22. R. Lotan, G. Beattie, W. Hubbel and G. L. Nickelson, *Biochemistry* **16**, 1787 (1977).
23. B. F. Kilpatrick and M. G. Caron, *J. biol. Chem.* **258**, 13528 (1983).
24. S. E. Leff and I. Creese, *Biochem. biophys. Res. Commun.* **108**, 1150 (1982).
25. G. L. Stiles, *J. biol. Chem.* **260**, 6728 (1985).
26. L. T. Williams, D. Mullikin and R. J. Lefkowitz, *J. biol. Chem.* **253**, 2984 (1978).
27. D. R. Sibley and I. Creese, *J. biol. Chem.* **258**, 4957 (1983).
28. M. Watanabe, S. R. George and P. Seeman, *J. Neurochem.* **45**, 1842 (1985).
29. S. Y. Cech and M. E. Maguire, *Molec. Pharmac.* **22**, 267 (1982).
30. K. A. Heidenreich, G. A. Weiland and P. B. Molinoff, *J. biol. Chem.* **257**, 804 (1982).

Biochemical Pharmacology, Vol. 35, No. 17, pp. 2977–2979, 1986.
Printed in Great Britain.

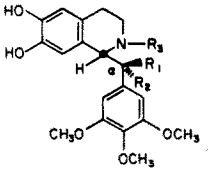
0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

Antiallergic effect of trimetoquinol analogs on actively sensitized guinea pig lung tissue, *in vitro*

(*Received* 28 *February* 1985; *accepted* 5 *February* 1986)

β-Adrenoceptor agonists are drugs widely used for the treatment of allergic bronchial asthma [1, 2]. Trimetoquinol [1-(3,4,5-trimethoxybenzyl)-6-7-dihydroxy-1,2,3,4 tetrahydroisoquinoline (TMQ)] exists as two optical isomers, and *S*(−)-TMQ, is a β_2-selective adrenoceptor stimulant which is currently used in Japan for treatment of moderate bronchial asthma [3, 4]. We found that TMQ is 7 times more selective than isoproterenol as a bronchial relaxant versus a cardiostimulant in guinea pig tracheal and atrial preparations respectively [5]. Our recent studies of chemical modification of TMQ at the α-benzyl carbon atom has led us to compounds with greater β_2-agonist potency and tissue selectivity [5, 6]. Using mono- and dimethyl substituted analogs of TMQ (see Fig. 1), we observed a stereoselectivity and rank order of potency as agonists of tracheal relaxation of *threo*-α-methylTMQ > TMQ > isoproterenol > *erythro*-α-methylTMQ > *N*-methylTMQ > α-dimethylTMQ [5]. Moreover, *N*-methylTMQ, *threo*-α-methylTMQ and *erythro*-α-methylTMQ were 6, 106 and 27 times more selective as β_2-agonists than isoproterenol.

Only a few reports on the effects of TMQ and congeners on antagonism of hypersensitivity reactions are available [7, 8]. Studies of passive cutaneous and systemic anaphylaxis in rodents *in vivo* show that TMQ is more potent than isoproterenol and newer β_2-selective agonists [7]. Blockade of antigen-sensitized histamine release in rat mast cells by TMQ is mediated by β-adrenoceptor activation [7]. Belcheva *et al.* [8] recently found that tetrahydroprotoberberin, a rigid congener of TMQ, is less potent as an antagonist of histamine release *in vivo*. These workers proposed that conformational orientation and flexibility of the 3,4,5-trimethoxybenzyl group of TMQ play an important role in the antiallergic activity of this chemical class of β-adrenoceptor agents.



Compd	R_1	R_2	R_3
Trimetoquinol (TMQ)	H	H	H
Threo-α-methyl TMQ	H	CH_3	H
Erythro-α-methyl TMQ	CH_3	H	H
α-Dimethyl TMQ	CH_3	CH_3	H
N-Methyl TMQ	H	H	CH_3

Fig. 1. Chemical structures of trimetoquinol (TMQ) and methyl-substituted TMQ analogs. The solid circle indicates the presence of the asymmetric carbon atom in the tetrahydroisoquinoline nucleus.

In the present work we have examined the concentration-dependent relationships of these promising TMQ analogs (Fig. 1) on antigen-sensitized histamine release from guinea pig lung tissue. Assessment of structure–activity relationships will allow us to determine if β-adrenoceptor activation is involved for the action of the TMQ analogs in this experimental system.

Male albino Hartley guinea pigs (300–400 g, Glenn Carr, Columbus, OH) were sensitized with chicken egg albumin (ovalbumin) as described by Wong and Buckner [9]. Guinea pigs were killed 3–4 weeks after sensitization. Endogenous catecholamines were depleted by a single intraperitoneal injection of reserpine (5 mg/kg) 12 hr before the experiments. Guinea pigs were killed by exsanguination, and lungs were removed, washed with physiological salt solution, excised free from large blood vessels and bronchi, and chopped into 1-cm fragments by a McIlwaine tissue chopper. Lung slices were filtered and washed thoroughly to remove excess blood. Samples of tissue (200–250 mg) were placed in polypropylene tubes containing 1 ml of Krebs–Henseleit solution of the following millimolar composition; NaCl, 118; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 6; $CaCl_2 \cdot 2H_2O$, 2.5; $NaHPO_4 \cdot 2H_2O$, 1; $NaHCO_3$, 25; and dextrose, 11. Tissues were incubated with 10^{-5} M phenoxybenzamine for 30 min in a metabolic incubator at 37° aerated with 95% O_2 and 5% CO_2. At this time medium was replaced with a fresh solution containing tropolone (10^{-4} M) and preincubated for 15 min, and then for another 15 min in the presence of various drug concentrations. These pretreatments ensured blockade of extraneuronal uptake and inhibition of catechol-*O*-methyl transferase activity. Histamine release was evoked by the addition of ovalbumin, and the samples were incubated at 37° for 15 min for maximum release of histamine. Preliminary experiments indicated that the addition of 50 μg/ml of ovalbumin was sufficient to induce maximum histamine release. The reaction was terminated by placing samples in an ice-cold bath. Each sample was centrifuged at 100 *g* for 15 min at 4° using a Sorvall refrigerated centrifuge. Histamine content in the supernatant fraction was extracted [10] and assayed according to the fluorometric method of Hakanson and Ronnberg [11]. Total histamine content of the lung slices was determined by homogenization of samples followed by boiling for 10 min and centrifugation. Aliquots of the supernatant fraction were assayed for total histamine content. In each experiment, histamine release from triplicate tissue samples for a given concentration of

the test compounds was determined. Data are expressed as the percentage of non-drug-treated control release within the same experiment. For each compound, three to five experiments were performed, and data are presented as the mean ± S.E. Total histamine content (mean ± S.E.) of lung slices was found to be 16.3 ± 1.3 μg/g of tissue (N = 30). The basal and net release of histamine by ovalbumin challenge were 5 and 25%, respectively, of the total histamine content.

Concentration–effect relationships of TMQ analogs for the antagonism of antigen-induced histamine release are shown in Fig. 2A. The rank order of potency for TMQ analogs in these experiments was TMQ > *erythro*-α-methylTMQ = *threo*-α-methylTMQ > *N*-methylTMQ > α-dimethylTMQ. The inhibitory effect of selected TMQ analogs was abolished by preincubation of the tissues with 10^{-6} M (±)-propranolol (Fig. 2B). We also examined the comparative concentration-dependent effects of the optical isomers of TMQ and isoproterenol in this system (Fig. 3). The data indicate that the (−)-isomers were more potent than the corresponding (+)-isomers. The calculated isomeric-activity ratios for TMQ and isoproterenol were 1584- and 1000-fold respectively. The greater inhibitory potency of *S*(−)-TMQ (pIC_{50} = 7.6) versus (−)-isoproterenol (pIC_{50} = 6.95) is in agreement with reports showing that TMQ is more potent than isoproterenol against anaphylactic release of histamine from minced lung slices, *in vitro* [7], on systemic anaphylaxis in guinea pigs [7], and on the activation of β_2-adrenoceptors in guinea pig trachea [3, 5].

Our data show that structurally related analogs and optical isomers of TMQ inhibited antigen-induced histamine release from guinea pig lung pieces by activation of β-adrenoceptors. The inhibition of antigen-induced histamine release by isoproterenol and TMQ in lung parenchymal slices was stereoselective in favor of the (−)-isomers which act as a β-adrenoceptor agonist. This finding was also identical to our previous reports [5, 6, 12, 13] in which

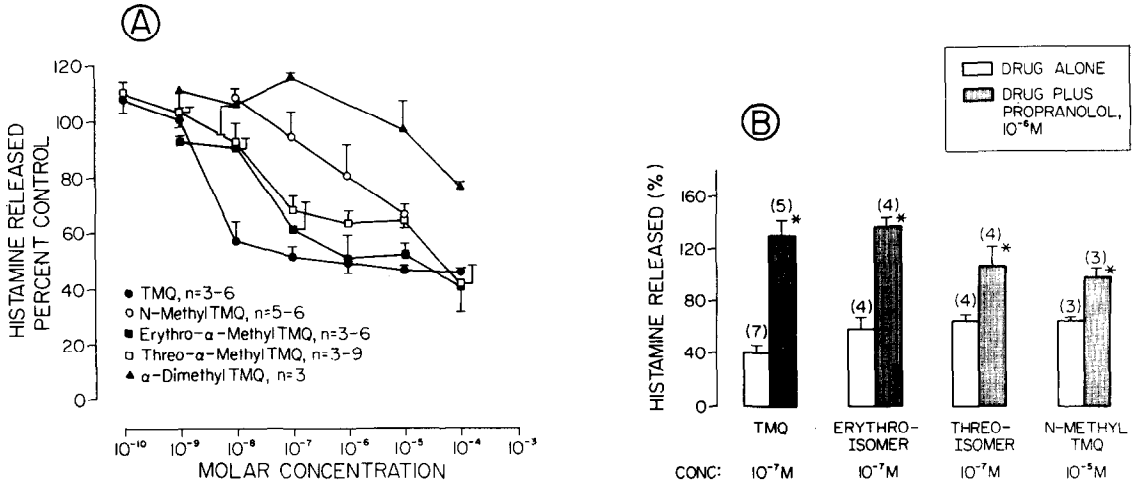


Fig. 2. Comparative concentration-dependent inhibition of antigen-induced histamine release by trimetoquinol analogs from actively sensitized guinea pig minced lung pieces *in vitro* (Fig. 2A), and the effect of (±)-propranolol on the inhibition of histamine release by selected TMQ analogs (Fig. 2B). Data are expressed as the percentage of the non-drug-treated release in each experiment. Mean control release of histamine was 4.07 ± 0.3 μg/g tissue. The concentration of (±)-propranolol was 10^{-6} M. Each data point represents mean ± S.E. The asterisk indicates that the means are significantly different ($P < 0.05$).

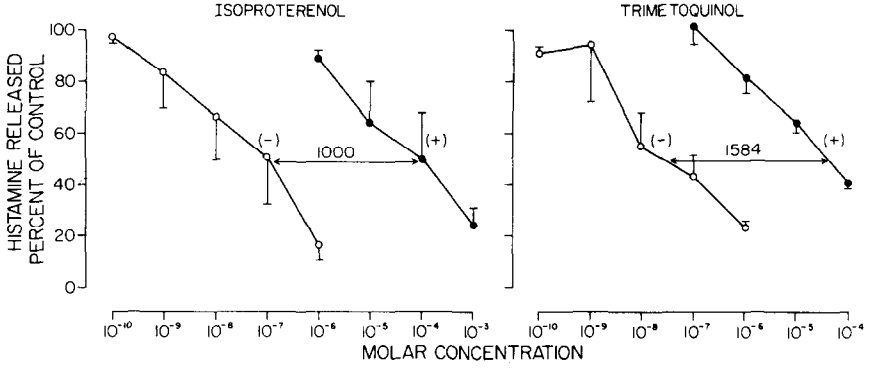


Fig. 3. Comparative concentration-dependent inhibitory effects of the optical isomers of isoproterenol (left panel) and trimetoquinol (right panel) on antigen-induced histamine release from sensitized minced guinea pig lung tissue *in vitro*. Data represent mean ± S.E. of N = 3–4. The numbers (1000 and 1584) are the ratio of the ED_{50} values for the optical isomers and represent the isomeric activity difference.

$S(-)$-TMQ is considerably more potent than $R(+)$-TMQ as a stimulant of β-adrenoceptor systems including lipolysis, relaxation of trachea, and chronotropic activity in heart. Furthermore, the blockade of antigen-sensitized histamine release by the TMQ analogs and the reversal of drug inhibition by propranolol are further evidence for involvement of β-adrenoceptors for this series of compounds. In this regard, Tsuzurahara *et al.* [7] demonstrated that the blockade of histamine release by $S(-)$-TMQ in rat peritoneal mast cells is directly related to elevations in cyclic-3′,5′-adenosine monophosphate and β-adrenoceptor activation.

The rank order of potency of TMQ analogs and isoproterenol for inhibition of antigen-induced histamine release was (−)-TMQ > (−)-isoproterenol > *erythro*-α-methylTMQ = *threo*-α-methylTMQ > *N*-methylTMQ > α-dimethylTMQ. With the exception of *threo*-α-methylTMQ, this rank order is identical to that reported for the activation of β_2-adrenoceptors in guinea pig trachea [5]. Previously, we observed a reduction in potency of TMQ analogs for the stimulation of β-adrenoceptors in guinea pig lung parenchymal versus tracheal strips, and suggested that these more lipophilic analogs of TMQ may bind to nonspecific (or nonreceptor) sites in lung parenchyma [5]. Similar to lung parenchymal strips, the minced lung pieces represent a heterogeneous cell population, and an increased nonspecific binding of *threo*-α-methylTMQ may account for the difference in rank order of potency of this compound in these two pharmacological systems. Further, the ability of propranolol to reverse the inhibitory effect of *threo*-α-methylTMQ on antigen-stimulated histamine release suggests that this drug, like the other TMQ analogs, produces its effect by the stimulation of β-adrenoceptors.

In summary, we conclude that the methyl-substituted TMQ analogs retain significant *in vitro* antiallergic activity and interact with a high degree of stereoselectivity and potency in β-adrenoceptor systems. Further evaluation of these TMQ analogs *in vivo* may provide useful leads to the development of agents for treatment of pulmonary and/or hypersensitivity disorders.

Acknowledgements—The authors gratefully acknowledge the partial support of this work by the National Institute of Health (HL-22533) and a Grant-in-Aid from the American Heart Association with funds contributed in part by the Central Ohio Heart Chapter, Inc. We also thank Mr. James M. Miller for his technical assistance.

Divisions of Pharmacology and Medicinal Chemistry
College of Pharmacy
The Ohio State University
Columbus, OH 43210, U.S.A.

ASOKE MUKHOPADHYAY*
DUANE D. MILLER
DENNIS R. FELLER†

REFERENCES

1. A. F. Wilson and J. J. McPhillips, *A. Rev. Pharmac.* **18**, 541 (1978).
2. D. D. Miller, in *Kirk–Othmer Encyclopedia of Chemical Technology* (Ed. Board: H. Mark, D. Othmer, C. Overberger and G. Seaborg), Vol. 2, 3rd edn, pp. 758–82. John Wiley, New York (1978).
3. Y. Iwasawa and A. Kiyomoto, *Jap. J. Pharmac.* **17**, 143 (1967).
4. Y. Yamamura and S. Kishmoto, *Ann. Allergy* **26**, 504 (1968).
5. A. Mukhopadhyay, D. J. Sober, J. Chang, R. T. Slenn, H. M. Amin, D. D. Miller and D. R. Feller, *Eur. J. Pharmac.* **77**, 209 (1982).
6. D. J. Sober, J. Chang, J. W. Fowble, A. Mukhopadhyay, D. R. Feller and D. D. Miller, *J. med. Chem.* **24**, 970 (1981).
7. K. Tsuzurahara, K. Ono, K. Ogiwara, T. Murata and S. Takeyama, *Chem. pharm. Bull. Tokyo.* **27**, 1715 (1979).
8. A. Belcheva, D. Zhelyakov, B. Manevska and Ch. Ivanov, *Biomed. Biochim. Acta* **43**, 803 (1984).
9. S. K. Wong and C. K. Buckner, *J. Pharmac. exp. Ther.* **214**, 152 (1980).
10. P. A. Shore, A. Burkhalter and V. J. Cohn, *J. Pharmac. exp. Ther.* **127**, 182 (1959).
11. R. Hakanson and A. L. Ronnberg, *Ann. Biochem.* **60**, 560 (1974).
12. D. R. Feller, M. T. Piascik and D. D. Miller, in *Recent Advances in Pharmacology of Adrenoceptors* (Eds. E. Szabadi, C. M. Bradshaw and P. Bevan), pp. 111–20. Elsevier North-Holland, Amsterdam (1978).
13. R. F. Shonk, D. D. Miller and D. R. Feller, *Biochem. Pharmac.* **20**, 3403 (1971).

* Present address: Department of Pharmacology, Smith Kline & French, 1500 Spring Garden, Philadelphia, PA 19101.

† Address correspondence to: Dennis R. Feller, Ph.D., College of Pharmacy, The Ohio State University, 500 West 12th Ave., Columbus, OH 43210.

Biochemical Pharmacology, Vol. 35, No. 17, pp. 2979–2982, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

The activity of UDP-glucuronyltransferase, sulphotransferase and glutathione-*S*-transferase in primary cultures of rat hepatocytes

(*Received* 12 *March* 1986; *accepted* 12 *March* 1986)

Primary cultures of hepatic parenchymal cells are widely used to study mechanisms of cytotoxicity and carcinogenesis of xenobiotics under defined conditions *in vitro* [1, 2]. The cytotoxic and carcinogenic potency of chemicals depends upon the balance between activation and detoxification processes in the cells. Activation is often associated with the cytochrome P-450 mixed function oxidase (MFO)* system, while the Phase II conjugation reactions are usually considered to detoxify xenobiotics. Alterations in the balance between these two processes during culture may limit the application of cultured hepatocytes to studies of cytotoxicity and carcinogenesis.

Very little is known about the maintenance of conjugation reactions during primary culture of hepatocytes, whereas MFO activity is known to decline to low levels within the first 24–48 hr of culture [3–5]. We have measured the activity of UDP-glucuronyltransferase (GT, EC 2.4.1.17) and sulphotransferase (ST, EC 2.8.2) in cultured hepatocytes using the model compounds 1-naphthol and

* Abbreviations used: GT UDP-glucuronyltransferase, ST sulphotransferase, GSH reduced glutathione, MFO mixed function oxidase, CDNB 1-chloro-2,4-dinitrobenzene, HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.