J. Pharm. Pharmacol. 1990, 42: 217–220
Communicated June 15, 1989

Ability of ketanserin to block different receptors in human placental vessels

JESUS MARIN, JESUS REVIRIEGO*, MARIA S. FERNANDEZ-ALFONSO, *Departamento de Farmacologia y Terapéutica, Facultad de Medicina, Universidad de Extremadura* and Departamento de Farmacologia y Terapéutica, Facultad de Medicina, Universidad Autónoma, c/ Arzobispo Morcillo 4, 28029 Madrid, Spain*

Abstract—5-Hydroxytryptamine (5-HT), noradrenaline (NA) and histamine induced concentration-dependent contractions in segments of human chorionic arteries and veins, whereas clonidine, an α_2-adrenoceptor agonist, had no effect. 5-HT and histamine induced strong contractions, while NA elicited weak contractions in some segments. The maximal response was similar for 5-HT and histamine. The order of potency (EC50 values) was: 5-HT ≥ NA ≥ histamine. These agonists induced tachyphylaxis, and single concentrations caused transient contractions. Contractions elicited by 5-HT were antagonized by ketanserin, a 5-HT_2-receptor antagonist, which also antagonized the responses to NA and histamine, but at greater concentrations than those needed for 5-HT responses. Contractions elicited by histamine were reduced by diphenydramine. Low concentrations of 5-HT amplified contractions caused by NA and histamine. The results indicate that: (i) 5-HT is the most potent constrictor agent tested in these vessels; (ii) its effects are mediated by 5-HT_2-receptors; and (iii) ketanserin at therapeutic plasma concentrations (10^{-7} M) seems to block mainly 5-HT_2-receptors, and α_1-adrenergic- and H_1-receptors to a small extent only.

Ketanserin is a potent antagonist of 5-hydroxytryptamine (5-HT) receptors of the subtype 5-HT_2, and has been shown to be an effective antihypertensive drug. This therapeutic effect was initially ascribed to its ability to block those receptors in peripheral vessels, thereby reducing vascular resistance (Van Nueten et al 1981; Vanhoutte 1982; Reimann & Frölich 1983). Later, it was observed that ketanserin possesses more complex actions, which lead to its antihypertensive effect (Cohen 1984; Saxena et al 1987). Among them, are its capacity to block α-adrenoceptors and the amplifying effect, mainly mediated by 5-HT_2-receptors, that 5-HT produces on contractions elicited by noradrenaline (NA) or other agonists (Cohen 1984; Van Nueten et al 1982, 1986; Saxena et al 1987; Van der Starre & Reneman 1988).

5-HT is a potent vasoconstrictor agent in umbilicoplacental vasculature in contrast to the small contraction elicited by NA (Altura et al 1972; Maigaard et al 1986). Furthermore, 5-HT concentration in maternal blood, placental circulation and placental tissue is enhanced from late pregnancy until spontaneous vaginal delivery (Koren et al 1965; O'Reilly & Loncin 1967; Jones & Rowsell 1973). 5-HT may be involved in the closure of umbilical vessels at birth (Tulenko 1979; Mak et al 1984) and in the pathogenesis of preeclampsia (Montenegro et al 1985). Also, NA concentration in human umbilical blood is enhanced in normal vaginal deliveries (Jones & Greiss 1982). This increase of 5-HT and NA in umbilicoplacental circulation suggests a physiological role for these amines, as well as a mutual interaction between their contractile effects.

The aim of the present study was to investigate in human chorionic arteries and veins: (i) the ability of 5-HT, NA and histamine to elicit contractions; (ii) whether 5-HT is able to amplify the responses induced by the other two agents, and (iii) the capacity of ketanserin to inhibit both the contractions caused by these agonists and the amplifying effects of 5-HT.

Correspondence to: J. Marin, Departamento Farmacologia y Terapéutica, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo 4, 28029 Madrid, Spain.

Material and methods

Solution and drugs used. The composition of Krebs-Henseleit solution (KHS, mM) was: NaCl 115; KCl 4·6; $CaCl_2$ 2·5; KH_2PO_4 1·2; $NaHCO_3$ 25; $MgSO_4$. $7H_2O$ 1·2; glucose 11·1; Na_2EDTA 0·03; pH 7·4. The concentrations of drugs used were obtained from stock solutions (10^{-2} M) in 0·9% NaCl (saline) containing 0·01% (w/v) ascorbic acid. All the stock solutions were kept at −20°C.

Drugs used were: 5-hydroxytryptamine creatinine sulphate (Sigma), ketanserin tartrate (Janssen), (−)-noradrenaline bitartrate (Sigma), and clonidine hydrochloride (Boehringer Ingelheim). Results are given as mean ± s.e.m. Statistical significance was evaluated by Student's *t*-test for paired or unpaired experiments and *P* values of 0·05 or less were considered significant.

Vascular preparations. Human chorionic arteries and veins (2·3–1·8 mm o.d.) placed near to the point of insertion of the umbilical cord were used. The placentas were obtained from full-term normal vaginal deliveries from apparently healthy women. Immediately following delivery, the vessels were carefully isolated and immersed in a beaker containing KHS at 4°C, cleaned of blood traces and adhered tissues and divided into cylindrical segments 5 mm in length in a Petri dish containing cold KHS.

Reactivity experiments. For isometric tension recording, each arterial cylinder was set up in an organ bath as described by Marín et al (1979). The bath contained 6 mL of KHS at 37°C continuously bubbled with 95% O_2–5% CO_2 mixture, which gave a pH of 7·4. Two stainless steel pins, 200 μm diameter, were passed through the lumen of the arterial segments. One pin was fixed to the organ bath wall while the other was connected to a strain gauge for isometric tension recording. The latter pin was placed parallel with the former and was movable, thus allowing the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder. The isometric contraction was recorded through a force-displacement transducer (Grass FT 03C) connected to a Grass model 7D polygraph. The segments were submitted to different tensions in order to obtain the optimal resting tone, which was of 1·5 g. This tone was readjusted every 15 min during a 120 min equilibration period.

Segments were exposed, at the onset of the experiment, to 75 mM K^+ to test their functional state. Subsequently, the bath medium was changed several times until the resting tone was recovered and the cumulative concentration-response curves to 5-HT (10^{-9} to 10^{-5} M); NA (3×10^{-8} to 10^{-4} M); histamine (3×10^{-7} to 10^{-3} M) and clonidine (10^{-4} to 10^{-3} M) were determined. Diphenhydramine (10^{-6} M) and ketanserin (10^{-8} to 10^{-6} M) were added 15 and 30 min, respectively, before the agonists.

Contractile responses induced by different vasoactive drugs were expressed in mg or in percentage of the response induced by a previous administration of 75 mM K^+. Concentrations of drugs producing fifty percent of maximum contractile responses (EC50) were calculated according to Fleming et al (1972).

Results

5-HT, NA and histamine induced concentration-dependent contractions in human chorionic arteries and veins (Figs 1–3). 5-HT and histamine elicited strong vasoconstrictor responses in all segments used, their maximal responses being similar. In contrast, NA produced weak responses in a small number of segments (12 segments of 40). The order of potency of these agonists (EC50, M) in arteries was: 5-HT [2·8 (1·1–8·0) × 10^{-7}, n = 15] ≥ NA [1·4 (0·7–2·5) × 10^{-6}, n = 20] ≥ histamine [3·5 (2·1–5·4) × 10^{-5}, n = 24], and in veins: 5-HT [2·6 (1·1–6·5) × 10^{-7}, n = 14] ≥ NA [1·0 (0·5–2·0) × 10^{-6}, n = 15] ≥ histamine [1·7 (1·2–2·7) × 10^{-5}, n = 24] (95% confidence interval in parentheses).

Single concentrations of 5-HT (3 × 10^{-7} M), NA (10^{-6} M) or histamine (10^{-6} M) induced transient contractions in chorionic arteries and veins [maximal responses (mg, n = 5 in each case): 5-HT, 910 ± 90; NA, 400 ± 50, and histamine 420 ± 60, which were reached in about 5 min; the basal tone was reached 20–35 min

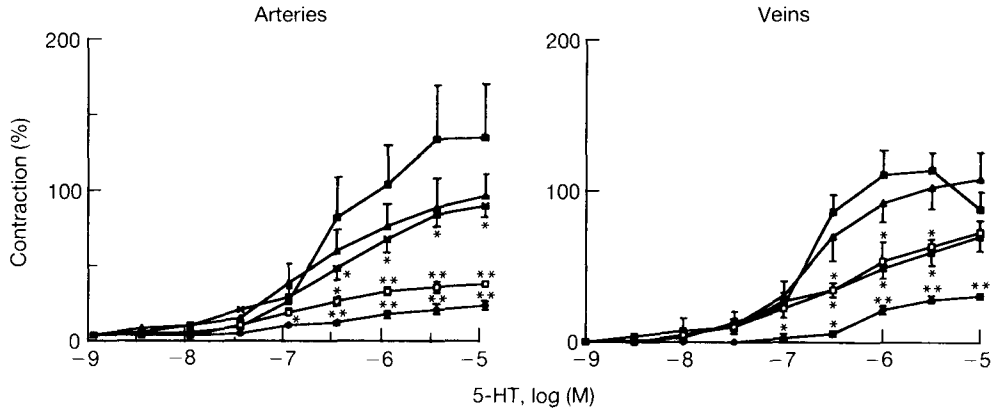

FIG. 1. Effect of ketanserin on contractions induced by 5-HT in segments of human chorionic arteries and veins. Responses (mean ± s.e.m.) were expressed as % of those induced by a previous contraction with 75 mM K^+. (1580 ± 150 in arteries and 1665 ± 125 mg in veins). * $P < 0.005$, ** $P < 0.01$. ■ Control (10), ▲ Ket. (10 nM) (6), →← Ket. (50 nM) (8), ⊟ Ket. (0·1 μM) (6), ◇ Ket. (1 μM) (6).

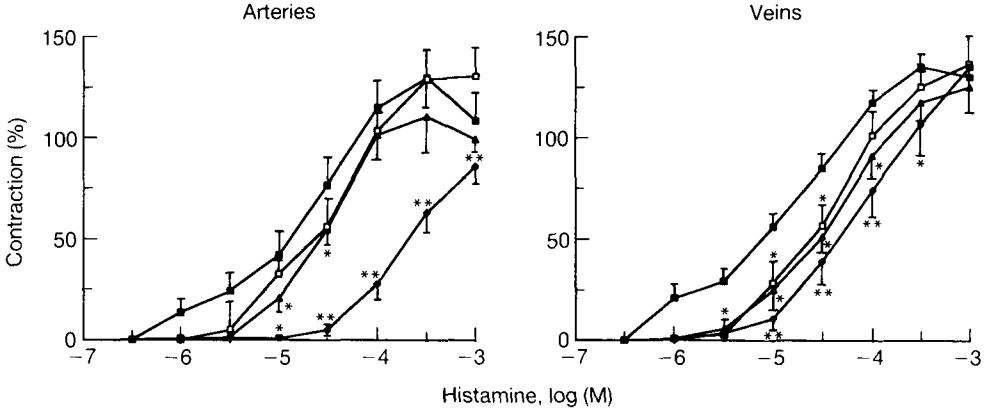

FIG. 2. Effect of diphenhydramine and ketanserin on the contractions elicited by histamine in segments of human chorionic arteries and veins. Expression of results and symbols as in Fig. 1. * $P < 0.05$, ** $P < 0.01$. ■ Control (22), ▲ Ket. (0·5 μM) (5), ⊟ Ket. (1 μM) (5), ● (10).

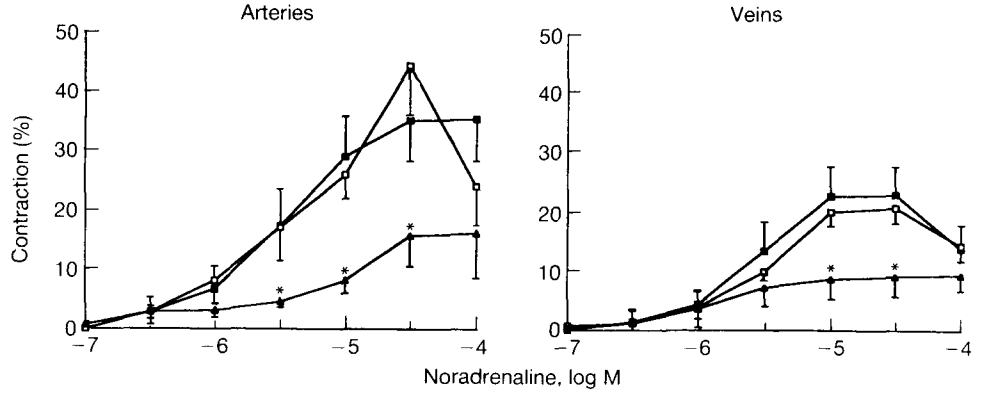

FIG. 3. Effect of ketanserin on the contractions induced by noradrenaline in segments of human chorionic arteries and veins. Expression of results and symbols as in Fig. 1. * $P < 0.05$. ■ Control (10), ⊟ Ket. (0·1 μM) (5), ▲ Ket. (0·5 μM) (5).

later]. These contractions, however, were not observed in segments in which a concentration-response curve to the same agonist had been previously determined.

To investigate whether the vessels had α_2-adrenoceptors, the α_2-agonist clonidine was used and even at very high concentrations (10^{-3} M) did not produce any effect in 12 segments.

Responses elicited by 5-HT were reduced in a concentration-dependent fashion by ketanserin (10^{-8} to 10^{-6} M) (Fig. 1); those induced by histamine were reduced by diphenhydramine (10^{-6} M) but only slightly by ketanserin (10^{-7} to 10^{-6} M) (Fig. 2). NA contractions were also weakly diminished by those concentrations of ketanserin (Fig. 3).

Low concentrations of 5-HT increased the contractions elicited by NA and histamine; this increase was antagonized by ketanserin (30 min preincubations).

Discussion

The results show that 5-HT, NA and histamine elicited concentration-dependent contractions in segments of human chorionic arteries and veins. 5-HT produced the greatest contractions when tested on both types of vessels, which agrees with the results obtained by others on umbilicoplacental vasculature (Altura et al 1972; Tulenko 1979; Mak et al 1984; Maigaard et al 1986).

Elevated concentrations of 5-HT, NA and histamine produced desensitization, which can be a physiological way of protecting chorionic vessels from a maintained contraction production by high concentrations of agonists, mainly 5-HT. Increased concentrations of 5-HT (around 10^{-7} M) have been reported at birth in maternal blood and placental circulation (O'Reilly & Loncin 1967; Jones & Rowsell 1973), i.e. similar to the EC50 value for 5-HT in those vessels. Furthermore, a contribution by 5-HT to the spasm of umbilical vessels after birth has also been suggested (Tulenko 1979; Mak et al 1984). These data indicate a possible role of this amine in umbilicoplacental circulation.

The concentration of histamine required to induce contractions was some 100 times greater than that of 5-HT, which indicates a reduced physiological role of histamine in placental circulation. The contraction it caused was reduced by the H_1-receptor antagonist diphenhydramine suggesting that this effect is mediated by H_1-type receptors. In human umbilical vessels, the H_1-receptor antagonist mepyramine, also antagonized the contraction induced by histamine (Altura et al 1972). The weak responses elicited by NA, an α_1- and α_2-adrenocepter agonist (Van Meel et al 1982) and the inability of clonidine, an α_2-adrenoceptor agonist (Van Meel et al 1982), to produce contractions suggests the existence of a population of α_1-adrenoceptors in these vessels, which appear to have little functional importance.

Ketanserin, a 5-HT_2-receptor antagonist (Van Nueten et al 1981, 1986), in some vessels, seems to possess the ability to block α_1-adrenoceptors (Cohen 1984; Saxena et al 1987; Van der Starre & Reneman, 1988; Nishimura et al 1987) and H_1-receptors (Van Nueten et al 1986). Our experiments show that this drug antagonized non-competitively the contractions caused by 5-HT, a finding suggesting that 5-HT_2-receptors mediate these responses. However, other receptors or mechanisms could be involved. It has also been observed that ketanserin may elicit competitive or non-competitive antagonism or unaffect 5-HT responses (Van Nueten et al 1982, 1986; Arneklo-Nobin et al 1985; Bradley et al 1986), indicating the existence of different populations of 5-HT-receptors in the vessels. Contractions induced by NA and histamine were antagonized by ketanserin at concentrations greater than those needed to inhibit 5-HT responses. This observation suggests that ketanserin, at therapeutic plasma concentrations (about 10^{-7} M, Williams et al 1986; Nishimura et al 1987), blocks mainly 5-HT_2-receptors and to a lesser extent α_1- and H_1-receptors in the placental vasculature. These results also confirm that the affinity of ketanserin for the first type of receptor is greater than that for the other two types (Cohen 1984; Phillips et al 1985; Van Nueten et al 1986).

Low concentrations of 5-HT amplified the contractions induced by NA and histamine, an effect blocked by ketanserin suggesting that the potentiation is mainly mediated by 5-HT_2-receptors. The amplifying effect of 5-HT on the contractions caused by these amines, and its inhibition by ketanserin, have also been observed in other vessels (Van Nueten et al 1982; Van Nueten 1985).

In conclusion, 5-HT induces potent vasoconstrictor effects in human placental vasculature, and amplifies the responses elicited by NA and histamine. The contractions caused by these agonists are partially mediated by 5-HT_2-, α_1-adrenergic- and H_1-receptors. The amplifying effect caused by 5-HT is mediated by 5-HT_2-receptors. Ketanserin has most affinity for the latter receptors and, at therapeutic plasma concentrations, blocks 5-HT_2-receptors.

We are grateful to the physicians and nurses of the Department of Obstetrics and Gynecology at the 'Hospital La Paz' of Madrid for their cooperation in this study. We thank Janssen and Boehringer Ingelheim laboratories for the generous supply of some drugs. This work was supported by Grants from F.I.S.S. (88/841) and C.A.I.C.Y.T. (327/84)

References

Altura, B. M., Malaviya, D., Reich, C. F., Orkin, L. R. (1972) Effects of vasoactive agents in isolated umbilical arteries and veins. Am. J. Physiol. 222: 345–355

Arneklo-Nobin, B., Nobin, A., Owman, C., Törnebrandt, K. (1985) Serotonergic mechanisms in isolated human peripheral arteries and veins. J. Cardiovasc. Pharmacol. 7 (Suppl. 7): S52–S55

Bradley, P. B., Humphrey, P. P. A., Williams, R. H. (1986) Evidence for the existence of 5-hydroxytryptamine receptors, which are not of the 5-HT_2 type, mediating contraction of rabbit isolated basilar artery. Br. J. Pharmacol. 87: 3–4

Cohen, M.L. (1984) Vascular serotonin receptors and blood pressure regulation. Drug Develop. Res. 4: 301–313

Fleming, W. W., Westfall, D. P., De La Lande, I. S., Jellet, L. B. (1972) Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. J. Pharmacol. Exp. Ther. 181: 340–345

Jones, J. B., Rowsell, A. (1973) Fetal 5-hydroxytryptamine levels in late pregnancy. J. Obst. Gynecol. 80: 687–689

Jones, C. M., Greiss, F. C. (1982) The effect of labor on maternal and fetal circulating catecholamines. Am. J. Obst. Gynecol. 144: 149–153

Koren, Z., Pfeifer, Y., Sulman, F. G. (1965) Serotonin content of human placenta and fetus during pregnancy. Ibid. 93: 411–415

Maigaard, S., Forman, A., Andersson, K.-E. (1986) Relaxant and contractile effects of some amines and prostanoids in myometrial and vascular smooth muscle within the human uteroplacental unit. Acta Physiol. Scand. 128: 33–40

Mak, K. K.-W., Gude, N. M., Walters, W. A. W., Boura, A. L. A. (1984) Effects of vasoactive autacoids on the human umbilical-fetal placental vasculature. Br. J. Obst. Gynecol. 91: 99–106

Marín, J., Salaices, M., Marco, E. J., Gómez, B., Lluch, S. (1979) Analysis of the contractile effect of 5-hydroxytryptamine on the isolated posterior communicating artery of the cat. J. Pharm. Pharmacol. 31: 456–459

Montenegro, R., Knuppel, R. A., Shah, D., O'Brien, W. F. (1985) The effect of serotonergic blockade in postpartum preeclamptic patients. Am. J. Obst. Gynecol. 153: 130–134

Nishimura, J., Kanaide, H., Shogakiuchi, Y., Nakamura, M. (1987) Ketanserin blocks alpha$_1$-adrenoceptors of porcine vascular smooth muscle cells. Eur. J. Pharmacol. 133: 235–238

O'Reilly, S., Loncin, M. (1967) Ceruloplasmin and 5-hydroxytryptamine metabolism in pregnancy. Am. J. Obst. Gynecol. 97: 8–12
Phillips, C. A., Mylecharane, E. J., Shaw, J. (1985) Mechanisms involved in the vasodilator action of 5-hydroxytryptamine in the dog femoral arterial circulation in vivo. Eur. J. Pharmacol. 113: 325–334
Reimann, I. W., Frölich, J. C. (1983) Mechanism of antihypertensive action of ketanserin in man. Br. Med. J. 287: 381–383
Saxena, P. R., Bolt, G. R., Dhasmana, K. M. (1987) Serotonin agonists and antagonists in experimental hypertension. J. Cardiovasc. Pharmacol. 10 (Suppl. 3): S12–S18
Tulenko, T. N. (1979) Regional sensitivity to vasoactive polypeptides in the human umbilicoplacental vasculature. Am. J. Obst. Gynecol. 135: 629–636
Van der Starre, P. J. A., Reneman, R. S. (1988) The alpha-adrenergic receptor blocking effect of ketanserin and the interaction between alpha-adrenergic and S_2-serotonergic receptor blockade. J. Cardiovasc. Pharmacol. 11 (Suppl. 1): S54–S61
Vanhoutte, P. M. (1982) Does 5-hydroxytryptamine play a role in hypertension? Trends Pharmacol. Sci. 3: 370–373
Van Meel, J. C. A., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1982) Interaction between calcium antagonists and vascular postsynaptic alpha-receptors. J. Physiol. (Paris) 13: 367–379
Van Nueten, J. M. (1985) Serotonin and the blood vessel wall. J. Cardiovasc. Pharmacol. 7 (Suppl. 7): S49–S51
Van Nueten, J. M., Janssen, P. A. J., Van Beek, J., Xhonneux, R., Verbeuren, T. J., Vanhoutte, P. M. (1981) Vascular effects of ketanserin (R 41468), a novel antagonist of 5-HT_2 serotonergic receptors. J. Pharmacol. Exp. Ther. 218: 217–230
Van Nueten, J. M., Janssen, P. A. J., De Ridder, W., Vanhoutte, P. M. (1982) Interaction between 5-hydroxytryptamine and other vasoconstrictor substances in the isolated femoral artery of the rabbit; effect of ketanserin (R 41468). Eur. J. Pharmacol. 77: 281–287
Van Nueten, J. M., Schuurkes, J. A. J., De Ridder, W. J. E., Kuyps, J. J. M. D., Janssens, W. J. (1986) Comparative pharmacological profile of ritanserin and ketanserin. Drug Develop. Res. 8: 187–195
Williams, F. M., Leeser, J. E., Rawlins, M. D. (1986) Pharmacodynamics and pharmacokinetics of single doses of ketanserin and propanolol alone and in combination in healthy volunteers. Br. J. Clin. Pharmacol. 22: 301–308

J. Pharm. Pharmacol. 1990, 42: 220–222
Communicated November 1, 1989

β-Adrenoceptor antagonists and human sperm motility

P. B. CURTIS-PRIOR, A. L. GADD*, *Cambridge Research Institute, Histon, Cambridge, CB4 4JE and Napp Research Centre, Cambridge Science Park, Cambridge, UK*

Abstract—Several β-adrenoceptor blocking agents have been evaluated for spermicidal activity using a transmembrane migration method. The rank order of potency of the active compounds was: penbutolol > (+) - propranolol > bufuralol > (−) - alprenolol > oxprenolol > metoprolol. Atenolol, pindolol, practolol, tolamolol were without activity. The observed potencies of spermicidal activity are believed to be unrelated to β-blocking activities, and we have shown that whilst they are not predictable from lipid solubility or non-specific membrane properties of the compound alone, both these aspects appear to play a role in this pharmacological activity.

Many β-adrenoceptor antagonists, in addition to their specific cardiovascular therapeutic effect, exert a non-specific action on membranes which has been termed 'membrane stabilizing activity'. This property has not been clearly defined and encompasses a spectrum of non-specific membrane effects unrelated to the β-receptor antagonist activity, including local anaesthetic or quinidine-like effects, physical stabilization of membranes and protection against cell lysis (Smith 1982). These properties have been demonstrated with propranolol, a chiral member of the β-adrenoceptor antagonist family. Both optical isomers, (+)- and (−)-propranolol possess membrane stabilizing activity, whereas the (+)-isomer is only a weak β-adrenoceptor antagonist (Barrett & Cullum 1968). Peterson & Freund (1973) demonstrated that both the racemic mixture and (+)-propranolol inhibited human sperm motility at millimolar concentrations, compatible with "membrane stabilizing activity" involvement. We have thus investigated the effects on human sperm motility of several β-adrenoceptor antagonists, with and without previously reported non-specific membrane stabilizing activity and having a broad range of lipid solubilities.

Correspondence to: P B. Curtis-Prior, Cambridge Research Institute, Histon, Cambridge, CB4 4JE, UK.
* Present address: Biosciences, ICI Pharmaceuticals, Macclesfield, Alderly Edge, Cheshire, UK.

Methods

The modified transmembrane migration method of Hong et al (1981a), as previously described (Gadd & Curtis-Prior 1988), was used to measure the effects on sperm motility of the following β-adrenoceptor antagonists: (±), (+), and (−)-propranolol hydrochloride (Sigma and ICI); bufuralol hydrochloride (Roche); (−)-alprenolol (Sigma); penbutolol sulphate (Hoechst); oxprenolol hydrochloride (Ciba-Geigy); metoprolol tartrate (Ciba-Geigy); tolamolol hydrochloride (Pfizer); pindolol (Sandoz); practolol (ICI) and atenolol (ICI). All drugs were dissolved in 0·9% w/v NaCl saline or phosphate buffered saline (Dulbecco "A"). Dose-response curves were constructed on each ejaculate with triplicate measurements of motility at each concentration. The data were analysed using PCONLIN, an iterative non-linear regression analysis program to fit the parameters of the following equation:

$$I = I_0 - \frac{I_0 C^s}{Q^s + C^s}$$

where I_0 is the percentage inhibition of motility at zero concentration of inhibitor drug, C is the variable drug concentration, Q (IC50) is the drug concentration at which 50% maximal inhibition occurs, and S is a parameter controlling the "sigmoidicity" of the response curves.

Results and discussion

In agreement with previous observations (Peterson & Freund 1973; Hong et al 1981a), the racemate and (+)-/(−)-isomers of propranolol (0·1 to 10 mM) produced a dose-dependent inhibition of sperm motility, the concentrations producing 50% inhibition of motility (IC50) being 1·26±0·09, 1·3±0·11 and 1·65±0·21 mM, respectively (Fig. 1). The IC50 value obtained