Effects of KB-5492, a New Anti-Ulcer Agent with a Selective Affinity for the Sigma-Receptor, on Aspirin-Induced Disruption of the Rat Gastric Mucosal Barrier

Yasuo Morimoto, Koichi Shimohara, Shinya Oshima, Hideaki Hara and Takayuki Sukamoto

Department of Pharmacology, New Drug Research Laboratories, Kanebo Ltd., 1-5-90, Tomobuchi-cho, Miyakojima-ku, Osaka 534, Japan

Received June, 30 1993 Accepted October 23, 1993

ABSTRACT—The effect of KB-5492, a new anti-ulcer agent with a selective affinity for the sigma-receptor, on aspirin-induced disruption of the gastric mucosal barrier was studied in rats. Intragastric instillation of aspirin at 200 mg/kg rapidly decreased the gastric transmucosal potential difference (PD) in anesthetized rats. The PD recovered gradually following the removal of aspirin from the instillation solution. Aspirin, administered orally at 200 mg/kg, also reduced the amount of gastric covering mucus and induced a decrease in gastric H⁺ concentration and an increase in gastric Na⁺ concentration in pylorus-ligated rats. KB-5492, administered intraduodenally at 200 mg/kg, significantly prevented the aspirin-induced decrease in PD and accelerated the recovery of PD. In addition, KB-5492 at 200 mg/kg significantly prevented the reduction of gastric covering mucus, the decrease in gastric H⁺ concentration and the increase in gastric Na⁺ concentration induced by aspirin. These effects were similar to those of 0.01 mg/kg of 16,16-dimethyl prostaglandin E₂ (dmPGE₂). Teprenone at 200 mg/kg did not show any effect except for the inhibitory effects on the changes in gastric H⁺ and Na⁺ concentration. In the histological study, marked reduction of PAS-positive epithelial mucus and the exfoliation of surface epithelial cells were observed in the gastric mucosa exposed to aspirin. KB-5492 and dmPGE₂ almost completely prevented the former, whereas both drugs prevented the latter incompletely. These findings indicate that KB-5492 protects the gastric mucosal barrier against the disruption by aspirin, which may be mainly exerted by retention of the gastric covering mucus.

Keywords: KB-5492, Aspirin, Gastric mucosal barrier, Gastric transmucosal potential difference, Gastric mucus

The gastric mucosal barrier is a physiological defensive mechanism to protect the gastric mucosa from the aggression of gastric acid (H⁺). It consists morphologically of surface epithelial cells and a mucous layer covering the mucosa, and gastric H⁺ diffusing into the mucosa is neutralized in this mucous layer with HCO₃⁻ secreted from surface epithelial cells (1, 2). It is well-known that aspirin disrupts the gastric mucosal barrier, induces the back diffusion of gastric H⁺ and finally produces hemorrhagic mucosal lesions (2).

KB-5492, 4-methoxyphenyl 4-(3,4,5-trimethoxybenzyl)-1-piperazinacectate monofumarate monohydrate, is a new anti-ulcer agent previously shown to prevent various experimental gastric mucosal lesions in rats including those induced by oral administration of aspirin or acidified aspirin (3, 4). Although KB-5492 at anti-ulcer doses did not affect either basal or histamine-stimulated gastric acid secretion in rats (3, 4), it increased the gastric mucosal blood flow (4) and prevented the reduction of hexosamine content induced by aspirin in rat gastric mucosa (4). These findings strongly suggest that KB-5492 enhances gastric mucosal defensive factors. Recently, KB-5492 was found to have a selective affinity for the sigma-receptor in guinea pig brain and porcine gastric fundic mucosal membranes (Y. Harada et al., unpublished data). The ulceroprotective effects of KB-5492 were antagonized by haloperidol, a sigma-receptor antagonist, but not by sulpiride, a dopamine D₂-receptor antagonist (5). However, the detailed mechanism of its action remains to be clarified.

In the present study, the effect of KB-5492 on aspirin-induced disruption of the gastric mucosal barrier was investigated in rats to clarify the mechanism by which KB-5492 enhances gastric mucosal defensive factors.
MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats weighing 180–240 g (Charles River Japan, Atsugi) were used. The animals were deprived of food for 24 hr before the experiments. Water was given freely during the fasting period, but withheld during the last 2 hr.

Drugs
KB-5492 (Kanebo, Osaka) and teprenone (Eisai, Tokyo) were suspended in 10% gum arabic solutions. 16,16-Dimethyl prostaglandin E2 (dmPGE2; Cayman Chemical, Ann Arbor, Michigan, USA) was dissolved initially in absolute ethanol at 0.2 mg/ml, and then diluted with 10% gum arabic solution 100 times. Other drugs used were aspirin (Wako, Osaka), urethane (Kishida, Osaka), alcian blue 8GX (AB, Wako) and sodium dioctyl sulfosucinate (DSS; Tokyo Kasei, Tokyo).

Measurement of gastric transmucosal potential difference
The experiment was performed according to the method of Nagashima et al. (6) with some modifications. Rats were anesthetized with urethane (1.25 g/kg, i.p.), and the abdomens were incised. The esophagus was ligated, and then a vinyl tube, which was used for the instillation of aspirin or saline, was inserted into the gastric lumen via the forestomach. A KCl-agar electrode, made by filling a polyethylene tube (SP-55; Natsume, Tokyo) with 3% agar saturated with KCl, was inserted into the gastric lumen via the pylorus (luminal electrode). The other KCl-agar electrode was inserted into the abdomen (reference electrode). The other end of each electrode was led into a saturated KCl solution in which an Ag-AgCl electrode, connected to a pH-millivoltmeter (F-7; Horiba, Kyoto), was immersed. Subsequently, saline was instilled into the gastric lumen and the potential difference (PD) was recorded continuously. After the PD had stabilized, drugs were administered intraduodenally, and then the PD was measured for 30 min. Subsequently, saline was removed and 20 mg/ml aspirin, suspended in saline, was instilled into the gastric lumen in a volume of 1 ml/100 g body weight. After 30 min, the aspirin suspension was replaced with normal saline, and then the PD was measured for 30 min.

Measurement of gastric covering mucus and gastric H+ and Na+ concentration
Rats were anesthetized with ether and their abdomen was incised. The pylorus was ligated and drugs were administered intraduodenally immediately after pylorus-ligation. Thirty minutes later, 20 mg/ml aspirin, suspended in 1% gum arabic solution, was administered orally in a volume of 1 ml/100 g body weight under the conscious condition. When investigating the effects of the drugs in normal rats, 1% gum arabic solution was administered orally instead of the aspirin suspension under the conscious condition. Thirty minutes later, the animals were killed by decapitation. The stomach was removed and the gastric contents were transferred to a centrifuge tube. The amount of gastric covering mucus was measured according to the method of Corne et al. (7) as modified by Kitagawa et al. (8). The stomach was incised along the lesser curvature and everted. The surface of the gastric mucosa was rinsed gently with 0.25 M sucrose, and then the stomach was soaked for 2 hr in 10 ml of AB dyeing reagent (0.1% AB dissolved in 0.16 M sucrose, buffered with 0.05 M sodium acetate and adjusted pH 5.8). Subsequently, the stomach was soaked for 1 hr in 20 ml of 0.25 M sucrose to remove free AB and then soaked for 2 hr in 10 ml of DSS reagent (30% DSS dissolved in 70% ethanol) to extract the AB. The optical density of the extract measured at 620 nm reflected the amount of gastric covering mucus.

Histological examination of gastric mucosa
The pylorus of the rat was ligated under ether anesthesia and drugs were administered intraduodenally. After 30 min, 20 mg/ml of aspirin, suspended in 1% gum arabic solution, was administered orally in a volume of 1 ml/100 g body weight, and the animals were killed by decapitation 30 min after aspirin treatment. The stomach was removed and fixed by inflation with 10 ml of 10% phosphate-buffered formalin (PBF), pH 7.0, and then incised along the greater curvature. The stomach was spread on cardboard and immersed in the PBF for 48–72 hr. A small specimen of the corpus was embedded in paraffin and sliced into 4-μm sections. Subsequently, the sections were stained with periodic acid-Schiff’s reagent (PAS) or with hematoxylin and eosin.

Statistics
The results were expressed as the mean±S.E. The statistical significance was determined by one-way analysis of variance followed by Dunnett’s or Duncan’s test.

RESULTS

Effects of drugs on aspirin-induced decrease in gastric transmucosal potential difference
As shown in Fig. 1, PD was rapidly decreased by in-
tragastric instillation of aspirin at 200 mg/kg and gradually recovered after removal of aspirin. KB-5492, administered intraduodenally at 200 mg/kg, significantly prevented the aspirin-induced decrease in PD and accelerated the recovery of PD following the removal of aspirin from the instillation solution. DmPGE2 at 0.01 mg/kg significantly prevented the aspirin-induced decrease in PD and accelerated the recovery of PD. Teprenone at 200 mg/kg did not affect either the decrease in PD or the recovery of PD. No drugs affected the PD before the instillation of aspirin.

Fig. 1. Effects of KB-5492, 16,16-dimethyl prostaglandin E2 and teprenone on aspirin (200 mg/kg, i.g.)-induced changes in gastric transmucosal potential difference in anesthetized rats. Each point represents the mean±S.E. of 6 animals. *P<0.05, **P<0.01, significantly different from the control (Dunnett’s test). ○: control (vehicle-treated group); ●: KB-5492, 100 mg/kg, i.d.; △: KB-5492, 200 mg/kg, i.d.; ▲: 16,16-dimethyl prostaglandin E2, 0.01 mg/kg, i.d.; ■: teprenone, 200 mg/kg, i.d.

Effects of drugs on aspirin-induced reduction of gastric covering mucus and changes in gastric H+ and Na+ concentration

As shown in Fig. 2, aspirin, administered orally at 200 mg/kg, significantly reduced the amount of gastric covering mucus in pylorus-ligated rats. Simultaneously, aspirin significantly decreased the gastric H+ concentration, but increased the gastric Na+ concentration. KB-5492, administered intraduodenally at 100 and 200 mg/kg, significantly and dose-dependently prevented aspirin-induced reduction of gastric covering mucus. In addition, KB-5492 at 200 mg/kg significantly prevented both the decrease in gastric H+ concentration and the increase in gastric Na+ concentration. DmPGE2 at 0.01 mg/kg significantly prevented the aspirin-induced reduction of gastric covering mucus, decrease in gastric H+ concentration.

Fig. 2. Effects of KB-5492 (KB), 16,16-dimethyl prostaglandin E2 (PG) and teprenone (TEP) on aspirin (200 mg/kg, p.o.)-induced changes in the amount of gastric covering mucus and in gastric H+ and Na+ concentration in pylorus-ligated rats. Control rats (Cont) were administered the vehicle. Vehicle-treated rats (Vehicle) were administered aspirin (p.o.) and vehicle (i.d.). Each column represents the mean±S.E. of 8 animals. *P<0.05 and **P<0.01 represent significant differences (Duncan’s test).
and increase in gastric Na+ concentration. Teprenone at 200 mg/kg did not prevent the aspirin-induced reduction of gastric covering mucus. Although teprenone significantly prevented the increase in gastric Na+ concentration, it significantly enhanced the decrease in gastric H+ concentration.

Effects of drugs on the amount of gastric covering mucus and gastric H+ and Na+ concentration in pylorus-ligated rats

As shown in Fig. 3, KB-5492 and dmPGE2, administered intraduodenally at 200 and 0.01 mg/kg, respectively, did not affect the amount of gastric covering mucus or gastric H+ and Na+ concentration. Teprenone at 200 mg/kg, which did not affect the amount of gastric covering mucus or gastric Na+ concentration, significantly decreased the gastric H+ concentration.

Histological appearance of gastric mucosa

Figures 4 and 5 show the typical histological appearances of the gastric mucosa 30 min after the oral administration of aspirin at 200 mg/kg. Marked reduction of PAS-positive epithelial mucus and the exfoliation of surface epithelial cells were observed in the gastric mucosa exposed to aspirin. KB-5492 and dmPGE2, administered intraduodenally at 200 and 0.01 mg/kg, respectively, almost completely prevented the reduction of PAS-positive epithelial mucus, whereas both drugs prevented the exfoliation of surface epithelial cells incompletely. In contrast, teprenone at 200 mg/kg neither prevented the reduction of PAS-positive epithelial mucus nor the exfoliation of surface epithelial cells.

DISCUSSION

In the present study, KB-5492 prevented the aspirin-induced decrease in PD and accelerated the recovery of PD following the removal of aspirin. The recovery of PD after damage has been reported to closely parallel the restitution process both in vitro and in vivo (9–11). Furthermore, the significant correlation between the histologic data and PD data supports the hypothesis that PD recovery is a good index of the recovery of epithelial integrity. In addition, KB-5492 prevented the aspirin-induced reduction of gastric covering mucus, decrease in gastric H+ concentration and increase in Na+ concentration. These effects of KB-5492 were similar to those of dmPGE2. These findings indicate that KB-5492 as well as dmPGE2 protects the gastric mucosal barrier and prevents the back diffusion of gastric H+ by retaining the gastric covering mucus.

Murakami et al. (12) demonstrated that teprenone, an anti-ulcer agent known to enhance gastric mucosal defensive factors, prevented the aspirin-induced gastric mucosal lesions in pylorus-ligated rats at 100 and 200 mg/kg, i.d. Terano et al. (13) have reported that intravenous teprenone (200 and 400 mg/kg) prevented the aspirin-induced decrease in PD in rats. However, in the present study, teprenone did not affect the aspirin-induced decrease in PD even at the anti-ulcer dose of 200 mg/kg, i.d. (12). Since teprenone was administered i.d. at 200 mg/kg in our study, it may be difficult to detect its preventive effect, if any, on the decrease in PD. However, teprenone is assumed to prevent the decrease in PD only at a higher dose than its anti-ulcer dose.

Teprenone also did not affect the aspirin-induced reduction of the gastric covering mucus, which supports the finding obtained by Oketani et al. (14) that teprenone (300 mg/kg, i.d.) did not prevent the aspirin-induced reduction of gastric glycoproteins in the surface mucous layer. Although teprenone prevented the aspirin-induced
Fig. 4. Histological appearance of rat gastric mucosa 30 min after the oral administration of aspirin (200 mg/kg). A: normal, B: vehicle (1% gum arabic) + aspirin, C: KB-5492 (200 mg/kg, i.d.) + aspirin, D: 16,16-dimethyl prostaglandin E₂ (0.01 mg/kg, i.d.) + aspirin, E: teprenone (200 mg/kg, i.d.) + aspirin. Periodic acid-Schiff staining (×100).

Fig. 5. Histological appearance of rat gastric mucosa 30 min after the oral administration of aspirin (200 mg/kg). The exfoliation of surface epithelial cells is indicated by arrows. A: normal, B: vehicle (1% gum arabic) + aspirin, C: KB-5492 (200 mg/kg, i.d.) + aspirin, D: 16,16-dimethyl prostaglandin E₂ (0.01 mg/kg, i.d.) + aspirin, E: teprenone (200 mg/kg, i.d.) + aspirin. Hematoxylin and eosin staining (×200).
increase in gastric Na⁺ concentration, it enhanced the decrease in gastric H⁺ concentration. This is partly inconsistent with the findings obtained by Murakami et al. (12) that teprenone (200 mg/kg, i.d.) prevented both the aspirin-induced decrease in gastric H⁺ concentration and increase in gastric Na⁺ concentration. However, they also reported that teprenone at doses higher than 50 mg/kg, i.d. inhibited gastric acid secretion in rats (15), which was confirmed in our previous study (4). In addition, the present finding demonstrated that teprenone significantly decreased the gastric H⁺ concentration in pylorus-ligated rats. These findings suggested that enhancement of the aspirin-induced decrease in gastric H⁺ concentration by teprenone may have resulted from its inhibitory effect on gastric acid secretion and not from aggravation of the damage to the gastric mucosal barrier. Indeed, teprenone did not enhance the aspirin-induced decrease in PD.

The detailed mechanism by which KB-5492 prevented aspirin-induced reduction of gastric covering mucus remains to be clarified. KB-5492 does not seem to enhance the production or secretion of gastric mucus by itself, because it did not affect the amount of gastric covering mucus in pylorus-ligated rats. Sarosiek et al. (16) have reported that aspirin enhanced the proteolytic activity of pepsin on mucus glycoprotein and reduced the gastric mucus viscosity. They also showed that dmPGE₂ markedly increased the viscosity of glycoprotein derived from the gastric mucus in vitro (17). Therefore, KB-5492 may retain the gastric covering mucus by directly or indirectly increasing the viscosity in the same manner as dmPGE₂. In the present study, dmPGE₂ did not affect the amount of gastric covering mucus in pylorus-ligated rats. On the other hand, Bickel and Kaufman (18) and McQueen et al. (19) have reported that dmPGE₂ increased the thickness of the gastric surface mucous layer in normal rats. This discrepancy may be due to the different method for the measurement of the amount of gastric covering mucus. Further studies are required to clarify the exact effects of KB-5492 on the gastric surface mucous layer.

KB-5492 as well as dmPGE₂ prevented the aspirin-induced decrease in PD and back diffusion of gastric H⁺ incompletely. In the histological study, both drugs almost completely prevented the aspirin-induced reduction of PAS-positive epithelial mucus, but their preventive effects on the exfoliation of surface epithelial cells were incomplete. This is consistent with our finding that both preventive effects of KB-5492 and dmPGE₂ on the exfoliation of surface epithelial cells induced by acidified aspirin were incomplete (20). Surface epithelial cells are indispensable for the gastric mucosal barrier to secrete HCO₃⁻ into the surface mucous layer. Therefore, the incomplete prevention by KB-5492 and dmPGE₂ of aspirin-induced decrease in PD and back diffusion of gastric H⁺ may be due to their incomplete protection of the surface epithelial cells.

Recently, sigma-receptors have been reported to exist in many organs such as the brain (21), liver (22) and spleen (23). Furthermore, sigma receptors are abundant in the mucosal and submucosal plexus of the gastric fundus and duodenal in guinea pigs and humans (24, 25). The sigma-receptor ligand 1,3-di(2-tolyl) guanidine (DTG) stimulates duodenal alkaline secretion and shows protective effects on various gastric lesions in rats through a sigma-receptor (26, 27). Thus, sigma-receptors may play an important role in the control of mucosal function. KB-5492 was found to have a selective affinity for the sigma-receptors in guinea pig brain and porcine fundic mucosal membranes (Y. Harada et al., unpublished data). Furthermore, protective effects of KB-5492 against ethanol- and water-immersion stress-induced gastric mucosal lesions in rats and stimulating effect on gastric alkaline secretion in rats were antagonized by haloperidol, but not by sulpiride, a dopamine D₂-receptor antagonist (5). Future studies to provide additional evidence on the functional role of KB-5492, a sigma-receptor ligand, in the gastric mucosal barrier are necessary.

In conclusion, KB-5492 protects the gastric mucosal barrier against the disruption by aspirin, which may be mainly exerted by retaining the gastric covering mucus. The protection of the gastric mucosal barrier is considered to play an important part in the mechanism by which KB-5492 enhances gastric mucosal defensive factors.

Acknowledgments

The authors are grateful to Miss Y. Sakita and Miss K. Tanaka for their technical assistance.

REFERENCES

1 Allen, A. and Garner, A.: Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. Gut 21, 249–262 (1980)
2 Garner, A., Allen, A. and Rowe, P.H.: Gastroduodenal mucosal defense mechanisms and the action of non-steroidal anti-inflammatory agents. Scand. J. Gastroenterol. 22, Supp. 127, 29–34 (1987)
3 Shimohara, K., Niida, H. and Okabe, S.: Effects of KB-5492, 1-(3,4,5-trimethoxybenzyl)-4-((4-methoxyphenyl)oxycarbonylmethyl)piperazine monofumarate monohydrate, on gastric lesions and gastric secretion in rats. Jpn. J. Pharmacol. 53, 275–279 (1990)
4 Morimoto, Y., Shimohara, K., Oshima, S. and Sukamoto, T.: Effects of the new anti-ulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cinetidine. Jpn. J. Pharmacol. 57, 495–505 (1991)
5 Hara, H., Tanaka, K., Harada, Y. and Sukamoto, T.: Effect of
KB-5492, a new selective sigma ligand, on gastric lesions and alkaline secretion in rats. Jpn. J. Pharmacol. 61, Supp. I, 194P (1993)

6 Nagashima, R., Hoshino, E., Hinohara, Y., Sakai, K., Hata, S. and Nakano, H.: Effect of sucralfate on ethanol-induced gastric mucosal damage in the rat. Scand. J. Gastroenterol. 18, Supp. 83, 17 – 20 (1983)

7 Corne, S.J., Morrissey, S.M. and Woods, R.J.: A method for the quantitative estimation of gastric barrier mucus. J. Physiol. (Lond.) 242, 116P – 117P (1974)

8 Kitagawa, H., Takeda, F. and Kohei, H.: A simple method for estimation of gastric mucus and effects of antiulcerogenic agents on the decrease in mucus during water-immersion stress in rats. Arzneimittelforschung 36, 1240 – 1244 (1986)

9 Morris, G.P. and Wallace, J.L.: The roles of ethanol and of acid in the production of gastric mucosal erosions in rats. Virchows Arch. [Cell Pathol.] 38, 23 – 38 (1981)

10 Rutten, M.J. and Ito, S.: Morphology and electrophysiology of guinea pig gastric mucosal repair in vitro. Am. J. Physiol. 244, G171 – G182 (1983)

11 Wallace, J.L., Morris, G.P., Krausse, E.J. and Greaves, S.E.: Reduction by cytoprotective agents of ethanol-induced damage to the rat gastric mucosa: a correlated morphological and physiological study. Can. J. Physiol. Pharmacol. 60, 1686 – 1699 (1982)

12 Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., Ohgo, T. and Okabe, S.: Effects of the antiulcer drug geranylgeranylacetone on aspirin-induced gastric ulcers in rats. Jpn. J. Pharmacol. 32, 299 – 306 (1982)

13 Terano, A., Matsumoto, K., Motoki, T., Murao, S. and Kamii, K.: Effect of prostaglandin E2, cimetidine and geranyl-geranylacetone on rat gastric mucosal damage. Jpn. J. Gastroenterol. 78, 1577 – 1584 (1981) (Abs. in English)

14 Oketani, K., Murakami, M., Fujisaki, H., Wakabayashi, T. and Hotta, K.: Effect of geranylgeranylacetone on aspirin-induced changes in gastric glycoproteins. Jpn. J. Pharmacol. 33, 593 – 601 (1983)

15 Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T. and Ohgo, T.: Antiulcer effect of geranylgeranylacetone, a new acyclic polyisoprenoid on experimentally induced gastric and duodenal ulcers in rats. Arzneimittelforschung 31, 799 – 804 (1981)

16 Sarosiek, J., Mizuta, K., Slomiany, A. and Slomiany, B.L.: Effect of acetylsalicylic acid on gastric mucin viscosity, permeability to hydrogen ion, and susceptibility to pepsin. Biochem. Pharmacol. 35, 4291 – 4295 (1986)

17 Sarosiek, J., Murty, V.L.N., Nadziejko, C., Slomiany, A. and Slomiany, B.L.: Prostaglandin effect on the physical properties of gastric mucus glycoprotein and its susceptibility to pepsin. Prostaglandins 32, 635 – 646 (1986)

18 Bickel, M. and Kauffman, G.L.: Gastric mucus thickness: Effect of distention, 16.16-dimethyl prostaglandin E3, and carbinoxolone. Gastroenterology 80, 770 – 775 (1981)

19 McQueen, S., Hutton, D., Allen, A. and Garner, A.: Gastric and duodenal surface mucus gel thickness in rats: effects of prostaglandins and damaging agents. Am. J. Physiol. 245, G388 – G393 (1983)

20 Morimoto, Y., Oshima, S., Hara, H. and Sukamoto, T.: Effects of KB-5492, a new anti-ulcer agent, on ethanol- and acidified aspirin-induced gastric mucosal damage in vivo and in vitro. Jpn. J. Pharmacol. 64, 41 – 47 (1994)

21 Weber, E., Sonders, M., Quarum, M., McLean, S., Pou, S. and Keana, J.F.W.: 1,3-Di(2-[5-3H]tolyl)guanidine: a selective ligand that labels σ-type receptors for psychotomimetic opiates and antipsychotic drugs. Proc. Natl. Acad. Sci. USA. 83, 8784 – 8788 (1986)

22 Samovilova, N.N., Nagornaya, I.V. and Vinogradov, V.A.: (+)[3H]SKF 10,047 binding sites in rat liver. Eur. J. Pharmacol. 147, 259 – 264 (1988)

23 Su, T.-P., Schell, S.E., Ford-Rice, F.Y. and London, E.D.: Correlation of inhibitory potencies of putative antagonists for μ receptors in brain and spleen. Eur. J. Pharmacol. 148, 467 – 470 (1988)

24 Roman, F.J., Pascaud, X., Chomette, G., Bueno, L. and Junien, J.L.: Autoradiographic localization of sigma opioid receptors in the gastrointestinal tract of the guinea pig. Gastroenterology 97, 76 – 82 (1989)

25 Roman, F.J., Pascaud, X., Salmon, R., Chomette, G. and Junien, J.L.: Localization and characterization of sigma receptors in the human gastrointestinal tract (abstract). Gastroenterology 100, A662 (1991)

26 Pascaud, X., Defaux, J.P., Roze, C. and Junien, J.L.: Effect of selective sigma ligands on duodenal alkaline secretion in the rat. J. Pharmacol. Exp. Ther. 255, 1354 – 1359 (1990)

27 Pascaud, X.B., Chomet, M., Soulard, P., Chevalier, E., Roze, C. and Junien, J.L.: Effects of a new σ ligand, JO 1784, on cytochrome ulcers and duodenal alkaline secretion in rats. Gastroenterology 104, 427 – 434 (1993)