ELSEVIER

European Journal of Pharmacology 364 (1999) 123–132

ejp

Activity and onset of action of reboxetine and effect of combination with sertraline in an animal model of depression

Andrew Harkin, John P. Kelly, Mairead McNamara, Thomas J. Connor, Keith Dredge, Anna Redmond, Brian E. Leonard *

Department of Pharmacology, National University of Ireland, Galway, Ireland

Received 24 August 1998; revised 3 November 1998; accepted 10 November 1998

Abstract

The limitations of antidepressant drugs to treat depression has warranted ongoing research to identify pharmacological agents and strategies which offer a faster onset of action and greater therapeutic efficacy. Noradrenaline and serotonin are widely reported to be involved in the mechanism of action of antidepressants and the recent development of selective reuptake inhibitors of these transmitters has provided the opportunity to determine the effects of targeting these transmitter systems, alone and in combination, in an antidepressant response. The present study investigated the effects of reboxetine, a new antidepressant that selectively inhibits noradrenaline reuptake, sertraline, a selective serotonin reuptake inhibitor and a combination treatment composed of the two drugs in the olfactory bulbectomized (OB) rat model of depression. Sub-acute (2 days) administration of reboxetine (2.5, 5, and 10 mg/kg, i.p.) to sham-operated and OB rats reduced the immobility time in the forced swim test. Repeated (14 days) reboxetine (10 mg/kg) treatment attenuated the OB-related behavioural hyperactivity in the 'open-field' test. Examination of the onset of the antidepressant effect in the 'open-field' test demonstrated that reboxetine (10 mg/kg), sertraline (5 mg/kg) and the combination reduced the behavioural hyperactivity after 14 days but not before this following 3, 7 or 10 days of treatment. Reduced 5-hydroxyindoleacetic acid (5-HIAA) concentrations in amygdaloid cortex of both sham and OB rats following sertraline and combination treatments are likely to be related to acute pharmacological effects on the reuptake of 5-hydroxytryptamine (5-HT). Attenuation of the hypothermia induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 0.05 mg/kg s.c.) and clonidine (0.1 mg/kg s.c.) occurred in the reboxetine and sertraline combination treated groups following both 7 and 14 days administration indicating changes to 5-HT_{1A} receptor and α_2-adrenoceptor sensitivity. The results indicate that changes to 8-OH-DPAT and clonidine-induced responses occur quicker with the combination treatment than with either reboxetine or sertraline treatments alone.

Keywords: Olfactory bulbectomy; Reboxetine; Sertraline; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); Clonidine; Depression

1. Introduction

Conventional antidepressant treatment has many limitations to the effective treatment of depression. While antidepressants are slow to take effect, some of which have a side effect profile that limits compliance, there are also a large number of treatment-resistant patients. Such a profile has warranted ongoing research to identify new agents and therapeutic strategies which may offer a faster onset of action and greater efficacy in a larger proportion of patients. One such approach has been to combine tricyclic antidepressants with selective serotonin reuptake inhibitors or to use dual acting antidepressants such as venlafaxine or mirtazepine, as it has been suggested that maximal antidepressant efficacy require a dual action on both serotonergic and noradrenergic systems (see Nelson, 1997; Pinder, 1997). In support of this, it has been reported that the selective serotonin reuptake inhibitors are not as effective as such dual action antidepressants as clomipramine particularly in the treatment of severe depression (Danish University Antidepressant Group, 1986, 1990; Nelson et al., 1991). Moreover, there is evidence that the dual 5-HT and noradrenaline reuptake inhibitor, venlafaxine, exerts a rapid onset of action and greater efficacy in major depression (Guelfi et al., 1995; Schweizer et al., 1991). In light of these reports, it was of interest to study the effects of selective uptake inhibitors of noradrenaline and 5-HT alone

* Corresponding author. Tel.: +353-91-524411 ext. 2624; Fax: +353-91-525300

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00838-3

and in combination in a relevant animal model of depression.

It is widely reported that noradrenaline is involved in the pathophysiology of depression and in the mechanism of action of antidepressant drugs (reviewed by Redmond and Leonard, 1997). Increasing noradrenaline availability by means of reuptake inhibition using desipramine or by means of antagonism of presynaptic α_2-adrenoceptors using mianserin or mirtazapine relieves depression (reviewed by Nutt and Pinder, 1996). Reboxetine, 2-[*a*(2-ethoxyphenoxy)benzyl] morpholine methane sulphonate, is a new agent shown to be active in pharmacological and biochemical models (antagonism of reserpine-induced bleopharospasm, reserpine-induced and clonidine-induced hypothermia, inhibition of noradrenaline reuptake, down-regulation of beta-adrenergic receptors) predictive of antidepressant action (Melloni et al., 1984; Riva et al., 1989). Moreover, the selectivity of reboxetine for noradrenaline reuptake inhibition makes it a useful tool for investigating the role of noradrenaline in the neurobiological mechanisms involved in antidepressant therapy (Riva et al., 1989).

Sertraline is a selective inhibitor of synaptosomal 5-HT uptake in vitro and in vivo and is only a weak inhibitor of noradrenaline and dopamine uptake (Koe et al., 1983). Furthermore, radioligand binding studies in vitro have shown sertraline to have negligible effects on a wide range of neurotransmitter receptors. Sertraline is well-tolerated in animals with no locomotor stimulant effects or the appearance of anticholinergic or cardiovascular side effects (Koe et al., 1983).

There are a number of ways to detect and characterize antidepressant activity in rodents. To date a series of biochemical and behavioural tests have provided an integrated concept of possible antidepressant effects (Leonard, 1997). While there are a number of models available, one of the most appropriate is the olfactory bulbectomized (OB) rat model of depression (reviewed by Kelly et al., 1997). The principal advantages of the OB model are the high degree of face similarity to depression and how the associated behavioural changes respond to antidepressant treatment. Moreover, the activity of antidepressant drugs can be selectively demonstrated in the model (Van Riezen and Leonard, 1991). The OB model has recently been used to test for rapid onset of antidepressant action by combining the β-adrenoceptor antagonist, pindolol, with the serotonin reuptake inhibitor, paroxetine (Cryan et al., 1998). Similar to the dual action noradrenaline and 5-HT approach, such a combination has generated much interest with claims of its ability to increase the speed of onset of the therapeutic effect (Artigas et al., 1994).

Recently, the behavioural despair or the 'forced swim test' (Porsolt et al., 1978) as a procedure for predicting the activity of acutely administered antidepressants has been successfully incorporated into a study design with olfactory bulbectomy (see Kelly and Leonard, 1994). All classes of antidepressants have shown activity in both of these models (reviewed by Borsini and Meli, 1988; Kelly et al., 1997).

In conjunction with experimental models, drug challenges may also be used to characterize antidepressant activity. Subcutaneous administration of 8-OH-DPAT to rats produces a dose-dependent hypothermic effect which is maximal at 30–45 min post-injection (Goodwin et al., 1987; Yu and Lewander, 1997). 8-OH-DPAT-induced hypothermia may be inhibited by the 5-HT_{1A} receptor antagonists (−) pindolol (O'Connell et al., 1992) and (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane carboxamide trihydrochloride) WAY-100635 (Forster et al., 1995) but not by pre-treatments with the serotonin depleting agent, *p*-chlorophenylalanine (O'Connell et al., 1992) indicating that the hypothermic response to 8-OH-DPAT is mediated by postsynaptic 5-HT_{1A} receptors. Modification of the hypothermic response induced by 8-OH-DPAT in rats has been used to assess adaptive changes in the 5-HT_{1A} receptor in response to antidepressant treatments (Goodwin et al., 1987; Wozniak et al., 1988).

In a similar fashion, the sensitivity of α_2-adrenoceptors may be pharmacologically monitored by challenging with low doses of clonidine followed by measurement of the rectal temperature response. A previous study of O'Donnell et al. (1996), demonstrated that clonidine dose-dependently reduces colonic temperature in rats (0.05–2 mg/kg s.c.). This effect may be inhibited by prior administration of the centrally acting α_2-adrenoceptor antagonists yohimbine or idazoxan but not by the peripherally-acting α_2-adrenoceptor antagonist L-659,066 (a spirocyclic-substituted benzofuroquinolizine). As central α_2-adrenoceptors and 5-HT_{1A} receptors are believed to be involved in the mechanism of action of antidepressants (reviewed by Blier and De Montigny, 1994; Potter, 1996) these challenges were introduced to complement the behavioural tests with the OB model.

The aims of the present study were to investigate activity of reboxetine in the OB rat model of depression and to compare its action to that of sertraline. In addition, the effect of combined treatment of reboxetine and sertraline was examined in the model. Immobility time in the forced swim test, locomotor activity in an 'open-field', hypothermic responses to 8-OH-DPAT and clonidine and neurochemical changes in amygdaloid cortex were used as indices of the antidepressant response.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats were obtained from Harlan Olac, UK (weight on arrival: 230–250 g). The animals were housed four per cage under standard colony conditions with a 12 h light:dark cycle (light period 0800–2000

h) and ad libitum food and water. The animals were allowed to acclimatize to the colony for at least 7 days prior to any experimentation. All procedures were carried out under the guidelines of the animal welfare committee of the National University of Ireland, Galway.

2.2. Forced swim test

This test is as described elsewhere (Porsolt et al., 1978). In brief, rats were placed individually in plexiglass cylinders (height 40 cm, diameter 18 cm), containing 20 cm of water at 25°C and 15 min later they were removed and dried before returning to their home cage. The animals were replaced in the cylinders 24 h later, and the procedure was repeated but on this occasion the duration (s) that the rats remained immobile during a 5 min observation period was recorded. Immobility was defined as the absence of active escape oriented behaviours such as swimming, diving, rearing and sniffing. The antidepressant was administered intraperitoneally 15 min after removal of the rats from the water on the first day, the second dose on the following morning, 5 h prior to the second immersion in the water, and a third dose 1 h prior to the second immersion in the water.

2.3. Olfactory bulbectomy

After a 1-week acclimatization period, bilateral olfactory bulbectomy was performed in rats anaesthetised with 2.5% w/v 2-2-2-tribromoethanol (10 ml/kg i.p.) essentially as described by Cairncross et al. (1977). The head was shaved and a midline sagittal incision was made extending at least 1 cm rostral to the bregma. Pressure was applied to ensure that the periosteum on the underlying bone had been penetrated. A burr hole was drilled at points 8 mm anterior to the bregma and 2 mm either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. For sham animals, the dura was carefully pierced and the wound closed. For OB animals, the olfactory bulbs were aspirated using a water suction pump. Care was taken not to damage the frontal cortex. After the operation, bleeding was controlled by plugging the holes with haemostatic sponge (Haemofibrine®, Specialities Septodont, France). Oxytetracycline dusting powder was sprinkled on the wound prior to closure. The animals were housed four per cage (two shams and two OBs) and were allowed to recover for 14 days following surgery. They were handled daily throughout the recovery period to eliminate any aggressiveness that would otherwise arise (Leonard and Tuite, 1981).

2.4. 'Open-field' test

The rats were tested approximately 18 h following their last drug treatment. Each rat was placed singly into the centre of the 'open-field' apparatus. This apparatus is essentially as described by Gray and Lalljee (1974). The 'open-field' consisted of a circular base, 90 cm in diameter which was divided into 10 cm squares by faint yellow lines. The wall surrounding the base consisted of a 75 cm high aluminium sheet. Illumination was provided by a 60 W bulb, positioned 90 cm above the floor of the apparatus. All measurements were carried out in a darkened room. Each animal was placed in the centre of the 'open-field' and the number of squares crossed were counted over a 3 min period for each animal between 0700 and 1100 h.

2.5. Hypothermic response to 8-OH-DPAT

Eighteen hours after their last antidepressant treatment, the colonic temperature was recorded by means of a digital thermometer. The probe was inserted 2 cm into the colon of the rat. Temperatures were recorded prior to and 40 min following an injection of 8-OH-DPAT (0.05 mg/kg s.c.). The dose of 8-OH-DPAT and the time of temperature measurement were based on previous dose and time-dependent curves. For these studies, the dose and time selected produced an intensity of response that allowed us to measure either increases or decreases in the response to a single injection.

2.6. Hypothermic response to clonidine

At 18 h after their last antidepressant treatment, the colonic temperature was recorded by means of a digital thermometer as described above. Temperatures were recorded prior to and 60 min following an injection of clonidine (0.1 mg/kg s.c.). The dose of clonidine and the time of temperature measurement were based on previous dose and time response curves. For these studies, the dose and time selected produced an intensity of response that allowed us to measure either increases or decreases in the response to a single injection.

2.7. Animal decapitation and brain dissection

Rats were sacrificed by decapitation immediately following the 'open-field' test on days 3, 7, 10 and 14 of antidepressant treatment. The brains were rapidly removed and were checked for signs of cortical damage or incomplete removal of the olfactory bulbs. Such subjects were excluded from the data analysis. The amygdaloid cortex was dissected on an ice-cold plate using a sharp dissection blade, weighed and homogenized by sonication in 1.0 ml elution buffer (pH 2.8) containing 2 ng/50 μl *N*-methyldopamine as an internal standard and stored at −20°C prior to assay.

2.8. Determination of brain biogenic amine concentrations

Concentrations of serotonin, noradrenaline, dopamine, di-hydroxyphenylacetic acid and 5-hydroxyindoleacetic acid were measured by high performance liquid chromatography with electrochemical detection (Seyfried et al.,

1986). The mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 1.4 mM octane-1-sulfonic acid, 0.1 mM ethylenediaminetetra-acetic acid and 9% v/v methanol. The pH of the mobile phase was adjusted to 2.8 with concentrated NaOH. The retention times of biogenic amines varied between 5 and 40 min on an LI Chrosorb RP-18 column. The flow rate of the mobile phase through the column was 1 ml/min at a pressure of approximately 200 bar. The column oven was maintained at 30°C. All standards were purchased from Sigma (Poole, Dorset, UK).

2.9. Drug administration

The drugs used were reboxetine HBr (Pharmacia and Upjohn, Milan, Italy), sertraline HCl (Pfizer, Sandwich, Kent, UK), 2-2-2-tribromoethanol (Aldrich Chemical, Gillingham, UK), 8-OH-DPAT HBr and clonidine HCl (RBI, Natwick, MA, USA).

The experiment was divided into two studies. In study 1, reboxetine was dissolved in saline to give a concentration of either 2.5, 5 or 10 mg/ml. These were administered intraperitoneally in a dosage volume of 1 ml/kg. The drug was administered daily in the morning (between 0900 and 1000 h) and afternoon (1600 and 1700 h) for 14 days. In study 2, reboxetine and sertraline were dissolved in distilled water at a concentration of 10 and 5 mg/ml, respectively. The reboxetine and sertraline combination consisted of 10 mg/ml of reboxetine and 5 mg/ml of sertraline. All treatments were administered intraperitoneally in a dosage volume of 1 ml/kg, twice daily (morning and evening as for study 1) for 3, 7, 10, and 14 days. Controls received injections of vehicle alone. There were four sets of eight groups, one set for each time interval (Days 3, 7, 10, and 14). Separate groups of animals were used in the 8-OH-DPAT and clonidine challenge tests.

Twice daily dosing was adopted because of the relatively short half-life of reboxetine in rodents (terminal half-life of elimination = 1–2 h) as reported by Dostert et al. (1997). Using a similar dosing schedule, Riva et al. (1989) reported that reboxetine (10 mg/kg i.p.) given twice daily down-regulates beta-adrenoceptors in rat cortical membranes after only a 5-day treatment with concomitant desensitization of the noradrenaline-dependent adenylate cyclase in cortical slices. Previous studies with sertraline (see Kelly and Leonard, 1994) also supported the use of a twice daily dosing schedule in the present study. Sertraline is extensively distributed into tissues and metabolized by the rat. The *N*-desmethyl metabolite is 10-fold less potent as an inhibitor of 5-HT and is inactive in animal models of antidepressant activity (Doogan and Caillard, 1988; Tremaine et al., 1989).

2.10. Statistical analysis of data

Data were initially analyzed using a Two-way analysis of variance (ANOVA) where drug treatment and bulbectomy were the first and second factors. Individual group differences were assessed with the Student–Newman–Keuls or Fishers (least significant difference) LSD multiple range tests. Data were deemed significant when $P < 0.05$.

3. Results

3.1. Effect of reboxetine in the forced swim test

In the forced swim test there was an effect of treatment [$F(3,53) = 4.78$, $P < 0.01$]. Reboxetine reduced the immobility time in both sham-operated and OB groups in a dose-related fashion (Fig. 1).

3.2. Effect of reboxetine in the 'open-field' test

In the 'open-field' test there was an effect of bulbectomy [$F(1,53) = 122.04$, $P < 0.001$], treatment [$F(3,53) = 5.12$, $P < 0.01$] and a treatment × bulbectomy interaction [$F(3,53) = 4.51$, $P < 0.01$]. Post-hoc comparisons revealed a typical hyperactivity with OB rats when compared to sham-operated controls ($P < 0.01$). There was a significant attenuation of the OB-related hyperactivity in the 10 mg/kg reboxetine treated group ($P < 0.01$) (Fig. 2).

3.3. Effect of reboxetine and sertraline, alone and in combination, in the 'open-field' test

In the 'open-field' test there was an effect of bulbectomy following 3 [$F(1,56) = 119.05$, $P < 0.001$], 7 [$F(1,56) = 96.00$, $P < 0.001$], 10 [$F(1,56) = 189.40$, $P < 0.001$] and 14 [$F(1,56) = 33.84$, $P < 0.001$] days of treatment. Post-hoc comparisons revealed a typical hyperactivity with OB rats when compared to sham-operated controls at each interval ($P < 0.01$) (Fig. 3). In addition, there was an effect of drug treatment [$F(3,56) = 3.12$, $P = 0.033$] and a drug treatment × bulbectomy interaction [$F(3,56) =$

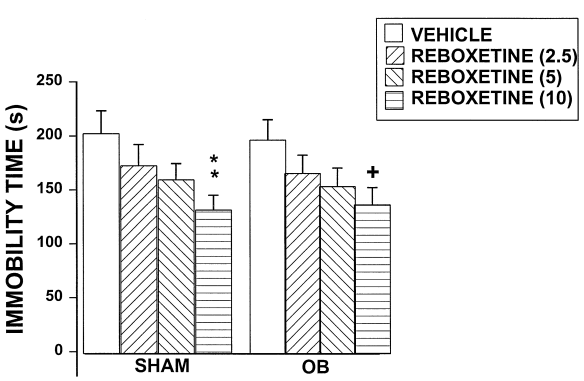

Fig. 1. Rats were administered reboxetine (2.5, 5 and 10 mg/kg i.p.) 24, 5 and 1 h prior to rating the forced swim test. Data is expressed as mean and standard error of seven to eight animals and analysed using a Two-way ANOVA followed by the Fishers LSD test. $^{**}P < 0.01$ vs. sham vehicle; $^{+}P < 0.05$ vs. OB vehicle.

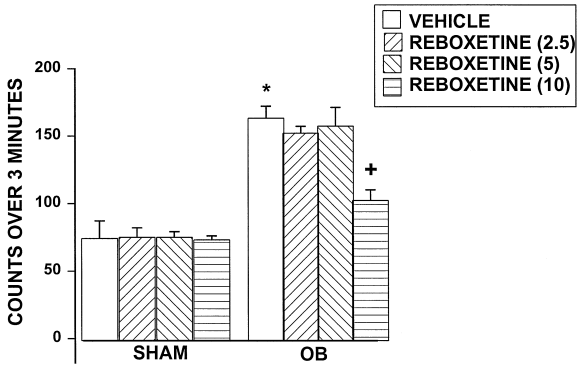

Fig. 2. Rats were administered reboxetine (2.5, 5 and 10 mg/kg i.p.) twice daily for 14 days prior to test. At 18 h following the last treatment ambulation scores were measured in the 'open-field'. Data is expressed as mean and standard error of seven to eight animals and analysed using a Two-way ANOVA followed by the Student–Newman–Keuls test. $^{*}P < 0.01$ vs. sham-operated vehicle; $^{+}P < 0.01$ vs. OB vehicle.

2.88, $P = 0.044$] following 14 days of treatment. Post-hoc comparisons revealed that the OB related hyperactivity was attenuated by reboxetine ($P < 0.01$), sertraline ($P < 0.05$) and the reboxetine + sertraline combination ($P < 0.05$) (Fig. 3).

3.4. 8-0H-DPAT challenge

There was no difference in temperatures prior to challenge in bulbectomized or drug treated groups at any interval. There was a significant drop in temperature recorded in control animals 40 min after the 8-OH-DPAT challenge following 3, 7, and 14 days. Analysis of the temperatures taken 40 min following the challenge injection of 8-OH-DPAT showed an effect of bulbectomy [$F(1,56) = 9.88$, $P < 0.01$] and antidepressant treatment [$F(3,56) = 12.89$, $P < 0.001$] in animals treated for 7 days. Post-hoc comparisons revealed that there was a significant attenuation of 8-OH-DPAT-induced hypothermia in OB animals that were treated with the reboxetine + sertraline combination ($P < 0.05$) with a corresponding trend towards significance in the sham-operated group. Following 14 days of treatment, there was an effect of antidepressant treatment [$F(3,56) = 15.77$, $P < 0.001$]. Post-hoc comparisons revealed that there was a significant attenuation of 8-OH-DPAT-induced hypothermia in sham-operated reboxetine ($P < 0.05$), sertraline ($P < 0.01$) and combination ($P < 0.01$) treated groups. Similarly, there was an attenuation of the hypothermic response in OB sertraline

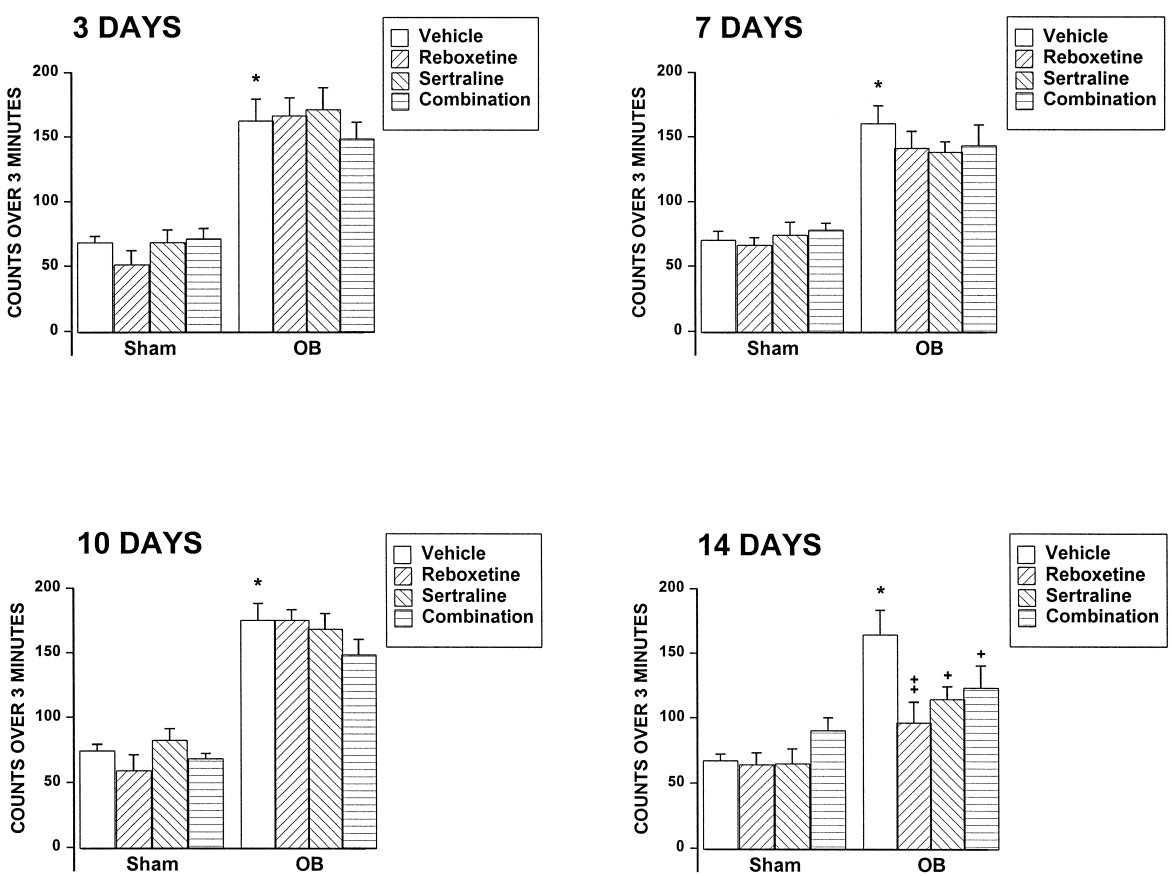

Fig. 3. Rats were administered reboxetine (10 mg/kg i.p., b.i.d.), sertraline (5 mg/kg, i.p., b.i.d.) or a combination of both for 3, 7, 10, and 14 days prior to test. At 18 h following the last treatment ambulation scores were measured in the 'open-field'. Data expressed as mean with standard error of eight rats/group and analysed using Two-way ANOVA followed by the Student–Newman–Keuls test. $^{*}P < 0.01$ vs. sham vehicle; $^{+}P < 0.05$; $^{++}P < 0.01$ vs. OB vehicle.

Table 1
Effect of reboxetine and sertraline alone and in combination on 8-OH-DPAT-induced hypothermia in sham- and OB-operated rats after different durations of treatment

	T (0)	T (40)
3 Days		
Sham vehicle	37.56 ± 0.17	36.30 ± 0.12
Sham reboxetine	37.35 ± 0.16	36.08 ± 0.13
Sham sertraline	37.32 ± 0.15	36.11 ± 0.19
Sham combination	37.33 ± 0.13	36.05 ± 0.03
OB vehicle	37.54 ± 0.06	36.42 ± 0.15
OB reboxetine	37.21 ± 0.09	36.05 ± 0.16
OB sertraline	37.70 ± 0.11	36.50 ± 0.14
OB combination	37.59 ± 0.20	36.44 ± 0.19
7 Days		
Sham vehicle	37.47 ± 0.09	36.05 ± 0.13
Sham reboxetine	37.08 ± 0.12	35.89 ± 0.15
Sham sertraline	37.50 ± 0.15	36.63 ± 0.13
Sham combination	37.39 ± 0.24	36.59 ± 0.16
OB vehicle	37.80 ± 0.08	36.41 ± 0.14
OB reboxetine	37.26 ± 0.18	36.21 ± 0.13
OB sertraline	37.87 ± 0.18	36.84 ± 0.18
OB combination	37.74 ± 0.16	37.03 ± 0.15[c]
14 Days		
Sham vehicle	37.56 ± 0.13	36.00 ± 0.12
Sham reboxetine	37.39 ± 0.10	36.50 ± 0.10[a]
Sham sertraline	37.70 ± 0.17	36.70 ± 0.11[b]
Sham combination	37.42 ± 0.12	36.89 ± 0.09[b]
OB vehicle	37.75 ± 0.11	36.39 ± 0.14
OB reboxetine	37.52 ± 0.17	36.36 ± 0.17
OB sertraline	37.64 ± 0.16	36.95 ± 0.13[c]
OB combination	37.79 ± 0.17	36.92 ± 0.05[c]

Rats were administered reboxetine (10 mg/kg i.p., b.i.d.), sertraline (5 mg/kg, i.p., b.i.d.) or a combination of both for 3, 7, or 14 days prior to the 8-OH-DPAT challenge test.
Temperatures were taken prior to [T (0)] and 40 min [T (40)] following a challenge injection of 8-OH-DPAT (0.05 mg/kg s.c.). Data expressed as mean with standard error of eight rats/group and analysed using Two-way ANOVA followed by the Student–Newman–Keuls test.
[a] $P < 0.05$.
[b] $P < 0.01$ vs. sham vehicle.
[c] $P < 0.05$ vs. OB vehicle.

($P < 0.05$) and combination ($P < 0.05$) treated groups (Table 1).

3.5. Clonidine challenge

There was no difference in temperatures prior to challenge in bulbectomized or drug treated groups at any interval. There was a significant drop in temperature recorded in control animals 60 min after the clonidine challenge following 3, 7, and 14 days. There was an effect of antidepressant treatment 60 min following the challenge injection of clonidine [$F(3,56) = 11.48$, $P < 0.001$] in animals treated for 7 days. Post-hoc comparisons revealed a significant attenuation of clonidine-induced hypothermia in sham ($P < 0.05$) and OB ($P < 0.05$) animals that were treated with the reboxetine + sertraline combination. There was an effect of bulbectomy [$F(1,56) = 9.38$, $P < 0.01$] and antidepressant treatment [$F(3,56) = 15.33$, $P < 0.001$] in animals treated for 14 days. Post-hoc comparisons revealed that there was a significant attenuation of clonidine-induced hypothermia in sham animals that were treated with the reboxetine + sertraline combination ($P < 0.05$). In OB animals, there was a significant attenuation of clonidine-induced hypothermia in animals that were treated with sertraline ($P < 0.05$), the combination ($P < 0.01$) with a similar trend towards significance in the reboxetine treated group (Table 2).

3.6. 5-HIAA concentrations in amygdaloid cortex

No significant changes in the concentrations of noradrenaline, dopamine, dihydroxyphenylacetic acid or 5-HT

Table 2
Effect of reboxetine and sertraline alone and in combination on clonidine-induced hypothermia in sham- and OB-operated rats after different durations of treatment

	T (0)	T (40)
3 Days		
Sham vehicle	37.45 ± 0.15	36.09 ± 0.11
Sham reboxetine	37.31 ± 0.13	35.96 ± 0.14
Sham sertraline	37.44 ± 0.12	36.00 ± 0.14
Sham combination	37.56 ± 0.13	36.14 ± 0.14
OB vehicle	37.39 ± 0.17	36.29 ± 0.24
OB reboxetine	37.62 ± 0.10	36.31 ± 0.18
OB sertraline	37.76 ± 0.15	36.26 ± 0.15
OB combination	37.62 ± 0.23	36.43 ± 0.25
7 Days		
Sham vehicle	37.56 ± 0.15	36.21 ± 0.15
Sham reboxetine	37.21 ± 0.19	36.06 ± 0.22
Sham sertraline	37.70 ± 0.15	36.35 ± 0.15
Sham combination	37.64 ± 0.07	37.07 ± 0.19[a]
OB vehicle	37.69 ± 0.09	36.11 ± 0.18
OB reboxetine	37.53 ± 0.18	36.36 ± 0.21
OB sertraline	37.72 ± 0.08	36.54 ± 0.19
OB combination	37.85 ± 0.11	37.20 ± 0.21[b]
14 Days		
Sham vehicle	37.63 ± 0.16	36.38 ± 0.18
Sham reboxetine	37.06 ± 0.13	36.25 ± 0.15
Sham sertraline	37.97 ± 0.16	36.81 ± 0.19
Sham combination	37.19 ± 0.13	37.18 ± 0.20[a]
OB vehicle	37.77 ± 0.08	36.22 ± 0.25
OB reboxetine	37.34 ± 0.15	36.96 ± 0.26
OB sertraline	38.08 ± 0.21	37.22 ± 0.08[b]
OB combination	37.74 ± 0.18	37.91 ± 0.19[c]

Rats were administered reboxetine (10 mg/kg i.p., b.i.d.), sertraline (5 mg/kg, i.p., b.i.d.) or a combination of both for 3, 7, or 14 days prior to the clonidine challenge test.
Temperatures were taken prior to [T (0)] and 60 min [T (60)] following a challenge injection of clonidine (0.1 mg/kg s.c.). Data expressed as mean with standard error of eight rats/group and analysed using Two-way ANOVA followed by the Student–Newman–Keuls test.
[a] $P < 0.05$ vs. sham vehicle.
[b] $P < 0.05$.
[c] $P < 0.01$ vs. OB vehicle.

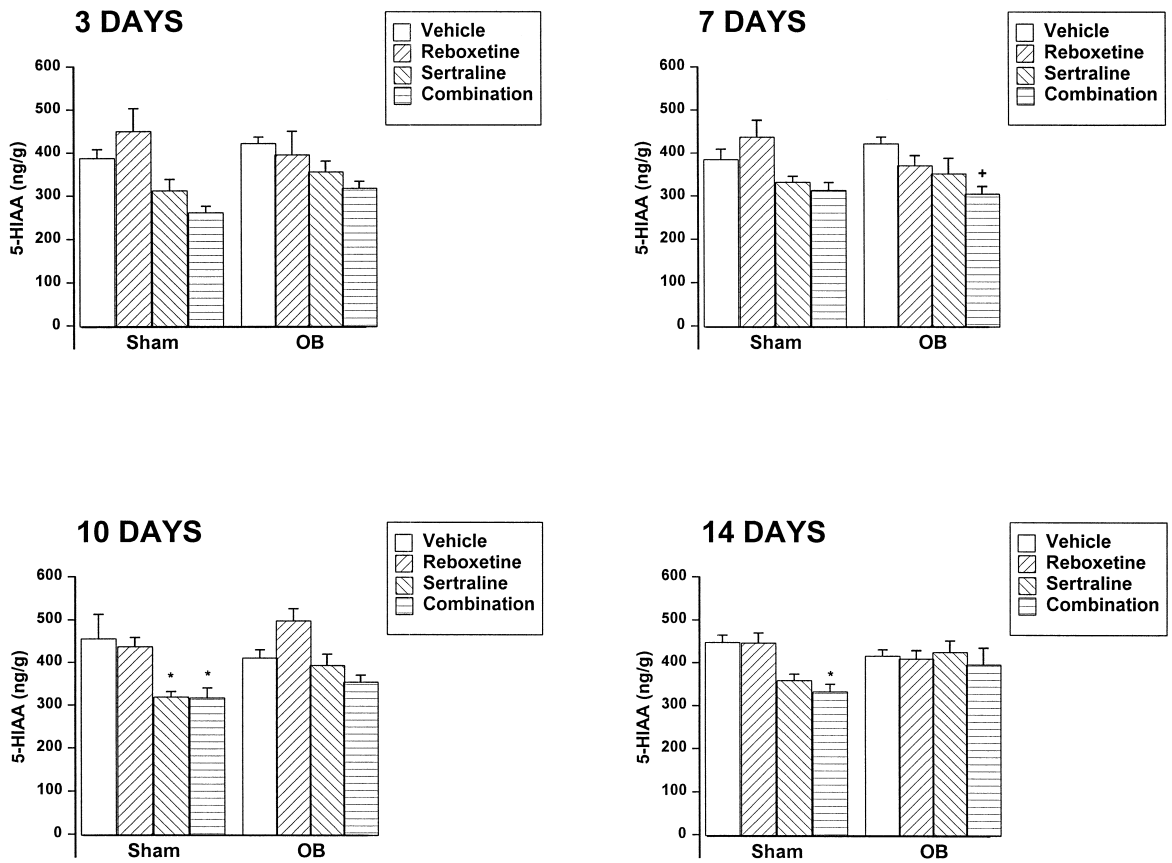

Fig. 4. Rats were administered reboxetine (10 mg/kg i.p., b.i.d.), sertraline (5 mg/kg, i.p., b.i.d.) or a combination of both for 3, 7, 10, and 14 days. Animals were decapitated 18 h following the last treatment. Data expressed as mean ng/g wet weight of tissue with standard error of seven to eight rats/group and analysed using Two-way ANOVA followed by the Student–Newman–Keuls test. $^{*}P < 0.05$; $^{+}P < 0.05$ vs. OB vehicle.

in the amygdaloid cortex were found (data not shown). There were effects of antidepressant treatment on 5-HIAA concentrations [$F(3,53) = 3.78$, $P = 0.016$] and a treatment × bulbectomy interaction [$F(3,53) = 3.03$, $P = 0.037$] after 14 days of treatment. Post-hoc comparisons revealed that sertraline and the sertraline + reboxetine combination reduced 5-HIAA concentrations ($P < 0.05$) in the amygdaloid cortex in sham-operated groups. Similarly, analysis of 5-HIAA concentrations showed effects of antidepressant treatment following 3 [$F(3,54) = 7.68$, $P < 0.001$], 7 [$F(3,54) = 7.12$, $P < 0.001$] and 10 [$F(3,55) = 9.36$, $P < 0.001$] days of treatment. Post-hoc comparisons revealed that the combination treatment significantly reduced 5-HIAA concentrations in amygdaloid cortex in the OB-treated group ($P < 0.05$) following 7 days of treatment. Sertraline and the combination treatment significantly reduced 5-HIAA concentrations in amygdaloid cortex in sham-operated animals following 10 days of treatment ($P < 0.05$) (Fig. 4).

4. Discussion

In the OB model, the primary index of potential antidepressant activity is reversal of increased ambulation scores in an 'open-field' test (Van Riezen and Leonard, 1991). Chronic treatment with antidepressants will illicit a response in bulbectomized rats which is manifested as a reduction of the behavioural hyperactivity in a stressful novel environment. This property is shared by a variety of antidepressants and strengthens the validity of the OB rat as a simulator of the action of antidepressants in the treatment of depression (reviewed by Kelly et al., 1997). Effective doses of reboxetine (10 mg/kg i.p.) in the forced swim test and the 'open-field' test were determined. While the antidepressants tested in the present study were effective in the model, no added response was observed by targeting the noradrenergic and 5-HT systems simultaneously. Moreover, the onset of the antidepressant effect in the 'open-field' was similar with reboxetine and sertraline alone or in combination. The recommended doses of reboxetine for human use are lower than those for sertraline. The discrepancy between relative effective doses of these drugs in humans and rats as reported here, may be due to different pharmacokinetics of reboxetine in animals compared to humans. The administration of a high dose of reboxetine twice daily was necessary given the short half-life of this drug in rodents. In addition, reboxetine has no inhibitory effect on the major enzymes involved in drug metabolism and shows no relevant drug interactions

(Dostert et al., 1997). The ability to alter 8-OH-DPAT-induced hypothermia suggests that sertraline and reboxetine can alter 5-HT_{1A} receptor sensitivity previously demonstrated with other antidepressants including desipramine and electroconvulsive shock therapy (Goodwin et al., 1987), clorgyline (Wozniak et al., 1988) and sertraline treatments (Kelly and Leonard, 1994). Following 14 days of treatment (but not following 3 and 7 days), all treatments attenuated the 8-OH-DPAT-induced hypothermic response in sham-operated animals, indicating a possible alteration to the sensitivity of 5-HT_{1A} receptors. A similar profile was observed for sertraline and the combination treatment in the OB-treated groups. In addition, following treatment for 7 days with the reboxetine + sertraline combination, OB animals displayed a reduced hypothermic response to 8-OH-DPAT. Such a reduction suggests a more rapid response in the test following 7 days of treatment.

Previously, it has been reported that chronic antidepressant treatments reduce the hypothermic effect of clonidine (Von Voigtlander et al., 1978; Bill et al., 1989; Redmond et al., 1995). The hypothermic effects of clonidine are mediated via central α_2-adrenoceptors, and chronic antidepressant treatment desensitizes to clonidine indicating a change in the status of these receptors (Pilc, 1987). Following 7 days of treatment with the reboxetine + sertraline combination, the clonidine-induced hypothermic response was attenuated in sham and OB animals. Similarly sertraline, the combination treatment and to a lesser degree reboxetine attenuated the clonidine-induced hypothermic response following 14 days administration. Such changes in the response to clonidine indicate that there is a reduction in the sensitivity to α_2-adrenoceptors following repeated administration of either sertraline or reboxetine and that a faster onset of the response is observed when sertraline is combined with reboxetine.

In the present study, the outcome of the challenge studies indicates similarities between the two classes of drug despite their different neurochemical properties and suggests that the mechanism underlying their antidepressant action may involve both serotonergic and noradrenergic systems. Sertraline with a selective action on central serotonergic transmission alters the response to 8-OH-DPAT and clonidine. Likewise, reboxetine with a selective action on central noradrenergic transmission modifies the response to 8-OH-DPAT, and to a lesser extent clonidine. Interestingly, treatment with a combination of both inhibitors is complementary and leads to a faster response in the challenge tests. Similarly, long-term adaptive changes in β-adrenoceptors are known to occur following both noradrenaline and serotonin reuptake inhibitors alike (Koe et al., 1983; Riva et al., 1989). Such changes following antidepressant treatment to experimental animals have been shown to require an interaction with a functional serotonergic system (see Baron et al., 1988). Moreover, a rapid down-regulation of β-adrenoceptors has been reported following co-administration of the tricyclic antidepressant, desipramine and selective serotonin reuptake inhibitor, fluoxetine to rats (Baron et al., 1988). Such reports of interactions between the serotonergic and noradrenergic systems in response to antidepressant treatments suggest that both noradrenaline and serotonin are involved in the response to antidepressant treatments and lend some support to the working hypothesis that a dual action on both biogenic amine uptake sites may have a broader spectrum of therapeutic action.

Olfactory bulbectomy is thought to result in neurochemical changes in many brain regions including the amygdala as a consequence of the disrupted connections with the olfactory bulbs (see Kelly et al., 1997). Reduced noradrenaline and 5-HT concentrations in limbic brain structures which can be reversed following repeated antidepressant treatments (Song and Leonard, 1995) are consistent with the OB rat being a model of hyposerotonergic depression (Lumia et al., 1992). However, studies to date on the underlying neurochemical mechanisms mediating the specific behavioural disturbances observed in the model, and their subsequent normalization by antidepressant treatment, are equivocal. In the present study, there was no effect of bulbectomy on amine concentrations in the amygdaloid cortex. However, treatment with sertraline and the combination reduced amygdaloid 5-HIAA concentrations following 3, 7, 10, and 14 days of treatment. By inhibiting the neuronal reuptake of 5-HT, decreased uptake of 5-HT leads to a reduction in its intracellular metabolism to 5-HIAA. Thus, these neurochemical changes are more likely to be related to acute pharmacological effects on the reuptake of 5-HT rather than onset of an antidepressant effect in the 'open-field' or the challenge tests.

In conclusion, reboxetine demonstrated antidepressant activity in the 'forced swim test' and olfactory bulbectomized rat model of depression. Reboxetine, sertraline and the combination treatment were effective in reducing the hyperactive response of the OB rat in the 'open-field' test following 14 days of treatment, but not at earlier time intervals. The results of the 8-OH-DPAT and clonidine challenge tests suggest that changes to 5-HT_{1A} receptor and α_2-adrenoceptor responsivity occur quicker with the combination treatment than with either antidepressant treatment alone. The effects emphasize the role of these receptor subtypes in the mechanism of action of antidepressants, as well as supporting the concept of targeting the 5-HT_{1A} receptor and α_2-adrenoceptors for the development of faster acting antidepressants. However, the lack of a bulbectomy effect in the hypothermic response in this and previous studies (Kelly and Leonard, 1994; Cryan et al., 1998) suggests that the challenge tests do not provide a neurochemical marker of either the behavioural deficits or antidepressant response in the model. It is unlikely that a disorder as complex as depression, or simulations of depression in animal models, are due solely to abnormalities in single neurotransmitter systems. Such a hypothesis is

supported by the diversity of associated behavioural and biochemical changes (for review of the OB rat model see Kelly et al., 1997). While the neurobiological basis for antidepressant reversal of bulbectomy-induced hyperactivity remains unknown, the approach of integrating several tests used to detect and characterize antidepressant activity, compliments the diverse nature of the disorder and corresponding mode of action of antidepressants.

Acknowledgements

The authors would like to thank Pharmacia and Upjohn, Kalamazoo, MI, USA for financial assistance towards the cost of the present studies and Pfizer UK for the generous gift of sertraline.

References

Artigas, F., Perez, V., Alverez, E., 1994. Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. Arch. Gen. Psychiatry 51, 248–251.

Baron, B.M., Ogden, A.M., Siegel, B.W., Stegeman, J., Ursillo, R.C., Dudley, M.W., 1988. Rapid down regulation of β-adrenoceptors by co-administration of desipramine and fluoxetine. Eur. J. Pharmacol. 154, 125–134.

Bill, D.J., Hughes, I.E., Stephens, R.J., 1989. The effects of acute and chronic desipramine on thermogenic and hypoactivity responses to α_2-agonists in reserpinized and normal mice. Br. J. Pharmacol. 96, 144–152.

Blier, P., De Montigny, C., 1994. Current advances in the treatment of depression. Trends Pharmacol. Sci. 15, 220–226.

Borsini, F., Meli, A., 1988. Is the forced swimming test a suitable model for revealing antidepressant activity?. Psychopharmacology 94, 147–161.

Cairncross, K.D., Wren, A.F., Cox, B., Schnieden, H., 1977. Effects of olfactory bulbectomy and domicile on stress induced corticosterone release in the rat. Physiol. Behav. 19, 485–487.

Cryan, J.F., McGrath, C., Leonard, B.E., Norman, T.R., 1998. Combining pindolol and paroxetine in an animal model of chronic antidepressant action—can early onset of action be detected?. Eur. J. Pharmacol. 352, 23–28.

Danish University Antidepressant Group, 1986. Citalopram: clinical effect profile in comparison with clomipramine. A controlled multicenter study. Psychopharmacology 90, 131–138.

Danish University Antidepressant Group, 1990. Paroxetine: a selective serotonin reuptake inhibitor showing better tolerance but weaker antidepressant effect than clomipramine in a controlled multicenter study. J. Affect. Disord. 18, 289–299.

Doogan, D.P., Caillard, V., 1988. Sertraline: a new antidepressant. J. Clin. Psychiatry 49, s46–s51.

Dostert, P., Benedetti, M.S., Poggesi, I., 1997. Review of the pharmacokinetics and metabolism of reboxetine, a selective noradrenaline reuptake inhibitor. Eur. Neuropsychopharmacol. 7, s23–s35.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281, 81–88.

Goodwin, G.M., DeSouza, R.J., Green, A.R., 1987. Attenuation by electroconvulsive shock and antidepressant drugs of the 5-HT_{1A} receptor-mediated hypothermia and serotonin syndrome produced by 8-OH DPAT in the rat. Psychopharmacology 91, 500–505.

Gray, J.A., Lalljee, B., 1974. Sex differences in emotional behaviour in the rat: correlation between the 'open field' defaecation and active avoidance. Anim. Behav. 22, 856–861.

Guelfi, J.D., White, C., Hackett, D., Gaichoux, J.Y., Magni, G., 1995. Effectiveness of venlafaxine in patients hospitalized for major depression and melancholia. J. Clin. Psychiatry 56, 450–458.

Kelly, J.P., Leonard, B.E., 1994. The effect of tianeptine and sertraline in three animal models of depression. Neuropharmacology 33, 1011–1016.

Kelly, J.P., Wrynn, A.S., Leonard, B.E., 1997. The olfactory bulbectomized rat as a model of depression: an update. Pharmacol. Ther. 75, 1–17.

Koe, B.K., Weissman, A., Welch, W.M., 1983. Sertraline 1*S*, 4-*S*-*N*-methyl-4-(3,4,dichlorophenyl)-1,2,3,4-tetrahydro-1-napthylamine, a new uptake inhibitor with selectivity for serotonin. J. Pharmacol. Exp. Ther. 226, 686–700.

Leonard, B.E., 1997. Animal models of depression. In: Briley, M., Montgomery, S. (Eds.), Antidepressant Therapy at the Dawn of the Third Millennium. Martin Dunitz, UK, pp. 87–109.

Leonard, B.E., Tuite, T.E., 1981. Anatomical, physiological and behavioural aspects of olfactory bulbectomy in the rat. Int. Rev. Neurobiol. 22, 251–286.

Lumia, A.R., Teicher, M.H., Slachli, F., Ayers, E., Possidente, B., 1992. Olfactory bulbectomy as a model of agitated hyposerotonergic depression. Brain Res. 587, 181–185.

Melloni, P., Carniel, G., Della Torre, A., Bonsignori, A., Buonamici, M., Pozzi, O., Ricciardi, S., Rossi, S.C., 1984. Potential antidepressant agents: a aryloxy–benzyl derivatives of ethanolamine and morpholine. Eur. J. Med. Chem. 19, 235–242.

Nelson, J.C., 1997. Synergistic effects of serotonergic and noradrenergic antidepressants in combination. In: Briley, M., Montgomery, S. (Eds.), Antidepressant Therapy at the Dawn of the Third Millennium. Martin Dunitz, UK, pp. 297–305.

Nelson, J.C., Mazure, C.M., Bowers, M.B., Jatlaw, P.I., 1991. A preliminary open study of the combination of fluoxetine and desipramine for rapid treatment of major depression. Arch. Gen. Psychiatry 48, 303–307.

Nutt, D.J., Pinder, R.M., 1996. α_2-adrenoceptors and depression. Journal of Psychopharmacology 10, s35–s42.

O'Connell, M.T., Sarna, G.S., Curzon, G., 1992. Evidence for postsynaptic mediation of the hypothermic effect of the 5-HT_{1A} receptor activation. Br. J. Pharmacol. 106, 603–609.

O'Donnell, J.M., Banyasz, T., Kovaks, T., 1996. Altered thermoregulatory responses to clonidine in streptozocin-diabetic rats. Br. J. Pharmacol. 117, 938–942.

Pilc, A., 1987. The role of alpha-2-adrenoceptors in the mechanism of action of antidepressant drugs. Pol. J. Pharmacol. Pharm. 39, 691–713.

Pinder, R.M., 1997. Designing a new generation of antidepressant drugs. Acta Psychiatrica Scandinavica 96, 7–13.

Porsolt, R.D., Bertin, A., Blavet, M., Jalfre, M., 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47, 379–391.

Potter, W.Z., 1996. Adrenoceptors and serotonin receptor function. Relevance to antidepressant mechanisms of action. J. Clin. Psychiatry 57, 4–8.

Redmond, A.M., Leonard, B.E., 1997. An evaluation of the role of the noradrenergic system in the neurobiology of depression: a review. Hum. Psychopharmacol. 12, 407–430.

Redmond, A.M., Kelly, J.P., Leonard, B.E., 1995. The effects of acute and chronic antidepressant treatments on temperature and locomotor activity responses to 8-OH DPAT, clonidine and apomorphine. J. Serotonin Res. 2, 139–148.

Riva, M., Brunello, N., Rosecalli, A.C., Galimberti, R., Carfragna, N., 1989. Effect of reboxetine, a new antidepressant drug, on the central noradrenergic system: behavioural and biochemical studies. J. Drug Dev. 1, 243–252.

Schweizer, E., Weise, C., Clary, C., Fox, I., Rickels, K., 1991. Placebo-

controlled trial of venlafaxine for the treatment of major depression. J. Clin. Psychopharmacol. 11, 233–236.

Seyfried, C.A., Adams, G., Greve, T., 1986. An automated direct-injection HPLC method for the electrochemical/fluorometric quantitation of monoamines and related compounds optimized for screening of large numbers of animals. Biomed. Chromatogr. 1, 78–88.

Song, C., Leonard, B.E., 1995. The effect of olfactory bulbectomy in the rat, alone or in combination with antidepressants and endogenous factors, on immune function. Hum. Psychopharmacol. 10, 7–18.

Tremaine, L.M., Welch, W.M., Ronfeld, R.A., 1989. Metabolism and distribution of the 5-hydroxytryptamine uptake blocker sertraline in the rat and dog. Drug Metab. Dispos. 17, 542–550.

Van Riezen, H., Leonard, B.E., 1991. Effects of psychotropic drugs on the behaviour and neurochemistry of olfactory bulbectomized rats. In: Psychopharmacology of Anxiolytics and Antidepressants. Pergammon, New York, pp. 231–250.

Von Voigtlander, P.F., Triezenberg, H.J., Losey, E.G., 1978. Interactions between clonidine and antidepressant drugs: a method for identifying antidepressant-like agents. Neuropharmacology 17, 375–381.

Wozniak, K.M., Aulakh, C.S., Hill, J.L., Murphy, D.L., 1988. The effect of 8-OH DPAT on temperature in the rat and its modification by chronic antidepressant treatments. Pharmacol. Biochem. Behav. 30, 451–456.

Yu, H., Lewander, T., 1997. Pharmacokinetic and pharmacodynamic studies of (*R*)-8 hydroxy-2-(di-*n*-propylamino)tetralin in the rat. Eur. Neuropsychopharmacol. 7, 165–172.