J. Pharm. Pharmacol. 1988, 40: 873–875
Communicated February 1, 1988



# Orally administered κ but not μ opiate agonists enhance gastric emptying of a solid canned food meal in dogs

M. GUÉ, J. FIORAMONTI, J. L. JUNIEN*, L. BUENO, *Department of Pharmacology INRA, 180 chemin de Tournefeuille, 31300 Toulouse, France, *Jouveinal Laboratoires, 1 rue des Moissons, 94260 Fresnes, France*

**Abstract**—The effects of oral administration of selective mu (D-Ala$^2$, *N*-Me-*p*-nitro-Phe$^4$, Gly$^5$-ol-DAGO, morphine) and/or kappa (3,4 dichloro-N-methyl N [2-(1. fyrrolidinyl) cyclohexyl]-benzene acetamide-U-50488, tifluadom) or mixed agonist (*N*-desmethyltrimebutine) opioid on gastric emptying have been evaluated using a radiolabelled [$^{57}$Co] canned food meal in dogs fitted with gastric cannulas. In control conditions (placebo) the percentage of solids emptied 1 h after feeding was 27·3 ± 4·1%. When given orally at doses of 0·01 to 0·5 mg kg$^{-1}$, U-50488 increased significantly ($P < 0·05$) by 29·1 to 60·8% in a dose-related manner (r = 0·94, $P < 0·01$) the amount of gastric emptying of the meal in 1 h. This effect was reproduced by oral administration of tifluadom (0·01 to 0·1 mg kg$^{-1}$) and by *N*-desmethyltrimebutine (0·1 to 1 mg kg$^{-1}$). In contrast, the gastric emptying was unaffected by DAGO and morphine at low doses (0·01 and 0·1 mg kg$^{-1}$) but significantly ($P < 0·05$) slowed with higher doses of morphine. The increases in amount of gastric emptying induced by tifluadom, U-50488 and *N*-desmethyltrimebutine were abolished by previous administration of naloxone (0·1 mg kg$^{-1}$ i.v.) and [(3-furylmethyl) noretazocine]-MR 22-66 (0·1 mg kg$^{-1}$ i.v.). These results indicate that orally administered kappa, but not mu agonists at doses not exceeding 1 mg kg$^{-1}$ enhance the amount of gastric emptying of a solid meal in dogs and suggest that this is due to a selective local stimulation of kappa mucosal or submucosal opiate receptors at antroduodenal level.

Morphine and its derivatives, as well as endogenous opiates, are considered to delay gastric emptying of both liquids and solids when systemically administered to dog and man (Sullivan et al 1981; Shea-Donohue et al 1983; Kostritsky-Pereira et al 1984; Mittal et al 1986). Consequently, although delta, mu and kappa agonists have different effects peripherally and centrally, on both gastric and intestinal motility (Ruckebusch et al 1984; Gué et al 1988a) the final result of their systemic administration seems to lead to a reduction in the extent of gastric emptying. Furthermore, the physiological involvement of those endogenous opiates in the regulation of gastric emptying is suggested by the tendency of naloxone to increase the amount of gastric evacuation of a solid meal in man (Mittal et al 1986).

However, the experiments were conducted mainly in man using test meals (Sullivan et al 1981; Mittal et al 1986) and intravenous administration of opiates. Moreover, histological studies in dogs revealed the presence of nerve cells containing enkephalin and dynorphin peptide immunoreactive materials in the submucosal plexus, muscularis mucosae and villi of the stomach, pylorus and proximal intestine (Daniel et al 1987). These observations led us to speculate that opiate efferent sensitive fibres located at the gastric and duodenal levels may influence the gastric emptying as well as the motor pattern (Gué et al 1988a).

Consequently, the present experiments were made to evaluate the influence of orally administered mu and kappa agonists on the gastric emptying of the solid phase of a spontaneously eaten radiolabelled [$^{57}$Co] normal meal in dogs.

## Materials and methods

Six female adult mongrel dogs 12–16 kg, were used. Under halothane (Fluothane) anaesthesia, a Thomas cannula was placed in the great curvature of the gastric body at about 10 cm from the pylorus, it was passed through and fixed to the left abdominal wall at 5 cm from the last rib and 10 cm from the midline. Animals were allowed 2 weeks to recover before tests were made.

Correspondence to: L. Bueno, Department of Pharmacology INRA, 180 chemin de Tournefeuille, 31300 Toulouse, France.

The meal presented to them consisted of 400 g of canned food (Fidèle, Quaker France) containing 21·7% dry matter, 7·7% protein, 4·5% fat, 6·9% carbohydrates and 2·6% of minerals. Ovine liver (20 g) labelled with [$^{1}$Co] cyanocobalamin (Amersham, specific activity 10 μCi μg$^{-1}$) was added and mixed to the canned food (solid phase) with 100 mL of tap water. Then the meal was presented to the animals for spontaneous eating which did not exceed 2–4 min.

One hour later the gastric contents were collected, weighed, and the volume measured. They were then blended to a liquid consistency.

Five samples (4–5 mL) were rapidly taken, precisely weighed and introduced into a gamma counter (MR252, Kontron, Switzerland) for counting $^{57}$Co.

In a first series of experiments, 20 min before the test meal the animals received, morphine (0·01, 0·1, 1 mg kg$^{-1}$) (D-Ala$^2$, *N*-Me-*p*-nitro-Phe$^4$, Gly$^5$-ol) enkephalin-DAGO (0·01, 0·1 mg kg), U-50488 (0·01, 0·1 and 0·5 mg kg$^{-1}$) and tifluadom (0·01, 0·1 mg kg$^{-1}$) given in a randomized order, twice to each dog. Experiments were performed on each dog at three day intervals.

In a second series of experiments, the oral administration of U-50488 (0·1 mg kg$^{-1}$) and tifluadom (0·1 mg kg$^{-1}$) was preceded (10 min) by the intravenous injection of nalaxone (0·1 mg kg$^{-1}$) or MR 22-66 (0·1 mg kg$^{-1}$).

In a further series of experiments, *N*-desmethyltrimebutine an active metabolite of trimebutine in man was also administered orally at dose of 0·1 to 5 mg kg$^{-1}$, 20 min before the test meal, with or without prior intravenous administration of naloxone or MR 22-66 (0·1 mg kg$^{-1}$). Gastric emptying of solids was measured 1 h after feeding and experiments were performed in duplicate on each dog. The dogs were allowed to recover for 7 days between consecutive series of experiments. Gastric emptying of the solid phase was expressed in per cent of meal (canned food) emptied as previously described (Gué et al 1988b). Because of the small number of dogs (n = 6), statistical analysis of the results was made using a Wilcoxon matched-pairs signed rank test and differences were considered significant for $P \leqslant 0·05$.

## Results

*Influence of oral administration of kappa (U-50488, tifluadom) vs mu (morphine, DAGO) agonists.* When measured 1 h after feeding under control (placebo) conditions, the gastric emptying of solids represented 27·3 ± 4·1% (mean ± s.d., n = 12) of the initial weight of the solid phase (Fig. 1).

When given orally at doses of 10 to 500 μg kg$^{-1}$, U-50488 increased significantly ($P < 0·01$) in a dose-related manner (r = 0·94, $P < 0·05$) the percentage of solid meal emptied after 1 h. This effect was reproduced by oral administration of tifluadom (10 and 100 μg kg$^{-1}$). Neither morphine nor DAGO given orally at doses of 10 and 100 μg kg$^{-1}$ affected the gastric emptying of the solid phase of a meal; however, at a higher dose (1 mg kg$^{-1}$) morphine induced a significant ($P < 0·05$) reduction in the amount emptied in 1 h (10·7 ± 9·2% of the test meal vs 27·3 ± 4·1% in controls (Fig. 1, Table 1).

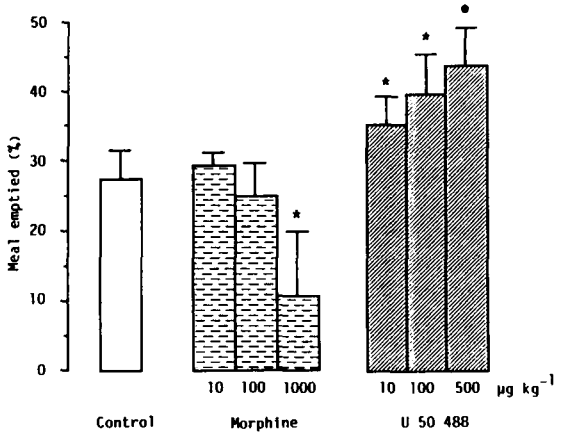


FIG. 1. Comparative effects of mu (morphine) and kappa (U-50488) opioid agonists orally administered on the 1 h values of gastric emptying of canned food meal in dogs (mean ± s.d., n = 6 dogs; *: significantly different from control at $P < 0.05$, W-test).

Table 1. Comparative effects of orally administered U-50488, tifluadom, morphine and DAGO on gastric emptying of a solid regular canned food meal in dogs (mean ± s.d. n = 6 dogs).

| | Dose mg kg$^{-1}$ | Gastric emptying(a) (%) |
|---|---|---|
| Placebo | — | 27·3 ± 4·1 |
| Tifluadom | 0·01 | 36·8 ± 5·1* |
| | 0·1 | 42·8 ± 6·8* |
| U 50488 | 0·01 | 35·2 ± 4·1 |
| | 0·1 | 39·6 ± 6·0* |
| Morphine | 0·01 | 29·4 ± 1·9 |
| | 0·1 | 25·0 ± 4·6 |
| | 1 | 10·7 ± 9·2* |
| DAGO | 0·01 | 31·6 ± 6·6 |
| | 0·1 | 25·4 ± 3·9 |

(a) Gastric emptying was expressed in percent of meal emptied 1 h after feeding
(*) Significantly different ($P < 0.05$) from placebo values, W-test

*Influence of naloxone and MR 22-66.* Naloxone and MR 22-66 (0·1 mg kg$^{-1}$ i.v.), given 40 min before the meal had no effect themselves on the 1 h values for gastric emptying, but when injected 10 min before U-50488 or tifluadom (0·1 mg kg$^{-1}$ p.o.), both naloxone and MR 22-66 abolished the stimulatory effect of those drugs on the evacuation of the meal (Fig. 2).

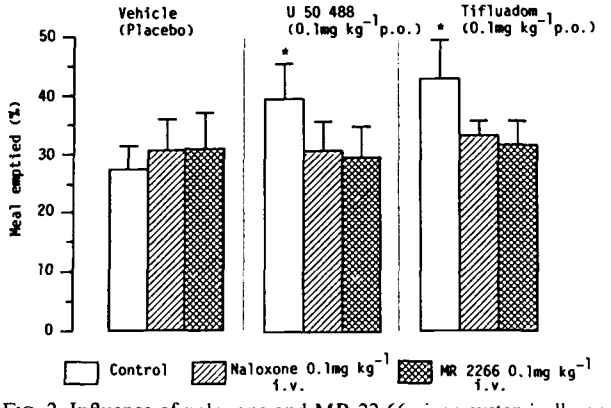


FIG. 2. Influence of naloxone and MR 22-66 given systemically on the stimulation of gastric emptying induced by oral administration of U 50488 and tifluadom (*significantly different from control values at $P < 0.05$, W-test).

*Effects of* N-*desmethyltrimebutine.* When administered orally at doses from 0·1 to 5 mg kg$^{-1}$, 20 min before the meal, *N*-desmethyltrimebutine significantly increased the percentage of the meal emptied at 1 h but the dose response curve presented a maximum of 0·25 mg kg$^{-1}$ (Table 2). Both naloxone and MR 22-66 (0·1 mg kg$^{-1}$ i.v.) abolished the stimulatory effects of *N*-desmethyltrimebutine (0·25 mg kg$^{-1}$) on gastric emptying (Fig. 3).

Table 2. Influence of increasing oral doses of *N*-desmethyltrimebutine on the 1 h gastric emptying of canned food meal in dogs (mean ± s.d., n = 12).

| | Dose mg kg$^{-1}$ | Gastric emptying at 1 h(1%) |
|---|---|---|
| Control (placebo) | 0 | 28·4 ± 1·1 |
| *N*-DesmethyTMB | 0·1 | 48·7 ± 6·4* |
| | 0·25 | 53·7 ± 9·4* |
| | 0·5 | 46·2 ± 5·3* |
| | 1 | 44·0 ± 4·1* |
| | 5 | 37·3 ± 5·3 |

(*) Significantly different from control at $P < 0.05$, W-test

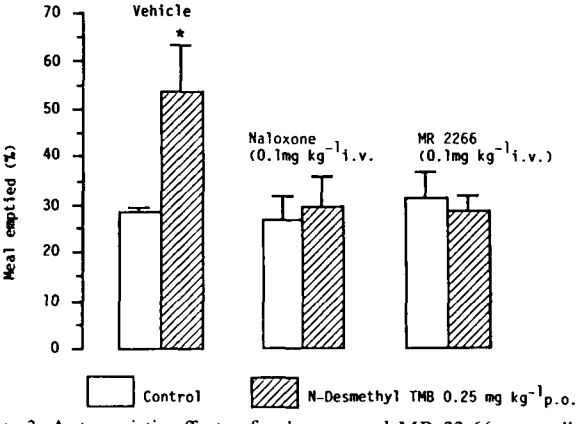


FIG. 3. Antagonistic effects of naloxone and MR 22-66 on orally given *N*-desmethyltrimebutine (TMB) induced-increase of gastric emptying in dogs (mean ± s.d., n = 6 dogs *: $P < 0.05$, W-test).

## Discussion

In agreement with literature showing that parenteral administration of morphine and its derivatives (Silbiger et al 1968; Kostritsky-Pereira et al 1984; Mittal et al 1986) as well as enkephalins and their analogues (Sullivan et al 1981; Shea-Donohue et al 1983; Kostritsky-Pereira et al 1984) reduce gastric emptying in dogs and man, the present experiments indicate that morphine, at a high dosage by mouth, also reduces the extent of emptying of a spontaneously eaten solid meal in dogs. The present work also establishes that opiate agonists like U-50488 and tifluadom, administered orally are able to increase the gastric evacuation of a solid canned food meal. The active doses of these opioid agonists given orally are 10 times lower than those required to affect gastrointestinal motility or transit by the systemic route (Porreca et al 1984; Ruckebusch et al 1984), suggesting that they act locally to enhance gastric emptying in dogs.

In agreement with this hypothesis, opiate receptors of different classes are present in the stomach and intestinal wall of the dog, a species presenting nerve cells containing enkephalin-and dynorphin-immunoreactive materials in the submucosal plexus, muscularis mucosae and villi of the stomach, pylorus and proximal intestine (Daniel et al 1987). Consequently, we postulate that opioid agonists of different classes may affect the gastric motor activity differently and in turn the emptying rate via local sites of action.

Tifluadom, a benzodiazepine with high affinity for kappa receptors (Roemer et al 1982), and U-50488, considered as a

highly potent and selective kappa agonist (Wood 1984), have the same stimulating effects on the gastric evacuation of solids. Blockade of those effects by both naloxone, a non-selective opiate antagonist, and MR 22-66 a selective kappa antagonist (Magnan et al 1982) strongly suggests that response is due to a stimulation of kappa receptors. Such stimulatory effects of kappa agonists on the gastric emptying of solids may be related to an enhancing action on post-prandial motility of the distal stomach (Ruckebusch et al 1984).

Trimebutine (2-dimethylamineo-2 phenylbutyl-3,4,5-trimethoxybenzoate hydrogen maleate) and its major metabolite *N*-desmethyltrimebutine possess a high affinity for mu and kappa peripheral and central receptors (Pascaud & Roman 1987). They induce peripheral opiate effects on gastrointestinal and colonic motility when administered systemically to dogs (Fioramonti et al 1984; Poitras et al 1986) and man (Frexinos et al 1985; Chaussade et al 1985). The present experiments demonstrate that, *N*-desmethyltrimebutine, orally administered at low doses stimulates gastric emptying probably by acting on kappa receptors since, as with U-50488 and tifluadom, its action is antagonized by MR 22-66. However, the lack of a linear dose-response relation associated with the presence of a bell-shape curve may be explained by the mixed mu and kappa properties of this drug. Consequently, at low dose *N*-desmethyltrimebutine may exhibit preferential kappa agonist activity contrasting with a predominant mu activity at higher dosages.

A local site of action has also been previously suspected for other opiate drugs such as loperamide (Altaparmakov & Wienbeck 1984). Given intraduodenally, loperamide stimulates jejunal and colonic contractions in the calf while opposite effects are observed for the same dose given subcutaneously (Fioramonti & Bueno 1987). Furthermore, agreement with the hypothesis of a local effect of kappa agonists, it has been shown in-vitro that such kappa agonists affect the 5-HT release in the myenteric plexus only in the presence of the submucosal layer (Cherubini & North 1985) and kappa receptor activation at submucosal level inhibits calcium conductance and depresses neurotransmitter release favouring smooth muscle relaxation (Werz & MacDonald 1984).

Finally the present work establishes that drugs with selective or predominantly kappa affinity, but not mu selective affinity, when given orally increase the extent of gastric emptying of a solid meal in dogs probably by acting locally on mucosal or submucosal opiate receptors at the gastric and(or) duodenal levels.

The authors are indebted to Mrs G. Bories and C. Delrio for their helpful technical assistance, Mrs C. Santamaria for typing the manuscript.

## References

Altaparmakov, I., Wienbeck, M. (1984) Local inhibition of myoelectrical activity of human colon by loperamide. Dis. Sci. 29: 232–238

Chaussade, S., Grandjouan, S., Couturier, D., Thierman-Duffaut, D. (1985) Trimebutine induces activity fronts of the interdigestive migrating motor complex in human jejunum. Ibid. 30: 762 p

Cherubini, E., North, R. A. (1985) $\mu$ and $\kappa$ opioids inhibit transmitter release by different mechanisms. Poc. Natn. Acad. Sci. USA. 82: 1860–1863

Daniel, E. E., Fox, J. E. T., Allescher, H. D., Ahamd, S., Kostalanska, F. (1987) Peripheral actions of opiates in canine gastrointestinal tract: actions on nerves and muscles. Gastroenterol. Clin. Biol. 11: 35–39

Fioramonti, J., Buéno, L. (1987) Effects of loperamide hydrochloride on experimental diarrhea and gastrointestinal myoelectrical activity in calves. Am. J. Vet. Res. 48: 415–419

Fioramonti, J., Fargeas, M. J., Buéno, L. (1984) The involvement of opiate receptors in the effects of trimebutine on intestinal motility in the conscious dog. J. Pharm. Pharmacol. 36: 618–621

Frexinos, J., Fioramonti, J., Buéno, L. (1985) Effect of trimebutine on colonic myoelectrical activity in IBS patients. Eur. J. Clin. Pharmacol. 28: 181–185

Gué, M., Fioramonti, J. Buéno, L. (1988a) A simple double radiolabeled technique to evaluate gastric emptying of canned food meal in dogs: Application to pharmacological tests. Gastroenterol. Clin. Biol. 12: 425–430

Gué, M., Pascaud, X., Hondé, C., Junien, J. L., Buéno, L. (1988b) Peripheral antagonistic action of trimebutine and kappa opioid substances on acoustic stress induced motor inhibition in dogs. Eur. J. Pharmacol. 146: 57–63

Kostritsky-Pereira, A., Wonssen-Coulle, M. C., DeGraef, J. (1984) Effects of morphine, enkephalins and naloxone on postprandial gastric acid secretion, gastric emptying and gastrin release in dogs. Arch. Int. Physiol. Biochim. 92: 19–26

Magnan, J. Paterson, S. J., Tavini, A., Kosterlitz, H. W. (1982) The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. Naunyn-Schmiedeberg's. Arch. Pharmacol. 319: 197–205

Mittal, R. K., Frank, E. B., Lange, R. C., McCallum, R. W. (1986) Effects of Metenkephalin and naloxone on esophageal motility and gastric emptying in man. Dig. Dis. Sci. 31: 936–942

Pascaud, X., Roman, F. (1987) Participation des récepteurs opioïdes dans le mode d'action de la trimébutine. Gastroenterol. Clin. Biol. 11: 77–81

Poitras, P., Hondé, C., Havrankowa, J., Lahaie, R. G., Trudel, L., Boyer, R., Junien, J. L., Pascaud X. (1986) Effect of trimebutine on intestinal motility and plasma motilin in the dog. Am. J. Physiol. 251: 349–356

Porreca, F., Mosberg, H. I., Hurst, R., Hruby, V. J., Burks, T. F. (1984) Role of mu, delta and kappa opioid recepts in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. J. Pharmacol. Exp. Ther. 230: 341–348

Roemer, K. N., Buscher, H. H., Hill, R. C., Maurer, R., Petcher, T. J., Zeugner, H., Bendon, W., Finner, E., Milkowski, W., Thies, P. W. (1982) An opioid benzodiazepine. Nature (Lond.) 298: 759–760

Ruckebusch, Y., Bardon, T., Pairet, M. (1984) Opioid control of the ruminant stomach motility: functional importance of mu, kappa and sigma receptors. Life Sci. 35: 1731–1738

Shea-Donohue, P. T., Adams, N., Arnold, J., Dubois, A. (1983) Effects of MetEnkephalin and naloxone on gastric emptying and secretion in rhesus monkeys. Am. J. Physiol. 245: G196–G200

Silbiger, M. L., Donner, M. W. (1968) Morphine in the evaluation of gastrointestinal disease. Radiology 90: 1086–1090

Sullivan, S. N., Lamki, L., Corcoran, P. (1981) Inhibition of gastric emptying by enkephalin analogue. Lancet 11: 86–87

Werz, M. A., MacDonald, R. L. (1984) Dynorphin reduces calcium-dependent action potentials by decreasing voltage-dependent calcium conductance. Neurosci. Lett. 46: 85–190

Wood, P. L. (1984) Kappa agonist analgesics: evidence for $mu_2$ and delta opioid receptor antagonism. Drug Develop. Res. 4: 429–432