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.

REFERENCES

Blanquet, F., Bouvier, M., Gonella, J. (1985) J. Pharmacol. Exp. Ther. 234: 708–712

Childers, S. R., Creese, I., Snowman, A. M., Snyder, S. H. (1979) Eur. J. Pharmacol. 55: 11–18

Fioramonti, J., Fargeas, M. J., Bueno, L. (1984) J. Pharmacol. 36: 618–621

Frexinos, J., Fioramonti, J., Bueno, L. (1985) Eur. J. Clin. Pharmacol. 28: 181–185

Gillan, M. G. C., Kosterlitz, H. W., Paterson, S. J. (1980) Br. J. Pharmacol. 70: 481–490

Grandjouan, S., Chaussade, S., Thierman-Duffaud, D., Couturier, D. (1985) Gastroentérol. Clin. Biol. 9: 2 bis, 8A

Honde, C., Pascaud, X., Junien, J. L., Bueno, L. (1986) Dig. Dis. Sci. 31: 472S

Kosterlitz, H. W., Paterson, S. J., Robson, L. E. (1981) Br. J. Pharmacol. 73: 939–949

Latour, A., Bueno, L. (1980) Int. J. Bio. Med. Comput. 11: 389–396

Luttecke, K. (1980) Curr. Med. Res. Opin. 6: 437–443

Malavaud, A. (1972) Anesth. Analy. Reanim. 29: 65–69

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

Pert, C. B., Snyder, S. H. (1974) Mol. Pharmacol. 10: 868–879

Poitras, P., Trudel, L., Goyer, R., Junien, J. L., Pascaud, X. (1985) Gastroentérol. Clin. Biol. 9: 2 bis 105A

Sasaki, D., Kido, A., Yoshida, Y. (1984) Int. J. Clin. Pharmacol. Ther. Toxicol. 22: 333–337

Valori, R., Shannon, S., Reddy, S. N., Campbell, D., Collins, S. M. (1985) Gastroenterology 88: 1622

J. Pharm. Pharmacol. 1987, 39: 407–408
Communicated October 26, 1986

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

Table 1. Pharmacological effects and binding characteristics of racemic Gö 4962 and its enantiomers relative to diazepam.

	Anticonflict[a] D100 (mg kg^{-1})	Metrazol s.c.[b] ED50 (mg kg^{-1})	Rotarod[b] TD50 (mg kg^{-1})	[^{3}H]FLU assay[c] K_i (nM)	GABA-ratio[d] R
(±)-Gö 4962	16·1	9·1	130	660	1·3
(+)-Gö 4962	120	70	430	>100 000	—
(−)-Gö 4962	8·3	9·0	17·3	420	1·4
Diazepam	1·1	0·2	4·5	23	2·1

[a] D100: dose which increased number of punished crossings by 100% compared with control.
[b] ED50, TD50: dose at which 50% of the animals were protected from clonic seizures in the metrazol s.c. test or fell down from the rotating rod, respectively.
[c] Values given are $\bar{x}$, n = 2–4. The K_i values refer to those obtained in the absence of 100 μM GABA, and were calculated from the Cheng-Prusoff equation: $K_i = IC50/(1 + \frac{[^3H]}{K_D})$ in which [^{3}H] was 0·5 nM and K_D was 5·5 nM (2·8 nM in the presence of GABA). Substances not included above but tested for comparative purposes were flunitrazepam (K_i = 6·5 nM, R = 2·0) and the BZ-antagonist Ro 15-1788, (K_i = 3·4 nM, R = 0·9).
[d] Ratio of K_i values in the absence and presence of GABA.

rotarod test (rotating rod, 2 cm diameter, 10 rev min^{-1}, equilibrium test for 2 min). Gö 4962 and diazepam were administered orally in a 0·8% hydroxypropyl methylcellulose suspension 30 min before the tests. Depending on the test, six or ten male mice of NMRI strain (Ivanovas, Kisslegg, FRG), 18 to 23 g, were used for each dose of the drugs in the pharmacological studies. To obtain comparable doses, equipotent doses were calculated from the dose-response curves.

The preparation of neocortical membranes (osmotically shocked to reduce endogenous GABA from rat brains) and the [^{3}H]flunitrazepam ([^{3}H]FLU, 86 Ci mmol^{-1}; New England Nuclear) binding assay have been described elsewhere (Dooley & Bittiger 1982).

Gö 4962 showed anticonflict and anticonvulsive activity without causing sedation or muscle relaxation. Additionally, it exhibited in-vitro and in-vivo affinity for the BZ-receptor. (±)-Gö 4962 was therefore considered to have the characteristics of a partial BZ-agonist both pharmacologically and in the in-vitro BZ-binding assay (Table 1).

The enantiomers of Gö 4962 were found to act stereoselectively both in the [^{3}H]FLU assay and in the pharmacological tests (Table 1). (−)-Gö 4962 was the active enantiomer having the binding characteristics of a partial agonist similar to those of the racemate. In general, this enantiomer was not more than twice as potent as the racemate. However, (−)-Gö 4962 no longer showed a separation between anticonflict and anticonvulsive activity and minimal neurotoxicity. Thus, pharmacologically, (−)-Gö 4962 must be considered as a full BZ-agonist with selectivity inferior to that of diazepam. (+)-Gö 4962, inactive in the [^{3}H]FLU assay, exhibited comparable pharmacological activity at doses 8- to 15-fold higher than those of (−)-Gö 4962. Cooper & Yerbury (1986) found no pharmacological activity of (*R*)-Ro 11-3624 at doses up to 100 times greater than those of the active *S*-enantiomer, methylclonazepam.

Whether (−)-Gö 4962 possesses the active *S*-configuration analogous to that of chiral BZs is open to speculation. Nevertheless, the results demonstrate that Gö 4962 behaves differently from classical BZs. This dissimilarity suggests that Gö 4962 interacts in a more complex fashion with BZ receptors and/or has sites of action additional to these receptors.

REFERENCES

Aron, C., Simon, P., Larousse, C., Boissier, J. R. (1971) Neuropharmacology 10: 459–469

Cooper, S. J., Yerbury, R. E. (1986) Psychopharmacology 89: 462–466

Dooley, D. J., Bittiger, H. (1982) J. Neurochem. 38: 1621–1626

Haefely, W., Kyburz, E., Gerecke, M., Möhler, H. (1985) in: Testa, B. (ed.) Advances in Drug Research 14, Academic Press, London, pp 165–322

Krall, R. L., Penry, J. K., White, B. G., Kupferberg, H. J., Swinyard, E. A. (1978) Epilepsia 19: 409–428

Williams, M. (1983) J. Med. Chem. 26: 619–628