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Antidepressant treatments: effects in rodents on dose-response curves of 5-hydroxytryptamine- and dopamine-mediated behaviours and 5-HT_2 receptor number in frontal cortex

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1 The effects of repeated electroconvulsive shock (ECS) administration, repeated desmethylimipramine injection (5 mg kg^{-1}, twice daily for 14 days) and acute administration of the β-adrenoceptor, clenbuterol, on 5-hydroxytryptamine (5-HT)- and dopamine-mediated behaviours in mice have been examined.

2 All three treatments enhanced the carbidopa/5-hydroxytryptophan (5-HTP)-induced head-twitch response at all doses of 5-HTP examined, producing a parallel shift in the dose-response curve. A single ECS administration or single dose of desmethylimipramine had no effect.

3 Only repeated ECS enhanced the locomotor response to injection of apomorphine. The dose-response curve shift was not parallel. A single ECS had no effect.

4 A 6-hydroxydopamine lesion of brain dopamine terminals also enhanced the apomorphine response, but again did not produce a parallel shift in the dose-response curve.

5 Both repeated ECS and repeated desmethylimipramine administration to rats increased the number of 5-HT_2 receptor sites in rat brain. Clenbuterol had no effect.

6 The enhancing effects of repeated ECS and clenbuterol administration on the 5-HTP-induced head-twitch response were additive.

7 Enhanced 5-HT-mediated behavioural responses are seen in both mice and rats after these treatments. If it is assumed, therefore, that similar receptor changes occur in both species it appears that there is no relationship in either behavioural system between the ability of the treatment to alter receptor number and the change in the dose-response curve (parallel or non-parallel).

8 All three antidepressant treatments (ECS, a tricyclic and a β-adrenoceptor agonist) increase 5-HT-mediated behavioural responses although clenbuterol did not increase 5-HT_2 receptor number. Only ECS increased dopamine-mediated responses.

Introduction

Following administration of repeated electroconvulsive shocks (ECS), rats and mice display enhanced behavioural responses in various 5-hydroxytryptamine (5-HT)-mediated behavioural models (see Green, 1980). Repeated ECS also increases the number of 5-HT_2 receptor sites in rat brain (Kellar, Cascio, Butler & Kurtzke, 1981; Vetulani, Lebrecht & Pilc, 1981; Green, Johnson & Nimgaonkar, 1983) and the enhanced behaviour appears to be a consequence of this increase in receptor number (Green *et al.*, 1983).

The β-adrenoceptor agonist salbutamol, has been shown to be an antidepressant (Lecrubier, Puech, Jouvent, Simon & Widlöcher, 1980; Lerer, Ebstein & Belmaker, 1981). In this study we used the more liposoluble β-adrenoceptor agonist, clenbuterol. Both drugs enhance 5-HT-mediated behaviour (Cowen, Grahame-Smith, Green & Heal, 1982; Nimgaonkar, Green, Cowen, Heal, Grahame-Smith & Deakin, 1983) but do not increase 5-HT_2 receptor number (Nimgaonkar *et al.*, 1983).

Some tricyclic antidepressants have also been shown to increase 5-HT-mediated behaviour following their long-term administration (Friedman & Dallob, 1979; Stolz & Marsden, 1982; Stolz, Marsden & Middlemiss, 1983). However, some of these drugs

have, in contrast, been found to decrease 5-HT_2 number in brain (Peroutka & Snyder, 1980; Kellar *et al.*, 1981).

Repeated ECS, but not clenbuterol, has also been shown to enhance dopamine-mediated behaviour (Green, 1980; Cowen *et al.*, 1982) although dopamine receptor number does not change after ECS (Bergstrom & Kellar, 1979; Deakin, Owen, Cross & Dashwood, 1981).

In all the behavioural studies reviewed above, the enhancement was shown using one or perhaps two doses of the monoamine agonist challenge. No investigation has been made as to whether there is a shift in the log dose-behavioural response curve after a range of drug concentrations.

In this investigation we have looked at log dose-response curves and also the ligand-receptor binding characteristics of the 5-HT_2 receptor in the frontal cortex to determine whether the shape of the dose-response curves reflects a receptor number change.

The conventional 5-HT-mediated behavioural models in rats do not lend themselves to quantifiable dose-response curves. The model used, therefore, was the 5-hydroxytryptophan (5-HTP) head-twitch response in mice, first described by Corne, Pickering & Warner (1963) which produced a reproducible dose-response curve. Radioligand-receptor binding, however, was performed on rat brain tissue both in order to obtain sufficient material for Scatchard analysis using the minimum number of animals and to compare with other binding data. Similarly, dopamine-induced locomotor behaviour was measured in mice, although previous binding data have been obtained in rats. Enhanced behavioural responses using the treatments listed above have been reported in both mice and rats (see Discussion).

Methods

Animals, drugs and ECS administration

Male Sprague Dawley derived rats (Charles River, Ramsgate, Kent), weighing 75–100 g at the start of experiment and male C57B16 Ola mice (Olac, Bicester, Oxon) weighing 18–20 g at the start of experiment were used in all studies. They were houses in groups in conditions of constant heating (21°C) and lighting (light period 08 h 00 min–20 h 00 min) and given food and water *ad libitum*.

Drugs were obtained from the following sources (in parentheses): clenbuterol (Boehringer-Ingelheim), desmethylimipramine (DMI) (Geigy Pharmaceuticals), apomorphine (MacFarlan Smith), carbidopa (Merck, Sharp & Dohme), 5-hydroxytryptophan (Sigma Chemical Co.).

Electroconvulsive shocks were administered to halothane-anaesthetized animals using a Theratronics small animal electroplexy unit. The ECS (90 V for mice, 120 V for rats, 1 s, 50 Hz) were given through ear-clip electrodes. Control animals were anaesthetized and electrodes placed but no current passed.

5-Hydroxytryptamine-induced behaviours

Mice were injected with carbidopa (25 mg kg^{-1} i.p.) followed 15 min later by various doses of 5-HTP 20–200 mg kg^{-1} i.p.). After a further 30 min, head-twitches were counted over a 2 min period by an observer who was 'blind' to the pretreatment (ECS, clenbuterol, desmethylimipramine or control). Both carbidopa and 5-HTP were dissolved in saline to which a few drops of concentrated HCl had been added and which was then back titrated with NaOH to pH 5.5

Apomorphine-induced behaviours

Two pairs of mice (control and experimental) were allowed to settle in cages on Animex activity meters (sensitivity and tuning both set at 30 μA). They were then injected with various doses of apomorphine (0.25–8 mg kg^{-1} i.p.) and the resultant activity changes measured on the meters during the following 40 min.

5-HT_2 receptor binding

5-HT_2-receptor binding studies were performed essentially by the method of Rosenfeld & Makman (1981) using [^{3}H]-spiperone (NEN, specific activity 26.3 Ci $mmol^{-1}$) as the radioligand and LSD (1 μM; Sandoz Pharmaceuticals, Feltham) as the displacing agent for measurement of specific binding. Rat frontal cortex, isolated by the method of Bacopoulos (1981) was homogenized in Tris buffer (pH 7.2) using a motor driven Teflon homogenizer; both total and non-specific binding was always measured in triplicate. In the experiments using tissue from rats treated with DMI, an extra wash of the membranes was included in the procedure. For saturation binding curves, three frontal cortices were combined for each plot and the radioligand concentrations used ranged from 0.3 nM to 5.0 nM.

Scatchard analysis of the data was performed using linear regression analysis by the method of least squares. Results reported are from at least 5 separate experiments, using different tissue.

6-Hydroxydopamine lesioning of brain dopamine systems

Mice weighing approximately 30 g were lightly

anaesthetized with a halothane/air mixture and the skull was located in a simple stereotaxic device. This was achieved by positioning the eyes against fixed reference marks in a triangular head restraint. The skull was then pierced by having a fixed length needle passed down a hinged guide and 50 μg of 6-hydroxydopamine (Sigma Chemical Co.: 2 μl of a 25 μg base μl^{-1} solution made up in ice-cold distilled water containing 2 μg μl^{-1} ascorbic acid) was injected intracerebroventricularly. Control mice were injected with ascorbic solution. Histological examination of the brains after dye injection showed that the success rate for needle positioning was >80%. Whole brain dopamine concentrations were depleted by an average of 80 ± 3% (mean ± s.e.mean; $n = 10$).

Statistics

Head-twitch data were analysed by use of the Mann-Whitney rank order test for non-parametric data. The apomorphine-locomotor activity results and binding data were analysed by use of Student's *t* test (unpaired).

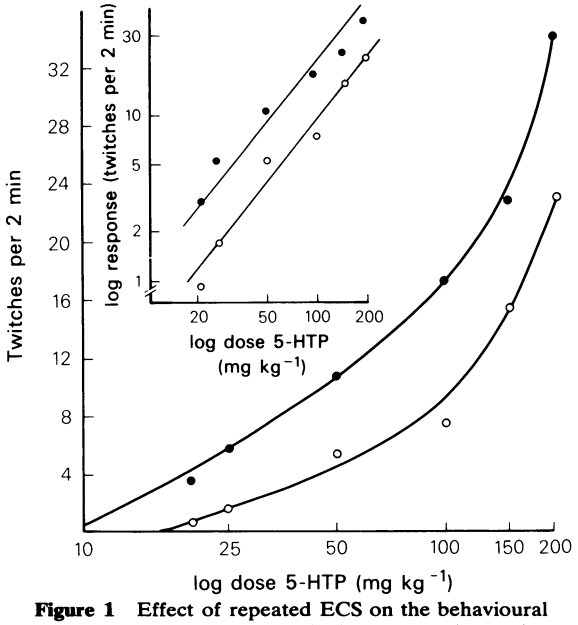

Figure 1 Effect of repeated ECS on the behavioural response to carbidopa and 5-hydroxytryptophan. Mice were given either 5 ECS spread over 10 days (●) or 5 anaesthetic exposures only (○). Twenty-four hours after the last treatment both groups were given carbidopa (25 mg kg^{-1}) followed by various doses of 5-hydroxytryptophan. Main graph shows the log dose of 5-HTP versus total number of head twitches in 2 min, 30 min after the 5-HTP. Small graph shows the log dose-log response curve. Experimental group significantly different from control group ($P < 0.05$ or better) at every dose. Eight animals used at each dose point.

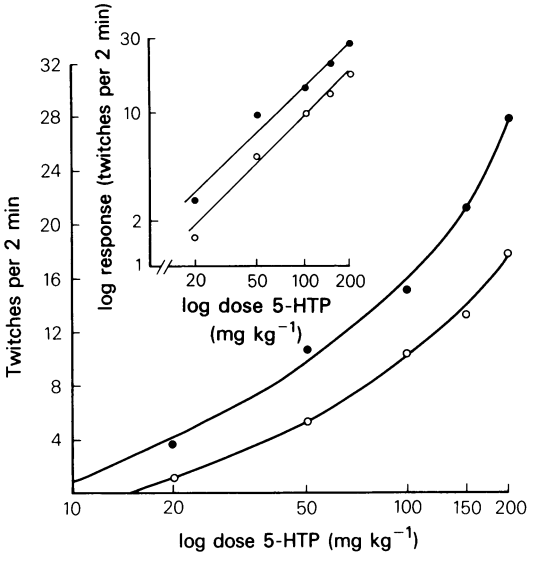

Figure 2 Effect of repeated desmethylimipramine (DMI) doses on the behavioural response to carbidopa and 5-hydroxytryptophan. Mice were given either DMI (5 mg kg^{-1}) twice daily for 14 days (●) or repeated saline injection (○). Eighteen hours after the last treatment both groups were given carbidopa (25 mg kg^{-1}) followed by various doses of 5-hydroxytryptophan. Main graph shows the log dose of 5-HTP versus total number of head twitches in 2 min, 30 min after the 5-HTP. Small graph shows the log dose-log response curve. Experimental group significantly different from control group ($P < 0.05$ or better) at every dose. Eight animals used at each dose point.

Results

5-Hydroxytryptophan dose head-twitch response following repeated ECS

Mice were given a course of ECS (5 over 10 days, see Methods) during halothane anaesthesia or halothane anaesthesia only (control). Twenty-four hours after the last treatment all animals were given carbidopa (25 mg kg^{-1} i.p.) followed 30 min later by 5-HTP at various doses (10 mg kg^{-1}–300 mg kg^{-1} i.p.) and the total number of head-twitches measured during a 2 min period 30 min after the 5-HTP.

At every dose of 5-HTP examined, ECS pretreatment enhanced the response (Figure 1). The maximum response could not be examined as very high doses of 5-HTP resulted in the animals convulsing.

The log dose-log response curves are parallel (Figure 1). Twenty-four hours after a single ECS, no enhancement of the head-twitch response was seen (control mean number of head-twitches: 7.3 (6); ECS × 1: 7.8 (6)).

5-HTP dose and head-twitch response following repeated desmethylimipramine administration

Mice were given either DMI (5 mg kg^{-1} i.p.) twice daily (09 h 30 min and 18 h 00 min) for 14 days or saline (control).

Eighteen hours after the final injection both groups were given carbidopa (25 mg kg^{-1} i.p.) followed 30 min later by 5-HTP at various doses and the total number of head-twitches measured during a 2 min period 30 min after the 5-HTP.

Like ECS, DMI pretreatment also enhanced the response at every dose of 5-HTP examined (Figure 2) and the log dose-log response curves were parallel (Figure 2). A single dose of DMI (5 mg kg^{-1}) did not enhance the head-twitch response (control mean head-twitch response: 7.8 (6), desmethylimipramine-treated: 7.8 (6)).

5-HTP dose and head-twitch response following administration of clenbuterol

Mice were injected with either clenbuterol (1 mg kg^{-1} i.p.) or saline (control) given 15 min before carbidopa (25 mg kg^{-1}) with 5-HTP being given 30 min later. The head-twitch response was measured after a further 30 min.

Enhancement was seen following clenbuterol at all doses of 5-HTP (Figure 3). The result was again a parallel shift in the log dose-log response curve (Figure 3).

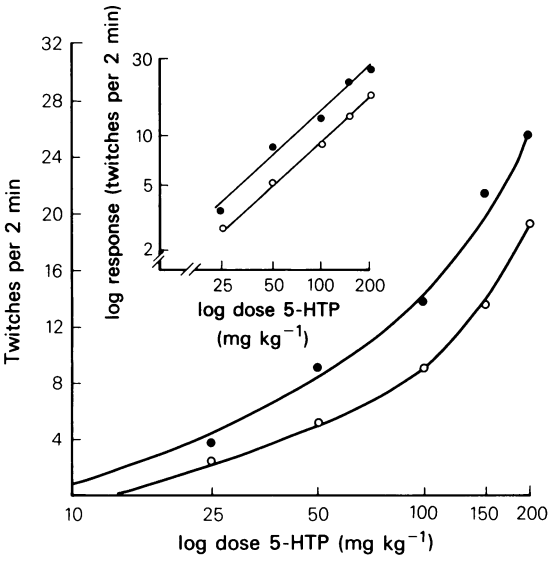

Figure 3 Effect of clenbuterol on the behavioural response to carbidopa and 5-hydroxytryptophan (5-HTP). Mice were given either clenbuterol (1 mg kg^{-1}) (●) or saline injection (○). Thirty min later both groups were given carbidopa (25 mg kg^{-1}) followed by various doses of 5-HTP. Main graph shows the log dose of 5-HTP versus total number of head twitches in 2 min, 30 min after the 5-HTP. Small graph shows the log dose-log response curve. Experimental group significantly different from control group ($P < 0.05$ or better) at every dose. Eight animals used at each dose point.

Table 1 Effects of repeated ECS, repeated desmethylimipramine and acute clenbuterol on the binding characteristics of [^{3}H]-spiperone on the 5-HT$_2$ receptor in rat frontal cortex

Treatment	*Time after last treatment*	*[^{3}H]-spiperone binding characteristics* B_{max}	K_d
Anaesthetic × 5/10 days	24 h	356 ± 17 (3)	1.32 ± 0.01 (3)
ECS × 5/10 days	24 h	494 ± 40 (4)†	1.65 ± 0.04 (4)*
Saline twice daily 14 days	18 h	235 ± 21 (6)	1.35 ± 0.35 (6)
Desmethylimipramine (5 mg kg^{-1}) twice daily 14 days	18 h	414 ± 20 (5)*	1.35 ± 0.15 (5)
Saline	30 min	371 ± 18 (4)	1.16 ± 0.08 (4)
Clenbuterol (5 mg kg^{-1})	30 min	335 ± 27 (4)	1.23 ± 0.15 (4)
Saline daily for 14 days	24 h	295 ± 59 (5)	0.99 ± 0.04 (5)
Clenbuterol (5 mg kg^{-1}) daily for 14 days	24 h	299 ± 43 (5)	1.20 ± 0.20 (5)

Rats were treated as described in Methods, frontal cortex tissue prepared and saturation analysis performed using [^{3}H]-spiperone as ligand. Tissue prepared from the desmethylimipramine-treated rats was given one more wash than that prepared following other treatments. Different from appropriate control: *$P < 0.05$; †$P < 0.01$.
ECS data taken from Green *et al.* (1983).

Effects of ECS, DMI and clenbuterol on 5-HT_2 receptor binding in rat frontal cortex

Rats were treated with repeated ECS, injected with DMI for 14 days or given a single injection of clenbuterol exactly as described for mice above. At the time of the behavioural studies (24 h after the last ECS; 18 h after the last DMI injection and 45 min after clenbuterol) the rats were killed and saturation binding performed.

Intermittent ECS increased the number of 5-HT_2 binding sites (B_{max}) and also the dissociation constant (K_d) (Table 1). Repeated DMI injections also increased the number of 5-HT_2 binding sites but did not alter the dissociation constant (Table 1). The number of binding sites in the control group was lower than in the other control groups. This is almost certainly due to the extra washing of the membranes. The extra work was included to try and ensure that the drug had been fully removed in the tissues of the experimental animals (see Methods). Clenbuterol changed neither the number of 5-HT_2 receptor sites nor the dissociation constant (Table 1). In a further set of experiments it was found that administration of clenbuterol (5 mg kg^{-1}) once daily for 14 days did not affect the 5-HT_2 receptor binding characteristics 24 h after the last dose (Table 1).

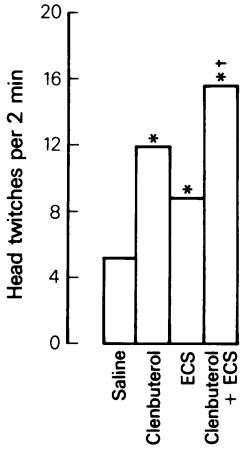

Figure 4 Effect of clenbuterol, ECS and their combination on 5-hydroxytryptophan (5-HTP)-induced head twitch. Mice were given either 5 ECS spread over 10 days during halothane anaesthesia or halothane only. Twenty-four hours after the last treatment all groups were given carbidopa (25 mg kg^{-1}). Fifteen min later half the anaesthetic group ($n = 6$) were given saline and half clenbuterol (1 mg kg^{-1}), whilst half ($n = 6$) the ECS group were given clenbuterol and half saline. Fifteen min later all groups were given 5-HTP (100 mg kg^{-1}). Head twitches were measured for 2 min after a further 30 min. Different from saline group: $^*P < 0.02$. Different from clenbuterol or ECS group: $\dagger P < 0.05$.

Additive effect of clenbuterol and repeated ECS on 5-HTP-induced head-twitch

The binding data suggested that ECS enhanced 5-HT-mediated behaviour by changing receptor characteristics whilst clenbuterol was enhancing the behaviour by acting 'beyond' the receptor. If this supposition is true one might expect the effects of ECS and clenbuterol to be additive. This was examined using the head-twitch response.

Mice were given 5 ECS spread over 10 days during halothane anaesthesia or halothane only. Twenty-four hours after the final treatment some of the control group were then given clenbuterol (1 mg kg^{-1}), others saline. Similar treatments were given to the ECS group. All groups were then given carbidopa followed by 5-HTP (100 mg kg^{-1}). Both clenbuterol and ECS enhanced the head-twitch compared with the control group. Together they produced a further enhancement (Figure 4).

Apomorphine dose-activity response following repeated ECS

Mice were given repeated ECS as in the head-twitch experiments described above. Twenty-four hours after the last treatment the mice were given various doses of apomorphine (0.25–8 mg kg^{-1} i.p.) and the activity response measured in pairs of mice over the subsequent 40 min using Animex activity meters. Even at the highest dose observation suggested that only locomotor type behaviour was present, though some sniffing was seen at higher doses.

No enhancement was seen at the lowest dose of apomorphine used and the enhancement at high doses was less than in the mid-range doses (Figure 5) resulting in a non-parallel shift in the log dose-log response curve (Figure 5). Twenty-four hours after a single ECS no enhancement of the apomorphine-induced activity was seen (Table 2).

Apomorphine-induced activity following repeated DMI, or a single injection of clenbuterol

Mice were given either repeated DMI (5 mg kg^{-1} twice daily for 14 days) or a single dose of clenbuterol (1 mg kg^{-1}) as described above.

Neither treatment enhanced the response to apomorphine (1 mg kg^{-1}) (Table 2).

Apomorphine dose-activity response following a 6-OHDA lesion of brain dopamine systems

The log apomorphine dose-response curve seen following ECS treatment (Figure 5) was of a different shape from that seen with the head-twitch responses (Figures 1–3). We therefore examined whether this

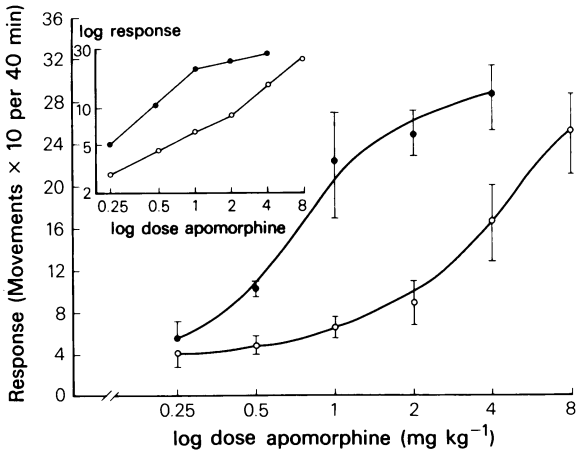

Figure 5 Effect of repeated ECS on the locomotor response to various doses of apomorphine. Mice were given either 5 ECS spread over 10 days (●) or 5 anaesthetic exposures only (○). Twenty-four hours after the last treatment both groups were given apomorphine. Main graph shows the log dose of apomorphine versus the mean (s.e.mean shown as bars) recorded locomotor activity on the Animex meters. Experimental group not different from control group at the 0.25 mg kg^{-1} dose of apomorphine. Experimental group significantly different ($P < 0.05$ or better) at all other doses.

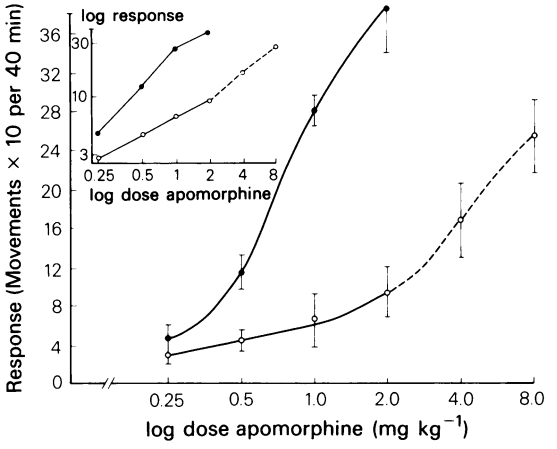

Figure 6 Effect of 6-hydroxydopamine (6-OHDA) lesion on the locomotor response to various doses of apomorphine. Mice were given either a 6-OHDA lesion (●) or a vehicle injection. Twelve days after the lesion or vehicle injection both groups were given apomorphine, Main graph shows the log dose of apomorphine versus the mean (s.e.mean shown as bars) recorded locomotor activity on the Animex meters. Experimental group not different from control group at the 0.25 mg kg^{-1} dose of apomorphine. Experimental group significantly different ($P < 0.05$ or better) at all other doses. Because the control curve was identical to that obtained in Figure 5 no values were obtained above 2 mg kg^{-1}. However, dotted line shows projection obtained from data in Figure 5.

was likely to be because a different type of behaviour was being investigated or because ECS is known not to alter the characteristics of the dopamine receptor, as judged by ligand-receptor binding (Bergstrom & Kellar, 1979; Deakin *et al.*, 1981). To do this mice were treated in a way known to increase dopamine receptor number, namely, denervation (Creese, Burt & Snyder, 1977).

Table 2 Activity response in mice to apomorphine (1 mg kg^{-1}) following single or repeated ECS administration, repeated desmethylimipramine (5 mg kg^{-1} twice daily for 14 days) or a dose of clenbuterol (1 mg kg^{-1})

Injected	*Total locomotor activity/40 min*
Saline	946 ± 629 (5)
Clenbuterol (1 mg kg^{-1})	605 ± 344 (3)
Saline 2 × daily, 14 days	675 ± 193 (4)
Desmethylimipramine (5 mg kg^{-1} 2 × daily, 14 days)	846 ± 459 (5)
Anaesthetic × 1	886 ± 284 (3)
ECS × 1	1102 ± 456 (3)
Anaesthetic × 5 (over 10 days)	649 ± 175 (4)
ECS × 5 (over 10 days)	2216 ± 857 (3)*

Results expressed as mean ± s.e.mean. *Different from control group: $P < 0.01$.

Mice were chemically lesioned with 6-OHDA (see Methods). Fourteen or more days after the lesioning or sham-lesioning they were injected with various doses of apomorphine (0.25–2 mg kg^{-1}).

No enhancement was seen in the lesioned mice at the lowest dose of apomorphine used; however at higher doses of apomorphine there was a marked increase in behavioural response (Figure 6) which, however, did not show a parallel shift in the log dose-response curve (Figure 6) but closely resembled that seen after ECS. Because the 6-OHDA lesioned mice showed a marked locomotor activity even at low doses of apomorphine, responses were not examined at doses above 2 mg kg^{-1}. The sham-lesioned mice were also not examined above this dose as their responses were identical to that seen in the control group in the ECS experiment.

Discussion

The fact that the 5-HT-mediated behavioural responses were measured in mice (because the re-

sponses are more easily quantified) whilst the binding was performed in rats (in order to compare with other data and in order not to use very large numbers of animals) means that firm conclusions cannot be drawn. Nevertheless all the treatments which enhanced the behavioural responses in mice (both to 5-HT and dopamine agonists) can be shown to enhance 5-HT- and dopamine-mediated behaviour in rats.

With the proviso above in mind, it nevertheless appears that it may not be possible to determine whether there has been a change in receptor number by looking at the way that the dose-behavioural response curve shifts. We obtained similar shifts in the head-twitch model with treatments that both did and did not increase receptor number. A similar situation held true for the apomorphine-induced locomotor response with similar curves for treatments thought both to increase and not change dopamine receptor number. Whilst it could be argued that with higher doses of apomorphine the behaviour is changed, this did not appear to be true with the doses used. Furthermore this would not explain the lack of significant enhancement at the low dose of apomorphine. Indeed, the shape of the shift seemed to be determined by the model being examined rather than any receptor change. It would seem, therefore, that the complexities of monoamine-mediated behavioural models prevent any simple deductions being made as to receptor changes occurring on the basis of dose-response curves.

As expected, repeated but not single ECS treatment enhanced the 5-HT-mediated behavioural response as has been shown using a single dose of agonist previously in both rats (Green, Heal & Grahame-Smith, 1977; Green & Deakin, 1980; Green, Sant, Bowdler & Cowen, 1982) and mice (Lebrecht & Nowak, 1981). Previous data have suggested that enhanced behavioural responses to 5-HT agonists are only seen in rats when ECS has been given in ways that increase 5-HT_2 receptor number (Green *et al.*, 1983).

Clenbuterol pretreatment also increased the behavioural response in both species confirming earlier reports (Cowen *et al.*, 1982; Nimgaonkar *et al.*, 1983).

The effect of tricyclic antidepressants on 5-HT function is at present confusing. Both De Montigny & Aghajanian (1978) and Gallagher & Bunney (1979) using microiontophoretic techniques have suggested an increased responsiveness of forebrain neurones to 5-HT following longer term tricyclic administration. De Montigny (1981) has also seen a similar change after repeated ECS. Friedman & Dallob (1979), Stolz & Marsden (1982) and Stolz *et al.* (1983) have shown that longer term pretreatment with some antidepressants can result in enhanced behavioural responses to 5-HT agonists and Cowen (personal communication) has observed enhanced quipazine-mediated behaviours in rats given DMI on the same protocol as used here for mice. Against this, Peroutka & Snyder (1980) and Kellar *et al.* (1981) have reported that 5-HT_2 receptor number is decreased following longer term tricyclic administration and Stolz *et al.* (1983) found no clear-cut change in binding characteristics.

Using a fairly low dose of DMI and a brief (18 h) period of withdrawal we have also observed in mice a marked increase in 5-HT-mediated behaviours following DMI. A similar change has also be observed in rats (Cowen, personal communication). However, in addition, an increase in 5-HT_2 receptor number was also seen. Thus there was an apparent correlation between an increase in 5-HT_2 receptor number and enhanced behaviour. This correlation is given further strength by the recent observations which suggest that the commonly used 5-HT-induced behavioural models, including the head-twitch response are 5-HT_2 receptor mediated (Peroutka, Lebkovitz & Snyder, 1981; Ortmann, Bischoff, Radeke, Buech & Delini-Stula, 1981; Green, O'Shaughnessy, Hammond, Schächter & Grahame-Smith, 1983). Clenbuterol, in contrast, seems to work 'beyond' the receptor to enhance the behavioural response and it would appear that β-adrenoceptor agonists act by modulating the response in some way.

The question arises, therefore, as to the reasons for the discrepancy between our binding data and those of Peroutka & Snyder (1980) and Kellar *et al.* (1981). We would suggest that the most plausible explanation is as follows.

Many of the tricyclic antidepressants are 5-HT_2 receptor antagonists (see Green & Nutt, 1983) and when rats were examined in a 5-HT behavioural model a short time after the last of a series of doses of some of these drugs, an inhibited response was seen (Stolz *et al.*, 1983). The decrease in binding seen previously (Peroutka & Snyder, 1980; Kellar *et al.*, 1981) therefore may result from the presence of drug still bound to the receptor, leading to an apparent decrease in the number of receptors. The dose, number of administrations of drug and withdrawal time could all, therefore, be crucial to the result obtained. ECS administration, of course, does not produce such complications.

Since ECS increases 5-HT_2 receptor number whilst clenbuterol acts 'beyond' the receptor, one might expect their effects in enhancing 5-HT-mediated behaviour to be additive and this was, in fact, seen.

Repeated ECS also increased the dopamine-mediated behavioural response, seen after administration of the dopamine agonist, apomorphine, in

both rats and mice. This does not appear to be a receptor-mediated change since several workers have been unable to find any change in either dopamine-receptor binding (Bergstrom & Kellar, 1979; Deakin *et al.*, 1981) or dopamine-sensitive adenylate cyclase (Green *et al.*, 1977). Neither clenbuterol nor DMI altered the apomorphine-induced behaviour.

In conclusion, therefore, all three antidepressant treatments studied enhanced 5-HT-mediated behaviour in mice as has previously been shown in rats. Binding studies in rats suggested that whilst ECS and desmethylimipramine increased 5-HT_2 receptor number, this change did not occur after clenbuterol. Only ECS increased dopamine-mediated behaviour.

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