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European Journal of Pharmacology 449 (2002) 105–111

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DR4004, a putative 5-HT$_7$ receptor antagonist, also has functional activity at the dopamine D2 receptor

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Received 28 March 2002; received in revised form 18 June 2002; accepted 25 June 2002

Abstract

The tetrahydrobenzindole, 2*a*-(4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl)-2*a*,3,4,5-tetrahydrobenzo[*cd*]indol-2(1*H*)-one (DR4004) has been described as a highly selective antagonist for the 5-hydroxytryptamine$_7$ (5-HT$_7$) receptor [J. Med. Chem. 42 (1999) 533]. Consistent with original data, DR4004 bound to rat hypothalamic membranes with an affinity of 7.3 ± 0.2 (pK_i ± S.E.M.) for the 5-HT$_7$ receptor. However, competition binding studies showed that DR4004 had poor receptor selectivity with the following affinity profile; dopamine D2 receptor, α_1-adrenoceptor ≥ 5-HT$_7$ receptor > histamine H$_1$ receptor, α_2-adrenoceptor>dopamine D1 receptor>β-adrenoceptor, muscarinic and 5-HT$_{2A/C}$ receptors. In conscious rats DR4004 (1, 5 or 10 mg/kg i.p.) produced a dose-dependent hyperglycaemia and hypothermia, but the former was reduced by the dopamine D2 receptor antagonist raclopride. Another 5-HT$_7$ receptor antagonist, (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970) produced hypothermia but no hyperglycaemia. This study confirms that DR4004 has high affinity for the 5-HT$_7$ receptor but suggests that dopamine D2 receptor activity contributes to some of the in vivo effects.

Keywords: 5-HT$_7$ receptor; 5-HT (5-hydroxytryptamine, serotonin); Body temperature; Plasma glucose; Dopamine D2 receptor; 5-HT$_7$ receptor antagonist

1. Introduction

The most recent 5-HT receptor to be identified by molecular cloning was the seven-transmembrane spanning G-protein-coupled 5-hydroxytryptamine$_7$ (5-HT$_7$) receptor (Bard et al., 1993; Lovenberg et al., 1993; Plassat et al., 1993; Ruat et al., 1993; Shen et al., 1993). Four splice variants of the 5-HT$_7$ receptor (a to d) have been identified which show species- and tissue-specific expression (Jasper et al., 1997; Heidmann et al., 1998) but little variation in agonist or antagonist affinity. Autoradiography, in situ hybridisation, radioligand binding and immunohistochemistry consistently show that the 5-HT$_7$ mRNA and receptor protein have a similar abundant distribution in the cerebral cortex, hippocampus, thalamus, amygdala and hypothalamus (Ruat et al., 1993; Heidmann et al., 1998; Hagan et al., 2000; Neumaier et al., 2001). Although a prolonged hypotensive phase of the response to intravenous 5-HT has been shown to be elicited by 5-HT$_7$ receptor-mediated vasodilatation, in particular, in skeletal muscle vasculature (De Vries et al., 1999; Vanhoenacker et al., 2000), the identification of any role for this receptor in the central nervous system (CNS) has been much more elusive. Increasing evidence suggests that 5-HT$_7$ receptor activation may regulate circadian rhythms (Lovenberg et al., 1993; Kawahara et al., 1994; Duncan et al., 1999) but it is unclear whether this receptor has any "physiological" role in this process (Gannon, 2001). There is also more speculative evidence for 5-HT$_7$ receptor involvement in acute stress (Yau et al., 2001), sensory processing (Vanhoenacker et al., 2000), schizophrenia (Roth et al., 1994), depression (Sleight et al., 1995; Mullins et al., 1999) and sleep disorders (Hagan et al., 2000).

Until recently, no selective 5-HT$_7$ receptor antagonist has been available, and characterisation of 5-HT$_7$ receptor function has relied on the use of non-selective ligands to exclude involvement of other 5-HT receptors (Eglen et al., 1997) or antisense oligonucleotide treatment (Clemett et al.,

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PII: S0014-2999(02)02003-4

1998). However, Kikuchi et al. (1999) recently described a tetrahydrobenzindole, 2*a*-(4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl)-2*a*,3,4,5-tetrahydrobenzo[*cd*]indol-2(1*H*)-one (DR4004), that displaced [^{3}H]5-carboxamidotryptamine from the cloned human 5-HT$_7$ receptor expressed in an African Green Monkey cell line (COS-7 cells) with an affinity of 8.7 ± 0.1 (pK_i ± S.E.M.) and had 47-fold selectivity over the three 5-HT$_2$ receptors. Although DR4004 was also reported to displace [^{3}H]spiperone from rat striatual dopamine D2 receptors with an affinity of 7.0 ± 0.1 (pK_i ± S.E.M., Kikuchi et al., 1999), the overall assessment of receptor selectivity was partial and no assessment of in vivo activity of DR4004 was given in this initial report.

As no selective 5-HT$_7$ receptor agonists are available, one approach has been to pharmacologically characterise the response produced by the non-selective 5-HT agonist 5-carboxamidotryptamine, which has high affinity for the 5-HT$_7$ receptor, in combination with a variety of selective 5-HT antagonists. 5-Carboxamidotryptamine induces hyperglycaemia, that can be prevented by pretreatment with metergoline or methysergide, two compounds with high 5-HT$_7$ receptor affinity (Yamada et al., 1998) and hypothermia in the guinea pig that is blocked by the selective 5-HT$_7$ receptor antagonist (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol, SB-269970 (Hagan et al., 2000).

The current study, therefore, performed more extensive competition binding studies with DR4004 and examined the effect on plasma glucose and body temperature following systemic administration to rats. To further characterise the functional response to DR4004, the change in body temperature and plasma glucose was compared with that of another reportedly selective 5-HT$_7$ receptor antagonist (SB-269970, Lovell et al., 2000) and following pretreatment with the dopamine D2 receptor antagonist raclopride and the α_1-adrenoceptor antagonist prazosin. The data suggest that DR4004-induced hypothermia may result from 5-HT$_7$ receptor antagonism but that the hyperglycaemia may, in part, be due to dopamine D2 receptor activity.

2. Materials and methods

2.1. Receptor binding assays

The affinity of DR4004 for a range of 5-HT receptors has previously been reported but that at many other monoamine receptors has not been examined. Competition curves were performed in triplicate to determine the affinity of DR4004 to the 5-HT$_7$ and eight other monoamine receptors according to the methods cited in Table 1, with the minor modifications indicated.

2.2. [^{3}H]5-HT binding to rat hypothalamic 5-HT$_7$ receptors

5-HT$_7$ receptor binding was determined using [^{3}H]5-HT binding to rat hypothalamic membranes in the presence of pindolol, as characterised previously (Sleight et al., 1995; Clemett et al., 1998, 1999) as more selective 5-HT$_7$ receptor ligands were not available to us. In brief, frozen brains obtained from adult male Lister hooded rats (250–300 g, Charles River) were allowed to defrost and the hypothalami were rapidly dissected out. These were immediately homogenised in 20 volumes of 50 mM Tris (pH 7.4 at 23 °C) and the homogenate centrifuged (Sigma, 3K20) at 36,000 × *g* for 15 min at 4 °C. The supernatant was removed and the resulting pellet resuspended in Tris before being centrifuged and resuspended twice more, with an intervening 15-min incubation at 37 °C to remove endogenous 5-HT. The final pellet was frozen at − 80 °C until required.

Prepared hypothalamic membranes were resuspended in assay buffer (50 mM) Tris containing 10 μM pargyline, 4 mM $CaCl_2$ and 0.58 mM ascorbic acid, pH 7.4 at 23 °C) to a concentration of approximately 500 μg protein in 50 μl. Aliquots of membranes were incubated in the presence of 2 μM pindolol with 3 nM [^{3}H]5-HT and at least seven concentrations of the displacing drug (DR4004 dissolved in 30% β-cyclodextrin, or two other 5-HT$_7$ receptor antagonists, ((*R*)-3,*N*-dimethyl-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzene-sulphonamide (SB-258719, Thomas et al., 1998) and SB-269970). After

Table 1
Comparison of the apparent pK_i (mean ± S.E.M.) obtained from at least three separate experiments performed in duplicate for DR4004 competition binding at the named receptors using the radioligand and rat brain tissue indicated

Receptor	[^{3}H] radioligand (nM)	Rat tissue source	Method reference	Non-specific binding (μM)	pK_i
5-HT$_7$	[^{3}H] 5-HT (2.0)	hypothalamus	(Clemett et al., 1999)	methiothepin (10.0)	7.3 ± 0.2
α_1-Adrenoceptor	[^{3}H] prazosin (0.2)	whole brain	(Greengrass and Bremner, 1979)	WB4101 (0.1)	7.4 ± 0.1
α_2-Adrenoceptor	[^{3}H] rauwolscin (0.4)	whole brain	(Boyajian and Leslie, 1987)	yohimbine (1.0)	6.3 ± 0.0
β-adrenoceptor	[^{125}I] ICP (0.05)	whole brain	(Byland and Snyder, 1976)	propranolol (1.0)	< 5
Dopamine D1	[^{3}H] SCH23390 (0.2)	corpus striatum	(Billard et al., 1984)	SCH23390 (0.03)	< 6
Dopamine D2	[^{3}H] raclopride (0.8)	corpus striatum	(Hall et al., 1988)	spiperone (0.03)	7.4 ± 0.2
Histamine H$_1$	[^{3}H] pyrilamine (2.0)	whole brain	(Tran et al., 1978)	Promethazine (1.0)	6.9 ± 0.4
Muscarinic	[^{3}H] QNB (1.0)	cortex	(Yamamura and Snyder, 1974)	atropine (1.0)	< 5
5-HT$_2$	[^{3}H] ketanserin (0.4)	cortex	(Battaglia et al., 1983)	Methysergide (10.0)	< 5

ICP = iodocyanopindolol, SCH23390 = *R*(+)-2,3,4,5-tetrahydro-8-iodo-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hydrochloride and QNB = quinuclidinyl benzilate.

incubation at 23 °C for 2 h, the reaction was terminated by rapid filtration through Whatman GF/B filters followed by three 4-ml washes with ice-cold Tris (50 mM, pH 7.4 at 4 °C). Radioactivity bound to the filters was measured by liquid scintillation counting (1214 RackBeta, LKB Wallac) in 4 ml scintillation fluid (Emulsifier Scintillator Plus, Packard).

2.3. Monoamine receptor binding assays

For each radioligand binding assay, frozen rat brain tissue (whole brain for histamine, α_1-, α_2- and β-adrenoceptors, cortex for muscarinic and 5-$HT_{2A/C}$ receptors, or striatum for the dopamine receptors, Table 1) was homogenised using a Brinkman Polytron PT-10 (setting 6, for 15 s, × 2). For the 5-$HT_{2A/C}$ receptor, histamine receptor and α_1-, α_2- and β-adrenoceptor assays, the tissue was homogenised in 25 volumes 0.25 M sucrose and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 40,000 × *g* for an additional 10 min, and the resultant pellet was resuspended in buffer and centrifuged for an additional 10 min at 40,000 × *g*. For muscarinic and dopamine receptor assays, the tissue was homogenized in 25 volumes buffer and centrifuged at 40,000 × *g* for 10 min. The resultant pellet was resuspended in buffer and centrifuged for an additional 10 min at 40,000 × *g*. For all assays, the final pellets were resuspended in the appropriate volume of assay buffers (Table 1).

The unlabelled ligand used to identify non-specific binding, the radiolabelled ligand and the concentration of these used for each receptor assay are listed in Table 1. Increasing concentrations of test agent were incubated with the appropriate concentration of radiolabel and membrane aliquots in a final volume of 1 ml. Spiperone, promethazine, methysergide and yohimbine were dissolved in dimethylsulfoxide (DMSO), with a final concentration of ≤ 1% and DMSO controls were run in conjunction with each assay. All other compounds were dissolved in purified water. Assays were terminated by filtration over Whatman GF/B glass-fiber filters on a Brandel cell harvester, followed by an ice-cold 10 ml saline wash. Filters were presoaked in 0.1% (w/v) polyethylenimine to reduce non-specific binding. Radioactivity bound was determined after a period of equilibration (at least 5 h) in Fisher ScintiSafe Econo 1 Scintillation Cocktail using a Beckman LS5000 TA counter with an efficiency of approximately 43%.

2.4. Rat behavioural and glucose studies

Two groups of adult male Lister hooded rats were given either DR4004 (1, 5 or 10 mg/kg i.p., $n = 18$) dissolved in 30% (v/v) β-cyclodextrin (vehicle) or SB-269970 (5, 10, 20 mg/kg i.p., $n = 18$) dissolved in saline (1 ml/kg i.p.), or the appropriate vehicle ($n = 6$ each). Twenty minutes post-injection, rats were stunned and decapitated to collect mixed arterio-venous trunk blood in heparinised vials that were centrifuged (5 min, 1500 × *g*, 4 °C, Harrier, 18/80) and plasma decanted to measure glucose levels in a β-glucose analyser (Hemocue). In addition, the core body temperature was measured using a rectal probe (inserted 8 cm, Portex, P9005) within 1 min of decapitation to prevent any stress-induced alteration in plasma glucose and corticosterone that would have occurred if these had been measured in the conscious rat.

A third group of 24 male Lister hooded rats were pretreated with either prazosin (0.3 mg/kg i.p.) or saline (0.154 M, 1 ml/kg i.p.) 20 min prior to receiving either DR4004 (10 mg/kg i.p.) or 30% (v/v) β-cyclodextrin. Twenty minutes after the second treatment, core body temperature and plasma glucose levels were measured as described above.

In a final group ($n = 24$), rats were pretreated with raclopride (0.1 mg/kg i.p.) or saline (0.154 M, 1 ml/kg i.p.) and body temperature and plasma glucose measured. Animal experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 by an observer who was blind to the treatment used.

2.5. Materials

DR4004 and SB-258719 were kindly synthesised by Eli Lilly and SB-269970 was a gift from SmithKline Beecham Pharmaceuticals. Radioligands, reagents and compounds used in these studies were obtained from commercial suppliers, New England Nuclear (Boston MA), Sigma (St. Louis, MO.) and Research Biochemicals (Natick, MA), respectively. All other laboratory reagents were obtained from Sigma.

2.6. Data analysis

Affinity constants (K_i) were determined using non-linear least-squares regression software, Graphad Inplot, San Diego, CA (Munson and Rodbard, 1980). Half-maximal inhibitor concentrations (IC_{50}) were calculated according to the following equation describing dose–response curves: $y = A + (B - A/1 + (10^x/10^c))$, where A is the bottom of the curve, B is the top of the curve, and c is the x value at the middle of the curve (IC_{50}). The affinity constant (K_i) was then calculated from the Cheng–Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$ assuming simple competitive interaction between radioligand and displacer (Cheng and Prussoff, 1973), where [L] is the concentration of the free radioligand used and K_d is the equilibrium dissociation constant of the radioligand for the receptor. Affinities (K_i) were determined from 11-point concentration curves and are the mean ± S.E.M. of at least three experiments.

Core body temperature and plasma glucose data were analysed by one-way analysis of variance (ANOVA) followed by Scheffé's *S*-test, $P < 0.05$ being considered significant.

3. Results

3.1. Radioligand binding studies

In membranes prepared from the rat hypothalamus, DR4004 competed with pindolol-insensitive [^{3}H]5-HT binding with an estimated affinity of 7.3 ± 0.2 (pK_i ± S.E.M.) and a Hill coefficient of 0.8 ± 0.3 (mean ± S.E.M.) S.E.M.) consistent with the previous report of binding to the 5-HT$_7$ receptor (Kikuchi et al., 1999). Results from the receptor binding assays revealed that DR4004 had similar affinity for the α_1-adrenoceptor (7.4 ± 0.1, pK_i ± S.E.M.) determined by [^{3}H]prazosin and the dopamine D2 receptor (7.4 ± 0.2, pK_i ± S.E.M.) measured by [^{3}H]raclopride in membranes prepared from rat whole brain and striatum, respectively (Table 1). Relatively high affinity was also observed for the histamine H$_1$ receptor (6.9 ± 0.4, pK_i ± S.E.M.), α_2 adrenoceptor (6.3 ± 0.0, pK_i ± S.E.M.) but not at the dopamine D1 receptor (pK_i < 6). In competitive binding assays, DR4004 showed little affinity for β-adrenoceptors, muscarinic receptors or the 5-HT$_2$ receptors. The overall affinity profile of DR4004 was thus: dopamine D2 receptor = α_1-adrenoceptor ≥ 5-HT$_7$ receptor>histamine H$_1$ receptor>α_2-adrenoceptor>dopamine D1 receptor, β-adrenoceptor, muscarinic receptor and 5-HT$_{2A/C}$ receptor.

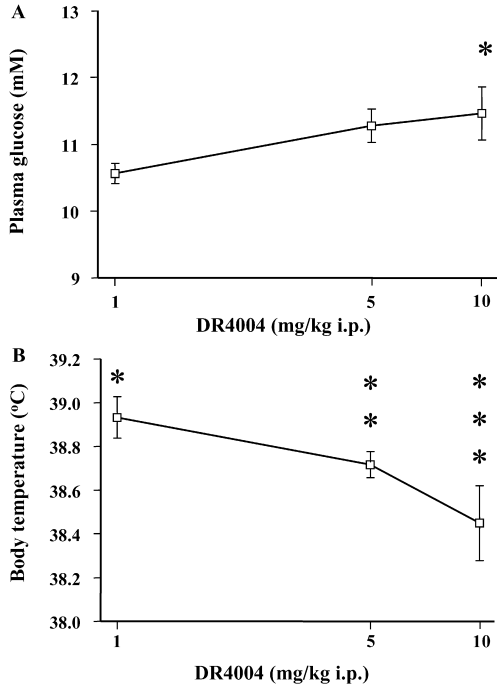

Fig. 1. Log$_{10}$ dose–response curves showing the effect of DR4004 (1, 5 and 10 mg/kg i.p.) on: (A) plasma glucose (mM, saline = 10.3 ± 0.3 mM) and (B) core body temperature (°C, saline = 39.3 ± 0.1 °C) in the rat (n = 6 at each dose, mean ± S.E.M.). * P < 0.05, ** P < 0.01 and *** P < 0.001 from vehicle, Scheffe's S-test following ANOVA.

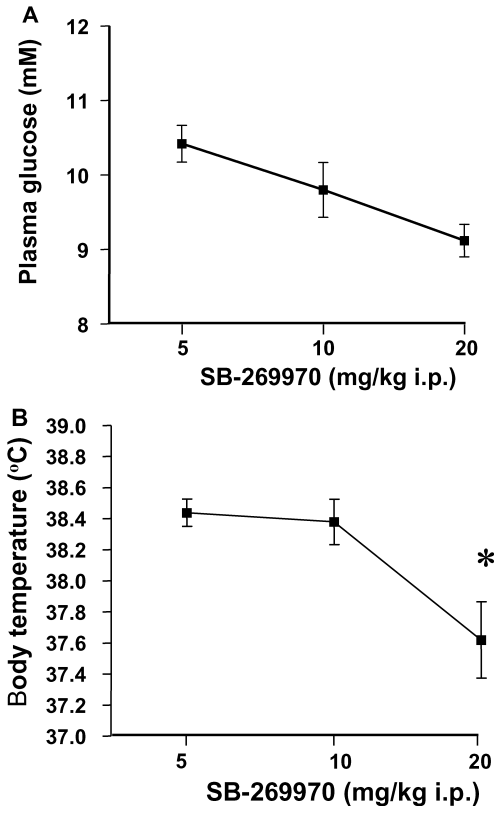

Fig. 2. Log$_{10}$ dose–response curves showing the effect of SB-269970 (5, 10 and 20 mg/kg i.p.) on: (A) plasma glucose (mM, saline = 10.2 ± 0.3 mM) and (B) core body temperature (°C, saline = 38.8 ± 0.7 °C) in the rat (n = 6 at each dose, mean ± S.E.M.). * P < 0.05 from vehicle, Scheffe's S-test following ANOVA.

3.2. Plasma glucose and core temperature

Given the high expression of the 5-HT$_7$ receptor mRNA and binding sites in hypothalamic, limbic and cortical areas (To et al., 1995; Heidmann et al., 1998; Hagan et al., 2000; Neumaier et al., 2001); this study focussed on the effect of DR4004 on homeostatic responses regulated by the hypothalamus. DR4004 (1, 5, 10 mg/kg i.p.) produced a small dose-related increase in plasma glucose that reached significance (P < 0.05) with the highest dose (Fig. 1A). DR4004 also produced a dose-dependent hypothermia (Fig. 1B) that was significant compared with vehicle at all three doses used. In addition, the selective 5-HT$_7$ receptor antagonist, SB-269970, produced a dose-dependent hypothermia that reached significance with the highest dose (Fig. 2B), consistent with the involvement of the 5-HT$_7$ receptor in this response. In contrast to DR4004, SB-269970 did not produce hyperglycaemia (Fig. 2A), but tended to decrease plasma glucose in the dose range used, although this was not significant even at the highest dose tested (20 mg/kg i.p.).

As the 5-HT$_7$ receptor antagonist, DR4004, also had a comparable affinity for both the dopamine D2 receptor

and α_1-adrenoceptor, the effect of pretreatment with the dopamine D2 receptor antagonist raclopride (Hall et al., 1988) and the α_1-adrenoceptor antagonist prazosin (Cavero and Roach, 1980) on the hyperglycaemia and the hypothermia induced by DR4004 was examined. DR4004 (10 mg/kg i.p.) produced a modest but significant increase in plasma glucose when given alone (Fig. 3A) while raclopride (0.1 mg/kg i.p.) had no effect when it was given alone. However, raclopride pretreatment prevented the hyperglycaemia elicited by DR4004 (Fig. 3A). DR4004 (10 mg/kg i.p.) also produced a small but significant reduction in body temperature when given alone (Fig. 3B), while raclopride, at the dose used, had no effect on body temperature. Although the apparent hypothermia elicited by DR4004 in the presence of raclopride just failed to reach significance, it was not different from the body temperature recorded with DR4004 alone (Fig. 3B).

When given alone, prazosin (0.3 mg/kg i.p) had no effect on plasma glucose levels (Fig. 4A). Furthermore, the hyperglycaemia produced by DR4004 (10 mg/kg i.p.) was unaltered by prazosin pretreatment (Fig. 4A). Prazosin pretreatment produced a modest (0.7 °C) but significant ($P<0.05$) reduction in body temperature when given alone but it also had an additive effect on the hypothermia produced by DR4004 (Fig. 4B).

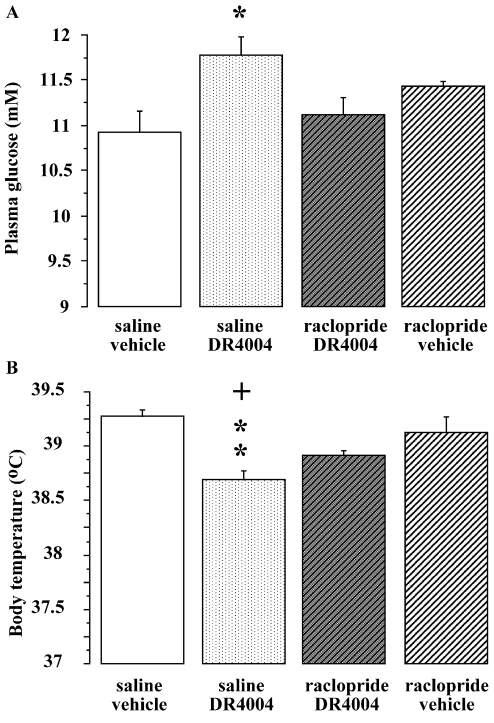

Fig. 3. Effect of DR4004 (10 mg/kg i.p.) and raclopride (0.1 mg/kg i.p.), either alone or in combination, on: (A) plasma glucose (mM) and (B) core body temperature (°C) in the rat ($n=6$ at each dose, mean ± S.E.M.). * $P<0.05$ and * * $P<0.01$ from vehicle and $^{+}P<0.05$ from raclopride/vehicle group, Scheffe's *S*-test following ANOVA.

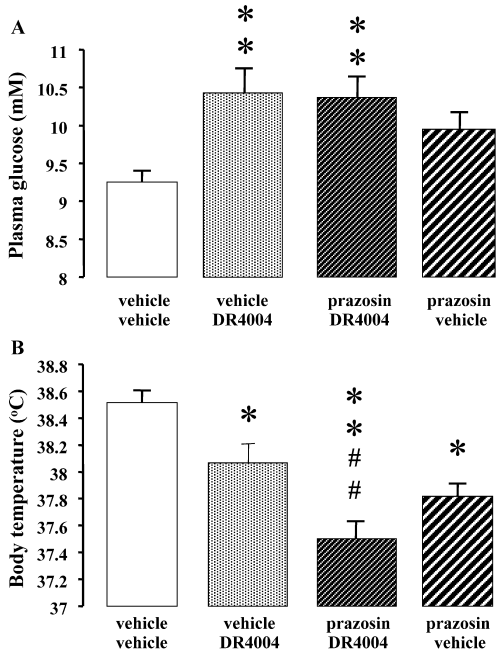

Fig. 4. Effect of DR4004 (10 mg/kg i.p.) and prazosin (0.3 mg/kg i.p.), either alone or in combination, on: (A) plasma glucose (mM) and (B) core body temperature (°C) in the rat ($n=6$ at each dose, mean ± S.E.M.). * $P<0.05$ and * * $P<0.01$ from vehicle and $^{\#\#}P<0.01$ from DR4004 alone Scheffe's *S*-test following ANOVA.

4. Discussion

The current competitive ligand binding data using pindolol-insensitive [^{3}H]5-HT binding in rat hypothalamic membranes confirmed the high affinity of DR4004 for the 5-HT$_7$ receptor (Kikuchi et al., 1999). The pK_i determined in the present binding assay is slightly less than that reported by Kikuchi et al. (1999) using a recombinant human 5-HT$_7$ receptor in a high expression cell line. In a previous comparison of binding affinities determined by pindolol-insensitive [^{3}H]5-HT binding in hypothalamic membranes with [^{3}H]5-HT binding in a recombinant rat receptor source, we found a similar, approximately 10-fold, reduction in affinity in the former assay for all nine structurally unrelated compounds tested (Clemett et al., 1999). This apparent discrepancy may be due to using an agonist radioligand or differences in 5-HT$_7$ G-protein coupling in the two preparations, as previously suggested by To et al. (1995). Furthermore, a similar 0.5 log unit reduction in the pK_i values for 5-HT$_7$ receptor antagonists has also been reported for [^{3}H]5-carboxamidotryptamine binding in membranes from guinea pig cortex compared with those obtained in a human 5-HT$_7$ receptor cell line (Hagan et al., 2000). As the pK_i values of DR4004, SB-258719 and SB-269970 have not been reported in a rat recombinant cell line, an extension of

the correlation to include comparison with the hypothalamic [^{3}H]5-HT binding values of these additional three ligands cannot be performed. Nonetheless, the values obtained for DR4004, SB-258719 and SB-269970 (pK_i 7.3 ± 0.2, 7.1 ± 0.3, 7.5 ± 0.2) in the current rat hypothalamic assay were in the same rank order as the respective pK_i values (8.7 ± 0.1, 7.5 ± 0.1, 8.9 ± 0.1) for [^{3}H]5-carboxamidotryptamine binding to the h5-HT$_{7(a)}$ receptor in cell lines (Kikuchi et al., 1999; Hagan et al., 2000). The Hill slopes for inhibition of hypothalamic [^{3}H]5-HT binding by DR4004, SB-258719 or SB-269970 were also not significantly different from unity, consistent with the previous proposed utility of this assay to measure 5-HT$_7$ receptor binding (Clemett et al., 1999). A more comprehensive analysis reported herein also shows that DR4004 has a comparable affinity for the α_1-adrenoceptor, dopamine D2 receptor and the 5-HT$_7$ receptor in rat CNS membrane preparations. The affinity reported herein for the dopamine D2 receptor is also comparable with that reported by Kikuchi et al. (1999).

Recently, Hagan et al. (2000) reported that 5-carboxamidotryptamine-induced hypothermia in the conscious guinea pig was prevented by pretreatment with the selective 5-HT$_7$ receptor antagonist SB-269970. This contrasts with the data from the current study, showing that both DR4004 and SB-269970 induce a modest but significant reduction in body temperature in the rat. A possible explanation for this apparent discrepancy is that there is a species difference in the role of various 5-HT receptors in thermoregulation in the guinea pig and the rat. For example, Siniscalchi et al. (1990) demonstrated that (+)-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), an agonist with affinity for both 5-HT$_{1A}$ and 5-HT$_7$ receptors, elevated body temperature in the guinea pig but reduced body temperature in the rat. Alternatively, as 5-carboxamidotryptamine has poor CNS penetration, it is possible that the hypothermia observed by systemic administration in the guinea pig by Hagan et al. (2000) was due to an action on peripheral rather than central 5-HT$_7$ receptors. Consistent with the current finding that prazosin induced hypothermia, the α_1-adrenoceptor antagonist terazosin has also been reported to produce hypothermia in the rat (Stone et al., 1999). The apparently additive effect of prazosin on DR4004-induced hypothermia observed herein could indicate a contribution of α_1-adrenoceptor antagonist action to this effect of DR4004. Alternatively, DR4004-induced hypothermia may involve a mechanism independent of the α_1-adrenoceptor, which could best be established by a full dose–response analysis of the interaction. As the hypothermia produced by systemic administration of either DR4004 or SB-269970 was modest, it is unlikely that the 5-HT$_7$ receptor has any physiological role in the maintenance of normal body temperature in the rat.

In contrast, although DR4004 produced a dose-related hyperglycaemia, this was not seen with another 5-HT$_7$ receptor antagonist, SB-269970. The hyperglycaemic effect of DR4004 is also unlikely to be mediated by 5-HT$_7$ receptor blockade since the hyperglycaemia elicited by the 5-HT agonist, 5-carboxamidotryptamine, involves facilitation of adrenaline release and is thought to be due to activation of 5-HT$_7$ receptors (Yamada et al., 1998). As raclopride appeared to prevent the hyperglycaemia produced by DR4004, it is possible that dopamine D2 receptor activity could contribute to the rise in plasma glucose produced by DR4004. Interestingly a range of antipsychotics have been shown to inhibit glucose transport in pheochromocytoma cells through dopamine D2 receptor antagonism (Dwyer et al., 1999), but the effect of dopamine D2 receptor agonists has not been examined to determine any relevance to the current hyperglycaemic effect of DR4004.

This study confirms that DR4004 has high affinity for the 5-HT$_7$ receptor in the rat. The modest hypothermia induced by both DR4004 and SB-269970 suggests that this effect may be due to 5-HT$_7$ receptor blockade in the rat. However, the high affinity of DR4004 for both the dopamine D2 receptor and the α_1-adrenoceptor limits the use of this compound to examine the in vivo role of the 5-HT$_7$ receptor.

Acknowledgements

We would like to thank Eli Lily for supplying DR4004 and financial support, SmithKline Beecham Pharmaceuticals for the gift of SB-269970, I. Topham for technical assistance and the European Social Fund for studentship support (H.A.K.). All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

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