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Desipramine administration in the olfactory bulbectomized rat: changes in brain β-adrenoceptor and 5-HT_{2A} binding sites and their relationship to behaviour

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1 The effects of repeated administration of the tricyclic antidepressant drug, desipramine (DMI), on behaviour (locomotor activity and rearing) and the number and affinity of brain β-adrenoceptor and 5-HT_{2A} receptor binding sites were examined in olfactory bulbectomized (OB) and sham-operated control rats.

2 Locomotor activity and rearing were increased in OB rats compared to sham-operated controls. The effect of various doses of DMI (administered orally twice daily for 21 days) on these behavioural measures was examined. A dose of 7.5 mg kg^{-1} provided optimal reversal of hyperlocomotion and increased rearing in OB rats, without changing these measures in sham-operated controls.

3 The time course of DMI (7.5 mg kg^{-1}) on behavioural and neurochemical measures was examined. Locomotion and rearing in OB rats were not significantly altered after 7 days, were significantly attenuated after 14 days and were normalized after 21 days.

4 After 7 days of DMI administration the number of β-adrenoceptors was lower in frontal and occipital cortex and hippocampus. This reduction was largely restricted to the β_1-adrenoceptor subtype. Administration of DMI for 14 or 21 days did not further reduce the number of β-adrenoceptors. The DMI induced reduction in β-adrenoceptors did not differ in OB and sham-operated control rats.

5 DMI administration for up to 21 days produced a progressive reduction in the number of 5-HT_{2A} receptors in frontal cortex, without significant alterations in occipital cortex.

6 The time course of the reduction in the number of 5-HT_{2A} receptors was similar to that of the DMI-induced behavioural changes whereas that for the reduction in β-adrenoceptors was clearly different.

7 The present results suggest that the action of DMI in this animal model is unlikely to be directly related to a reduction in β-adrenoceptors but may be related to a reduction in frontal cortical 5-HT_{2A} receptors.

Keywords: Antidepressants; desipramine; β-adrenoceptors; 5-HT_{2A} receptors; radioligand binding; olfactory bulbectomy; animal models of antidepressant drug action; rat brain

Introduction

The mechanims(s) by which drugs are able to relieve the symptoms of depression have not been convincingly established. Acutely many, but not all, drugs increase the availability of monoamine neurotransmitters, particularly noradrenaline and 5-hydroxytryptamine (5-HT), by inhibiting reuptake or blocking metabolism by monoamine oxidase. However clinically it is established that there is a 2-3 week delay from the initiation of antidepressant drug therapy before an improvement in symptoms is apparent. This suggests that antidepressant action depends on some adaptive response which develops slowly and this has prompted many studies of the effects of repeated administration of antidepressants in laboratory animals. Changes in the number of several classes of central monoamine receptor binding sites have been reported. Decreases in the number of cortical β-adrenoceptor binding sites have been consistently reported following tricyclic antidepressants, monoamine oxidase inhibitors, atypical drugs (such as mianserin and viloxazine) and repeated electroconvulsive shocks (ECS, a model in animals of electroconvulsive therapy) although not following selective 5-HT (serotonin) reuptake inhibitors (SSRIs) (Sugrue, 1983; Baker & Greenshaw, 1989; Caldecott-Hazard *et al.*, 1991; Johnson, 1991). Similar findings have been reported in respect of cortical 5-HT_{2A} receptors, except that repeated ECS increases the number of 5-HT_{2A} receptors (Bergstrom & Kellar, 1979) and SSRIs do not consistently alter the number of 5-HT_{2A} receptors (Sanders-Bush *et al.*, 1989; Johnson, 1991; Hrdina & Vu, 1993; Klimek *et al.*, 1994).

It is not clear if, or to what extent, such changes in monoamine receptor binding sites contribute to antidepressant action. Most studies have been performed in normal laboratory animals without any attempt to relate neurochemical changes to altered behaviour. One approach to this problem is to study neurochemical effects in an animal test system in which abnormal behaviours can be reversed by antidepressants. One such model is the olfactory bulbectomized (OB) rat. Bilateral destruction of the olfactory bulbs induces a number of behavioural changes, including increased locomotor activity in a novel environment and deficits in active and passive avoidance tasks (Leonard & Tuite, 1981). Antidepressants (including atypical drugs) when administered chronically, but not acutely, are able to normalize these behaviours, without altering responses in sham-operated controls. Other psychoactive drugs, including tranquillisers and amphetamine can modify the behavioural effects following olfactory bulbectomy but only at doses which also cause behavioural effects in sham-operated controls (Cairncross *et al.*, 1978a,b; Janscar & Leonard, 1983; O'Connor *et al.*, 1985; Jesberger & Richardson, 1986; O'Connor & Leonard, 1988).

The aim of the present study was to examine the time course of the reversal of abnormal behaviours in OB rats by repeated administration of the tricyclic antidepressant, desipramine (DMI) and to compare this with the time course of changes in β-adrenoceptor and 5-HT_{2A} receptor binding sites within the

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same animals. Behavioural and neurochemical studies were also performed concurrently in sham-operated controls receiving repeated DMI.

Methods

Olfactory bulbectomy

Male Sprague-Dawley rats (200–250 g) were housed four to a cage under standardized conditions of temperature and lighting, with free access to food and water and were handled daily. After 7 days of acclimatization, bilateral olfactory bulbectomy was performed as described by O'Connor & Leonard (1986). Briefly, rats were anaesthetized with tribomoethanol (250 mg kg^{-1}, i.p.) and a mid-line skin incision made to expose the skull overlying the bulbs. Two 2 mm diameter holes were drilled above the bulbs (7 mm anterior to bregma and 2 mm on either side of the mid-line, at a point corresponding to the posterior margin of the orbit of the eye). The olfactory bulbs were removed by aspiration and the resulting spaces filled with haemostatic sponges, the wound was sprinkled with antibiotic dusting powder (Cicatrin, Wellcome) and closed with three Michel clips. Sham-operated rats were treated the same, including piercing of the dura but the bulbs were left intact. The rats were re-housed four to a cage (2 OB and 2 sham) and received buprenorphine (50 $\mu g\ kg^{-1}$, s.c.) twice daily for two days to provide post surgical analgesia. Rats continued to be handled daily.

Drug administration

Two weeks after surgery, DMI was administered by gastric tube twice daily (between 07 h 00 min and 10 h 00 min and 14 h 00 min and 19 h 00 min) for up to 21 days in a volume of 5 ml kg^{-1}. Control rats received an equivalent volume of saline vehicle. In each cage, one sham-operated and one OB rat received saline and one sham-operated and one OB rat received DMI.

Behavioural testing

Twenty four hours after the last treatment, rats (selected randomly from each cage) were placed singly in a brightly illuminated circular (90 cm diameter) open field apparatus, consisting of an aluminium wall (70 cm high) and a wooden based divided into 10 cm squares (Janscar & Leonard, 1983). Beginning immediately, locomotor activity (number of line crossings made) and rearing (number of times both forepaws were raised from the floor) were measured during a 3 min period. The apparatus was cleaned with a damp cloth between each rat. Following the behavioural measurements, rats were killed by cervical dislocation, their brains removed, dissected over dry ice and stored frozen at −80°C until assayed.

Tissue preparation and binding assays

Brain membranes were prepared according to Cheetham *et al.* (1988). Briefly, frozen tissue was twice homogenized in 0.25 M sucrose and the supernatants combined, diluted with 50 mM Tris-HCl (pH 7.6 for β-adrenoceptor binding and pH 7.5 for 5-HT_{2A} binding) and centrifuged at 35000 *g* for 10 min. The pellets were resuspended in buffer and again centrifuged at 35000 *g* for 10 min. The pellets were resuspended in Tris-HCl at 5 mg ml^{-1} for β-adrenoceptor binding and at 12.5 mg ml^{-1} for 5-HT_{2A} receptor binding. Saturation binding of β-adrenoceptors was carried out according to De Paermentier *et al.* (1989) with [^{3}H]-CGP 12177 (6 concentrations, 0.03–1 nM). Specific total β-adrenoceptor and β_1-adrenoceptor binding were defined as radioactivity displaced by 200 μM (−)-isoprenaline and 100 nM CGP 20712A, respectively. Saturation binding of 5-HT_{2A} receptors was carried out according to Cross & Horton (1987) with [^{3}H]-ketanserin (8 concentrations, 0.1–5 nM). Specific binding was defined as radioactivity displaced by 1 μM methysergide. Aliquots of membrane were stored at −25°C for subsequent protein determination as described by Lowry *et al.* (1951) with bovine serum albumin used as the standard. Equilibrium dissociation constants (K_d) and the maximum number of binding sites (B_{max}) were determined by computerized non-linear regression fitting to a one site binding model.

Analysis

Behavioural and binding data were analysed by multiple analysis of variance. When the F ratio was significant ($P<0.05$), individual group means were compared by Student's t test.

Results

Preliminary behavioural experiments

Behavioural testing was performed following administration of DMI (5, 7.5 and 10 mg kg^{-1}) for 21 days. Locomotor activity and rearing were significantly higher in OB rats that received saline compared to sham-operated controls (Table 1). DMI at a dose of 5 mg kg^{-1} did not alter the hyperlocomotion or increased rearing, whereas both were normalized following 7.5 mg kg^{-1}. DMI at 10 mg kg^{-1} did not reduce the hyperlocomotion in OB rats but increased locomotion in sham-operated rats. A dose of 7.5 mg kg^{-1} DMI was selected for further experiments in which the time course of behavioural and neurochemical changes were examined.

The time course of the behavioural effects of DMI (7.5 mg kg^{-1})

In OB rats, DMI administration for 7 days did not significantly alter locomotor activity or rearing. After 14 and 21 days locomotor activity and rearing were significantly attenuated compared with saline-administered OB rats. Locomotor activity and rearing in OB rats that had received DMI for 21 days did not differ significantly from saline administered sham-operated controls (Table 2). In sham-operated rats, DMI administration for 7, 14 or 21 days did not significantly alter locomotor activity or rearing (Table 2).

β-Adrenoceptors

The number of total β- and β_1-adrenoceptors did not differ significantly between OB and sham-operated control rats in any of the four brain regions studied (Tables 3 and 4).

The number of total β- and β_1-adrenoceptors in frontal cortex, occipital cortex and hippocampus was significantly lower in rats receiving DMI (Tables 3 and 4). In the striatum, DMI administration had no significant effect on the number of

Table 1 Locomotor activity and rearing in sham and OB rats following saline or DMI for 21 days

		DMI		
	Saline	*5 mg kg^{-1}*	*7.5 mg kg^{-1}*	*10 mg kg^{-1}*
Locomotor activity				
Sham	111±19	107±15	107±12	139±48
OB	155±46*	160±28**	102±15	144±27
Rearing				
Sham	18±5	17±7	18±4	23±8
OB	23±7	26±7*	19±9	21±7

Values are means±s.d., $n=9-12$. Significant differences between sham and OB rats are denoted by *$P<0.05$, **$P<0.001$.

total β- and β_1-adrenoceptors (Table 4). There were no significant interactions between surgery (OB or sham), drug (DMI or saline) and day (7, 14 or 21 days) and the number of β-adrenoceptors in any of the brain regions i.e. the effects of DMI did not differ significantly between sham and OB rats and between 7, 14 and 21 days of administration. The K_d of total β-adrenoceptor (0.05–0.1 nM) and β_1-adrenoceptor (0.03–0.07 nM) binding was unaffected by OB or DMI administration (results not shown).

5-HT$_{2A}$ receptors

The number of 5-HT$_{2A}$ receptors did not differ significantly between OB and sham-operated control rats in frontal or occipital cortex (Table 5). In frontal cortex, the number of 5-HT$_{2A}$ receptors was significantly lower in rats receiving DMI (7, 14 and 21 days, Table 4). There was a significant interaction between drug and day and the number of 5-HT$_{2A}$ receptors i.e. the effect of DMI differed significantly with the duration of

Table 2 Locomotor activity and rearing in sham and OB rats following saline or DMI (7.5 mg kg^{-1}) for 7, 14 and 21 days

	Sham		*OB*	
	Saline	*DMI*	*Saline*	*DMI*
7 days				
Locomotor activity	99±16	101±15	184±27**	183±28††
Rearing	12±4	11±3	28±3**	28±3††
14 days				
Locomotor activity	100±18	98±15	165±29**	130±38†$^{\#}$
Rearing	13±5	16±4	25±4**	19±4$^{\#}$
21 days				
Locomotor activity	94±22	95±18	168±32**	94±19$^{\#\#}$
Rearing	12±3	13±3	21±5**	15±8$^{\#\#}$

Values are means±s.d., $n=8-12$. Significant differences between sham and OB rats following saline are denoted by *$P<0.05$, **$P<0.01$. Significant differences between sham and OB rats following DMI are denoted by †$P<0.05$, ††$P<0.01$. Significant differences in OB rats between saline and DMI are denoted by $^{\#}P<0.05$, $^{\#\#}P<0.01$.

Table 3 β-Adrenoceptor binding sites (B_{max}) in frontal and occipital cortex of sham and OB rats following saline or DMI (7.5 mg kg^{-1}) administration for 7, 14 and 21 days

	Frontal cortex				*Occipital cortex*			
	Sham		*OB*		*Sham*		*OB*	
	Saline	*DMI*	*Saline*	*DMI*	*Saline*	*DMI*	*Saline*	*DMI*
7 days								
Total β-	67±6	47±6**	77±9	49±8**	73±13	51±8**	72±11	41±9**
β_1	54±7	31±6**	53±5	31±6**	55±12	36±7**	54±10	27±6**
14 days								
Total β-	83±14	43±11**	78±11	53±12**	64±9	44±5**	67±16	45±9**
β_1	60±7	29±8**	55±10	33±9**	48±7	31±4**	44±11	29±6**
21 days								
Total β-	79±13	48±8**	81±4	52±12**	62±12	44±13**	72±13	52±12**
β_1	55±9	31±4**	56±5	34±9**	44±11	31±11**	53±13	31±8*

B_{max}, fmol mg^{-1} protein. Values are means±s.d., $n=5-6$. Significant differences between rats following DMI or saline are denoted by *$P<0.05$, **$P<0.01$.

Table 4 β-Adrenoceptor binding sites (B_{max}) in hippocampus and striatum of sham and OB rats following saline or DMI (7.5 mg kg^{-1}) administration for 7, 14 and 21 days

	Hippocampus				*Striatum*			
	Sham		*OB*		*Sham*		*OB*	
	Saline	*DMI*	*Saline*	*DMI*	*Saline*	*DMI*	*Saline*	*DMI*
7 days								
Total β-	32±7	23±4*	36±5	24±5*	75±18	76±14	75±12	65±6
β_1	23±5	16±4*	28±5	15±3*	48±8	51±10	48±10	44±5
14 days								
Total β-	35±7	21±4*	37±5	26±7*	66±10	70±17	62±18	76±16
β_1	27±3	12±3*	25±3	15±3*	44±3	31±12	42±10	55±11
21 days								
Total β-	36±4	24±6*	31±11	24±7*	79±20	68±12	85±20	77±11
β_1	26±3	13±3*	24±10	16±6*	51±15	48±8	57±13	52±7

B_{max}, fmol mg^{-1} protein. Values are means±s.d., $n=5-6$. Significant differences between sham and OB rats following DMI or saline are denoted by *$P<0.05$, **$P<0.01$.

Table 5 5-HT_{2A} receptor binding sites (B_{max}) in sham and OB rats following saline or DMI (7.5 mg kg^{-1}) administration for 7, 14 and 21 days

	Frontal cortex				*Occipital cortex*			
	Sham		*OB*		*Sham*		*OB*	
	Saline	*DMI*	*Saline*	*DMI*	*Saline*	*DMI*	*Saline*	*DMI*
7 days	296±21	212±33*	285±77	230±58*	143±27	141±35	143±39	156±47
14 days	325±58	262±25**	347±31	234±26**	164±32	122±16	139±51	128±32
21 days	353±39	188±57**	343±69	219±37**	162±22	122±38	154±47	134±44

B_{max}, fmol mg^{-1} protein. Values are means±s.d., $n=5-6$. Significant differences between sham and OB rats following DMI or saline are denoted by $*P<0.05$, $**P<0.01$.

DMI administration. There was no significant interaction between surgery (OB or sham) and drug i.e. the effect of DMI did not differ between OB and sham-operated control rats.

In occipital cortex, the number of 5-HT_{2A} receptors was not significantly altered by DMI administration (Table 5). Following 14 and 21 days DMI administration, the number of 5-HT_{2A} receptors appeared lower than in saline-administered rats; these differences were not statistically significant. There was no significant interactions between surgery and drug or between drug and day and the number of 5-HT_{2A} receptors. The K_d of [^{3}H]-ketanserin binding was unaffected by OB or DMI administration in either cortical area (range 0.4–0.6 nM).

Discussion

Preliminary studies demonstrated that DMI at a dose of 7.5 mg kg^{-1} twice daily for 21 days reversed the hyperlocomotor activity and increased rearing in OB rats, without significantly altering either measure in sham-operated controls. A dose of 5 mg kg^{-1} was ineffective and a dose of 10 mg kg^{-1} while only slightly reducing the locomotor activity in OB rats increased locomotor activity in sham-operated controls. Thus the effective dose-range of DMI in this model is fairly narrow under the conditions used (twice daily oral administration for 21 days with testing 24 h after the last administration).

On the basis of these preliminary experiments, a dose of 7.5 mg kg^{-1} was selected for further study. At this dose, there was no behavioural reversal in OB rats at 7 days, a partial reversal at 14 days and normalization of behaviour by 21 days. No significant effects of DMI were seen on locomotor activity or rearing in sham-operated controls. Thus the action of DMI in this animal model shows a lag period and is thus broadly in parallel to the action of antidepressants in depressed patients.

Although the possibility of a fundamental relationship between antidepressant action and decreases in cortical β-adrenoceptors has been advocated, the evidence is largely indirect. The greatest weight of evidence derives from consistent reports of decreased numbers of cortical β-adrenoceptors in rats following repeated ECS and monoamine oxidase inhibitors, atypical and tricyclic antidepressants, although the SSRIs do not share this property (Surgue, 1983; Baker & Greenshaw, 1989; Caldecott-Hazard *et al.*, 1991). The current experiments clearly demonstrate a different time course for the behavioural reversal and the decrease in cortical β-adrenoceptors in OB rats during DMI administration. The decrease in the number of β-adrenoceptors in frontal and occipital cortex and hippocampus was marked and near maximal following 7 days of DMI administration, before the effect on OB-related behaviours had developed. Full reversal of OB-related behaviours was seen following 21 days of DMI, but there was no associated change in the number of β-adrenoceptors between 7 and 21 days. As previously reported in normal rats receiving antidepressants, the decrease in β-adrenoceptors was largely restricted to the β_1-adrenoceptors (Minneman *et al.*, 1979; Dooley & Bittiger, 1987; Heal *et al.*, 1989) and was not seen in the striatum.

The rapid decrease in the number of β-adrenoceptors is in apparent conflict with many of the earlier studies of antidepressants in normal rats, in which progressive decreases in β-adrenoceptors over a 2–3 week period were demonstrated (Banerjee *et al.*, 1977; Minneman *et al.*, 1979; Sugrue, 1983). This rapid decrease appears not to be related to OB *per se*, since the density of β-adrenoceptors did not differ between OB and sham controls in any of the brain regions studied, or to OB rats being more sensitive to DMI since the effects of DMI on β-adrenoceptors did not differ between OB rats and sham controls. The solution to this apparent conflict lies in the work of Riva & Creese (1989a,b). They demonstrated that [^{3}H]-dihydroalprenolol ([^{3}H]-DHA), the ligand used in the early studies of β-adrenoceptors, labels sites in addition to β-adrenoceptors when specific binding is defined with high concentrations of propranolol or alprenolol (Riva & Creese, 1989a). They also compared the cortical binding of [^{3}H]-DHA with that of [^{3}H]-CGP 12177, a more selective ligand for β-adrenoceptors, following DMI (15 mg kg^{-1}, i.p.). With [^{3}H]-DHA, binding was not significantly decreased until 7 days of DMI administration (because of an increase in the non-β-adrenoceptor component), whereas with [^{3}H]-CGP 12177, the number of β-adrenoceptor binding sites was reduced by 20% after 2 days and by 34% after 7 days. The present results after 7 days of DMI administration are in good agreement with those of Riva & Creese (1989b). Others have also reported a rapid decrease in cortical β-adrenoceptors following antidepressants in normal rats (Wolfe *et al.*, 1978; Heal *et al.*, 1989; Hosoda & Duman, 1993).

The present results indicate that there is no direct temporal relationship between the reversal of behaviour and the decrease in β-adrenoceptors during DMI administration in this animal model. Although the results are from a single antidepressant drug and thus need to be treated with caution, they suggest that antidepressant drug action is not critically and directly linked to a decrease in β-adrenoceptor binding sites. A more direct involvement of β-adrenoceptor function, such as a decrease in adrenoceptor stimulated adenylyl cyclase, has not been addressed in these studies.

In contrast to the effects on β-adrenoceptors, the number of 5-HT_{2A} receptor binding sites in frontal cortex decreased with the duration of DMI administration, with an indication of an inverse relationship to the behavioural changes. OB alone was not associated with a change in the number of 5-HT_{2A} receptor binding sites and the effects of DMI did not differ between OB and sham controls. There was a differential effect of DMI in frontal cortex compared to occipital cortex, where the number of 5-HT_{2A} receptors following DMI did not differ significantly from saline administration in OB or sham controls. The results indicate that an association between the action of DMI and a reduction in 5-HT_{2A} receptor binding sites in this model is more likely than an association with reduced β-adrenoceptor binding sites.

Many antidepressant drugs when administered repeatedly decrease cortical 5-HT_{2A} receptors although increases, decreases and no alterations have been reported with the SSRIs (Sanders-Bush *et al.*, 1989; Johnson, 1991; Hrdina & Vu, 1993; Klimek *et al.*, 1994) and ECS causes an increase in cortical 5-

HT_{2A} binding sites (Bergstrom & Kellar, 1979). A number of studies of brain tissue obtained at post-mortem examination has provided evidence that people dying by violent suicide have increased numbers of frontal cortical 5-HT_{2A} receptors compared with matched controls (Stanley & Mann, 1983; Mann *et al.*, 1986; Arora & Meltzer, 1989; Arango *et al.*, 1990; Hrdina *et al.*, 1993). As it is likely that some of these subjects would be suffering from depression, these studies provide indirect evidence that depression is associated with increased numbers of frontal cortical 5-HT_{2A} receptors. However, not all studies have reached this conclusion. We have studied 5-HT_{2A} receptor binding in a large sample of suicides and controls and found no evidence for an increase in suicides, or when only those with a clear retrospective diagnosis of depression were considered (Lowther *et al.*, 1994). The effects of clinically relevant doses of antidepressants on 5-HT_{2A} receptors in human brain are unclear and are difficult to address experimentally. We have compared 5-HT_{2A} binding sites in post-mortem brain samples from subjects with a retrospective diagnosis of depression who had been prescribed antidepressant drugs. While such studies are difficult to interpret and subject to a great number of confounding variables, we found no evidence to suggest that 5-HT_{2A} receptor binding was significantly altered in such subjects than in matched controls, even when we considered only subjects who had been prescribed antidepressants for over one year (Lowther *et al.*, 1994).

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