Vasoconstrictor Effect of Endothelin in Isolated Perfused Stomach of the Rat in Comparison with Noradrenaline and Serotonin

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Abstract—In the isolated rat stomach perfused with Krebs-Henseleit solution at 36 °C, endothelin induced vasoconstriction in a dose-dependent manner. The threshold dose for inducing vasoconstriction was very small, and the pressor response to a bolus injection at 0.1 nmol lasted more than 1 hr. In contrast, the vasoconstrictor effects of noradrenaline and serotonin were transient. The magnitude of the maximal response to endothelin was almost the same as that to noradrenaline, but greater than that to serotonin. The pressor response to serotonin, but not noradrenaline, was greatly augmented after pretreatment with endothelin. These results suggest that endothelin causes a long-lasting vasoconstriction, which would be associated with its ulcerogenic activity, especially in combination with other vasoactive agents under some pathophysiological conditions.

Endothelin, a 21-amino acid peptide isolated from the medium of cultured endothelial cells (1), has a potent vasoconstrictor action on the porcine coronary artery (1) and the renal artery (2). On the other hand, endothelin has been demonstrated to induce gastric lesions by a close intraarterial injection to the stomach in anesthetized rats (3) and to potentiate formation of ethanol-induced gastric lesions in an ex vivo rat stomach chamber model (4). It is supposed that endothelin profoundly decreases gastric mucosal blood flow, resulting in ischemia of the tissue. Furthermore, generation of oxygen radicals by blood reperfusion will damage the gastric mucosal vessels (5). So far, vasoactive substances such as noradrenaline, serotonin (6) and platelet-activating factor (PAF) (7, 8) have been considered to play a significant role in the etiology of gastric hemorrhage and erosions. From the same standpoint, endothelin is predicted to be involved in gastric mucosal vascular disturbances under pathophysiological conditions such as stress. The present study was undertaken to document the vasoconstrictor effect of endothelin, comparing it with those of noradrenaline and serotonin, in the isolated perfused stomach of the rat.

Male Wistar rats (200–300 g) were fasted overnight but allowed free access to water. They were anesthetized with urethane (1.35 g/kg, i.p.), and the trachea was cannulated. The method for measuring vascular perfusion pressure in isolated stomachs was principally the same as that described by Salvati and Whittle (9). Briefly, a cannula for perfusion was inserted into the celiac artery; its branching vessels such as the splenic artery, pancreatic duodenal artery and common hepatic artery were ligated in advance so that a solution could drain into the left gastric artery. After section of the vessels, the stomach was isolated and placed in an organ bath (Petri dish) for perfusion. Modified Krebs-Henseleit solution was perfused with a peristaltic pump (TMP-10H, Toyo Kagaku Sangyo). The flow rate was kept constant at 2 ml/min throughout the experiments. The basal perfusion pressure averaged 52.8±1.6 mmHg (mean±S.E.M., N=15). The pressure changes were measured with a pressure transducer (MPU-0.5-290-0-III, Toyo Boldwin) connected to a polygraph system (amplifier, Type 1236, Sanei; recorder, Recti-Horiz 8K, Sanei). The drug

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solution was either administered as a bolus into the rubber tube connecting the cannula in a volume of 0.1 ml over a period of 5 sec by a microinjector (Terumo Co.) or infused together with perfusing nutrient solution. Vasoconstriction was recorded as an increase in perfusion pressure. Modified Krebs-Henseleit solution contained 112 mM NaCl, 5.9 mM KCl, 1.12 mM MgCl₂, 2.5 mM CaCl₂, 1.45 mM NaH₂PO₄, 25 mM NaHCO₃, 11.5 mM glucose, 0.114 mM ascorbic acid and 0.03 mM disodium EDTA; it was gassed with a mixture of 95% O₂ and 5% CO₂ and maintained at 36°C. The experiments were started after the preparation was equilibrated for 1 hr.

Drugs used were l-noradrenaline bitartrate (Wako Pure Chemical Industry), serotonin creatinine sulfate (Merck) and endothelin (porcine/human; Peptide Inst.). The former two drugs were dissolved in saline, and the latter was dissolved in saline containing 0.01% Triton X 305 (Sigma).

Typical response patterns to noradrenaline, serotonin and endothelin in the gastric vasculature of the isolated perfused stomach are illustrated in Fig. 1 (A, B and C). The summarized data are shown in Fig. 2. When noradrenaline or serotonin was bolusly administered, an increase in perfusion pressure was produced dose-dependently in the range of 1–1000 nmol or 1–100 nmol, respectively. The pressor responses were transient and disappeared within 5 min after administration. The magnitude of the maximal response to noradrenaline was much greater than that exerted by serotonin; the maximal net increase in perfusion pressure was 129±7 mmHg (N=6) with noradrenaline and 44±7 mmHg (N=4) with serotonin. Endothelin also exerted an increase in perfusion pressure. The pressor response was

![Fig. 1. Vasoconstrictor effects of increasing doses of noradrenaline (NE), serotonin (5-HT) and endothelin (ET) and of their combinations on the isolated perfused stomach of rats. Effects of agents alone are shown in A, B and C, and effects of their combinations are shown in D.](image-url)
characteristic in that the threshold dose was extremely small, and the vasoconstriction was long-lasting. At a dose of 0.1 nmol, its response lasted more than 1 hr. The increase in perfusion pressure occurred dose-dependently in a range of 0.01–0.1 nmol. The maximal net increase in perfusion pressure was 107±19 mmHg (N=5). From these findings, it is suggested that the order of potency in terms of sensitivity and duration in vasoconstriction is endothelin>>noradrenaline=serotonin, and the magnitude of the maximal response is in the following order: noradrenaline=endothelin>serotonin.

Typical response patterns to each combination of endothelin with noradrenaline or serotonin are illustrated in Fig. 1D. As compared with the pressor response to noradrenaline alone at a dose of 10 nmol (62±8 mmHg of net increase; N=4), there were no significant changes in its pressor response after pretreatment with a bolus injection of endothelin at 0.01 nmol (65±9 mmHg of net increase; N=4) or together with an infusion of noradrenaline at 3×10^{-7} M (59±8 mmHg of net increase, N=4). In contrast, the pressor response to serotonin at a dose of 10 nmol (32±8 mmHg of net increase, N=4) was significantly augmented in combination with noradrenaline infusion (106±15 mmHg of net increase, N=4) or pretreatment with endothelin (76±6 mmHg of net increase, N=4).

It is of particular interest that the vasoconstrictor action of endothelin lasts more than 1 hr after elimination of endothelin by continuously perfusing endothelin-free nutrient solution. Similar phenomena have been reported with the rat hypertensive response in vivo (1). On the other hand, Wallace et al. (4) have demonstrated the potent vasoconstrictor action of endothelin in the isolated perfused rat stomach, but the disappearance of action after the end of endothelin infusion occurs relatively quickly as compared with our results noted after its bolus injection. However, the difference in the persistence of action may not be explained by the difference in calcium concentration of the nutrient solution, because the duration of action of endothelin was not greatly changed by the reduction of the calcium concentration from 2.5 mM (in the present study) to 1.0 mM (in the study of Wallace et al.) (data not shown).

The sustained effect of endothelin would be at least partly associated with the difficulty in washing it out from the vasculatures. As for its vasoconstricting mechanism, it is sug-
gested that endothelin contracts vascular smooth muscles through mechanisms such as increase in calcium influx (10) and release of calcium from its store sites (11). On the other hand, it is possible that the perfusion pressure rises indirectly through contraction of smooth muscles of the stomach by endothelin. However, this involvement would be relatively small, because a marked decrease in vascular perfusion pressure takes place after an injection of acetylcholine (data not shown), which is known to contract gastric smooth muscles.

It is not clear what kind of roles endothelin plays in physiological and pathological conditions. At present, however, it has been postulated that endothelin relates to a certain type of hypertension because of its long-lasting and potent vasoconstrictor effect (1). Moreover, the present results apparently suggest the possibility that release or formation of endothelin in the gastric microcirculation precipitates mucosal ischemia leading to hemorrhagic or necrotic damage. As the vasoconstrictor effect of endothelin may persist even after it disappears from the microcirculation, it is predictable that this endogenous substance is potentially ulcerogenic to gastric mucosa. Such possibilities have also been emphasized by the present finding that the vasoconstrictor effect of serotonin was greatly augmented by combination with endothelin.

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