The effects of a selective 5-HT₂ receptor antagonist (ICI 170,809) on platelet aggregation and pupillary responses in healthy volunteers

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- 1 ICI 170,809 (2-(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline hydrochloride) is a potent 5-hydroxytryptamine (5-HT) type 2 postsynaptic receptor antagonist.
- 2 Effects of ICI 170,809 as single oral doses (3, 7, 15 and 30 mg) or placebo were studied on the duration of antagonism for the *ex vivo* platelet aggregatory response to 5-HT and to the pupillary light constrictor response in eight healthy male volunteers.
- 3 Pupillary dark adapted responses to a 0.5 s light stimulus were measured using a portable infrared pupillometer, for up to 24 h after dosing.
- 4 The *in vitro* platelet 5-HT aggregation response was reduced by ICI 170,809, with depression of the dose-response curve to 5-HT at all concentrations of 5-HT and with no evidence for a parallel shift.
- 5 The *ex vivo* platelet 5-HT response demonstrated a dose related significant ($P < 0.02$) decrease in aggregation reaching a maximum at 2 h after dosing with the effect persisting for at least 8 h after dosing with the 7 and 15 mg doses.
- 6 Resting pupil diameter (RPD), and light induced pupillary responses in the dark adapted pupil, showed a significant ($P < 0.01$) dose related reduction with significant ($P < 0.05$) effects still present with the 15 and 30 mg doses at 8 h after dosing.
- 7 We conclude that, changes in both *ex vivo* platelet aggregation to 5-HT and dark adapted pupil size, are significantly correlated ($P < 0.0001$) with log plasma concentrations (ng ml^{-1}) of ICI 170,809, enabling the assessment of 5-HT₂-receptor antagonism in man.

Keywords 5-HT₂-receptor antagonist ICI 170,809 platelet aggregation pupil response

Introduction

5-Hydroxytryptamine-2 (5-HT₂) receptors have been identified in both rat (Goldstein *et al.*, 1989; Peroutka *et al.*, 1981) and human central nervous system (Biegon *et al.*, 1987; Pazos, 1987; Reimann *et al.*, 1986). Subsequent work has demonstrated similarities of the 5-HT₂ site with the 'D' receptor of Gaddum & Picarelli (1957). The 5-HT₂ receptor is thought to be homogeneous, but to have structural homology with the 5-HT_{1c} site (Pritchett *et al.*, 1988), both of which are linked to a second messenger system stimulating phosphatidyl inositol turnover (Conn & Sanders-Bush, 1985). 5-HT₂ receptors are located also in humans on platelets (Bevan & Heptinstall, 1986; Gotta *et al.*, 1987) and in vascular smooth muscle (Blauw *et al.*, 1988; Houston & Vanhoutte, 1989) where they mediate some of the excitatory actions of 5-HT (De Clerk & Van Nueten, 1982).

Recently, human volunteer studies with ICI 169,369 (2-(2-dimethylamino ethylthio)-3-phenyl quinoline) a 5-HT₂-receptor antagonist which is structurally related to ICI 170,809 (Blackburn *et al.*, 1988, 1990), raised the possibility that 5-HT₂ receptors may be directly involved in the neuronal control of human pupillary responses (Millson *et al.*, 1991). This is supported by human data where fenfluramine (a 5-HT releasing agent), both topically applied and systemically administered, caused a mydriasis (Kramer & Turner, 1973); and animal data where 5-HT₂ receptors have been identified in rabbit iris (Tobin *et al.*, 1988).

ICI 170,809, whose structure is shown in Figure 1 is a potent selective 5-HT₂-receptor antagonist, which expresses its activity at vascular, neuronal and gastrointestinal 5-HT₂ receptors in both *in vitro* and *in vivo* animal models (Blackburn *et al.*, 1988). It is necessary

5-hydroxytryptamine (5-HT)

2-(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline hydrochloride (ICI 170,809).

Figure 1 Structure of ICI 170,809 illustrating its relationship to 5-HT.

to demonstrate that ICI 170,809 has 5-HT₂-receptor antagonist properties in man. Two pharmacodynamic responses will be explored together with measurements of plasma ICI 170,809 to investigate the ensuing pharmacodynamic/kinetic relationships.

- 1) The *in vitro* antagonist activity of ICI 170,809 at functional 5-HT₂ receptors on human platelets, together with the duration of action of any effect, using 5-HT induced *ex vivo* platelet aggregation.
- 2) Changes in light induced pupillary reflexes and dark adapted resting pupil diameter resulting from dosing with ICI 170,809, together with the duration of action of any effect.

The ability to detect both neuronal and platelet disaggregatory effects in volunteers might provide a useful model in which to predict an active dose of 5-HT₂-receptor antagonist at the human 5-HT₂ receptor. This would allow assessment of potential efficacy in diseases of both the cardiovascular and central nervous system, and provide confirmatory evidence for the involvement of 5-HT₂ receptors in the control of human pupillary responses.

Methods

Volunteer selection and study design

The study was a double-blind, randomised, ascending dose, partial cross over design in which eight male volunteers (aged 18–45 years, weighing between 63 and 91 kg) received three out of a possible four single oral doses (3, 7, 15 and 30 mg) of ICI 170,809, or placebo on four dosing occasions 7 days apart.

Before commencing the study, the protocol was approved by an ethics committee and written informed consent was obtained. Volunteers were only allowed to proceed into the study if no abnormalities were found after medical examination, laboratory blood and urinary safety screens.

Protocol followed

ICI 170,809 was administered orally to volunteers who had fasted overnight. *Ex vivo* platelet sensitivity to 5-HT measured as the extent of aggregation, was determined on three separate occasions before, and 2, 5, 8 and 24 h after oral administration of ICI 170,809 or placebo.

Infra-red pupillometry was performed at 3, 5, 8 and 24 h after dosing, six consecutive artefact free recordings were made and mean values derived for resting (RPD), light constricted (MPD) and the final recovered (FPD) pupil diameter. Except for the 3 mg dose (where sampling was limited to 24 h) venous blood sampling for estimation of ICI 170,809 plasma concentration was performed predose and at 1, 1.5, 2, 3, 5, 8, 12, 15, 24, 30 and 48 h after dosing.

Assessments

Platelet aggregation tests Platelet rich plasma was prepared as a preliminary to examining the aggregatory effects of 5-HT. Subjects recruited into the study were bled from an antecubital vein into tubes containing tri-sodium citrate solution (3.2 w/v, one part to nine parts whole blood) and platelet rich plasma (PRP) was harvested after centrifugation (200 g for 15 min) on each of three separate occasions.

In vitro platelet aggregation was carried out using human PRP in a Payton aggregometer. Aliquots (250 µl) of PRP were stirred (900 rev min⁻¹) and incubated (37° C) for 60 s in an aggregometer before ICI 170,809 or vehicle were added. Platelets were incubated for a further 60 s and a single concentration of 5-HT was then added. This procedure was repeated using a range of 5-HT concentrations (5 × 10⁻⁹ to 5 × 10⁻⁵ M). The extent of 5-HT induced aggregation was measured and expressed as a percentage of the maximum obtained with vehicle controls (Figure 2).

Ex vivo platelet aggregation was performed on PRP from volunteers following oral dosing with either ICI 170,809 or placebo. Aliquots of PRP (250 µl) were incubated for 120 s in an aggregometer (900 rev min⁻¹, 37° C) before concentrations of 5-HT were added. The extent of 5-HT induced aggregation, following oral

Figure 2 5-HT induced platelet aggregation of human PRP in the presence of ICI 170,809: 10⁻⁷ M (○, n = 8); 4 × 10⁻⁷ M (△, n = 7) and vehicle (●, n = 8). The extent of the 5-HT induced aggregation was expressed as a percentage of the mean maximum aggregation response obtained with vehicle, on three occasions prior to dosing.

Table 1 The maximum velocity and acceleration associated with the light induced pupillary constriction at 3 h after dosing with ICI 170,809 (3, 15, 30 mg or placebo), expressed as the mean with s.e. mean and 95% confidence intervals for the differences from placebo values and associated *P* values

Treatment	Mean	Maximum constriction			Mean	s.e. mean	CI
		Velocity (mm s ⁻¹)	s.e. mean	CI	Acceleration (mm s ⁻²)	s.e. mean	CI
Placebo	7.87	1.2			64.9	13.9	
ICI 170,809 3 mg	5.68	0.9	1.25		48.0	8.9	22.26
15 mg	3.93	0.5	0.66**		47.0	10.4	22.62
30 mg	4.00	0.4	0.58**		24.2	1.2	8.64**

CI = 95% CI for the differences from placebo mean. ** = *P* < 0.01.

dosing with ICI 170,809 or placebo, was expressed as a percentage of the mean individual maximum platelet aggregation response as previously described (Figure 2).

Pupil responses

A 'pupilsan' hand held infra-red pupillometer (Millson *et al.*, 1988) was used to measure dark adapted resting pupil diameter (RPD) after 15 min dark adaptation wearing dark goggles (BS 6975EW). A 0.5 s (565 nm; 36 cd m⁻²) light stimulus induced pupillary reflex, minimally light constricted (MPD) and recovered (FPD) pupil diameter after a 3 s cycle were measured together with dynamic pupillary changes every 10 ms. A 'pupilsan' data analysis template was used to analyse the maximum velocity (mm s⁻¹) and acceleration (mm s⁻²) associated with the pupillary constriction.

Plasma assay of ICI 170,809

A 7 ml sample of venous blood was taken into a tube containing oxalate as anticoagulant, and after thorough mixing centrifuged at 2000 rev min⁻¹ for 10 min. The plasma was decanted off and stored at -20° C prior to assay.

After thawing stored plasma a 1 ml aliquot was mixed with 1 ml pH 10 buffer and agitated with 10 ml 1.5% amyl alcohol in hexane for 15 min. After centrifuging to separate the phases, 9 ml of the solvent layer was transferred to a tube containing 2 ml 0.1 N hydrochloric acid. After shaking and centrifugation the solvent layer was discarded. The aqueous layer was mixed with 2.5 ml 0.1 N sodium hydroxide to which 10 ml 1.5% amyl alcohol was added. After mixing and centrifugation 9 ml of the solvent layer was evaporated to dryness at 40° C. The residue was redissolved in 0.2 ml eluent (20% pH 4.0 triethylamine buffer (0.28%):80% acetonitrile) and aliquots analysed by high pressure liquid chromatography at a 1.0 ml min⁻¹ flow rate using a 10 cm × 3 mm (internal diameter) Hyperil ODS column with detection by ultraviolet absorption at 259 nm.

A calibration series covering the expected samples concentration range was constructed for each set of analyses. The unknown samples were determined by a least square fit calculation from the standard curve. The limit of quantification was 2.0 ng ml⁻¹ with an intraassay coefficient of variation of < 10% over the concentration range 2.0–100 ng ml⁻¹.

Determination of pharmacokinetic parameters for ICI 170,809

For each dose and plasma ICI 170,809 concentration time curve: the maximum measured concentration of ICI 170,809 in plasma (*C*_{max}), and the time corresponding to the maximum concentration in plasma (*t*_{max}) were obtained by inspection. The areas under the curve up to the last measured time point (AUC_t) for ICI 170,809 concentrations were calculated using trapezoidal and log trapezoidal rules and extrapolated to infinite time (AUC_∞) employing a terminal exponential rate constant (*λ*_z) estimated by log-linear regression of selected data points describing a terminal log-linear phase, wherever possible. The exponential elimination rate constant was used to extrapolate the area under the curve to infinite time (AUC_∞) and to provide an estimate of the elimination half-life (*t*_{1/2,z}) where possible. Thus *t*_{1/2,z} = 0.693/*λ*_z. A similar approach was adopted to calculate a half-life for the absorption and distribution phase.

Statistical methods

A multifactor analysis of variance was used to analyse pupillometry and platelet data using the following terms in the analysis: volunteer, treatment and time after dosing. The main objective of this repeated measures analysis was to test whether significant differences were detectable in measurements of 5-HT induced platelet aggregation, and pupil diameter parameters between the placebo treated and ICI 170,809 treated groups, at various times after dosing. The Greenhouse Geisser correction factor for repeated measures analysis was applied. If a statistically significant (*P* < 0.05) difference was found between the profiles, then analysis of covariance was performed at each timepoint separately, using the pre-dose value as the covariate.

A least square regression analysis was used to examine the correlation between pharmacodynamic parameters (such as pupil diameter or % change in platelet responses to 5-HT) and pharmacokinetic measurements (i.e. plasma concentrations of ICI 170,809). The 95% confidence intervals (CI) for the line of best fit with 95% CI for the parameter estimate are displayed graphically together with associated correlation coefficient, slope and intercept values.

Differences in velocity and acceleration between placebo and ICI 170,809 treated volunteers at 3 h after

dosing, were analysed with values derived from the median pupillary light constriction curves. Median curves were plotted for each volunteer, which were summated to give a mean $3 \text{ h} (\pm \text{s.e. mean})$ curve for each dose.

Results

Effects of ICI 170,809 on the *in vitro* platelet aggregation

The percentage maximum aggregation achieved to (10^{-5} M) 5-HT was reduced to less than 40% with both 10^{-7} and $4 \times 10^{-7} \text{ M}$ concentrations of ICI 170,809,

compared with the vehicle when added to PRP *in vitro* (Figure 2). The dose-response curve to 5-HT, was significantly depressed at all concentrations of 5-HT, with no evidence of a parallel shift.

Effects of ICI 170,809 on (ex vivo) platelet aggregation

When dosed orally to human volunteers ICI 170,809 caused dose dependent inhibition of 5-HT induced platelet aggregation *ex vivo* (Figure 3). ICI 170,809 (3 mg) did not significantly ($P > 0.05$) modify aggregation responses. Two hours after administration of higher doses of the drug (7, 15, 30 mg) attenuation of 5-HT induced platelet aggregation was evident yielding mean

Figure 3 The effects of ICI 170,809 (3 mg, $\circ$, $n = 5$; 7 mg, $\triangle$, $n = 5$; 15 mg, $\square$, $n = 6$; 30 mg, ∇ , $n = 5$) and placebo ($\bullet$, $n = 7$), on *ex vivo* 5-HT induced human platelet aggregation at 2, 5, 8 and 24 h after oral dosing, with data expressed as inhibition of the mean maximum aggregation response to 5-HT in the presence of vehicle, on three occasions prior to dosing. The histograms depict the maximum inhibition achieved for each treatment (ICI 170,809 3 mg $\square$, 7 mg $\square$, 15 mg $\blacksquare$, 30 mg $\boxtimes$, placebo $\boxplus$) at 2, 5, 8 and 24 h, with appropriate P values for values significantly different from placebo. * $P < 0.02$.

Figure 4 Relationship between 5-HT-induced platelet aggregation (5×10^{-6} M) expressed as % inhibition compared with baseline values, vs log plasma (ng ml^{-1}) ICI 170,809 after oral dosing ($r = 0.797$, $P < 0.0001$, slope = -46.30 , intercept = 106.43). Least squares regression line is given with 95% confidence intervals for the line of best fit (---) and parameter values.

($\pm$ s.e. mean percentage inhibitions of 59.5 ± 4.6 , 73.8 ± 4.2 and 82.4 ± 4.0 respectively, which were significantly different from placebo ($P < 0.05$). This activity persisted for 5 h with 7 and 15 mg doses (mean $\pm$ s.e. mean percentage inhibition of 42.4 ± 4.8 and 54.6 ± 4.2 respectively). The minimal effective oral dose in human volunteers was 7 mg. Data for the 30 mg dose of ICI 170,809 was not collected beyond 2 h after dosing since this dose was considered to be in excess of the anticipated clinical doses, and was included in this study principally to provide tolerability data.

5-HT₂ induced platelet aggregation (5×10^{-6} M) was significantly correlated ($r = 0.797$, $P < 0.0001$, slope = -46.30 , intercept = 106.4) with log concentrations (ng ml^{-1}) of ICI 170,809 (Figure 4).

Effects of single oral doses of ICI 170,809 on pupillary responses

When dosed orally to human volunteers 3, 15 and 30 mg doses produced a dose dependent pupillary miosis (Figure 5). No significant baseline changes were evident when pre-treatment groups were compared with placebo. Data from the 7 mg dose was not available due to a technical failure with the infrared sensing equipment on week 2 of the study.

A significant ($P < 0.01$) reduction in mean RPD and

Figure 5 Effects of ICI 170,809 (3 mg ○, 15 mg ■, 30 mg □ and placebo ●) on mean ($\pm$ 95% confidence intervals for treatment differences from placebo) for dark adapted resting pupil diameter (RPD), minimally constricted pupil diameter (MPD) and final resting pupil diameter (FPD), predose, 3, 5, 8 and 24 h after dosing. Significant differences from placebo are highlighted with appropriate P values. * $P < 0.05$, ** $P < 0.01$.

Table 2 Pharmacokinetic parameters for ICI 170,809 after single oral doses

Dose (mg)	$C_{\max}$ (ng ml^{-1})	$t_{1/2, \lambda_1}$ (h)	$t_{1/2, z}$ (h)	AUC_{∞} ($\text{ng ml}^{-1} \text{ h}$)
3.0	8.0 (1.5)	NC	NC	NC
7.0	15.9 (3.0)	2.6 (0.8)	18.0 (5.5)	197.0 (47)
15.0	37.7 (7.0)	2.6 (0.2)	40.0 (8.6)	1030.0 (388)
30.0	104.0 (2.6)	3.5 (0.2)	43.0 (7.8)	2259.0 (807)

Values are expressed as the mean $\pm$ s.e. mean.

$C_{\max}$ = peak plasma concentration achieved.

$t_{1/2, \lambda_1}$ and $t_{1/2, z}$ are the distribution and elimination half-lives respectively.

AUC_{∞} is the area under the plasma concentration-time curve extrapolated to infinity.

Figure 6 Effects of ICI 170,809 (3 mg ▼, 15 mg ▲, 30 mg ●, or placebo ■) on pupillary response curves. Each curve represents the change in mean dark adapted vertical pupil diameter ($\text{mm} \pm$ s.e. mean) from resting values at 3 h after dosing, following a light stimulus (0.5 s, 565 nm, 36 cd m^{-2}) and with reflected infrared recordings every 10 ms.

FPD occurred at 3 and 5 h after dosing, with reductions in vertical pupil diameter of up to 50% compared with placebo. MPD was also significantly reduced ($P < 0.01$) by approximately 50% with 15 and 30 mg doses of ICI 170,809 at 5 h after dosing. Significant reductions in RPD, MPD and FPD were still present at 8 h ($P < 0.05$) with recovery to baseline values occurring 24 h after dosing (Figure 5).

Mean pupil light response curves (Figure 6) derived from summation of median plots from each volunteer show a reduction in reflex amplitude together with a shift in RPD. Analysis of the kinetics of pupillary light constriction following oral ICI 170,809 demonstrated a reduction in both the velocity and acceleration of pupillary constriction in a dose dependent manner (Table 1).

Pupillary miosis as a function of plasma ICI 170,809 concentration

Percentage changes in resting pupil diameter (compared with baseline values) were linearly related (Figure 7) to log plasma concentrations (ng ml^{-1}) in a similar manner to the platelet aggregatory response ($r = 0.55$, $P < 0.0001$, slope = -26.13 , intercept = 98.89).

Figure 7 The relationship between resting pupil diameter (RPD) expressed as a percentage change from baseline values, vs log concentration of ICI 170,809 (ng ml^{-1}). Least squares regression ($r = 0.55$, $P < 0.0001$, slope = -26.13 , intercept = 98.89) line is given with 95% confidence intervals for the fitted line (---) and parameter estimates.

Figure 8 Pharmacokinetic profiles over a 50 h period of treatment with single oral doses of ICI 170,809 3 ($\circ$), 7 ($\blacktriangle$), 15 ($\bullet$) and 30 mg ($\blacksquare$). Values are shown as mean and s.e. mean ($n = 6$).

Pharmacokinetic analysis

Pharmacokinetic profiles for plasma ICI 170,809 with respect to time after dosing are given in Figure 8, together with the derived pharmacokinetic parameters in Table 2. Insufficient data was generated for the 3 mg dose to calculate all parameters. The absorption phase was similar for all doses, with a median time to peak concentration of 2 h (range 1–3 h). The rise in peak plasma concentration (C_{max}) was approximately linear with increased dose, whereas the area under the concentration-time curve displayed non-linearity above 7 mg. The elimination phase was variable and became more prolonged with increasing dose. After the peak, the concentrations declined rapidly until about 15 h post-dose, following which there was a slower decline or beta phase.

Discussion

Single oral doses of ICI 170,809, a potent selective 5-HT₂ receptor antagonist (Blackburn *et al.*, 1988), produced

dose related, plasma concentration dependent changes in both 5-HT₂ induced *ex vivo* platelet aggregation and pupillary responses in healthy volunteers. In both cases, a log linear dose-response relationship was demonstrated. The effective dose of ICI 170,809, expressed as the ID₅₀ for both platelet and pupillary responses (inhibitory dose 50%) was approximately 0.1 mg kg^{-1} . This is in agreement with the effective range of doses active centrally (Cox *et al.*, 1988), and in the cardiovascular system at the antiplatelet and antiarrhythmic 5-HT₂ receptor using *in vivo* animal models (Coker & Ellis; 1990).

Antagonism of both the 5-HT induced *ex vivo* platelet aggregation, and pupillary miosis following the single 15 mg doses of ICI 170,809 were apparent for greater than 8 h. The pharmacokinetic profile of ICI 170,809 following single oral doses displayed a variable and prolonged plasma elimination half-life, which increased from a mean of 16 h with the 7 mg dose to over 40 h with the 15 and 30 mg doses. There was a linear increase in peak plasma concentration (C_{max}). The apparent increase in elimination half-life and AUC _{∞} with increasing dose, are probably related to the better definition and resolution of the beta elimination phase. Therefore, proposed oral dosing with either 7 or 15 mg as single, or divided daily doses, would be appropriate for use in clinical trials.

Bevan & Heptinstall (1986) have subdivided 5-HT₂-receptor antagonists into two categories 'competitive' and 'non-competitive', based on their behaviour at the platelet 5-HT₂ receptor. The *in vitro* and *ex vivo* effects of ICI 170,809 in this study would suggest non-competitive or high affinity antagonism at the platelet 5-HT₂ receptor. An alternative possibility, is that the 5-HT₂ receptor exists in one of two conformations with both high and low affinity for ligands, since this would produce a similar dose-response curve, as suggested for ICI 170,809 by Frenken & Kaumann (1989). From the clinical point of view, this apparently unsurmountable antagonism with ICI 170,809 may be beneficial, when antagonising high local concentrations of 5-HT released from platelets, in disease states such as migraine (Davies & Steiner, 1990; Jansen *et al.*, 1991) or coronary artery spasm (Noble *et al.*, 1990).

Recently, antagonism of the 5-HT₂ platelet response by ketanserin has been used to monitor efficacy in patients with Raynaud's phenomenon (Marasini *et al.*, 1990). They were able to recommend increased dosing frequency to improve clinical efficacy. This demonstrates the utility of being able to measure a pharmacodynamic endpoint, and the desirability of a prolonged duration of action for a drug designed to treat such cardiovascular disorders. ICI 170,809 has such a profile of activity.

The demonstration of a pupillary miosis with ICI 170,809, which was dose and concentration dependent, provides evidence to support the hypothesis that 5-HT₂ receptors may be involved in controlling human pupillary responses (Millson *et al.*, 1991). Animal evidence to support this comes from observations that intraocular injection of 5-6 dihydroxy 5-HT (a 5-HT depleting agent) produced a denervation supersensitivity, affecting both pupillary responses to 5-HT and production of aqueous humour (Moro *et al.*, 1987). This is supported by the biochemical findings of Tobin *et al.* (1988), Palkama

et al. (1984), Uusitalo *et al.* (1982, 1984) and Cutcliffe & Osborne (1987) demonstrating selective 5-HT uptake, post synaptic 5-HT binding sites, functional responses and second messenger generation linked to 5-HT₂ receptors. Thus compelling evidence exists for a serotonergic influence in the iris-pupillary body (Cutcliffe & Osborne, 1987).

Therefore, this study has demonstrated two pharmacodynamic responses which reflect pharmacokinetic changes in ICI 170,809 following single oral doses in healthy volunteers. Both *ex vivo* platelet and pupillary responses are thus techniques readily applicable to monitor the effects of 5-HT₂-receptor antagonists in man.

References

- Bevan, J. & Heptinstall, S. (1986). Effects of combinations of 5-HT antagonists on 5-HT induced platelet aggregation. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **334**, 341–345.
- Blackburn, T. P., Cox, B., Thornber, C. V. & Pearce, R. J. (1990). Pharmacological studies *in vivo* with ICI 169,369 a chemically novel 5-HT₂/5-HT_{1C} receptor antagonist. *Eur. J. Pharmacol.*, **180**, 229–237.
- Blackburn, T. P., Thornber, C. V., Pearce, R. J. & Cox, B. (1988a). *In vitro* pharmacology of ICI 169,369, a chemically novel 5-HT antagonist. *Eur. J. Pharmacol.*, **150**, 247–256.
- Blackburn, T. P., Thornber, C. V., Pearce, R. J. & Cox, B. (1988b). *In vitro* pharmacology of ICI 170,809—A new 5-HT₂ antagonist. *FASEB J.*, **2**, A1404 (P6441).
- Biegen, A., Weizman, A., Karp, L., Ram, A., Tiano, S. & Wolff, M. (1987). Serotonin 5-HT₂ receptor binding on blood platelets—a peripheral marker for depression? *Life Sci.*, **41**, 2485–2492.
- Blauw, G. J., Van Brummelen, P., Chang, P. C., Vermeij, P. & Van Weiten, P. A. (1988). Regional vascular effects of serotonin and ketanserin in young healthy subjects. *Hypertension*, **11**, 256–263.
- Coker, S. J. & Ellis, A. M. (1990). Antiarrhythmic and antiplatelet activity of the 5-HT₂ antagonist ICI 170,809. *J. Mol. Cell. Cardiol.*, **22**, S35.
- Conn, J. P. & Sanders-Bush, E. (1985). Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to serotonin 5-HT₂ binding sites. *J. Neurosci.*, **6**, 3669–3675.
- Cox, B., Thornber, C. W., Pearce, R. J. & Blackburn, T. P. (1988). *In vivo* pharmacology of ICI 170,809—A new 5-HT₂ antagonist. *FASEB J.*, **2**, A1404 (P6442).
- Cutcliffe, N. & Osborne, N. N. (1987). Serotonergic and cholinergic stimulation of inositol phosphate formation in the rabbit retina. Evidence for the presence of 5-HT₂ and muscarinic receptors. *Brain Res.*, **421**, 95–104.
- Davies, P. T. G. & Steiner, T. J. (1990). Serotonin S₂ receptors and migraine: A study with the selective antagonist ICI 169,369. *Headache*, **30**, 340–343.
- De Clerck, F. & Van Nueten, J. M. (1982). Platelet-mediated vascular contractions: inhibition of the serotonergic component by ketanserin. *Thromb. Res.*, **27**, 713–727.
- Frenken, M. & Kaumann, A. J. (1989). Dimethylation of the activator ICI 169,369 results in a high affinity partial deactivator ICI 170,809 of the arterial 5-HT₂ receptor system. *J. Pharmac. exp. Ther.*, **250**, 707–713.
- Gaddum, J. H. & Picarelli, Z. P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmacol.*, **12**, 323–328.
- Goldstein, J. M., Litwin, L. C., Sutton, E. B. & Malick, J. B. (1989). Effects of ICI 169,369 a selective serotonin-2 antagonist, in electrophysiological tests predictive of antipsychotic activity. *J. Pharmac. exp. Ther.*, **249**, 673–680.
- Gotta, A. W., Capuano, C., Hartnung, J. & Naryanan, M. (1987). Ketanserin inhibits human platelet aggregation. *Clin. Pharmac. Ther.*, **218**, PIIB-6.
- Houston, D. S. & Vanhoutte, P. M. (1989). Pathophysiological significance of 5-hydroxytryptamine in the periphery. In *The peripheral actions of 5-HT*, Chapter 16, pp. 377–395. ed. Fozard, J. Oxford: Oxford Medical Publications.
- Jansen, I., Blackburn, T., Eriksen, K. & Edvinsson, L. (1991). 5-HT antagonist effects of ICI 169,369, ICI 170,809 and methysergide in human temporal and cerebral arteries. *Pharmac. Tox.*, **68**, 8–13.
- Kramer, R. & Turner, P. (1973). The topical and systemic effects of fenfluramine and its metabolite norfenfluramine on pupil size in healthy volunteers. *J. Pharm. Pharmacol.*, **25**, 573–576.
- Marasini, B., Biodini, M. L. & Mollica, B. (1990). Effect of chronic ketanserin treatment of serotonin-induced platelet aggregation in patients with Raynaud's phenomenon. *Eur. J. Pharmacol.*, **39**, 289–290.
- Millson, D. S., Harry, J. D. & Wilkinson, D. (1988). Measurement of pupillary responses to miotic and mydriatic drugs in human volunteers, using a portable infrared pupillometer. *Br. J. clin. Pharmacol.*, **26**, 200P.
- Millson, D. S., Haworth, S. J., Rushton, A., Wilkinson, D. & Harry, J. (1991). The effects of a 5-HT₂ receptor antagonist (ICI 169,369) on changes in waking EEG, pupillary response and state of arousal in human volunteers. *Br. J. clin. Pharmacol.*, **32**, 447–454.
- Moro, F., Scapagnini, U., Scaletta, S. & Drago, F. (1987). Serotonin nerve endings and regulation of pupillary diameter. *Ann. Ophthalmology*, **13**, 487–490.
- Noble, M. I. M. & Drake-Holland, A. J. (1990). Is serotonin involved in the formation of thrombi at critical coronary arterial stenoses in man? *J. Mol. Cell. Cardiol.*, **22** (Suppl. III) PT24.
- Palkama, A., Lehtosalo, J. & Uusitalo, H. (1984). 5-hydroxytryptamine receptors in the cornea and ciliary processes of the rabbit and human eyes. *Ophthalmic Res.*, **16**, 14.
- Pazos, A., Probst, A. & Palacios, J. M. (1987). Serotonin receptors in the human brain—IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience*, **21**, 123–139.
- Peroutka, S. J., Lebovitz, R. M. & Snyder, H. S. (1981). Two distinct central serotonin receptors with different physiological functions. *Science*, **212**, 827–829.
- Pritchett, D. B., Bach, A. W., Wozny, M., Dal-Toso, R., Shih, J. C. & Seeburg, P. H. (1988). Isolation of 5-HT₂ and 5-HT_{1C} receptor c-DNA from rat brain by homology screening. *EMBO J.*, **7**, 4135.
- Reimann, W., Ziegler, G., Ludwig, L. & Frohlich, J. C.

Addendum

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- (1986). Central and autonomic nervous system side effects of ketanserin. *Drug Res.*, **36**, 1681–1684.
- Tobin, A. B., Ungar, W. & Osborne, N. N. (1988). Evidence for the presence of serotonergic nerves and receptors in the iris-ciliary body complex. *J. Neurosci.*, **8**, 3713–3721.
- Uusitalo, H., Lehtosalo, J., Laakso, J., Harkonen, M. & Palkama, A. (1982). Immunohistochemical and biochemical evidence for 5-HT containing nerves in the anterior part of the eye. *Exp. Eye Res.*, **35**, 671–675.
- Uusitalo, H., Raji, K., Lehtosalo, J., Harkonen, M. & Palkama, A. (1984). 5-HT binding in anterior segment of the eye. *Ophthalmic Res.*, **16**, 207.

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