column and similar elution profiles were observed for two groups. The recovery of cadmium as metallothionein was only a few per cent of the applied amount in both groups and this suggested the possibility that the recovered cadmium as metallothionein 30 min after the injection was incorporated by exchange with zinc in the endogenous zinc-thionein. The result indicated that the injected cadmium-thioneins were degraded at least to a certain extent within 30 min after the injection.

The amount of cadmium recovered as metallothionein from the kidneys one day after the injection increased more than that for 30 min after the injection. The recovered metallothioneins in both groups were mixtures of the two forms which bonded not only with cadmium but also with zinc.

Figure 1 shows the elution patterns of kidney metallothioneins obtained three days after the injection of cadmium-thionein-I or -II. The two elution profiles were similar and both forms of metallothionein bonded not only with cadmium but also with a large amount of zinc.

Although the primary amino acid sequences of both forms of rat metallothionein have not been determined, the sequences of the two forms were assumed to be different as in the case of equine kidney metallothioneins [7] and mouse liver metallothioneins [8, 9]. Therefore, the interconversion of the two forms cannot occur and the two forms cannot be obtained unless the synthesis occurs *de novo*. As the affinity of zinc to thionein is 3000 times weaker than that of cadmium [10], the replacement of cadmium in metallothionein by zinc is not possible unless the synthesis of metallothionein occurs *de novo*. Therefore, degradation of the injected cadmium-thioneins and resynthesis of metallothionein in the kidneys were confirmed. The metallothionein induced by the injection of cadmium-thionein was rich in zinc (and low in copper content) as liver metallothionein induced by injection of cadmium ion and was different from kidney metallothionein (which is rich in copper) induced by injection of cadmium ion [11].

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Beta-adrenoceptor blocking activity of 1-substituted trimetoquinol analogs

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Trimetoquinol (TMQ; 6,7-dihydroxy-1-[3′,4′,5′-trimethoxybenzyl]-1,2,3,4-tetrahydroisoquinoline) was first reported to be a potent beta-adrenoceptor stimulant by Iwasawa and Kiyomoto [1]. Later work with the stereoisomers of TMQ demonstrated that much of the pharmacological activity in beta-adrenoceptor systems resides in the *S*(−)-isomer [2–4]. TMQ has been shown to be equipotent at both $beta_1$- and $beta_2$-adrenoceptors and nearly equipotent to isoproterenol on lipolysis [2], cAMP accumulation in isolated rat adipocytes [4] and the stimulation of adenylate cyclase from isolated rat adipocyte plasmalemma.*

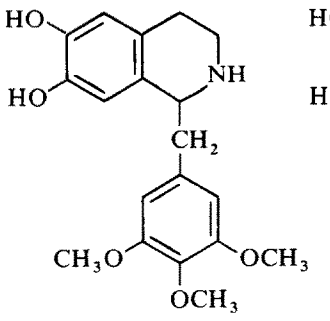
In an attempt to develop more potent and/or more selective adrenergic agents within the tetrahydroisoquinoline (THI) series, Miller *et al.* [5] examined the effect of increasing the size of the substituent at the 1-carbon position of TMQ by synthesizing 1-methyltrimetoquinol (1-methyl TMQ) and 1-benzyltrimetoquinol (1-benzyl TMQ). Miller *et al.* [5] found neither compound capable of inducing significant tracheal relaxation or lipolysis ($beta_2$ and $beta_1$ respectively). In the right atrium preparation, 1-methyl TMQ was a weak agonist while 1-benzyl TMQ was inactive. Of interest was the fact that, in the guinea pig, 1-benzyl TMQ was a competitive antagonist of TMQ-induced increases in chronotropy (right atria) and was ineffective in tracheal relaxation [5].

This study was performed to obtain a clearer profile of the structural aspects of the adrenoceptor activity and biochemical mechanisms possessed by 1-methyl TMQ and 1-benzyl TMQ. The adrenoceptor blocking properties of 1-methyl TMQ and 1-benzyl TMQ were characterized in three pharmacological systems (glycerol release, cAMP accumulation and adenylate cyclase activation) associated with rat adipocytes.

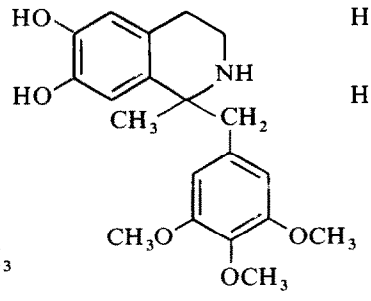
Experimental

General. Male Sprague–Dawley (Harlan) rats weighing between 160 and 220 g were employed in all experiments. Animals (eight to fifteen per experiment) were stunned and killed by cervical dislocation. Epididymal fat pads were removed and placed in Krebs–Ringer bicarbonate buffer. Fat cells were isolated by the method of Rodbell [6] employing crude bacterial collagenase (Worthington Biochemicals,

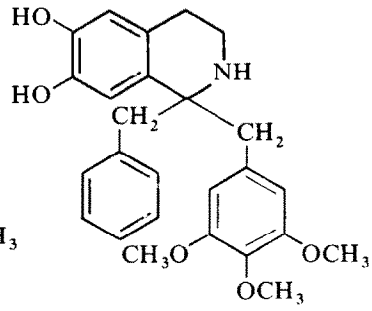
* M. T. Piascik and D. R. Feller, manuscript in preparation.



Trimetoquinol



1-Methyltrimetoquinol



1-Benzyltrimetoquinol

Freehold, NJ). Following several washings with the Krebs–albumin buffer (37°), a fat cell suspension was obtained by centrifugation at 750 *g* for 15 secs. This packed cell suspension was used undiluted as a starting material in all experiments.

Lipolysis. The ability of 1-methyl and 1-benzyl TMQ to produce an antagonism of (±)-TMQ and (−)-isoproterenol-induced glycerol release (as an index of lipolysis) was studied as described previously [4]. Mixtures contained 0.2 ml of the packed cell suspension, 1-substituted THIs, and Krebs–albumin buffer, pH 7.4, in a final volume of 2.5 ml. After a 15-min preincubation, appropriate concentrations of agonist were added and the flasks were incubated for an additional 30 min in an atmosphere of 95% O_2–5% CO_2 at 37° (100 oscillations/min). Reactions were terminated by the addition of an equal volume of trichloroacetic acid (TCA, 10%, w/v) and the amount of glycerol released was measured by procedures described previously [7, 8]. In each experiment, a maximal rate of glycerol release was obtained in the presence of 10^{-7} M (±)-TMQ or 10^{-5} M (−)-isoproterenol. This maximal response was employed to calculate per cent inhibition observed by the 1-methyl and 1-benzyl TMQ analogs. Rates of drug-induced lipolysis were corrected for basal activity.

Cyclic AMP accumulation studies. The ability of 1-methyl TMQ and 1-benzyl TMQ to antagonize intracellular cAMP accumulation in rat adipocytes induced by (±)-TMQ and (−)-isoproterenol was determined essentially as described previously [4]. Reaction mixtures contained 0.3 ml of the undiluted packeted cell suspension, 0.1 ml of theophylline (10^{-6} M), 0.05 ml of 1-substituted TMQ analogs, and 1.95 of the Krebs–albumin buffer, pH 7.4. All flasks were preincubated for 15 min prior to initiation of the reaction by the addition of (±)-TMQ or (−)-isoproterenol. The reaction was allowed to proceed for 10 min and TCA was added to terminate the reaction. The isolation and quantitation of cyclic AMP were determined by the procedures outlined by Gilman [9]. In each experiment, a maximal cAMP accumulation was obtained in the presence of 10^{-7} M (±)-TMQ or 10^{-5} M (−)-isoproterenol, and this value was used to calculate the dose–response relationships for each drug.

Adenylate cyclase activity in plasmalemma of rat adipocytes. The packeted cell suspension was used as a starting point to isolate rat plasmalemma. Adipocytes were placed in a hypotonic lysing medium (1:10 dilution of the Krebs–albumin buffer) and adipocyte plasmalemma was isolated by low speed centrifugation (G. Krishna, personal communication, 1977) and resuspended in 40 mM Tris–HCl (pH 7.4). Final concentrations in the 0.1-ml reaction mixture were: 40 mM Tris–HCl buffer (pH 7.6); 3.0 mM $MgCl_2$; 1 mM cold cyclic AMP; 7 mM theophylline; 0.1 mg creatine phosphokinase (140 units/mg); 1 μCi α-[^{32}P]ATP (Amersham Searle, Arlington Heights, IL, 80–250 Ci/m-mole); blocking agent (1-substituted TMQ analog) and (±)-TMQ (10^{-7}–10^{-4} M) or (−)-isoproterenol (10^{-8} M). Reactions were initiated with the addition of 0.03 ml of the plasmalemma preparation. Protein concentration was determined by the method of Lowry *et al.* [10] and the amount of plasmalemma used was between 0.03 and 0.1 mg protein/assay. Flasks were incubated and reactions stopped by the addition of 0.1 ml of 100 mM EDTA. 8-[^{3}H]-cAMP (2×10^4 dis./min) was added to each flask in a 0.3 ml volume to monitor the extent of product recovery during the isolation procedure. The double column chromatographic method outlined by Salomon *et al.* [11] was used to isolate [^{3}H]- and [^{32}P]-cAMP from the reaction mixture. The ^{3}H and ^{32}P present in the final column eluates were determined simultaneously on a Beckman LS-355 scintillation counter using external standardization as a quench monitoring method. Efficiencies of ^{3}H and ^{32}P detection were 30 and 98 per cent respectively. Recovery of cAMP varied from 25 to 45 per cent, and linearity in product formation was maintained for the 10-min period.

The basal rate of cAMP formation was subtracted from drug-induced rates to obtain the net pmoles of cAMP formed/mg of protein/min. In each experiment, a maximal cAMP

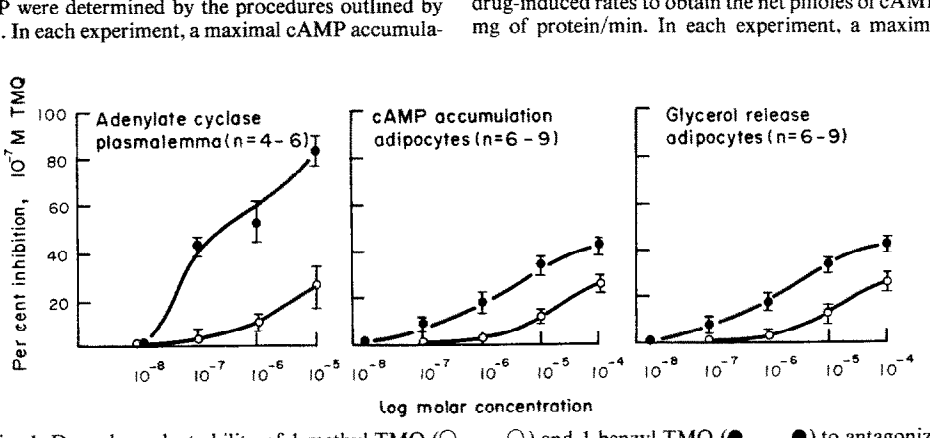


Fig. 1. Dose-dependent ability of 1-methyl TMQ (○——○) and 1-benzyl TMQ (●——●) to antagonize (±)-TMQ-induced (10^{-7} M) glycerol release (right panel), cAMP accumulation (middle panel) and adenylate cyclase activation (left panel) in rat adipocyte preparations. Each point represents the mean ± S.E.M. of N = 4–9.

Table 1. Inhibitory actions of 1-methyl TMQ (10^{-4} M) and 1-benzyl TMQ (10^{-4} M) on (−)-isoproterenol-stimulated lipolysis, cAMP accumulation and adenylate cyclase activation in isolated rat adipocyte preparations. *

Experimental system	% Inhibition†	
	1-Methyl TMQ	1-Benzyl TMQ
Lipolysis		
(−)-Isoproterenol (10^{-7} M)	40 ± 7‡	45 ± 10‡
cAMP accumulation		
(−)-Isoproterenol (10^{-7} M)	25 ± 6‡	42 ± 12‡
Adenylate cyclase activation		
(−)-Isoproterenol (10^{-8} M)	10 ± 4	75 ± 15‡

* Methods are as described in the text.
† Each value represents the mean ± S.E.M. of N = 3.
‡ Significant difference ($P < 0.05$) from corresponding control value.

formation rate was generated in the presence of 10^{-7} M (±)-TMQ or 10^{-8} M isoproterenol and this value was used to calculate the per cent of the maximal cAMP response for each test drug concentration and to calculate the per cent inhibition by each antagonist.

Results

The effect of structural modification at the 1-carbon position of TMQ was investigated by examining the ability of 1-methyl TMQ and 1-benzyl TMQ to promote and/or inhibit glycerol release, cAMP accumulation and adenylate cyclase activity in rat adipocyte preparations. In none of the test systems did the 1-substituted derivatives demonstrate agonist activity in concentrations ranging from 10^{-7} to 10^{-4} M. Figure 1 (right panel) shows that both the 1-methyl and 1-benzyl analogs were able to produce a dose-dependent inhibition of TMQ-induced lipolysis. Further examination of the dose–response curves revealed that the 1-benzyl analog was more potent than 1-methyl TMQ at antagonizing TMQ-stimulated lipolysis. Similar results were obtained on the ability of the 1-methyl and 1-benzyl analogs to antagonize TMQ-induced cAMP accumulation in isolated rat adipocytes (see Fig. 1, middle panel). In these studies, 1-benzyl TMQ produced approximately 45 per cent inhibition at 10^{-4} M, whereas 1-methyl TMQ produced a 25 per cent inhibition of TMQ-induced cAMP accumulation.

The 1-methyl and 1-benzyl TMQ analogs were examined for their differential ability to produce antagonism of TMQ-stimulated adenylate cyclase from isolated plasmalemma (see Fig. 1, left panel). 1-Benzyl TMQ was a more potent inhibitor of TMQ-induced activation of adenylate cyclase than the 1-methyl analog. The 1-benzyl compound produced greater than 80 per cent inhibition of TMQ-induced activation of adenylate cyclase at 10^{-5} M.

In another experiment (Table 1), the 1-substituted TMQ compounds were found to inhibit (−)-isoproterenol-induced glycerol release, cAMP accumulation and adenylate cyclase activation in rat adipocyte preparations. These data reveal that (a) both TMQ analogs are capable of producing an inhibition of (−)-isoproterenol-induced responses and (b) 1-benzyl TMQ is a more effective inhibitor of isoproterenol than 1-methyl TMQ.

The antagonism of TMQ-stimulated adenylate cyclase activity and glycerol release by 1-benzyl TMQ appears to be competitive in nature. As indicated in Fig. 2, 1-benzyl TMQ is capable of producing parallel shifts in the dose–response curve of TMQ on the stimulation of glycerol release (Fig. 2, left panel) and on the activation of adenylate cyclase (Fig. 2, right panel). The pA_2 value [12] for 1-benzyl TMQ as an antagonist of glycerol release was calculated to be 4.5.

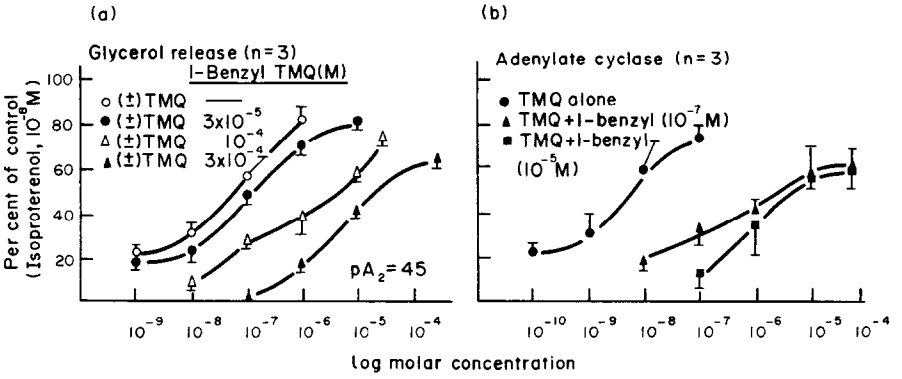


Fig. 2. Dose-dependent ability of 1-benzyl TMQ to produce shifts in the dose–response curves of (±)-TMQ on glycerol release (left panel) and adenylate cyclase activation (right panel) in rat adipocyte preparations. Each point represents the mean ± S.E.M. of N = 3. Key: Left panel, ○——○, TMQ alone; ●——●, TMQ and 3×10^{-5} M 1-benzyl TMQ; △——△, TMQ and 10^{-4} M 1-benzyl TMQ; and ▲——▲, TMQ and 3×10^{-4} M 1-benzyl TMQ. Right panel, ●——●, TMQ alone; ▲——▲, TMQ and 10^{-7} M 1-benzyl TMQ; and ■——■, TMQ and 10^{-5} M 1-benzyl TMQ

Discussion

In these experiments, several levels of investigation were employed to characterize the adrenergic blocking properties of the 1-substituted TMQ analogs in rat adipocytes, a $beta_1$-adrenoceptor system. The present investigation has confirmed that both the 1-methyl and 1-benzyl TMQ compounds are inactive as agonists and are inhibitors of lipolysis. 1-Methyl TMQ was also found to be a weak inhibitor of cAMP accumulation and adenylate cyclase activity induced by TMQ and isoproterenol, suggesting that this tetrahydroisoquinoline is an antagonist at the beta-adrenoceptor. It is most interesting to note that the 1-methyl TMQ was an agonist in one $beta_1$-adrenoceptor system (positive chronotropy in the atria [5]) and an antagonist in another $beta_1$ system (lipolysis). This apparent discrepancy is difficult to reconcile. It could be that in the atria, a relatively intact tissue preparation, 1-methyl TMQ could have exhibited properties other than direct beta-receptor activation, e.g. blockage of normal catecholamine reuptake (cocaine-like effect), stimulation of catecholamine release (amphetamine-like effect) or modification of metabolizing enzymes (COMT, MAO) for catecholamines. As noted previously [13, 14], these properties have been observed for other THIs. In this experimental system, however, there are no sympathetic nerve endings or high activity of metabolizing enzymes [15]. Therefore, if 1-methyl TMQ interfered with these steps, then in the isolated fat cell these agonist properties would not be exhibited. Such an observation has been noted previously with a pharmacological study of substituted phenethylamine mediated effects in rat adipose tissue [16]. However, an alternate and equally plausible explanation exists in that 1-methyl TMQ is an agonist at the $beta_1$-receptor of the atria and an antagonist at the $beta_1$-receptor of the adipocyte.

Relative to 1-methyl TMQ, 1-benzyl TMQ was found to be more effective as an inhibitor of TMQ-induced glycerol release, cAMP accumulation and adenylate cyclase activity in rat adipocyte preparations. No qualitative differences in antilipolytic activity were observed between these 1-substituted TMQ analogs. However, 1-benzyl TMQ was quantitatively different in the blockade of adenylate cyclase activity. The blockade of TMQ-induced adenylate cyclase activation and glycerol release by 1-benzyl TMQ was competitive in nature. This is evidenced by the parallel shifts in the dose–response curves of TMQ (see Fig. 2). Our findings are in agreement with the results of Miller *et al.* [5] who demonstrated that 1-benzyl TMQ is a competitive antagonist of TMQ-induced chronotropy in guinea pig atria, a $beta_1$-system. Miller *et al.* [5] also reported that 1-benzyl TMQ was ineffective as an antagonist of guinea pig trachea relaxation, a $beta_2$-adrenoceptor system. These results taken together would indicate that 1-benzyl TMQ has the properties of a selective $beta_1$-adrenoceptor blocker.

In the present study, the 1-substituted TMQ analogs antagonized both (±)-TMQ and (−)-isoproterenol mediated phenomena in rat adipocytes (see Table 1). The comparative inhibitory effects of the 1-benzyl TMQ and 1-methyl TMQ against these beta-agonists were quantitatively similar and lend further support to the proposal that the 1-substituted TMQ analogs are acting at the level of the beta-adrenoceptor rather than at a nonselective site involving an interaction between structurally related tetrahydroisoquinolines.

We have also reported that 1-benzyl TMQ was a competitive inhibitor ($pK_B = 5.1$) of phenylephrine-induced contractions of the isolated rabbit aorta [17]. Therefore, in addition to beta-adrenoceptor blockade, 1-benzyl TMQ also exhibits the properties of an alpha-adrenoceptor antagonist. In this regard, the isomers of TMQ were shown to be equipotent as competitive inhibitors of phenylephrine-induced rabbit aortic contractions [17]. If, indeed, THIs and catecholamines interact at the same alpha- and/or beta-adrenoceptor, this would substantiate the hypothesis of Belleau [18] that the presence of bulky *N*-substituents on catecholamines would hinder ion-pair formation with an alpha-adrenoceptor leading to agents which are antagonists whereas their presence facilitates the formation of an effective drug–receptor complex in beta systems [18]. The 1-substituted TMQ analogs would appear to represent a novel drug entity which possesses nearly equivalent alpha- and $beta_1$-adrenoceptor blocking actions. Clearly, pharmacological investigations into the cardiovascular actions of these 1-substituted TMQ analogs are warranted.

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