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Short communication

Effects of co-administration of a monoamine oxidase inhibitor and a 5-HT_{1A} receptor antagonist on 5-hydroxytryptamine cell firing and release

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Abstract

We report the effects of the monoamine oxidase inhibitor, tranylcypromine, combined with the 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl cyclohexanecarboxamide (WAY 100635), on both 5-hydroxytryptamine (5-HT) cell firing and cortical extracellular 5-HT in the rat. Tranylcypromine inhibited 5-HT cell firing in the dorsal raphe nucleus dose-dependently (ED_{50} 5 mg/kg i.v.). In microdialysis experiments, tranylcypromine (5 mg/kg i.v.) increased extracellular 5-HT in the frontal cortex. WAY 100635 (0.1 mg/kg i.v.) both reversed the inhibition of 5-HT cell firing and facilitated the increase in extracellular 5-HT. In conclusion, WAY 100635 enhances the effect of tranylcypromine on presynaptic 5-HT function. These data are relevant to clinical evidence that co-therapy with a 5-HT_{1A} receptor antagonist improves the antidepressant efficacy of a monoamine oxidase inhibitor.

Keywords: 5-HT_{1A} receptor; WAY 100635; Monoamine oxidase inhibitor; Dorsal raphe nucleus; Microdialysis; Extracellular recording

1. Introduction

Monoamine oxidase inhibitors, in common with other antidepressant drugs, need to be administered for 2–3 weeks to achieve their full therapeutic effect, and even then, up to one third of patients do not respond (Merson and Tyrer, 1991). Pharmacological strategies which speed up the onset of action of antidepressant drugs and improve their efficacy would be of considerable benefit to patient and clinician. In recent clinical studies the time of onset of the antidepressant effect of monoamine oxidase inhibitors, as well as selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors, was significantly reduced by co-administration of pindolol, a β-adrenoceptor and 5-HT_{1A} receptor antagonist (Artigas et al., 1994; Blier and Bergeron, 1995). Current thinking is that pindolol facilitates the antidepressant effect of monoamine oxidase inhibitors and selective serotonin reuptake inhibitors by enhancing the action of these treatments on brain 5-HT function.

More specifically, in the case of selective serotonin reuptake inhibitors, microdialysis studies in the rat show that 5-HT_{1A} receptor antagonists potentiate the selective serotonin reuptake inhibitor-induced rise in extracellular 5-HT in the forebrain (Hjorth, 1993; Gartside et al., 1995a). It is thought that this potentiation is associated with findings that 5-HT_{1A} receptor antagonists prevent the inhibition of 5-HT cell firing typically induced by selective serotonin reuptake inhibitors (Arborelius et al., 1995; Hajós et al., 1995a; Gartside et al., 1995a). Normally, this inhibition of 5-HT cell firing would decrease the release of 5-HT in the forebrain, and hence limit the ability of the selective serotonin reuptake inhibitor to increase 5-HT transmission.

Monoamine oxidase inhibitors also inhibit 5-HT neuronal activity in the rat midbrain raphe nuclei (Aghajanian et al., 1970) although the receptor mechanisms underlying this effect have been little investigated. Here we determined whether the selective 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl cyclohexanecarboxamide (WAY 100635; Fletcher et al., 1995), might prevent the inhibition of 5-HT cell firing induced by a commonly used monoamine oxidase inhibitor, tranylcypromine. In parallel microdialysis

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experiments, we tested whether the 5-HT_{1A} receptor antagonist would potentiate the ability of the monoamine oxidase inhibitor to increase extracellular 5-HT in the frontal cortex. This work was presented in a preliminary form to the British Association of Psychopharmacology (Gartside et al., 1995b)

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan-Olac, Bicester, UK) were housed under conditions of controlled temperature (21 ± 1°C) and lighting (lights on 08.00–20.00 h) with food pellets and water freely available.

2.2. Surgical procedure

Rats (260–300 g) were anaesthetised with chloral hydrate (500 mg/kg i.p.), and placed in a stereotaxic frame. The skull was exposed and a burr hole drilled for the implantation of either a recording electrode or microdialysis probe. A lateral tail vein was cannulated for administration of drugs. Supplementary doses of chloral hydrate were administered (i.v.) as required to maintain full general anaesthesia. Core temperature was maintained at 35–36°C throughout the experiment using a thermoregulated blanket connected to a rectal thermometer.

2.3. Recording of 5-HT neuronal activity

Extracellular recordings of the activity of 5-HT neurones in the dorsal raphe nucleus were made as reported previously (Hajós et al., 1995b). Glass microelectrodes (filled with 2 M NaCl saturated with Pontamine sky blue; 4–8 MΩ impedance in vitro) were lowered into the dorsal raphe nucleus (co-ordinates in mm: anterior-posterior −7.9, medial-lateral 0, dorso-ventral −4.5–6.5; relative to bregma and dura surface) under stereotaxic control and with the aid of a hydraulic microdrive. Signals were amplified (×1000) and filtered (300–3000 Hz band-pass), and fed to an audio speaker, an oscilloscope, and a chart recorder. The signal was also recorded on digital audio tape for off-line analysis.

5-HT neurones were identified on the basis of their electrophysiological characteristics (broad action potentials with positive-negative or positive-negative-positive deflections, regular firing pattern, 0.5–3 Hz firing rate) as described previously (Aghajanian et al., 1970; Hajós et al., 1995b). Burst-firing, presumed 5-HT neurones (Hajós et al., 1995b) were not recorded in this study. Drugs were given after a baseline recording period of at least 3 min. Tranylcypromine was injected i.v. at 2 min intervals in accumulating doses. A bolus injection of WAY 100635 was administered after tranylcypromine, once the firing was reduced by 70% or more. Only one 5-HT neurone was studied per rat.

The position of the recorded neurone was verified by the iontophoretic ejection of a small amount of Pontamine sky blue and subsequent inspection of cresyl violet stained brain sections.

2.4. Microdialysis measurement of extracellular 5-HT

Microdialysis probes (single cannula type, 3 mm tip length, Gambro membrane) were stereotaxically implanted into the frontal cortex (co-ordinates in mm: anterior-posterior +3.3, medial-lateral −3.0, dorso-ventral −4.5) and perfused (2 μl/min) with artificial cerebrospinal fluid (composition in mM: NaCl 140, KCl 3, $CaCl_2$ 2.4, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.27, glucose 7.2, pH 7.4). Samples of dialysate were collected over 20 min and analysed for 5-HT by high-performance liquid chromatography with electrochemical detection (Gartside et al., 1995a). Drugs were administered (i.v.) in a bolus injection once a stable baseline level of 5-HT had been established (typically 2–3 h after probe implantation). WAY 100635 was administered 10 min before tranylcypromine.

2.5. Data analysis

For the electrophysiological studies, the percentage inhibition induced by each total dose of tranylcypromine was calculated as the firing rate (in a 30 s period) after tranylcypromine, relative to the firing rate (100%) in a 60 s baseline period immediately before administration of the first dose of the drug. An ED_{50} value for each cell was determined by interpolation on a semi-log plot.

For the microdialysis experiments, data are expressed as a percentage of the mean amount of 5-HT in the final three 20 min samples collected prior to injection of tranylcypromine. Microdialysis data were analysed by two-way analysis of variance with post-hoc Student's unpaired *t*-test. Statistical significance at the 95% level is reported.

2.6. Drugs

WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide 3HCl; Wyeth Pharmaceuticals) and tranylcypromine HCl (Sigma) were dissolved in either water (electrophysiological experiments) or 5% glucose (microdialysis experiments). Drugs were administered i.v. either in a single dose at 1.0 ml/kg (microdialysis experiments) or in doubling doses with an initial volume of 0.1 ml (electrophysiological experiments).

3. Results

3.1. Effect of tranylcypromine on 5-HT neuronal activity: reversal by WAY 100635

Cumulative doses of tranylcypromine (0.5–16 mg/kg i.v.) inhibited the firing of 5-HT neurones in the dorsal

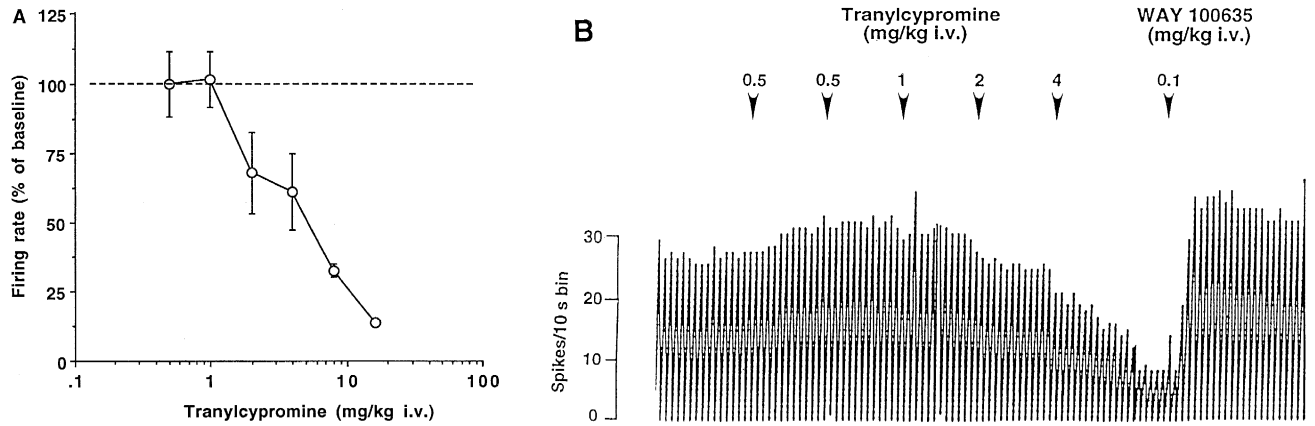

Fig. 1. Inhibitory effect of tranylcypromine on the firing rate of 5-HT neurones in the rat dorsal raphe nucleus, and its reversal by WAY 100635. (A) Dose–response curve. Tranylcypromine was given in doubling doses at approximately 2-min intervals. The firing rate was determined over a 30-s period after each dose and is expressed relative to the firing rate in a 60 s baseline period. Data are mean ± S.E.M. ($n = 6$). (B) Rate meter recording of a single 5-HT neurone showing the inhibitory effect of cumulative doses of tranylcypromine, and its reversal by WAY 100635.

raphe nucleus (Fig. 1A). The effect was dose-related, with an estimated ED_{50} of 5.1 ± 0.7 mg/kg i.v., and a total cessation of cell firing was achieved at the higher doses. At lower doses of tranylcypromine (0.5–2 mg/kg i.v.), an increase in firing rate of about 20% was observed in some cells (Fig. 1B) but not all (4/6). The inhibition of 5-HT cell firing induced by tranylcypromine was completely reversed by subsequent administration of WAY 100635 (0.1 mg/kg i.v.) in all five neurones in which it was tested (Fig. 1B). In the example shown in Fig. 1B, WAY 100635 returned firing to a slightly higher rate than the baseline rate. This phenomenon was observed on one other cell, but not seen in a further three.

3.2. Effect of tranylcypromine and WAY 100635 on extracellular 5-HT in frontal cortex

Injection of tranylcypromine (5 mg/kg i.v.) induced a rise in 5-HT levels in dialysates of the frontal cortex which was slow in onset, but large (300–400%) and long-lasting (> 120 min). As shown in Fig. 2, the effect of tranylcypromine (5 mg/kg i.v.) on extracellular 5-HT was significantly greater in rats pretreated with WAY 100635 (0.1 mg/kg i.v., 10 min previously) than in those which received vehicle (two-way analysis of variance treatment × time interaction, $F = 2.0$; $P < 0.05$). Post-hoc analysis revealed statistically significant differences in the increase in 5-HT at the 20 and 40 min time points ($P < 0.05$).

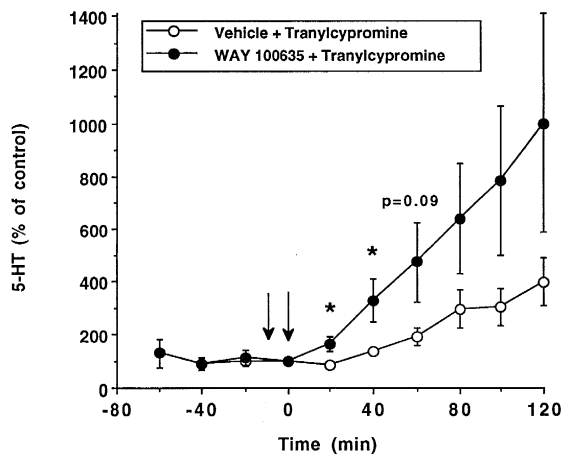

Fig. 2. Effect of tranylcypromine (5 mg/kg i.v.) on extracellular 5-HT in the frontal cortex of rats pretreated (10 min) before with WAY 100635 (0.1 mg/kg) or vehicle. Data are mean ± S.E.M. ($n = 8$). * $P < 0.05$ (Student's unpaired *t*-test following two-way analysis of variance).

Injection of vehicle alone had no effect on basal output of 5-HT (data not shown). We have previously shown that injection of WAY 100635 (0.1 mg/kg i.v.) alone has no effect on 5-HT levels in dialysates of the frontal cortex (Gartside et al., 1995a).

4. Discussion

In this study, we examined the effects of the monoamine oxidase inhibitor, tranylcypromine, and the selective 5-HT_{1A} receptor antagonist, WAY 100635, on both the firing rate of midbrain raphe 5-HT neurones, and extracellular 5-HT in the frontal cortex, in the anaesthetised rat. Our data show that the 5-HT_{1A} receptor antagonist prevents the inhibition of 5-HT cell firing induced by the monoamine oxidase inhibitor and, under similar experimental conditions, increases the effect of the monoamine oxidase inhibitor on extracellular 5-HT in the terminal region. These results may be relevant to recent clinical evidence that pindolol, which is a 5-HT_{1A} receptor antagonist (as well as a β-adrenoceptor antagonist), facilitates the antidepressant effect of monoamine oxidase inhibitors (Artigas et al., 1994; Blier and Bergeron, 1995).

In confirmation of earlier electrophysiological studies (Aghajanian et al., 1970), we found that administration of tranylcypromine inhibits 5-HT neuronal activity in the dorsal raphe nucleus: we show that this inhibition is

dose-related (ED_{50} 5 mg/kg i.v.), and complete at high doses. The earlier electrophysiological studies demonstrated that the inhibition of 5-HT cell firing by tranylcypromine is blocked by treatment with a 5-HT synthesis inhibitor (Aghajanian et al., 1970), indicating that the effect is mediated indirectly by increased 5-HT availability. Indeed, tranylcypromine has been shown to increase extracellular 5-HT in the region of the dorsal raphe nucleus (Celada and Artigas, 1993), the site of the somatodendritic 5-HT_{1A} autoreceptor.

In the present study, we found that the inhibition of 5-HT cell firing induced by tranylcypromine was reversed by administration of WAY 100635 in every cell tested. WAY 100635 is a potent and selective 5-HT_{1A} antagonist (Fletcher et al., 1995) and has been shown previously to block the inhibition of 5-HT cell firing induced by the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*N*-propylamino)tetralin, and the 5-HT reuptake inhibitor, paroxetine, but not the α_1-adrenoceptor antagonist, prazosin (Fletcher et al., 1995; Gartside et al., 1995a, 1997). Taken together these findings strongly suggest that tranylcypromine inhibits 5-HT cell firing via activation of 5-HT_{1A} autoreceptors in the dorsal raphe nucleus, secondary to an increase in extracellular 5-HT in this region.

In a proportion of cells (4/6), we found that tranylcypromine (1–5 mg/kg i.v.) transiently increased 5-HT cell firing before full inhibition set in, as has been observed previously (Aghajanian et al., 1970). Although we have not fully investigated the mechanism underlying this effect, we know from recent microdialysis experiments (Sharp et al., unpublished observation) that tranylcypromine (1–5 mg/kg i.v.) causes a 2- to 3-fold increase in extracellular noradrenaline in the raphe region. One possibility is that the transient activation relates to stimulation of α_1-adrenoceptors in the dorsal raphe nucleus which are well known to be excitatory on 5-HT neurones in this region (Baraban and Aghajanian, 1980).

In our microdialysis experiments we found that tranylcypromine increased extracellular 5-HT in the frontal cortex as previously reported (e.g., Celada and Artigas, 1993), but now we show that this effect is facilitated by pretreatment with WAY 100635. Tranylcypromine alone probably causes a rise in cortical extracellular 5-HT (at least in part) by increasing the size of the pool of releasable (vesicular?) 5-HT following inhibition of 5-HT metabolism. Our electrophysiological data show, however, that the same dose of tranylcypromine which increased extracellular 5-HT (5 mg/kg i.v.) inhibits 5-HT cell firing by 50%. It seems entirely plausible that this decrease in 5-HT neuronal activity induced by tranylcypromine offsets its ability to mobilise the releasable pool of 5-HT, and thereby raise extracellular 5-HT. Accordingly, our finding that WAY 100635 potentiated the tranylcypromine-induced increase in extracellular 5-HT is likely to be directly associated with the ability of the 5-HT_{1A} receptor antagonist to block the inhibition of cell firing. This association is emphasised by the fact that WAY 100635 facilitated the tranylcypromine-induced rise in cortical extracellular 5-HT at the same dose (0.1 mg/kg i.v.) which blocked the effect of tranylcypromine on 5-HT cell firing.

It should be noted that part of the effect of tranylcypromine alone on extracellular 5-HT may be mediated by an amphetamine-like releasing effect of the drug (the chemical structure of tranylcypromine resembles that of amphetamine) and not blockade of monoamine oxidase. This component of the tranylcypromine-induced increase in extracellular 5-HT probably would not be dependent on the level of 5-HT neuronal activity and not be facilitated by WAY 100635.

In addition to tranylcypromine, other classes of monoamine oxidase inhibitors inhibit 5-HT cell firing in the dorsal raphe nucleus (Aghajanian et al., 1970). It is reasonable to assume that a 5-HT_{1A} receptor antagonist would also block the inhibition of 5-HT neuronal activity induced by these monoamine oxidase inhibitors, and facilitate their effect on extracellular 5-HT in the forebrain.

In summary, the present study shows that a selective 5-HT_{1A} receptor antagonist blocks the inhibition of 5-HT cell firing induced by a monoamine oxidase inhibitor, and increases the effect of the same monoamine oxidase inhibitor on extracellular 5-HT in the forebrain. This effect may underlie clinical evidence that the antidepressant effect of an monoamine oxidase inhibitor can be facilitated by co-administration of a 5-HT_{1A} receptor antagonist (Artigas et al., 1994; Blier and Bergeron, 1995).

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