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Pharmacological activities of trimetoquinol and 1-benzyl halogen-substituted analogues on rat β-adrenoceptor subtypes

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Abstract

The β-adrenoceptor activity profile of trimetoquinol and its 1-benzyl halogen-substituted analogues was studied in rat tissues containing primarily β_1 (atria)-, β_2 (trachea)- and atypical β/β_3 (distal colon and brown adipose tissue)-adrenoceptors. Functional biological activity resided in the (−)-isomer of trimetoquinol which was 112-, 275-, 372- and 513-fold more potent than (+)-trimetoquinol in trachea, right atria, distal colon and brown adipose tissue, respectively. (±)-Trimetoquinol was equally or slightly less active than (−)-trimetoquinol. The 1-benzyl halogen-substituted analogues of trimetoquinol exhibited differential activation of β-adrenoceptor subtypes. In functional assays, 3′-iodotrimetoquinol was a potent activator of all β-adrenoceptor subtypes. 3′,5′-Diiodotrimetoquinol was 10-fold more potent as an agonist in tissues containing atypical β/β_3-adrenoceptors than those tissues containing β_1- and β_2-adrenoceptor sites. Furthermore, this drug was a partial agonist as compared to (±)-trimetoquinol and 3′-iodotrimetoquinol on β_1-adrenoceptors. Pharmacological properties of the compounds on rat β_3-adrenoceptors expressed in Chinese hamster ovary (CHO) cells were consistent with results observed in functional assays. 3′,5′-Diiodotrimetoquinol possessed the greatest potency for activation of adenylyl cyclase. Rank order of affinity for rat β_3-adrenoceptor was 3′-iodotrimetoquinol = 3′,5′-diiodotrimetoquinol > (±)-trimetoquinol > (−)-isoprenaline. These results suggest that 3′,5′-diiodotrimetoquinol is a promising drug for further chemical modification in the development of selective β_3-adrenoceptor ligands.

Keywords: β-Adrenoceptor; β-Adrenoceptor, atypical; β_3-Adrenoceptor; Isomeric activity ratio; Trimetoquinol; Atrium, rat; Trachea, rat; Distal colon, rat; Brown adipose tissue, rat

1. Introduction

β-Adrenoceptors are cell membrane-bound G-protein-coupled receptors that mediate physiological functions of the endogenous catecholamines adrenaline and noradrenaline. These adrenoceptors were initially classified as β_1 (heart, white adipose tissue)- and β_2 (lung, vasculature)-adrenoceptors by Lands et al. (1967) based on rank order of sympathomimetic amine potencies. Studies with non-selective β-adrenoceptor antagonists (Fassina, 1967) as well as the selective β_1-adrenoceptor antagonist practolol (Stanton, 1972) gave results inconsistent with the classification of β-adrenoceptor in rat adipocytes as β_1-adrenoceptor subtype. Synthesis of novel compounds such as ICI D7114 [(*S*)-4-(2-hydroxy-3-phenoxypropylaminoethoxy)-*N*-(2-methoxy-ethyl)phenoxyacetamide] and BRL 37344 [(*R***R**)-(±)-4-[2′-{2-hydroxy-2-(3-chlorophenyl)ethyl-amino}propyl]phenoxyacetic acid] and its derivatives that selectively elicited thermogenic and lipolytic responses in rat adipose tissue suggested that a β-adrenoceptor with atypical properties is present in rat brown adipocytes (Holloway et al., 1991; Arch et al., 1984; Wilson et al., 1984).

Cloning and characterization of a β-adrenoceptor with atypical properties from a human genomic library conclusively demonstrated the existence of a third subtype of β-adrenoceptor designated as β_3-adrenoceptor (Emorine et al., 1989). Later, the rat homologue of the human β_3-adrenoceptor was cloned from a rat brown adipose tissue cDNA library (Granneman et al., 1991). Rat β_3-adrenoceptors expressed in Chinese hamster ovary (CHO) cells

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exhibited pharmacological properties similar to the atypical β-adrenoceptor present in rat adipocytes (Muzzin et al., 1991). Functional atypical β-adrenoceptors have also been identified in other rat tissues such as distal colon, ileum, oesophageal smooth muscle and skeletal muscle (McLaughlin and MacDonald, 1990; Growcott et al., 1993; Buckner and Christopherson, 1974; Challiss et al., 1988).

Atypical β-adrenoceptors play an important role in lipid metabolism and compounds that selectively activate these receptors may have potential for the treatment of obesity (Arch et al., 1984; Howe, 1993). Additionally, administration of these compounds to diabetic rats and mice decreased their blood glucose levels and improved insulin sensitivity (Smith et al., 1985; Yoshida et al., 1994). These results suggest that β-adrenoceptor agonists may be useful for the treatment of non-insulin-dependent diabetes.

Trimetoquinol is a prototype of the tetrahydroisoquinoline class of compounds (Fig. 1). It has one center of asymmetry and the (−)-isomer of trimetoquinol has been shown to be the biologically active isomer on β-adrenoceptors (Iwasawa and Kiyomoto, 1967; Fraundorfer et al., 1994) whereas the (+)-isomer possesses thromboxane A_2/prostaglandin H_2 antagonist activity (Shin et al., 1993). The chemical structure of trimetoquinol differs significantly from classical β-adrenoceptor agonists such as adrenaline and isoprenaline. It lacks the β-hydroxy group of catecholamines and the amino nitrogen is contained within a semirigid tetrahydroisoquinoline ring. However, it possesses a 3′,4′,5′-trimethoxybenzyl ring at the 1-carbon of the tetrahydroisoquinoline ring that is required for biological activity (Feller et al., 1978). Trimetoquinol has been shown to be a highly potent activator of β-adrenoceptors in guinea pig atria, guinea pig trachea and rat epididymal white adipose tissue (Piascik et al., 1978). However, two structurally related analogues, 3′-iodotrimetoquinol and 3′,5′-diiodotrimetoquinol (see Fig. 1) exhibited partial agonist activity in guinea pig atria and trachea (Shams et al., 1990). Recent studies indicate that compounds which are potent activators of atypical β/β_3-adrenoceptors are either partial agonists or antagonists at β_1- and β_2-adrenoceptor sites (Sugasawa et al., 1992; Blin et al., 1993; Mohell and Dicker, 1989). Moreover, the structure of trimetoquinol closely resembles those of ICI D7114 and BRL 37344, prototypical β_3-adrenoceptor agonists (Fig. 1). Therefore, we have evaluated the functional activities of trimetoquinol and halogenated analogues in rat tissues containing predominantly β_1- or β_2- or atypical β/β_3-adrenoceptor subtypes, and on adenylyl cyclase activation and receptor binding in CHO cells expressing rat β_3-adrenoceptors.

2. Materials and methods

2.1. Pharmacological experiments using isolated tissues and cells

2.1.1. Rat right atria and trachea

Male Sprague Dawley rats (Harlan Industries, Cumberland, IN, USA) weighing between 200–425 g were killed by cervical dislocation and the tissues were quickly removed according to standard procedures (Staff of the

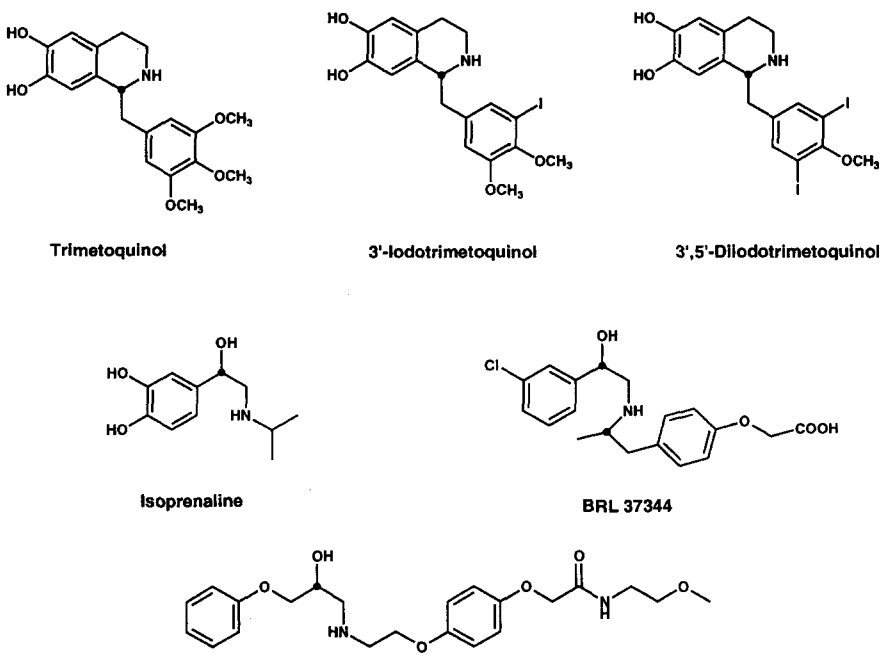

Fig. 1. Chemical structures of trimetoquinol, 1-benzyl halogen-substituted analogues of trimetoquinol, isoprenaline, BRL 37344 [(*R***R**)-(±)-4-[2′-{2-hydroxy-2-(3-chlorophenyl)ethylamino}propyl]phenoxyacetic acid] and ICI D7114 [(*S*)-4-(2-hydroxy-3-phenoxypropylaminoethoxy)-*N*-(2-methoxyethyl)phenoxyacetamide]. The presence of an asymmetric carbon atom is denoted by ● in the chemical structure.

Department of Pharmacology, 1968). Spontaneously beating right atria and trachea were prepared and mounted under a resting tension of 1 g in 10 ml water-jacketed tissue baths containing physiological salt solution (composition in mM: $CaCl_2 \cdot 2H_2O$, 2.5; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 5; NaCl, 118; $NaHCO_3$, 25; $NaH_2PO_4 \cdot H_2O$, 1; and dextrose, 11.1) warmed to 37°C and gassed with 95% O_2-5% CO_2. Both tissues were preincubated with hydrocortisone (10^{-4} M), to inhibit extraneuronal uptake and 10^{-5} M U-0521 (3′,4′-dihydroxy-2-methyl-propiophenone), to inhibit the enzyme catechol-*O*-methyl transferase for 30 min before the construction of concentration-response curves (Trendelenburg, 1980). In tracheal experiments, cocaine (3×10^{-5} M) was also present to inhibit adrenergic neuronal uptake (Iversen, 1967). In some experiments, 10^{-7} M pindolol was added as a β-adrenoceptor antagonist. Both right atrial and tracheal tissues were used to construct concentration-response curves only once for each agonist.

Cumulative concentration-response curves were generated on spontaneously beating right atria, after an initial equilibration period of 60 min, to measure the chronotropic effects of trimetoquinol and its analogues.

Spirally cut tracheal strips were equilibrated for 60 min and then precontracted with carbachol (3×10^{-7} M) to induce a response equal to 60–70% of the maximum. After stabilisation of the tracheal contraction (approximately 10–15 min), cumulative concentration-relaxation curves were constructed for each drug. In preliminary experiments, no significant changes in the carbachol-induced contraction of tracheal strips were observed over the time period used for studies with agonists.

In both tissues, drug-induced effects were expressed as a percentage of the maximal response to 10^{-5} M (−)-isoprenaline, added to tissue baths on completion of each concentration-response curve.

2.1.2. Rat distal colon and brown adipose tissue

Harlan male rats (180–400 g) were killed by CO_2 asphyxiation, tissues were dissected out and prepared according to the procedures described by McLaughlin and MacDonald (1990) for rat distal colon and Rodbell (1964) as modified by Bukowiecki et al. (1981) for brown adipocytes, respectively. Pindolol (10^{-6} M) was present in some experiments to block the β_1- and β_2-adrenoceptor-mediated responses in these tissues.

Rat distal colon segments (about 3 cm) were mounted under 2 g tension in an organ bath containing 10 ml physiological salt solution and pretreated for 30 min with hydrocortisone (10^{-5} M), cocaine (3×10^{-5} M), U-0521 (10^{-5} M) and the α-adrenoceptor antagonist phentolamine (10^{-5} M). An approximate 75% maximal tone was induced in these segments with 30 mM KCl. Cumulative concentration-response curves were constructed for relaxation of colon segments by trimetoquinol and its analogues. Following addition of the highest concentration of each compound, 10^{-5} M (−)-isoprenaline was added to determine the maximal relaxation response in each tissue.

Interscapular brown adipose tissue (approximately 0.5 g wet weight/animal) was dissected free of surrounding white adipose and finely minced with scissors. The tissue was then suspended in physiological salt solution (pH 7.4) containing 4% bovine serum albumin and bacterial collagenase (5 mg/ml). Following an incubation period of 45–60 min at 37°C, the suspension was filtered through a nylon mesh screen. The filtrate was centrifuged for 1 min at 200 × g and adipocytes floating on top of the solution were recovered. Adipocytes ($0.5–1.5 \times 10^6$ cells) were incubated with varying concentrations of drugs in 1 ml (final volume) of physiological salt solution containing 4% bovine serum albumin for 30 min at 37°C. Trichloroacetic acid (5% final concentration) was added to quench the reactions. Aliquots of the medium were assayed for glycerol release by the method of Nash (1953).

2.1.3. Radioligand binding and other assays

Radioligand binding assays were carried out as described before (Fraundorfer et al., 1994). CHO cells expressing rat β_3-adrenoceptors were harvested into Ham's F-12 solution following digestion with trypsin. Cells were centrifuged and the cell pellet was washed three times with Tris-EDTA (composition in mM: TRIZMA HCl, 75 mM; NaCl, 154 mM; and disodium EDTA $\cdot 2H_2O$, 20 mM) buffer (pH 7.4). Competition binding assays were performed in duplicate by incubating $0.8–1 \times 10^6$ cells/130–200 pM [^{125}I]iodocyanopindolol and varying concentrations of competitors for 1 h at 37°C. Non-specific binding was determined in the presence of propranolol (10^{-4} M). Incubations were terminated by filtration over Whatman GF/B glass fiber filters using a Brandel model 12-R cell harvester. The radioactivity in the filter discs was quantitated by gamma scintillation counting (Beckman gamma counter Model 8000). The PC version of the radioligand binding program LIGAND (McPherson, 1985) was used to calculate dissociation constants (K_i) for each drug for displacing [^{125}I]-iodocyanopindolol (K_d = 1.3 nM, Muzzin et al., 1991) from rat β_3-adrenoceptors.

Accumulation of cAMP in confluent cultures of CHO cells expressing rat β_3-adrenoceptors was determined as described previously (Fraundorfer et al., 1994). Briefly, CHO cells were grown to confluence and washed with Hank's balanced salt solution. Cells were then incubated with Hank's buffer (pH 7.4) containing 1 mM 3-isobutyl-1-methylxanthine and 20 mM Hepes for 20 min at 37°C. Various concentrations of drugs were then added and cells incubated for an additional 30 min. Hank's buffer was then removed and the cAMP generated in these cells was extracted by trichloroacetic acid (6% w/v) and the precipitated cellular protein was dissolved in 0.1 N NaOH. cAMP content in samples and standards was estimated by radioimmunoassay as described by Brooker et al. (1979). cAMP was measured as the amount of ^{125}I-labelled suc-

cinyl-cAMP tyrosine methyl ester/antibody precipitated by addition of ammonium sulfate solution (60% of saturation). A standard curve generated by GraphPad InPlot was used to interpolate sample cAMP concentrations. Protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

2.1.4. Data analysis

Data are expressed as means ± S.E.M. of the given number of experiments. Statistical difference between two data sets was determined by Student's *t*-test (paired: trachea, distal colon and brown adipose tissue; unpaired: right atria) at a significance level of 5%.

2.1.5. Drugs and cell system

Chemicals used in this study were procured from the following sources: hydrocortisone sodium succinate (Abbott laboratories, Chicago, IL, USA), carbamyl choline chloride (Aldrich Chemical Co., Milwaukee, WI, USA), (−)-3-[^{125}I]iodocyanopindolol (2000 Ci/mmol, Amersham, Arlington Heights, IL, USA), (±)-propranolol (Ayerst Laboratories, New York, NY, USA), fetal bovine serum, Geneticin, L-glutamine, Ham's F-12 culture medium, Hank's balanced salt solution, penicillin-streptomycin solution and trypsin-EDTA solution (Gibco, Gaithersburg, MD, USA), cocaine HCl (Mallinckrodt Chemical Works, St. Louis, MO, USA), (±)-pindolol (Receptor Research Biochemicals, Baltimore, MD, USA), bovine serum albumin, 3-isobutyl-1-methylxanthine, (−)-isoprenaline-(+)-bitartrate, phentolamine HCl and TRIZMA HCl (Sigma Chemical Co., St. Louis, MO, USA) and bacterial collagenase (Worthington Biochemicals Corp., Freehold, NJ, USA). The isomers of trimetoquinol [(−)-(*S*)-trimetoquinol, $\alpha_D = -28.5$ (99.9% stereochemical purity) and (+)-(*R*)-trimetoquinol, $\alpha_D = +29.0$ (99.78% stereochemical purity)] were generously provided by Dr Yoshio Iwasawa (Tanabe Seiyaku Co., Osaka, Japan) and U-0521 was provided by Dr Popat N. Patil (College of Pharmacy, The Ohio State University, Columbus, OH, USA). ICI D7114 was a gift from ICI Pharmaceuticals (Macclesfield, UK). (±)-Trimetoquinol and the 1-benzyl halogen-substituted analogues of trimetoquinol were provided by Dr Duane D. Miller (Department of Pharmaceutical Sciences, University of Tennessee, Memphis, TN, USA). Drugs were dissolved at their highest concentration (1–10 mM) in double distilled water or Tris-EDTA buffer solution. All other chemicals used were of reagent grade.

Chinese hamster ovary (CHO) cells transfected with and expressing a homogeneous population of rat β_3-adrenoceptors were a gift from Dr. Claire Fraser (Institute of Genomic Research, Gaithersburg, MD, USA). Cells were grown in sterile culture flasks containing Ham's F-12 culture medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 μg/ml Geneticin and 50 μg/ml penicillin-streptomycin in a humidified atmosphere of 5% CO_2-balance air at 37°C.

3. Results

3.1. Pharmacological studies in isolated tissues and rat brown adipocytes

3.1.1. Functional activities of trimetoquinol isomers on rat β-adrenoceptor subtypes

The isomers of trimetoquinol elicited functional responses in rat tissues containing predominantly β_1 (atria)-, β_2 (trachea)- and atypical β/β_3 (distal colon, brown adipocytes)-adrenoceptors in a stereoselective manner (Table 1). The (−)-isomer was 112-, 275-, 372- and 513-fold more potent than the (+)-isomer and (−)-trimetoquinol possessed agonist potencies (pEC_{50} values) of 7.53, 8.44, 8.42 and 8.08 in trachea, atria, distal colon and brown adipose tissue, respectively (Table 1).

On rat atria and trachea, the non-selective antagonist pindolol (at 10^{-8} M and 10^{-7} M) produced concentration-dependent parallel rightward shifts of the response curves to (−)-trimetoquinol without change in the maximal response (data not shown). Experimentally determined pK_B values for 10^{-7} M pindolol on rat atria and trachea were 8.83 and 8.6, respectively (Table 2). However, pindolol at 10^{-6} M, exhibited a low antagonist potency to drug-induced responses in rat distal colon and brown adipose tissue. Experimentally derived pK_B values for 10^{-6} M pindolol on distal colon and brown adipocytes were 6.16 and 6.37, respectively (Table 2).

3.1.2. Functional activities of trimetoquinol analogues on rat β-adrenoceptor subtypes

Responses to (±)-trimetoquinol, 1-benzyl halogen-substituted analogues, and the standard agonist (−)-isoprenaline in rat tissues are illustrated in Fig. 2. (−)-Isoprenaline and (±)-trimetoquinol were potent agonists of β-adrenoceptor subtypes present in atria, trachea, distal colon and brown adipocytes. Corresponding pEC_{50} ± S.E.M. values of (−)-isoprenaline and (±)-trimetoquinol on these tissues were 8.20 ± 0.03 and 8.17 ± 0.11; 7.75 ± 0.11 and 7.89 ± 0.09; 7.89 ± 0.09 and 8.18 ± 0.03; 7.99 ± 0.12 and

Table 1
Agonist activities of the isomers of trimetoquinol on β-adrenoceptor subtypes in rat tissues

Receptor subtype (tissue)	pEC_{50} [a] ± S.E.M.		Isomeric activity ratio [b]
	(−)-Isomer	(+)-Isomer	
β_1 (right atria)	8.44 ± 0.10	6.00 ± 0.13 [c]	2.44 ± 0.13
β_2 (trachea)	7.53 ± 0.12	5.48 ± 0.16 [c]	2.05 ± 0.16
β_3 (distal colon)	8.42 ± 0.13	5.85 ± 0.11 [c]	2.57 ± 0.11
β_3 (brown adipose)	8.08 ± 0.19	5.47 ± 0.17 [c]	2.61 ± 0.17

[a] $pEC_{50} = -\log EC_{50}$; EC_{50} = concentration required to produce a response equal to 50% of the maximal response elicited by the drug. Data are expressed as the mean ± S.E.M. of $n = 4$–7 experiments. [b] Isomeric activity ratio = antilog [(pEC_{50} (−)-isomer − pEC_{50} (+)-isomer)] ± S.E.M. [c] Significant difference ($P < 0.05$) from the mean value of the corresponding (−)-isomer.

Table 2
Effect of pindolol on concentration-response curves to (−)-trimetoquinol in rat tissues

Parameter	Atria	Trachea	Distal colon	Brown adipose
pEC_{50} [a] ± S.E.M.	8.42 ± 0.08	7.31 ± 0.01	8.29 ± 0.19	8.08 ± 0.19
I.A. [b] ± S.E.M.	0.94 ± 0.04	0.92 ± 0.03	1.00 ± 0.00	1.21 ± 0.08
pEC_{50} [c] ± S.E.M.	6.62 ± 0.15 [e]	5.70 ± 0.20 [e]	7.88 ± 0.20 [e]	7.53 ± 0.11 [e]
I.A. ± S.E.M.	1.00 ± 0.02	0.98 ± 0.01	1.00 ± 0.00	1.03 ± 0.04
pK_B [d] ± S.E.M.	8.83 ± 0.13	8.60 ± 0.18	6.16 ± 0.16	6.37 ± 0.21

[a] $pEC_{50} = -\log EC_{50}$; EC_{50} = concentration required to produce a response equal to 50% of the maximal response elicited by the drug. Data are expressed as the mean ± S.E.M. of $n = 4–5$ experiments. Experiments were performed in the absence of pindolol. [b] I.A. = intrinsic activity: maximal drug-induced response relative to the response to 10^{-5} M(−)-isoprenaline. [c] $pEC_{50} = -\log EC_{50}$; data are expressed as the means ± S.E.M. of $n = 4–5$ experiments. Experiments were performed in the presence of 10^{-7} M (rat atria and trachea) or 10^{-6} M (rat distal colon and brown adipose tissue) pindolol. [d] $pK_B = -\log [A]/(CR - 1)$; where [A] = molar concentration of antagonist and CR = concentration ratio = EC_{50} (plus antagonist)/EC_{50} (control). [e] Significant difference ($P < 0.05$) from mean value in the absence of pindolol.

7.96 ± 0.11, respectively. Mono- and di-iodinated analogues of trimetoquinol exhibited differential effects in these tissue systems. 3′-Iodotrimetoquinol was a potent agonist in rat atria, trachea, distal colon and brown adipose tissue, possessing potencies (pEC_{50} ± S.E.M.) of 8.26 ± 0.06, 7.67 ± 0.16, 8.21 ± 0.37 and 8.05 ± 0.14, respectively. On the other hand, 3′,5′-diiodotrimetoquinol was a more potent agonist in tissues containing atypical β/β_3-

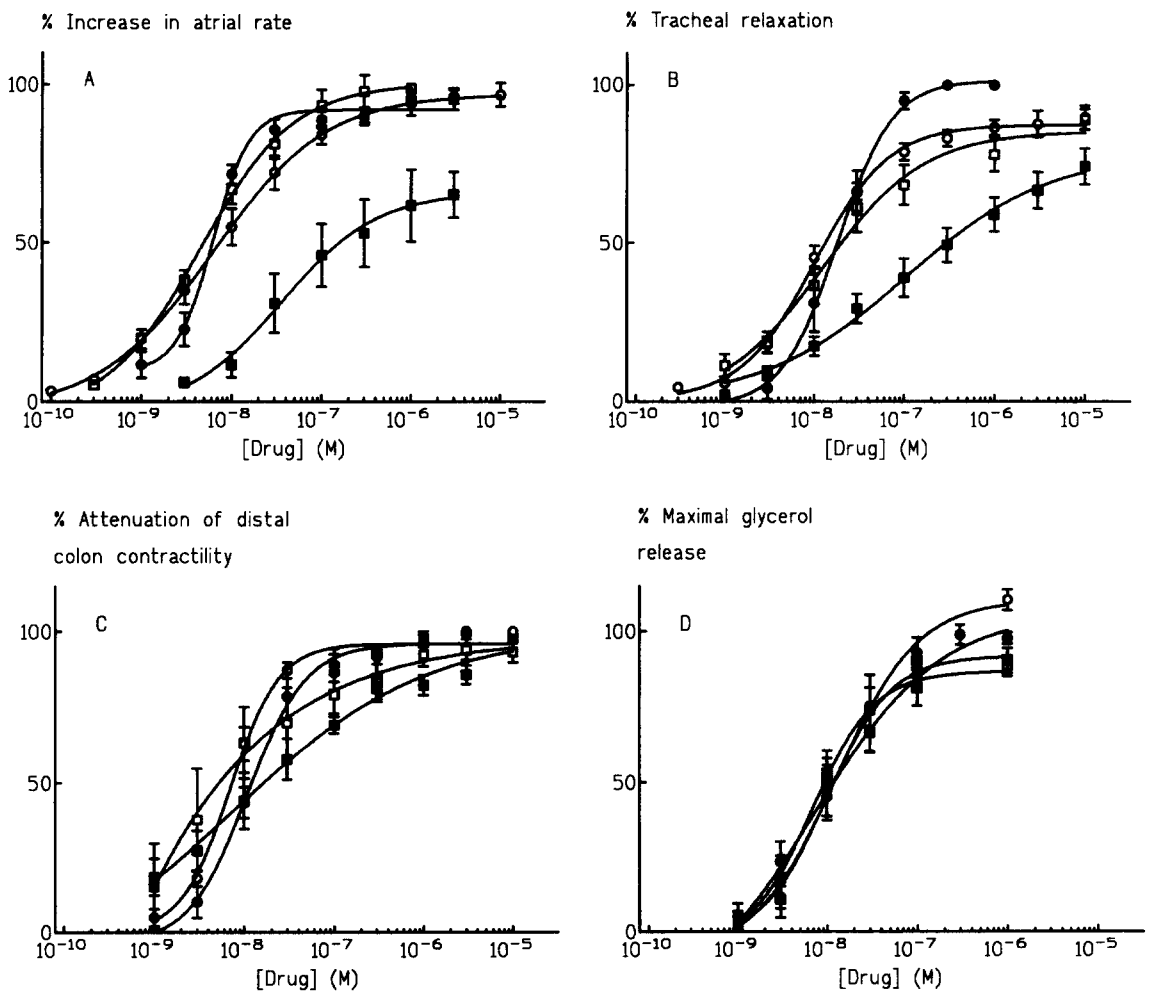

Fig. 2. Concentration-response curves of (±)-trimetoquinol (open circles); 3′-iodotrimetoquinol (open squares); 3′,5′-diiodotrimetoquinol (closed squares); and (−)-isoprenaline (closed circles) in spontaneously beating right atria (panel A), tracheal strips (panel B), distal colon (panel C) and brown adipose tissue (panel D). Responses are expressed relative to the maximal response produced by 10^{-5} M (−)-isoprenaline, and data represent the mean percentage response ± S.E.M. of $n = 3–9$ experiments.

adrenoceptor sites. In spontaneously beating right atria and precontracted tracheal strips, 3',5'-diiodotrimetoquinol was observed to be a partial agonist as compared to (±)-trimetoquinol and 3'-iodotrimetoquinol. Moreover, the potencies for increasing atrial chronotropy and relaxation of trachea (pEC_{50} ± S.E.M.: 7.08 ± 0.31 and 6.98 ± 0.19, respectively) were about 10-fold lower than its functional activities in distal colon and brown adipocytes (pEC_{50} ± S.E.M.: 7.92 ± 0.21 and 8.00 ± 0.11, respectively), tissues that contain primarily atypical β/β_3-adrenoceptors (Fig. 2).

3.2. Studies in Chinese hamster ovary (CHO) cells transfected with rat β_3-adrenoceptors

3.2.1. Radioligand binding experiments

Trimetoquinol and its analogues exhibited high affinities for the rat β_3-adrenoceptors (Fig. 3A). Rank order for the displacement of [^{125}I]iodocyanopindolol from rat β_3-adrenoceptors by the compounds was 3'-iodotrimetoquinol = 3',5'-diiodotrimetoquinol > ICI D7114 > (±)-trimetoquinol > (−)-isoprenaline and the affinity constants (pK_i values) for displacement of the radioligand were 6.21, 6.17, 5.90, 5.11 and 4.29, respectively (Table 3). In this cell system, slope values for the concentration inhibition displacement curves of each compound were less than unity and varied between 0.72–0.81.

3.2.2. cAMP accumulation studies

The effect of trimetoquinol and its analogues on cAMP accumulation in CHO cells expressing rat β_3-adrenoceptors is shown in Fig. 3B. (±)-Trimetoquinol and the halogenated analogues of trimetoquinol were potent activators of cAMP accumulation in these cells. Similar to its high potency in functional assays, 3',5'-diiodotrimetoquinol exhibited the highest potency for cAMP accumulation. Rank order of potencies for the compounds tested was 3',5'-diiodotrimetoquinol > 3'-iodotrimetoquinol = (±)-trimetoquinol > (−)-isoprenaline > ICI D7114 and the potencies (pK_{act} values) for β_3-adrenoceptor activation were 9.40, 8.72, 8.69, 8.27 and 7.28, respectively (see Table 3).

Table 3
β_3-Adrenoceptor binding affinities (pK_i values) and potencies for stimulation of cAMP accumulation of (−)-isoprenaline, (±)-trimetoquinol and 1-benzyl halogen-substituted analogues of trimetoquinol on rat β_3-adrenoceptors expressed in Chinese hamster ovary (CHO) cells

Compound	pK_i value [a]	Hill slope [b]	pK_{act} value [c]
(−)-Isoprenaline	4.29 ± 0.11	0.81 ± 0.04	8.27 ± 0.13
(±)-Trimetoquinol	5.11 ± 0.12	0.80 ± 0.08	8.69 ± 0.14
3'-Iodotrimetoquinol	6.21 ± 0.05	0.79 ± 0.05	8.72 ± 0.09
3',5'-Diiodotrimetoquinol	6.17 ± 0.08	0.72 ± 0.06	9.40 ± 0.08
ICI D7114	5.90 ± 0.19	0.79 ± 0.06	7.28 ± 0.23

[a] $pK_i = -\log K_i$; K_i values were calculated using the LIGAND computer program. Data are expressed as the means ± S.E.M. of $n = 3–10$ experiments. [b] Hill slope = slope of a graphically determined Hill plot for agonist-induced displacement of [^{125}I]iodocyanopindolol from rat β_3-adrenoceptors expressed in CHO cells, calculated assuming binding to one receptor site. [c] $pK_{act} = -\log K_{act}$; K_{act} values were calculated as the molar concentration needed to produce a half maximal stimulation of cAMP accumulation by each compound. Data represent the means ± S.E.M. of $n = 5–11$ experiments.

4. Discussion

Trimetoquinol, a prototypical tetrahydroisoquinoline derivative, has been actively studied for its β-adrenoceptor agonist properties. Earlier studies with guinea pig tissues focused on elucidating functional activities of trimeto-

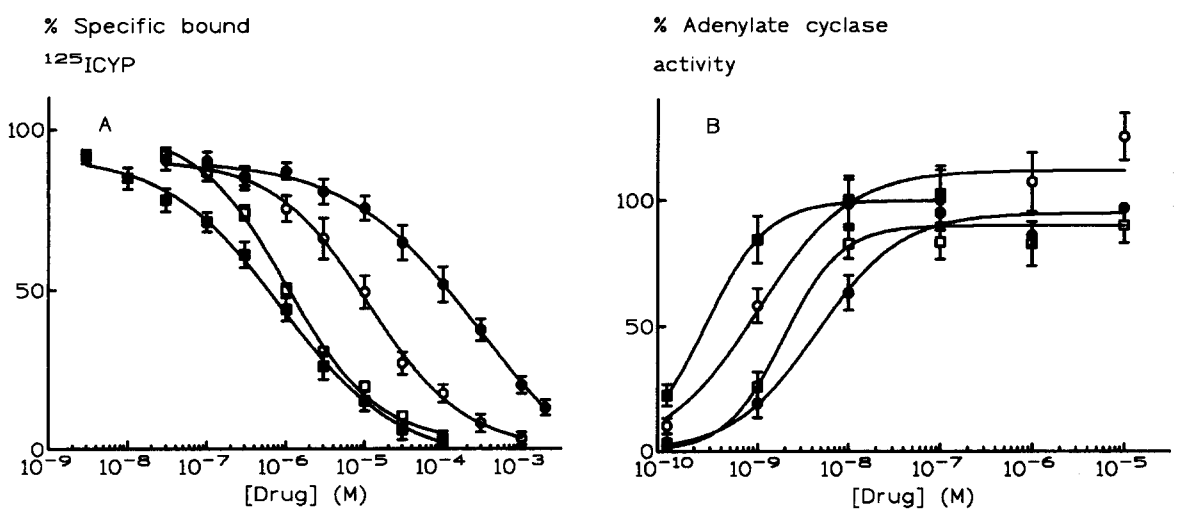

Fig. 3. Pharmacological activities of (±)-trimetoquinol (open circles), 3'-iodotrimetoquinol (open squares), 3',5'-diiodotrimetoquinol (closed squares) and (−)-isoprenaline (closed circles) on rat β_3-adrenoceptors. Concentration-dependent inhibition of specific [^{125}I]iodocyanopindolol binding to rat β_3-adrenoceptors (panel A) and agonist-stimulated increases in cAMP accumulation (panel B) in Chinese hamster ovary cells expressing rat β_3-adrenoceptors. Data are expressed as the means ± S.E.M. of $n = 5–10$ experiments (panel A) and mean percentage ± S.E.M. of the maximal response induced by (−)-isoprenaline of $n = 5–11$ experiments (panel B).

quinol and its analogues at β_1- and β_2-adrenoceptor subtypes to determine their selectivity for β_2-adrenoceptors (Shams et al., 1990). However, until recently, no attempt was made to define the interaction of trimetoquinol with atypical β/β_3-adrenoceptors (Fraundorfer et al., 1994). In this study, we have investigated the atypical β/β_3-adrenoceptor agonist activities of trimetoquinol and related analogues in rat tissues. The β_1- and β_2-adrenoceptor agonist activities of these analogues were also examined in rat tissues to avoid variability in response due to species differences.

Our results demonstrate that trimetoquinol is a potent agonist of all β-adrenoceptor subtypes in rat tissues. Activation of β-adrenoceptor subtypes by trimetoquinol in these tissues was highly stereoselective and (−)-trimetoquinol was the more potent isomer. Stereoselectivity ratios of chiral compounds have been proposed to be useful indicators for distinguishing receptor subtypes (Patil, 1969). It has been reported that stereoisomers of β-adrenoceptor ligands typically exhibit low stereoselectivity indices at atypical β/β_3-adrenoceptors as opposed to their activities at β_1- and β_2-adrenoceptor subtypes (Patil et al., 1971; Shonk et al., 1971; Harms et al., 1977). In contrast, our data from functional studies show that isomeric activity ratios of trimetoquinol at atypical β/β_3-adrenoceptors are higher than at β_1- and β_2-adrenoceptor subtypes. High isomeric activity ratios for trimetoquinol isomers were also obtained in biochemical studies on Chinese hamster ovary (CHO) cells expressing a homogeneous population of rat β_3-adrenoceptors. Similar results have been demonstrated in rat oesophageal smooth muscle, a tissue that predominantly contains atypical β/β_3-adrenoceptor sites (Fraundorfer et al., 1994). These results suggest that trimetoquinol, as opposed to other catecholamines, interacts with β-adrenoceptor subtypes in a unique manner. A three point interaction between catecholamine substituents and β-adrenoceptor structural domains has been hypothesised to be critical for functional activity of catecholamines (Easson and Stedman, 1933). Trimetoquinol lacks the corresponding β-hydroxy group implicated in the three point interaction and has the amino nitrogen constrained within the semirigid tetrahydroisoquinoline ring. In addition, it has a rather large 3′,4′,5′-trimethoxybenzyl substituent at the 1-carbon of the tetrahydroisoquinoline ring that appears to compensate for the absence of the β-hydroxy group and is essential for functional activity (Feller et al., 1978). While allowing the (−)-isomer to interact with the atypical β/β_3-adrenoceptor, these structural features of trimetoquinol might obstruct interaction of the (+)-isomer with this receptor subtype. The large 3′,4′,5′-trimethoxybenzyl ring as well as the relatively inflexible amino group may not allow (+)-trimetoquinol to adopt a suitable conformation within the binding pocket necessary for functional activity.

Trimetoquinol-induced responses in atrial and tracheal tissues that predominantly contain β_1- and β_2-adrenoceptor subtypes, respectively, were sensitive to blockade by pindolol. In contrast, pindolol (10^{-6} M) exhibited a low potency for antagonism of drug-induced responses in rat distal colon and brown adipose tissues. These tissues are postulated to contain atypical β/β_3-adrenoceptors that mediate drug-induced responses and yield low antagonist potency values for blockade by typical β-adrenoceptor antagonists (McLaughlin and MacDonald, 1990; Arch, 1989). Studies have also reported the presence of mRNA for the rat β_3-adrenoceptor in the brown adipose tissue as well as distal colon and the rat β_3-adrenoceptor has been isolated and cloned from both these tissues (Granneman et al., 1991; Muzzin et al., 1991; Bensaid et al., 1993). Trimetoquinol-induced relaxation of colon segments and glycerol release from brown adipocytes is therefore mediated by the activation of these atypical sites. Further corroboration was provided by our studies on CHO cells expressing a homogeneous population of rat β_3-adrenoceptors in which trimetoquinol increased intracellular cAMP concentrations in a concentration-dependent manner and competitively inhibited binding of the radiolabelled β-adrenoceptor ligand [^{125}I]iodocyanopindolol. The Hill slope value was less than unity as has been previously observed for potent β-adrenoceptor agonists (Harden et al., 1982) and may indicate the existence of a high and low affinity state of the β_3-adrenoceptor.

We further attempted to characterise the activities of trimetoquinol analogues on β-adrenoceptor subtypes to determine their β-adrenoceptor activation profile. Earlier pharmacological investigations of trimetoquinol analogues in guinea pig tissues revealed that substitution of a single or two methoxy groups on the 3′,4′,5′-trimethoxybenzyl ring with the iodo group gave compounds which exhibited partial β_1- and β_2-adrenoceptor agonist activity (Shams et al., 1990). Recent investigations have shown that compounds with large substituents on the amino nitrogen exhibit antagonist activity or partial agonist activity at β_1- and β_2-adrenoceptors and are potent agonists at atypical β/β_3-adrenoceptors (Sugasawa et al., 1992; Blin et al., 1993; Mohell and Dicker, 1989). Thus, the halogenated derivatives of trimetoquinol were tested for their β-adrenoceptor agonist activities on various subtypes. Like the parent compound, 3′-iodotrimetoquinol activated β-adrenoceptor subtypes in all tissues in a non-selective manner. 3′,5′-Diiodotrimetoquinol exhibited differential activities at β-adrenoceptor subtypes. Similar to its pharmacological profile in guinea pig atria and trachea, 3′,5′-diiodotrimetoquinol was a partial agonist in the corresponding rat tissues with lower potencies than trimetoquinol. In contrast, the compound was a full and potent agonist in atypical β/β_3-adrenoceptor-containing tissues. We further defined the atypical activity of this analogue and other compounds in CHO cells expressing rat β_3-adrenoceptors. 3′,5′-Diiodotrimetoquinol was the most potent compound tested in cAMP accumulation assays and also exhibited a high affinity for the rat β_3-adrenoceptor. In both biochemical

and binding assays, the activities of 3′,5′-diiodotrimetoquinol closely paralleled those reported earlier for the reference β_3-adrenoceptor-selective agonist, BRL 37344 (Muzzin et al., 1991). These functional and biochemical studies indicate that 3′,5′-diiodotrimetoquinol exhibits high affinity and selectivity for the atypical β/β_3-adrenoceptors.

Our studies demonstrate that trimetoquinol induces potent functional responses at all β-adrenoceptor subtypes in rat tissues and the interaction is highly stereoselective. The 3′,5′-diiodotrimetoquinol analogue exhibited moderate selectivity for the atypical β/β_3-adrenoceptor in rat tissues, and a high binding affinity and potency for the cloned rat β_3-adrenoceptors. Stereoselective synthesis and chemical modifications of this diiodo analogue of trimetoquinol may lead to development of compounds with greater β_3-adrenoceptor selectivity.

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