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5-HT_2 receptor characteristics in frontal cortex and 5-HT_2 receptor-mediated head-twitch behaviour following antidepressant treatment to mice

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1 The effects of repeated administration of antidepressant drugs or electroconvulsive shock on the binding of [^{3}H]-spiperone to the 5-hydroxytryptamine$_2$ (5-HT_2) receptor in mouse frontal cortex and the 5-HT-mediated head-twitch response have been examined.

2 Repeated electroconvulsive shock increased both the head-twitch response and the number of 5-HT_2 binding sites (B_{max}).

3 After 35 d but not 24 h or 14 d oral tranylcypromine (5.6 mg kg^{-1} per day) there was a marked decrease in both the behavioural response and the number of 5-HT_2 receptors.

4 Repeated oral doses of zimeldine (20 mg kg^{-1} per day, 14 days) also decreased the head-twitch response and the number of 5-HT_2 binding sites and these effects persisted after 48 h withdrawal.

5 Oral mianserin (2.1 mg kg^{-1} per day, 14 days) decreased both the behaviour and the number of 5-HT_2 binding sites, but this change was also seen after acute (1 day) administration. After 48 h withdrawal from chronic treatment the head-twitch response was still decreased but the B_{max} had returned to control values.

6 Desipramine given orally (27 mg kg^{-1} per day, 14 days) decreased both the behaviour and number of 5-HT_2 binding sites. After 48 h withdrawal, binding was still decreased but the head-twitch response was enhanced above control values.

7 In contrast to repeated electroconvulsive shock (ECS), all drugs decreased both 5-HT_2 binding and the head-twitch response, while the mice were still on treatment. Binding and behaviour did not correlate after withdrawal. It is concluded that antidepressant treatments do not produce a common alteration in 5-HT_2 receptor number and function.

Introduction

Repeated administration of electroconvulsive shock (ECS) increases 5-hydroxytryptamine- (5-HT) mediated behavioural responses in rats and mice and [^{3}H]-spiperone binding to the presumed 5-HT_2 receptor in the frontal cortex of rats (Evans *et al.*, 1976; Lebrecht & Nowak, 1980; Vetulani *et al.*, 1981; Kellar *et al.*, 1981; Green *et al.*, 1983a, b). Since electroconvulsive therapy (ECT) is an effective treatment for severe depressive illness (see, for example, Royal College of Psychiatrists, 1977; Kendell, 1981; Brandon *et al.*, 1984) and abnormalities of 5-HT neuronal function may be an important aetiological factor in depression (Goodwin & Post, 1983), it is tempting to endow the ECS-induced increases in 5-HT-mediated function with therapeutic significance (Grahame-Smith *et al.*, 1978). However, comparison must then be made with the effects of antidepressant drugs, and it is their effects on both 5-HT-mediated behaviour and binding that are the subject of the present work.

The head-twitch behaviour evoked in mice by 5-HT or 5-HT receptor agonists appears to be mediated by 5-HT_2 receptors (Peroutka *et al.*, 1981; Ortmann *et al.*, 1982; Green *et al.*, 1983c). This behaviour has been used previously to investigate both the acute and longer term effects of antidepressant drugs on 5-HT function. For example, following acute administration, several antidepressant drugs inhibit the head-twitch response (Ogren *et al.*, 1979) and Friedman *et al.*, (1983) also described inhibition following 14 days treatment. However, after 48 h withdrawal from chronic administration, increased head-twitch responses were observed (Stolz & Marsden, 1982; Friedman *et al.*, 1983; Stolz *et al.*, 1983).

Most studies on 5-HT_2 receptor binding have been conducted in rats with varying periods of drug withdrawal and most reports have described reduced binding (for example, Peroutka & Snyder, 1980; Kellar *et al.*, 1981).

We have now studied both head-twitch behaviour and 5-HT_2 receptor binding in the same species and under identical conditions of drug treatment. This has allowed a more definitive comparison between behavioural and molecular indices of 5-HT function, both whilst the animals were on drug treatment and after withdrawal. In addition, we have administered all antidepressant drugs orally in line with both clinical use and drug development practice.

Methods

Animals, drugs and ECS administration

Adult male C57/B16/Ola mice (Olac, Bicester) weighing 20–30 g were used in all experiments. They were housed in groups of ten under conditions of controlled lighting (dark period 20 h 00 min–08 h 00 min) and temperature (20° ± 1 °C) and given an *ad libitum* diet of modified 41B pellets and tap water. All behavioural observations and killings for biochemical determinations were performed between 09 h 00 min and 17 h 30 min.

The following drugs were used (abbreviations and source in brackets): 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT, Sigma), 5-hydroxytryptophan (5-HTP, Sigma), carbidopa (Merck Sharp & Dohme, Hoddesdon), zimeldine dihydrochloride (Astra Pharmaceuticals, Södertälje, Sweden), tranylcypromine sulphate (Smith, Kline & French, Welwyn), desipramine hydrochloride (Geigy Pharmaceuticals, Horsham) mianserin hydrochloride (Organon, Lanark), halothane (ICI Pharmaceuticals, Macclesfield).

Doses are quoted as the salt where appropriate. Drugs given orally were made up in distilled water and the solution provided *ad libitum* as the only source of drinking water. Consumption was measured by bottle weighing.

Injected drugs were dissolved in 0.9% w/v NaCl solution (saline), sometimes with the aid of sonication.

Mice were anaesthetized with halothane and ECS (90 V, 1 s, 50 Hz) was given via ear-clip electrodes from a Theratronics small animal electroplexy unit. Control animals were given halothane only.

Behavioural measurements

Head-twitch behaviour was provoked either by administration of the 5-HT agonist 5-MeODMT or the 5-HT precursor 5-HTP. In the former case 5-MeODMT was injected i.p. and head-twitches counted over the next 6 min. In the latter case mice were injected with carbidopa (25 mg kg^{-1} i.p.) followed 15 min later by 5-HTP (100 mg kg^{-1} i.p.) with the number of head twitches in a 2 min period being counted 15 min later. Head-twitches were counted by an observer unaware of the treatment condition whenever possible. Testing with an agonist was used when it was felt that the administered drug would alter the effect of the precursor (monoamine oxidase (MAO) inhibitors or 5-HT uptake inhibitors).

Ligand receptor binding

5-HT_2 receptor binding studies were performed essentially by the method of Rosenfeld & Makman (1981) using [^{3}H]-spiperone (N.E.N., specific activity 26.3 Ci $mmol^{-1}$) as the radioligand and lysergic acid diethylamide (LSD, 1 μM; Sandoz Pharmaceuticals, Feltham) as the displacing agent for measurement of specific binding. Frontal cortex tissue dissected from four mice by the method of Bacopoulos (1981) was combined for each assay. It was homogenized in Tris buffer (pH 7.2) with a motor driven Teflon homogenizer; both total and non-specific binding was always measured in triplicate. Saturation binding curves used radioligand at 6 concentrations from 0.3 nM to 5.0 nM and Scatchard analysis of the data was performed using linear regression analysis by the method of least squares. Assays were repeated on different tissues at least three times for each experimental condition. Results were rejected if the correlation coefficient was less than 0.7, but the great majority were above 0.85. Brain protein concentration was determined by the method of Lowry *et al.* (1951).

MAO activity measurement

Mouse brains were homogenized in 0.32 M sucrose and MAO activity towards [1-^{14}C]-5-hydroxytryptamine (Amersham International) measured by the method of Southgate and Collins (1969). Enzyme activity was calculated as nmol deaminated product formed in 1 mg protein per 30 min incubation and results calculated as the percentage inhibition compared with the control group.

Statistics

All behavioural data were compared by use of the Mann-Whitney rank order test for non-parametric data. Binding data were compared using Student's *t* test (2-tail, unpaired).

Table 1 Effect of repeated electroconvulsive shock (ECS) on 5-hydroxytryptophan (5-HTP)-induced head-twitch and 5-HT$_2$ receptor binding

	Behaviour	*5-HT$_2$ receptor binding*	
	Total head-twitches per 2 min	B_{max}	K_D
Control	5.9 (8)	207 ± 28 (3)	1.57 ± 0.23 (3)
ECS	14.2 (8)**	248 ± 25 (7)*	1.48 ± 0.92 (7)

Groups of mice received ECS during halothane anaesthesia or halothane alone (controls). Results are shown as mean head-twitch response and mean ± s.e.mean for binding data (B_{max}: fmol mg^{-1} protein; K_D:nM) with number of observations in parentheses. Different from control: *$P < 0.05$; **$P < 0.01$.

Results

Effect of repeated ECS on 5-HTP-induced head-twitch and 5-HT$_2$ receptor binding

Mice were given 5 ECS spread over 10 days (Mon, Wed, Fri, Mon, Wed) with both behavioural and biochemical measures made 48 h after the last treatment.

In confirmation of previous studies there was a significant increase in the head-twitch response of the mice following repeated ECS (Table 1). An increase in the number of 5-HT$_2$ binding sites (B_{max}) in the frontal cortex was also seen after repeated ECS (Table 1).

Effect of tranylcypromine on head-twitch behaviour and 5-HT$_2$ receptor binding

Mice were given either distilled water (control group) or distilled water containing tranylcypromine (30 mg l^{-1}) following a loading dose on day 1 of 6 mg kg^{-1}. The experimental group consumed a dose of 5.6 mg kg^{-1} per day. This regime produced almost complete inhibition of MAO activity when tested at 24 h and 14 d (> 90% inhibition compared with control group, $n = 6$ in each group).

Following either acute drug administration (24 h) or 14 d continuous treatment there were no changes in either the characteristics of 5-HT$_2$ receptor binding or the behavioural responses to 5-MeODMT (Figure 1). However, by 35 days of treatment there was a marked decrease in both the head-twitch response and the number of 5-HT$_2$ binding sites (B_{max}) whilst the dissociation constant was unaffected (Figure 1). The animals were not withdrawn from the drug at any time of testing.

Effect of zimeldine on head-twitch behaviour and 5-HT$_2$ receptor binding

Mice were given either distilled water (control group) or distilled water containing zimeldine (100 mg l^{-1})

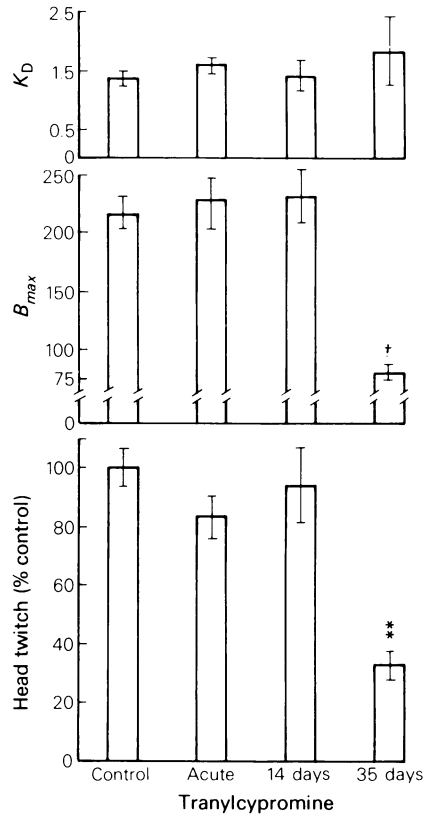

Figure 1 The parameters of [^{3}H]-spiperone binding (K_D:nM; B_{max}:fmol mg^{-1} protein), together with mean head-twitch response evoked by 5-methoxy-*N,N*-dimethyltryptamine (15 or 20 mg kg^{-1} i.p.), expressed as a percentage of untreated controls run simultaneously. Tranylcypromine given as loading dose (6 mg kg^{-1} i.p.), then orally (5.6 mg kg^{-1} per day). Binding and behaviour determined under control (untreated) condition, 24 h, 14 d and 35 d after starting drug treatment. Results shown as mean of at least 8 observations (behaviour) and 3 observations (binding); vertical lines show s.e.mean. Different from control groups: †$P < 0.025$; **$P < 0.01$.

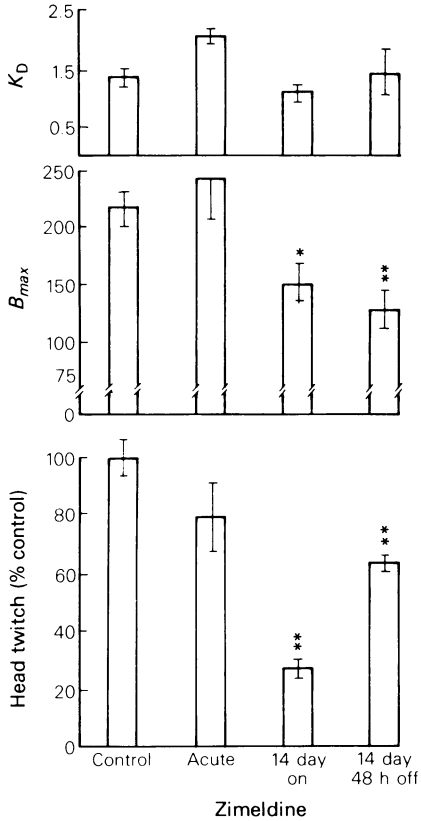

Figure 2 The parameters of specific [^{3}H]-spiperone binding (K_D:nM; B_{max}:fmol mg^{-1} protein), together with head-twitch response evoked by 5-methoxy-*N,N*-dimethyltryptamine (20 mg kg^{-1} i.p.) expressed as a percentage of untreated controls run simultaneously. Zimeldine given as loading dose (20 mg kg^{-1} i.p.) then orally (20 mg kg^{-1} per day). Binding and behaviour determined in control (untreated) condition, 24 h and 14 d after starting drug treatment and 48 h after discontinuing chronic (14 d) treatment. Results shown as mean of at least 8 observations (behaviour) and 3 observations (binding); s.e.mean shown by vertical lines. Different from control groups: $^*P<0.05$; $^{**}P<0.01$.

following a loading dose on day 1 of 20 mg kg^{-1}. The experimental group consumed a dose of 20 mg kg^{-1} per day. Twenty-four hours after acute drug administration there were no differences between the control and experimental groups in terms of either head-twitch behaviour or 5-HT$_2$ receptor binding (Figure 2). Following 14 d drug administration, whilst the mice were still undergoing treatment, the head-twitch response to 5-MeODMT had decreased by over 70% (Figure 2). The number of 5-HT$_2$ receptor sites had also declined although there was no change in the dissociation constant (Figure 2). These changes were still maintained 48 h after drug withdrawal (Figure 2).

Effect of mianserin on head-twitch behaviour and 5-HT$_2$ receptor binding

Mice were given either distilled water or distilled water containing mianserin (10 mg l^{-1}) following a loading dose on day 1 of 2 mg kg^{-1}. The experimental group consumed a dose of 2.1 mg kg^{-1} per day. Twenty-four hours after acute administration, whilst the mice were still undergoing treatment they showed

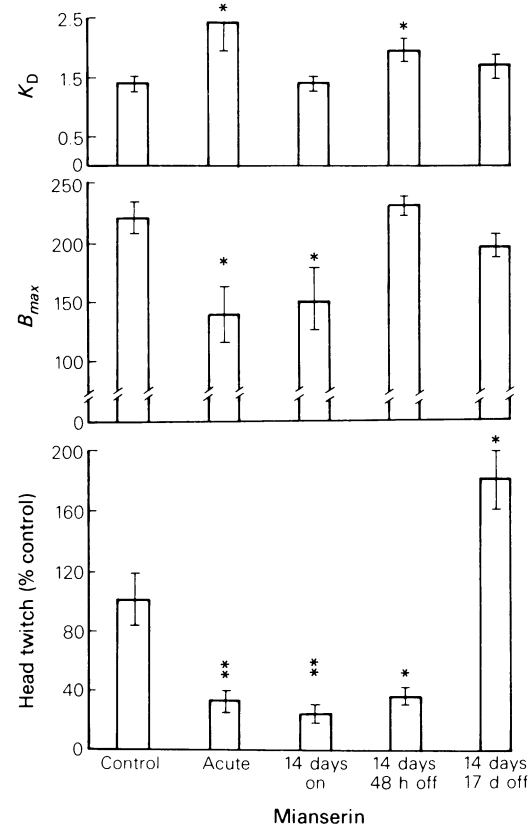

Figure 3 The parameters of [^{3}H]-spiperone binding (K_D:nM; B_{max}:fmol mg^{-1} protein), together with mean head-twitch response evoked by 5-hydroxytryptophan (100 mg kg^{-1} i.p.) expressed as a percentage of untreated controls run simultaneously. Mianserin given as loading dose (2 mg kg^{-1} i.p.), then orally (2.1 mg kg^{-1} per day). Binding and behaviour determined under control (untreated) condition, 24 h and 14 d after starting drug treatment and 48 h and 17 d after discontinuing chronic (14 d) treatment. Results shown as mean of at least 8 observations (behaviour) and 3 observations (binding); s.e.mean shown by vertical lines. Different from control groups: $^*P<0.05$; $^{**}P<0.01$.

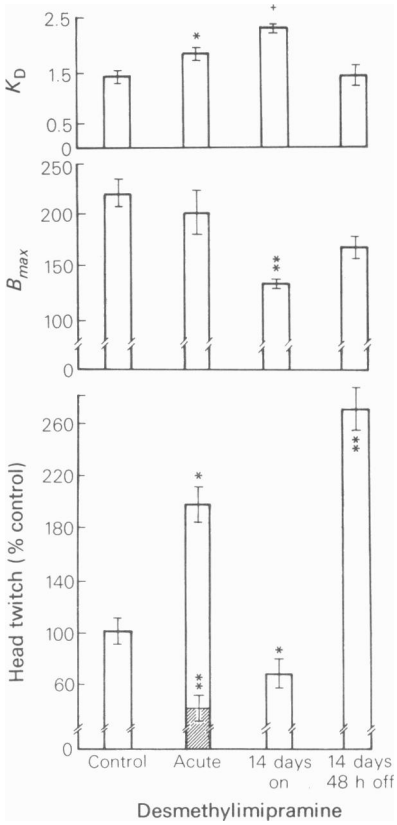

Figure 4 The parameters of [^{3}H]-spiperone binding (K_D:nM; B_{max}:fmol mg^{-1} protein), together with the mean head-twitch response evoked by 5-hydroxytryptophan (100 mg kg^{-1} i.p.) or 5-MeoDMT (20 mg kg^{-1} i.p.); shaded column) expressed as a percentage or mean of untreated controls run simultaneously. Desipramine given as loading dose (30 mg kg^{-1} i.p.), then orally (27 mg kg^{-1} per day). Behaviour and binding determined under control (untreated) condition, 24 h and 14 d after starting drug treatment and 48 h after discontinuing chronic (14 d) treatment. Results shown as mean of at least 8 observations (behaviour) and 3 observations (binding); s.e.mean shown by vertical lines. Different from control groups: $*P<0.05$; $**P<0.01$.

decreased head-twitch responses following administration of carbidopa and 5-HTP (see Methods). The number of 5-HT$_2$ receptor sites was also decreased and there was an increase in the dissociation constant (Figure 3). The head-twitch response was similarly decreased both after 14 days treatment with mianserin (and still on the drug) and after 48 h withdrawal (Figure 3). The B_{max} for 5-HT$_2$ binding was decreased whilst the mice were on longer term treatment, but had returned to control values 48 h after withdrawal. The K_D was not found to be increased after longer term treatment although this was seen after 48 h withdrawal (Figure 3).

Effect of desipramine on head-twitch behaviour and 5-HT$_2$ receptor binding

Mice were given either distilled water or distilled water containing desipramine (150 mg l^{-1}) following a loading dose of 30 mg kg^{-1}. The experimental group consumed a dose of 27 mg kg^{-1} per day. Following acute drug administration the mice showed an enhanced head-twitch response when the response was elicited with carbidopa and 5-HTP. However, they showed an inhibited response when it was produced by injection of 5-MeODMT (Figure 4). There was a modest increase in the K_D for 5-HT$_2$ receptor binding but no change in the number of binding sites (Figure 4). After 14 days drug administration whilst the mice were still on treatment there was a decrease in both the head-twitch response and the 5-HT$_2$ receptor number while the K_D was increased (Figure 4). After 48 h withdrawal the K_D had returned to control values but the B_{max} remained increased, although this just failed to reach statistical significance ($P<0.06$). In contrast, the head-twitch response was now increased considerably above control values (Figure 4).

Discussion

The present results allow behavioural data and ligand-receptor binding parameters to be compared in the same species under identical treatment conditions.

ECS increased both the B_{max} of 5-HT$_2$ binding and the head-twitch response. Enhanced 5-HT-mediated behaviour after ECS has been shown several times in both mice and rats (see Introduction and Green, 1984, for review). However, whilst ECS has been shown to increase [^{3}H]-spiperone binding in rat frontal cortex (Vetulani *et al.*, 1981, Kellar *et al.*, 1981; Green *et al.*, 1983a, b), this is the first report of intermittent ECS increasing the B_{max} or 5-HT$_2$ binding in mouse brain. Increased [^{3}H]-spiperone binding following ECS can therefore be regarded as an authentic finding. We regard it, and the other pharmacological changes produced by ECS, as important pointers towards the understanding of the effects of other antidepressant treatments; not least because ECS avoids both the uncertainties of dose and the possible spurious interference with binding techniques that are unavoidable with drug treatment.

[^{3}H]-spiperone binding as used in this study is believed to reflect binding to the 5-HT$_2$ receptor (Peroutka & Snyder, 1979; Leysen *et al.*, 1982) and

head-twitch behaviour is probably mediated through 5-HT_2 receptors (Peroutka *et al.*, 1981; Ortmann *et al.*, 1982; Green *et al.*, 1983c). It is therefore natural to wish to relate changes in binding to changes in behaviour even though it is unlikely that the frontal cortex is directly involved in the head-twitch response.

The antidepressants used in this study were chosen to reflect a variety of pharmacological actions rather than their clinical importance. Desipramine is a relatively selective noradrenaline uptake blocker while zimeldine shows marked selectivity towards inhibition of 5-HT uptake (see Iversen & Mackay, 1979). In contrast, mianserin has little effect on monoamine uptake (Baumann & Maitre, 1977) and tranylcypromine is a potent MAO inhibitor.

For ease of discussion, the effects of the drugs on binding and behaviour are considered separately initially.

Effects of antidepressants on [^{3}H]-spiperone binding

By contrast with ECS, none of the drugs studied increased B_{max} at any stage of drug exposure. All of them decreased B_{max} but with very different time courses. Mianserin produced a reduction in B_{max} within 24 h; an effect comparable with that seen for other antagonists at the 5-HT_2 receptor (Blackshear *et al.*, 1983) and consistent with previous observations on the effect of this drug on 5-HT_2 binding in rats (Blackshear & Sanders-Bush, 1982). Zimeldine and desipramine produced comparable effects on the B_{max} after 14 d treatment, and the reduction persisted after withdrawal of the drugs (Figures 3 and 4). However, the mechanism of the effect of these two drugs seems unlikely to be their binding to the 5-HT_2 receptor since the IC_{50} values for desipramine and zimeldine (3.5 μM and 11 μM respectively) are much higher than that of mianserin (0.1 μM) (Hall & Ogren, 1981). The argument using these *in vitro* values is, of course, weakened by the existence of active metabolites *in vivo* (Ross & Renyi, 1977) and the observation that acute administration of desipramine inhibited the head-twitch response elicited by the 5-HT agonist, 5-MeODMT (Figure 4). The increase in K_D observed in our experiments with mianserin and desipramine argues for the presence of the drugs in the tissue and their interaction with the receptor. In contrast, no evidence was obtained for tranylcypromine binding to the receptor but when given for a sufficiently long time, this compound also increased the B_{max}.

It seems reasonable to suggest that the decrease in B_{max} following zimeldine, tranylcypromine and possibly desipramine results from an increase in the concentration of 5-HT in the synaptic cleft, due either to uptake blockade or MAO inhibition. Zimeldine has unquestioned 5-HT uptake blocking effects and while desipramine is a weak 5-HT uptake inhibitor, the fact that it potentiated the head-twitch response when this was provoked by precursor loading suggests that at the dose given 5-HT uptake inhibition is occurring. The decrease in 5-HT_2 binding following zimeldine, desipramine and tranylcypromine treatment therefore appears to be a 'down-regulation' of the number of receptors. In the case of mianserin this may also be true, given the long-lasting change observed by Blackshear & Sanders-Bush (1982). However, in mice we observed a decrease in B_{max} only when the animals were on drug treatment. It could be, therefore, that the decrease is the result of the antagonist properties of this drug at the 5-HT receptor, although antagonists added *in vitro* have not been shown to decrease the B_{max} of [^{3}H]-spiperone binding (Blackshear *et al.*, 1983).

In summary, all the antidepressant drugs investigated reduced [^{3}H]-spiperone binding in mouse frontal cortex, in accord with other studies in rats (Peroutka & Snyder, 1980; Kellar *et al.*, 1981) and is here shown to be associated with a reduced B_{max} in each case. An increase in K_D also sometimes occurred, limiting interpretation of any single point binding studies (e.g. Peroutka & Snyder, 1980; Stolz *et al.*, 1983).

Effects of antidepressants on 5-HT_2-mediated behaviour

If the changes in binding seen *in vitro* reflect a functional change in the brain, then the behavioural responses evoked *in vivo* via 5-HT_2-receptor stimulation should show parallel changes to the binding and this was broadly true under conditions of chronic and continuing drug treatment.

Drug withdrawal produced changes in 5-HT-mediated behaviour in the case of both desipramine and mianserin that were not deducible from changes in receptor number. Desipramine increased head-twitch following withdrawal without an increase in receptor number. The enhanced behavioural response following tricyclic withdrawal has been previously described by other workers (Friedman & Dallob, 1979; Stolz & Marsden, 1982; Friedman *et al.*, 1983; Stolz *et al.*, 1983; Green *et al.*, 1983b). Following mianserin withdrawal the behaviour remained depressed while B_{max} returned to normal. In addition, there was a delayed facilitation of head-twitch, seen 2–3 weeks following withdrawal (Figure 3). Changes in noradrenergic transmission may be involved in the behavioural responses seen on withdrawal since changes in α_2-adrenoceptor function alter the head-twitch response (Handley & Brown, 1982). Administration of drugs with reasonable selectivity for inhibiting 5-HT uptake produce an

inhibited head-twitch response even after withdrawal (zimeldine, this paper; fluoxetine and clomipramine, Stolz *et al.*, 1983).

Implications for the mechanisms of antidepressant effect

All antidepressant drugs examined produced reduced [^{3}H]-spiperone binding and a reduced head-twitch response after chronic and continuing drug treatment. The net consequence of this change for the physiological function of 5-HT neurones is uncertain since neither parameter takes into account changes in concomitant presynaptic function. Zimeldine alters uptake, tranylcypromine inhibits metabolism, mianserin and desipramine interfere with presynaptic receptors associated with release mechanisms.

In any event, the end result for the postsynaptic receptor during continuing drug therapy is the opposite of that seen with ECS. This contrasts with the more consistent changes seen in the noradrenergic system. Most antidepressant treatments, including ECS, can be shown to down-regulate β-adrenoceptors and reduce α_2-adrenoceptor function (Bergstrom Kellar, 1979; Sellinger-Barnette *et al.*, 1980; Sulser & Mobley, 1981; Heal *et al.*, 1981; 1983; and see Green & Nutt, 1983 for review). Thus, whatever antidepressant treatments have in common, it is not a consistent change in 5-HT$_2$ function. However, this is not to deny any involvement of 5-HT in the action of antidepressant treatments since intact 5-HT innervation is required for the decrease in β-adrenoceptor number produced by desipramine (Brunello *et al.*, 1982; Janowsky *et al.*, 1982).

Lastly, the functional changes seen during drug withdrawal are of interest in examining neurotransmitter interactions but are, perhaps, of little relevance to the therapeutic effect of antidepressant drugs.

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