British Journal of Pharmacology (2005) 145, 587–592
www.nature.com/bjp

Effects of amantadine and budipine on antidepressant drug-evoked changes in extracellular 5-HT in the frontal cortex of freely moving rats

[1]Jenny C.E. Owen & *,[1]Peter S. Whitton

[1]Department of Pharmacology, The School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX

1 Evidence has recently suggested that NMDA receptors may play a role in the aetiology and possible treatment of depression and that weak noncompetitive NMDA receptor antagonists such as amantadine can synergize with conventional antidepressants in a model of the illness.

2 To try to obtain a neurochemical rationale for these findings, we have studied the effects of acute and chronic administration of amantadine or the related drug budipine on cortical release of 5-hydroxytryptamine (5-HT) following the antidepressants reboxitine (REB), paroxetine (PAROX) and clomipramine (CLOM) in freely moving rats by using microdialysis.

3 Acute administration of amantadine (40 mg kg^{-1}), budipine (10 mg kg^{-1}), REB (10 mg kg^{-1}), PAROX (10 mg kg^{-1}) or CLOM (10 mg kg^{-1}) all failed to significantly alter extracellular 5-HT in the cortex. However, when either amantadine or budipine was administered 30 min prior to any of the three antidepressants, a significant rise in 5-HT was observed.

4 For chronic studies, the effects of the drugs were studied at 4, 7, 14 and 21 days. Amantadine and budipine did not significantly alter extracellular 5-HT at any time point. The three antidepressant drugs all elicited a gradual increase in 5-HT, which became significant after 14 days and tended to plateau thereafter. When either amantadine (20 mg kg^{-1}) or budipine (5 mg kg^{-1}) was coadministered with any of the three antidepressants, two differences were seen compared with the effects of the antidepressants alone. Firstly, the time required for significant increases in cortical 5-HT was reduced with elevated levels now being observed by 7 days. Secondly, the absolute magnitude of the increase in extracellular 5-HT was markedly greater in these rats from day 7 until the end of the experiment.

5 If, as is widely considered, an increase in extracellular 5-HT represents a critical step in the mechanism of action of antidepressants, these data suggest that combined treatment with clinically tolerated NMDA antagonists such as amantadine could reduce the delay in therapeutic onset of antidepressants as well as possibly enhance their efficacy.
British Journal of Pharmacology (2005) **145,** 587–592. doi:10.1038/sj.bjp.0706188
Published online 18 April 2005

Keywords: Antidepressants; amantadine; budipine; 5-HT; cortex; microdialysis

Abbreviations: ADs, antidepressant drugs; CLOM, clomipramine; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; PAROX, paroxetine; REB, reboxetine; SNRI, selective noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor

Introduction

For several decades, the monoamine theory of depression has been predominant with regard to the aetiology of the illness itself as well as the rationale behind the bulk of treatments available in the clinic. Currently, some of the most widely prescribed antidepressant drugs (ADs) are those that have high degrees of selectivity for the 5-hydroxytryptamine (5-HT) transporter, the selective serotonin reuptake inhibitors (SSRIs) and, to a lesser extent, those with a high degree of selectivity for the noradrenaline transporter, the selective noradrenaline reuptake inhibitors (SNRIs). Despite the potency of drugs in these classes, they offer little therapeutic improvement on earlier generations of antidepressants. Although generally having a markedly superior side-effect profile, they are similarly not clinically effective in a significant proportion of patients (Lieberman *et al*., 2005). Furthermore, like earlier generations of antidepressants, both SSRIs and SNRIs require a period of several weeks for full therapeutic effect to occur (Blier & De Montigny, 1994). This time lag is clearly emotionally undesirable for the patient and can be a serious consideration in those patients at high risk of suicide. (Lieberman *et al*., 2005). Although the exact mechanism responsible for this delay in therapeutic onset is still under debate, there is a general agreement that this must involve neuroadaptive changes at the cellular and/or receptor level, leading to net alterations in neurotransmission (e.g. see Blier & De Montigny, 1994).

Evidence has now suggested that the mechanism of AD action may be partly dependent on a reduction in NMDA receptor function (Skolnick, 1999). For example, both competitive and noncompetitive NMDA receptor antagonists show AD-like activity when investigated in animal paradigms of the illness (Maj *et al*., 1992; Layer *et al*., 1995). More recently, it has been demonstrated that treatment with weak

*Author for correspondence; E-mail: peter.whitton@ulsop.ac.uk

NMDA noncompetitive antagonists has a synergistic type of response with a number of different ADs when assessed in the forced swim test (Rogoz *et al*., 2002; 2004). This suggests that neurochemical effects beyond alterations in monoaminergic transmission may be mechanistically significant in AD drug action. Amantadine and also budipine are clinically used compounds, which have been found to be weak noncompetitive antagonists at the NMDA receptor. We have speculated that since these compounds may induce a decrease in NMDA receptor function and appear, in the case of amantadine at least, to potentiate the behavioural effects of ADs (Maj & Rogoz, 2000; Rogoz *et al*., 2002; 2004), it seems logical that they may potentiate the neurochemical effects of ADs. To study this possibility, we have investigated the effects of coadministration of amantadine and budipine with three ADs on extracellular 5-HT. To this end, we have used the SSRI paroxetine (PAROX), the SNRI reboxitine (REB) or clomipramine (CLOM), a relatively typical tricyclic antidepressant having a somewhat higher affinity for the 5-HT transporter but with a metabolite possessing greater affinity for the NA site. The effects of these drugs have been tested both acutely and chronically, for periods of up to 21 days, on extracellular 5-HT in the frontal cortex of freely moving rats using *in vivo* microdialysis.

Methods

Experiments were carried out in accordance with the Animals Scientific Procedures Act U.K. (1986). Male Wistar rats (210–240 g) were group housed and had access to food and water *ad libitum*. Rats, $n=6$–12 per experimental group, were anaesthetized with isofluorane and implanted with concentric dialysis probes of a construction described previously (Whitton *et al*., 1992), having an active membrane length of 4.0 mm and diameter of 0.2 mm. Probes were implanted into the frontal cortex (A 3.2 mm, 1.5 mm L from bregma and 6.0 mm below dura) using stereotaxic coordinates from the Atlas of Paxinos & Watson (1982). The following day, rats were perfused at a rate of 0.5 μl min^{-1} with an artificial cerebrospinal fluid (composition in mM^{-1}: 2.5 KCl; 125 NaCl; 1.18 $MgCl_2$; 1.26 $CaCl_2$) as previously used (Whitton *et al*., 1992) but without the addition of the 5-HT reuptake inhibitor citalopram. Following a 1 h equilibration period, four 30 min basal samples were collected prior to drug or vehicle administration in acute experiments (see Results), while only basalsamples were used for 5-HT determination after chronic treatment. All drugs were dissolved in 0.9% saline and, in chronic studies, either drug or vehicle was administered between 09:00 and 10:00, while control animals were given two vehicle injections only. Drugs were administered intraperitoneally (i.p.) in a volume of 1.0 ml kg^{-1} body weight of animal. During chronic treatment, different groups of rats were used at each time point (4, 7, 14 and 21 days) and the final dose of drug was administered the day before dialysis. Rats were administered REB (10 mg kg^{-1}; a kind gift from Pharmacia Upjohn, U.S.A.), PAROX, CLOM (10 mg kg^{-1}; Sigma, U.K.), budipine (5 or 10 mg kg^{-1}; a kind gift from Professor M.S. Starr) or amantadine (20 or 40 mg kg^{-1}; Sigma). Dialysates were analysed for 5-HT as described previously (Whitton & Fowler, 1991). Briefly, 5-HT was separated on a C18 reverse phase column maintained at 40°C (Rainin Dynamax Instrument Co., U.S.A.) using a buffer composed of 90 mM^{-1} sodium acetate, 35 mM^{-1} citrate, 0.34 mM^{-1} EDTA, 0.06 mM^{-1} 1-octane-sulphonic acid with 5.5 % methanol at pH 4.2. 5-HT was determined using an Amec-Intro electrochemical detector (Amtec Leyden BV, the Netherlands). Mean dialysate levels of 5-HT were 9.51 $\pm$ 0.69 fmol 10 μl^{-1}, $n=80$, uncorrected for *in vitro* recovery. In the case of acute treatments, data were converted into percentages in order to reduce the variability, and therefore minimize the number of animals used. In chronic studies, data are represented as absolute values of 5-HT. Data were analysed using two-way analysis of variance followed by Bonferonni's multiple comparison test to compare mean values at different time points.

Results

Acute experiments

Acutely REB, PAROX, CLOM, amantadine or budipine did not significantly alter cortical extracellular 5-HT (Figures 1–3). However, when amantadine or budipine was given 30 min prior to REB, a clear and significant change in frontal cortex dialysate 5-HT was observed over time (F (5, 12) = 20.73, $P<0.0001$; Figure 1) and between treatments (F (5, 12) = 96.59, $P<0.0001$; Figure 1). Furthermore, Bonferonni's multiple comparison test revealed that either amantadine or budipine given prior to REB elicited significant increases in extracellular 5-HT when compared with either vehicle + vehicle or vehicle + REB-treated animals (Figure 1). Indeed, these drug treatment combinations were significantly different from all other groups from sample 7 up to the end of the experiment. As shown in Figure 2 amantadine and budipine had a similar effect on PAROX-evoked changes in extracellular 5-HT in the cortex over time (F (5, 12) = 7.883, $P<0.0001$) and between treatments (F (5, 12) = 80.78,

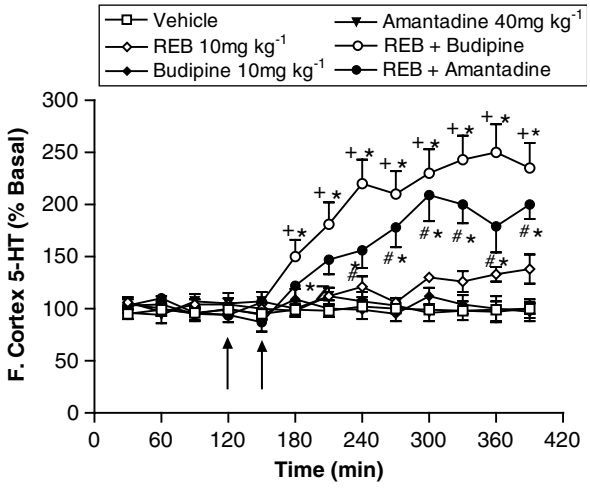

Figure 1 Effect of amantadine or budipine on REB-induced changes in extracellular 5-HT in the frontal cortex of freely moving rats. The first arrow indicates amantadine, budipine or vehicle injection and the second vehicle or REB administration. Data are the mean $\pm$ s.e.m. of six rats in each group. *Significant differences from vehicle-treated controlsl; $^{+}$Significant differences from REB-only treated rats.

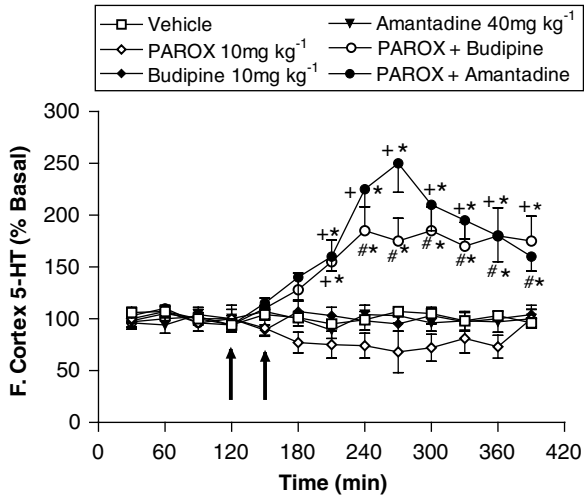

Figure 2 Effect of amantadine or budipine on PAROX-induced changes in extracellular 5-HT in the frontal cortex of freely moving rats. The first arrow indicates amantadine, budipine or vehicle injection and the second PAROX or vehicle administration. Data are the mean ± s.e.m. of six rats in each group. *Significant differences from vehicle treated controls; +Significant differences from PAROX-only treated rats.

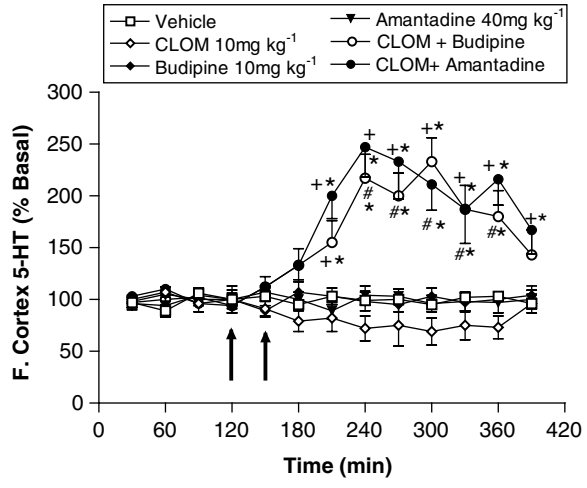

Figure 3 Effect of amantadine or budipine on CLOM-induced changes in extracellular 5-HT in the frontal cortex of freely moving rats. The first arrow indicates amantadine, budipine or vehicle injection and the second CLOM or vehicle administration. Data are the mean ± s.e.m. of six rats in each group. *Significant differences from vehicle-treated controls; +Significant differences from CLOM-only treated rats.

$P<0.0001$). Again *post hoc* analysis of the data revealed significantly increased extracellular 5-HT following amantadine or budipine with PAROX compared with any of the other drug combinations (Figure 2). Very similar results were observed with the tricyclic drug CLOM (Figure 3), in which two way analysis of variance again revealed highly significant changes in extracellular 5-HT over time (F (5, 12) = 9.19, $P<0.0001$) and between treatments (F (5, 12) = 85.20, $P<0.0001$). Once more, *post hoc* analysis showed that the amantadine or budipine plus CLOM combination significantly increases frontal cortex extracellular 5-HT compared with all other treatment groups (Figure 3).

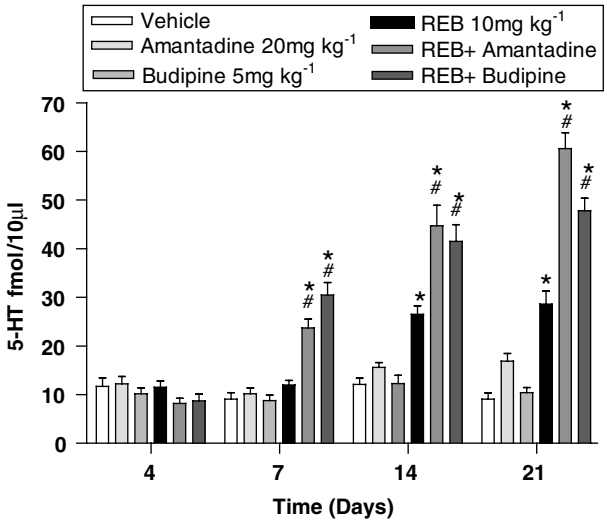

Figure 4 Effect of amantadine or budipine on REB-induced changes in extracellular 5-HT in the frontal cortex of freely moving rats over 21 days. Data are the mean ± s.e.m. of eight to 12 rats in each group. *Significant differences from vehicle-treated controls; #Significant differences from rats administered REB only.

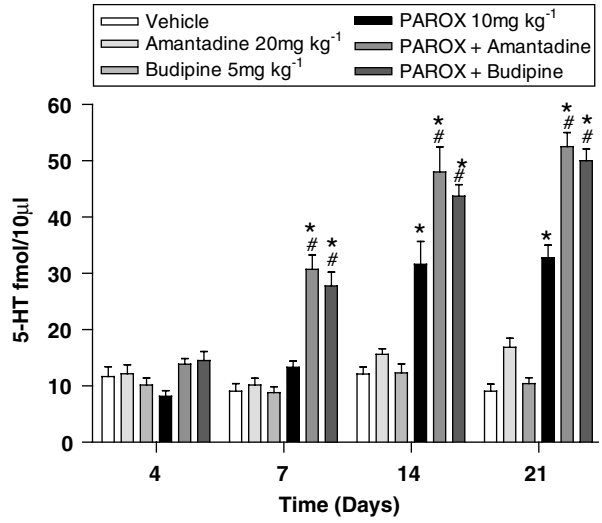

Figure 5 Effect of amantadine or budipine on PAROX-induced changes in extracellular 5-HT in the frontal cortex of freely moving rats over 21 days. Data are the mean ± s.e.m. of eight to 12 rats in each group. *Significant differences from vehicle-treated controls; #Significant differences from rats administered PAROX only.

Chronic studies

In these investigations, the three ADs were studied at separate times for reasons of practicality with regard to performing dialysis experiments. Each AD had its own vehicle group, but these were pooled for final data analysis as was the amantadine or budipine. Data have been divided into groups according to the AD (Figures 4–6). Administration of either amantadinne or budipine did not significantly alter extracellular 5-HT at any of the time points studied (Figures 4–6). When administered alone, all three of the antidepressants elicited a progressive increase in 5-HT levels over the course of the experiments. Each of the antidepressant groups displayed

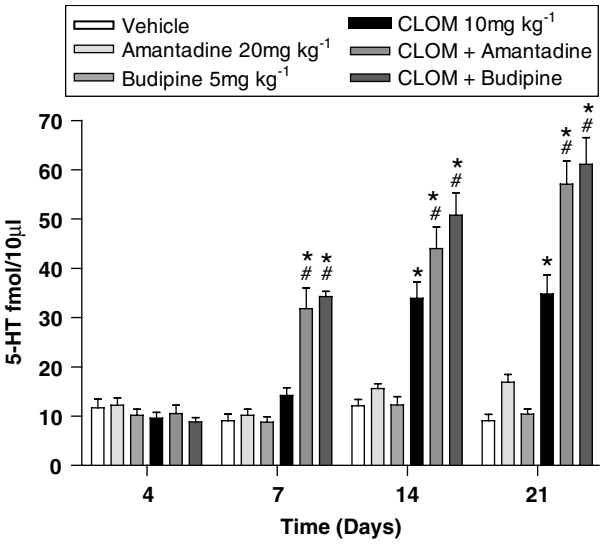

Figure 6 Effect of amantadine or budipine on CLOM-induced changes in extracellular 5-HT in the frontal cortex of freely moving rats over 21 days. Data are the mean ± s.e.m. of eight to 12 rats in each group. *Significant differences from vehicle-treated controls; #Significant differences from rats administered CLOM only.

highly significant effects over time and between treatment groups (REB, over time and between treatments F (3, 5) = 99.32 and 134.2, respectively, $P < 0.0001$; PAROX, over time and between treatments F (3, 5) = 116.6 and 111.5, respectively, $P < 0.0001$; CLOM, over time and between treatments F (3, 5) = 93.82 and 80.22, respectively, $P < 0.0001$; Figures 3–6). When either amantadine or budipine was coadministered with any of the three ADs, two clear effects emerged. Firstly, as revealed by *post hoc* analysis, the time required for significant differences to be evident from both vehicle and antidepressant plus amantadine/budipine treatment was reduced to 7 as opposed to 14 days. Importantly, throughout the period of the study, with the exception of day four, multiple group analysis revealed statistically significant differences between vehicle plus AD and amantadine/budipine plus AD for all three ADs at each time point (Figures 3–6). Secondly, the absolute amount of extracellular 5-HT measured after the two-drug combination was clearly greater than that seen for any of the three ADs given alone with vehicle (Figures 3–6).

Discussion

The monoamine hypothesis of depression has provided an essential grounding for the basis of AD action and has been considered to provide an insight into the genesis of depressive illness. Thus, the majority of ADs increase monoaminergic transmission in the CNS and this appears to be particularly the case for 5-HT (Blier & De Montigny, 1994). However, it should be remembered that both NA and DA play, particularly in the case of the former, highly significant roles in the aetiology of depression and response to ADs (Millan *et al*., 2000). In the present study, we have not observed increases in extracellular 5-HT following acute AD treatment. This observation is in accord with some other findings (Adell & Artigas, 1991; Gartside *et al*., 1999; Pallotta *et al*., 2001; Beyer *et al*., 2002) but differ from other authors (Bymaster *et al*., 2002; Nakayama, 2002). These differences have been attributed to differences in the behavioural state of the animals, altering responsiveness of somatodendritic 5-HT_{1A} receptors, in the raphe nuclei (e.g. see Ceglia *et al*., 2004). The mechanism behind the failure to observe acute increases in dialysate 5-HT following acute systemic administration of drugs with a high or relatively high selectivity for the serotonergic transporter appears to be that the elevation of 5-HT in the raphe nuclei of these animals activates local somatodendritic 5-HT_{1A} receptors, leading to a net decrease in serotonergic transmission in ascending pathways (Adell & Artigas, 1991; Ceglia *et al*., 2004). This event leads to an acute diminution in terminal 5-HT release (Hjorth & Auerbach, 1996) in the absence of which transporter blocking drugs have limited efficacy in these regions. For example, Adell & Artigas (1991) observed that systemic CLOM failed to elevate cortical extracellular 5-HT, but an increase was observed when CLOM was infused *via* dialysis probes into the cortex itself, thereby bypassing an effect on somatodendritic receptors. A progressive desensitization of somatodendritic 5-HT_{1A} receptors and thereby disinhibition of serotonergic activity in ascending pathways is considered by a number of authors to underlie the progressive effects of these ADs (Le Poul *et al*., 1995; 2000). Although REB is an SNRI, we have found it to increase raphe extracellular 5-HT (our unpublished data), raising the possibility that such a process could be active in the case of this drug and the 5-HT system. Overall, therefore, it is considered that this progressive disinhibition of activity in ascending serotonergic pathways could account for the initial failure to observe increased extracellular cortical 5-HT after acute AD treatment but accounts for the steady increase observed during chronic treatment, as we have seen in the present study (Blier & De montigny, 1994; Pallotta *et al*., 2001).

Despite the wide range of ADs altering monoaminergic transmission available and the wealth of research into the illness itself, the monoamine theory does not seem to provide an all-encompassing explanation for the disease or its treatment (Skolnick, 1999). Over the past decade, interest has turned to a potential role of the glutamatergic system in depression, particularly with regard to the NMDA receptor (Trullas & Skolnick, 1990). It has been found that a variety of NMDA receptor antagonists demonstrate antidepressant activity comparable to conventional ADs in animal models of the illness. These include both competitive (Maj *et al*., 1992) and noncompetitive (Papp & Moryl, 1994) NMDA receptor antagonists. Moreover, conversely, a significant number of ADs have been demonstrated to alter the NMDA receptor in a manner that would be consistent with a resulting decrease in functional activity at this site (Paul *et al*., 1994; Boyer *et al*., 1998; Pallotta *et al*., 1999a, b; 2001). Unfortunately, in the case of NMDA receptor antagonists, many of these compounds have very limited value in patients, partly as a result of extremely poor CNS penetration in some cases or unacceptable side effects in others, although quite recently Berman *et al*. (2000) demonstrated a long-lasting antidepressant effect of ketamine following intravenous (i.v.) infusion of the drug into patients. It appears that clinical tolerability of noncompetitive NMDA receptor antagonists may depend upon their having low affinity and possibly subunit specificity within the NMDA complex (Porter & Greenamyre, 1995). Recently, amantadine and memantine, both weak NMDA

receptor antagonists, which are currently in use in the clinic, have been shown to have a synergistic effect with conventional ADs in a model of depression (Rogoz *et al*., 2002; 2004). These authors observed that fluoxetine, venlafaxine and imipramine all synergized with amantadine or memantine to give improved performance by rats in the forced swim test (Maj & Rogoz, 2000; Rogoz *et al*., 2002; 2004). Significantly, particularly in light of our observations, Stryjer *et al*. (2003) have reported that, in a small open-label trial, amantadine given with conventional ADs caused a 50% improvement in treatment-resistant patients and, interestingly, a decrease in the time required for therapeutic improvement in some of the patients. This suggests that the observations made in animal models may have some validity in the clinic and as such it is possible that our current neurochemical observations could also be significant.

The mechanism by which noncompetitive NMDA receptor antagonists potentiate the effect of ADs with regard to increased extracellular 5-HT is yet to be elucidated. A pharmacokinetic interaction cannot be discounted, possibly as a result of either ADs or amantadine/budipine inhibiting the enzymes responsible for each other's metabolism. We are unaware, however, of any study that has demonstrated that any of the drugs used in the present study increase the brain concentrations of the others. Moreover, the fact that both amantadine and budipine had such similar effects on all three ADs would suggest a remarkable coincidence and indicates to us that a true pharmacodynamic effect takes place. Although the underlying neurochemical mechanisms by which weak NMDA antagonists are able to potentiate cortical 5-HT levels by antidepressants are therefore unclear, they could involve direct alterations in serotonergic transmission or effects on synthesis of the transmitter. Thus, infusion of NMDA into either the raphe nuclei or frontal cortex has been shown to alter local 5-HT release and also serotonergic transmission (Lejeune *et al*., 1994; Tao & Auerbach, 1996; Pallotta *et al*., 1998). Although we did not observe effects of either amantadine or budipine given alone on 5-HT release either acutely or during long-term treatment, it may be that the presence of these weak NMDA receptor antagonists alter glutamatergic tone at NMDA receptors in a manner that facilitates 5-HT transmission in ascending pathways or locally in the vicinity of the 5-HT terminals. Alternatively, budipine and amantadine have both been shown to alter monoamine metabolism. These studies have largely focused on the effects of these drugs on the dopaminergic system due to their clinical role in Parkinson's disease. Both have been shown to potentiate the activity of L-DOPA decarboxylase as well as inhibiting monoamine oxidase B (Eltze, 1999; Fisher & Starr, 2000) and to increase extracellular DA. Fisher & Starr (2000) have also reported regional effects of these drugs on the activity 5-hydroxy-tryptophan decarboxylase in the substantia nigra and striatum of rats. Additionally, amantadine has been reported to increase cerebral 5-HT turnover (Altagracia *et al*., 1993). It would therefore be of interest to investigate the effects of these drugs on monoamine oxidase A and 5-HT decarboxylase activities in brain regions, such as the cortex, which are associated with the aetiology of depression.

Overall there is a general agreement that current AD treatment has significant limitations. Despite the existence of both SSRIs and SNRIs, which are pharmacologically very highly selective for their respective transporter sites, these drugs show therapeutic efficacy in a maximum of only around 70% of patients (Stryjer *et al*., 2003). Moreover, these current therapies are no more effective than those previously employed such as monoamine oxidase inhibitors or tricyclic antidepressants, but do show considerable improvement with regard to side-effect profile and overall safety. Although drug-resistant depression is treated using combinations of Ads, this form of treatment has at best moderate therapeutic success (Anath, 1998). As such, a comparison of the efficacy of ADs with, for example that of benzodiazepines such as anxiolytics suggests a relatively ineffective class of drugs. Moreover, even in those patients in whom ADs are effective, there is a delay in therapeutic onset of several weeks. This suggests a need for alternative treatment strategies for depressed patients.

Our current findings indicate both qualitative and quantitative effects of the two weak NMDA antagonists on REB-, PAROX- and CLOM-induced increases in cortical extracellular 5-HT. Firstly, in all cases, the combination of treatments led to a more rapid increase in dialysate 5-HT than that observed for the AD given alone. Secondly, the magnitude of the increase was consistently greater with the drug combination. This may well constitute an obvious possible mechanism by which amantadine and memantine show a synergistic effect with antidepressants in the forced swim test (Rogoz *et al*., 2002; 2004). It is also possible that these findings, were they to occur in patients, could account for the greater efficacy of amantadine given with an AD compared with the AD alone, in treatment-resistant patients (Stryjer *et al*., 2003), and could therefore offer an alternative treatment strategy in drug-resistant patients as an adjunct to current therapies.

References

ADELL, A. & ARTIGAS, F. (1991). Differential effects of clomipramine on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **343,** 237–244.

ALTAGRACIA, M., ROJAS, P., KRAVZOV, J. & RIOS, C. (1993). Amantadine enhances dopamine and serotonin turnover in the MPTP model of Parkinson's disease. *Proc. West. Pharmacol. Soc.,* **36,** 289–291.

ANATH, J. (1998). Treatment-resistant depression. *Psychother. Psychosomatic,* **67,** 61–70.

BERMAN, R.M., CAPPIELLO, A., ANNAND, A., OREN, D.A., HENINGER, G.R., CHARNEY, D.S. & KRYSTAL, J.H. (2000). Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry,* **47,** 351–354.

BEYER, C.E., BOIKESS, S., LUO, B. & DAWSON, L.A. (2002). Comparison of the effects of antidepressants on norepinephrine and serotonin concentrations in the rat frontal cortex: an *in vivo* microdialysis study. *J. Psychopharmacol.,* **16,** 297–304.

BLIER, P. & DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.,* **15,** 230–236.

BOYER, P.A., SKOLNICK, P. & FOSSUM, L.H. (1998). Chronic administration of imipramine and citalopram alters the expression of NMDA subunit mRNAs in mouse brain. *J. Mol. Neurosci.,* **10,** 219–233.

BYMASTER, F.P., ZHANG, W., CARTER, P.A., SHAW, J., CHERNET, E., PHEBUS, L., WONG, D.T. & PERRY, K.W. (2002). Fluoxetine, but not other selective serotonin reuptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology,* **160,** 353–361.

CEGLIA, I., ACCONCIA, S., FRACASSO, C., COLOVIC, M., CACCIA, S. & INVERNIZZI, R.W. (2004). Effects of chronic treatment with escitalopram or citalopram on extracellular 5-HT in the prefrontal cortex of rats: role of 5-HT_{1A} receptors. *Br. J. Pharmacol.,* **142,** 469–478.

ELTZE, M. (1999). Multiple mechanisms of action: the pharmacological profile of budipine. *J. Neural Trans. Suppl.,* **56,** 83–105.

FISHER, A. & STARR, M.S. (2000). Opposite effects of glutamate antagonists and antiparkinsonian drugs on the activities of DOPA decarboxylase and 5-HTP decarboxylase in the rat brain. *Brain Res.,* **868,** 268–274.

GARTSIDE, S.E., CLIFFORD, E.M., COWEN, P.J. & SHARP, T. (1999). Effects of (−)-tertatolol, (−)-penbutolol and (+−)-pindolol in combination with paroxetine on presynaptic 5-HT function: an *in vivo* microdialysis and study. *Br. J. Pharmacol.,* **127,** 145–152.

HJORTH, S. & AUERBACH, S.S. (1996). 5-HT1A autoreceptors and the mode of action of selective serotonin reuptake inhibitors (SSRI). *Behav. Brain. Res.,* **73,** 281–283.

LAYER, R.T., POPIK, P., OLDS, T. & SKOLNICK, P. (1995). Antidepressant-like action of the polyamine site antagonist, eliprodil (SL 82015). *Pharmacol. Biochem. Behav.,* **52,** 615–620.

LEJEUNE, F., GOBERT, A., RIVET, J.M. & MILAN, M.J. (1994). Blockade of transmission of NMDA receptors facilitates the electrical and synthetic activity of ascending serotonergic neurones. *Brain Res.,* **656,** 427–431.

LE POUL, E., BONI, C., HANOUN, N., LAPORTE, A.M., LAZARIS, N., CHAUVEAU, J. & LANFUMEY, I. (2000). Differential adaptation of brain 5-HT1A and 5-HT1B receptors and 5-HT transporter in rats treated chronically with fluoxetine. *Neuropharmacology,* **39,** 110–122.

LE POUL, E., LAZARIS, N., DOUCET, E., LAPORTE, A.M., HAMON, M. & LANFUMEY, I. (1995). Early desensitisation of somato-dendritic 5-HT_{1A} autoreceptors in rats treated with fluoxetine or paroxetine. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 141–148.

LIEBERMAN, J.A., GREENHOUSE, J., HAMER, R.M., KRISHNAN, K.R., NEMEROFF, O., SHEENAN, D.V., THASE, M.E. & KELLER, M.B. (2005). Comparing the effects of antidepressants: Consensus guidelines for evaluating quantitative reviews of antidepressant efficacy. *Neuropsychopharmacology,* **30,** 445–460.

MAJ, J. & ROGOZ, Z. (2000). Synergistic effect of amantadine and imipramine in the forced swimming test. *Pol. J. Pharmacol.,* **52,** 111–114.

MAJ, J., ROGOZ, Z., SKUZA, G. & SOWINSKA, H. (1992). The effect of CGP 37849 and CGP 39551, competitive NMDA receptor antagonists, in the forced swimming test. *Pol. J. Pharmacol,* **44,** 337–346.

MILLAN, M.J., LEJEUNE, F. & GOBERT, A. (2000). Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. *J. Psychopharmacol.,* **142,** 114–138.

NAKAYAMA, K. (2002). Effect of paroxetine on extracellular serotonin and dopamine levels in the prefrontal cortex. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **365,** 102–105.

PALLOTTA, M., SEGIETH, J. & WHITTON, P.S. (1998). *N*-methyl-D-aspartate receptors regulate 5-HT release in the raphe nuclei and frontal cortex of freely moving rats: differential role of 5-HT_{1A} autoreceptors. *Brain Res.,* **675,** 157–164.

PALLOTTA, M., SEGIETH, J. & WHITTON, P.S. (1999a). Chronic but not acute clomipramine alters the effect of NMDA receptor regulation of dopamine release in the rat frontal cortex. *Neurosci. Lett.,* **262,** 187–190.

PALLOTTA, M., SEGIETH, J. & WHITTON, P.S. (1999b). Chronic clomipramine administration reverses NMDA-evoked decreases in dopamine release in the raphe nuclei. *Brain Res.,* **823,** 217–220.

PALLOTTA, M., SEGIETH, J., SADIDEEN, F. & WHITTON, P.S. (2001). Repeated but not acute clomipramine decreases the effect of *N*-methyl-D-aspartate receptor activation on serotonergic transmission between the raphe nuclei and frontal cortex. *Neuropharmacology,* **41,** 294–300.

PAPP, P. & MORYL, E. (1994). Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. *Eur. J. Pharmacol.,* **263,** 1–7.

PAUL, I.A., NOWAK, G., LAYER, R.T., POPIK, P. & SKOLNICK, P. (1994). Adaptation of the *N*-methyl-*D*-aspartate complex following chronic antidepressant treatments. *J. Pharmacol. Exp. Ther.,* **269,** 95–102.

PAXINOS, G. & WATSON, C. (1982). *The rat brain in stereotaxic co-ordinates*. Academic Press, New York.

PORTER, R.H. & GREENAMYRE, J.T. (1995). Regional variations in the pharmacology of NMDA receptor channel blockers: implications for therapeutic potential. *J. Neurochem.,* **64,** 614–623.

ROGOZ, Z., SKUZA, G., MAJ, J. & DANYSZ, W. (2002). Synergistic effect of uncompetitive NMDA receptor antagonists and antidepressant drugs in the forced swimming test in rats. *Neuropharmacology,* **42,** 1024–1030.

ROGOZ, Z., SKUZA, G., KUSMIDERN, M., WOIJICOWSKI, J., KOR, M. & DANYSZ, W. (2004). Synergistic effect of imipramine and amantadine in the forced swimming test in rats. Behavioural and pharmacokinetic studies. *Polish J. Pharmacol.,* **56,** 179–185.

SKOLNICK, P. (1999). Antidepressants for the new millennium. *Eur. J. Pharmacol.,* **375,** 31–40.

STRYJER, R., STROUSS, R.D., SHAKED, G., BAR, F., FELDMAN, B., KOTLER, M., POLAK, L., ROSENSCWAIG, S. & WEIZMANN, A. (2003). Amantadine as augmentation therapy in the management of treatment-resistant depression. *Int. Clin. Psychopharmacol.,* **18,** 93–96.

TAO, R. & AUERBACH, S.B. (1996). Differential effect of NMDA on extracellular serotonin in midbrain raphe and forebrain sites. *J. Neurochem.,* **66,** 1067–1075.

TRULLAS, R. & SKOLNICK, P. (1990). Functional antagonists of the NMDA receptor complex exhibit antidepressant action. *Eur. J. Pharmacol.,* **185,** 1–10.

WHITTON, P.S. & FOWLER, L.J. (1991). The effects of valproic acid on 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentration in hippocampal dialysates *in vivo*. *Eur. J. Pharmacol.,* **200,** 167–169.

WHITTON, P.S., BIGGS, C.S., PEARCE, B.R. & FOWLER, L.J. (1992. MK-801 increases extracellular 5-hydoxytryptamine in rat hippocampus and striatum *in vivo*. *J. Neurochem.,* **58,** 1573–1575.

(Received November 26, 2004
Revised January 18, 2005
Accepted January 28, 2005
Published online 18 April 2005)