J. Pharm. Pharmacol. 1984, 36: 618–621
Communicated November 18, 1983

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The involvement of opiate receptors in the effects of trimebutine on intestinal motility in the conscious dog

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The effects of intravenous (i.v.) vs intracerebroventricular (i.c.v.) administration of trimebutine on the motility of the small intestine and colon of fasted dogs were assessed using chronic electromyography. Trimebutine, injected intravenously, stimulated small intestinal motility, by inducing a propagated phase of regular spike activity, and inhibited colonic motility for some 4 h. These effects were not reproduced by i.c.v. administration which disrupted the cyclic motor profile of the small intestine for about 2·5 h and did not modify colonic motility. The stimulation of the small intestine motility induced by i.v. administration of the drug was blocked by previous i.v. but not by i.c.v. administration of naloxone. It was concluded that in the dog, the effects of trimebutine on the small intestine but not on the colon, involve peripheral opiate receptors.

Trimebutine (2-dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate hydrogen maleate) is used in the treatment of gastrointestinal disorders including gastritis, abdominal pain, dyspepsia, nausea, emesis, irritable bowel syndrome and post-operative ileus. Despite its use in disorders including disturbances of gastrointestinal motility, its effects on digestive motility have not been established. However, the efficacy of trimebutine in the spastic colon (Luttecke 1978; Moshal 1979) supposes an inhibitory effect on digestive motility while the more rapid recovery of the digestive transit time after abdominal surgery (Malavaud 1972) supports a stimulatory effect. These opposite effects may be due to a stimulation of the small intestine motility associated with inhibition of colonic motility. The aim of the present study was to investigate the effects of trimebutine on both small intestine and colonic motility using chronic electromyographic methods in the conscious dog, a species with a well defined pattern of intestinal and colonic motility (Szurszewski 1969; Fioramonti et al 1980).

Since trimebutine is supposed to stimulate intestinal motility in the postoperative period, we have compared the effects of trimebutine to those of morphine which stimulates small intestinal motility in the conscious dog (Sarna et al 1982) as well as after abdominal surgery in man (Ingram & Catchpole 1981). Some effects of opiates on intestinal motility have been found to be centrally mediated in various species (Bueno & Ruckebusch 1978; Stewart et al 1977) including the dog (Weisbrodt et al 1982). Consequently the present work was performed (i) to evaluate in dog the central vs peripheral effects of trimebutine (ii) to see if these effects are mediated through opiate receptors.

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Material and methods

Animals. Four female mongrel dogs, 10–15 kg were housed in individual cages and received a daily meal of 500 g canned food. After an overnight fast, pairs of electrodes made of insulated nichrome wire were implanted under halothane anaesthesia along the small intestine and the large bowel. Electrodes were implanted on the duodenum and the jejunum at 10, 30, 50 and 70 cm from the pylorus and on the transverse colon at 10 cm from the ileo-colonic junction. In addition, a small polyethylene cannula was inserted into the right ventricle of the brain.

Electromyographic recordings. Recordings were started 6–8 days after surgery and the electrical activity was recorded from 8.00 am to 7.00 pm each day on an electroencephalograph machine (Reega XII, Alvar, Paris). At 20 s intervals, concurrent summation of spike activity of the duodenum and the transverse colon during consecutive 20 s periods was obtained continuously by a linear integrator circuit (Latour 1973) connected to a potentiometric recorder with a paper speed of 5 cm h^{-1}. The temporal distribution of spike activity was monitored directly from the integrated record. For a phase of irregular spike activity the bursts of spike potentials were superimposed on 20–70% of the slow waves in a random fashion so that the deflection (μ coulomb) on the axis varied from 20–70% of the maximal deflection observed for the phases of regular spike activity.

Experimental procedure. In a first series of experiments, trimebutine maleate (Debridat ND, Jouveinal Laboratoires, Fresnes, France) was injected in each animal three times intravenously (i.v.) at a dose of 5 mg kg^{-1} and intracerebroventricularly (i.c.v.) at a dose of 0·1 mg kg^{-1}. Injections were performed in a randomized order at 2–3 days intervals, 15 to 18 h after the last daily meal and 30 min after the occurrence of a phase of regular spike activity in the duodenum.

In a second series of experiments, trimebutine was injected at a dose of 5 mg kg^{-1} i.v. under the same conditions but 20 min after i.v. or i.c.v. administration of naloxone (Endo Laboratories Garden City, New

York) at a dose of 0·1 mg kg^{-1} in a randomized order. All injections were repeated 3 times in each animal.

Results

Control studies. The fundamental motor profile of the small intestine in the dog fasted for 10–15 h consists of the cyclic occurrence at hourly intervals of migrating myoelectric complexes which include successive periods of irregular spike activity and regular spike activity slowly propagating from the duodenum to the ileum followed by a period of quiescence (Szurzsewski 1969; Bueno et al 1975). Colonic motility in the dog is characterized by phases of contractile activity lasting 5–10 min occurring at 20 min intervals along the length of the colon (Fioramonti et al 1980).

Intestinal and colonic motor profiles observed were similar to those previously described. In the fasting dog migrating myoelectric complexes recurred in the small intestine at a rate of 0·6 ± 0·07 h^{-1}. The colonic electromyogram consisted of phases of propulsive long spike bursts, lasting 6·3 ± 2·5 min, recurring at 27 ± 5 min intervals on the transverse colon and of short spike bursts occurring irregularly and without cyclic organization.

Intravenous administration. Intravenous administration of trimebutine at a dose of 5 mg kg^{-1} after a delay of 30–60 s, induced a phase of regular spike activity in the small intestine lasting 7·3 ± 1·6 min, identical to that observed for a migrating myoelectric complex (Fig. 1). This phase, first seen at the duodenum level and then propagated in the jejunum at a velocity of 3·7 ± 0·8 cm min^{-1}, was followed by a period of quiescence lasting 95 ± 12 min. The onset of the next spontaneous phase of regular spike activity was delayed, appearing 175 ± 22 min after the trimebutine induced phase of regular spike activity (Table 1).

The same administration of trimebutine caused a total inhibition of colonic motility during 105 ± 17 min. Then, colonic spike activity expressed as per cent time or as total spike level was nearly halved during 3 h (Fig. 2, Table 2).

Table 1. Comparative effects of intravenous vs intracerebroventricular administration of trimebutine and naloxone antagonism on the duration of regular spike activity (RSA) cycle of the jejunum in fasted dog.

Trimebutine dose	Duration of RSA cycle (min)[a] Control[b]	1st phase[c]	2nd phase
5 mg kg^{-1} i.v.	98 ± 6	39 ± 2[d]	175 ± 22[d]
0·1 mg kg^{-1} i.c.v.	93 ± 15	162 ± 17[d]	98 ± 5
5 mg kg^{-1} i.v. after naloxone 0·1 mg kg^{-1} i.v.	86 ± 7	101 ± 10	92 ± 7
5 mg kg^{-1} i.v. after naloxone 0·1 mg kg^{-1} i.c.v.	105 ± 9	38 ± 2	163 ± 12[d]

[a] Time elapsed between the ends of two consecutive phases of regular spike activity.
[b] Time between the two last phases of RSA preceding the administration of trimebutine.
[c] Trimebutine was administered 30 min after the end of a phase of RSA.
[d] Significantly different ($P \leq 0·01$) from control.

Intracerebroventricular administration. The cyclic motor profile of the small intestine was disrupted during 162 ± 17 min after i.c.v. administration of trimebutine at a dose of 0·1 mg kg^{-1} (Table 1) and replaced by continuous irregular spike activity. No phase of regular spike activity similar to that observed after i.v. injection was induced. No modification of colonic motility was observed.

Naloxone antagonism. Naloxone on its own whether i.v. or i.c.v. administered at 0·1 mg kg^{-1}, did not affect the motor profile of the small intestine and the colon for 4 h after its injection.

The effects of trimebutine (5 mg kg^{-1} i.v.) on small intestine motility were blocked by previous administration of naloxone at a dose of 0·1 mg kg^{-1} by the i.v. route but not by the i.c.v. route (Table 1; Fig. 2). The inhibition of colonic motility induced by trimebutine was not blocked by naloxone i.v. or i.c.v.

Discussion

Our results indicate two peculiarities of the action of trimebutine on digestive motility: (i) its differential action on the small intestine and the large bowel, (ii) its different mechanisms and/or pathways according to the segment of the digestive tract considered.

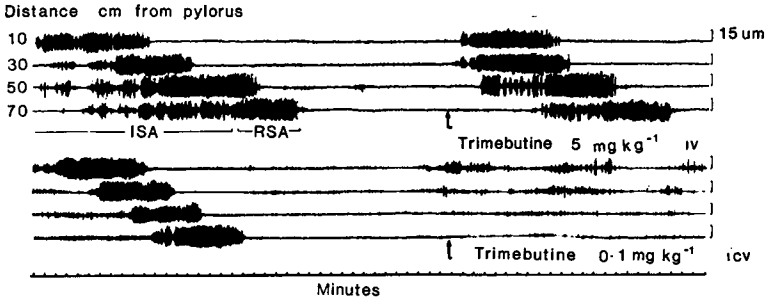

FIG. 1. Influence of intravenous (i.v.) vs intracerebroventricular (i.c.v.) administration of trimebutine on the electrical activity of the small intestine. Note that only i.v. administration induces a propagated phase of regular spike activity (RSA).

Table 2. Influence of intravenous vs intracerebroventricular administration of trimebutine and naloxone antagonism on the duration and the level of colonic spike activity in fasted dog.

Trimebutine dose	Duration (% time)			Total spike level (mC)		
	Control	0/60	60/120	Control	0/60	60/120
5 mg kg^{-1} i.v.	31 ± 9	0	12 ± 3*	2·1 ± 0·4	0·4 ± 0·3	0·9 ± 0·2*
0·1 mg kg^{-1} i.c.v.	27 ± 10	33 ± 7	16 ± 5	2·4 ± 0·5	2·9 ± 0·7	1·8 ± 0·5
5 mg kg^{-1} i.v. after naloxone 0·1 mg kg^{-1} i.v.	33 ± 7	0	10 ± 4*	2·9 ± 0·7	0	1·2 ± 0·6*
5 mg kg^{-1} i.v. after naloxone 0·1 mg kg^{-1} i.c.v.	30 ± 6	0	9 ± 2*	2·6 ± 0·3	0·9 ± 0·7*	0·8 ± 0·3*

* Significantly different ($P \leq 0{\cdot}01$) from control.

Colonic motility was inhibited after administration of trimebutine while motility of the small intestine was stimulated by the induction of a phase of regular spike activity—an alteration of the digestive motor profile that seems to be unique. Other kinds of stimulation of the small intestine by increasing the irregular spike activity associated with an inhibition of colonic motility have already been described for other compounds such as anthraquinone derivatives (Garcia-Villar et al 1980) or for some antidopaminergic substances such as sulpiride (Bueno & Fioramonti 1983), but trimebutine has been found to be without antidopaminergic properties (Berga et al 1981). In the dog, only morphine induces a similar phase of regular spike activity on the small intestine (Sarna et al 1982) but it also induces a long lasting stimulation of colonic motility (Bueno & Fioramonti 1982). As with trimebutine, the effects of central vs peripheral administration of morphine differed. In anaesthetized dogs, morphine administered via the carotid artery stimulated small intestinal motility while an inhibition has been observed with an 8 times higher dose after administration into the superior mesenteric artery (Weisbrodt et al 1982).

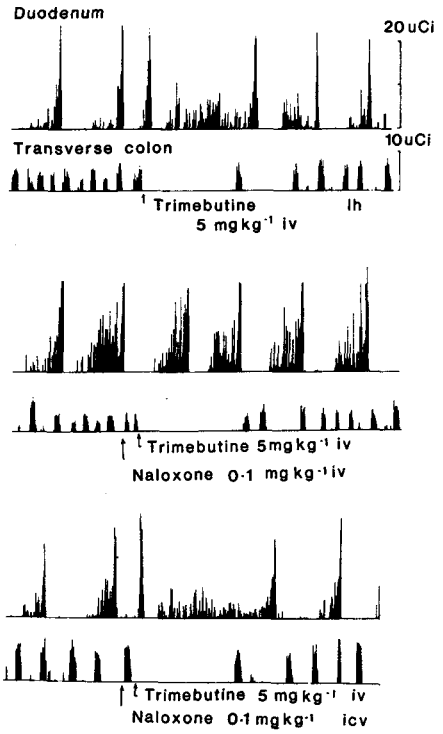

FIG. 2. Effects of intravenous administration of trimebutine on duodenal and colonic spike activity. Integrated records showing that naloxone i.v. blocked the effects of trimebutine only on the small intestine. Naloxone i.c.v. did not modify changes in intestinal and colonic spike activity induced by trimebutine.

The action of trimebutine in the dog seems to be mediated through different mechnanisms, and opiate receptors are only involved in the action on the small intestine since the effects of trimebutine are similar to those of morphine (Sarna et al 1982) and are blocked by naloxone. The phase of regular activity induced by morphine i.v. has been found to involve peripheral receptors since it is blocked by nalorphine methiodide, a peripheral μ receptor antagonist (Pinnington & Wingate 1982). As for morphine the effects of i.v. trimebutine on small intestinal motility seem to be peripherally mediated since they are blocked by naloxone administered at the same dose only by the i.v. but not the i.c.v. route.

The inhibition of colonic motility indicates a spasmolytic property of trimebutine which was probably masked in the small intestine by the opiate like stimulation. Such an inhibition confirms the peripheral action of trimebutine: since opiates have been found to increase colonic motility through central pathways (Bueno & Fioramonti 1982), a central action of i.v. administered trimebutine would lead to stimulation of colonic motility instead of inhibition.

In conclusion, trimebutine, a drug largely used in digestive disturbances, induces by the i.v. route typical modifications of the motility of the small intestine and the large bowel. Only the effects on small intestine involve opiate receptors and are mediated through peripheral pathways.

REFERENCES

Berga, P., Beckett, P. R., Masso, J. L., Roberts, D. J. (1981) Arch. Farmacol. Toxicol. 7: 189–192

Bueno, L., Fioramonti, J., Ruckebusch, Y. (1975) J. Physiol. (London) 249: 69–85

Bueno, L., Ruckebusch, Y. (1978) C. R. Soc. Biol. 172: 972–977

Bueno, L., Fioramonti, J. (1982) Eur. J. Pharmacol. 82: 147–153

Bueno, L., Fioramonti, J. (1983) in: Ruckebusch, Y., Toutain, P. L., Koritz, G. D. (eds) Veterinary Pharmacology and Toxicology. MTP Press, Lancaster, 283–298

Fioramonti, J., Garcia-Villar, R., Bueno, L., Ruckebusch, Y. (1980) Dig. Dis. Sci. 25: 641–646

Garcia-Villar, R., Leng-Peschlow, E., Ruckebusch, Y. (1980) J. Pharm. Pharmacol. 32: 323–329

Ingram, D. M., Catchpole, B. N. (1981) Dig. Dis. Sci. 26: 989–992

Latour, A. (1973) Ann. Rech. Vet. 4: 347–353

Luttecke, K. (1978) J. Int. Med. Res. 6: 86–88

Malavaud, A. (1972) Anesth. Anal. Réan. 29: 65–69

Moshal, M. G. (1979) J. Int. Med. Res. 7: 231–234

Pinnington, J., Wingate, D. L. (1982) Life Sciences 31: 2217–2219

Sarna, S. Northcott, P., Belbeck, L. (1982) Am. J. Physiol. 242: G588–G595

Stewart, J. J., Weisbrodt, N. W., Burks, T. F. (1977) J. Pharmacol. Exp. Ther. 202: 174–181

Szurszewski, J. H. (1969) Am. J. Physiol. 217: 1757–1763

Weisbrodt, N. W., Thor, P. J., Anderson, J., Copeland, E. M. (1982) in: Wienbeck, M. (ed.), Motility of the Digestive Tract, Raven Press, New York, 461–466

J. Pharm. Pharmacol. 1984, 36: 621–622
Communicated January 23, 1984

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Increased number of brain benzodiazepine receptors after in-vivo administration of estazolam to rats

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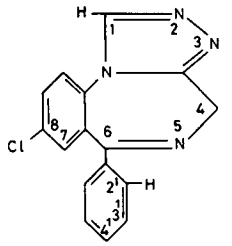
Estazolam significantly increased the K_d of [^{3}H]flunitrazepam in-vitro, like other benzodiazepines (BDZs) acting competitively at the receptor site. At variance with other BDZs, estazolam significantly raised the B_{max} for [^{3}H]flunitrazepam, at concentrations lower than its K_i for BDZ receptors. This effect may be responsible for the observed increase in [^{3}H]diazepam binding after in-vivo administration of estazolam to rats.

Benzodiazepines (BDZ) exert their different pharmacological effects by occupying specific receptor sites in the cns to various extents (Mennini & Garattini 1982). Under standard conditions, 50% protection against pentetrazol (leptazol, metrazol)-induced seizures in rats (ED50 AP) is achieved when 20–25% of total brain receptors are occupied by various BDZs corresponding to ≈20% displacement of [^{3}H]flunitrazepam (Braestrup & Nielsen 1983) or ≈50% displacement of [^{3}H]diazepam (Garattini et al 1981) bound in-vivo to cerebral membranes.

There are some exceptions to this general rule. For example, estazolam, an *s*-triazolo benzodiazepine with a chloro-substituent in position 8 (see Fig. 1) with high affinity for BDZ receptors in-vitro (Cotecchia et al 1981) when injected to rats at a dose corresponding to its ED50 AP, raises the amount of [^{3}H]diazepam bound (Mennini & Garattini 1982).

The present findings further describe the in-vivo and in-vitro interactions of estazolam with BDZ receptors in rat brain.

FIG. 1.



* Correspondence.

Materials and methods

Male rats (CD-COBS, Charles River, Italy), 200 ± 20 g, were used. In-vivo binding of [^{3}H]diazepam was determined as previously described (Mennini et al 1982a), by injecting iv 50 μCi/rat of [^{3}H]diazepam (spec. act. 78 Ci mmol^{-1}, NEN) 1 min before the animals were killed. Non-specific binding was determined by incubating the homogenates at 0 °C for 30 min in the presence of 3 μM unlabelled diazepam. In-vitro [^{3}H]flunitrazepam binding (spec. act. 84 Ci mmol^{-1}, NEN) concentration range 0·5–10 nM, was determined on thoroughly washed, thawed crude membrane preparations in 0·15 M Tris HCl, pH 7·4 as detailed elsewhere (Mennini et al 1982b). Non-specific binding was determined in the presence of 3 μM unlabelled diazepam, and represented about 10% of total. GABA levels were measured by radioreceptor assay as described by Herschel & Baldessarini (1979) on brain tissues from animals killed by microwave irradiation.

Results and discussion

Table 1 reports the in-vivo displacement of [^{3}H]diazepam bound to hippocampus or cerebellum of rats after pretreatment with equiactive doses (ED50 AP) of