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Comparison of the functional potencies of ropinirole and other dopamine receptor agonists at human $D_{2(long)}$, D_3 and $D_{4.4}$ receptors expressed in Chinese hamster ovary cells

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1 The aim of the present study was to characterize functional responses to ropinirole, its major metabolites in man (SKF-104557 (4-[2-(propylamino)ethyl]-2-(3H) indolone), SKF-97930 (4-carboxy-2-(3H) indolone)) and other dopamine receptor agonists at human dopamine $D_{2(long)}$ (hD_2), D_3 (hD_3) and $D_{4.4}$ (hD_4) receptors separately expressed in Chinese hamster ovary cells using microphysiometry.

2 All the receptor agonists tested (ropinirole, SKF-104557, SKF-97930, bromocriptine, lisuride, pergolide, pramipexole, talipexole, dopamine) increased extracellular acidification rate in Chinese hamster ovary clones expressing the human D_2, D_3 or D_4 receptor. The pEC_{50}s of ropinirole at hD_2, hD_3 and hD_4 receptors were 7.4, 8.4 and 6.8, respectively. Ropinirole is therefore at least 10 fold selective for the human dopamine D_3 receptor over the other D_2 receptor family members.

3 At the hD_2 and hD_3 dopamine receptors all the compounds tested were full agonists as compared to quinpirole. Talipexole and the ropinirole metabolite, SKF-104557, were partial agonists at the hD_4 receptor.

4 Bromocriptine and lisuride had a slow onset of agonist action which precluded determination of EC_{50}s.

5 The rank order of agonist potencies was dissimilar to the rank order of radioligand binding affinities at each of the dopamine receptor subtypes. Functional selectivities of the dopamine receptor agonists, as measured in the microphysiometer, were less than radioligand binding selectivities.

6 The results show that ropinirole is a full agonist at human D_2, D_3 and D_4 dopamine receptors. SKF-104557 the major human metabolite of ropinirole, had similar radioligand binding affinities to, but lower functional potencies than, the parent compound.

Keywords: Ropinirole; D_2 receptors; D_3 receptors; D_4 receptors; human; functional potency

Abbreviations: alpha MEM, alpha minimum essential medium; CHO, Chinese hamster ovary; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethylsulphoxide; FBS, Foetal Bovine Serum; G418, Geneticin; hD_2, human dopamine D_2 receptor; hD_3, human dopamine D_3 receptor; hD_4, human dopamine D_4 receptor; L-DOPA, 3-hydroxytyrosine; MEM, Minimum Essential Medium; pEC_{50}, $-\log_{10}$ of the concentration to elicit the half maximal response; PEG, polethyleneglycol; pK_i, $-\log_{10}$ of the inhibition constant; SKF-104557, 4-[2-(propylamino)ethyl]-2-(3H) indolone; SKF-97930, 4-carboxy-2-(3H) indolone

Introduction

L-DOPA (3-hydroxytyrosine), combined with peripherally active amino acid decarboxylase inhibitors, is successfully used to treat the symptoms of Parkinson's disease (Mierau *et al*., 1995; Camacho-Ochoa *et al*., 1995; Hagan *et al*., 1997). Unfortunately, chronic L-DOPA treatment is accompanied by the development of severe motor side effects after a period of maximal benefit which usually lasts 3–5 years (Sage & Mark, 1994). There follows a progressive loss of efficacy ('wearing-off'), rapid 'on/off' fluctuations in symptom control and the development of dyskinesias in 60–80% of patients (Cedarbaum *et al*., 1991). Evidence that the onset of adverse effects is related to the dose and duration of treatment with L-DOPA (Marsden & Parkes, 1997; Lesser *et al*., 1979) led to the suggestion that delaying L-DOPA treatment and limiting the dose might delay the onset of dyskinesias (Lesser *et al*., 1979). Accordingly, dopamine receptor agonists have been investigated for efficacy in Parkinson's Disease. When prescibed as adjuncts to L-DOPA, dopamine agonists reduce 'off' time and motor fluctuations and allow reductions in the maintenance dose of L-DOPA (Rabey, 1995), a profile which has been established for bromocriptine (Lieberman & Goldstein, 1985), pergolide (Goetz & Diederich, 1992) and lisuride (Rinne, 1989; Goetz & Diederich, 1992). These ergoline derivatives have been tested as monotherapies with varying degrees of success (Hagan *et al*., 1997 for references) but are relatively non-selective with respect to a variety of non-dopaminergic receptors and have the potential to produce adverse events related to their ergot structure (Tulloch, 1997).

The 'second generation' selective, dopamine receptor agonists ropinirole, pramipexole and talipexole are non-ergolines which have recently entered clinical use. Ropinirole (Eden *et al*., 1991) is selective for human dopamine D_3 receptors over human D_2 and human $D_{4.4}$ receptors as measured by radioligand binding studies (Boyfield *et al*., 1996). In animal models of Parkinson's disease, ropinirole is an effective anti-Parkinsonian drug with a low propensity to induce dyskinetic side effects (Eden *et al*., 1991, Jenner & Tulloch, 1997). Ropinirole was effective and well tolerated as monotherapy for 12 months in patients with early Parkinson's

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disease (Sethi *et al*., 1998). Ropinirole was superior to bromocriptine as monotherapy in patients with early Parkinson's disease when improvements in Unified Parkinson's Disease Rating Scale total motor score, proportion of 'responders', or 'improvers' on the Clinical Global Impression scale were determined (Korczyn *et al*., 1998). In man the major metabolite of ropinirole is SKF-104557 (4-[2-(propylamino)ethyl]-2-(3H) indolone) and SKF-97930 (4-carboxy-2-(3H) indolone) is a secondary metabolite (Jenner & Tulloch, 1997).

Pramipexole (Mierau, 1995; Mierau *et al*., 1995) is selective for the human dopamine D_3 receptor as measured by radioligand binding (Mierau *et al*., 1995). It was active in animal models of Parkinson's disease (Mierau, 1995) and improved 'off' time when used as an adjunct to L-DOPA in patients with advanced Parkinson's disease (Molho *et al*., 1995). Talipexole is also selective for the dopamine D_3 in receptor radioligand binding studies (Sautel *et al*., 1995), active in animal models of Parkinsons disease (Domino *et al*., 1998) and effective in parkinsonian patients (Mizuno *et al*., 1993).

Microphysiometry is a technique to measure extracellular acidification rates in live cells by detecting changes in cellular catabolism induced by a variety of ligand-receptor interactions, including G-protein linked receptors (Owicki *et al*., 1990; McConnell *et al*., 1992). This technique has been used to characterize functional responses at hD_2, hD_3 and hD_4 receptors expressed in Chinese hamster ovary (CHO) cells (Coldwell *et al*., in press). We now report studies on the functional selectivity and potency of ropinirole and its major metabolites in man (SKF-104557, SKF-97930) at the human dopamine D_2 receptor subtypes as compared with those obtained for a range of other dopamine receptor agonists used in the treatment of Parkinson's disease.

Methods

Cloned cell lines expressing hD_2, hD_3 or hD_4 receptors

Cloned human $D_{2(long)}$ receptors expressed in CHO cells were obtained from the Garvan Institute of Medical Research, Sydney, Australia (Selbie *et al*., 1989). Human D_3 receptors expressed in CHO or NG108-15 cells were obtained from Unite de Neurobiologie et Pharmacologie (U.109) de I'INSERM, Paris, France (Sokoloff, 1990; Sautel *et al*., 1995). Human $D_{4.4}$ receptors expressed in CHO cells were obtained from the Laboratory for Molecular Neurobiology, Clarke Institute of Psychiatry, Toronto, Canada.

Measurement of affinty constants by radioligand binding

Radioligand binding assays were carried out using membranes from CHO cells transfected with hD_2, hD_3 or $hD_{4.4}$ receptors as described previously (Coldwell *et al*., in press). Briefly, membranes (5–15 μg protein) were incubated with [^{125}I]-iodosulpride (0.1 nM) in a buffer containing (mM): Tris 50, NaCl 120, KCl 5, $CaCl_2$ 2 and $MgCl_2$ 1 (pH 7.4) for 30 min at 37°C in the presence or absence of competing ligands. Non-specific binding was defined with 0.1 mM iodosulpride. Curves were fitted to the data using an iterative four parameter logistic model (Bowen & Jerman, 1995)

Growth of cells expressing hD_2, hD_3 or hD_4 receptors

CHO cells expressing hD_2 receptors were grown in 50 : 50 Dulbecco's modified Eagle Medium (DMEM; without sodium pyruvate, with glucose): Ham's F-12 containing 10% (v v^{-1}) foetal bovine serum (FBS). The medium for growth of hD_3 CHO clones was DMEM (without sodium pyruvate, with glucose) containing 10% FBS, 100 nM methotrexate, 2 mM glutamine, 500 nM (−)-sulpiride and 1% (v v^{-1}) essential amino acids. $hD_{4.4}$ CHO clones were cultured in alpha minimum essential medium (alpha MEM) containing 10% FBS and 400 μg ml^{-1} geneticin (G418). Cells were removed from confluent plates by scraping and harvested by centrifugation (200 × *g*, 5 min, room temperature). Following resuspension in 10 ml of fresh culture medium, an aliquot was counted and the cells passaged at 12,500 or 25,000 cells cm^2. Cultures between passages 5 and 30 were used for functional studies.

Determination of extracellular acidification rates

Cells were seeded into 12 mm transwell inserts (Costar) at 300,000 cells cup^{-1} in FBS-containing growth medium. The cells were incubated for 6 h at 37°C in 95%O_2/5%CO_2, before changing to FBS- and sulpiride-free medium. After a further 16–18 h, cups were loaded into the sensor chambers of the microphysiometer and the chambers perfused with bicarbonate-free Dulbecco's modified Eagles medium (containing 2 mM glutamine and 44 mM NaCl) at a flow rate of 100 ul min^{-1} and temperature of 37°C. Each pump cycle lasted 90 s. The pump was on for the first 60 s and the acidification rate determined between 68 and 88 s, using the Cytosoft programme (Molecular Devices).

Assessment of agonist potencies and intrinsic activities

Cells were exposed (4.5 min for hD_2, 7.5 min for hD_3, 6 min for hD_4) to increasing concentrations (at half log unit intervals) of the agonist at half hourly intervals. Thirty minutes after the highest test concentration of agonist, the cells were perfused with the dopamine receptor agonist, quinpirole (1000 nM for hD_2 and hD_4 cells, 100 nM for hD_3 cells). We have shown previously that responses to these maximal concentrations of quinpirole are the same whether obtained at the start or end of experiments (Coldwell *et al*., in press). For bromocriptine and lisuride, concentration-response curves were prepared by application of a single concentration of each agonist in each chamber, followed by exposure to quinpirole.

Changes in acidification rates (calculated as the difference between the maximum effect after agonist addition and the average of three measurements taken immediately before agonist exposure) to each agonist concentration were determined and concentration-response curves analysed using Robofit (Tilford *et al*., 1995). The intrinsic activity for each agonist was calculated as the maximum increase in acidification rate obtained expressed as a percentage of the quinpirole internal standard.

Statistical analysis was carried out by means of Student's unpaired two-tailed *t*-test. A P value of less than 0.05 was considered significant.

Drugs

Stock solutions of drugs were prepared in bicarbonate-free Dulbecco's modified Eagles medium containing 2 mM glutamine and 44 mM NaCl. For compounds of poor solubility (bromocriptine, lisuride, pergolide and talipexole), stock solutions were prepared in 50 : 50 PEG:DMSO containing 100 μl glacial acetic acid. The pH of these latter solutions was readjusted to that of the bicarbonate-free Dulbecco's modified Eagles medium (containing 2 mM glutamine and 44 mM NaCl).

All cell culture reagents were obtained from Gibco (Paisley, U.K.). Quinpirole, was purchased from RBI (Poole, U.K.). Dopamine was supplied by Sigma (Poole, U.K.). Bromocriptine was purchased from Becpharm Ltd (Harlow, U.K.). Pergolide was a generous gift from Eli Lilly (Indianapolis, U.S.A.). Ropinirole, SKF-104557, SKF-97930, pramipexole, talipexole and lisuride were synthesized at SmithKline Beecham (Harlow, U.K.).

Results

Agonist response characteristics

Basal acidification rates (mean ± s.e.mean (range)) were 149 ± 8 (120–220) μV s^{-1} in hD_2 clones, 154 ± 7 (90–200) μV s^{-1} in hD_3 clones and 127 ± 17 (60–220) μV s^{-1} in hD_4 clones ($n = 10$ in each case). Maximal stimulation of acidification rates (mean ± s.e.mean (range)) by quinpirole were 45 ± 3 (27–59) μV s^{-1} in hD_2 clones, 13 ± 1 (9–17) μV s^{-1} in hD_3 clones and 50 ± 7 (27–62) μV s^{-1} in hD_4 clones.

All of the drugs tested increased acidification rates in each of the cell lines. Ropinirole, SKF-104557, SKF-97930, pergolide, pramipexole, talipexole and dopamine produced transient increases in acidification rates which returned towards basal in the continued presence of the ligand (Figure 1a). However, responses to bromocriptine and lisuride were slower in onset, were maintained longer and had slow recovery. The duration of these experiments meant that cell viability (determined as responsiveness to maximal concentrations of quinpirole) deteriorated (data not shown). For bromocriptine and lisuride concentration-response relationships were therefore determined using a single concentration of the agonist in each chamber and exposure to the drug was prolonged to 12 min. However, even under these conditions, responses to bromocriptine at hD_2, hD_3 and hD_4 receptors were slow in onset requiring up to 35 min to reach maximum after perfusion with the drug had ceased (Figure 1b). Responses persisted for >1 h and the concentration-response relationship to bromocriptine was not dose related (Figure 1b). The kinetics of the response to bromocriptine were similar at all three D_2-like receptors.

The potency of lisuride at the hD_2 receptor was very high (threshold concentration 10 pM). At hD_2, hD_3 and hD_4 receptors, responses to lisuride were quicker than bromocriptine in onset, but still reached maximum after perfusion with the drug had ceased (up to 17 min to reach maximum; Figure 1c). Lisuride also caused prolonged increases in acidification rate (>>1 h) and the concentration-response relationship to lisuride was not dose related. The time course of the responses to lisuride were similar at each of the receptors. Accordingly it was not possible to assess the concentration-response relationships to bromocriptine and lisuride.

Intrinsic activities

Ropinirole, SKF-104557, SKF-97930, pergolide, pramipexole and talipexole produced sigmoidal concentration response curves at hD_2, hD_3 and hD_4 dopamine receptors with Hill slopes in the range 0.9–1.9 (Figure 2; Table 1). Dopamine had very steep sigmoidal concentration-response curves (Hill slopes >2 at all three receptors; Table 1).

All the ligands tested were full agonists at hD_2, hD_3 and hD_4 receptors with the exception of SKF-104557 (41 ± 5%) and talipexole (57 ± 8%), which had less than full efficacy at the hD_4 receptor (Figure 2 and Table 1).

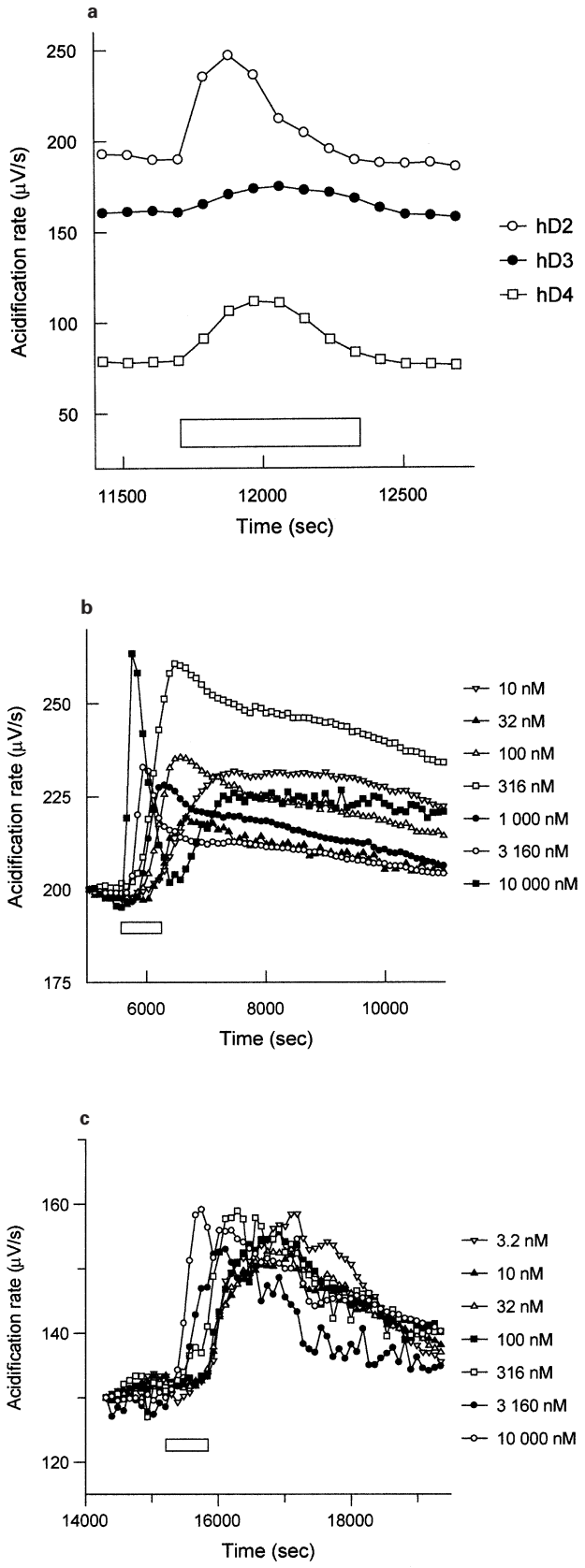

Figure 1 Acidification rate traces from human dopamine D_2, D_3 and D_4 clones. (a) Typical time course of agonist response to ropinirole (316 nM) in all three cell lines. (b) Single chamber concentration-response curve to bromocriptine at the hD_2 receptor, representative of the bromocriptine response in all three receptor clones. (c) Single chamber concentration-response curve to lisuride at the hD_3 receptor, representative of the lisuride response in all three receptor clones. Exposure to agonist indicated by open bars.

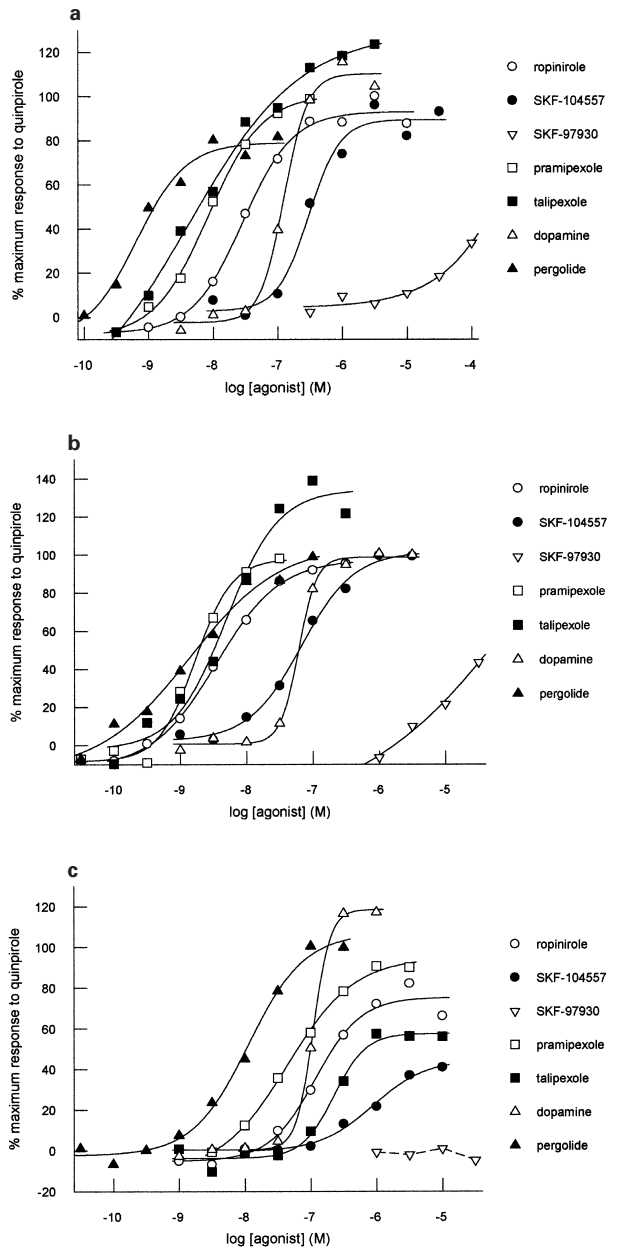

Figure 2 Extracellular acidification rate concentration-response curves to agonists in cloned cell lines expressing the human dopamine (a) D_2, (b) D_3 or (c) D_4 receptors. Results are expressed as a percentage of the response to a maximal concentration of quinpirole in each experiment. $n=5-9$ experiments.

Agonist affinities, potencies and selectivities

The radioligand binding affinities for hD_2 and hD_3 receptors obtained in these studies (Table 1) were similar to those described elsewhere (Sautel *et al*., 1995; Mierau *et al*., 1995), with the exception that, at both the hD_2 and hD_3 receptor, the affinity of lisuride was 10 fold higher than reported by Sautel *et al*. (1995). The radioligand binding affinities of ropinirole, SKF-104557, SKF-97930, pergolide and talipexole at the human D_4 receptor have not been reported previously.

There was no consistent relationship between radioligand binding affinities and functional potencies, for any given compound across the receptor subtypes (Table 1). Dopamine showed lower functional selectivity (approximately 4 fold) for hD_3 over hD_2, with pEC_{50}s ($-\log_{10}$ of the concentration to elicit the half maximal response) of 7.5 and 6.9 respectively, than the selectivity (approximately 20 fold) obtained in radioligand binding studies (pK_is ($-\log_{10}$ of the inhibition constant) of 7.4 and 6.1 respectively).

The pEC_{50}s of ropinirole at the human D_2, D_3 and D_4 dopamine receptor subtypes were 7.4 ± 0.1, 8.4 ± 0.1 and 6.8 ± 0.1 respectively (Figure 3). Ropinirole was therefore

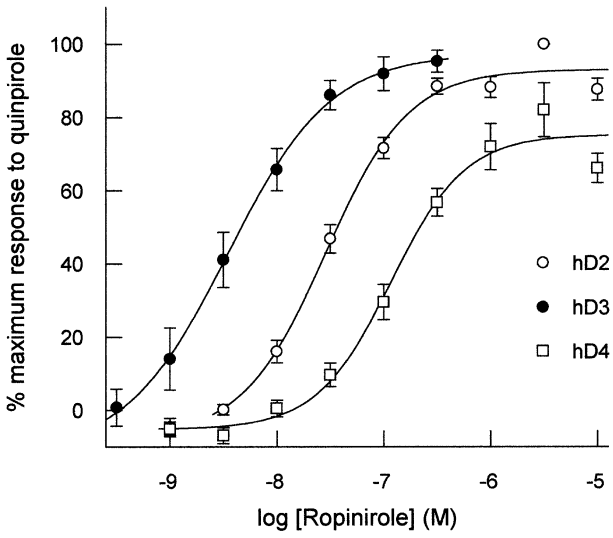

Figure 3 Concentration-response curves to ropinirole in hD_2, hD_3 or hD_4 cloned cell lines. Data expressed as a percentage of quinpirole internal standard. Results are shown as mean$\pm$s.e.mean from 7–9 experiments.

Table 1 Radioligand binding affinities (pK_i) and functional potencies (microphysiometer; pEC_{50} and % max.) at human dopamine receptor subtypes

	hD2				*hD3*				*hD4*			
Agonist	*pK_i*	*pEC_{50}*	*% max*	*Slope*	*pK_i*	*pEC_{50}*	*% max*	*Slope*	*pK_i*	*pEC_{50}*	*% max*	*Slope*
Ropinirole	5.8±0.1	7.4±0.1	100±1	1.2±0.1	7.1±0.1	8.4±0.1	97±2	1.3±0.1	5.4±0.1	6.8±0.1	81±6	1.4±0.2
SKF-104557	5.7±0.1	6.2±0.2	96±1	1.7±0.7	7.0±0.1	7.2±0.2	100±7	1.0±0.2	4.8±0.1	5.9±0.2	41±5*	1.4±0.3
SKF-97930	<5	<4	NF	NF	<5.5	<4.5	NF	NF	<5.5	<4	NF	NF
Bromocriptine	8.5±0.1	NF	NF	NF	8.7±0.1	NF	NF	NF	6.6±0.1	NF	NF	NF
Lisuride	9.8±0.1	NF	NF	NF	9.7±0.1	NF	NF	NF	8.7±0.1	NF	NF	NF
Pergolide	8.1†	9.0±0.2	82±8	1.7±0.2	8.8†	8.6±0.4	87±15	1.7±0.8	6.9±0.1	8.2±0.3	100±1	1.7±0.3
Pramipexole	6.0±0.1	8.0±0.1	99±1	1.3±0.1	7.8±0.1	8.7±0.1	98±2	1.9±0.2	6.4±0.1	7.3±0.1	91±5	1.2±0.2
Talipexole	5.8±0.2	7.9±0.2	118±11	0.9±0.1	7.0±0.1	8.2±0.1	139±9	1.2±0.4	5.2±0.1	6.5±0.1	57±8*	1.8±0.2
Dopamine	6.1†	6.9±0.2	115±14	3.9±1.4	7.4†	7.5±0.3	101±10	4.2±1.0	6.1±0.1	7.0±0.1	117±7	2.1±0.4

Results are mean$\pm$s.e.mean except for †from Brown *et al*. (1993), where values were generated from mean curves. $n=3-6$ for radioligand binding affinities. $n=5-9$ for functional responses. % max. is the maximum stimulation caused by the agonist expressed as a percentage of the quinpirole internal standard. NF = not fitted. *$P<0.01$ compared to quinpirole (Student's *t*-test).

Table 2 The hD_3/hD_2 and hD_4/hD_2 selectives of drugs in radioligand binding studies (pK_i) and functional assays (pEC_{50})

	hD_3/hD_2		*hD_4/hD_2*	
Agonist	*pK_i*	*pEC_{50}*	*pK_i*	*pEC_{50}*
Ropinirole	20	10	0.4	0.3
SKF-104557	20	10	0.1	0.5
Bromocriptine	2	–	0.01	–
Lisuride	1	–	0.06	–
Pergolide	5	0.4	0.06	0.2
Pramipexole	63	5	3	0.2
Talipexole	16	2	0.3	0.06
Dopamine	20	4	1	1

The numbers are derived from the data given in Table 1.

selective ($P<0.01$ [Student's *t*-test]) for hD_3 receptors over hD_2 receptors in both radioligand binding (20 fold) and functional (10 fold) studies. The major human metabolite of ropinirole, SKF-104557, had affinities for hD_2 and hD_3 receptors in radioligand binding studies (pK_i 5.7 and 7.0 respectively) which were similar to those of ropinirole. Whilst the functional hD_3 selectivity of SKF-104557 remained similar to the functional selectivity of ropinirole (10 fold, Table 1) the secondary human metabolite, SKF-97930, had much lower affinity than SKF-104557 in radioligand binding at all three human dopamine receptor subtypes (Table 1) and in functional studies showed only weak activity at hD_2 and hD_3 receptors at the higher concentrations tested (threshold 10 and 3 μM respectively; Figure 2). SKF-97930 was not an agonist at hD_4 receptors at concentrations up to 30 μM.

Pramipexole was 5 fold selective for the hD_3 over the hD_2 receptor in functional studies but the other ligands showed little or no functional selectivity for hD_3 over hD_2 (Table 1). Pergolide and talipexole were both hD_3-selective in radioligand binding studies but hD_2-selective and non-selective respectively in functional studies. The hD_3/hD_2 selectivities of drugs in functional assays were generally lower than the selectivities measured by radioligand binding (Table 2). The rank orders of radioligand binding affinities at the hD_2, hD_3 and hD_4 receptors did not match their rank orders of functional potencies (Table 1). At the hD_3 receptor, the rank order of radioligand binding affinities was pergolide > pramipexole > dopamine > ropinirole > SKF-104557 = talipexole > SKF-97930, whereas the rank order of functional potencies was pramipexole > pergolide > ropinirole > talipexole > dopamine > SKF-104557 > SKF-97930.

The rank orders of selectivities in radioligand binding and functional studies were also different. The hD_3/hD_2 rank order of radioligand binding selectivities was pramipexole > ropinirole = SKF-104557 = dopamine > talipexole > pergolide, whereas the rank order for functional selectivities was ropinirole = SKF-104557 > pramipexole > dopamine > talipexole > pergolide.

Discussion

Ropinirole and its major human metabolite, SKF-104557, were found to be full agonists at human D_2 and D_3 receptors. Ropinirole is also a full agonist at human D_4 receptors, but SKF-104557 has lower intrinsic activity at this receptor. In radioligand binding studies, the affinity of ropinirole and SKF-104557 were similar at hD_2 and hD_3 receptors, but in functional studies ropinirole was 10 fold more potent than its major human metabolite. These results show different intrinsic efficacy of the two compounds at the hD_2 and hD_3 receptors. The secondary human metabolite, SKF-97930, had very low affinity and potency at all three dopamine D_2 family receptor subtypes. Ropinirole also showed selectivity for human D_3 receptors over human D_2 receptors in radioligand binding studies (20 fold), although selectivity was less in functional assays (10 fold). SKF-104557 had lower functional hD_3 selectivity (10 fold), as compared to radioligand binding selectivity (20 fold).

Concentration-response curves to dopamine were very steep, confirming previous findings (Coldwell *et al*., in press) and may reflect oxidation / metabolism of dopamine under the assay conditions used. Nevertheless dopamine, like ropinirole, was selective for the hD_3 over the hD_2 receptor with selectivity ratios in the radioligand binding assays (20 fold) exceeding those in functional assays (4 fold).

Pramipexole was 5 fold selective for the hD_3 receptor over hD_2 receptors in the microphysiometry assay. The affinities and functional potencies of pramipexole were comparable with those obtained at the human D_2 and D_3 receptors reported by Sautel *et al*. (1995) and at the human and rat D_2, D_3 and D_4 receptors reported by Mierau *et al*. (1995).

Agonist potencies were higher than their corresponding radioligand binding affinities. This difference probably reflects receptor reserve and/or amplification within the signalling cascade in clonal cell lines (Coldwell *et al*., in press). Selectivities in the functional assays were lower than those in radioligand binding experiments, confirming previous observations (Coldwell *et al*., in press) and may reflect differences in coupling efficacy for the agonists studied.

The basal acidification rates and increased agonist-induced acidification rates which were observed in this study were similar to those reported previously with other dopamine receptor agonists (Boyfield *et al*., 1996; Coldwell *et al*., in press). For most of the agonists tested, the response times at each receptor were similar, with the exception of bromocriptine and lisuride, which had much slower response onsets. In their mitogenesis assays with cloned dopamine receptors Sautel *et al*. (1995) did not report slow onset with these compounds, although in that study, agonist incubation was overnight, which may have masked the effects observed here. Further investigations are required to elucidate the reasons for these differences.

The distribution of mRNA for D_2, D_3 and D_4 receptors has been described in many studies and D_3 receptor mRNA has been localized to specific areas, (e.g. the islands of Calleja and nucleus accumbens in rat brain, Diaz *et al*., 1995). Autoradiographic and immunohistochemistry studies have been used to identify dopamine receptor protein distribution in rat (Levesque *et al*., 1992; Ariano & Sibley, 1994), marmoset (Hurley *et al*., 1996) and human (Herroelen *et al*., 1994; Hall *et al*., 1996) brain. The specificity of agonist radiolabels used in such studies has been questioned (Burris *et al*., 1995; Gonzalez & Sibley, 1995) but appropriate assay conditions (Hall *et al*., 1996) or the use of antagonist labels (Murray *et al*., 1994) have circumvented these problems, and rat D_3 receptor distribution, as measured by radioligand binding assays is consistent with D_3 mRNA localization (Sokoloff & Schwartz, 1995). In rat brain, the highest levels of D_3 receptor were found primarily in limbic areas, whereas in marmoset and human brain tissue there was more extensive distribution of the D_3 receptor in the basal ganglia (Hurley *et al*., 1996; Herroelen *et al*., 1994,).

It has been proposed that the presence of dopamine D_3 receptors in areas of the primate brain involved in the control of movement, as well as parts of the limbic system, might

indicate that the D_3 receptor is a target for anti-Parkinsonian drugs (Hurley *et al*., 1996). Ropinirole and pramipexole show some D_3 selectivity in both radioligand binding and functional studies in clonal cell lines, whereas pergolide and talipexole are not functionally D_3-selective. Further work on the efficiency of hD_3 receptor coupling in human brain, and the relative abundance of hD_3 and hD_2 receptors in Parkinson's disease tissue, is needed to determine if this translates to functional selectivity *in vivo*. The functional consequences of D_3 receptor activation are unknown, and the impact of dopamine D_3 receptor selectivity of these drugs in long-term therapy in Parkinsons disease is being assessed.

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