Centrally Mediated Inhibitory Effect of 5-[2-(Diethylamino)ethyl]-amino-5,11-dihydro[1]benzoxepino[3,4-b]pyridine Trihydrochloride (KW-5805) on Gastric Acid Secretion in Rats

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Abstract—KW-5805, 5-[2-(diethylamino)ethyl]amino-5,11-dihydro[1]benzoxepino[3,4-b]pyridine trihydrochloride, is a new tricyclic compound with antiulcer activities. Its effect on stimulated gastric acid secretion was investigated in the perfused stomach of anesthetized rats. KW-5805 at 0.3–10 mg/kg, i.v., dose-dependently inhibited gastric acid secretion stimulated by 2-deoxy-D-glucose (2-DG). On the other hand, the compound at 10–20 mg/kg, i.v., exerted a moderate decrease in gastric acid secretion stimulated by bethanechol; and at 10 mg/kg, i.v., it produced no change in gastric acid secretion evoked peripherally by vagal electrical stimulation. When applied intracerebroventricularly at 1–5 µg/rat, this compound dose-relatedly reduced gastric acid secretion stimulated by 2-DG. Three main metabolites (KF-10504, KF-9530 and KF-10847) of KW-5805 at 1 mg/kg, i.v., caused no significant decrease in gastric acid secretion stimulated by 2-DG. Doxepin, a tricyclic compound, definitely depressed the 2-DG stimulated gastric acid secretion at 1 mg/kg, i.v. It is suggested that intravenous administration of KW-5805 inhibits gastric acid secretion stimulated by 2-DG, mainly via centrally mediated mechanisms, and that biotransformation of KW-5805 to the metabolites contributes little to the development of the antisecretory effect.
with that of pirenzepine in the receptor binding assay (1). This compound showed weak antisecretory activity after i.d. administration of 30 mg/kg in pylorus-ligated rats (1, 2). Interestingly, however, KW-5805 has been found to potently inhibit gastric acid secretion stimulated by 2-deoxy-D-glucose (2-DG) in our preliminary study. This finding implies the possibility that KW-5805 reduces the centrally stimulated gastric acid secretion more selectively than does the peripherally stimulated one.

The purpose of the present study is to investigate the characteristic effect of KW-5805 in blocking centrally stimulated gastric acid secretion and also to see if any active metabolite of KW-5805 exerts an antisecretory effect in anesthetized rats.

Materials and Methods

Animals: Male Wistar rats weighing 220–270 g were used. The animals were housed under controlled environmental conditions (kept at 24±1°C and lighted between 7:00 a.m. and 7:00 p.m.) and fed commercial rat chow (Oriental Yeast Co., Ltd., Japan). The rats were fasted for 24 hr before each experiment, but were allowed free access to water.

Measurement of gastric acid secretion: The rats were anesthetized with urethane (1.35 g/kg, i.p.). Gastric acid secretion was measured by a previously described procedure (8). Briefly, the trachea was exposed and cannulated. After ligation of the pylorus and esophagus, a dual polyethylene gastric cannula was inserted into the gastric lumen from a small incision in the forestomach. The stomach lumen was perfused with saline solution (adjusted to pH 5.0 with diluted HCl and maintained at 37±1°C) through the inlet tube of the dual cannula connected to a perfusion pump (Minipump TMP-10H, Toyo Kagaku Sangyo, Ltd., Japan) at a rate of 1 ml/min. After at least 30 min of perfusion for equilibration, the perfusate from the outlet of the cannula was recovered as a 10 min fraction with a fraction collector (Eyela DC-20, Tokyo Rikakikai Co., Ltd., Japan) and titrated to pH 5.0 with 20 mM NaOH using an automatic titrator (HSS-10A, Toa Electronics Ltd., Japan). The acid output was expressed in terms of μEq H⁺. The femoral vein was cannulated for administration of drugs. Rectal temperature was maintained at 36±1°C by intermittently heating with an infrared lamp.

Cannulation for intracerebroventricular injection: A rat was mounted in a stereotaxic instrument. The bregma or interaural line served as a reference point, and stereotaxic coordinates were referred to the atlas of Paxinos and Watson (9). A stainless steel cannula (outer diameter of 0.35 mm) was stereotaxically implanted into the lateral cerebroventricle (with skull flat: 1.0 mm behind the bregma; 1.5 mm lateral to the sagittal suture and 4.5 mm vertical) and fixed with dental cement. Each drug solution was applied in a volume of 10 μl over a period of 2 min. At the end of the experiments, pontamine sky blue solution was injected into the lateral ventricle to make sure that the solution diffused into the cerebral cavities.

Electrical stimulation of the vagus nerve: Bilateral cervical nerves were exposed in the neck, separated from the carotid artery and severed centrally after ligation. The distal end of the left nerve was stimulated electrically by applying square pulses of 5 V, 10 Hz and 1 msec for 10 min with an electrical stimulator (Electrostimulator 3F31, Sanei Instrument Co., Ltd., Japan) through a pair of silver electrodes.

Experimental procedures: Collection of the perfusate was started 30 min before injection of each test compound and was continued for 120 min after injection of secretagogues. Test compounds were administered i.v. or i.c.v. 10 min before injection of secretagogues.

In the vagus nerve stimulation experiments, peripheral electrical stimulation was repeated three times at intervals of 90 min. KW-5805 or saline was administered i.v. 10 min before the second electrical stimulation. The acid output for 30 min before electrical stimulation was referred to as the basal secretory control, while the acid output for 60 min after electrical stimulation was compared for evaluation of drug effects.

Drugs: KW-5805 and its three main metabolites (KF-10504, KF-9530 and KF-10847), whose chemical structures are shown in Fig. 1, were gifts from Kyowa Hakko, Ltd., Tokyo, Japan. Other drugs used were doxepin hydrochloride and bethanechol chloride.
(Sigma Chemical Co., St. Louis, U.S.A.) and 2-deoxy-D-glucose (Nacalai Tesque Co., Ltd., Kyoto, Japan). These drugs were dissolved in saline, and their doses were expressed in terms of their salts.

**Statistical analysis:** Results were expressed as a mean±S.E.M. Statistical analysis was made by one-way ANOVA followed by Dunnett's test or the paired t-test.

**Results**

**Effect of intravenous injection of KW-5805 on 2-deoxy-D-glucose stimulated gastric acid secretion:** 2-Deoxy-D-glucose (2-DG; 200 mg/kg, i.v.) produced a sustained increase in gastric acid secretion which reached a plateau in 1 hr (Fig. 2). The acid output for 120 min after 2-DG administration was compared between saline and KW-5805 pretreated groups. KW-5805 definitely inhibited the 2-DG stimulated gastric acid secretion at 1 mg/kg, and this blocking action was dose-dependent in the dose range of 0.3–10 mg/kg. Inhibition of the control response by 1 mg/kg of KW-5805 was approx. 75%.

**Effect of intravenous injection of KW-5805 on bethanechol stimulated gastric acid secretion:** Bethanechol (BeCh; 1 mg/kg, s.c.) elicited a long lasting increase in gastric acid secretion with a peak response at 10–20 min after injection (Fig. 3). The acid output for 120 min after BeCh administration was compared between saline and KW-5805 pretreated groups. KW-5805 at 10 mg/kg produced a small but significant decrease in gastric acid secretion stimulated by BeCh, and its inhibition of the saline control response was about 40%. The antisecretory extent became somewhat greater at 20 mg/kg of KW-5805.

**Effect of intravenous injection of KW-5805 on gastric acid secretion evoked by vagal electrical stimulation:** In rats undergoing an operation for bilateral cervical vagotomy, the distal end of the left nerve was electrically stimulated for 10 min. A marked increase in gastric acid secretion occurred in 10–30 min and disappeared in 60 min after vagal electrical stimulation (Fig. 4). These secretory patterns were almost the same as that by three repeated vagal electrical stimulations. The drug dosing was carried out 10 min before the second stimulation. KW-5805 (10 mg/kg, i.v.) neither inhibited the acid output over a period of 60 min after vagal electrical stimulation (Table 1) nor changed the secretory patterns shown by vagal electrical stimulation (Fig. 4).

**Effect of intracerebroventricular injection of KW-5805 on 2-deoxy-D-glucose stimulated gastric acid secretion:** KW-5805 was injected intracerebroventricularly (i.c.v.) at doses of 1 and 5 µg/rat 10 min before 2-DG administration (200 mg/kg, i.v.). This compound reduced the 2-DG stimulated gastric...
Table 1. Effect of intravenous injection of KW-5805 on gastric acid secretion evoked by vagal electrical stimulation

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>(N)</th>
<th>Acid output ($\mu$Eq H+/60 min) after electrical stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>(4)</td>
<td>45.0±13.7 57.5±15.2 55.2±13.5</td>
</tr>
<tr>
<td>KW-5805 10 mg/kg, i.v.</td>
<td>(4)</td>
<td>56.4±4.1 49.2±8.0 50.2±9.7</td>
</tr>
</tbody>
</table>

Electrical stimulation (5 V, 10 Hz and 1 msec for 10 min) was repeated three times at intervals of 90 min. Drugs were i.v. administered 10 min before the onset of the second stimulation. Gastric acid secretion was estimated as acid output for 60 min after electrical stimulation. N: number of animals. Each value represents the mean±S.E.M. No significant changes were noted between drug treatments or between applications of electrical stimulation.

Fig. 4. Typical recordings of effect of intravenous injection of KW-5805 on gastric acid secretion evoked by vagal electrical stimulation. Electrical stimulation (5 V, 10 Hz and 1 msec for 10 min) was repeated three times at intervals of 90 min. Gastric acid secretion was estimated as acid output per 10 min. Drugs were i.v. administered 10 min before the onset of the second stimulation. SAL: saline. Arrow mark: drug injection. Striped column: electrical stimulation.

Effect of intravenous injection of three metabolites of KW-5805 and doxepin on 2-deoxy-D-glucose stimulated gastric acid secretion: The acid output for 2 hr after 2-DG administration (200 mg/kg, i.v.) was compared between saline and drug pretreated groups. The three metabolites (KF-10504, KF-9530 and KF-10847) of KW-5805 caused no significant decrease in the 2-DG stimulated gastric acid secretion at a dose of 1 mg/kg, i.v. (Fig. 6). On the other hand, doxepin, a tricyclic antidepressant, definitely reduced the 2-DG stimulated gastric acid secretion at a dose of 1 mg/kg, i.v.
Fig. 6. Effect of intravenous injection of three metabolites (KF-10504, KF-9530 and KF-10847) of KW-5805 and doxepin on 2-deoxy-D-glucose (2-DG) stimulated gastric acid secretion. Gastric acid secretion was estimated as acid output for 120 min after injection of 2-DG at 200 mg/kg, i.v. Drugs were administered at 1 mg/kg, i.v. Each value represents the mean±S.E.M. of 4-5 animals. **P<0.01, significantly different from the saline group.

Discussion

It is well-known that decreased intracellular glucose, i.e., cytoglucopenia, in the brain induces gastric acid secretion (10). Administration of glucose analogues such as 2-DG, which are not metabolized in the cells, elicits functional cytoglucopenia, leading to stimulation of gastric acid secretion by way of the vagus nerve. The acid stimulatory action of 2-DG is considered to be central in origin (11). In the present study, i.v. administration of KW-5805 inhibited the gastric acid secretion stimulated by 2-DG more sensitively and more potently than did that stimulated by BeCh; the 2-DG stimulated gastric acid secretion was definitely inhibited at a dose of 1 mg/kg, while the BeCh stimulated gastric acid secretion was moderately reduced at a dose of 10 mg/kg. These findings led us to the assumption that KW-5805 inhibits gastric acid secretion by acting on its central controlling mechanisms.

The centrally mediated antisecretory effect of KW-5805 was further elucidated in the experiments of vagal electrical stimulation and in the experiments of i.c.v. administration of KW-5805. At a dose of 10 mg/kg, i.v., KW-5805 produced no change in gastric acid secretion stimulated peripherally by vagal electrical stimulation. In contrast, i.c.v. administration of KW-5805 definitely depressed the 2-DG stimulated gastric acid secretion at a dose of 5 μg/rat. Accordingly, the i.v. vs. i.c.v. dose ratio required for inhibition of the 2-DG stimulated acid secretion was roughly 50. This estimation provides support for the assumption that the antisecretory effect of intravenous KW-5805 is largely exerted by its central action.

Several tricyclic antidepressants are known to have antisecretory properties. In the literature, the antisecretory effect of imipramine was considered to be mediated through blockade of histamine H2-receptors or muscarinic receptors and through uptake inhibition of noradrenaline or serotonin (6). However, the 2-DG or BeCh stimulated gastric secretion was not greatly affected by s.c. administration of imipramine in rats (12). Desipramine, an active metabolite of imipramine, was found to inhibit gastric acid secretion via centrally mediated mechanisms (13–15), probably through noradrenaline uptake inhibition (15). Trimipramine, another tricyclic compound, has been used clinically in the treatment of gastric and duodenal ulcers (16, 17) on the basis of its antidepressant effect along with antisecretory activity (18). However, trimipramine did not affect the 2-DG or insulin stimulated gastric acid secretion (12, 19), suggesting that its antisecretory effect would not result from its central action. Doxepin, a tricyclic antidepressant, was also reported to be effective in the treatment of peptic ulcer disease (20). The compound acts centrally to reduce gastric acid secretion at low doses presumably through uptake inhibition of noradrenaline (14). Furthermore, it causes a blockade of histamine H1-receptors (21), H2-receptors (6) and adrenoceptors (6) in the brain or possesses K+ antagonistic activity at the H+/K+ ATPase site of the parietal cell (5). Pirenzepine, which is a tricyclic compound without antidepressant properties, definitely inhibits gastric acid secretion by blocking muscarinic receptors in the stomach (4). Its central effects are negligible because of its inability to cross the blood brain barrier. In the present study, doxepin was selected as a reference drug of tricyclic compounds because of its antiulcerogenic and antisecretory activities which may be exerted through cen-
tral mechanisms. On the other hand, it has been found that KW-5805 is an antiulcerogenic compound (1) without antidepressive activity (K. Watanabe and S. Yano, unpublished observation). It elicited weak inhibition of gastric acid secretion in pylorus-ligated rats (1, 2). However, the present results showed that KW-5805, as well as doxepin, potently inhibited the 2-DG stimulated gastric acid secretion. The difference in antisecretory activity of KW-5805 seems to result from its potent inhibition of centrally activated vagal tone, but not basal vagal tone. By comparing the antisecretory features of tricyclic compounds described above, it may well be deduced that their antisecretory mechanisms are different from each other and are independent of their antidepressive activities. Of these tricyclic compounds, KW-5805 is considered to be characteristic in that its antisecretory mechanisms are predominantly central in origin.

It has been reported that desipramine is an active metabolite of imipramine and is more potent in exerting antidepressant effect than the latter (22). In general, monomethylated tricyclic antidepressants (e.g., desipramine) were more potent in inhibiting catecholamine uptake than dimethylated ones (e.g., imipramine) (23). Thus, we investigated the antisecretory activity of three main metabolites (KF-10504, KF-9530 and KF-10847) of KW-5805. KW-5805, a diethylated compound, was found to be metabolized to KF-10504 and to KF-9530 by oxidative N-dealkylation and also metabolized to KF-10847 by aromatic hydroxylation in the body. The order of plasma concentration shown at 5–6 hr after oral administration of KW-5805 in the rat was the monoethylated compound (KF-10504) > the dealkylated compound (KF-9530) > the hydroxylated compound (KF-10847) (24). However, the inhibitory effect of these metabolites on the 2-DG gastric acid secretion was much less potent than that of KW-5805. Accordingly, it is suggested that KW-5805 mainly acts directly, and not via its metabolites.

It has been reported that KW-5805 is an antiulcerogenic compound that acts on the defensive mechanisms (3), as its antisecretory activity is weak in pylorus-ligated rats (1, 2). However, the present results indicate that KW-5805 could inhibit gastric acid secretion through centrally mediated mechanisms. At present, it remains to be elucidated whether or not KW-5805 inhibits gastric acid secretion stimulated centrally by drugs such as baclofen and thyrotropine releasing hormone (TRH) or by electrical stimulation in the brain. In therapeutic application, compounds such as KW-5805 which selectively inhibit the centrally stimulated gastric acid secretion would be beneficial to the prevention of stress-induced gastric ulceration. It is known that stress exposure of experimental animals may elicit an increase in acid secretion (25) which is closely associated with stress ulceration. On these pharmacological aspects, the antiulcerogenic effects of KW-5805 will be further investigated.

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