhibited PNS-induced responses without affecting noradrenaline-induced responses. Telithromycin significantly reduced PNS (12 Hz)-evoked noradrenaline release in the perfusate. In pre-constricted preparations with or without endothelium, telithromycin (0.1 nM–10 μM) caused a concentration-dependent vasodilation. Telithromycin (10 nM) inhibited calcium-induced vasoconstriction in high KCl and calcium-free medium. None of the antibiotics used affected PNS (0.5–2 Hz)-induced calcitonin gene-related peptide (CGRP) nerve- and exogenous CGRP-mediated vasodilation. Intravenous injection of telithromycin significantly lowered blood pressure in anaesthetized rats.
Conclusions and implications: These results suggest that telithromycin causes not only strong inhibition of perivascular adrenergic neurotransmission but also a vasodilator action in mesenteric vascular beds and hypotension. It is thus possible that telithromycin increases visceral blood flow, consequently reducing cerebral blood flow and resulting in a temporary loss of consciousness.
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Keywords: telithromycin; ketolide antibiotics; adrenergic nerve neurotransmission; vasodilation; calcitonin gene-related peptide-containing nerves; rat mesenteric arteries

Abbreviations: CGRP, calcitonin gene-related peptide; PNS, periarterial nerve stimulation

Introduction

Telithromycin (Nguyen and Chung, 2005) is the first ketolide (a macrolide derived from erythromycin) antibiotic to enter clinical use. Worsening of myasthaenia gravis has been reported as an adverse event in the clinical use of telithromycin (Nieman *et al.*, 2003; Perrot *et al.*, 2006), suggesting that it has neurogenic effects. Furthermore, the United States Food and Drug Administration and European Medicines Agency reported the side effects of syncope or loss of consciousness in clinical use of telithromycin (FDA, 2006a; European Medicines Agency, 2007). These events have usually been reported as transient, occurring shortly after the first or second dose, with rapid and full recovery generally observed. Although the mechanism for these events has not been clarified, a frequent association with hypotension and/or bradycardia in these reports suggests a vagally mediated phenomenon that is usually secondary to other more common adverse events such as nausea,

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diarrhoea or dizziness. However, the involvement of CNS in telithromycin-induced loss of consciousness has been excluded (FDA, 2006b). Therefore, it is assumed that a sudden decrease in cerebral blood flow, probably induced by acute hypotension, may be responsible for the events. However, the precise mechanisms underlying the loss of consciousness have not been clarified.

There is strong evidence that the vascular tone is regulated not only by sympathetic adrenergic vasoconstrictor nerves but also by non-adrenergic non-cholinergic vasodilator nerves in various species (Bevan and Brayden, 1987; Kawasaki *et al*., 1988; Toda and Okamura, 1992; Lee *et al*., 1996; Hatanaka *et al*., 2006). The main non-adrenergic non-cholinergic nerves innervating the rat mesenteric artery are nerves releasing calcitonin gene-related peptide (CGRP) as the neurotransmitter (peptidergic (CGRP) nerves). These induce potent vasodilation when activated by electrical field stimulation (Kawasaki *et al*., 1988). Additionally, peptidergic (CGRP) nerves have been reported to function to inhibit adrenergic vasoconstrictor nerves and control vascular tone, whereas adrenergic nerves inhibit the function of peptidergic (CGRP) nerves (Kawasaki *et al*., 1988; Takenaga *et al*., 1995). Thus, malfunction in the control system of these nerves causes a loss of control of vascular tone and results in hypertension or hypotension (Kawasaki *et al*., 1990a, b; Takenaga and Kawasaki, 1999). Accordingly, telithromycin may cause a malfunction in perivascular nerves, leading to reduced vascular tone and therefore to hypotension.

This study was therefore designed to investigate the influence of telithromycin on perivascular nerve function in the mesenteric vascular beds of the rat *in vitro* and blood pressure *in vivo* and to clarify the possible mechanisms underlying loss of consciousness induced by telithromycin. To compare the effects of telithromycin, we used the macrolide antibiotics, erythromycin and clarithromycin, and the new quinolones, levofloxacin and gatifloxacin, as active controls. Here, we report evidence that telithromycin, but not other related antibiotics, induces a potent inhibition of adrenergic neurotransmission without affecting peptidergic (CGRP) nerve function, has a potent vasodilator activity and causes hypotension.

Methods

Animals

This study was carried out in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, Japanese Government Animal Protection and Management Law (no. 115) and the Japanese Government Notification on Feeding and Safekeeping of Animals (no. 6). Every effort was made to minimize the number of animals used and their suffering. A total of 224 male Wistar rats (purchased from Shimizu Experimental Animals, Shizuoka, Japan), weighing 230–330 g, were used in this study. Animals were given food and water *ad libitum*. They were housed in the Animal Research Center of Okayama University at a controlled ambient temperature of 22 ± 2 °C with 50 ± 10% relative humidity and with a 12-h light/12-h dark cycle (lights on at 0800 hours).

Blood pressure measurement

The animals were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.). A polyethylene catheter (PE-10) was positioned in the left jugular vein to administer drugs, and a polyethylene catheter (PE-50) was inserted into the left carotid artery and connected to a pressure transducer (model DX-100; Nihon Kohden, Tokyo, Japan). The arterial and mean blood pressures were recorded using a polygraph (model RM-6000; Nihon Kohden). The heart rate triggered by arterial pulses was measured using a cardiotachometer (model AT-600G; Nihon Kohden) and was recorded with a polygraph. Telithromycin at doses of 1 and 2 μmol kg^{-1} was intravenously administered in bolus doses (1.0 mL kg^{-1}).

Perfusion of mesenteric vascular beds and perfusion pressure measurement

Each animal was anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), and the mesenteric vascular bed was isolated and prepared for perfusion as described previously (Kawasaki *et al*., 1988, 1990a). The isolated mesenteric vascular bed was placed on a water-jacketed organ bath maintained at 37 °C and perfused with Krebs solution at a constant flow rate of 5 mL min^{-1} with a peristaltic pump and superfused with the same solution at a rate of 0.5 mL min^{-1} to prevent drying. The Krebs solution was bubbled with a mixture of 95% O_2 plus 5% CO_2 before passage through a warming coil maintained at 37 °C. The modified Krebs solution had the following composition (mM): NaCl 119.0, KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, EDTA-2Na 0.03 and glucose 11.1 (pH 7.4). Changes in the perfusion pressure were measured with a pressure transducer (model TP-400T; Nihon Kohden) and recorded using a pen recorder (model U-228; Nippon Denshi Kagaku, Tokyo, Japan).

Periarterial nerve stimulation and bolus injection of agonists

Periarterial nerve stimulation (PNS) at 4, 8 and 12 Hz was applied at 7-min intervals using bipolar platinum ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 1 ms and supramaximal voltage (50 V) were applied for 30 s using an electronic stimulator (model SEN 3301; Nihon Kohden).

Some agonists such as ACh, noradrenaline and CGRP were injected directly into the perfusate proximal to the arterial cannula with an infusion pump (model 975; Harvard Apparatus, Holliston, MA, USA). A volume of 100 μL was injected over a period of 12 s.

Experimental protocols for vasoconstrictor responses

In perfused mesenteric preparations with resting tension, vasoconstrictor responses to the first PNS (S_1) and noradrenaline injection (I_1) were obtained as the control. Thereafter, the Krebs solution was switched to Krebs solution containing telithromycin (1, 10, 100 nM, 1 or 10 μM), clarithromycin (1 or 10 μM), erythromycin (1 or 10 μM) or gatifloxacin (1 or 10 μM), levofloxacin (1 or 10 μM) or no drug, and then the second PNS (S_2) and noradrenaline injection (I_2) were

carried out. The concentrations of telithromycin used were chosen on the basis of clinical data showing that the therapeutic blood concentration of telithromycin ranged from 1 to 3 $\mu g\,mL^{-1}$, that is, 1.23–3.69 μM (Sanofi-Aventis, 2007). Also, the concentrations of the other antibiotics used were similarly chosen on the basis of clinical data. To assess the effects of the antibiotics, changes in perfusion pressure in response to PNS or noradrenaline injection were expressed as the ratio between the vasoconstriction induced by PNS (S_2) and (S_1), or between the vasoconstriction induced by noradrenaline injection (I_2) and (I_1), respectively. At the end of each experiment, the vascular tone of preparations was increased by substituting Krebs solution containing methoxamine and guanethidine without antibiotics, and the integrity of the intact endothelium was verified by the relaxant effect after a bolus injection of 1 nmol of ACh. Also, the vasodilation mediated by peptidergic (CGRP) nerves was confirmed by applying 2 Hz PNS.

Experimental protocols for vasodilator responses

The preparations were perfused with Krebs solution containing methoxamine (7 μM), a selective α_1-adrenoceptor agonist, to increase vascular tone and guanethidine (5 μM) to block adrenergic neurotransmission. After responses to the first PNS (S_1) and CGRP injection (I_1) were obtained as the control, the Krebs solution containing methoxamine and guanethidine was switched to a Krebs solution containing methoxamine, guanethidine and an antibiotic (either telithromycin (10 nM, 1 or 10 μM), clarithromycin (1 or 10 μM), erythromycin (1 or 10 μM), gatifloxacin (1 or 10 μM) or levofloxacin (1 or 10 μM)) or without any antibiotic, and then the second PNS (S_2) and CGRP injection (I_2) were carried out. To estimate the effects of the antibiotics, changes in perfusion pressure in response to PNS or CGRP injection were expressed as the ratio between the vasodilation induced by PNS (S_2) and (S_1), or between vasodilation induced by CGRP injection (I_2) and (I_1), respectively. In the experiment using telithromycin, vascular tone was maintained by increasing the concentration of methoxamine to 10 μM, as telithromycin at 10 nM induced a decrease in perfusion pressure due to vasodilation.

At the end of each experiment, 100 μM papaverine was perfused to produce complete relaxation. Vasodilation was expressed as a percentage of the perfusion pressure at maximum relaxation induced by papaverine.

Experimental protocols for vascular responses to perfusion of telithromycin

The preparations with or without intact endothelium were perfused with Krebs solution containing methoxamine and guanethidine. After stabilization of the elevated perfusion pressure, the Krebs solution was switched to Krebs solution containing the final concentration of telithromycin (0.1 nM–10 μM) and perfused for 10 min. After the perfusion pressure had stabilized, the Krebs solution containing methoxamine (7 μM), guanethidine (5 μM) and a higher concentration of telithromycin was perfused. At the end of each experiment, the preparations were perfused with 100 μM papaverine to induce complete relaxation. Vasodilation was expressed as a percentage of the perfusion pressure at maximum relaxation induced by papaverine.

To remove the vascular endothelium, preparations with resting tone were perfused with a solution of sodium deoxycholate in saline (1.8 $mg\,mL^{-1}$) for 30 s as described previously (Takenaga and Kawasaki, 1999; Shiraki *et al*., 2000; Hatanaka *et al*., 2006). The preparations were then washed with sodium deoxycholate-free Krebs solution for 1 h. Thereafter, the preparations were perfused with Krebs solution containing methoxamine (2 μM) and guanethidine (5 μM). After confirming the removal of endothelium by the lack of a relaxant effect after 1 nmol ACh injection, telithromycin perfusions were performed.

Experimental protocols for Ca^{2+}-induced vasoconstriction in high KCl and Ca^{2+}-free medium

The preparations at resting tension were perfused with Krebs solution containing Ca^{2+}-free medium. Thereafter, the Krebs solution was switched to Ca^{2+}-free Krebs solution containing a high concentration of KCl (60 mM) for 20 min. Next, the preparations were subjected to perfusion of Krebs solution containing high KCl and $CaCl_2$ (0.8, 1.6 or 2.4 mM) for 10 min as the control. After that, the preparation was perfused with Ca^{2+}-free Krebs solution for 20 min and then perfused with Ca^{2+}-free Krebs solution containing high KCl (60 mM) for 20 min. Next, the Krebs solution was switched to Krebs solution containing $CaCl_2$ (0.8, 1.6 or 2.4 mM), high KCl (60 mM) and telithromycin (10 μM) for 10 min to observe vasoconstriction. The effect of telithromycin on the Ca^{2+}-induced vasoconstriction was evaluated relative to the control effect (vasoconstriction without telithromycin).

Measurement of noradrenaline in the perfusate

In preparations with resting tone, the perfusate was collected before and after the first PNS (S_1, 12 Hz) for 3 min. Then, the Krebs solution was switched to Krebs solution containing telithromycin (10 μM), clarithromycin (10 μM) or erythromycin (10 μM) and the perfusate was collected before and after the second PNS (S_2). The net release of noradrenaline (post-PNS concentration minus pre-PNS concentration) was expressed as the PNS-evoked release of noradrenaline, and the effect of antibiotics on the PNS-induced noradrenaline release was evaluated by the S_2/S_1 ratio.

Noradrenaline in the perfusate was adsorbed onto alumina, and the extract obtained with acetic acid was assayed by HPLC with an electrochemical detector (model HTEC-500; Eicom, Kyoto, Japan) as described by Hatanaka *et al*. (2006). 3,4-Dihydroxybenzylamine hydrobromide (Sigma-Aldrich, St Louis, USA) was added as the internal standard.

Statistics

All values are expressed as the mean ± s.e.mean. Statistical analysis was performed using one-way ANOVA followed by Tukey's test for multiple comparisons and the unpaired and paired Student's *t*-test for two groups. $P < 0.05$ was considered significant.

Drugs

The following drugs were used: ACh chloride (Daiichi Sankyo, Tokyo, Japan), clarithromycin (Wako Pure Chemical Industries, Osaka, Japan), erythromycin (Sigma-Aldrich Japan, Tokyo, Japan), gatifloxacin (LKT Laboratories, St Paul, MN, USA), guanethidine sulphate (Tokyo Kasei, Tokyo, Japan), levofloxacin hydrochloride (LKT Laboratories), methoxamine hydrochloride (Nihon Shinyaku, Kyoto, Japan), noradrenaline hydrochloride (Daiichi Sankyo), papaverine hydrochloride (Sigma-Aldrich), rat CGRP (Peptide Institute, Osaka, Japan), sodium deoxycholate (Sigma-Aldrich) and telithromycin (Sequoia Research Products, Pangbourne, UK). All drugs, except for erythromycin, gatifloxacin, telithromycin and sodium deoxycholate, were dissolved in pure water and diluted with Krebs solution. Erythromycin, gatifloxacin and telithromycin were dissolved in dimethylsulphoxide (Nacalai Tesque, Tokyo, Japan) and diluted with Krebs solution (final dimethylsulphoxide concentration, 0.1%), which did not alter the perfusion pressure at the resting and active tone or affect the methoxamine-induced increase in perfusion pressure or the telithromycin-induced decrease in perfusion pressure. In the blood pressure measurement study, telithromycin was dissolved in 1% dimethylsulphoxide, which did not affect blood pressure levels. Sodium deoxycholate was dissolved in 0.9% saline. ACh and rat CGRP were diluted with Krebs solution containing 2–7 μM methoxamine and 5 μM guanethidine when injected directly. The drug/molecular target nomenclature in this study conforms to the BJP's Guide to Receptors and Channels (Alexander *et al*., 2008).

Results

Effects of antibiotics on vasoconstrictor responses to PNS and noradrenaline injection

As illustrated in Figure 1a, PNS (4, 8 and 12 Hz) of rat perfused mesenteric vascular beds with resting tone increased the perfusion pressure due to vasoconstriction in a frequency-dependent manner: 4 Hz, 2.1 ± 0.1 mm Hg ($n=6$); 8 Hz, 9.5 ± 0.8 mm Hg ($n=6$) and 12 Hz, 49.1 ± 3.8 mm Hg ($n=6$). Bolus injection of noradrenaline (4 or 8 nmol) into the perfusate also caused concentration-dependent vasoconstriction: 4 nmol, 11.0 ± 0.7 mm Hg ($n=6$); 8 nmol, 19.7 ± 0.3 mm Hg ($n=6$) (Figures 1a and 2a). Repeated PNS and noradrenaline injection caused reproducible vasoconstrictor responses. In the control response, the ratios of vasoconstrictor responses to PNS (S_1) and (S_2) at 4, 8 and 12 Hz, and noradrenaline injection (I_1) and (1_2) at 4 and 8 nmol were 1.03 ± 0.03, 1.05 ± 0.06 and 1.11 ± 0.03, and 1.15 ± 0.03 and 1.17 ± 0.04, respectively (Figure 2a).

At the end of the experiment, the preparation was perfused with Krebs solution containing methoxamine, a selective α_1-adrenoceptor agonist, to increase vascular tone, and guanethidine to block adrenergic neurotransmission. In this preparation, the intact endothelium was confirmed by the presence of a relaxant effect after a bolus injection of 1 nmol ACh. Also, PNS at 2 Hz caused a decrease in perfusion pressure due to vasodilation. We previously reported that PNS-induced vasodilation is mediated by endogenous CGRP released due to the stimulation of peptidergic (CGRP) nerves (Kawasaki *et al*., 1988; Hatanaka *et al*., 2006).

In rat perfused mesenteric vascular veds, as shown in Figure 1b, the ketolide antibiotic telithromycin (1 nM–10μM) did not affect the resting tone. A low concentration of telithromycin (1 nM) did not affect the vasoconstrictor response to PNS. However, telithromycin at concentrations higher than 10 nM markedly reduced vasoconstrictor responses to PNS at 4, 8 and 12 Hz (Figures 1b and 2a). The reduction in PNS-induced vasoconstriction with telithromycin was concentration dependent and reached a maximum of 80–90% (Figure 2a). In rat perfused mesenteric vascular beds, perfusion of the macrolide antibiotics erythromycin (10 μM) (Figure 1c) or clarithromycin (10 μM) did not alter the resting tone. Perfusion of erythromycin or clarithromycin at concentrations of 1 and 10 μM caused an approximately 20% reduction in the PNS-induced vasoconstriction at 12 Hz (Figures 1c, 2b and c).

The new quinolone antibiotics levofloxacin (10 μM) and gatifloxacin (10 μM) did not alter the resting tone (data not shown). Levofloxacin at 10 μM, but not 1 μM, and gatifloxacin at 1 or 10 μM, significantly reduced vasoconstrictor responses to PNS at higher frequencies of 8 and 12 Hz (Figures 2d and e). The reduction in the PNS (12 Hz)-induced response by levofloxacin and gatifloxacin at a concentration of 10 μM was 35.0 ± 12.3 and 28.5 ± 6.8%, respectively.

Neither erythromycin, clarithromycin, levofloxacin nor gatifloxacin affected the vasoconstrictor responses to exogenously applied noradrenaline (Figures 1 and 2). Low concentrations of telithromycin (1–100 nM) did not affect the vasoconstrictor responses to noradrenaline (Figure 2a). However, as shown in Figures 1b and 2a, telithromycin at 1 and 10 μM significantly inhibited noradrenaline (4 and 8 nmol)-induced vasoconstriction.

Effect of antibiotics on PNS-evoked noradrenaline release

In rat perfused mesenteric vascular beds with resting tone, the spontaneous release of noradrenaline was detected in the perfusate (34.4 ± 7.7 pg mL^{-1}, $n=10$). PNS at 12 Hz evoked a significant increase in the net release of noradrenaline (post-PNS minus pre-PNS; 111.9 ± 8.7 pg mL^{-1}, $n=10$) in the perfusate. The perfusion of erythromycin (10 μM) caused a small, but not significant, decrease in the PNS-induced net release of noradrenaline (98.0 ± 8.0 pg mL^{-1}, $n=5$). The perfusion of clarithromycin (10 μM) significantly reduced the PNS-evoked noradrenaline net release ($n=5$) compared with that of the control (85.2 ± 5.5 pg mL^{-1}, $n=5$, $P<0.05$). The perfusion of telithromycin significantly decreased the PNS-evoked net release of noradrenaline compared with that of the control (59.4 ± 3.5 pg mL^{-1}, $n=5$, $P<0.01$). The ratios of the net noradrenaline release of S_1 and S_2 with the control, telithromycin (10 μM), clarithromycin (10 μM) and erythromycin (10 μM) perfusion are shown in Figure 3.

Effects of antibiotics on vasodilation in response to PNS and CGRP injection

As shown in Figure 4a, in perfused mesenteric vascular beds with active tone produced by perfusion of Krebs solution

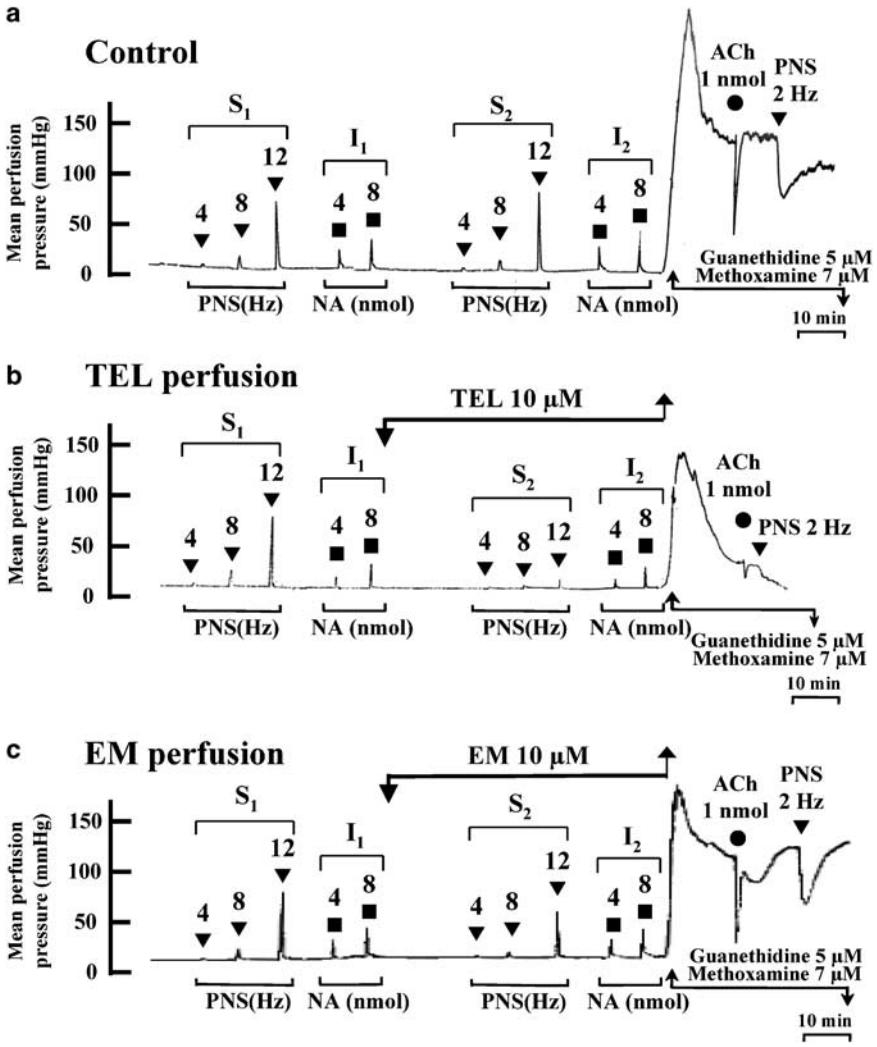

Figure 1 Typical records showing the effects of various antibiotics on vasoconstrictor responses to periarterial nerve stimulation (PNS; 4, 8 and 12 Hz) and bolus injections of noradrenaline (NA; 4 and 8 nmol) in rat perfused mesenteric vascular beds with resting tone. (**a**) Control responses in the absence of antibiotics. (**b**) Responses in the presence of telithromycin (TEL). (**c**) Responses in the presence of erythromycin (EM). S_1 and S_2 indicate the responses to the first PNS in the control and second PNS in the presence of an antibiotic, respectively. I_1 and I_2 indicate the responses to the first (control) and second (antibiotic) bolus injections of NA. Solid inverted triangles, solid squares and solid circles indicate PNS, bolus injections of noradrenaline and bolus injection of ACh, respectively.

containing 7 μM methoxamine and 5 μM guanethidine, PNS (0.5, 1 and 2 Hz) induced a frequency-dependent decrease in perfusion pressure due to vasodilation. A bolus injection of CGRP (50 and 100 pmol) into the perfusate also caused vasodilation. As shown in Table 1, repeated PNS and CGRP injections caused reproducible vasodilation. Perfusion of erythromycin (1 and 10 μM), clarithromycin (1 and 10 μM), levofloxacin (1 and 10 μM) or gatifloxacin (1 and 10 μM) did not alter the perfusion pressure, indicating that these antibiotics had no effect on the vascular tone (Table 1). Perfusion of erythromycin (1 and 10 μM), clarithromycin (1–10 μM), levofloxacin (1–10 μM) and gatifloxacin (1–10 μM) did not alter the PNS-induced or CGRP-induced vasodilation (Table 1).

In the perfused preparation with active tone, as shown in Figure 4b, perfusion of 1 μM telithromycin caused a sharp fall (88.4 ± 1.2% reduction; $n = 3$) of perfusion pressure to the resting level. On account of the strong vasodilation induced by telithromycin, the telithromycin concentration decreased to 10 nM, which had no effect on the perfusion pressure. Perfusion of telithromycin at 10 nM, which significantly reduced vasoconstrictor responses to PNS (Figure 2a), did not alter the PNS-induced or CGRP-induced vasodilation (Table 1).

Vasodilator responses to telithromycin perfusion

As shown in Figure 5, in perfused mesenteric vascular beds with active tone and an intact endothelium, the perfusion of telithromycin (0.1 nM–10 μM). for 10 min concentration-dependently decreased the perfusion pressure due to vasodilation. Other antibiotics did not cause vasodilation (data not shown). In preparations without endothelium, the perfusion of telithromycin (0.1 nM–10 μM) for 10 min also induced concentration-dependent vasodilation, as shown in Figure 5. The effective concentrations that induced 50%

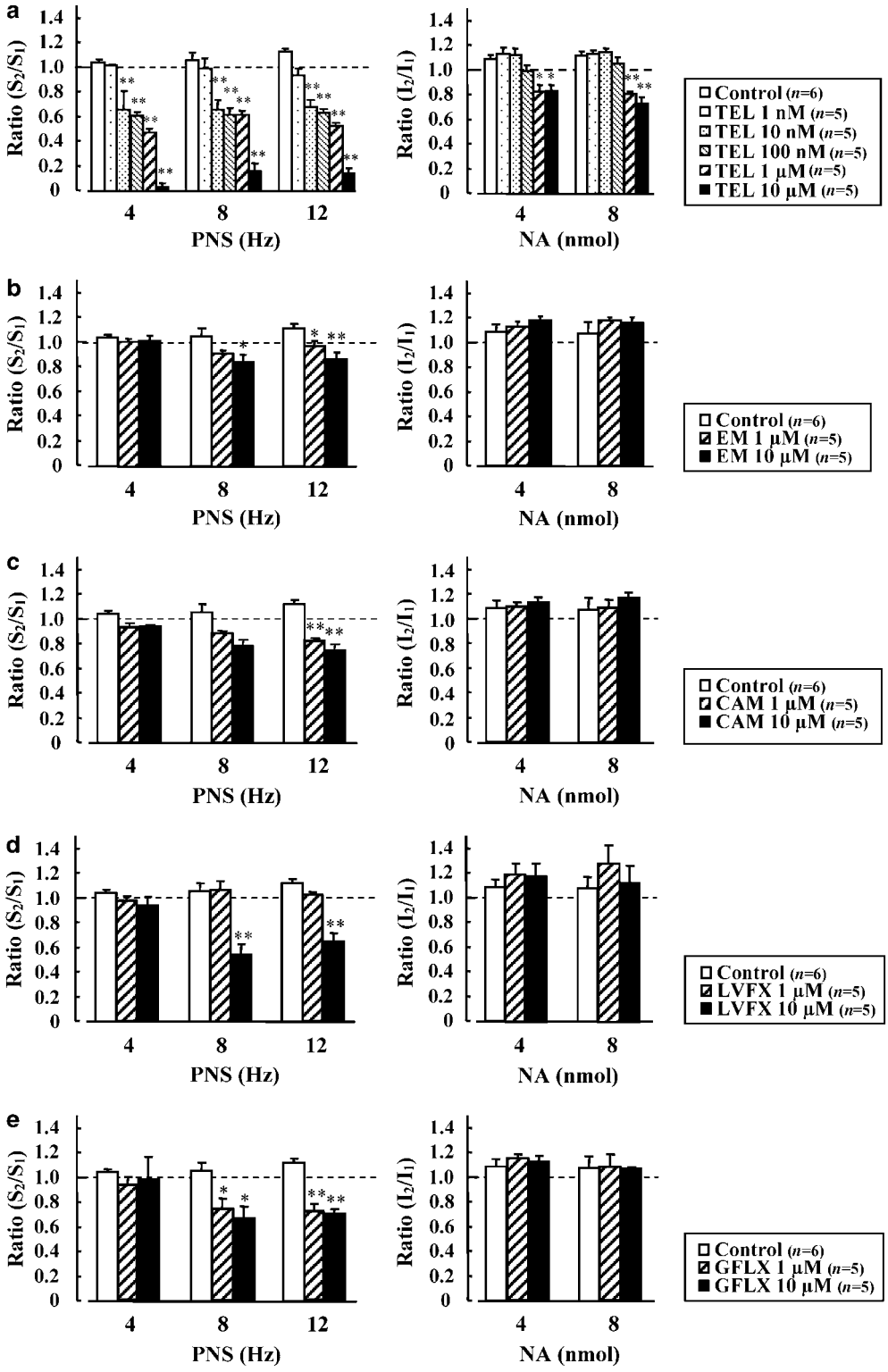

Figure 2 Effects of telithromycin (TEL) (**a**), erythromycin (EM) (**b**), clarithromycin (CAM) (**c**), levofloxacin (LVEX) (**d**) and gatifloxacin (GFLX) (**e**) on vasoconstrictor responses to periarterial nerve stimulation (PNS; 4, 8 and 12 Hz) (left) and bolus injection of noradrenaline (NA; 4 and 8 nmol) (right) in rat perfused mesenteric vascular beds with resting tone. S_1 and S_2 indicate the responses to the first PNS in the control and second PNS in the presence of an antibiotic, respectively. I_1 and I_2 indicate the responses to the first (control) and second (antibiotic) bolus injections of NA. Values represent the mean ± s.e.mean. *$P<0.05$, **$P<0.01$, compared with control.

vasodilation (EC_{50}) in preparations with intact endothelium and without endothelium were 4.6 ± 0.8 nM ($n=5$) and 5.4 ± 1.5 nM ($n=5$), respectively. The maximum vasodilation (E_{max}) at the highest concentration of telithromycin in intact and denuded preparations was 93.8 ± 2.1% ($n=5$) and 89.7 ± 3.7% ($n=5$), respectively.

Effect of telithromycin on the Ca^{2+}-induced vasoconstrictor response

Each preparation with intact endothelium was perfused with Krebs solution containing a high concentration of KCl (60 mM) and Ca^{2+}-free medium. The perfusion of $CaCl_2$ (0.8–2.4 mM) induced a concentration-dependent increase in perfusion pressure due to vasoconstriction (0.8 mM, 38.6 ± 11.0 mm Hg; 1.6 mM, 56.9 ± 12.4 mm Hg and 2.4 mM, 78.8 ± 10.9 mm Hg). Perfusion of telithromycin (10 μM) induced an approximately 50% reduction in $CaCl_2$-induced vasoconstriction, as shown in Figure 6.

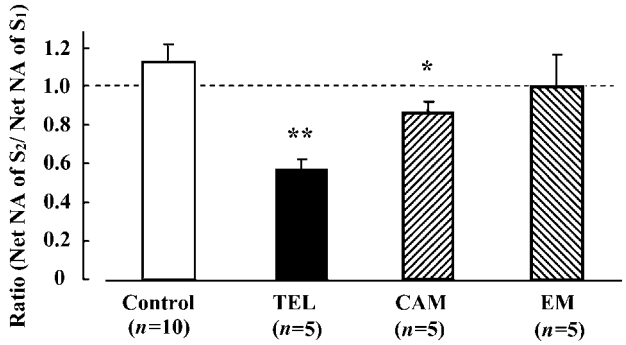

Figure 3 Effect of telithromycin (TEL) (10 μM), clarithromycin (CAM) (10 μM) and erythromycin (EM) (10 μM) on noradrenaline (NA) release evoked by periarterial nerve stimulation (PNS; 12 Hz) in rat perfused mesenteric vascular beds. Values represent the mean ± s.e.mean. *$P<0.05$, **$P<0.01$, compared with control.

Mean blood pressure responses to telithromycin in anaesthetized rats

As shown in Table 2, the intravenous injection of telithromycin at doses of 1 and 2 μmol kg^{-1} induced a sharp decrease in blood pressure in anaesthetized rats, without affecting the heart rate, in a dose-dependent manner. The blood pressure-lowering action started immediately after the injection, reached a maximum within 2 min, lasted for 10–30 min and returned to pre-administration levels at 30–60 min. However, vehicle injection had no effect on blood pressure. Significant differences were found in the mean blood pressure between pre-administration levels and vehicle administration (Table 2).

Discussion

This study demonstrated that the ketolide antibiotic telithromycin markedly reduced the vasoconstrictor response

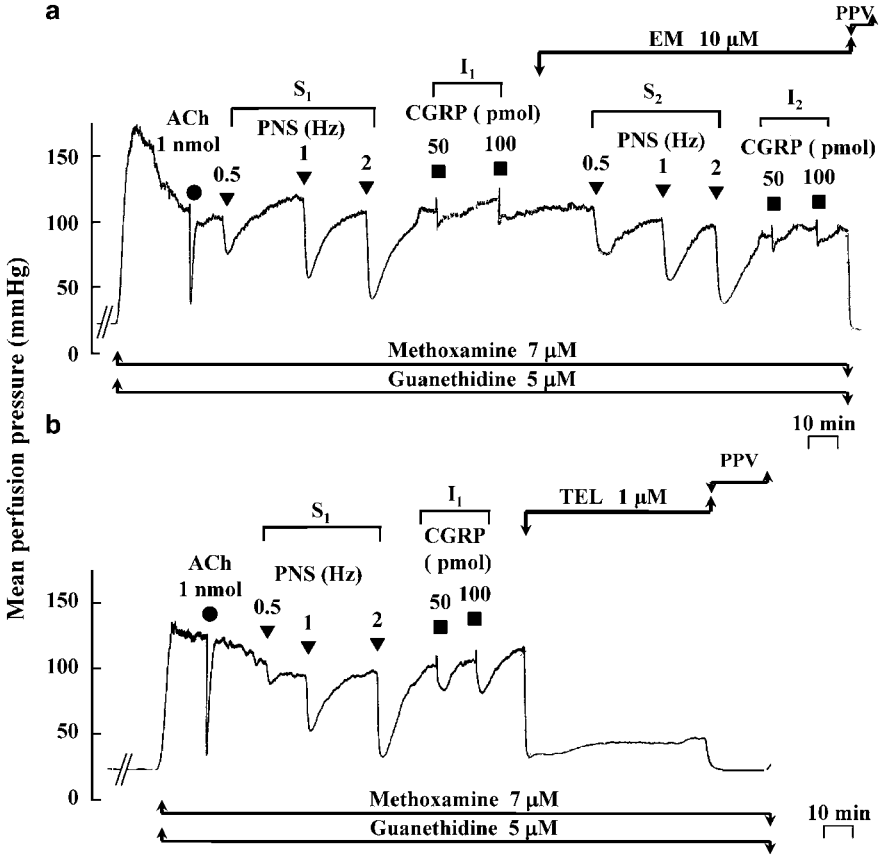

Figure 4 Typical records showing the effects of erythromycin (**a**) (EM; 10 μM) on the vasodilator responses to periarterial nerve stimulation (PNS; 0.5, 1 and 2 Hz) and bolus injections of calcitonin gene-related peptide (CGRP) (50 and 100 pmol), and vasodilation induced by telithromycin (**b**) (TEL; 1 μM) in rat perfused mesenteric vascular beds with active tone. S_1 and S_2 indicate the responses to the first PNS in the control and second PNS in the presence of an antibiotic (EM), respectively. I_1 and I_2 indicate the responses to the first (control) and second (antibiotic; EM) bolus injections of CGRP. In (**b**), note that addition of 1 μM TEL caused a sharp fall in perfusion pressure to the resting level. Symbols are the same as in Figure 1. PPV, perfusion of papaverine (100 μM).

Table 1 Effects of various antibiotics on vasodilation induced by periarterial nerve stimulation (PNS) and bolus injection of calcitonin-gene related peptide (CGRP) in perfused mesenteric vascular beds with active tone

Treatment	*PNS (Hz)*			*CGRP (pmol)*	
	0.5 S_2/S_1	*1* S_2/S_1	*2* S_2/S_1	*50* I_2/I_1	*100* I_2/I_1
Control	1.32 ± 0.05	1.18 ± 0.08	1.15 ± 0.04	1.27 ± 0.03	1.34 ± 0.07
Erythromycin					
1 μM	1.21 ± 0.02	1.15 ± 0.03	1.14 ± 0.04	1.26 ± 0.02	1.21 ± 0.05
10 μM	1.22 ± 0.03	1.12 ± 0.02	1.13 ± 0.04	1.20 ± 0.05	1.22 ± 0.03
Clarithromycin					
1 μM	1.37 ± 0.09	1.11 ± 0.04	1.10 ± 0.06	1.36 ± 0.07	1.23 ± 0.11
10 μM	1.26 ± 0.01	1.11 ± 0.02	1.10 ± 0.06	1.17 ± 0.08	1.23 ± 0.03
Levofloxacin					
1 μM	1.19 ± 0.02	1.22 ± 0.04	1.14 ± 0.03	1.26 ± 0.05	1.18 ± 0.06
10 μM	1.22 ± 0.04	1.25 ± 0.12	1.16 ± 0.04	1.12 ± 0.06	1.11 ± 0.08
Gatifloxacin					
1 μM	1.26 ± 0.07	1.18 ± 0.08	1.15 ± 0.05	1.12 ± 0.06	1.13 ± 0.09
10 μM	1.23 ± 0.06	1.04 ± 0.07	1.07 ± 0.06	1.21 ± 0.06	1.27 ± 0.09
Telithromycin					
10 nM	1.35 ± 0.08	1.21 ± 0.05	1.16 ± 0.07	1.27 ± 0.13	1.31 ± 0.07

Values represent the mean ± s.e.mean of five rats and the ratio of the first PNS (S_1; control)- and second PNS (S_2; antibiotics)-induced vasodilation and the ratio of the first CGRP injection (I_1; control)- and second CGRP injection (I_2; antibiotics)-induced vasodilation.

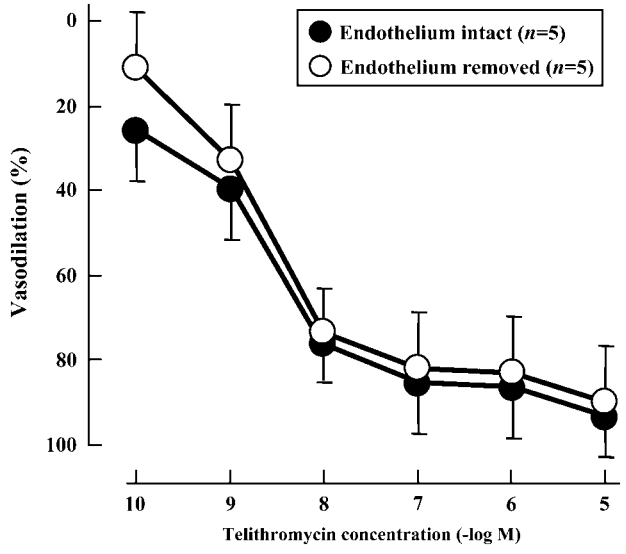

Figure 5 The concentration–response curve for vasodilation induced by telithromycin in rat perfused mesenteric vascular beds with intact endothelium and without endothelium. Values represent the mean ± s.e.mean.

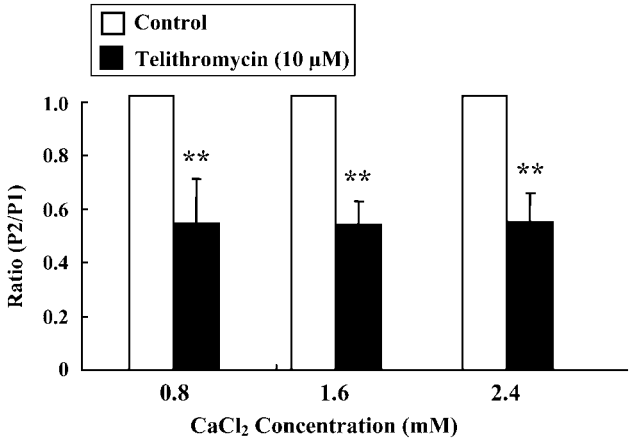

Figure 6 The effect of telithromycin (10 μM) on $CaCl_2$-induced vasoconstriction in rat perfused mesenteric vascular beds with intact endothelium and resting tone. The preparation was perfused with Krebs solution containing a high concentration of KCl (60 mM). P1 and P2 indicate the responses to the first $CaCl_2$ perfusion in the control and the second $CaCl_2$ perfusion in the presence of telithromycin. The ordinate shows the ratio of $CaCl_2$ (P1)- and (P2)-induced vasoconstriction. Values represent the mean ± s.e.mean. $^{**}P<0.01$, compared with the control.

induced by PNS in the rat perfused mesenteric vascular bed. Vasoconstriction in response to PNS of the rat mesenteric artery has been shown to be abolished by tetrodotoxin (neurotoxin), guanethidine (adrenergic neuron blocker), prazosin (α_1-adrenoceptor antagonist) and 6-hydroxydopamine (toxic to adrenergic neurons) (Kawasaki and Takasaki, 1984; Kawasaki *et al.*, 1987). Additionally, PNS of the rat perfused mesenteric artery increases the release of noradrenaline in the perfusate (Hatanaka *et al.*, 2006). Therefore, it is likely that noradrenaline released by PNS of periarterial sympathetic adrenergic nerves is responsible for the vasoconstriction induced by PNS. These findings indicate that telithromycin strongly inhibits the release of the perivascular adrenergic neurotransmitter noradrenaline from perivascular adrenergic nerves in the mesenteric vascular bed. This notion is supported by this finding that telithromycin significantly decreased the release of noradrenaline evoked by PNS.

Table 2 Effect of telithromycin (TEL) on mean blood pressure (MBP) and heart rate (HR) in anaesthetized rats

MBP	*Pre-treatment (mm Hg)*	*After vehicle and TEL injection (% change from the baseline)*						*%max*
		1 min	*3 min*	*5 min*	*10 min*	*30 min*	*60 min*	
Vehicle ($n=4$)	115.0 ± 3.7	1.4 ± 3.0	4.4 ± 4.8	6.2 ± 3.3	4.3 ± 3.5	4.5 ± 2.2	7.1 ± 0.5	6.2 ± 3.3
TEL 1 μmol kg^{-1} ($n=4$)	115.0 ± 3.6	−17.2 ± 3.7*,††	−14.5 ± 3.3*,††	−11.5 ± 4.1†	−7.8 ± 3.8†	−4.9 ± 2.1†	−5.4 ± 2.2†	−17.2 ± 3.7*,††
TEL 2 μmol kg^{-1} ($n=4$)	114.2 ± 4.1	−18.7 ± 0.8**,††	−17.4 ± 2.0**,††	−15.3 ± 2.5**,††	−16.7 ± 3.7*,††	−17.2 ± 6.1††	−8.5 ± 4.3†	−18.7 ± 0.8**,††
HR	*Pre-treatment (b.p.m.)*	*After vehicle and TEL injection (% change from the baseline)*						*%max*
		1 min	*3 min*	*5 min*	*10 min*	*30 min*	*60 min*	
Vehicle ($n=4$)	357.8 ± 18.3	−0.2 ± 1.3	0.3 ± 1.4	0.1 ± 1.4	−0.7 ± 2.1	−0.2 ± 2.1	−1.2 ± 1.8	−0.7 ± 2.1
TEL 1 μmol kg^{-1} ($n=4$)	353.5 ± 16.5	−0.2 ± 1.0	−0.3 ± 1.2	−1.2 ± 1.5	−1.9 ± 1.7	−1.7 ± 1.6	−2.3 ± 0.9	−1.9 ± 1.7
TEL 2 μmol kg^{-1} ($n=4$)	383.7 ± 22.4	−1.3 ± 0.5	−0.1 ± 2.2	−2.1 ± 0.4	−3.1 ± 2.4	1.0 ± 2.3	−2.9 ± 1.5	−3.1 ± 2.4

Abbreviations: b.p.m., beats per minute; %max, %maximum change.
$^{*}P<0.05$, $^{**}P<0.01$ vs baseline; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ vs vehicle control.
Telithromycin (TEL) was dissolved in the vehicle (1% dimethylsulphoxide) and administered intravenously.

The macrolide antibiotic erythromycin has been reported to inhibit the cholinergic and non-cholinergic transmitters in longitudinal and circular muscle preparations of the guinea-pig small intestine (Minocha and Galligan, 1991). In the airway smooth muscle, macrolide antibiotics (erythromycin, clarithromycin and roxithromycin) and a new quinolone (ciprofloxacin) have inhibitory effects on cholinergic neuro-effector transmission (Tagaya *et al*., 1994; Tamaoki *et al*., 1995a, b). Hong (2001) reported that the macrolide antibiotics bafilomycin A and concanamycin A suppressed neuromuscular transmission in the mouse diaphragm. Furthermore, bafilomycin A has been shown to inhibit not only cholinergic neurotransmitter release, but also adrenergic neurotransmitter release in the guinea-pig atrium (Hong, 2002). In this study, the macrolide antibiotics erythromycin and clarithromycin and two new quinolones, levofloxacin and gatifloxacin, at high concentrations (10 μM), inhibited the PNS-induced vasoconstriction in the rat mesenteric-resistant artery without affecting the noradrenaline-induced vasoconstriction. Additionally, clarithromycin attenuated the PNS-evoked noradrenaline release in the rat mesenteric vascular bed. Taken together, it is very likely that the macrolide and new quinolone antibiotics exert inhibitory actions on presynaptic neurotransmitter release in the peripheral nervous system.

On the other hand, the ketolide antibiotic telithromycin, with a chemical structure related to those of macrolide antibiotics, caused a marked reduction in PNS-induced vasoconstriction. It should be noted that the inhibitory effect of telithromycin appeared at concentrations lower than the therapeutic concentration (1 μM), and a high concentration (10 μM) of telithromycin almost abolished the PNS-induced vasoconstriction, whereas this antibiotic moderately inhibited noradrenaline-induced vasoconstriction. These findings suggest that telithromycin, at concentrations achieved clinically, strongly inhibits the release of the adrenergic neurotransmitter noradrenaline from perivascular adrenergic nerves in the mesenteric vascular bed. This notion was confirmed by the present finding that telithromycin reduced the PNS-evoked release of noradrenaline in the rat perfused mesenteric artery. Therefore, it should be emphasized that telithromycin at blood level concentrations used clinically presynaptically inhibits adrenergic neurotransmission in rat mesenteric vascular beds.

Telithromycin inhibited the vasoconstriction in response to exogenously applied high concentrations of noradrenaline, whereas other antibiotics did not alter the noradrenaline-induced responses. This finding suggests that telithromycin affects postsynaptic α-adrenoceptors in vascular smooth muscles. Colbert *et al*. (1991) reported that a macrolide antibiotic (LY281389) at a very high concentration of 1 mM inhibited α-adrenoceptor activity on the rat vas deferens. Additionally, the macrolide antibiotic erythromycin has been shown to have a direct inhibitory effect on the rat urinary bladder smooth muscle (Nissan *et al*., 1999). This study demonstrated that in the pre-contracted mesenteric artery, telithromycin induced concentration-dependent and endothelium-independent vasodilation, whereas perfusion pressure after perfusion of other antibiotics even at a concentration of 10 μM left them unchanged. These results indicate that telithromycin has strong vasodilator activity in the mesenteric artery. Additionally, this study showed that perfusion of telithromycin in a high K^+ medium inhibited Ca^{2+}-induced vasoconstriction, which is mediated by voltage-gated calcium channels, suggesting that telithromycin has a calcium channel blocking action. Aminoglycoside antibiotics have been reported to reduce entry of Ca^{2+} through voltage-gated channels in the axon terminals of the rat forebrain (Atchison *et al*., 1988). In addition, erythromycin has been shown to block calcium channels on longitudinal and circular muscle preparations of the guinea-pig small intestine (Minocha and Galligan, 1991). Taken together, these findings indicate that telithromycin causes inhibition of transmitter release by reducing entry of Ca^{2+} into vascular adrenergic nerves.

In this study, the degree of inhibition of PNS-induced vasoconstriction by telithromycin was greater than that of

the inhibition of PNS-induced noradrenaline release by telithromycin. Therefore, it seems likely that telithromycin not only causes inhibition of adrenergic neurotransmission but also induces strong relaxation of vascular smooth muscle. This finding suggests that telithromycin induces loss of vascular tone by inhibiting the function of perivascular adrenergic nerves and by relaxing blood vessels in the rat mesenteric vascular bed. This notion is supported by this *in vivo* study where telithromycin had a hypotensive effect in anaesthetized rats. It should be noted that telithromycin had no effect on heart rate even when hypotension appeared. This implies that telithromycin inhibits heart rate increase through baroreflex, by attenuating transmitter release from sympathetic adrenergic nerves.

This study demonstrated that in the pre-contracted mesenteric artery, PNS-induced vasodilation, which is mediated by peptidergic (CGRP) nerves, as well as the vasodilator response to exogenous CGRP, was not altered in the presence of erythromycin, clarithromycin, levofloxacin or gatifloxacin. Therefore, it appears that these antibiotics have no effect on peptidergic (CGRP) nerve function or postsynaptic CGRP receptor activity. As telithromycin at a low concentration of 1 μM resulted in a sharp fall in perfusion pressure and failure to maintain active tone, we had to use a further 100-fold dilution of telithromycin concentration (10 nM) to maintain active tone. This concentration (10 nM) of telithromycin, which significantly inhibited the PNS-induced adrenergic nerve-mediated vasoconstriction, did not affect the PNS-induced or exogenous CGRP-induced vasodilation, suggesting that telithromycin has no effect on peptidergic (CGRP) nerve function. However, it is unknown whether higher concentrations (1 and 10 μM) of telithromycin would affect the function of peptidergic (CGRP) nerves.

These findings that telithromycin, at a clinically attained blood concentration, induces potent inhibition of perivascular adrenergic nerve function in the rat mesenteric vascular bed and causes hypotension implying that functional loss of adrenergic nerves, which has an important function in maintaining and regulating vascular tone, could occur in the clinical use of telithromycin. To increase the blood supply in skeletal muscles and the brain during the daytime, adrenergic nerves sustain visceral vasoconstriction to decrease the circulating blood volume in the visceral area. Therefore, it is possible that inhibition of vascular adrenergic nerve function by telithromycin in clinical use may cause visceral vasodilation to increase the blood flow in the viscera. This may become prominent when patients who take telithromycin perform skeletal muscle activities associated with daily living and may result in a decrease in cerebral blood flow leading to loss of consciousness.

In conclusion, these results suggest that telithromycin causes not only strong inhibition of perivascular adrenergic neurotransmission but also vasodilator action in the rat mesenteric arteries. Therefore, it is very likely that these vascular effects of telithromycin could induce an increase in the visceral blood flow and hence reduce the cerebral blood flow, causing a temporary loss of consciousness.

Conflict of interest

The authors state no conflict of interest.

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