ORIGINAL ARTICLE

The affinity and selectivity of α-adrenoceptor antagonists, antidepressants, and antipsychotics for the human α1A, α1B, and α1D-adrenoceptors

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Abstract
α1-adrenoceptor antagonists are widely used for hypertension (eg, doxazosin) and benign prostatic hypertrophy (BPH, eg, tamsulosin). Some antidepressants and antipsychotics have been reported to have α1 affinity. This study examined 101 clinical drugs and laboratory compounds to build a comprehensive understanding of α1-adrenoceptor subtype affinity and selectivity. [3H]prazosin whole-cell binding was conducted in CHO cells stably expressing either the full-length human α1A, α1B, or α1D-adrenoceptor. As expected, doxazosin was a high-affinity nonselective α1-antagonist although other compounds (eg, cyclazosin, 3-MPPI, and ARC239) had higher affinities. Several highly α1A-selective antagonists were confirmed (SNAP5089 had over 1700-fold α1A selectivity). Despite all compounds demonstrating α1 affinity, only BMY7378 had α1D selectivity and no α1B-selective compounds were identified. Phenoxybenzamine (used in pheochromocytoma) and dibenamine had two-component-binding inhibition curves at all three receptors. Incubation with sodium thiosulfate abolished the high-affinity component suggesting this part is receptor mediated. Drugs used for hypertension and BPH had very similar α1A/α1B/α1D-adrenoceptor pharmacological profiles. Selective serotonin reuptake inhibitors (antidepressants) had low α1-adrenoceptor affinity. Several tricyclic antidepressants (eg, amitriptyline) and antipsychotics (eg, chlorpromazine and risperidone) had high α1-adrenoceptor affinities, similar to, or higher than, α blockers prescribed for hypertension and BPH, whereas others had poor α1 affinity (eg, protriptyline, sulpiride, amisulpride, and olanzapine). The addition of α blockers for the management of hypertension or BPH in people already taking tricyclic antidepressants and certain antipsychotics may not be beneficial. Awareness of the α-blocking potential of different antipsychotics may affect the choice of drug for those with delirium where additional hypotension (eg, in sepsis) may be detrimental.

KEYWORDS
affinity, antidepressant, antipsychotic, benign prostatic hypertrophy, hypertension, α antagonist

Abbreviations: BPH, benign prostatic hypertrophy; CHO, Chinese hamster ovary; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.
The α1-adrenoceptors are expressed in a wide range of tissues including blood vessels, kidney, spleen, liver, brain, and lower urinary tract.1-3 There are three subtypes: α1A, α1B, and α1D-adrenoceptors.1-4 All are present in blood vessels, and whilst α1A and α1D and are both important in smooth muscle contraction (and control of blood pressure), the role of the α1B-adrenoceptors is less certain.2,3,5,6

α-adrenoceptor antagonists (α blockers) were first used to reduce systemic blood pressure with dibenamine, phentolamine, and phenoxybenzamine used in the diagnosis and management of pheochromocytoma, an adrenal catecholamine-secreting tumor.7,8 While phenoxybenzamine is still important for pheochromocytoma, longer acting, nonselective α1-antagonists were developed (doxazosin, terazosin, indoramin, and prazosin) and remain important in the management of resistant hypertension.

α blockers are also used in benign prostatic hypertrophy (BPH) where α1A blockade induces prostate and lower urinary tract smooth muscle relaxation, improving urinary flow.9 Phenoxybenzamine was the first α blocker to be used in BPH10 although its α2 effects limited its use.11 The nonselective α1-antagonists doxazosin, terazosin, indoramin, and prazosin were used effectively for BPH, but caused hypotension, particularly postural hypotension, and required dose titration to manage this problematic side effect.9,12 Selective α1A-antagonists were developed, hoping to minimize hypotension by reducing α1B-antagonism.11,13 Tamsulosin, alfuzosin, and silodosin were developed as prostate-specific (α1A selective) drugs and are used without dose titration.9 Despite reports of “better tolerability,”11,14 alfuzosin is reported to be a nonselective α1-antagonist and tamsulosin to have equal α1A- and α1D-adrenoceptor affinity,15,16 suggesting they may be pharmacologically indistinguishable from drugs used for hypertension. Indeed, tamsulosin (the most commonly prescribed α blocker for BPH) is associated with increased hypotension, falls, and fractures.12,13,17 Although effective for BPH, silodosin appears to have more sexual side effects, whereas its cardiovascular effects remain uncertain.18

α1-adrenoceptors are the most abundant adrenoceptors in the brain and modulate neurotransmitter release.3 Many antidepressants prevent the reuptake of neurotransmitters (serotonin and noradrenaline), and therefore increase neurotransmitter concentration. However, several antidepressants have significant α1-adrenoceptor affinity.19-21 This high affinity is seen in brain homogenates.22 In theory these two effects (increased neurotransmitter presence, but receptor blockade) could cancel each other out.20 However, antidepressants cause hypotension, particularly postural hypotension (up to 58% users23,24). Not surprisingly therefore, antidepressant use is associated with twice the risk of falls.25

Several antipsychotics (neuroleptics) bind to α1-adrenoceptors in blood vessels and brain homogenates.6,26,27 Many antipsychotics cause postural hypotension,28,29 and again, rates are high (eg, 48% taking risperidone26) including postural hypotension in those taking long-term antipsychotics (77%30). Interestingly, the degree of postural hypotension seen with several antipsychotics correlates well with the α1-adrenoceptor affinity.29 Antipsychotic drug use is also associated with falls and hip fractures and regular use is associated with twice the risk of falls (even after controlling for other risks31,32).

There are many studies examining the affinity of α1-adrenoceptor ligands. Many are older studies before the identification of the three subtypes and many are in whole tissue where multiple subtypes will be present. Most studies only report the two or three ligands under investigation. Here we aimed to investigate the subtype selectivity of a wide range of α-antagonists including those used in hypertension, BPH, antidepressants, antipsychotics as well as laboratory compounds. Human α1A, α1B, and α1D-adrenoceptors were expressed in intact mammalian cells, in order to build a comprehensive and directly comparable picture of α1-subtype selectivity in living cells.

2 | METHODS

2.1 | Materials

A list of all of the compounds studied, together with the source and supplier code from which it was purchased, is given in Table S1. White-sided view plates were from Greiner Bio-one, Kremsmunster, Austria; and [3H]prazosin, Microscint 20, and scintillation fluid from PerkinElmer (Buckinghamshire, UK). Fetal calf serum was from Gibco (Thermo-Fisher), Lipofectamine, and OPTIMEM were from Life Technologies, Thermo-Fisher, Massachusetts USA. All other cell culture reagents were from Sigma Chemicals (Poole, Dorset, UK).

2.2 | Cell lines

CHO-K1 (RIDD: CVCL_0214) were stably transfected with the DNA of the human α1A-adrenoceptor, human α1B-adrenoceptor (DNAs from Guthrie DNA Resource Centre), or human α1D-adrenoceptor (full-length DNA from Andre Pupo33; using Lipofectaime and Optinem according to the manufacturers’ instructions. Transfected cells were selected for 3 weeks using resistance to neomycin (at 1mg/ml). Single clones from each transfection were then isolated by dilution cloning giving rise to the stable cell lines CHO-α1A, CHO-α1B, and CHO-α1D.

2.3 | Cell culture

CHO cells were grown in Dulbecco’s modified Eagle’s medium nutrient mix F12 (DMEM/F12) containing 10% fetal calf serum and 2 mmol/L L-glutamine in a 37°C humidified 5% CO₂: 95% air
atmosphere. Cells were seeded into white-sided, clear bottomed 96-well view plates and grown to confluence.

2.4 | [3H]prazosin binding—saturation binding

The $K_D$ value for [3H]prazosin was determined in each cell line by saturation binding. [3H]prazosin was diluted in serum-free media. Media were removed from each well and replaced with either 100 µL serum-free media (total binding) or 100 µL 20 µmol/L tamsulosin ($\alpha1A$ and $\alpha1B$) or 200 µmol/L tamsulosin ($\alpha1D$) to determine nonspecific binding. [3H]prazosin was then added to the wells (quadruplicates per condition, 1 in 2 dilution in well), and the plates incubated for 2 hours at 37°C in a humidified 5% CO$_2$: 95% air atmosphere. After 2 hours, the cells were washed twice by the addition and removal of 2 x 200 µL cold (4°C) phosphate-buffered saline. 100 µL Microscint 20 was added to each well and a white base applied to the plate to convert the wells into white-sided/white-bottomed wells. Plates were left at room temperature for at least 6 hours before being counted on a Topcount (PerkinElmer), with a counting time of 2 minutes per well.

2.5 | [3H]prazosin whole-cell binding—competition binding

Ligands were serially diluted in serum-free media (DMEM/F12 containing 2 mmol/L L-glutamine only) to twice their final required concentration. Media was removed from each well of the 96-well view plate and 100 µL ligand added to triplicate wells. This was immediately followed by the addition of 100 µL [3H]prazosin (diluted in serum-free media) and the cells incubated for 2 hours at 37°C (5% CO$_2$, humidified atmosphere). After 2 hours the plates were washed as above. Cells were inspected under a light microscope to ensure cells were still present after the wash and before the addition of Microscint 20. In a few cases, high concentrations of competing ligand caused the cells to round up and be washed off the plates. These concentrations were excluded from the analysis. Total binding (6 wells/plate) and nonspecific binding (6 wells/plate) determined by the presence of 10 µmol/L tamsulosin ($\alpha1A$ and $\alpha1B$) or 100 µmol/L tamsulosin ($\alpha1D$) was defined in every plate.

Sodium thiosulfate reacts with 2-chloroethylamines in a 1:1 stoichiometry to inactivate the ethyleniminium ions generated in solution (see Discussion). Sodium thiosulfate had no effect on [3H]prazosin binding up to concentrations of 10 mmol/L. Therefore, to ensure that all ethyleniminium ions were inactivated, sodium thiosulfate was used in excess, with a final well concentration of 1 mmol/L. When used, competing ligands were serially diluted in serum-free media (just as above) in the absence and presence of thiosulfate and both dilution series were then incubated for 30 minutes at 37°C (5% CO$_2$, humidified atmosphere). Media was then removed from the cells and competing ligand (in the presence or absence of thiosulfate) added to the wells immediately followed by [3H]prazosin (thus thiosulfate was present with the competing ligand for 30 minutes before addition to the cells, and then throughout the 2-hour incubation with cells at 1 mmol/L).

[3H]prazosin concentrations were determined from taking the average of triplicate 50 µL samples of each [3H]prazosin concentration used and counted on a PerkinElmer Scintillation counter and were in the range from 0.22 to 1.40 nmol/L.

All experiments have been conducted in intact living mammalian cells expressing human $\alpha1A$ or $\alpha1B$ or $\alpha1D$-adrenoceptors. Unlike membrane-binding studies, physiological levels of intracellular endogenous GTP will therefore always have been present. Although it should not make much difference for antagonists, the receptors (and therefore measurements taken) in this living system are therefore more akin to how drugs bind in people, than studies conducted in membrane preparations.

2.6 | Data analysis

In all cases where a $K_D$ value is stated, increasing concentrations of the competing ligand fully inhibited the specific binding of [3H]prazosin (unless otherwise annotated in the tables).

The following equation was then fitted to the data using Graphpad Prism 7 and the IC$_{50}$ was then determined as the concentration required to inhibit 50% of the specific binding.

$$\%\text{specific binding} = 100 - \frac{100 \times [A]}{[A] + IC_{50}}$$

where [A] is the concentration of the competing ligand and IC$_{50}$ is the concentration at which half of the specific binding of [3H]prazosin has been inhibited.

From the IC$_{50}$ value, the known concentration of [3H]prazosin and the known $K_D$ for [3H]prazosin at each receptor, a $K_D$ (concentration at which half the receptors are bound by the competing ligand) value was calculated using the Cheng–Prusoff equation:

$$K_D = \frac{IC_{50}}{1 + \left(\frac{[3H]prazosin}{K_D}\right)}$$

In some cases, the maximum concentration of competing ligand was not able to inhibit all of the specific binding. Where no inhibition of [3H]prazosin binding was seen, even with maximum concentration of competing ligand possible, “no binding” is given in the tables. Where the inhibition produced by the maximum concentration of the competing ligand was 50% or less, an IC$_{50}$ could not be determined and thus a $K_D$ value not calculated. This is shown in the tables as IC$_{50}$ > top concentration used (ie, IC$_{50}$ > 100 µmol/L means that 100 µmol/L inhibited some but less than 50% of the specific binding). In cases where the competing ligand caused a substantial (greater than 60%, but not 100%) inhibition of specific binding, an IC$_{50}$ value was determined by extrapolating the curve to nonspecific levels and assuming that a
greater concentration would have resulted in 100% inhibition. These values are given as apparent $K_D$ values in the tables.

For some ligands, the inhibition of [3H]prazosin binding was best described by a two-component curve, using the equation below:

$$\%\text{specific binding} = \frac{[A]N}{([A]+IC_{501})} + \frac{[A](100-N)}{([A]+IC_{502})}$$

where [A] is the concentration of the competing ligand, $IC_{501}$ and $IC_{502}$ are the respective $IC_{50}$ values for the two components and N is the percentage of the response occurring through the first component ($IC_{501}$). $K_D$ values were calculated from $IC_{50}$ values as above.

Selectivities are given as a ratio of the $K_D$ values for the different receptors.

3 | RESULTS

Saturation binding yielded a $K_D$ value for [3H]prazosin of 0.71 nmol/L ± 0.07 (1552 ± 166 fmol/mg protein, $n = 11$) at the human $\alpha_1A$-adrenoceptor, 0.87 nM ± 0.11 (4350 ± 317 fmol/mg protein, $n = 12$) at the human $\alpha_1B$-adrenoceptor, and 1.90 ± 0.31 nmol/L (417 ± 48 fmol/mg protein, $n = 9$) at the full-length human $\alpha_1D$-adrenoceptor. As the lower expression of the $\alpha_1D$-receptor meant that a larger proportion of the experimental window was nonspecific binding, the affinity of prazosin was also determined by competing prazosin with [3H]prazosin. The log $K_D$ values obtained were $-9.07 ± 0.04 (=0.85 \text{ nmol/L}, n = 9)$ at the $\alpha_1A$-adrenoceptor, $-8.74 ± 0.06 (=1.82 \text{ nmol/L}, n = 8)$ at the $\alpha_1B$-adrenoceptor, and $-9.07 ± 0.23 (=0.85 \text{ nmol/L}, n = 10)$ at the $\alpha_1D$-adrenoceptor.
TABLE 1  Log K<sub>D</sub> values and selectivity ratios of α-antagonists binding to the human α<sub>1A</sub>, α<sub>1B</sub> and α<sub>1D</sub>-adrenoceptors. Values represent mean ± SE mean of n separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. Thus, SNAP5089 has 1778 fold higher affinity for the α<sub>1A</sub>-adrenoceptor than the α<sub>1B</sub>-adrenoceptor. Compounds are arranged in order of α<sub>1A</sub>-selectivity.

| Ligand          | Log K<sub>D</sub> α<sub>1A</sub> | n  | Log K<sub>D</sub> α<sub>1B</sub> | n  | Log K<sub>D</sub> α<sub>1D</sub> | n  | α<sub>1A</sub> vs α<sub>1B</sub> | α<sub>1A</sub> vs α<sub>1D</sub> | α<sub>1B</sub> vs α<sub>1D</sub> |
|-----------------|----------------------------------|----|----------------------------------|----|----------------------------------|----|-------------------------------|-------------------------------|-------------------------------|
| SNAP5089        | −8.89 ± 0.03                     | 6  | −5.64 ± 0.04<sup>app</sup>      | 6  | −5.65 ± 0.09<sup>app</sup>      | 5  | 1778                          | 1738                          | 1.0                           |
| Silodosin       | −9.61 ± 0.08                     | 9  | −5.50 ± 0.09                    | 9  | −6.94 ± 0.16                    | 9  | 1288                          | 468                           | 2.8                           |
| RS100329        | −9.60 ± 0.05                     | 10 | −6.67 ± 0.07                   | 7  | −7.63 ± 0.17                    | 6  | 851                           | 93                            | 9.1                           |
|                |                                  |    |                                  |    | −5.13 ± 0.15                    |    |                               |                               | 70.5 ± 30%<sup>site 1</sup>  |
| Niguldipine     | −9.24 ± 0.11                     | 9  | −6.33 ± 0.08                   | 5  | −5.92 ± 0.06                    | 6  | 813                           | 2089                          | 2.6                           |
| S-methyl-urapidil | −8.23 ± 0.05                     | 5  | −6.06 ± 0.04                   | 5  | −5.61 ± 0.07                    | 5  | 148                           | 417                           | 2.8                           |
| Lisuride        | −7.94 ± 0.06                     | 5  | −6.07 ± 0.04                   | 5  | −6.93 ± 0.11                    | 7  | 74                            | 10                            | 7.2                           |
| Benoxathian     | −9.08 ± 0.05                     | 6  | −7.32 ± 0.03                   | 6  | −7.91 ± 0.10                    | 7  | 58                            | 15                            | 3.9                           |
| Anisodamine     | −5.21 ± 0.03                     | 5  | −3.45 ± 0.04<sup>app</sup>     | 5  | −4.21 ± 0.05                    | 5  | 58                            | 10                            | 5.8                           |
| RS17053         | −8.33 ± 0.09                     | 11 | −6.61 ± 0.09                   | 11 | −6.84 ± 0.13                    | 6  | 52                            | 31                            | 1.7                           |
| Urapidil        | −7.21 ± 0.02                     | 5  | −5.50 ± 0.07                   | 7  | −6.37 ± 0.10                    | 6  | 51                            | 6.9                           | 7.4                           |
| 2-MPMDQ         | −9.06 ± 0.07                     | 6  | −7.37 ± 0.04                   | 6  | −9.01 ± 0.16                    | 8  | 49                            | 1.1                           | 44                            |
| WB4104          | −9.03 ± 0.04                     | 10 | −7.39 ± 0.05                   | 7  | −8.63 ± 0.11                    | 9  | 44                            | 2.5                           | 17                            |
| Indoramin       | −8.43 ± 0.07                     | 5  | −6.82 ± 0.04                   | 5  | −6.29 ± 0.07                    | 5  | 41                            | 138                           | 3.4                           |
| Phentolamine    | −8.15 ± 0.08                     | 8  | −6.55 ± 0.05                   | 5  | −6.84 ± 0.11                    | 6  | 40                            | 20                            | 1.9                           |
| Tamsulosin      | −9.67 ± 0.06                     | 17 | −8.12 ± 0.04                   | 15 | −9.18 ± 0.08                    | 13 | 35                            | 3.1                           | 11                            |
| Amitraz         | −5.52 ± 0.05                     | 5  | Log IC<sub>50</sub> > −4       | 6  | −5.08 ± 0.05                    | 5  | >33                           | 2.8                           | >12                           |
| Labetolol       | −7.33 ± 0.04                     | 7  | −5.91 ± 0.03                   | 7  | −6.12 ± 0.07                    | 6  | 26                            | 16                            | 1.6                           |
| Domperidone     | −6.85 ± 0.12                     | 6  | −5.50 ± 0.05                   | 5  | −5.98 ± 0.05                    | 5  | 22                            | 7.4                           | 3.0                           |

(Continues)
| Ligand       | Log $K_D$ $\alpha_1A$ | $n$ | Log $K_D$ $\alpha_1B$ | $n$ | Log $K_D$ $\alpha_1D$ | $n$ | $\alpha_1A$ vs $\alpha_1B$ | $\alpha_1A$ vs $\alpha_1D$ | $\alpha_1B$ vs $\alpha_1D$ |
|--------------|------------------------|-----|------------------------|-----|------------------------|-----|-----------------------------|-----------------------------|-----------------------------|
| Dibenamine   | $-7.91 \pm 0.06$       | 15  | $-6.57 \pm 0.07$       | 14  | $-7.37 \pm 0.15$       | 9   | 3.5                         | 6.3                         | 6.3                         |
| Atipamezole  | $-5.99 \pm 0.03$       | 5   | $-4.68 \pm 0.08$       | 6   | $-5.33 \pm 0.04$       | 5   | 4.6                         | 4.5                         |
| MK-912       | $-6.76 \pm 0.03$       | 5   | $-5.46 \pm 0.05$       | 5   | $-7.30 \pm 0.16$       | 7   | 3.5                         | 69                          |
| 2-PMDQ       | $-8.19 \pm 0.09$       | 5   | $-6.95 \pm 0.05$       | 6   | $-8.42 \pm 0.12$       | 9   | 1.7                         | 30                          |
| BRL44408     | $-5.92 \pm 0.09$       | 9   | $-4.68 \pm 0.07$       | 9   | $-5.06 \pm 0.05$       | 5   | 7.2                         | 2.4                         |
| ARC239       | $-9.35 \pm 0.08$       | 8   | $-8.15 \pm 0.07$       | 9   | $-8.74 \pm 0.12$       | 7   | 4.1                         | 3.9                         |
| Efaroxan     | $-5.47 \pm 0.03$       | 5   | $-4.27 \pm 0.07$       | 5   | $-4.97 \pm 0.06$       | 5   | 3.2                         | 5.0                         |
| Ifenprodil   | $-7.66 \pm 0.11$       | 9   | $-6.49 \pm 0.07$       | 6   | $-8.12 \pm 0.18$       | 8   | 2.9                         | 43                          |
| Naftopidil   | $-7.97 \pm 0.03$       | 6   | $-6.82 \pm 0.06$       | 6   | $-7.06 \pm 0.11$       | 7   | 8.1                         | 1.7                         |
| SKF846466    | $-6.06 \pm 0.05$       | 5   | $-4.93 \pm 0.05$       | 5   | $-5.16 \pm 0.09$       | 5   | 7.9                         | 1.7                         |
| Sunepitron   | $-5.78 \pm 0.06$       | 5   | $-4.65 \pm 0.06$       | 5   | $-5.33 \pm 0.23$       | 6   | 2.8                         | 4.8                         |
| RX821002     | $-6.51 \pm 0.09$       | 6   | $-5.46 \pm 0.06$       | 6   | $-5.31 \pm 0.13$       | 7   | 16                          | 1.4                         |
| 3-MPPI       | $-9.57 \pm 0.06$       | 6   | $-8.59 \pm 0.03$       | 6   | $-9.76 \pm 0.15$       | 7   | 1.5                         | 15                          |
| S32212       | $-5.90 \pm 0.06$       | 5   | $-4.92 \pm 0.02^{\text{app}}$ | 5   | $-5.69 \pm 0.13^{\text{app}}$ | 5   | 1.6                         | 5.9                         |
| Promethazine | $-7.00 \pm 0.10$       | 11  | $-6.06 \pm 0.05$       | 10  | $-5.75 \pm 0.07$       | 5   | 18                          | 2.0                         |
| AH11110A     | $-6.48 \pm 0.03$       | 5   | $-5.65 \pm 0.09$       | 5   | $-4.98 \pm 0.06$       | 5   | 32                          | 4.7                         |
| Yohimbine    | $-6.23 \pm 0.03$       | 5   | $-5.44 \pm 0.05$       | 5   | $-6.20 \pm 0.08$       | 8   | 1.1                         | 5.8                         |
| Idazoxan     | $-5.67 \pm 0.07$       | 5   | $-4.88 \pm 0.03$       | 5   | $-5.23 \pm 0.11$       | 5   | 2.8                         | 2.2                         |
| Bromocriptine| $-8.73 \pm 0.06$       | 5   | $-7.96 \pm 0.07$       | 5   | $-7.31 \pm 0.15^{\text{early plateau}}$ | 9   | 26                          | 4.5                         |
| Phenoxymexine| $-8.45 \pm 0.12$       | 12  | $-7.69 \pm 0.06$       | 13  | $-8.43 \pm 0.19$       | 10  | 1.0                         | 5.5                         |
|              | $-6.02 \pm 0.08$       |     | $-5.57 \pm 0.06$       |     | $-5.42 \pm 0.08$       |     |                             |                             |
|              | 77.7 ± 5.2% site 1     |     | 67.5 ± 2.5% site 1     |     | 39.1 ± 2.0% site 1     |     |                             |                             |
The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). An IC\textsubscript{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible. Thus an app K\textsubscript{D} was calculated.

An early plateau was reached when competition was complete. The inhibition curve reached a plateau of maximal inhibition of binding at 71.0\% ± 3.4\% inhibition of specific binding (n = 9).

**TABLE 1**

| Ligand      | Log K\textsubscript{D} \(\alpha1A\) | \(n\) | Log K\textsubscript{D} \(\alpha1B\) | \(n\) | Log K\textsubscript{D} \(\alpha1D\) | \(n\) | \(\alpha1A\) vs \(\alpha1B\) | \(\alpha1A\) vs \(\alpha1D\) | \(\alpha1D\) vs \(\alpha1D\) |
|-------------|-----------------------------------|------|-----------------------------------|------|-----------------------------------|------|-----------------|-----------------|-----------------|
| RS79948     | -5.75 ± 0.05                      | 5    | -4.99 ± 0.05                      | 5    | -6.07 ± 0.11                      | 6    | 5.8             | 2.1             | 12              |
| JP1302      | -6.21 ± 0.04                      | 5    | -5.46 ± 0.02                      | 5    | -5.58 ± 0.09                      | 5    | 5.6             | 4.3             | 1.3             |
| Imiloxan    | -4.60 ± 0.05                      | 5    | IC\textsubscript{50} > -4        | 5    | -5.02 ± 0.07                      | 5    | >4.0            | 2.6             | >10             |
| HEAT        | -8.57 ± 0.06                      | 5    | -8.04 ± 0.04                      | 5    | -8.11 ± 0.18                      | 8    | 3.4             | 2.9             | 1.2             |
| Carvedilol  | -8.35 ± 0.06                      | 12   | -7.84 ± 0.06                      | 6    | -7.87 ± 0.12                      | 7    | 3.2             | 3.0             | 1.1             |
| A80426      | -6.57 ± 0.05                      | 5    | -6.08 ± 0.02                      | 5    | -6.07 ± 0.07                      | 5    | 3.1             | 3.1             | 1.0             |
| Rec15-2615  | -8.26 ± 0.10                      | 6    | -7.79 ± 0.09                      | 6    | -7.89 ± 0.07                      | 7    | 3.0             | 2.3             | 1.3             |
| Spiroxatrine| -6.86 ± 0.06                      | 5    | -6.41 ± 0.06                      | 5    | -7.86 ± 0.13                      | 6    | 2.8             | 10              | 28              |
| BMY7378     | -6.61 ± 0.05                      | 5    | -6.23 ± 0.05                      | 6    | -8.60 ± 0.13                      | 9    | 2.4             | 98              | 234             |
| Prazosin    | -9.07 ± 0.04                      | 9    | -8.74 ± 0.06                      | 8    | -9.07 ± 0.23                      | 10   | 2.1             | 1.0             | 2.1             |
| Alfuzosin   | -7.82 ± 0.11                      | 8    | -7.56 ± 0.08                      | 6    | -7.66 ± 0.11                      | 6    | 1.8             | 1.4             | 1.3             |
| Cyclazosin  | -8.89 ± 0.06                      | 7    | -8.68 ± 0.08                      | 5    | -9.87 ± 0.06                      | 7    | 1.6             | 9.5             | 15              |
| Doxazosin   | -8.58 ± 0.09                      | 6    | -8.46 ± 0.05                      | 8    | -8.33 ± 0.13                      | 11   | 1.3             | 1.8             | 1.3             |
| Terazosin   | -7.93 ± 0.05                      | 6    | -7.95 ± 0.05                      | 6    | -7.71 ± 0.13                      | 7    | 1.0             | 1.7             | 1.7             |
| \(\beta\)-blockers |
| Carazolol   | -6.57 ± 0.03                      | 5    | -4.68 ± 0.05                      | 6    | -5.04 ± 0.08\textsuperscript{app} | 5    | 78              | 34              | 2.3             |
| SDZ21009    | -5.24 ± 0.07                      | 6    | Log IC\textsubscript{50} > -4     | 6    | -5.09 ± 0.18                      | 6    | >17             | 1.4             | >12             |
| CGP12177    | -5.14 ± 0.05                      | 6    | Log IC\textsubscript{50} > -4     | 5    | -4.20 ± 0.11                      | 5    | >14             | 8.7             | >1.6            |
| Bucindolol  | -7.57 ± 0.07                      | 5    | -6.46 ± 0.04                      | 5    | -6.45 ± 0.09                      | 5    | 13              | 13              | 1.0             |
| ICI118551   | -5.23 ± 0.03                      | 5    | -4.20 ± 0.06\textsuperscript{app} | 5    | -4.96 ± 0.03                      | 5    | 11              | 1.9             | 5.8             |
| CGP20712A   | -4.93 ± 0.10                      | 5    | Log IC\textsubscript{50} > -4     | 5    | -4.96 ± 0.07\textsuperscript{app} | 5    | >8.5            | 11              | >9.1            |
| Propranolol | -4.91 ± 0.02                      | 6    | -3.98 ± 0.04\textsuperscript{app} | 6    | -4.89 ± 0.09                      | 5    | 8.5             | 1.0             | 8.1             |
| Cyanopindolol| -5.59 ± 0.05                     | 8    | -4.91 ± 0.09                      | 7    | -5.40 ± 0.07                      | 5    | 4.8             | 1.5             | 3.1             |

\(\text{app}\) The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). An IC\textsubscript{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible. Thus an app K\textsubscript{D} was calculated.

\(\text{early plateau}\) Bromocriptine did not fully inhibit specific binding at the \(\alpha1D\)-adrenoceptor. The inhibition curve reached a plateau of maximal inhibition of binding at 71.0\% ± 3.4\% inhibition of specific binding (\(n = 9\)).

app, apparent.
These values are all within twofold of the value obtained from saturation studies. The values from saturation studies were used for further $K_D$ calculations. A lower receptor expression level for the full-length $\alpha_1$D-adrenoceptors is a common finding\textsuperscript{15,33} and reports suggest truncation of the N-terminus results in higher receptor expressions.\textsuperscript{33-35} Doxazosin, a commonly used $\alpha$ blocker in the treatment of hypertension, inhibited all three receptors with high affinity ($\log K_D$ $-8.58$, $-8.46$, and $-8.33$ at the $\alpha_1$A, $\alpha_1$B, and $\alpha_1$D-adrenoceptor, respectively, Figure 1, Table 1). Of all the compounds studied, SNAP 5089 had the highest receptor selectivity, being over 1700-fold selective for the $\alpha_1$A-adrenoceptor over the $\alpha_1$A and $\alpha_1$B-adrenoceptors, respectively (Figure 1, Table 1). Several compounds had affinities of less than 0.25nM, including ligands with $\alpha_1$A selectivity (silodosin, RS100329, and tamsulosin), cyclazosin with slight $\alpha_1$D selectivity, and nonselective 3-MPPI (Table 1).

Two compounds were best described by a two-component-binding inhibition curve at all three receptors—phenoxybenzamine and dibenamine (Figure 2, Table 1). Both of these are N,N-disubstituted-2-chloroethylamines. Preincubation of phenoxybenzamine and dibenamine with sodium thiosulfate before addition to the cells yielded a single-component-binding inhibition (Figure 2, Table 2), whereby the high-affinity-binding component of the parent curve had been abolished. Sodium thiosulfate had no effect on the binding of tamsulosin (Figure 2, Table 2). At the $\alpha_1$D-adrenoceptor, several
TABLE 2 Log $K_D$ values of phenoxybenzamine, dibenamine and tamsulosin binding to the human $\alpha_1A$, $\alpha_1B$ and $\alpha_1D$-adrenoceptors obtained in the absence and presence of 1 mmol/L sodium thiosulphate (Figure 2). Values represent mean ± SE mean of $n$ separate experiments.

|                  | Control                  | +1 mmol/L sodium thiosulphate |
|------------------|--------------------------|-------------------------------|
|                  | Log $K_D$ site 1 | Log $K_D$ site 2 | % site 1 | n  | Log $K_D$ site 1 | Log $K_D$ site 2 | % site 1 | n  |
| **CHO-α1A**      |                  |                            |         |    |                  |                            |         |    |
| Phenoxybenzamine | −8.45 ± 0.12 | −6.02 ± 0.08 | 77.7 ± 5.2 | 12 | −5.43 ± 0.07 |       |         |    |
| Dibenamine       | −7.91 ± 0.06 | −5.32 ± 0.08 | 83.0 ± 1.8 | 15 | −5.16 ± 0.10 |       |         |    |
| Tamsulosin       | −9.67 ± 0.06 |          |         | 17 | −9.75 ± 0.16 |       |         |    |
| **CHO-α1B**      |                  |                            |         |    |                  |                            |         |    |
| Phenoxybenzamine | −7.69 ± 0.06 | −5.57 ± 0.06 | 67.5 ± 2.5 | 13 | −5.18 ± 0.05 |       |         |    |
| Dibenamine       | −6.57 ± 0.07 | −4.66 ± 0.06 | 67.6 ± 2.6 | 14 | −4.85 ± 0.05 |       |         |    |
| Tamsulosin       | −8.12 ± 0.04 |          |         | 15 | −8.13 ± 0.08 |       |         |    |
| **CHO-α1D**      |                  |                            |         |    |                  |                            |         |    |
| Phenoxybenzamine | −8.43 ± 0.19 | −5.42 ± 0.08 | 39.1 ± 2.0 | 10 | −4.93 ± 0.10 |       |         |    |
| Dibenamine       | −7.37 ± 0.15 | −5.00 ± 0.14 | 47.8 ± 3.2 | 9  | −4.74 ± 0.09 |       |         |    |
| Tamsulosin       | −9.18 ± 0.08 | −5.67 ± 0.15 | 54.6 ± 3.7 | 13 | −9.11 ± 0.12 | −5.60 ± 0.08 | 44.0 ± 2.8 | 7  |

FIGURE 3 Inhibition of [3H]prazosin binding to whole cells by two commonly prescribed antidepressants amtriptyline (A–C) or trazodone (D–F) to CHO-α1A cells (A, D), CHO-α1B cells (B, E), or CHO-α1D cells (C, F). Bars represent total [3H]prazosin binding and nonspecific binding was determined in the presence of 10 $\mu$mol/L tamsulosin (CHO-α1A and CHO-α1B) or 100 $\mu$mol/L tamsulosin (CHO-α1D). The concentration of [3H]prazosin was a) 0.39 nmol/L, (B) 0.45 nmol/L, (C) 0.57 nmol/L, (D) 0.66 nmol/L, (E) 0.45 nmol/L, and (F) 0.66 nmol/L. Data points are mean ± SE mean of triplicate determinations.
other ligands were best described by a two-component-binding inhibition curve. Just as with tamsulosin (Figure 2), preincubation with sodium thiosulfate had no effect on either component of any of these other two-component ligands.

The affinity of several antidepressants and antipsychotics was then examined. Several of these were found to have high α1-adrenoceptor affinity (Figures 3 and 4, Table 3 and 4). Risperidone (previously suggested to have α1B selectivity, had slight α1A selectivity, in keeping with the findings of 37. There have also been discrepancies in the affinity of olanzapine: Richelson and Souder 37 found it to have high affinity (44nM for α1-adrenoceptor) and Nourain et al. 39 had conflicting data with low rat α1-adrenoceptor affinity, but significant hypotension in rats. However, here, olanzapine had low affinity, in keeping with 38 and the findings of 39 where olanzapine was described as having low postural hypotension potential. WB4104, was also initially thought to have α1B selectivity, however, it had higher and equal affinity for α1A and α1D-adrenoceptors (in keeping with 40,41).

Tables combing all of these ligands are presented in Supplementary Data. Table S1 has the ligands arranged in alphabetical order (together with their suppliers and individual ligand codes). Table S2 has the ligands organized in order of α1A affinity.

**DISCUSSION**

Dibenamine, phentolamine, and phenoxybenzamine were the first clinical α blockers and phenoxybenzamine is still used in the management of pheochromocytoma, particularly during surgery where catastrophic catecholamine release can cause hypertensive crises and arrhythmias. Both phenoxybenzamine and dibenamine are N,N-disubstituted-2-chloroethylamines containing a nitrogen mustard group. Both compounds were best described by a two-component-binding inhibition curve at all three α1-adrenoceptors (Figure 2, Table 1). In aqueous solution at physiological pH, the nitrogen mustard group cyclizes to form ethyleniminium ions. These highly reactive, unstable ions are pharmacologically active and covalently bind to a cysteine in transmembrane 3 of the α adrenoceptors, giving these compounds their "irreversible" properties.

Phenoxybenzamine has a longer duration of action in clinical studies than phentolamine 7 and hence its continued use in pheochromocytoma (although similar outcomes have been reported with doxazosin, terazosin, and prazosin. Sodium thiosulfate also rapidly reacts with the ethyleniminium ions thus prevents them from interacting with α adrenoceptors. Pretreatment with intravenous sodium thiosulfate prevented dibenamine binding to α adrenoceptors (in cats, and pretreatment with sodium thiosulfate prevented the
Table 3  Log Kᵣ values and selectivity ratios of antidepressants binding to the human α₁A, α₁B and α₁D-adrenoceptors. Values represent mean ± SE mean of n separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. Thus, amitriptyline has 93 fold higher affinity for the α₁A-adrenoceptor than the α₁B-adrenoceptor. Compounds are arranged in order of α₁A-selectivity.

| Ligand                                      | Log Kᵣ α₁A | n  | Log Kᵣ α₁B | n  | Log Kᵣ α₁D | n  | α₁A vs α₁B | α₁A vs α₁D | α₁B vs α₁D | α₁A | α₁B |
|---------------------------------------------|------------|----|------------|----|------------|----|------------|------------|------------|-----|-----|
| Noradrenaline and serotonin reuptake inhibitors |            |    |            |    |            |    |            |            |            |     |     |
| Tricyclic antidepressants                   |            |    |            |    |            |    |            |            |            |     |     |
| Amitriptyline                               | −8.19 ± 0.02 | 9  | −6.22 ± 0.05 | 9  | −6.25 ± 0.05 | 5  | 93         | 87         | 1.1        |     |     |
| Doxepin                                     | −7.11 ± 0.04 | 5  | −5.28 ± 0.11 | 7  | −5.58 ± 0.05 | 5  | 68         | 34         | 2.0        |     |     |
| Clonipram                                   | −8.12 ± 0.10 | 9  | −6.34 ± 0.07 | 9  | −6.15 ± 0.09 | 5  | 60         | 93         | 1.5        |     |     |
| Imipram                                     | −7.47 ± 0.04 | 6  | −5.76 ± 0.05 | 6  | −5.89 ± 0.05 | 5  | 51         | 38         | 1.3        |     |     |
| Norclonipram                                 | −7.52 ± 0.08 | 11 | −5.84 ± 0.04 | 12 | −5.84 ± 0.06 | 5  | 48         | 48         | 1.0        |     |     |
| Nortriptyline                                | −7.74 ± 0.03 | 6  | −6.07 ± 0.07 | 5  | −5.81 ± 0.05 | 5  | 47         | 85         | 1.8        |     |     |
| Doxepin                                     | −7.74 ± 0.04 | 5  | −6.18 ± 0.03 | 6  | −6.27 ± 0.11 | 5  | 36         | 30         | 1.2        |     |     |
| Desipramine                                 | −7.07 ± 0.05 | 6  | −5.57 ± 0.05 | 5  | −5.46 ± 0.07 | 5  | 32         | 41         | 1.3        |     |     |
| Lofepramine                                  | −6.94 ± 0.06 | 6  | −5.44 ± 0.07 | 6  | −5.37 ± 0.04 | 5  | 32         | 37         | 1.2        |     |     |
| Trimipramine                                | −7.37 ± 0.08 | 5  | −6.10 ± 0.06 | 5  | −5.99 ± 0.05 | 5  | 19         | 24         | 1.3        |     |     |
| Protriptyline                               | −6.67 ± 0.03 | 5  | −5.57 ± 0.02 | 5  | −5.46 ± 0.12 | 5  | 13         | 16         | 1.3        |     |     |
| Tetra cyclic antidepressants                |            |    |            |    |            |    |            |            |            |     |     |
| Mirtazapine                                 | −6.36 ± 0.02 | 5  | −5.36 ± 0.03 | 5  | −5.94 ± 0.05 | 5  | 10         | 2.6        | 3.8        |     |     |
| Other noradrenaline and serotonin reuptake inhibitors |            |    |            |    |            |    |            |            |            |     |     |
| Duloxetine                                  | −5.65 ± 0.05 | 5  | −4.71 ± 0.03<sup>app</sup> | 5  | −5.58 ± 0.12 | 7  | 8.7        | 1.2        | 7.4        |     |     |
| Venlafaxime                                 | −3.69 ± 0.02<sup>app</sup> | 5  | No binding to 1 mmol/L | 5  | −4.38 ± 0.16 | 7  | >4.9       | 4.9        | >24        |     |     |
| Noradrenaline reuptake inhibitors           |            |    |            |    |            |    |            |            |            |     |     |
| Reboxetine                                  | −4.91 ± 0.08 | 5  | Log IC<sub>50</sub> > −4 | 5  | −4.67 ± 0.12 | 5  | >8.1       | 1.7        | >4.7       |     |     |
| Selective serotonin reuptake inhibitors (SSRI) |            |    |            |    |            |    |            |            |            |     |     |
| Fluvoxamine                                 | −6.10 ± 0.03 | 5  | Log IC<sub>50</sub> > −4 | 5  | −4.97 ± 0.03 | 5  | >126       | 14         | >9.3       |     |     |
| Citalopram                                  | −5.95 ± 0.06 | 4  | Log IC<sub>50</sub> > −4 | 4  | −4.91 ± 0.11 | 5  | >89        | 11         | >8.1       |     |     |
| Fluoxetine                                  | −5.45 ± 0.04 | 5  | −4.41 ± 0.06 | 5  | −4.90 ± 0.13 | 5  | 11         | 3.5        | 3.1        |     |     |
| Paroxetine                                  | −5.59 ± 0.09 | 5  | Log IC<sub>50</sub> > −5 | 5  | −5.63 ± 0.13 | 5  | >3.9       | 1.1        | >4.3       |     |     |
| Sertaline                                   | −5.72 ± 0.04 | 5  | −5.45 ± 0.05 | 5  | −5.61 ± 0.04 | 5  | 19         | 1.3        | 1.4        |     |     |
| Serotonin reuptake inhibitors               |            |    |            |    |            |    |            |            |            |     |     |
| Vortioxetine                                | −6.32 ± 0.05 | 5  | −5.42 ± 0.02 | 5  | −5.43 ± 0.08 | 5  | 79         | 7.8        | 1.0        |     |     |
| Trazodone                                   | −7.33 ± 0.04 | 6  | −6.56 ± 0.07 | 6  | −6.38 ± 0.15 | 7  | 59         | 8.9        | 1.5        |     |     |
| Metalonin agonist                           | −4.57 ± 0.11<sup>app</sup> | 5  | No binding to 100 µmol/L | 5  | IC<sub>50</sub> > −4.5 | 6  | >3.7       | 1.1        | >3.2       |     |     |

<sup>app</sup>The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). An IC<sub>50</sub> was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible. Thus an app Kᵣ was calculated.
**TABLE 4** Log $K_O$ values and selectivity ratios of antipsychotics binding to the human $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$-adrenoceptors. Values represent mean $\pm$ SE mean of $n$ separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. Thus, chlorpromazine has 13 fold high affinity for the $\alpha_{1A}$-adrenoceptor than the $\alpha_{1B}$-adrenoceptor. Compounds are arranged in order of $\alpha_{1A}$-selectivity.

| Ligand          | Log $K_O\alpha_{1A}$ | n | Log $K_O\alpha_{1B}$ | n | Log $K_O\alpha_{1D}$ | n | $\alpha_{1A}$ vs $\alpha_{1B}$ | $\alpha_{1A}$ vs $\alpha_{1D}$ | $\alpha_{1B}$ vs $\alpha_{1D}$ |
|-----------------|----------------------|---|----------------------|---|----------------------|---|------------------|------------------|------------------|
| **First generation antipsychotics** |          |   |                      |   |                      |   |                  |                  |                  |
| Sulpiride       | $-4.50 \pm 0.07$     | 5 | $-7.84 \pm 0.05$     | 5 | $-3.66 \pm 0.09^{app}$ | 5 | >32              | 6.9              | >4.6             |
| Chlorpromazine  | $-8.94 \pm 0.06$     | 5 | $-8.00 \pm 0.08$     | 6 | 8.7                  | 5  | 6.9              | 1.4              |
| Flupenthixol    | $-8.35 \pm 0.05$     | 5 | $-7.47 \pm 0.07$     | 5 | $-6.96 \pm 0.12$     | 7  | 7.6              | 25               |
| Trifluoperazine | $-7.75 \pm 0.03$     | 5 | $-6.88 \pm 0.07$     | 5 | $-6.36 \pm 0.08$     | 5  | 7.4              | 25               |
| Prochlorperazine| $-7.61 \pm 0.12$     | 4 | $-6.88 \pm 0.06$     | 5 | $-6.53 \pm 0.13$     | 8  | 5.4              | 12               |
| Perphenazine    | $-8.15 \pm 0.09$     | 5 | $-7.43 \pm 0.08$     | 5 | $-7.86 \pm 0.10$     | 5  | 5.2              | 1.9              |
| Pimozide        | $-7.44 \pm 0.16$     | 5 | $-6.79 \pm 0.05$     | 5 | $-5.95 \pm 0.08$     | 6  | 4.5              | 31               |
| Haloperidol     | $-7.70 \pm 0.03$     | 5 | $-7.21 \pm 0.07$     | 6 | $-6.42 \pm 0.06$     | 5  | 3.1              | 19               |
| **Second generation antipsychotics** |          |   |                      |   |                      |   |                  |                  |                  |
| Amisulpiride    | $-5.05 \pm 0.04$     | 5 | No binding to 100 µmol/L | 5 | $-4.55 \pm 0.08^{app}$ | 5 | 11               | 3.2              |
| Ziprasidone     | $-8.73 \pm 0.05$     | 7 | $-7.70 \pm 0.07$     | 8 | $-7.20 \pm 0.09$     | 5  | 11               | 34               |
| Paliperidone    | $-8.36 \pm 0.09$     | 5 | $-7.36 \pm 0.08$     | 5 | $-7.47 \pm 0.10$     | 6  | 10               | 7.8              |
| Sertindole      | $-9.27 \pm 0.09$     | 9 | $-8.28 \pm 0.11$     | 8 | $-6.93 \pm 0.12$     | 8  | 9.8              | 219              |
| Risperidone     | $-8.74 \pm 0.06$     | 7 | $-7.77 \pm 0.05$     | 7 | $-7.14 \pm 0.07$     | 6  | 9.3              | 40               |
| Clozapine       | $-8.27 \pm 0.04$     | 5 | $-7.39 \pm 0.07$     | 5 | $-6.41 \pm 0.05$     | 5  | 7.6              | 72               |
| Quetiapine      | $-7.89 \pm 0.10$     | 5 | $-7.21 \pm 0.04$     | 5 | $-6.48 \pm 0.10$     | 5  | 4.8              | 26               |
| Lurasidone      | $-7.80 \pm 0.11$     | 5 | $-7.17 \pm 0.09$     | 5 | $-8.19 \pm 0.10$     | 7  | 4.3              | 25               |
| Aripiprazole    | $-7.32 \pm 0.07$     | 6 | $-6.69 \pm 0.03$     | 6 | $-6.15 \pm 0.11$     | 5  | 4.3              | 15               |
| Olanzapine      | $-6.61 \pm 0.11$     | 7 | $-6.00 \pm 0.10$     | 10 | $-5.86 \pm 0.06$     | 5  | 4.1              | 5.6              |

*app The maximum concentration of competing ligand inhibited most but not all of specific binding (as in Figure 1E). An IC$_{50}$ was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible. Thus an app $K_O$ was calculated.

app, apparent.
harmful interactions of the chemical weapon mustard gas in humans. Here, preincubation of phenoxybenzamine or dibenamine with sodium thiosulfate yielded single-component-binding inhibition curves (Figure 2, Table 2). Abolishment of the high-affinity-binding component suggests that it was due to specific α1-adrenoceptor interaction. The $K_D$ values of the low-affinity components were very similar to those obtained in the presence of thiosulfate, suggesting that this component is a non-orthosteric site or non-receptor-mediated effect.

Several other ligands were found to have a [3H]prazosin inhibition best described a two-component curve at the α1D-adrenoceptor, including tamsulosin (and hence why 100 μM was used to define nonspecific binding in CHO-α1D cells, rather than 10 μM used in α1A and α1B cells), and the only α1D-selective ligand, BMY7378. As expected for these nonmustard compounds, preincubation with sodium thiosulfate had no effect on binding. The reason for the second component is therefore unknown. Affinity ($K_D$ value) obtained for the high-affinity component the α1D-adrenoceptor has been used to determine receptor selectivity.

α1-adrenoceptor antagonists (α blockers, especially doxazosin) have been used for hypertension for decades. Doxazosin had high affinity for all three subtypes, similar to previous [3H]prazosin-binding studies. Terazosin and prazosin were also nonselective ligands (as in $^{45}$) as was phentolamine. Indoramin (licensed for hypertension), had an α1A selectivity of 40-fold (similar to $^{26}$). Of the α blockers that are used for the treatment of BPH in the UK, alfuzosin was nonselective, whereas tamsulosin with its α1A vs α1B selectivity of 35-fold, was equipotent at α1A and α1D receptors (as in $^{15,16,51}$). Thus, drugs used for hypertension and BPH include nonselective α1 blockers and those with up to 40-fold α1A selectivity. It would therefore be expected that drugs like tamsulosin and alfuzosin, licensed for BPH, are likely to have as much of an effect on blood pressure as α blockers intentionally prescribed for hypertension. Several other high-affinity non/poorly selective ligands were also identified that have higher affinity than doxazosin, for example, cycloazosin, 3-MPPI, and ARC239.

Carvedilol (commonly used in heart failure) is considered a dual α/β blocker. Carvedilol was nonselective, with high affinity at all three α1-adrenoceptors, however the α1A affinity (log $K_D$ of −8.35) was still 10-fold less than that for the β2 adrenoceptor. $^{52}$ Labetolol (used in hypertension particularly in pregnancy, and intravenously in hypertensive emergencies), is also considered a dual α/β blocker. Labetolol has lower affinity than carvedilol for all β $^{52}$ and α1-adrenoceptors (log $K_D$ −7.33 at α1A), but very poor affinity for the α1B and α1D-adrenoceptors. Labetolol should be considered a β1/β2/α1A blocker rather than dual pan α1 and β blocker. Given these dual α/β ligands, the affinity of a few β blockers with very high β-adrenoceptor affinity were examined (Table 1). With the exception of bucardol, the affinity was poor at all three α1-adrenoceptors, confirming their β selectivity. Although the affinity of bucardol was reasonably high (log $K_D$ at α1A −7.57), this is 54-fold and 263-fold lower than that for the human β1 and β2 adrenoceptor, respectively. $^{53}$

The most selective ligand detected here was SNAP5089, with 1700-fold selectivity for the α1A over the α1B or α1D-adreceptors. Other α1A-selective ligands were silodosin, R5100329, and niguldipine (in keeping with $^{90,54}$). As well as tamsulosin, several ligands had higher affinity for the α1A and α1D receptors than the α1B—for example, 2-MPMDQ, MK-912, 2-PMDQ, and ifenprodil. BMY7378 was the only compound with substantial α1D selectivity (the 100- to 200-fold selectivity is similar to $^{20,12041,50,55}$). No α1B-selective ligand was identified. To pharmacologically infer the presence of α1B-adrenoceptors in cells or tissues, several different compounds with different patterns of selectivity would be required, for example, SNAP5089, doxazosin, 2-MPMDQ, and BMY7378.

Several tricyclic antidepressants (TCA) had significant affinity for the α1-adrenoceptors. Amitriptyline, clomipramine, doxepin, and nortriptyline have similar α1-adrenoceptor affinities and selectivities to α blockers prescribed for hypertension or BPH. Thus patients taking these TCAs should be considered to be α blocked and are at risk from postural hypotension (as in $^{23}$). Furthermore, the addition of an α blocker for concomitant hypertension or BPH may not have any additional clinical benefit and may actually cause significant postural problems. Other TCA had lower affinity, for example, protriptyline and lofepramine and would therefore be expected to have less effect on blood pressure. The selective serotonin reuptake inhibitors (SSRIs) had very poor affinity for any of the α adrenoceptors and are therefore less likely to have significant α1-mediated hypotension.

Several antipsychotics (including first-generation chlorpromazine and flupenthixol and second-generation sertindole, risperidone, and clozapine) had high α1-adrenoceptor affinity. The very high affinity of sertindole (and 300-fold selectivity for α1A over α1D-adrenoceptors) was similar to previous reports. $^{6,29}$ The degree of α1A-adrenoceptor affinity observed here correlates well with the rankings for observations in rats. $^{29}$ The high α1A affinity of sertindole, risperidone, and ziprasidone (log $K_D$ −9.3 to −8.7) is similar to studies, $^{38}$ including in brain tissue, $^{27}$ and similar to (or even higher than) that for many drugs used to treat hypertension. The high rate of postural hypotension observed with these drugs $^{31,32}$ is therefore not surprising. A similar hypotensive effect would be expected with other antipsychotics α1 affinities equal or greater than that for α1 blockers used for hypertension, for example, chlorpromazine, flupenthixol, perphenazine, paliperidone, quetiapine, and lurasidone. Aripiprazole had lower α1 affinity (in keeping with $^{34}$ and indeed has a relative lack of reported postural hypotension in clinical studies $^{57}$). However, sulpiride and amisulpride would be expected to have even less hypotensive effect.

Thus, the high α1 affinity and selectivity profile of many antipsychotics is comparable to the α1 blockers intentionally prescribed for hypertension. Equivalent reductions in blood pressure are a likely very common side effect. These drugs are used to manage schizophrenia where their effect on blood pressure in agitated patients is less likely to be an issue. However, antipsychotics are also widely used to manage delirium in sick patients including the intensive care
The drugs used for hypertension and BPH have a very similar effect on blood pressure. (BMY7378), however no α1D-selective antagonist (eg, SNAP5089), and one already low or labile blood pressures. This study suggests that sulpiride, amisulpiride, ariprazole, and olazepine should have the least effect on blood pressure.

In conclusion, there are several highly α1A-selective antagonists (eg, SNAP5089), and one α1D-selective antagonist (BMY7378), however no α1B-selective ligand has been identified. The drugs used for hypertension and BPH have a very similar pharmacological profile in terms of α1-selectivity and selectivity. Several antidepressants and antipsychotics have high α1-selectivities, similar to, or even greater than, those seen for α blockers prescribed for hypertension and BPH. The addition of further α blockers for the management of hypertension or BPH in these patients may not be beneficial. The excellent correlation between the affinity values determined from this cell studies with the affinities measured in blood vessels, brain tissue, and whole animals (including humans) means that many, but not all antipsychotics and antidepressant may cause significant peripheral α-adrenoceptor blockade and associated hypotension. Finally, awareness of the α-blocking potential of certain, but not all antipsychotics may affect the choice of drug used for the management of delirium in the intensive care unit where additional α blockade and blood pressure lowering in a sick patient may be detrimental.

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DISCLOSURE

JGB has been on the Scientific Advisory Board for CuraSen Therapeutics since 2019.

AUTHORS CONTRIBUTIONS

JGB designed the research study. ASP contributed the α1D DNA and aided discussions. RGWP and JGB performed the research. JGB analyzed the data. JGB and ASP wrote the paper.

ETHICAL STATEMENT

No animals, human tissue, human volunteers, or patients were used in this study.

DATA SHARING

Further information and requests for data and reagents should be directed to and will be fulfilled by the corresponding author, Jillian Baker. Please contact jillian.baker@nottingham.ac.uk.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results.

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REFERENCES

1. Bylund DB. Subtypes of alpha 1- and alpha 2-adrenergic receptors. FASEB J. 1992;6:832-839.
2. Docherty JR. Subtypes of functional alpha1-adrenoceptor. Cell Mol Life Sci. 2010;67:405-417.
3. Piascik MT, Perez DM. Alpha1-adrenergic receptors: new insights and directions. J Pharmacol Exp Ther. 2001;298:403-410.
4. Ford AP, Williams TJ, Blue DR, Clarke DE. Alpha 1-adrenoceptor classification: sharpening Occam’s razor. Trends Pharmacol Sci. 1994;15:167-170.
5. Akinaga J, García-Sáinz JA, Pupo SA. Updates in the function and regulation of α1 adrenoceptors. Br J Pharmacol. 2019;176:2343-2357.
6. Ipsen M, Zhang Y, Dragsted N, Han C, Mulvany MJ. The antipsychotic drug sertindole is a specific inhibitor of alpha1A-adrenoceptors in rat mesenteric small arteries. Eur J Pharmacol. 1997;336:29-35.
7. Ross EJ, Prichard BN, Kaufman L, Robertson AI, Harries BJ. Preoperative and operative management of patients with phaeochromocytoma. Br Med J. 1967;1:91-98.
8. Spear HC, Griswold D. The use of dibenamine in phaeochromocytoma; report of a case. N Engl J Med. 1948;239:736-739.
9. Hollingsworth JM, Wilt TJ. Lower urinary tract symptoms in men. BMJ. 2014;349:g4474.
10. Caine M, Perlberg S, Meretyk S. A placebo-controlled double-blind study of the effect of phenoxbenzamine in benign prostatic obstruction. Br J Urol. 1978;50:551-554.
11. Lowe FC. Role of the newer alpha, -adrenergic-receptor antagonists in the treatment of benign prostatic hyperplasia-related lower urinary tract symptoms. Clin Ther. 2004;26:1701-1713.
12. Bird ST, Delaney JA, Brophy JM, Eltmann M, Skeldon SC, Hartzema AG. Tamsulosin treatment for benign prostatic hyperplasia and risk of severe hypotension in men aged 40–85 years in the United States: risk window analyses using between and within patient methodology. BMJ. 2013;347:f6320.
13. Welk B, McArthur E, Fraser LA, et al. The risk of fall and fracture with the initiation of a prostate-selective α antagonist: a population based cohort study. BMJ. 2015;351:h5398.
14. Michel MC. The forefront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction: α blockers in the treatment of male voiding dysfunction - how do they work and why do they differ in tolerability? J Pharmacol Sci. 2010;112:151-157.
15. Kenny BA, Miller AM, Williamson JJ, O’Connell J, Chalmers DH, Naylor AM. Evaluation of the pharmacological selectivity profile of alpha 1 adrenoceptor antagonists at prostatic alpha 1 adrenoceptors: binding, functional and in vivo studies. Br J Pharmacol. 1996;118:871-878.
16. Quaresma BMCS, Pimenta AR, Santos da Silva AC, et al. Revisiting the pharmacodynamic uroselectivity of α 1-adrenergic receptor antagonists. J Pharmacol Exp Ther. 2019;371:106-112.
17. Oelke M, Gericke A, Michel MC. Cardiovascular and ocular safety of α1-adrenoceptor antagonists in the treatment of male lower urinary tract symptoms. Expert Opin Drug Saf. 2014;13:1187-1197.
18. Jung JH, Kim J, MacDonald R, Reddy B, Kim MH, Dahm P. Silodosin for the treatment of lower urinary tract symptoms in men with benign prostate hyperplasia. Cochrane Database Syst Rev. 2017(11):CD012615.
19. Cusack B, Nelson A, Richelson E. Binding of antidepressants to human brain receptors: focus on newer generation compounds. Psychopharmacology. 1994;114:559-565.
20. Nojimoto FD, Mueller A, Hebeler-Barbosa F, et al. The tricyclic antidepressants amitriptyline, nortriptyline and imipramine are weak antagonists of human and rat alpha1B-adrenoceptors. Neuropharmacology. 2010;59:49-57.
21. van Zwieten PA. Inhibition of the central hypotensive effect of clonidine by trazodone, a novel antidepressant. Pharmacology. 1977;15:331-336.
22. Hall H, Sven-Ove Ö. Effects of antidepressant drugs on different receptors in the brain. Eur J Pharmacol. 1981;70:393-407.
23. Koppera H. Anticholinergic and blood pressure effects of mianserin, amitriptyline and placebo. Br J Clin Pharmacol. 1978;5:295-345.
24. Poon IO, Braun U. High prevalence of orthostatic hypotension and its correlation with potentially causative medications among elderly veterans. J Clin Pharmacol Ther. 2005;30:173-178.
25. Thapa PB, Gideon P, Cost TW, Milam AB, Ray WA. Antidepressants and the risk of falls among nursing home residents. N Engl J Med. 1998;339:875-882.
26. Richelson E, Nelson A. Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. Eur J Pharmacol. 1984;103:197-204.
27. Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci. 2000;68:29-39.
28. Buckley NA, Sanders P. Cardiovascular adverse effects of antipsychotic drugs. Drug Saf. 2000;23:215-228.
29. Nourian Z, Mow T, Muftic D, et al. Orthostatic hypotension effect of antidepressant drugs in Wistar rats by bag in vitro and in vivo studies of alpha1-adrenoceptor function. Psychopharmacology. 2008;199:15-27.
30. Silver H, Kogan H, Zlotogorski D. Postural hypotension in chronically medicated schizophrenics. J Clin Psychiatry. 1990;51:459-462.
31. Ray WA, Griffin MR, Schaftner W, Baugh DK, Melton LJ 3rd. Psychotrop drug use and the risk of hip fracture. N Engl J Med. 1987;316:363-369.
32. Thapa PB, Gideon P, Fought RL, Ray WA Psychotropic drugs and risk of recurrent falls in ambulatory nursing home residents. Am J Epidemiol. 1995;142:202-211.
33. Pupo AS, Uberti MA, Minneman KP. N-terminal truncation of human alpha1D-adrenoceptors increases expression of binding sites but not protein. Eur J Pharmacol. 2003;462:1-8.
34. Hague C, Chen Z, Pupo AS, Schulte NA, Toews ML, Minneman KP. The N terminus of the human alpha1D-adrenergic receptor prevents cell surface expression. J Pharmacol Exp Ther. 2004;309:388-397.
35. Kountz TS, Lee KS, Aggarwal-Howarth S, et al. Endogenous N-terminal domain cleavage modulates α1D-adrenergic receptor pharmacodynamics. J Biol Chem. 2016;291:18210-18221.
36. Sleight AJ, Koek W, Bigg DC. Binding of antipsychotic drugs at alpha1A- and alpha1B-adrenoceptors: risperidone is selective for the alpha1B-adrenoceptors. Eur J Pharmacol. 1993;238:407-410.
37. Eltz M. In functional experiments, risperidone is selective, not for the B, but for the A subtype of alpha1-adrenoceptors. Eur J Pharmacol. 1996;295:69-73.
38. Schmidt AW, Lebel LA, Howard HR Jr, Zorn SH. Ziprasidone: a novel antipsychotic agent with a unique human receptor binding profile. Eur J Pharmacol. 2001;425:197-201.
39. Beasley CM Jr, Hamilton SH, Crawford AM, et al. Olanzapine versus haloperidol: acute phase results of the international double-blind olanzapine trial. Eur Neuropsychopharmacol. 1997;7:125-137.
40. Fumagalli L, Pallavicini M, Budriesi R, et al. Affinity and activity profiling of unichiral 8-substituted 1,4-benzodioxane analogues of WB4101 reveals a potent and selective α1B-adrenoceptor antagonist. Eur J Med Chem. 2012;58:184-191.
41. Kenny BA, Chalmers DH, Philpott PC, Naylor AM. Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br J Pharmacol. 1995;115:981-986.
42. Schwind DA, Johnston GI, Page SO, et al. Cloning and pharmacological characterization of human alpha1-adrenergic receptors: sequence corrections and direct comparison with other species homologues. J Pharmacol Exp Ther. 1995;272:134-142.
43. Franh H, Cockcroft V, Karskela T, Scheinin M, Marjamäki A. Phenoxybenzamine binding reveals the helical orientation of the third transmembrane domain of adrenergic receptors. J Biol Chem. 2001;276:31279-31284.
44. Graham JD. The ethyleneiminium ion as the active species in 2-haloalkylamine compounds. Br J Pharmacol. 1957;12:489-497.
45. Buitenwerf E, Oisinga TE, Timmers HJLM, et al. Efficacy of α-blockers on hemodynamic control during pheochromocytoma resection - a randomized controlled trial. J Clin Endocrinol Metab. 2019;105:dgz188. https://doi.org/10.1210/clinem/dgz188
46. Liu C, Lv Q, Chen X, et al. Preoperative selective vs non-selective α-blockade in PPGL patients undergoing adrenalectomy. Endocr Connect. 2017;6:830-838.
47. Malek K, Miškiewicz P, Witkowska A, et al. Comparison of phenoxybenzamine and doxazosin in perioperative management of patients with pheochromocytoma. Kardiol Pol. 2017;75:1192-1198.
48. Graham JD, Lewis GP. The role of the cyclic ethyleneiminium ion in the pharmacological activity of the 2-haloethylenamines. Br J Pharmacol. 1954;9:68-75.
49. McKinley MD, McKinley FR, McGown LE. Thiosulfate as an antidote for mustard poisoning: a review of the literature. 1982 Institute report 127, Division of Research Support, Letterman Army Institute of Research, Presidio of San Francisco, Ca, 94129.
50. Ford AP, Arredondo NF, Blue DR Jr, et al. RS-17053 (N-[2-cyclopropylmethoxyphenoxylethyl]-5-chloro-alpha, alpha-di-methyl-1H-indole-3-ethanamine hydrochloride), a selective alpha1A-adrenoceptor antagonist, displays low affinity for functional alpha 1-adrenoceptors in human prostate: implications for adrenoceptor classification. Mol Pharmacol. 1996;49:209-215.
51. Silva RO, de Oliveira AS, Nunes Lemes LF, et al. Synthesis and structure-activity relationships of novel ary1piperezines as potent antagonists of α1-adrenoceptor. Eur J Med Chem. 2016;122:601-610.
52. Baker JG. The selectivity of β-adrenoceptor antagonists at the β1, β2 and β3 adrenoceptors. Br J Pharmacol. 2005;144:317-322.
53. Baker JG. The selectivity of β-adrenoceptor agonists at the human β1, β2 and β3 adrenoceptors. Br J Pharmacol. 2010;160:148-161.
54. Williams TJ, Blue DR, Daniels DV, et al. In vitro alpha1-adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel alpha1A-adrenoceptor selective antagonists. Br J Pharmacol. 1999;127:252-258.
55. Goetz AS, King HK, Ward SD, True TA, Rimele TJ, Saussy DL Jr. BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. Eur J Pharmacol. 1995;272:R5-R6.
56. Goodnick PJ,erry JM, Aripiprazole: profile on efficacy and safety. Expert Opin Pharmacother. 2002;3:1773-1781.
57. Keck PE Jr, McElroy SL. Aripiprazole: a partial dopamine D2 receptor agonist antipsychotic. Expert Opin Investig Drugs. 2003;12:655-662.
58. Burry L, Hutton B, Williamson DR, et al. Pharmacological interventions for the treatment of delirium in critically ill adults. Cochrane Database Syst Rev. 2019;9:CD011749.
59. Nikooie R, Neufeld KJ, Oh ES, et al. Antipsychotics for treating delirium in hospitalized adults: a systematic review. Ann Intern Med. 2019;171:485. https://doi.org/10.7326/M19-1860
60. Gaertner J, Eychmueller S, Leyhe T, Bueche D, Savaskan E, Schlögl M. Benzodiazepines and/or neuroleptics for the treatment of delirium in palliative care?—a critical appraisal of recent randomized controlled trials. Ann Palliat Med. 2019;8:504-515.
61. Skelton L, Guo P. Evaluating the effects of the pharmacological and nonpharmacological interventions to manage delirium symptoms in palliative care patients: systematic review. Curr Opin Support Palliat Care. 2019;13:384-391.
62. Girard TD, Exline MC, Carson SS, et al. Haloperidol and ziprasidone for treatment of delirium in critical illness. N Engl J Med. 2018;379:2506-2516.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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