Possible Mechanisms Underlying the Suppression of Gastric Vagal Afferents Due to Ecabapide (DQ-2511), a Gastroprokinetic Agent, in Rats

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ABSTRACT—We examined the implication of a nitric oxide (NO)-guanosine 3',5'-cyclic monophosphate (cGMP) cascade in the suppression of gastric vagal afferents due to ecabapide in anesthetized rats using a standard extracellular method of multi-unit recording. Sodium nitroprusside (SNP, 0.5 mg/kg), an NO donor, depressed the afferent discharge rate of the vagus nerve, like ecabapide (60 μg/kg). On the other hand, N⁶-nitro-L-arginine (L-NNA, 5 mg/kg), an NO biosynthesis inhibitor, significantly elevated its discharge rate. Pretreatment with L-NNA completely blocked the action of ecabapide. Atropine (0.05 mg/kg), a competitive antagonist of muscarinic cholinoreceptors, showed no effect on the afferent firing. These results suggest that ecabapide may suppress the activation of vagal afferents in gastric inhibitory vago-vagal reflex pathways through the NO-cGMP cascade.

Keywords: Ecabapide (DQ-2511), Gastric vagal afferent, Nitric oxide-cGMP cascade

Ecabapide (3-[2-(3,4-dimethoxyphenyl)ethyl]carbamoylmethyl]-amino-N-methylbenzamide: DQ-2511) improves experimentally-induced delays in gastric emptying without affecting gastric acid secretion or gastric mucosal blood flow (1–3) and also enhances gastric motility (3). Electrophysiological approaches revealed that the drug elicited a facilitatory effect on the gastric efferent activity as a result of a disinhibitory reflex action originating from suppressed gastric afferents (4). Although the binding affinity of ecabapide to selective receptors has been exhaustively explored, it showed no specific binding (2, 5). On the basis of the above studies, ecabapide was considered to exert its action via suppression of activation of afferents in the sensory component of gastric inhibitory vago-vagal reflex pathways (3, 4). More recently, ecabapide was reported to stimulate the intracellular production of guanosine 3',5'-cyclic monophosphate (cGMP) content (5, 6) at a concentration as low as 10⁻⁷ M when rabbit gastric parietal cells were used (7). To date, stimulation of guanylate cyclase and accumulation of intracellular cGMP have been proposed to induce smooth muscle relaxation related to nitric oxide (NO), which plays a pivotal role as a key mediator in these events (8, 9). In the present communication, to elucidate the possible role of NO in the suppression of gastric vagal afferents due to ecabapide, we examined the effect of either an NO donor sodium nitroprusside (SNP) or the NO biosynthesis inhibitor N⁶-nitro-L-arginine (L-NNA) on the afferent discharge rate in anesthetized rats using a standard extracellular method of multi-unit recording (10). Furthermore, we assessed the effect of pretreatment with L-NNA on the suppression of gastric vagal afferents due to ecabapide and then estimated the effect of changes in gastric motility on afferent discharges using atropine, a competitive antagonist of muscarinic cholinoreceptors.

Eight- to nine-week-old male Wistar rats weighing 250 to 350 g were used for the investigation. The animals were fasted overnight but allowed free access to water until the beginning of the experiments. Ecabapide was synthesized at our laboratory. SNP and L-NNA were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and atropine supplied from Tanabe Co. (Osaka). Ecabapide (60 μg/kg), SNP (0.5 mg/kg), L-NNA (5 mg/kg) and atropine (0.05 mg/kg) were dissolved in or diluted with sterile 0.9% saline solution and injected into the abdominal vein at a constant volume of 0.6 ml/kg. In an additional study using the same dose, L-NNA was administered to rats 1 hr prior to ecabapide treatment. The dosage level of each agent used in the present investigation was selected on the basis of preliminary studies or previously published reports (3, 4, 11, 12). Multi-unit neural discharge in the vagal afferent fibers was recorded extracellularly. Stan...
Fig. 1. Quantitative analysis of the effect of sodium nitroprusside (SNP) or $N^G$-nitro-L-arginine (L-NNA) on the afferent discharge rate. Data were calculated 30 and 60 min after injection of SNP (left: 0.5 mg/kg, i.v.) or L-NNA (right: 5 mg/kg, i.v.). Each point and vertical bar represents the mean±S.E.M. of 5 animals. *P<0.05 vs value at 0 min.

Fig. 2. A typical trace of the effect of NG-nitro-L-arginine (L-NNA) and/or ecabapide on the afferent discharge (impulses/5 sec in afferent fibers recorded from the ventral abdominal vagus). A: L-NNA (5 mg/kg, i.v.); B: ecabapide (60 pg/kg, i.v.); C: L-NNA plus ecabapide. The arrows show the time of injection of each test solution.
These experimental protocols (data not shown). The finding that the depressed afferent discharges provoked by blocked the action of ecabapide (Fig. 2: A–C), suggest in rat arteries (5). Pretreatment with L-NNA completely concentration (10⁻¹⁰ M) of ecabapide, however, caused excitations at 10⁻¹⁰ M (7), which almost corresponded to the suppression induced by SNP in response to the NO-cGMP cascade, a cyclic GMP analog, elicits the suppression of gastric afferent activity in this test system (S. Hatanaka et al., unpublished data).

In conclusion, ecabapide may suppress activation of gastric vagal afferents through the NO-cGMP cascade, and this phenomenon may be brought about by the action of the drug on the gastric innervation.

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