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Pharmacological study of atypical β-adrenoceptors in rat esophageal smooth muscle

Edwin J. Lezama [a,b], Anish A. Konkar [b], M. Margarita Salazar-Bookaman [a], Duane D. Miller [c], Dennis R. Feller [b,d,*]

[a] *Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela*
[b] *Division of Pharmacology, College of Pharmacy, Ohio State University, Columbus, OH 43210, USA*
[c] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee, Memphis, TN 38163, USA*
[d] *Department of Pharmacology, University of Mississippi, University, MS 38677, USA*

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Abstract

The chemical specificity for the β-adrenoceptor mediated relaxation of rat esophageal smooth muscle was evaluated using selective and non-selective β-adrenoceptor agonists and antagonists. Pindolol, ICI 89,406, ICI 118551 [erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol] and the β-adrenoceptor alkylating agent, pindobind, produced only small rightward shifts in the concentration-response curves of (−)-isoprenaline and (−)-trimetoquinol in this preparation. Rank order potency (pD_2 values) of agonists was: (±)-trimetoquinol [1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] (8.34) = (−)-trimetoquinol (8.26) = BRL 37344 [(*R***R**)-(±)-4-[2′-{2-hydroxy-2-(3-chlorophenyl)ethylamino}propyl]phenoxyacetic acid] (8.16) = ICI D7114 [(*S*)-4-(2-hydroxy-3-phenoxy-propylamino-ethoxy)-*N*-(2-methoxyethyl)phenoxyacetamide] (8.03) ≥ (−)-isoprenaline (7.82) > 3′,5′-diiodotrimetoquinol [1-(3′,5′-diiodo-4′-methoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] (7.28) > 3′-iodotrimetoquinol [1-(3′-iodo-4′,5′-dimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] (7.04) > ractopamine (6.84) = 5,8-difluorotrimetoquinol [5,8-difluoro-6,7-dihydroxy-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline] (6.82) > 8-fluorotrimetoquinol [6,7-dihydroxy-8-fluoro-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline] (6.56) ≥ (−)-noradrenaline (6.46) ≥ (−)-adrenaline (6.36) > (±)-noradrenaline (6.24) > (±)-adrenaline (6.00) > clenbuterol (5.83) > (−)-1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (5.75). Isomeric activity ratios of trimetoquinol isomers [(−)-(*S*)-≫(+)-(*R*)-] in esophageal smooth muscle in the presence and absence of 1 μM pindolol were 1995- and 2951-fold, respectively; and were much greater than those in rat atria (282-fold) and rat trachea (107-fold). The atypical β/β_3-adrenoceptor partial agonist, ICI D7114, produced concentration-dependent rightward shifts of the concentration-response curves of (−)-isoprenaline, (−)-trimetoquinol and the reference atypical β/β_3-adrenoceptor agonist, BRL 37344. Schild plot analysis of ICI D7114 against trimetoquinol gave slope and pA_2 values of 0.91 and of 7.9, respectively. These results clearly demonstrate that the relaxant effects of these agonists in rat esophageal smooth muscle are primarily mediated through the activation of atypical β/β_3-adrenoceptors. (−)-Trimetoquinol was as potent as (−)-isoprenaline and BRL 37344, and was the most stereoselective agonist evaluated in this tissue system.

Keywords: β/β_3-Adrenoceptor, atypical; β_3-Adrenoceptor; Smooth muscle, esophageal, rat; Trimetoquinol; Isomeric activity ratio; BRL 37344

1. Introduction

β-Adrenoceptors were initially classified into β_1- and β_2-adrenoceptor subtypes by Lands et al. (1967). Pharmacological studies have indicated the presence of a third β-adrenoceptor subtype, referred to as the atypical β/β_3-adrenoceptor (Arch et al., 1984). The presence of this additional subtype has been demonstrated in tissues from several species (Arch and Kaumann, 1993). The responses mediated by atypical β/β_3-adrenoceptors are characterised by a resistance to blockade by classical β_1- and β_2-adrenoceptor antagonists, low eudismic ratios to antagonist enantiomers, and activation by novel β-adrenoceptor agonists (Zaagsma and Nahorski, 1990). Cloning and expression of the β_3-adrenoceptor from rat brown adipose tissue in Chinese hamster ovary cells revealed a pharmaco-

* Corresponding author. Department of Pharmacology, Research Institute of Pharmaceutical Sciences, School of Pharmacy, 303 Faser Hall, University of Mississippi, University, MS 38677, USA.

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logical profile similar to the atypical β/β_3-adrenoceptor in rat adipocytes (Granneman et al., 1991; Muzzin et al., 1991).

In rat esophageal smooth muscle, Buckner and Christopherson (1974) reported unusually low potencies of classical β-adrenoceptor antagonists for the inhibition of relaxations induced by isoprenaline and trimetoquinol [1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline]. Recently, De Boer et al. (1993) investigated the nature of the β-adrenoceptor in rat esophageal smooth muscle, and reported that this tissue contains mainly an atypical β/β_3-adrenoceptor population in addition to presence of the β_2-adrenoceptor subtype.

Trimetoquinol, is a prototype cyclized phenethylamine of the tetrahydroisoquinoline chemical class which differs chemically from β-adrenoceptor agonists such as isoprenaline, adrenaline and noradrenaline. Trimetoquinol lacks a corresponding β-hydroxy group on the ethylamino side chain and has an amino nitrogen confined within a semi-rigid tetrahydroisoquinoline ring (Fig. 1). This molecule is a potent β-adrenoceptor agonist (Sato et al., 1967; Mukhopadhyay et al., 1982), and its activity on β_1-, β_2- (Iwasawa and Kiyomoto, 1967) and atypical β/β_3- (Fraundorfer et al., 1994; Konkar et al., 1994) adrenoceptors is attributed to the (−)-(*S*)-isomer. Only a few reports (Buckner and Christopherson, 1974; Fraundorfer et al., 1994) exist on the effects of trimetoquinol and its isomers on atypical β/β_3-adrenoceptor systems. No information is available which has examined the chemical specificity of the interactions of trimetoquinol analogues with β-adrenoceptors in rat esophageal smooth muscle. Further, trimetoquinol bears a structural resemblance to the prototypical atypical β/β_3-adrenoceptor agonists BRL 37344 (Fig. 1). Thus, we have included the isomers and halogenated analogues of trimetoquinol, and catecholamine isomers in our studies to further characterise the agonist specificity for activation of the atypical β/β_3-adrenoceptors present in rat esophageal smooth muscle.

The main objective of this study was to establish whether the relaxant responses to β-adrenoceptor agonists (catecholamine and trimetoquinol chemical classes) in rat esophageal smooth muscle are mediated via selective interaction with atypical β/β_3-adrenoceptors. Selective atypical β/β_3-adrenoceptor agonists may have value in the treatment of obesity (Arch et al., 1984), non-insulin dependent (type II) diabetes (Arch et al., 1991), diarrhea, and gastrointestinal disorders such as hiatal hernia and gastro-esophageal reflux (Goyal, 1991). The determination and comparison of the stereochemical specificity of isomers and analogues of trimetoquinol and catecholamines for eliciting rat esophageal smooth muscle relaxation will be important to establish the stereochemical requirements of

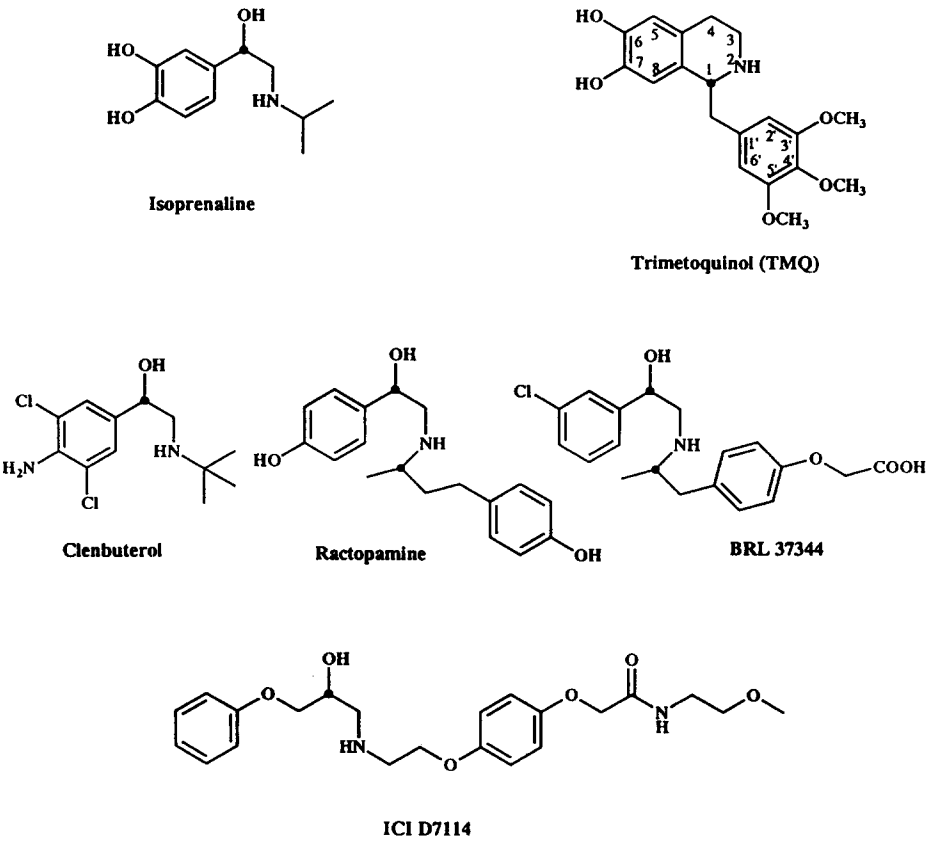

Fig. 1. Chemical structures of trimetoquinol, its analogues and other β-adrenoceptor agonists. The presence of a chiral center is indicated by ● in the chemical structure.

these chemical classes for activating the atypical β/β_3-adrenoceptor populations. The selective β_3-adrenoceptor agonists, BRL 37344 (Arch et al., 1984) and ICI D7114 (Holloway et al., 1991); and the β_1/β_2-adrenoceptor agonists, ractopamine (Anderson et al., 1990) and clenbuterol (Yang and McElligott, 1989) were included as comparison standards (Fig. 1). A preliminary report of this work has appeared (Lezama et al., 1994).

2. Materials and methods

2.1. Isolation and preparation of rat esophageal smooth muscle

Harlan male, albino Sprague-Dawley rats weighing 250–350 g were housed under a 12 h alternating light/dark cycle and with access to chow and water ad libitum. Animals were killed by exposure to carbon dioxide. A 4–5 cm segment from the esophagus body, measured from the diaphragm, was removed, cleaned and prepared as described by Buckner and Christopherson (1974). Each segment was suspended in a 10 ml water jacketed tissue bath containing physiological salt solution of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; $MgCl_2 \cdot 6H_2O$, 5.0; $NaH_2PO_4 \cdot H_2O$, 1.0; $NaHCO_3$, 25; and dextrose, 11.1; pH 7.4, maintained at 37°C and bubbled with 95% O_2/5% CO_2. One extreme of the esophageal segment was tied to a glass hook and at the other end to a Grass FT-03C isometric force-displacement transducer and tissue responses were recorded on a Grass Polygraph Model 7C. A baseline tension of 200 mg was applied to the tissues. Tissues were washed with physiological salt solution every 15 min for 1 h or until stabilisation was achieved before addition of drugs to the tissue baths.

2.2. Isolation and preparation of rat atria and trachea

Male and female albino Sprague-Dawley rats (200–300 g) were killed by a blow to the head followed by cervical dislocation. Spirally cut tracheal strips and spontaneously beating right atria were quickly removed, prepared and mounted in 10 ml tissue baths filled with physiological salt solution (see composition above) maintained at 37°C and aerated with a mixture of 95% O_2/5% CO_2. Tissue responses were recorded under 1 g resting tension on a Grass Polygraph Model 7C.

2.3. Carbachol contraction

2.3.1. Rat esophageal smooth muscle

A contraction equal to 70–80% of maximal carbachol-induced contraction was achieved by addition of 1 μM carbachol. The contraction reached a maximum within 10–15 min and then started to fade. A sustained plateau was observed 30–45 min after the induction of contraction. The plateau was used as the baseline for studying drug-induced relaxation responses. Tissues were incubated for 45 min with the following drugs: cocaine, 30 μM; hydrocortisone, 100 μM; U-0521, 10 μM; and phentolamine, 10 μM. Cumulative concentration-response curves were constructed by the method of Van Rossum (1963) using a concentration range of 10^{-10}–10^{-5} M for most drugs. Drugs were added every 8–10 min or until no further relaxation response was observed. When antagonists were used, the tissue was incubated with the requisite blocker for 45 min prior to the addition of agonists. In other experiments, tissues were incubated with 10 μM pindobind for 90 min following which tissues were washed with physiological salt solution every 5 min for 30 min. After a stable baseline tension was achieved, cumulative concentration-response curves to agonists were constructed.

2.3.2. Rat right atria and trachea

Tissues were equilibrated for 30–45 min followed by incubation with blockers U-0521 (10 μM) and hydrocortisone (100 μM) in atria, and U-0521 (10 μM), hydrocortisone (100 μM) and cocaine (30 μM) in trachea for a period of 30 min. After this preincubation time, contraction (approximately 70% of maximum, reached in about 10–15 min) was generated in tracheal strips with 0.3 μM carbachol. Cumulative concentration-response curves of drugs were constructed in both tissues, measuring over a concentration range of 10^{-10}–10^{-5} M, as described by Van Rossum (1963).

2.4. Quantification of drug responses

2.4.1. Rat esophageal smooth muscle

Relaxation response to each agonist was calculated as a percentage of the maximal change from the steady-state contraction produced by carbachol in each tissue. The EC_{50} value for each drug was determined as the molar concentration required to produce 50% of the maximal relaxation elicited by the drug in the carbachol contracted tissue. Maximal tissue relaxation was determined by the addition of a supramaximal concentration of isoprenaline (10 μM) at the end of each experiment.

Potency differences between optical isomers of agonists and different agonists were expressed as the antilog of the difference between pD_2 values. The pA_2 value of an antagonist, defined as the negative logarithm of the concentration of antagonist that produces a rightward shift in the concentration-response curve of an agonist by a factor of two, was calculated by the method of Schild (1947). The pK_B value of an antagonist, defined as the negative logarithm of the molar concentration of antagonist that produces a concentration ratio of two, was determined by the method of Furchgott and Bursztyn (1967).

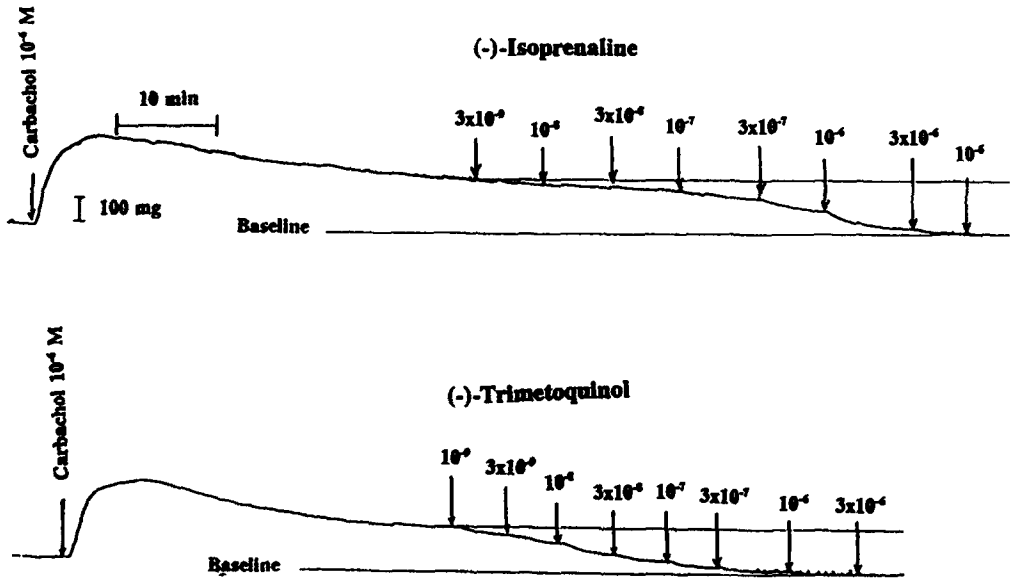

Fig. 2. Typical tracings of (−)-isoprenaline (upper curve)- and (−)-trimetoquinol (lower curve)-induced relaxations of rat esophageal smooth muscle.

2.4.2. Rat atria and trachea

After construction of the concentration-response curve for each drug, a supramaximal concentration of (−)-isoprenaline (10 μM) was added to elicit maximal response in each tissue. The EC_{50} values and potency differences of trimetoquinol isomers were determined as described above.

2.5. Data analysis

Drug-induced rat esophageal smooth muscle and tracheal relaxations, and right atrial chronotropic increases were expressed as a percent of the maximal response to 10 μM (−)-isoprenaline. Results were expressed as the geometric mean ± S.E.M. of the negative logarithm of the molar EC_{50} value. Statistical differences between two data sets were determined by the Student's *t*-test at 5% level of significance.

2.6. Chemicals

The compounds used and their sources are as follows: hydrocortisone sodium succinate (Abbott Laboratories, Chicago, IL); carbamyl choline chloride (Aldrich Chemical Co., Milwaukee, WI); (±)-pindolol (Receptor Research Biochemical, Baltimore, MD); phentolamine HCl, (±)-adrenaline HCl, (−)-isoprenaline-(+)-bitartrate, (±)-noradrenaline HCl, (±)-isoprenaline HCl (Sigma Chemical Co., St. Louis, MO); (±)-pindobind, (+)-isoprenaline-(+)-bitartrate, (−)-adrenaline-(+)-bitartrate, (−)-noradrenaline-(+)-bitartrate (Research Biochemical Int., Natick, MA); (+)-adrenaline-(+)-bitartrate, (+)-noradrenaline-(+)-bitartrate (Sterling-Winthrop Research Institute,

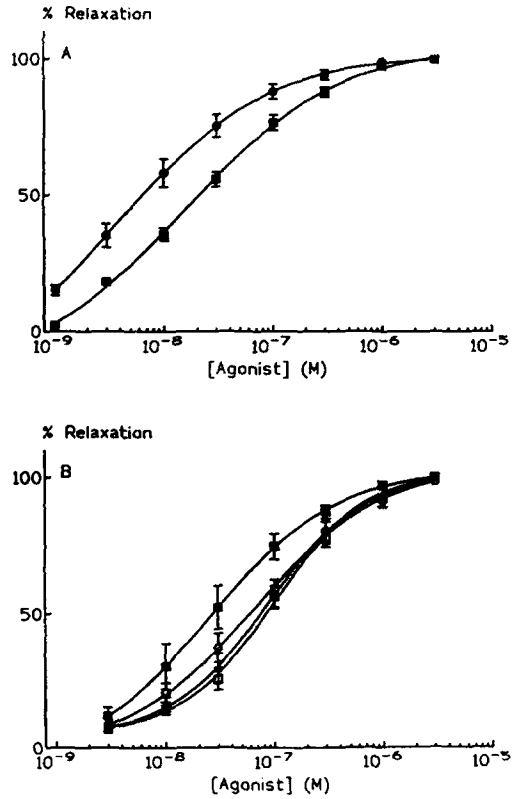

Fig. 3. Agonist-induced relaxations in rat esophageal smooth muscle (A) and the effect of varying concentrations of (±)-pindolol on relaxations induced by (−)-isoprenaline in rat esophageal smooth muscle (B). A: (−)-trimetoquinol (●); (−)-isoprenaline (■). B: control (■); pindolol 10 nM (○); pindolol 100 nM (△), pindolol 1 μM (□). See Section 2 for details of tissue pretreatments. Data are presented as the mean ± S.E.M. of $n = 15$ (A) and $n = 3$–5 experiments (B).

Rensselaer, NY); isomers of trimetoquinol [1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] {(−)-(*S*)-trimetoquinol, $\alpha_D = -28.5$ and (+)-(*R*)-trimetoquinol, $\alpha_D = +29.0$, in methanol; hereafter designated as the (−)- and (+)-isomers, respectively} (HCl salts) and 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline were provided by Yoshio Iwasawa (Tanabe Seiyaku Co., Osaka, Japan); (±)-trimetoquinol and its halogenated analogues were provided by Duane D. Miller (College of Pharmacy, University of Tennessee, Memphis, TN); U-0521 was provided by Popat N. Patil (College of Pharmacy, The Ohio State University, Columbus, OH); ractopamine HCl (Lilly Corporate Center, Indianapolis, IN); ICI 89406, ICI 118551 [erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol] and ICI D7114 [(*S*)-4-(2-hydroxy-3-phenoxy-propylamino-ethoxy)-*N*-(2-methoxyethyl) phenoxyacetamide] (ICI Pharmaceuticals, Macclesfield, England); clenbuterol HCl (Boehringer Ingelheim Animal Health, St. Joseph, MO); BRL 37344 [(*R***R**)-(±)-4-[2′-{2-hydroxy-2-(3-chlorophenyl)ethylamino}propyl]phenoxyacetic acid] (SmithKline Beecham Pharmaceuticals, Surrey, England). All other chemicals were of reagent grade. Drugs were dissolved at the highest concentration (10^{-2} M) in double distilled water and diluted in double distilled water or physiological salt solution.

3. Results

3.1. Effects of the adrenoceptor antagonists pindolol (β_1/β_2), ICI 89,406 (β_1) and ICI 118,551 (β_2) on relaxation of rat esophageal smooth muscle induced by (−)-isoprenaline and (−)-trimetoquinol

Typical relaxation responses to increasing concentrations of the (−)-isomers of isoprenaline and trimetoquinol on carbachol-induced contractions in rat esophageal smooth muscle are illustrated in Fig. 2. Initial experiments indicated that the maximal relaxations induced by these compounds were not significantly different from each other ($P > 0.05$, paired *t*-test), and addition of 10 μM (−)-isoprenaline at the end of concentration-response curves to (−)-trimetoquinol did not result in any further change in the relaxation response. Data of these compounds were expressed as percent of the maximal response to 10 μM (−)-isoprenaline, and (−)-trimetoquinol was about 3-fold more potent than (−)-isoprenaline (Fig. 3A).

The concentration-response curve to (−)-isoprenaline was only slightly shifted to the right in the presence of varying concentrations (10^{-8}–10^{-6} M) of pindolol (Fig. 3B). Similar results were observed when propranolol was used as an antagonist (data not presented). Further, the β-adrenoceptor antagonists ICI 89,406 (β_1-subtype selec-

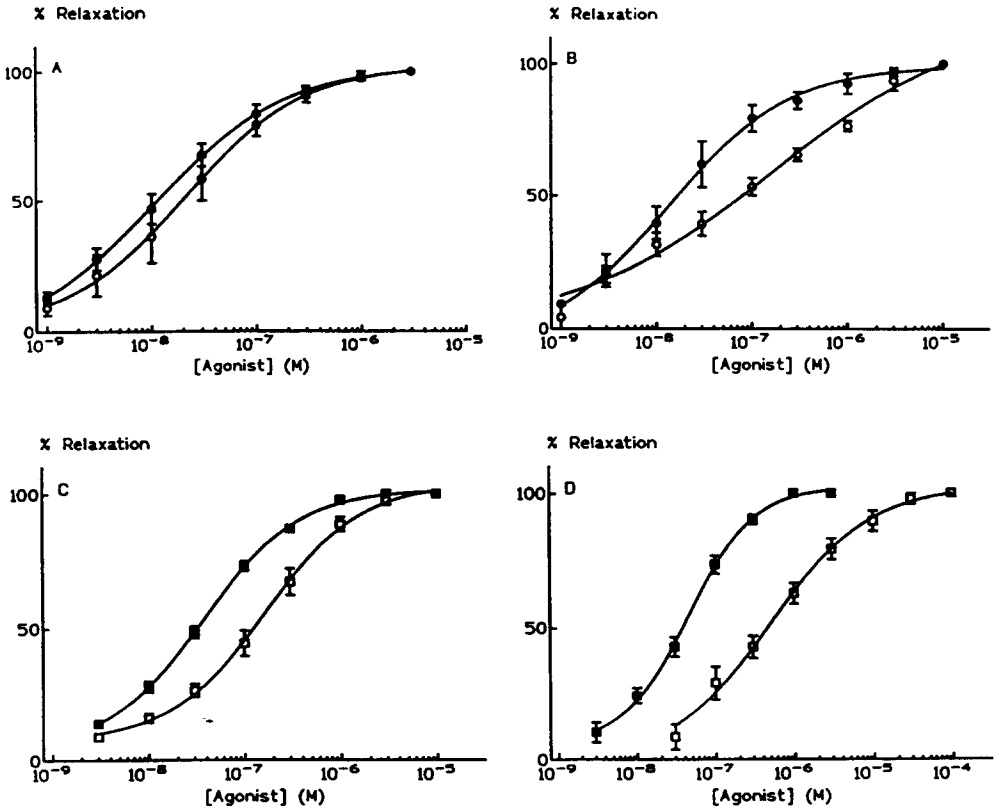

Fig. 4. Concentration-response curves to (−)-trimetoquinol (A and B) and (−)-isoprenaline (C and D) in the presence (□, ○) and absence (■, ●) of 10 μM pindobind in rat esophageal smooth muscle (A and C) and rat trachea (B and D). See Section 2 for tissue pretreatments. Data represent the mean ± S.E.M. of $n = 3$–4 experiments.

Table 1
Comparison of affinity constants (pK_B values) for pindolol, ICI 89,406 and ICI 118,551 against (−)-isoprenaline- and (−)-trimetoquinol-induced relaxations of rat esophageal smooth muscle

Antagonist [a]	$pK_B \pm$ S.E.M. [b]	
	(−)-Isoprenaline	(−)-Trimetoquinol
Pindolol	6.60 ± 0.07	6.15 ± 0.19
ICI 89,406	6.30 ± 0.09	6.19 ± 0.10
ICI 118,551	6.10 ± 0.05	5.99 ± 0.09

[a] Antagonist concentration used was 1 μM. [b] pK_B = negative logarithm of the molar concentration of antagonist that produces a concentration ratio (cr) of two. Values were calculated using the formula: $pK_B = \log(cr-1) - \log[\text{antagonist}]$, and expressed as the mean ± S.E.M. of $n = 3$–4 experiments.

tive) and ICI 118,551 (β_2-subtype selective), at 1 μM, produced small rightward shifts (approximately 2-fold) of the concentration-response curves of (−)-isoprenaline and (−)-trimetoquinol. The calculated affinity constants (pK_B values) for these β-adrenoceptor antagonists against (−)-isoprenaline and (−)-trimetoquinol-induced relaxations ranged from 5.99 to 6.60 (Table 1).

3.2. Effect of pindobind on relaxation responses to (−)-isoprenaline and (−)-trimetoquinol in rat esophageal smooth muscle and trachea

In rat esophageal smooth muscle, the β-adrenoceptor alkylating agent, pindobind (10 μM) shifted the concentration-response curve of (−)-trimetoquinol and (−)-isoprenaline to the right by 1.4- and 3.4-fold, respectively without producing a reduction in the maximal relaxant responses to these drugs (see Fig. 4A and Fig. 4C). In contrast, 4- and 11-fold rightward shifts were observed for (−)-trimetoquinol and (−)-isoprenaline in isolated tracheal strips (see Fig. 4B and Fig. 4D).

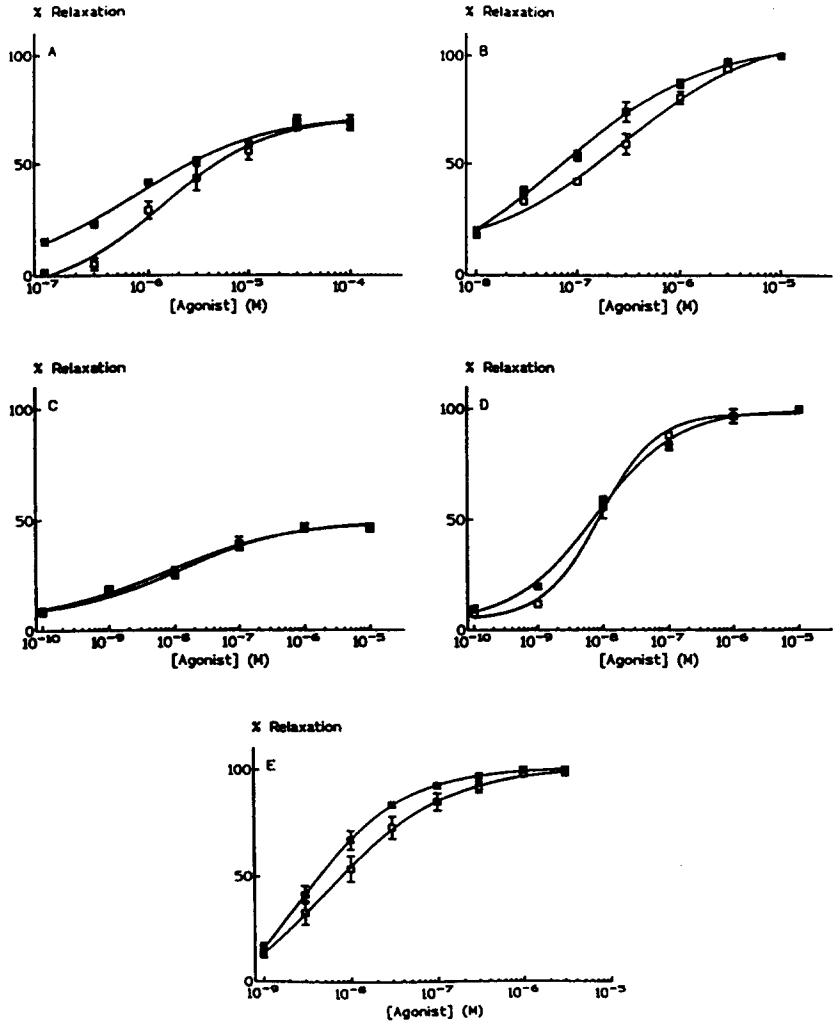

Fig. 5. Concentration-response curves of the atypical β-adrenoceptor agonists clenbuterol (A), ractopamine (B), ICI D7114 (C), BRL 37344 (D) and (±)-trimetoquinol (E) in rat esophageal smooth muscle in the absence (■) and presence (□) of 1 μM pindolol. See Section 2 for details of tissue pretreatments. Data represent the mean ± S.E.M. of $n = 3$–4 experiments.

Table 2
Comparative potencies and intrinsic activities of trimetoquinol analogues, catecholamines and atypical β/β_3-adrenoceptor agonists on rat esophageal smooth muscle

Agonist	pD_2 [a] ± S.E.M	IA [b] ± S.E.M.	pD_2 [c] ± S.E.M.	IA [b] ± S.E.M.
(−)-Isoprenaline	7.82 ± 0.08	1.00	7.58 ± 0.07	1.00
(±)-Trimetoquinol	8.34 ± 0.01	1.00	8.00 ± 0.20	0.98 ± 0.02
8-Fluorotrimetoquinol	6.56 ± 0.07	0.98 ± 0.03	6.35 ± 0.08	0.97 ± 0.01
5,8-Difluorotrimetoquinol	6.82 ± 0.10	0.89 ± 0.01	6.45 ± 0.15	0.90 ± 0.02
3′-Iodotrimetoquinol	7.04 ± 0.06	0.96 ± 0.01	6.97 ± 0.19	0.94 ± 0.01
3′,5′-Diiodotrimetoquinol	7.28 ± 0.05	0.84 ± 0.02	6.75 ± 0.11	0.76 ± 0.01
(−)-1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	5.75 ± 0.10	0.83 ± 0.01	5.69 ± 0.20	0.79 ± 0.02
(+)-1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	NA [d]		ND [e]	
BRL 37344	8.16 ± 0.01	1.00	8.05 ± 0.05	1.00
ICI D7114	8.03 ± 0.15	0.47 ± 0.01	7.99 ± 0.10	0.45 ± 0.01
Ractopamine	6.84 ± 0.07	1.00	6.57 ± 0.05	1.00
Clenbuterol	5.83 ± 0.04	0.69 ± 0.03	5.72 ± 0.05	0.68 ± 0.03
(−)-Adrenaline	ND [e]		6.36 ± 0.06	1.00
(±)-Adrenaline	ND [e]		6.00 ± 0.02	1.00
(−)-Noradrenaline	ND [e]		6.46 ± 0.02	1.00
(±)-Noradrenaline	ND [e]		6.24 ± 0.06	1.00
Pindolol	3.22 ± 0.13	0.97 ± 0.03		

[a] $pD_2 = -\log EC_{50}$; EC_{50} value for each drug was determined as the molar concentration required to produce 50% of maximal relaxation elicited by the drug in carbachol contracted tissue. Data are expressed as the mean ± S.E.M. of $n = 3$–5 experiments performed in the absence of pindolol. [b] IA = intrinsic activity; values were calculated as the ratio of the maximal relaxant response for each drug to the maximal relaxant response elicited by 10 μM (−)-isoprenaline added at the end of each experiment. [c] $pD_2 = -\log EC_{50}$; data are expressed as the mean ± S.E.M. of $n = 3$–5 experiments performed in the presence of 1 μM pindolol. [d] NA = not active up to 100 μM. [e] ND = not determined.

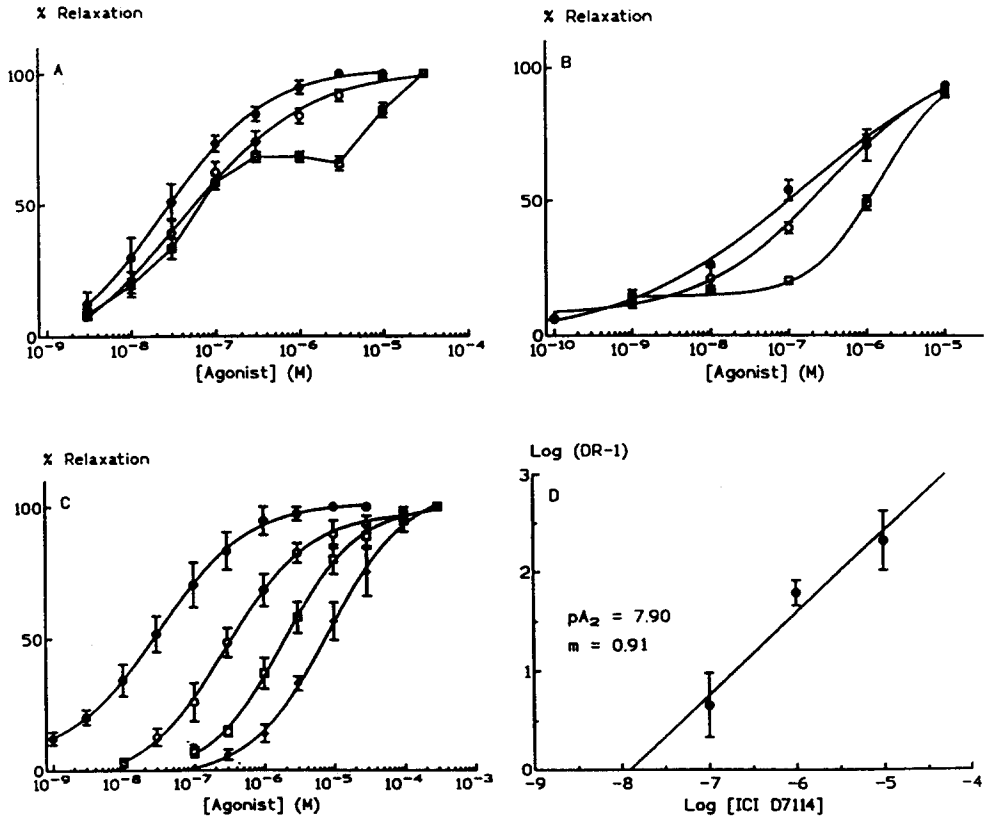

Fig. 6. Effect of ICI D7114 on relaxation responses induced by (−)-isoprenaline (A), BRL 37344 (B), (−)-trimetoquinol (C) and Schild plot analysis of the rightward shifts in the relaxation responses to (−)-trimetoquinol in rat esophageal smooth muscle (D). See Section 2 for details of tissue pretreatments. Data represent the mean ± S.E.M. of $n = 4$ experiments. Control (●), 100 nM ICI D7114 (○), 1 μM ICI D7114 (□) and 10 μM ICI D7114 (◇). pA_2 is defined as the negative logarithm of the concentration of antagonist that produces a rightward shift in the concentration-response curve of an agonist by a factor of two, and m represents the slope value of the Schild plot.

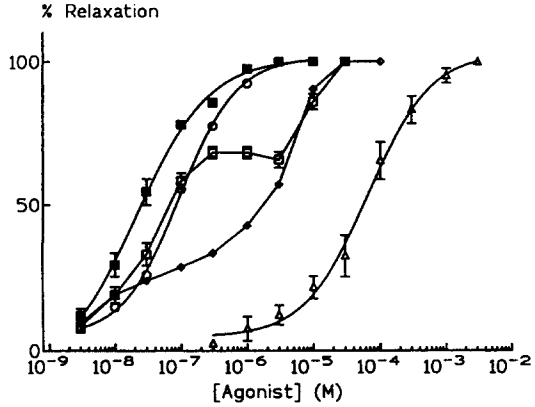

Fig. 7. Relaxation-response curves of (−)-isoprenaline in the presence of (±)-pindolol and ICI D7114 or both in rat esophageal smooth muscle. Control (■), 1 μM pindolol (○), 1 μM ICI D7114 (□), 1 μM ICI D7114 + 1 μM pindolol (◇) and 10 μM ICI D7114 (△). See Section 2 for details of tissue pretreatments. Data represent the mean ± S.E.M. of $n = 4–5$ experiments.

3.3. Relaxation responses of trimetoquinol analogues and selected atypical β/β_3-adrenoceptor agonists in rat esophageal smooth muscle

The potencies (pD_2 values) for a series of trimetoquinol analogues and reference β-adrenoceptor agonists were determined in the absence and presence of 1 μM pindolol. Only small changes in the potencies of these agonists for eliciting relaxation of esophageal smooth muscle were noted in the presence of pindolol (Fig. 5, Table 2). Pindolol itself displayed agonist activity at very high concentrations (10^{-5}–10^{-2} M) giving a pD_2 value of 3.22 (Table 2). The rank order of potency for trimetoquinol analogues was (±)-trimetoquinol [1-(3′,4′,5′-tri-

Table 3
Comparative potencies of trimetoquinol isomers on β-adrenoceptors in rat atria (β_1), trachea (β_2) and esophageal smooth muscle (atypical β/β_3)

Receptor subtype	pD_2 [a] ± S.E.M.		Isomeric activity ratio [b]
	(−)-Isomer	(+)-Isomer	
β_1	8.49 ± 0.13	6.04 ± 0.18	2.45 ± 0.19
β_2	7.56 ± 0.14	5.53 ± 0.21	2.03 ± 0.13
Atypical β/β_3	8.05 ± 0.13	4.69 ± 0.11	3.36 ± 0.15 [d]
Atypical β/β_3 [c]	8.26 ± 0.11	4.80 ± 0.12	3.46 ± 0.11 [d]

[a] $pD_2 = -\log EC_{50}$; EC_{50} value for each drug was determined as the molar concentration required to produce 50% of maximal relaxation elicited by the drug in carbachol contracted tissue. Data are expressed as the mean ± S.E.M. of $n = 3–5$ experiments. With the exception of rat atria, potencies of isomers were determined on paired tissues. [b] Isomeric activity ratio: $-\log [(pD_2$ (−)-isomer $- pD_2$ (+)-isomer)] ± S.E.M. [c] Values determined in the presence of 1 μM pindolol. [d] Means are significantly different ($P < 0.05$) from those in rat atria and trachea.

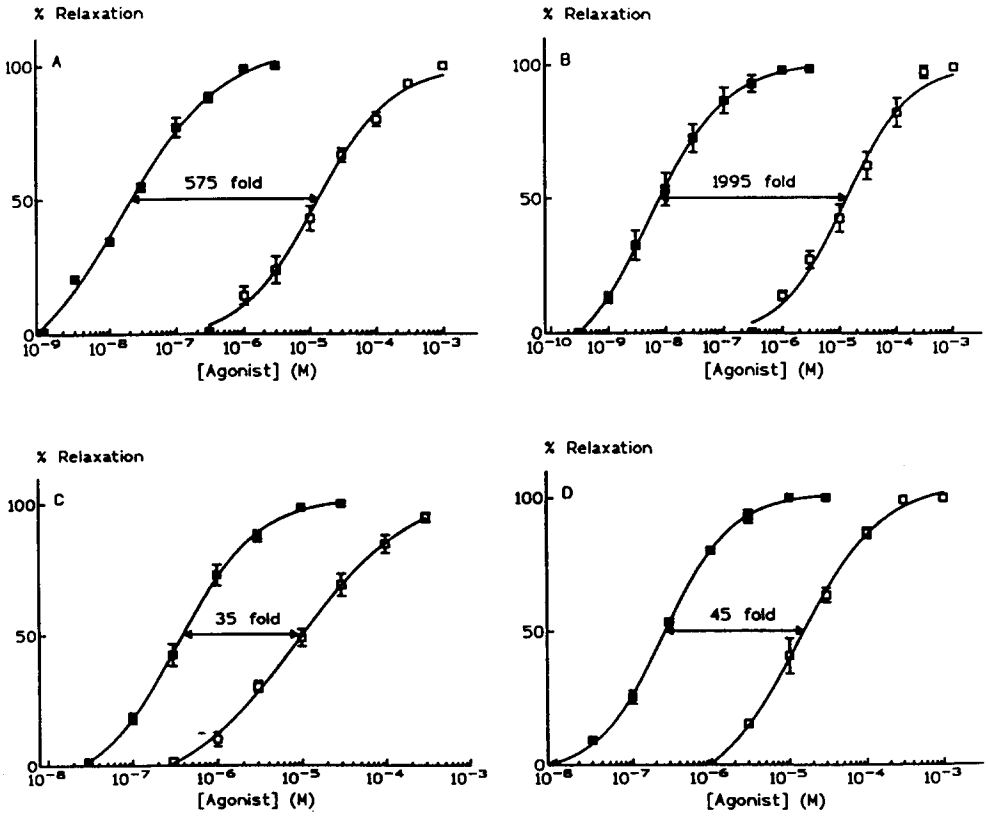

Fig. 8. Comparative functional activities of (−)-isomers (■) and (+)-isomers (□) of isoprenaline (A), trimetoquinol (B), adrenaline (C) and noradrenaline (D) in rat esophageal smooth muscle. See Section 2 for details of tissue pretreatments. Agonist-induced responses were measured in the presence of 1 μM pindolol. The number above the solid lines indicates the antilog of drugs isomeric activity ratio. Data represent the mean ± S.E.M. of $n = 3–5$ experiments.

methoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] > 3′,5′-diiodotrimetoquinol [1-(3′,5′-diiodo-4′-methoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] > 3′-iodotrimetoquinol [1-(3′-iodo-4′,5′-dimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] > 5,8-difluorotrimetoquinol [5,8-difluoro-6,7-dihydroxy-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline] > 8-fluorotrimetoquinol [6,7-dihydroxy-8-fluoro-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline] > (−)-1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline ≫ (+)-1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (inactive).

The β-adrenoceptor agonists, ractopamine, BRL 37344, ICI D7114 and clenbuterol elicited concentration-dependent relaxations of rat esophageal smooth muscle (Fig. 5). Clenbuterol and ICI D7114 behaved as a partial agonists, whereas both ractopamine and BRL 37344 were full agonists. A summary of the pD_2 values for these β-adrenoceptor agonists is provided in Table 2.

ICI D7114 at concentrations ranging from 10^{-7} to 10^{-5} M, produced concentration-dependent rightward shifts of the concentration-response curves of (−)-isoprenaline, (−)-trimetoquinol and BRL 37344 (Fig. 6). (−)-Isoprenaline-induced relaxation of esophageal smooth muscle exhibited a biphasic response when 1 μM ICI D7114 was used as an antagonist. This unusual response was absent when tissues were preincubated with a combination of ICI D7114 (1 μM) and pindolol (1 μM). The biphasic response was also abolished in the presence of a high concentration of ICI D7114 (10 μM) (see Fig. 7). Unlike (−)-isoprenaline, the relaxation responses to (−)-trimetoquinol and BRL 37344 were shifted in a progressive competitive and monophasic manner by ICI D7114. The calculated slope and pA_2 value (mean ± S.E.M.) of ICI D7114 against (−)-trimetoquinol were 0.91 and 7.90 ± 0.09 ($n = 4$), respectively (Fig. 6). The corresponding pK_B value for BRL 37344 was 7.14 ± 0.08 calculated using a concentration of 1 μM ICI D7114.

3.4. Stereoselective interactions of selected trimetoquinol and catecholamine analogues

The concentration-response curves to catecholamine and trimetoquinol isomers on rat esophageal smooth muscle are shown in Fig. 8. For each of these agonists, the (−)-isomer was markedly more potent than the (+)-isomer (Tables 2 and 3). The isomeric activity ratios for the isomers of trimetoquinol, isoprenaline, adrenaline and noradrenaline in the presence of 1 μM pindolol were 1995-, 575-, 35- and 45-fold, respectively. A similarly high eudismic ratio (2951-fold) was observed for esophageal smooth muscle relaxations induced by trimetoquinol isomers in the absence of pindolol (Table 3). As shown in Table 3, the isomeric activity ratios of trimetoquinol for the β-adrenoceptors in rat esophageal smooth muscle were 8.1- to 26.9-fold greater than those in rat atria and trachea. Although less active than trimetoquinol, isomers of 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline also displayed high stereoselectivity [(−)-isomer ≫ (+)-isomer] for relaxation of rat esophageal smooth muscle (Table 2).

4. Discussion

In our studies, (−)-isoprenaline- and (−)-trimetoquinol-induced relaxations of the rat esophageal smooth muscle were resistant to blockade by the β-adrenoceptor antagonists, pindolol (nonselective β_1/β_2), ICI 89,406 (β_1) and ICI 118,551 (β_2) (Table 2). The concentration-response curves to the above agonists were also resistant to blockade by pindobind, a pindolol analogue which is a β-adrenoceptor alkylating agent that blocked 41 and 47% of the total β-adrenoceptors in guinea pig atria and trachea, respectively (Molenaar et al., 1988). In our experiments, pindobind produced greater rightward shifts of the concentration-response curves to (−)-isoprenaline and (−)-trimetoquinol in rat tracheal strips than on rat esophageal smooth muscle (Fig. 4). In addition, (−)-trimetoquinol-induced relaxations of esophageal smooth muscle were more resistant to blockade by pindobind than those of (−)-isoprenaline. Thus, pindobind appears to block a small β_1-/β_2-adrenoceptor population present in rat esophageal smooth muscle, and the remaining atypical β/β_3-adrenoceptor population that mediated functional responsiveness of this tissue is resistant to alkylation by pindobind. The relaxations of carbachol-contracted rat esophageal smooth muscle strips induced by trimetoquinol and isoprenaline seem to be mediated mainly through the activation of a β-adrenoceptor with atypical characteristics. Our results are consistent with an earlier report by De Boer et al. (1993) that esophageal smooth muscle contains predominantly atypical β/β_3-adrenoceptors along with a smaller β_1/β_2-adrenoceptor population.

Several β-adrenoceptor agonists were used in this study to characterise the atypical β/β_3-adrenoceptors present in rat esophageal smooth muscle. BRL 37344, ICI D7114, ractopamine and clenbuterol elicited concentration-dependent relaxations of esophageal smooth muscle that were resistant to blockade by the β-adrenoceptor antagonist pindolol. Further, pindolol itself exhibited a weak agonist activity in esophageal smooth muscle effect at high concentrations. Pindolol has been shown to exhibit weak agonist activity in atypical β/β_3-adrenoceptor containing tissues (Zaagsma and Nahorski, 1990). Thus, our results clearly demonstrate the atypical nature of the β-adrenoceptor present in this tissue and that this receptor population primarily mediates functional responses to catecholamines and other β-adrenoceptor agonists in rat esophageal smooth muscle.

ICI D7114 elicited only a partial relaxation of the carbachol contracted rat esophageal smooth muscle, and behaved as a partial agonist. ICI D7114 was previously

observed to behave as a partial agonist and blocks responses to (−)-isoprenaline in rat distal colon, an atypical β/β_3-adrenoceptor containing tissue (MacDonald and Lamont, 1993). Partial agonists need to occupy a large proportion of receptors in order to produce an agonist effect, and the effect of full agonists can be antagonized by partial agonists (Kenakin, 1993). Accordingly, ICI D7114 produced concentration-dependent rightward shifts in the concentration-response curves of (−)-isoprenaline, (−)-trimetoquinol and BRL 37344 (Fig. 6). (−)-Isoprenaline-induced relaxation of esophageal smooth muscle was observed to exhibit an unusual biphasic pattern in the presence of 1 μM ICI D7114 (Figs. 6 and 7). This biphasic response was abolished when pindolol, a non-selective β_1/β_2-adrenoceptor antagonist was added along with ICI D7114 (Fig. 7). In addition, pindolol-induced shifts of the concentration-response curves to (−)-isoprenaline were not biphasic in nature (Fig. 2). Moreover, ICI D7114 at 10 μM did not exhibit a biphasic displacement of the (−)-isoprenaline concentration-response curve (Fig. 7) and thus we propose that at high concentrations, ICI D7114 (> 1 μM) is able to block both β_1/β_2- and atypical β/β_3-adrenoceptors present in rat esophageal smooth muscle. In contrast, the antagonistic effects of different concentrations of ICI D7114 on the relaxations induced by (−)-trimetoquinol and BRL 37344 were not biphasic in nature (Fig. 6), and the relaxation curves to these agonists were progressively shifted to the right in a concentration-dependent manner. The absence of a biphasic response for ICI D7114-induced shifts of trimetoquinol and BRL 37344 relaxations of esophageal smooth muscle may be explained by reports that these compounds are partial β_1-/β_2-adrenoceptor agonists and full agonists on atypical β/β_3-adrenoceptor populations (Shams et al., 1990; Arch et al., 1984). Thus, we conclude that the relaxation responses induced by trimetoquinol and BRL 37344 in this tissue are principally due to the activation of atypical β/β_3-adrenoceptors.

Isomeric activity ratios for agonists have been used as a criterion for the classification of receptors (Patil, 1969; Patil et al., 1974). Therefore, we further characterised the atypical nature of the receptor in this tissue further by investigating the interactions of the isomers of catecholamine and trimetoquinol analogues. The activation of the β-adrenoceptor population in the rat esophageal smooth muscle by isomers of adrenaline, noradrenaline, isoprenaline, trimetoquinol and 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline was highly stereodependent (Fig. 8 and Table 2). The isomeric activity ratio values obtained for the isomers of isoprenaline, adrenaline and noradrenaline [(−)-(*R*)-isomer ≫ (+)-(*S*)-isomer] in esophageal smooth muscle do not differ significantly from those reported before for these catecholamines in β_1 (guinea pig atria)- and β_2 (guinea pig trachea)-adrenoceptor systems (Buckner and Patil, 1971). These results suggest that catecholamines are not able to differentiate between β_1-, β_2- and atypical β/β_3-adrenoceptors. In contrast, the isomeric activity ratio of trimetoquinol isomers [(−)-(*S*)-isomer ≫ (+)-(*R*)-isomer] in rat esophageal smooth muscle was 8- and 27-fold greater than that obtained for activation of β_1- and β_2-adrenoceptors in rat atria and trachea, respectively (Table 3). Trimetoquinol isomers seem to be able to differentiate between the β-adrenoceptor subtypes present in rat atria, trachea and esophageal smooth muscle. Furthermore, the isomeric activity ratio of trimetoquinol isomers (3.29–3.46-log units) for their interaction with atypical β/β_3-adrenoceptors in rat esophageal smooth muscle was much greater than that observed for interaction with atypical β/β_3-adrenoceptors present in rat distal colon and rat brown adipose tissue (575- and 398-fold, respectively (Konkar et al., 1994; Fraundorfer et al., 1994)). In addition, the isomers of 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, a trimetoquinol analogue, displayed a very high stereoselectivity for interaction with the atypical β/β_3-adrenoceptors. Therefore, trimetoquinol and its analogues may represent important stereospecific probes for characterisation of the atypical β/β_3-adrenoceptor subtype.

A series of halogenated trimetoquinol analogues were evaluated for their agonist properties and chemical specificity of their interaction with rat esophageal β-adrenoceptors. The analogues differed in the position at which halogen group was substituted on the catechol and 1-benzyl ring systems of the trimetoquinol molecule (see Fig. 1). This enabled us to examine the effects of substituting an electronegative halogen group on the β-adrenoceptor activity of trimetoquinol. These compounds have been previously evaluated on β_1 (guinea pig atria)- and β_2 (guinea pig trachea)-adrenoceptor systems (Clark et al., 1987; Shams et al., 1990). Substitution of fluoro-atoms in the 8- or 5,8-positions of the 6,7-dihydroxy tetrahydroisoquinoline ring (Clark et al., 1987; Markovich et al., 1992) or replacement of methoxy groups with iodo-atoms on the 3'- or 3',5'-positions of the 1-(3',4',5'-trimethoxybenzyl) ring (Shams et al., 1990) of trimetoquinol gave compounds which possessed equal or reduced potencies and decreased maximal functional effects as compared to trimetoquinol and isoprenaline on β_1- and β_2-adrenoceptors. All halogenated analogues of trimetoquinol elicited relaxation of the carbachol-induced contraction of rat esophageal smooth muscle. The analogues exhibited reduced potencies and their maximal relaxant activities ranged from 76–97% of that obtained for (−)-isoprenaline and (−)-trimetoquinol. The rank order of potencies for these analogues was, 3',5'-diiodotrimetoquinol ≥ 3'-iodotrimetoquinol > 5,8-difluorotrimetoquinol = 8-fluorotrimetoquinol. The rank order of potencies for the iodinated analogues in comparison to trimetoquinol and isoprenaline differed markedly from that observed in other atypical β/β_3-adrenoceptor containing tissues (rat distal colon, brown adipose tissue). On rat esophageal smooth muscle, the agonist potencies for the 3'- and 3',5'-iodinated analogues of trimetoquinol

were about 10-fold less than that of trimetoquinol. In contrast, on rat distal colon and brown adipose tissue these compounds were equal in potency to trimetoquinol (Konkar et al., 1994). Taken together with our observation of a much greater stereoselectivity ratio (1995-fold) for trimetoquinol isomers on rat esophageal smooth muscle in comparison with those observed on rat distal colon and brown adipose tissue (Konkar et al., 1994; Fraundorfer et al., 1994) we propose that heterogeneous populations of atypical β/β_3-adrenoceptors may exist in rat tissues.

In summary, rat esophageal smooth muscle appears to contain a heterogeneous β-adrenoceptor population, and the functional responses are mediated predominantly by the atypical β/β_3-adrenoceptor subtype. Trimetoquinol was the most potent and stereoselective agonist evaluated in esophageal smooth muscle. Thus, trimetoquinol and isomeric analogues represent a new chemical entity (tetrahydroisoquinoline ring system) which exhibit high functional potency and stereodependency for interaction with atypical β/β_3-adrenoceptors.

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