The effects of BTS 54 505, a metabolite of sibutramine, on monoamine and excitatory amino acid-evoked responses in the rat dorsolateral geniculate nucleus *in vivo*

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1 The effects of BTS 54 505, the primary amine metabolite of the *non-tricyclic* putative antidepressant sibutramine, on the responses evoked by visual stimulation and ionophoretic application of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and excitatory amino acids (EAAs) in the rat dorsolateral geniculate nucleus (dLGN) have been investigated.

2 Ionophoretic application of 5-HT to dLGN neurones attenuated visually-evoked ($n = 46$), NMDA-evoked ($n = 21$) and AMPA-evoked responses ($n = 21$), while ionophoretic application of NA potentiated visually-evoked activity in these cells ($n = 27$).

3 Simultaneous application of BTS 54 505 with 5-HT (over 120 s) resulted in a prolongation of the recovery time (i.e. the period required by a neurone to recover by 50%, RT_{50}) from the 5-HT-mediated suppression of discharge activity ($\approx 275\%$ increase in RT_{50}). BTS 54 505 also prolonged the recovery time from a NA-mediated potentiation of firing ($\approx 450\%$ increase in RT_{50}). These effects on recovery time are attributed to the inhibition of uptake of both 5-HT and NA by BTS 54 505. The *amplitude* of the response to 5-HT or NA was unaffected by co-ejection of BTS 54 505.

4 Ionophoretic application of N-methyl-D-aspartate (NMDA) produced a current-dependent increase in neuronal firing, as did application of the non-NMDA receptor agonist α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). A simultaneous 120 s application of BTS 54 505 inhibited the NMDA response in all cells studied (mean $ED_{50} = 16 \pm 5$ nA) but had no effect on AMPA-evoked activity in the majority of the same cells ($n = 15/21$).

5 Short 10 s applications of BTS 54 505, at ejecting currents (≥ 30 nA) that attenuated NMDA-evoked activity in all cells studied, had no effect on either response amplitude or recovery time from ionophoretic application of 5-HT, suggesting that inhibition of NMDA-evoked activity by BTS 54 505 was not mediated by 5-HT uptake blockade.

6 These results suggest that BTS 54 505 inhibits NMDA-evoked activity, and the observation that this effect is unlikely to be due to raised levels of endogenous 5-HT following monoamine uptake blockade indicate that BTS 54 505 may interact directly with the NMDA receptor ionophore complex.

Keywords: Dorsolateral geniculate nucleus; excitatory amino acids; antidepressants; sibutramine HCl; BTS 54 505

Introduction

Sibutramine HCl (BTS 54 524; N-1-(1-(4-chlorophenyl)cyclobutyl)-3-methylbutyl-N,N-dimethylamine hydrochloride monohydrate) (Figure 1) is a non-tricyclic putative antidepressant. In models indicative of potential antidepressant activity, sibutramine potently counteracted the behavioural effects of reserpine and downregulated cortical β -adrenoceptors after only three days treatment (Buckett *et al.*, 1988a). This profile is characteristic of monoamine reuptake inhibitors, but sibutramine is a relatively weak inhibitor of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and dopamine uptake *in vitro* (Cheetham *et al.*, 1990). The primary and secondary amine metabolites (BTS 54 505 and BTS 54 354 respectively; Figure 1) of sibutramine have a similar pharmacological profile to the parent compound *in vivo*, but are up to 100 fold more potent than sibutramine as monoamine uptake inhibitors *in vitro* (Luscombe *et al.*, 1989; Cheetham *et al.*, 1990). This indicates that the pharmacological effects of sibutramine *in vivo* are mainly due to the activity of its primary and secondary amine metabolites, so the present studies have utilised the primary amine metabolite, BTS 54 505.

It has recently been reported that tricyclic antidepressants have antagonistic behaviour at N-methyl-D-aspartate (NMDA) receptors. Reynolds & Miller (1988) demonstrated that imipramine and desmethylimipramine (DMI) inhibited [3 H]-

MK-801 binding in rat brain membranes and prevented the NMDA-induced Ca^{2+} influx into cultured rat cortical neurones. It has also been demonstrated, in whole-cell and single-channel recording techniques, that DMI attenuates NMDA receptor channel activity in a dose- and voltage-dependent manner, suggesting that DMI inhibits NMDA receptors by open channel block in a manner similar to MK-801 (Sernagor *et al.*, 1989; White *et al.*, 1990). Tricyclic

Figure 1 Chemical structure of sibutramine (BTS 54 524) and its active amine metabolites.

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antidepressants have also been shown to block NMDA-induced lethality in mice (Leander, 1989) and the NMDA receptor antagonists MK-801 and 2-amino-7-phosphonopentanoic acid (AP7) have been shown to exhibit antidepressant-like actions in an inescapable stress paradigm (Trullas & Skolnick, 1990). Since these observations suggest that tricyclic antidepressants may act as NMDA receptor channel blockers, we have used electrophysiological techniques to elucidate whether BTS 54 505, an active metabolite of the non-tricyclic putative antidepressant sibutramine (Luscombe *et al.*, 1989), has any effect on NMDA receptor activity.

In this study we examined the effects of BTS 54 505 on NMDA-, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-, 5-HT- and NA-evoked activity in the dorso-lateral geniculate nucleus (dLGN) of the rat thalamus. It has been shown that direct application of NA to geniculate visual relay cells in the rat by microiontophoresis produces a facilitation of firing (Rogawski & Aghajanian, 1980), whereas in contrast to NA, ionophoretic application of 5-HT depresses the spontaneous and evoked activity of dLGN neurones in the rat (Rogawski & Aghajanian, 1980). These contrasting responses to NA and 5-HT in this nucleus therefore allow the effects of BTS 54 505 on both 5-HT and NA uptake to be readily investigated. It has been demonstrated that retinogeniculate neurotransmission is mediated by excitatory amino acid receptors in the cat (Sillito *et al.*, 1990a,b) and in the rat (Scott & Mason, 1992b,c). Recording from the dLGN thus affords the opportunity to investigate the effects of BTS 54 505 both on monoamine and on NMDA- and AMPA-evoked responses in one site. Preliminary reports of these studies have appeared elsewhere in abstract form (Scott *et al.*, 1991; 1992).

Methods

Male Lister hooded rat (200–250 g) were housed in groups of 2–5 with a 12 h light:12 h dark lighting cycle (lights on at 07h 00 min GMT). The animals were anaesthetized with urethane (1.3–1.5 g.kg⁻¹, i.p.; Sigma) and mounted in a David Kopf stereotaxic frame. Following subcutaneous injection under the scalp of local anaesthetic (2% w/v xylocaine; Astra), a mid-line scalp incision was made and the scalp muscle retracted. A craniotomy of 4–5 mm diameter was then performed with a hand-held trephine, 3 mm posterior to Bregma and 3.6 mm lateral to the midline suture in accordance with the stereotaxic atlas of Pellegrino *et al.* (1979). Silicone-coated seven-barrelled microelectrodes were used to record extracellular neuronal discharge activity and to eject drugs by the process of iontophoresis (Scott & Mason, 1992a). The silicone-coated seven-barrelled microelectrodes were made from borosilicate glass tubing with an inner filament ('Kwik-fil', 1.5 mm o.d. $\times$ 0.86 mm i.d.; Clark Electromedical Instruments, UK) and the tips broken back to $\approx$ 4–6 μ m. The centre barrel and one outer barrel were filled with pontamine sky blue (5% w/v in 500 mM NaCl) for extracellular recording and automatic current balancing respectively. The remaining barrels were each filled with one of the following: N-methyl-D-aspartate (NMDA: 10 mM: Sigma), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA: 1 mM: Tocris Neuramin), L-glutamate (10 mM: Sigma), 5-hydroxytryptamine (5-HT: 10 mM: Sigma), nor-adrenaline (NA: 10 mM: Sigma) or N-1-(1-[4-chlorophenyl]-cyclobutyl)-3-methylbutylamine hydrochloride (BTS 54 505: 10 mM: Boots Pharmaceuticals Research Department); all drugs were dissolved in 165 mM NaCl.

After surgical preparation of the animals for recording, the multibarrelled electrode was stereotaxically positioned and driven under remote hydraulic control to the dLGN. Hippocampal neurones were encountered 2–4 mm below the cortical surface and physiologically identified from their large spike amplitude ($\geq$ 800 μ V) and regular spontaneous firing rate (Kandel & Spencer, 1961). The dLGN neurones were

recorded below the level of the hippocampus (between 4.5–6 mm below the cortical surface), and were physiologically identified by their response to visual stimulation. This comprised a diffuse whole-field flash of 1 s duration followed by an off period of 1 s presented to the contralateral eye. Cells were classified according to their response as either light-activated (ON-cells), light suppressed (OFF-cells) or ON-OFF responsive cells. The recording sites were marked by ejection of pontamine sky blue (5% w/v in 500 mM NaCl; 10 μ A over 20 min) from the recording electrode and subsequent histological preparation of 50 μ m thick coronal brain sections to enable identification of the recording sites.

Timing of ionophoretic ejection of drugs was controlled by an electronic clock and retaining currents of $\pm$ 10 nA were used between ejections with continuous automatic current balancing. The effect of ionophoresed compounds on EAA-evoked responses in the dLGN were evaluated from integrated firing rate histograms. Spike waveform was monitored to ensure drugs did not exert anaesthetic effects. For each cell recorded a 'dose' (ejection current)-response profile was plotted for the various agonists used (e.g. Figure 2a for 5-HT; Figure 2b for NA). Subsequent modulatory effects of BTS 54 505 on agonist-evoked responses were then routinely assessed using agonist ejection currents that elicited 50–80% maximal response. Electrophysiological recording, display and processing of discriminated signals was conventional (Mason, 1986). Discriminated neuronal activity was plotted as integrated firing rate histograms over successive 5 s epochs onto a chart recorder. The chart records were then digitised with the aid of a digitising tablet (Summasketch Professional) using Sigmascan (Jandel, U.S.A.) software and imported into Sigmaplot (Jandel, U.S.A.) for data analysis.

Results

Effects of BTS 54 505 on 5-HT- and NA-evoked responses in dLGN

Ionophoretic application of 5-HT and NA onto dLGN neurones revealed opposite effects on visually-evoked firing, similar to their reported actions on electrical stimulation of the optic nerve in rat dLGN (Rogawski & Aghajanian, 1980). Ejection of 5-HT (–4 to 50 nA; 10–120 s duration) potentially inhibited visually-evoked firing in a current-dependent, reversible manner with a rapid onset (1–10 s) in all cells studied (n = 46) (Figure 2a). In contrast to 5-HT, ionophoretic application of NA (0 to 32 nA; 10–30 s duration), produced a current-dependent, reversible and rapid (1–10 s onset) potentiation of visually-evoked firing in all cells studied (n = 27) (Figure 2b). Both 5-HT- and NA-evoked responses were evident on the same dLGN cells.

As BTS 54 505, the primary amine metabolite of the monoamine uptake inhibitor sibutramine, is a more potent inhibitor of NA uptake than of 5-HT uptake (Cheetham *et al.*, 1990), ionophoretic application of BTS 54 505 might be predicted to have a more profound effect on exogenously applied NA than on exogenously applied 5-HT. This effect on monoamine uptake processes may be reflected in changes in response amplitude and/or recovery from agonist-evoked responses. The recovery time (RT), i.e. the period required by the neurone to recover by 50% (RT₅₀) from termination of the ionophoretic ejection, was used as an index of the efficacy of the transmitter uptake process (Wang *et al.*, 1979). In order to dissociate clearly the effects of BTS 54 505 on 5-HT receptor from NMDA receptor (see later results) responses, we examined the effects of BTS 54 505 on 5-HT-evoked responses in OFF cells, since visually-evoked responses in light-suppressed (OFF) cells in the rat *in vivo* appear to be mediated selectively by non-NMDA receptors (Scott & Mason, 1992b,c). BTS 54 505 (0 to 30 nA; 120 s duration) was ejected continuously at a current that had no effect on the basal visually-evoked firing rate (Figures 3a,b) while

Figure 2 (a) Integrated firing rate histogram accumulated over successive 5 s epochs for a visually-driven dLGN cell. Ionophoretic application of 5-hydroxytryptamine (5-HT, 1 to 5 nA; 60 s duration) resulted in a current-dependent and reversible inhibition of the visually-evoked response. In this and subsequent figures the duration of drug ejection is indicated by the bars above the histogram record; the numbers above the bars indicate the ionophoretic current used. (b) Integrated firing rate histogram accumulated over successive 5 s epochs for a visually-driven dLGN cell. Ionophoretic application of noradrenaline (NA, 1 to 16 nA; 30 s duration) resulted in a current-dependent and reversible potentiation of the visually-evoked response.

regular ejections of either 5-HT or NA were repeated at 2 or 4 min intervals. BTS 54 505 prolonged the suppression of firing induced by ionophoretically applied 5-HT [mean $RT_{50} \pm$ s.e.mean: (control) 14 ± 4 s; (BTS 54 505) 35 ± 4 s; $n = 13$] (Figure 3a). Similarly, BTS 54 505 prolonged the potentiation of firing by ionophoretically applied NA [mean $RT_{50} \pm$ s.e.mean: (control) 29 ± 2 s; (BTS 54 505) 131 ± 7 s; $n = 6$] (Figure 3b). In all of these cells, it is notable that *only* the recovery time of the response to both 5-HT and NA was affected by application of BTS 54 505; the amplitude of the 5-HT- and NA-evoked responses remained unaltered (Figures 3a, b). The observation that the response amplitudes were unaffected is unlikely to be due to saturation of agonist receptors since the effects of BTS 54 505 were assessed on 5-HT and NA responses which were between 50–80% of maximum (see Methods). In the same cells when 120 s applications of BTS 54 505 prolonged the recovery time following cessation of 5-HT ejection, short 10 s application of BTS 54 505, had no effect on the recovery time (0–30 nA; $n = 6$ cells) (Figure 3a).

Figure 3 (a) Integrated firing rate histogram accumulated over successive 5 s epochs for a visually-driven dLGN cell. Ionophoretic application of 5-hydroxytryptamine (5-HT, 20 nA; 30 s duration) (hatched bars) resulted in a reversible inhibition of the visually-evoked response. Application of BTS 54 505 (open bar) (30 nA for 10 s) had no effect on either response amplitude or recovery time of the 5-HT-evoked response, whereas application of BTS 54 505 (30 nA) for 120 s prolonged the total recovery time from the 5-HT-evoked inhibition, but had no effect on response amplitude. (b) Integrated firing rate histogram accumulated over successive 5 s epochs for a visually-driven dLGN cell. Ionophoretic application of noradrenaline (NA, 16 nA; 30 s duration) (hatched bars) resulted in a reversible potentiation of the visually-evoked response. Application of BTS 54 505 (20 nA; 120 s) (open bar) prolonged the total recovery time from NA-evoked excitation, but had no effect on response amplitude.

Effect of BTS 54 505 on excitatory amino acid-evoked responses in the dLGN

The effects of BTS 54 505 on NMDA- and AMPA-evoked responses were compared in order to evaluate the specificity of BTS 54 505 on NMDA and non-NMDA receptors. Ionophoretic application of NMDA (0 to 40 nA; 10 s duration; $n = 21$), AMPA (0 to 40 nA; 10 s duration; $n = 21$) or glutamate (20 to 100 nA; 10 s duration; $n = 9$) (data not shown) produced a current-dependent potentiation of activity in all cells studied.

BTS 54 505 (0 to 40 nA; 120 s duration) produced a current-dependent and reversible inhibition of the NMDA-evoked (2 to 40 nA; 10 s duration) response in all cells studied (mean $ED_{50} \pm$ s.e.mean: 16 ± 5 nA; $n = 21$) (Figure 4a). Ionophoretically applied 5-HT (5 to 20 nA; 30 s duration) also resulted in a current-dependent attenuation of the

Figure 4 (a) Integrated firing rate histogram accumulated over successive 5 s epochs for a dLGN cell. Ionophoretic application of N-methyl-D-aspartate (NMDA, 20 nA; 10 s duration) (●) at regular 120 s intervals resulted in a consistent potentiation of baseline activity. Co-application of 5-HT (5 to 10 nA; 30 s duration) (hatched bars) resulted in a current-dependent and reversible inhibition of the NMDA-evoked response. Co-application of BTS 54 505 (BTS; 5 to 20 nA; 120 s duration) (open bars) also resulted in a current-dependent and reversible inhibition of the NMDA-evoked response. (b) Integrated firing rate histogram accumulated over successive 5 s epochs for the same dLGN cell as in (a). Ionophoretic application of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 20 nA; 10 s duration) at regular 120 s intervals resulted in a consistent potentiation of baseline activity. AMPA applications are denoted by the filled circles below the histogram. Co-application of 5-HT (5 to 20 nA; 30 s duration) (hatched bars) resulted in a current-dependent and reversible inhibition of the NMDA-evoked response. Co-application of BTS 54 505 (BTS; 10 to 30 nA; 120 s duration) (open bars) had no effect on AMPA-evoked activity.

NMDA-evoked response in the same cells (mean $ED_{50} \pm$ s.e.mean: 9 ± 3 nA; $n = 21$) (Figure 4a).

The process mediating the block of NMDA-evoked activity by BTS 54 505 was addressed by applying BTS 54 505 and NMDA *simultaneously* for the 10 s period of NMDA ejection only. It would be unlikely that a 10 s application of BTS 54 505 would raise synaptic levels of endogenous 5-HT to a level sufficient to inhibit the simultaneous NMDA-evoked response, since a 10 s application of BTS 54 505 had no effect on the response amplitude or recovery time to ionophoretically applied 5-HT (Figure 3a). As illustrated in Figure 5, co-ejection of NMDA and BTS 54 505 (12–25 nA; 10 s duration) produced a current-dependent and reversible inhibition of the NMDA-evoked potentiation of firing in all cells studied (mean $ED_{50} \pm$ s.e.mean: 18 ± 4 nA; $n = 11$). Therefore, our observations that BTS 54 505 produced a current-dependent inhibition of the NMDA-evoked response following simultaneous application, strongly suggest that

Figure 5 Integrated firing rate histogram accumulated over successive 5 s epochs for a dLGN cell. Ionophoretic application of N-methyl-D-aspartate (NMDA, 40 nA; 10 s duration) at regular 120 s intervals (●) resulted in a consistent potentiation of baseline activity. Co-application of BTS 54 505 (25 nA; 120 s duration) (open bars) resulted in a reversible inhibition of the NMDA-evoked response. Shorter applications of BTS 54 505 (12 to 25 nA; 10 s duration) had the same effect as the longer application.

BTS 54 505 blockade of 5-HT uptake alone is unlikely to be responsible for this effect on NMDA-evoked responses.

If BTS 54 505 acts specifically at the NMDA receptor, then one prediction is that the responses evoked by the non-NMDA receptor agonist, AMPA, should remain unaffected by simultaneous application of BTS 54 505. In the same cells ($n = 21$) that 5-HT and BTS 54 505 attenuated NMDA-evoked activity (Figure 4a) ionophoresed 5-HT (5–20 nA; 30 s duration) produced a current-dependent and reversible inhibition of the AMPA-evoked response (mean $ED_{50} \pm$ s.e.mean: 11 ± 4 nA) (Figure 4b). Ejection of BTS 54 505 (10–30 nA; 120 s duration), however, had no effect on the AMPA-evoked response in $\approx 75\%$ of the same 21 cells. BTS 54 505 did show a weak ($<15\%$) current-dependent and reversible inhibition of the AMPA-evoked response in the remaining cells ($n = 6$) (Figure 4b).

Discussion

The present studies have shown that ionophoretic application of 5-HT attenuates both EAA- and visually-evoked firing in the rat dLGN, whereas NA potentiates EAA- and visually-evoked firing in these cells. Such activity has been reported previously (Rogawski & Aghajanian, 1980). BTS 54 505 prolonged the recovery time of dLGN cells from responses elicited by application of 5-HT and NA, but did not alter the amplitude of the responses to 5-HT and NA. Ionophoretic application of NMDA, AMPA or glutamate produced a current-dependent and reversible potentiation of firing in all cells studied. BTS 54 505 inhibited NMDA-evoked activity in a current-dependent manner, but had no effect on AMPA-evoked activity in $\approx 75\%$ of cells studied.

It may be considered that monoamine uptake inhibition by BTS 54 505 raises synaptic levels of 5-HT sufficiently to inhibit the NMDA-evoked or AMPA-evoked responses. However, BTS 54 505 is more potent at inhibiting NA uptake than 5-HT uptake (Cheetham *et al.*, 1990), yet at no time was there a potentiation of visually-evoked or NMDA-evoked firing by BTS 54 505, which would be mediated by blockade of uptake of endogenously released NA from the locus coeruleus noradrenergic innervation of the dLGN. It is possible that a dominance in 5-hydroxytryptaminergic over noradrenergic neurotransmission to the dLGN may explain

the absence of a potentiation of firing by BTS 54 505, but it is known that under anaesthesia, locus coeruleus neurones fire spontaneously at 0.5–5 Hz (Marwaha & Aghajanian, 1982) while dorsal raphe neurones fire spontaneously at 0.5–2.5 Hz (Marwaha & Aghajanian, 1982). The similarity between the firing rates, and hence the presumed release of neurotransmitter by the two monoaminergic projections, alone cannot be used as an index of the efficacy of synaptic activation in the dLGN. Nerve impulse traffic in the locus coeruleus or dorsal raphe pathways afferent to the dLGN does not necessarily imply release of neurotransmitter in the dLGN, since there may be physiological or modulatory synaptic influences that may prevent the ultimate release of transmitter at the terminal site. Even if there were release of transmitter in the dLGN, a similar frequency of host cell activation cannot imply a similar quantitative measure of neurotransmitter release. However, the observation that a 10 s application of BTS 54 505, at an ejecting current (30 nA) that was sufficient to inhibit NMDA-evoked activity in the cells studied (Figure 4a), had no effect on the response amplitude or recovery time from 5-HT application (Figure 3a), suggests that BTS 54 505 does not exert this attenuation of NMDA-evoked activity via 5-HT uptake blockade and that this rapid effect is probably mediated via NMDA receptor blockade.

In electrophysiological studies in the rat dLGN it has been observed that ionophoretic application of the tricyclic antidepressant desipramine also attenuated NMDA-evoked responses (Scott & Mason, unpublished observations), and that these inhibitory effects were equivalent to those observed using BTS 54 505. DMI is well established as a NMDA receptor antagonist; recent studies using [³H]-MK-801 binding (Reynolds & Miller, 1988; Scott *et al.*, unpublished observations), whole-cell and single-channel recording techniques (Sernagor *et al.*, 1989; White *et al.*, 1990) suggest that DMI blocks the open channel of the NMDA receptor ionophore. In addition, substitution of a single amino acid in the NMDA receptor channel by site-directed mutagenesis markedly alters the sensitivity to MK-801, Mg²⁺ and DMI (Sakurada *et al.*, 1993), providing further evidence that DMI acts in the NMDA receptor channel. It has also been demonstrated that DMI and imipramine prevent the Ca²⁺ influx produced by NMDA in rat cultured cortical neurones (Reynolds & Miller, 1988), that imipramine, amitriptyline and nortriptyline protect against NMDA-induced toxicity in rat cultured cerebellar granule cells (McCaslin *et al.*, 1992) and that TCA's block the induction of long-term potentiation in the rat hippocampus by inhibiting activity at NMDA receptors (Watanabe *et al.*, 1993).

The inhibition of EAA-evoked responses by BTS 54 505 may suggest that BTS 54 505 is a non-specific EAA antagonist, blocking both NMDA and AMPA receptor channels. However, the present studies have demonstrated that BTS 54 505 and 5-HT attenuate NMDA-evoked activity in all cells studied (Figure 4a). In contrast, 5-HT attenuated AMPA-evoked activity in the same cells (Figure 4b), whereas BTS 54 505 was ineffective at inhibiting the AMPA-evoked response in the majority of these cells (Figure 4b). In addition,

the NMDA receptor antagonist, APV, was observed to attenuate the AMPA-evoked response in a number of dLGN cells ($n = 4/9$; data not shown). Therefore a more plausible explanation may be that part of the AMPA-evoked response is in fact due to the recruitment of NMDA receptors following the removal of the voltage-dependent block by physiological levels of Mg²⁺ after AMPA-mediated depolarization, thereby allowing the activation of the NMDA receptors by endogenous EAAs. This postulated NMDA receptor-mediated component of the AMPA-evoked response would then be open to blockade by NMDA antagonists. While both NMDA- and AMPA-evoked responses were similarly affected by 5-HT, the EAA-evoked responses displayed differential sensitivity to application of BTS 54 505 in the same cells. This observation further supports the notion that attenuation of the NMDA-evoked activity is not due to a blockade of 5-HT uptake but is mediated by a direct action at the NMDA receptor. Indeed, this view is supported by receptor binding studies that show a displacement of [³H]-MK-801 binding to rat hippocampal membranes by BTS 54 505 with a K_i value equivalent to those of the known NMDA receptor channel blockers DMI and ketamine (Scott *et al.*, unpublished observations).

Open channel blockers and compounds such as DMI may be useful in protecting against NMDA-induced toxicity and in the treatment of neurodegenerative diseases induced by NMDA receptor hyperactivity (Lodge, 1988). These compounds, due to their use-dependent activity (Kemp *et al.*, 1987; Sernagor *et al.*, 1989), would have little or no effect on NMDA receptor mediated neurotransmission in situations where the NMDA receptor would be inactive due to the voltage-dependent block by Mg²⁺ (Mayer *et al.*, 1984; Nowak *et al.*, 1984). Alleviation of the voltage-dependent Mg²⁺ block of NMDA receptor activity by membrane depolarization would then allow channel blockers to exert their antagonistic properties. However, it is known that the channel blockers have psychotomimetic side-effects (Wheal & Thomson, 1991; Oye *et al.*, 1992). Tricyclic antidepressants also have a number of side effects which arise from the affinity of these drugs for muscarinic cholinergic receptors (Synder & Yamamura, 1977; Nomura *et al.*, 1987) and histamine receptors (Alvares *et al.*, 1988). These side-effects may therefore limit their therapeutic use in the treatment and/or prevention of NMDA-induced toxicity and neurodegeneration. Sibutramine and its active metabolite BTS 54 505, however, have no significant affinity for muscarinic receptors, α_1 , α_2 and β adrenoceptors, dopamine D₁ and D₂ receptors, and 5-HT₁ and 5-HT₂ receptors (Buckett *et al.*, 1988b; Luscombe, unpublished observations), suggesting that sibutramine and BTS 54 505 may result in fewer and less pronounced side-effects than the tricyclic antidepressants. Further investigations, including [³H]-MK-801 receptor binding and single-channel studies, are presently in progress to elucidate the precise mechanism of action of BTS 54 505 on NMDA receptor activity.

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