Benzodiazepine-induced intestinal motor disturbances in rats: mediation by ω_2 (BZ₂) sites on capsaicin-sensitive afferent neurones

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1 The central and peripheral effects of the ω (benzodiazepine) site ligands, clonazepam, alpidem, zolpidem, triazolam, flumazenil, ethyl β carboline-3-carboxylate (β -CCE) and N-methyl β carboline-3-carboxylate (β -CCM) on intestinal myoelectrical activity were evaluated in conscious rats, chronically fitted with Nichrome electrodes implanted on the duodenum and jejunum. The localization of the ω (benzodiazepine) receptors involved in these effects was evaluated by use of systemic and perivagal capsaicin treatments.

2 When administered intraperitoneally (i.p.) the ω site inverse agonists β -CCE and β -CCM, and the ω site antagonist flumazenil, did not affect the duodeno-jejunal motility. Alpidem and zolpidem, two selective ω_1 site agonists induced an inhibition of migrating myoelectric complexes (MMCs) only at a high dose (5 mg kg⁻¹). In contrast, clonazepam (a mixed ω_1/ω_2 agonist) and triazolam (a preferential ω_2 site agonist) disrupted the MMC-pattern at doses as low as 0.05 mg kg⁻¹, the effect of triazolam being of much longer duration than that of clonazepam. None of these drugs altered MMC-pattern when administered centrally (i.c.v.).

3 Administered i.p. or i.c.v. prior to triazolam, alpidem blocked the effect of triazolam on duodeno-jejunal spike activity. Administered i.p. prior to triazolam, flumazenil suppressed the triazolam-induced MMC-disruption. Previous systemic but not perivagal capsaicin treatment suppressed the effects of clonazepam on MMCs.

4 It is concluded that ω -site agonists but not antagonist or inverse agonists, administered systemically induce intestinal motor disturbances which may be linked to activation of ω_2 (BZ₂) sites located on nonvagal capsaicin-sensitive afferent neurones.

Keywords: Benzodiazepine-sites; intestinal motility; capsaicin-sensitive neurones

Introduction

The benzodiazepines (BZs) have been the most widely prescribed drugs for the treatment of anxiety and related neurotic disorders during the past three decades. The neuropharmacological effects of BZ derivatives are mediated by their interaction with a specific recognition site within the GABA_A receptor macromolecular complex, the so-called ω (BZ) modulatory site. Activation of the ω site increases the chloride conductance of the GABA_A receptor complex leading to hyperpolarization of the target cells.

Several lines of evidence have suggested a heterogeneity of the ω modulatory sites. Binding sites with high affinity for the triazolopyridazine, CL 218,872 (Klepner *et al.*, 1979), the imidazopyridines, zolpidem and alpidem (Arbilla *et al.*, 1985; Langer *et al.*, 1990), and several β carbolines (Braestrup & Nielsen, 1981; Braestrup *et al.*, 1982) are termed ω_1 (BZ₁) and sites with low affinity for these ligands are termed ω_2 (BZ₂) sites (Langer & Arbilla, 1988). Recent advances in molecular biology have demonstrated that GABA_A receptors are made up of different subunits (α, β, γ) probably associated in pentameric complexes and that ω sites are associated with the α subunits (see Olsen & Tobin, 1990 for review). At least six variants of this subunit have been reported and this heterogeneity appears to be responsible for the existence of different ω site subtypes. Ligands that possess affinity for the ω sites can induce different functional effects at GABA_A receptors: while agonists or partial agonists have positive intrinsic activity and enhance the functional response to GABA. The antagonists have no intrinsic activity and the

inverse agonists have negative intrinsic activity i.e. inhibit the functional response to GABA.

The ω sites are essentially located in the central nervous system but 'central type' ω sites can also be found in the myenteric plexus (Cherubini & North, 1985; Taniyama *et al.*, 1988). They differ from peripheral type BZ receptors (p sites) located in various organs and in non-neuronal brain tissues which are not associated with the GABA_A receptor macromolecular complex (Benavides *et al.*, 1989; 1990).

The benzodiazepines, in the range of their therapeutic doses have been shown to alter the motility of the small intestine in rats, producing an increased motor activity associated with a disruption of the migrating myoelectric complexes (MMCs) characterizing the fasted state (Martinez *et al.*, 1992; Bonnafeous *et al.*, 1993). Similarly, in fasted dogs, diazepam administered intravenously (i.v.) disrupts the MMC-pattern in a time-related manner (Fargeas *et al.*, 1984). Furthermore, in man, vomiting, nausea, diarrhoea and epigastric distress often occur following administration of high doses of benzodiazepines (Harvez *et al.*, 1985).

The purpose of the present study was to determine which subtype of ω site mediates these intestinal motor disturbances. To this end, we studied the effects of a number of ω site ligands with differing selectivity for the ω_1 and ω_2 subtypes on intestinal myoelectrical activity in conscious rats. Secondly, we investigated the central vs peripheral localization of those ω sites involved in this effect. The following ligands were used: clonazepam (a non selective ω site agonist), alpidem (a partial ω_1 receptor agonist), zolpidem (a full ω_1 site agonist), triazolam (a preferential ω_2 site agonist), flumazenil (a non selective ω site antagonist), ethyl β carboline-3-carboxylate (β -CCE) and N-methyl β carboline-3-carboxylate (β -CCM) (two ω site inverse agonists).

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Methods

Animal preparation

One hundred and twelve male Wistar rats, weighing 200–250 g, were prepared for long-term electromyographic recordings (Ruckebusch & Fioramonti, 1975). Briefly, following induction of anaesthesia with 100 mg kg⁻¹ i.p. of ketamine (Imalgene-1000, Rhône-Mérieux, Lyon, France), nichrome wire electrodes (80 µm in diameter) were implanted in the wall of the proximal and distal duodenum (3 and 6 cm from the pylorus) and in the wall of the jejunum (10 and 20 cm from the Treitz' ligament). The insulated electrode wires (80 cm in length) were exteriorized on the back of the neck and protected by a glass tube attached to the skin. Rats were also fitted with permanent polyethylene catheters (PE 10) inserted into the lateral ventricles of the brain (2 mm from the sagittal suture and 1 mm from the coronal suture).

Myoelectric recordings and analysis

Electromyographic recordings were initiated five days after surgery. The spiking activity was amplified with an electroencephalograph machine (minihuit ALVAR, France), summated every 20 s by an integrator circuit and automatically plotted 24 h day⁻¹ on a potentiometric recorder with a paper speed of 5 cm h⁻¹ (Latour, 1973). This 'integrated record' allowed a clear determination of the different patterns of intestinal activity and, particularly, the identification of the migrating myoelectric complexes (MMCs), characterizing the fasted pattern. Intestinal myoelectric activity was analysed over a daily 10 h period, from 09 h 00 min to 19 h 00 min. Modifications appearing after drug injections were appraised by measuring the duration of motor changes i.e. disruption of MMCs. It has been demonstrated that the duration of motor changes is directly correlated to the intensity of electrical activity and mechanical activity (Buéno *et al.*, 1981).

Capsaicin treatments

Systemic capsaicin treatment Capsaicin (Sigma, La Verpillière, France) was systemically administered to a group of eight rats for 4 days. Capsaicin, dissolved in 80% saline, 10% ethanol and 10% Tween 80, was administered subcutaneously. Injections were performed at increasing doses to reach a total dose of 125 mg kg⁻¹. The efficacy of capsaicin treatment was assessed by the eye-wiping test (Holzer *et al.*, 1990): impaired chemosensitivity of corneal afferents which are no longer sensitive to a solution of 1% NH₄OH. Another group of rats (*n* = 8) was treated with the capsaicin vehicle only.

Perivagal capsaicin treatment Eight rats were anaesthetized with ketamine (100 mg kg⁻¹, i.p.) and received atropine sulphate (1 mg kg⁻¹, i.p.) to decrease the acute effects of capsaicin on the respiratory and cardiovascular systems. Cervical vagi were carefully dissected from the carotid artery and isolated. A 1% solution of capsaicin (10 mg ml⁻¹) was applied to each vagus nerve in turn for 30 min. At 10 min intervals, the nerves were swabbed and capsaicin reapplied. The maximum amount of capsaicin applied was 0.5–0.6 mg per rat. After application, the area was thoroughly rinsed with sterile saline (Raybould *et al.*, 1990). A group of sham animals (*n* = 8) was treated in the same manner but with the capsaicin vehicle alone.

The efficacy of perivagal capsaicin treatment was assessed 15 days later by the CCK satiety test (South & Ritter, 1988). CCK8S (20 µg kg⁻¹) was administered i.p.; on sham animals CCK8S induced satiety as it did on control rats, whereas on perivagal capsaicin-treated rats CCK8S had no effect: animals ate as naive rats. Systemic and perivagal capsaicin treatments were performed 15–20 days before electromyographic recordings.

Drugs

The drugs used for this study were: clonazepam, (Roche, Paris, France), alpidem, zolpidem and triazolam (gifts from Synthelabo, Bagneux, France), flumazenil (Hoffman La Roche, Paris, France); β-CCE and β-CCM (RBI, Natick, U.S.A.).

Experimental procedure

In a first series of experiments, 32 rats were randomly treated by intraperitoneal (i.p.) administration of clonazepam and triazolam at doses of 0.05, 0.1, 0.3 and 0.5 mg kg⁻¹, zolpidem and alpidem at the doses of 0.05, 0.1, 0.3, 0.5 and 5 mg kg⁻¹ or their vehicle (dimethylsulphoxide: DMSO) (0.3 ml). These drugs or their vehicle (3 µl) were also administered intracerebroventricularly (i.c.v.) to the same rats at the dosage of 10 and 100 µg kg⁻¹.

In a second set of experiments, 24 rats were randomly treated i.p. with either β-CCE, β-CCM at doses of 0.1 and 1 mg kg⁻¹, and either flumazenil at doses of 0.1, 1 and 5 mg kg⁻¹, or their vehicle. They also received DMSO i.c.v. alone or containing one of the three previous drugs at a dose of 10 and 100 µg kg⁻¹.

In the third series of experiments, 16 rats were used and divided into two groups. In the first group (*n* = 8), rats received alpidem at a dose of 0.1 mg kg⁻¹, i.p. and 10 min later, triazolam at a dose of 0.1 mg kg⁻¹, i.p. in the second group (*n* = 8), rats received alpidem i.c.v. at a dose of 10 µg kg⁻¹ and 10 min later triazolam at a dose of 0.1 mg kg⁻¹, i.p.

In the fourth series of experiments, eight rats received triazolam at a dose of 0.1 mg kg⁻¹, i.p. preceded or not by the administration of flumazenil at a dose of 5 mg kg⁻¹, i.p.

In the last series of experiments, systemic capsaicin-treated rats (*n* = 8), perivagal capsaicin-treated rats (*n* = 8) and corresponding sham-treated rats (2 × *n* = 8) were randomly treated with clonazepam and triazolam at a dose of 0.1 mg kg⁻¹ or with their vehicle.

Statistical analyses

The duration of MMC-disruption, determined on integrated records from the last MMC to the first MMC reappeared, were expressed as means ± s.e.mean and compared using ANOVA followed by Student's unpaired *t* test when appropriate. Differences were considered significant for *P* < 0.05.

Results

Control studies

Rats fed only during the night presented a typical pattern of alternating intestinal motility. During day-time, fasted rats exhibited an electrical activity of the duodenum and the jejunum characterized by the cyclic occurrence of migrating myoelectric complexes (MMCs). Each MMC consisted of an irregular spiking activity (phase II) lasting 6–8 min, followed by a short period (4–5 min) of intense and regular spiking activity (phase III), separated by a 3 to 5 min quiescent period (phase I). These MMCs occurred firstly on the proximal duodenum and were propagated aborally at a mean rate of 3.5 ± 0.6 cm min⁻¹.

Comparative effects of *ω* site subtype agonists on duodenal and jejunal MMC-pattern

When injected i.p., clonazepam and triazolam induced dose-related disturbances of the small intestine fed pattern. These disturbances corresponded to a strong stimulation of duodenal and jejunal spiking activity associated with a transient suppression of the MMC-pattern (Figure 1). At all doses

Figure 1 Effect of vehicle (a), clonazepam (b), triazolam (c) and alpidem (d) administered i.p. at a dose of 0.1 mg kg^{-1} , on duodenal myoelectric activity (integrated records) in a fasted rat. Triazolam induced a more prolonged stimulation of duodenal spiking activity than clonazepam; alpidem had no effect.

tested, jejunal electrical pattern was more affected than that of the duodenum: for example, at a dose of 0.1 mg kg^{-1} , clonazepam disrupted MMC for $86.7 \pm 15 \text{ min}$ and $51.3 \pm 23.3 \text{ min}$ at the jejunum and duodenum levels, respectively (Figure 2). Triazolam, at similar doses, was always more potent than clonazepam on both duodenum and jejunum (Figure 2). Alpidem and zolpidem had no effect on the fasted pattern (Figure 2) except at the highest dose tested (5 mg kg^{-1}) (alpidem: duodenum: 52 ± 11 ; jejunum: 56 ± 8 ; zolpidem: duodenum: 189.1 ± 9.2 ; jejunum: 151.6 ± 6.6); even at this high dose the effect of these drugs was much smaller in magnitude than that elicited by clonazepam or triazolam. None of the drugs had any effect on the fasted pattern when injected centrally at a dose of 10 and $100 \mu\text{g kg}^{-1}$. Values obtained were not significantly different from the time between two consecutive MMCs corresponding to a control fasted pattern. For example, zolpidem at a dose of $10 \mu\text{g kg}^{-1}$: duodenum: 14.2 ± 3.1 vs $11.1 \pm 2.2 \text{ min}$ for control; jejunum: 12.5 ± 0.5 vs $12.3 \pm 2.1 \text{ min}$ for control.

Effects of i.p. administration of ω site inverse agonists and antagonists on duodenal and jejunal MMC-pattern

Neither β -CCM, β -CCE or flumazenil altered duodenal and jejunal fasted patterns at doses of 0.1, and 1 mg kg^{-1} . For example: respectively β -CCE, β -CCM and flumazenil at a dose of 1 mg kg^{-1} , duodenum: 11.4 ± 4.1 , 12.8 ± 2.7 , $12.7 \pm 4.5 \text{ min}$; jejunum: 10.5 ± 5.5 , 13.3 ± 1.1 , $15.1 \pm 3.2 \text{ min}$ vs 12.5 ± 2.4 and $13.1 \pm 2.2 \text{ min}$ for controls. The same range of values was obtained after 0.1 mg kg^{-1} . In the same way flumazenil at 5 mg kg^{-1} did not modify the MMC-pattern: duodenum: 13.2 ± 1.9 , jejunum: 12.7 ± 3.4 vs 12.5 ± 2.4 and 13.1 ± 2.2 for controls. Similarly, none of these compounds had any influence on MMCs when injected i.c.v. at 10 and $100 \mu\text{g kg}^{-1}$.

Figure 2 Comparative effects of i.p. administration of clonazepam (■), triazolam (◆), alpidem (●) and zolpidem (▲) on duodenal (a) and jejunal (b) fasted pattern. Clonazepam and triazolam induced a dose-related disruption of the MMC pattern whereas alpidem and zolpidem had no effect. Significantly different (* $P < 0.05$) from control values i.e. vehicle-injected i.p. ($n = 8$).

Antagonism by alpidem of triazolam induced MMC-disruption

When administered either i.p. at a dose of 0.1 mg kg^{-1} or i.c.v. at a dose of $10 \mu\text{g kg}^{-1}$, 10 min prior to triazolam (0.1 mg kg^{-1} , i.p.), alpidem suppressed triazolam-induced disruption of the duodeno-jejunal MMC-pattern (Figure 3).

Antagonism by flumazenil of triazolam induced MMC-disruption

When administered at a dose of 5 mg kg^{-1} i.p., 10 min prior to triazolam (0.1 mg kg^{-1}), flumazenil antagonized the triazolam induced MMC-disruption on either duodenum or jejunum (Figure 4). Flumazenil had no effect at this dose when administered alone (data not shown).

Effect of systemic and peri-vagal capsaicin treatment on clonazepam-induced MMC-disruption

In sham (vehicle) systemic capsaicin-treated rats, clonazepam (0.1 mg kg^{-1} , i.p.) induced MMC disruption of the duodenum and the jejunum lasting $53.1 \pm 23.7 \text{ min}$ and $89.4 \pm 15.4 \text{ min}$, respectively. However, in systemic capsaicin-treated rats, clonazepam at the same dosage did not alter the small intestine MMC-pattern. In the same way, triazolam (0.1 mg kg^{-1} , i.p.) induced MMC disruption of the duodenum and the jejunum lasting 160 ± 12 and $152.3 \pm 19.1 \text{ min}$, respectively (Figure 5).

In contrast, clonazepam and triazolam (0.1 mg kg^{-1} , i.p.) administered to sham perivagal capsaicin-treated rats, disrupted duodenal and jejunal MMCs as it did in control and sham-operated animals (Figure 6).

Figure 3 Antagonistic effect of alpidem on triazolam (0.1 mg kg^{-1} , i.p.) induced MMC-disruption at duodenal (solid columns) and jejunal (open columns) levels. Alpidem, administered i.p. or i.c.v. 10 min prior to triazolam, blocked the triazolam-induced MMC-disruption on the small intestine. Significantly different ($*P < 0.05$) from vehicle values ($n = 8$).

Figure 4 Antagonistic effect of flumazenil on triazolam (0.1 mg kg^{-1} , i.p.) induced MMC-disruption at duodenal (a) and jejunal (b) levels. Flumazenil, administered i.p. 10 min prior to triazolam, blocked the triazolam-induced MMC-disruption on the small intestine. Significantly different ($*P < 0.05$) from triazolam alone. ($n = 8$).

Figure 5 Effect of systemic capsaicin treatment on clonazepam and triazolam-induced small intestine MMC disruption: (a) duodenum; (b) jejunum. Solid columns, sham capsaicin; open columns, systemic capsaicin-treated rats. In vehicle capsaicin-treated rats clonazepam and triazolam induced a significant stimulation of the spiking activity, but had no effect in systemic capsaicin-treated rats. Significantly different (* $P < 0.05$) from sham capsaicin-treated rats + vehicle i.p.; significantly different († $P < 0.05$) from sham capsaicin-treated rats + clonazepam (0.1 mg kg⁻¹, i.p.) ($n = 8$).

Figure 6 Effect of perivagal capsaicin treatment on clonazepam and triazolam-induced MMC-disruption of the small intestine in fasted rats: (a) duodenum; (b) jejunum. In sham capsaicin-treated rats (solid columns) clonazepam and triazolam induced a significant stimulation of the spiking activity and had the same effect in perivagal capsaicin-treated rats (open columns). Significantly different (* $P < 0.05$) from sham capsaicin-treated rats + vehicle i.p. ($n = 8$).

Discussion

In agreement with previous studies (Martinez *et al.*, 1992; Bonnafous *et al.*, 1993), systemic administration of the benzodiazepines, clonazepam and triazolam, induced disturbances of the intestinal MMCs in rats. In contrast, the ω site inverse agonists (β -CCM and β -CCE) and antagonist (flumazenil) had no effect on MMCs. Thus, positive allosteric modulation of GABA_A receptor-mediated transmission is

associated with intestinal disturbances of MMCs, whereas negative allosteric modulation of the GABA_A receptor complex does not appear to induce opposite alterations. In agreement with these findings, flumazenil by itself possesses little intrinsic pharmacological activity in animals and man (Hunkeler *et al.*, 1981; Darragh *et al.*, 1983). However, the lack of effect of the inverse agonists β -CCM and β -CCE when administered peripherally or centrally may appear paradoxical since the inverse agonists β carbolines investigated are

known to elicit behavioural and electrophysiological effects that are opposite to those of ω site agonists (Oakley *et al.*, 1980; Braestrup *et al.*, 1982; Thiebot *et al.*, 1988). This discordance may be related to our experimental conditions which do not allow a demonstration of negative functional effects on intestinal motility.

One of the goals of the present study was to identify which subtype of ω sites is involved in the intestinal motor disturbances caused by ω site agonists. To this end, various compounds with differing affinities for ω_1 and ω_2 sites were studied. The present results show that clonazepam, which does not discriminate between ω_1 and ω_2 sites or triazolam which recognizes preferentially ω_2 sites *in vivo* (Benavides *et al.*, 1992) disrupt the jejunal and duodenal MMC-pattern. In contrast, zolpidem and alpidem which are respectively full and partial agonists at ω_1 sites (Langer *et al.*, 1990) do not affect this parameter except at high doses. These data suggest that ω_2 but not ω_1 sites may be involved in the effect of benzodiazepines on small intestine motility. This view is supported by previous *in vivo* binding studies which showed that doses of triazolam that cause marked disturbances of MMC-pattern (e.g. 0.1 mg kg⁻¹) selectively displace by about 50% the *in vivo* binding of [³H]-flumazenil in ω_2 -enriched brain areas in the rat (Benavides *et al.*, 1992). Moreover, the three fold lower potency of clonazepam as compared to triazolam in disturbing MMCs, accords with the relative potency of these drugs at displacing [³H]-flumazenil from central ω_2 sites (respectively ED₅₀'s = 0.34 and 0.1 mg kg⁻¹, i.p.) (Benavides *et al.*, 1992). Finally, within the range of doses where zolpidem do not produce MMC-disturbances (0.1–1 mg kg⁻¹, i.p.), this compound was inactive at displacing [³H]-flumazenil from ω_2 sites, yet partially occupied ω_1 sites (Benavides *et al.*, 1992). However, caution should be exercised when making such comparisons as all of these data refer to occupation of ω sites in the CNS and may be different for the myenteric plexus. Binding studies are clearly needed to confirm the pharmacological characteristics of those ω sites located in the myenteric plexus.

When administered by the central or peripheral route prior to triazolam, alpidem antagonizes the triazolam-induced MMC-disruption. The explanation for this finding is not obvious. It may be that ω_1 sites have an opposing effect against ω_2 -site-mediated MMC-disruption. Alternatively, alpidem may behave as an antagonist or inverse agonist at ω_2 sites thereby antagonizing the effect of ω_2 site stimulation. In support of this possibility, recent studies have shown that the direction of the modulatory effect of ω site agonists changes in relation to the molecular form of the γ subunit of the GABA_A receptor and that alpidem behaves as an inverse partial agonist in embryonic kidney 293 cells expressing the

subunit combination $\alpha_3\beta_1\gamma_1$, which may correspond to a subtype of ω_2 site (Puia *et al.*, 1991). Complementary experiments are clearly needed to clarify this issue.

In the present study, we have also attempted to investigate the localization of those ω sites involved in MMC-disruption. When administered centrally, none of the ω site agonists investigated altered intestinal motility. Since all these substances easily cross the blood-brain barrier, these observations suggest that they act peripherally to induce intestinal motor changes. This view is supported by recent studies which demonstrated the presence of GABA in guinea-pig myenteric plexus and that myenteric neurones themselves are endowed with ω sites which are akin to 'central type' sites (Cherubini *et al.*, 1985; Taniyama *et al.*, 1988). The fact that flumazenil antagonizes triazolam-induced MMC-disruption confirms that BZ receptors involved in this mechanism are of the 'central type'. We have recently demonstrated that peripheral clonazepam-induced MMC-disruption is linked to central release of cholecystokinin triggered by stimulation of peripherally located ω sites (Bonnafoous *et al.*, 1993). Altogether, these data suggest that ω -site agonists when administered i.p. act on 'central type' ω_2 sites located on afferent endings of the enteric nerves, thus activating afferent neurones involved in a long reflex responsible for digestive motor changes.

Capsaicin causes a long-term sensory receptor-blocking action which can be used in functional investigations on the sensory pathways (Jancso *et al.*, 1967; Maggi & Meli, 1988). In the digestive tract, capsaicin-sensitive afferent innervation participates in nociception and intestino-intestinal activation of inhibitory reflexes (Holzer *et al.*, 1987; Maggi, 1991). Moreover, the vagus nerve and the vagus afferents play a major role in the regulation of the gastrointestinal functions. Several studies have demonstrated the existence of a central relay in peripheral stimulation of the gastrointestinal tract; for example ileus or peritonitis share a common feature which is the activation of an inhibitory nervous reflex leading to the inhibition of small intestinal motility and gastrointestinal transit (Holzer *et al.*, 1986; Rivière *et al.*, 1993). The fact that peripheral but not perivagal capsaicin treatment prevents clonazepam and triazolam-induced MMC-disruption, suggests that capsaicin-sensitive non vagal afferent neurones are involved in the effects of clonazepam and triazolam on intestinal motility and that MMC-pattern may be triggered by an inhibitory reflex.

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References

- ARBILLA, A., DEPOORTERE, H., GEORGE, P. & LANGER, S.Z. (1985). Pharmacological profile of the imidazopyridine zolpidem at benzodiazepine receptors and electrocorticogram in rats. *Naunyn Schmied. Arch. Pharmacol.*, **330**, 248–251.
- BENAVIDES, J., DUBOIS, A., DENNIS, T., HAMEL, E. & SCATTON, B. (1989). ω (Peripheral-type benzodiazepine binding) site distribution in the rat immune system: an autoradiographic study with the photoaffinity ligand [³H]-PK14105. *J. Pharmacol. Exp. Ther.*, **249**, 333–339.
- BENAVIDES, J., DUBOIS, A., GOTTI, B., BOURDIOL, F. & SCATTON, B. (1990). Cellular distribution of ω_3 (peripheral type benzodiazepine) binding sites in the normal and ischemic rat brain: an autoradiographic study with the photoaffinity ligand [³H]-PK14105. *Neurosci. Lett.*, **144**, 32–38.
- BENAVIDES, T., PENY, B., DURAND, A., ARBILLAS, S. & SCATTON, B. (1992). Comparative *in vivo* and *in vitro* regional selectivity of central ω (benzodiazepine) site ligands in inhibiting [³H]-flumazenil binding in the rat central nervous system. *J. Pharmacol. Exp. Ther.*, **263**, 884–896.
- BONNAFOOUS, C., MARTINEZ, J., FARGEAS, M.J. & BUENO, L. (1993). Clonazepam-induced intestinal motor disturbances are linked to central nervous system release of cholecystokinin in rats. *Eur. J. Pharmacol.*, **237**, 237–242.
- BRAESTRUP, C. & NIELSEN, M. (1981). [³H] propyl β -carboline-3-carboxylate as a selective radioligand for BZ₁ benzodiazepine receptor subclass. *J. Neurochem.*, **37**, 333–341.
- BRAESTRUP, C., SCHMIECHEN, R., NEEF, G., NIELSEN, M. & PETERSEN, E.N. (1982). Interaction of convulsive ligands with benzodiazepine receptors. *Science*, **216**, 1241–1243.
- BUENO, L., FERRE, J.P., RUCKEBUSCH, M., GENTON, M. & PASCAUD, X. (1981). Continuous electrical and mechanical activity recording in the gut of the conscious rat. *J. Pharmacol. Methods*, **6**, 129–136.
- CHERUBINI, E. & NORTH, R.A. (1985). Benzodiazepines both enhance γ -aminobutyrate responses and decrease calcium action potentials in guinea-pig myenteric neurones. *Neuroscience*, **14**, 309–315.

- DARRAGH, A., LAUBE, R., KENNY, M. & BRICK, J. (1983). Tolerance of health volunteers to intravenous administrations of the benzodiazepine antagonist RO 15-1788. *Eur. J. Clin. Pharmacol.*, **14**, 569–570.
- FARGEAS M.J., FIORAMONTI, J. & BUENO, L. (1984). Time-related effects of benzodiazepines on intestinal motility on conscious dogs. *J. Pharm. Pharmacol.*, **36**, 130–134.
- HARVEZ, S.C., GOODMAN GILMAN, A., GOODMAN, L.S., RALL, T.W. & MURAD, F. (1985). Hypnotics and sedatives. In *The Pharmacological Basis of Therapeutics*. (Goodman and Gilman's) pp. 339–351. New York: Macmillan Publishing Company.
- HOLZER, P., LIPPE, I.Th. & HOLZER-PETSCH. (1986). Inhibition of gastrointestinal transit due to surgical trauma or peritoneal irritation is reduced in capsaicin-treated rats. *Gastroenterology*, **91**, 360–363.
- HOLZER, P., PABST, M.A., LIPPE, I.T., PESKAR, B.M., PESKAR, B.A., LIVINGSTON, E.H. & GUTH, P.H. (1990). Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. *Gastroenterology*, **98**, 839–849.
- HOLZER, P., SCHUET, W., LIPP, I.T. & SAMETZ, W. (1987). Involvement of capsaicin-sensitive sensory neurons in gastrointestinal function. *Acta Physiol. Hung.*, **69**, 403–411.
- HUNKELER, W., MOHLER, H., PIERI, L., POLC, P., BONETTI, E.P., CUMIN, R., SCHAFFNER, R. & HAEFELY, W. (1981). Selective antagonists of benzodiazepines. *Nature*, **290**, 514–516.
- JANSO, N., JANSO-GABOR, A. & SZOLCSANYI, J. (1967). Direct evidence for neurogenic inflammation and its prevention and by pretreatment with capsaicin. *Br. J. Pharmacol.*, **31**, 138–151.
- KLEPNER, C.A., LIPPA, A.S., BENSON, D.I., SANO, M.C. & BEER, B. (1979). Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. *Pharmacol. Behav.*, **11**, 457–462.
- LANGER, S.Z. & ARBILLA, A. (1988). Imidazopyridines as a tool for the characterization of benzodiazepine receptors: a proposal for a pharmacological classification as omega-receptors. *Pharmacol. Biochem. Behav.*, **29**, 763–767.
- LANGER, S.Z., ARBILLA, S., TAN, S., LLOYD, K.G., GEORGE, P., ALLEN, J. & WICK, A.E. (1990). Selectivity for omega-receptor subtypes as a strategy for the development of anxiolytic drugs. *Pharmacopsychiatry*, **23**, 102–107.
- LATOUR, A. (1973). Un dispositif simple d'analyses quantitative de l'électromyogramme intestinal chronique. *Ann. Rech. Vet.*, **4**, 347–353n.
- MAGGI, C.A. (1991). The pharmacology of the efferent function of sensory nerves. *J. Auton. Pharmacol.*, **11**, 173–208.
- MAGGI, C.A. & MELI, A. (1988). The sensory-efferent function of capsaicin-sensitive neurons. *Gen. Pharmacol.*, **19**, 1–43.
- MARTINEZ, J., FARGEAS, M.J. & BUENO, L. (1992). Gastrointestinal motor alterations induced by precipitated benzodiazepines withdrawal in rats. *J. Pharmacol. Exp. Ther.*, **260**, 1067–1070.
- OAKLEY, N.R. & JONES, B.J. (1980). The proconvulsant and diazepam-reversing effects of ethyl- β -carboline-3-carboxylate. *Eur. J. Pharmacol.*, **68**, 381–382.
- OLSEN, R.W. & TOBIN, A.J. (1990). Molecular biology of GABA_A receptors. *FASEB*, **4**, 1469–1480.
- PUJA, G., VICINI, S., SEEBURG, P.H. & COSTA, E. (1991). Influence of the recombinant γ -aminobutyric acid Λ -receptor subunit composition on the action of allosteric modulators of γ -aminobutyric acid-gated Cl⁻ currents. *Mol. Pharmacol.*, **39**, 691–696.
- RAYBOULD, H.E., HOLZER, P., REDDY, N. & TACHE, Y. (1990). Capsaicin-sensitive vagal afferents contribute to gastric acid and vascular responses to intracisternal TRH analog. *Peptides*, **11**, 789–795.
- RIVIERE, P.J.M., PASCAUD, X., CHEVALIER, E., LE GALLOU, B. & JUNIEN, J.L. (1993). Fedotozine reverses ileus induced by surgery or peritonitis: action at peripheral κ -opioid receptors. *Gastroenterology*, **104**, 724–731.
- RUCKEBUSCH, M. & FIORAMONTI, J. (1975). Electrical spiking activity and propulsion in small intestine in fed and fasted rats. *Gastroenterology*, **68**, 1500–1508.
- SOUTH, E.H. & RITTER, R.C. (1988). Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety. *Peptides*, **9**, 601–612.
- TANIYAMA, K., HASHIMOTO, S., HANADA, S. & TANAKA, C. (1988). Benzodiazepines and barbiturate potentiate the pro- and post-synaptic γ -aminobutyric acid (GABA_A) receptor-mediated response in the enteric nervous system of guinea-pig small intestine. *J. Pharmacol. Exp. Ther.*, **245**, 250–256.
- THIEBOT, M.H., SOUBRIE, P. & SANGER, D. (1988). Anxiogenic properties of β -CCE and FG7142: a review of promises and pitfalls. *Psychopharmacology*, **94**, 452–463.

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