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Interactions of trimebutine with guinea-pig opioid receptors

FRANÇOIS ROMAN*, XAVIER PASCAUD, JOHN E. TAYLOR†, JEAN-LOUIS JUNIEN, *Jouveinal Laboratories, 7, allée des Jachères, 94263 Fresnes Cedex, France, † Biomeasure Inc., Hopkinton Industrial Park, 11–15 E. Avenue, Hopkinton, Ma 01748, USA*

Affinities of trimebutine (TMB) and *N*-desmethyl trimebutine (NDTMB) for mu, delta and kappa opioid receptor subtypes have been examined using specific ^{3}H-ligands and guinea-pig membrane. TMB and NDTMB showed a relative higher affinity for the mu receptor subtype although they were, respectively, 30-and 48-fold less active than morphine. The receptor selectivity index for mu, delta and kappa were 100 : 12 : 14·4 for TMB, 100 : 32 : 25 for NDTMB and 100 : 5 : 5 for morphine. The sodium shift ratio was 14 for TMB, 10 for NDTMB and 37 for morphine. These data show that (unlike morphine, a pure mu agonist) TMB and NDTMB can be classified as weak opioid agonists and confirm that peripheral opioid receptors mediate their gastrointestinal motility effects.

Trimebutine (2-dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate hydrogen maleate) (TMB) is used in the treatment of various digestive tract disorders including dyspepsia, abdominal pain, irritable bowel syndrome (Moshal & Herron 1979; Luttecke 1980) and postoperative ileus (Malavaud 1972). Studies have shown that TMB stimulates small intestine motility by inducing regular spike activity in man (Grandjouan et al 1985, Valori et al 1985) and dog (Fioramonti et al 1984; Poitras et al 1985). The stimulatory effects of TMB are blocked by previous intravenous administration of naloxone, but not after intracerebroventricular (i.c.v.) injection of the mu antagonist, and are not reproduced after i.c.v injection of the drug. These results suggest that TMB acts on the small intestine through peripheral opioid receptors (Fioramonti et al 1984).

Moreover, recent work (Blanquet et al 1985) has shown that TMB induces a naloxone-sensitive spiking activity in cat and rabbit colon.

Since no data have been reported so far on the direct interaction of TMB and *N*-desmethyl trimebutine (NDTMB), its main metabolite, with opioid receptors, we have investigated the in-vitro affinity of both compounds for mu, delta and kappa opioid receptor subtypes using guinea-pig brain membranes and specific ligands.

* Correspondence.

Methods

Membrane preparation. Male tri-coloured guinea-pigs (30–325 g) were decapitated, brains rapidly removed, and the cerebellum discarded. The brains were then chilled in cold 0·05 M Tris buffer (pH = 7·5). Membranes for binding assays were prepared according to Kosterlitz et al (1981). Briefly, the brain tissue was homogenized in Tris buffer 0·05 M (pH = 7·5 at 0 °C), centrifuged at 49 000 *g* for 10 min, the pellet resuspended in Tris buffer, incubated at 37 °C for 45 min and centrifuged again. After a last washing of the pellet, the final homogenate was used for binding assays.

Binding assay. Aliquots of homogenate suspension (0·1 mL corresponding to 100 mg brain tissue), cold ligands and tritiated ligands (total volume 1 mL) were incubated for 40 min at 25 °C, filtered through Whatman GF/B glass fibre filter discs and washed three times with 4 mL ice-cold Tris buffer solution. Specific binding corresponded to the difference between total binding and non-specific binding obtained by addition of high concentration of levallorphan (10 μM).

The following ligands (New England Nuclear Corp) were used at the indicated final concentration: [^{3}H]dihydromorphine ([^{3}H]DHM: 73·6 Ci mmol^{-1}), 0·7 nM; [^{3}H]naloxone ([^{3}H]NAL: 42·7 Ci mmol^{-1}), 1 nM; [^{3}H]D-ala^2-D-leu^5-enkephalin ([^{3}H]DADLE: 46·9 Ci mmol^{-1}), 0·5 nM and [^{3}H]ethylketocyclazocine ([^{3}H]EKC: 18·7 Ci mmol^{-1}), 0·5 nM.

Calculations. The shift of the competition curves with [^{3}H]NAL by 100 mM NaCl was used to determine whether TMB and NDTMB behave as agonist or antagonist according to Pert & Snyder (1974). The equilibrium dissociation constant (KD) and the maximum number of binding sites (B_{max}) were determined by Scatchard analysis of saturation curves.

IC50 were determined according to the Hofstee method from the least squares linear regression curves of log(B/Be − B) versus log I where: I was the concentration of test compound in mol L^{-1}, Be the total

amount of ^{3}H-ligand bound and B the amount specifically bound at a given concentration of inhibitor.

For each test compound the receptor selectivity index (RSI) for each receptor subtype was determined according to the following equation:

$$RSI = \frac{IC50H}{IC50} \times 100$$

where IC50H is homogeneous displacement (for example, DADLE displacing [^{3}H]DADLE) and IC50 is the displacement of the primary ligand (for example, [^{3}H]DADLE) by the compound.

Morphine displacement of [^{3}H]DHM is considered to be a homogeneous displacement. Tritiated test compounds were not available, so they could not be used as primary ligands. Values of RSI for these compounds were referred to their lowest IC50. For a given primary ligand, the relative potency of competing compounds was calculated versus homologous compound, assuming arbitrarily that its IC50 is equivalent to 1.

Results

Tritiated ligands used in our binding assays displayed kinetic parameters very close to those described by Gillan et al (1980); [^{3}H]DHM: KD = 0·94 ± 0·09 nM, B_{max} = 339 ± 30 fmol mg^{-1} protein; [^{3}H]NAL: KD = 1·7 ± 0·28 nM, B_{max} = 935 ± 118 fmol mg^{-1} protein; [^{3}H]DADLE: KD = 1·2 ± 0·09 nM, B_{max} = 417 ± 23 fmol mg^{-1} protein. [^{3}H]EKC presented two different sites: a high affinity site (KD = 0·65 ± 0·02 nM, B_{max} = 423 ± 13 fmol mg^{-1} protein) and a low affinity site (KD = 2·1 ± 0·3 nM, B_{max} = 577 ± 34 fmol mg^{-1} protein).

IC50 relative potency and receptor selectivity index of tested compounds are reported in Table 1.

The IC50 of TMB and NDTMB were 212 ± 40 and 345 ± 90 nM, respectively, for the displacement of [^{3}H]DHM and 385 ± 47 and 339 ± 46 nM for [^{3}H]naloxone. Morphine had a 30- and 48-fold higher affinity in displacing [^{3}H]DHM than TMB and NDTMB, respectively. Both compounds showed weaker capacities to displace [^{3}H]DADLE (1·74 ± 0·26 and 1·08 ± 0·33 μM) and [^{3}H]EKC (1·45 ± 0·12 μM and 1·37 ± 0·16 μM). Receptor selectivity indexes for mu, delta and kappa subtypes were thus 100 : 12 : 14 for TMB, 100 : 32 : 25 for NDTMB, 100 : 5 : 6 for morphine, 18 : 100 : 0·1 for DADLE and 37 : 12 : 100 for EKC.

In Table 2 the values of IC50 for [^{3}H]NAL displacement are given. The sodium index is calculated as the ratio IC50 in presence and IC50 in absence of 100 mM NaCl.

Fig. 1 shows that [^{3}H]NAL displacement curves of TMB and NDTMB were shifted to the right when incubation was carried out with 100 mM NaCl.

The sodium index ratio was 14·4 for TMB, 10 for NDTMB and 37 for morphine. The naloxone displacement curve was not modified by higher sodium concentration.

Discussion

Several studies have shown that TMB, when given intravenously, stimulated small intestine motility by inducing migrating phase III type activity in man (Grandjouan et al 1985; Valori et al 1985) and dog (Fioramonti et al 1984). A net increase of blood motilin was also reported after drug administration in dog either in the fed or fasted state (Poitras et al 1985). A lack of effect (Latour & Bueno 1980; Sasaki et al 1984; Valori et al 1985) or a short period of stimulation followed by inhibition have been described in dog (Fioramonti et al 1984) and human colon (Frexinos et al 1985) while only stimulation was seen after i.v. perfusion in dog (Poitras

Table 1. Interactions of trimebutine with [^{3}H]opiate binding to cerebral guinea-pig membrane receptors. For each compound, IC50 values are expressed in 10^{-9} M and are the means ± s.e.m. of (n) determinations; RP = relative potency; RSI = receptor selectivity index.

Compounds		[^{3}H]DHM	[^{3}H]NAL	[^{3}H]DADLE	[^{3}H]EKC
Trimebutine	IC50	212 ± 40 (11)	385 ± 47 (11)	1747 ± 265 (7)	1455 ± 120 (6)
	RP	0·033	0·0078	0·00086	0·00036
	RSI	100	55	12·13	14·57
NDM-trimebutine	IC50	345 ± 90 (5)	339 ± 46 (4)	1080 ± 330 (10)	1372 ± 157 (6)
	RP	0·010	0·0089	0·0014	0·00038
	RSI	98·2	100	31·4	24·7
Morphine	IC50	7·10 ± 1·08 (4)	11·8 ± 2·46 (9)	152 ± 51·5 (6)	127·7 ± 15·3 (6)
	RP	1	0·27	0·0098	0·0041
	RSI	100	64	4·67	5·59
Naloxone	IC50	1·94 ± 0·31 (4)	3·02 ± 0·29 (4)	108·95 ± 10·02 (4)	7·75 ± 0·66 (6)
	RP	3·66	1	0·0137	0·067
	RSI	155·6	100	2·77	38·97
DADLE	IC50	8·4 ± 1·5 (4)	20·0 ± 4·2 (4)	1·5 ± 0·1 (4)	2187 ± 688 (4)
	RP	0·84	0·15	1	0·00024
	RSI	17·8	7·5	100	0·07
EKC	IC50	1·4 ± 0·41 (4)	0·7 ± 0·1 (4)	4·4 ± 0·5 (4)	0·52 ± 0·09 (4)
	RP	5·07	4·31	0·34	1
	RSI	37·1	74·3	11·8	100

Table 2. Potencies of TMB and NDTMB in displacing [^{3}H]naloxone binding in the presence and absence of sodium ion.

Compound	IC50 (nM) No NaCl	IC50 (nM) + 100 mM NaCl	IC50 + NaCl / IC50 − NaCl
TMB	331 ± 50	4770 ± 1146	14·4
NDTMB	339 ± 46	3395 ± 321	10·02
Morphine	11·41 ± 2·6	422 ± 188	37
Naloxone	3·02 ± 0·28	2·28 ± 0·22	0·75

Each value is the mean ± s.e.m. of 4–11 independent observations.

et al 1985) and after i.v. injection in cat and rabbit (Blanquet et al 1985).

Finally, Fioramonti et al (1984), have shown that TMB injected intravenously in the dog, stimulated small intestinal motility, by inducing a propagated phase of regular spiking activity and inhibited colonic motility for some 4 h. These effects were not reproduced by i.c.v. administration. 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, suggesting that, in the dog, the effects of TMB on the small intestine involve peripheral opiate receptors.

The fact that this opioid activity is of peripheral activity was also confirmed by the inhibition of the intestinal motility response to TMB observed with naloxone methyl bromide, a compound which does not cross the blood brain barrier (unpublished results).

Moreover, Blanquet et al (1985), working with two preparations, cat colon and rabbit proximal colon in-vivo and in-vitro, found that TMB blocked the cholinergic excitatory pathway through a mechanism reversed by naloxone. In both types of preparations, TMB induced an increase in the number and frequency of spike potentials, probably due to the activation of intramural non-cholinergic excitatory neurons, for this effect was atropine- and hexamethonium-resistant.

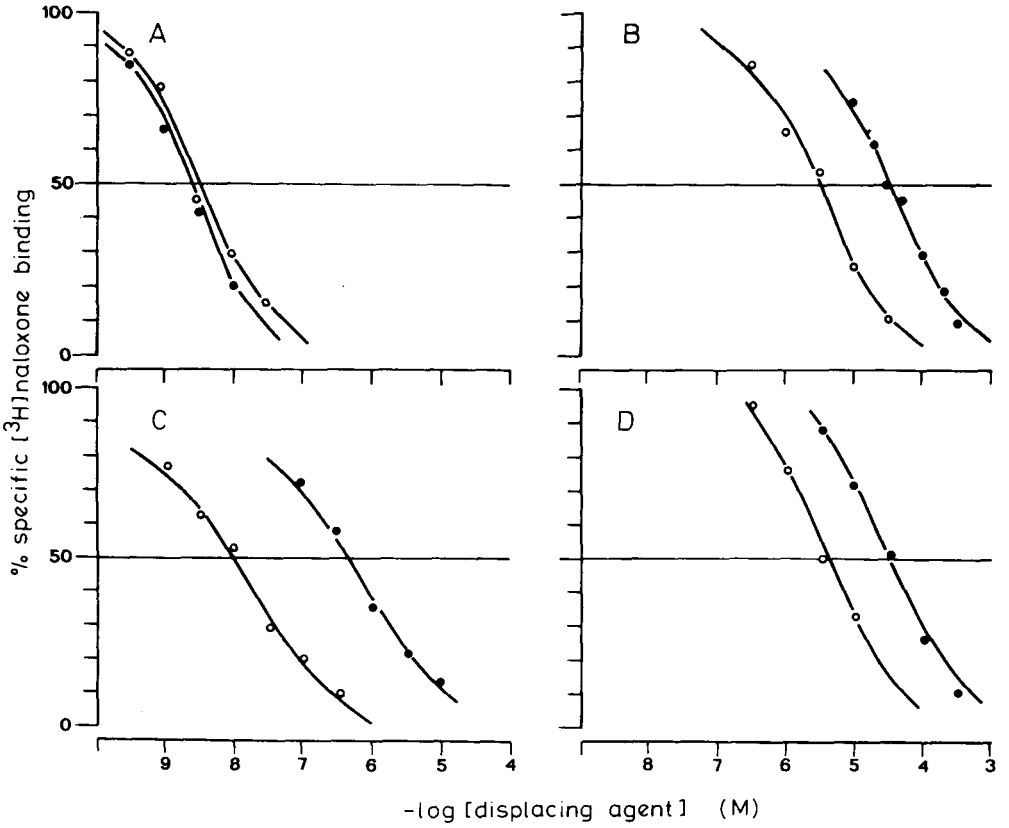

FIG. 1. Effect of 100 mM NaCl on [^{3}H]naloxone binding displacement by increasing concentration (M) of (A) naloxone, (B) trimebutine, (C) morphine and (D) NDM-trimebutine ○——○ [NaCl] = 0; ●——● [NaCl] = 100 mM. For experimental conditions see methods section. The sodium index value is the ratio of the IC50 value obtained in presence of 100 mM NaCl and that in absence of NaCl. Values are the means of at least 4 determinations. The sodium index ratio was 0·75 for naloxone, 14·4 for trimebutine, 37 for morphine and 10·0 for NDM-trimebutine.

Such a stimulating activity was also observed in the dog, when TMB was administered either by i.v. infusion or by the oral route (Honde et al 1986).

According to these results, the authors concluded that the atropine-resistant excitatory effects of TMB on colonic spiking activity could be due to an activation of intramural non-cholinergic excitatory neurons.

The results here reported indicate that TMB and NDTMB, its main metabolite, are able to compete with ligands of endogenous opioid receptors. Both compounds show a better affinity for mu receptor subtype although their affinities were 30- and 48-fold less than that of morphine. However, this selectivity is relatively low in comparison with reference drugs, and NDTMB has an even lower selectivity than TMB. According to Childers et al (1979), displacement curves in the absence and presence of 100 mM NaCl indicate that TMB and NDTMB can be characterized as agonists of the B class; this class includes partial agonists like levorphanol or Met^5-enkephalin in contrast to morphine, a full agonist of the A class, and to naloxone, a pure antagonist.

In conclusion, these data are in good agreement with the in-vivo pharmacological results confirming the hypothesis that peripheral opioid receptors are involved in the activity of this compound. However, since the colon responses to TMB are not unequivocal, other mechanisms could be implied in the mode of action of the drug.

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Letter to the Editor

Stereoselective pharmacological effects and benzodiazepine receptor affinity of the enantiomers of Gö 4962

G. D. Bartoszyk*, D. J. Dooley, H. Barth, J. Hartenstein, G. Satzinger, *Gödecke Research Institute, Mooswaldalee 1–9, D-7800 Freiburg, West Germany*

The search for anxioselective drugs has established new concepts of the benzodiazepine (BZ) receptor–GABA receptor–chloride ionophore complex. In particular, drugs acting on BZ receptors are divided both pharmacologically and biochemically into full agonists, partial agonists, antagonists and inverse agonists (Williams 1983; Haefely et al 1985). In the few examples of chiral 1,4-BZs, the in-vivo and in-vitro activity was restricted to the *S*-enantiomers (Haefely et al 1985; Cooper & Yerbury 1986).

* Correspondence.

The triazolobenzothiadiazine Gö 4962 ((±)-7-chloro-2-methyl-5-phenyl-[1,2,4]triazolo[5,1-c][1,2,4]benzothiadiazine-5-oxide, CAS 103541-73-7) was tested for BZ-like activity, and since it can be resolved into its enantiomers (I. Mergelsberg, unpublished) we also tested whether or not stereospecific pharmacological and biochemical effects would be present with these enantiomers.

Anticonflict activity was measured according to Aron et al (1971) in the four-plate test (footshock parameters are 1·5 mA for 0·5 s) and anticonvulsant activity (Krall et al 1978) in the s.c. metrazol test (85 mg kg^{-1}). Minimal neurotoxicity was determined by the common