British Journal of Pharmacology (2003) 139, 1480–1486
www.nature.com/bjp

$GABA_B$ receptor agonists reverse akinesia following intranigral or intracerebroventricular injection in the reserpine-treated rat

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1 This study examined whether $GABA_B$ receptor agonists injected directly into the substantia nigra pars reticulata (SNr) and globus pallidus (GP), or given intracerebroventricularly, could reverse reserpine-induced akinesia in the rat.

2 Male Sprague–Dawley rats, stereotaxically cannulated above the SNr, GP or third ventricle, were rendered akinetic by injection of reserpine ($5\,mg\,kg^{-1}$ s.c.). After 18 h, the locomotor effects of the $GABA_B$ receptor agonists, baclofen or SKF 97541 were examined.

3 Unilateral injection of baclofen (1–5 μg in 0.5 μl) into the GP failed to evoke any locomotor response ($n=6$). In contrast, unilateral intranigral injection of baclofen (0.08–1.6 μg in 0.5 μl) produced a dose-dependent increase in net contraversive rotations reaching a maximum of 162 ± 24 turns $90\,min^{-1}$ ($n=6$–8). Pretreatment with the selective $GABA_B$ receptor antagonist, CGP 46381 (2.4 μg in 0.5 μl), inhibited the effects of baclofen (0.8 μg) by $68\pm9\%$ ($n=6$).

4 Following intracerebroventricular injection, baclofen (0.8–4 μg in 2 μl) produced a dose-dependent increase in net arbitrary locomotor units (ALUs), reaching a maximum of 447 ± 154 ALUs in 35 min ($n=6$–7). SKF 97541 (4–32 μg in 2 μl) similarly reversed akinesia, reaching 129 ± 69 ALUs in 15 min ($n=6$).

5 These data show that activation of $GABA_B$ receptors within the SNr, but not the GP, reverses reserpine-induced akinesia. The success of intracerebroventricular injection of baclofen suggests a potential for systemically active $GABA_B$ receptor agonists in the treatment of akinesia in Parkinson's disease.

British Journal of Pharmacology (2003) **139,** 1480–1486. doi:10.1038/sj.bjp.0705372

Keywords: Akinesia; baclofen; globus pallidus; substantia nigra pars reticulata; $GABA_B$ receptor; rat, reserpine; Parkinson's disease

Abbreviations: BG, basal ganglia; CGP 35348, 3-aminopropyl (diethyoxymethyl) phosphinic acid; CGP 463813, 3-aminopropyl (cyclohexylmethyl)phosphinic acid; GABA, γ-aminobutyric acid; GP, globus pallidus; PD, Parkinson's disease; SKF 97541, 3-aminopropyl (methyl) phosphinic acid; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; STR, striatum

Introduction

Parkinson's disease (PD) is characterised by unrelenting degeneration of the dopaminergic nigrostriatal tract and resultant symptoms of akinesia, rigidity and tremor. Existing dopamine replacement therapies produce a successful alleviation of these symptoms but, in most cases, long-term use is associated with a reduction in efficacy, coupled with the appearance of debilitating dyskinesias (Bezard *et al*., 2001). For these reasons, alternative treatments are sought.

Striatal dopamine denervation leads to a multitude of downstream changes within the reciprocally linked nuclei of the basal ganglia (BG). Among these is the pronounced overactivity of striatopallidal projections as evidenced by an increase in GABA efflux in the globus pallidus (GP; rodent homologue of the external globus pallidus, GPe) of parkinsonian rodents (Segovia *et al*., 1986). Dysfunction of the GP is coupled with, and most likely contributes to, overactivity of the glutamatergic projections from the subthalamic nucleus (STN) to the output regions of the BG such as the substantia nigra pars reticulata (SNr) (e.g. Vila *et al*., 1997). These two overactive pathways ultimately contribute to inhibition of thalamocortical feedback that underlies the generation of parkinsonian motor deficits such as akinesia. Accordingly, surgical pallidotomy or subthalamotomy aimed at restoring normal activity of the BG circuits has been shown to reverse the motor deficits in PD patients. Moreover, surgical inactivation of the STN *via* high-frequency deep brain stimulation is effective in relieving symptoms as well as reducing both the 'off' periods and incidence of dyskinesia resulting from long-term L-Dopa treatment (reviewed by Lozano, 2003). However, a pharmacological means of achieving similar correction of the BG circuits would clearly be more desirable.

One pharmacological approach to correcting the activity of the BG motor circuit in PD might be to lessen the amount of neurotransmitter released from these overactive pathways. For

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example, reducing glutamate release from STN efferents in the SNr or reducing GABA release from striatopallidal efferents in the GP might be expected to achieve this. Targeting the inhibitory metabotropic $GABA_B$ receptor may provide a novel means of producing this desired inhibition of neurotransmitter release. $GABA_B$ mRNA is densely expressed in the striatum (STR) and STN, while corresponding $GABA_B$ receptor binding is high in the terminal fields, the GP and SNr, respectively (Kaupmann *et al*., 1997; Ambardekar *et al*., 1999; Durkin *et al*., 1999; Fritschy *et al*., 1999; Margeta-Mitrovich *et al*., 1999). Taken together, these data infer a presynaptic localisation of $GABA_B$ receptors in the GP and SNr that has since been confirmed by electron microscopic immunogold studies (Charara *et al*., 2000). Whether activation of these receptors leads to inhibition of GABA release from striatopallidal neurones or glutamate release from STN efferent terminals in the SNr remains to be determined. However, electrophysiological data support this action in the SNr at least, where $GABA_B$ receptor activation has been shown to reduce the size of glutamate-mediated excitatory postsynaptic potentials (Shen & Johnson, 1997). Therefore, $GABA_B$ receptor activation may offer a means of providing relief of motor deficits in PD.

The aim of this study was to assess whether injection of $GABA_B$ receptor agonists either discretely into the GP or SNr, or more diffusely into the third cerebral ventricle, could reverse akinesia in the reserpine-treated rat model of PD. Some of this work has been published previously in abstract form (Johnston *et al*., 2001; Johnston & Duty, 2002).

Methods

Intracerebral cannulation and induction of akinesia

Male, Sprague–Dawley rats (270–300 g) were housed in a temperature- and humidity-maintained environment with a 12 h light/dark cycle and free access to food and water. All procedures were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and all efforts were made to minimise animal suffering and the number of animals used. Following induction of general anaesthesia (Halothane, 4% in O_2), rats were stereotaxically implanted with 23-gauge stainless-steel guide cannulae positioned 1 mm above either the GP (0.9 mm anterior, 3.0 mm lateral and 5.8 mm ventral to bregma) or third ventricle (4.3 mm anterior, 0 mm lateral and 3.7 mm ventral to bregma) or 2 mm above the SNr (3.7 mm anterior, 2.0 mm lateral and 3.6 mm ventral to the interaural line), according to the standard rat brain atlas of Paxinos & Watson (1986). After 1 week, animals were rendered akinetic with the catecholamine-depleting agent reserpine (5 mg kg^{-1}, s.c.). A further 18 h later, when animals displayed a stable level of akinesia, the effects of $GABA_B$ receptor agonists on locomotor activity were assessed.

Assessment of rotational behaviour following intrapallidal or intranigral drug administration

For both intrapallidal and intranigral studies, visual behavioural assessments were made using video-recorded observations. Following a 15-min acclimatisation period in 40-cm diameter, flat-bottomed hemispheric bowls, baseline activity was videotaped for 30 min. Animals then received a single, unilateral injection of the $GABA_B$ receptor agonist baclofen (0.08–1.6 μg in SNr; 1–5 μg in GP) in 0.5 μl phosphate-buffered saline (PBS; mM): NaCl 137, KCl 2.7, KH_2PO_4 1.8, Na_2HPO_4 10; pH 7.4 or vehicle (0.2 μl PBS) into the GP or SNr. Injections were made over a 1-min period *via* 30-gauge stainless steel needles inserted through, and extending 1 mm (GP) or 2 mm (SNr) below the tip of the guide cannula and attached with flexible tubing (Portex) to a 10-μl Hamilton microsyringe. Animals were videotaped for a further 90 min. Net contraversive rotations (360°) were measured as an index of unilateral relief of akinesia. These rotations were counted manually from the videotape recordings, in 5-min timebins over the entire 90-min period ($n = 6–8$ animals per dose). In regions displaying convincing relief of akinesia following injection of baclofen, receptor specificity of these effects was confirmed using the $GABA_B$ receptor antagonist, 3-aminopropyl (cyclohexylmethyl) phosphinic acid (CGP 46381) (Olpe *et al*., 1993). The effects of CGP 46381 were examined against a single, submaximally effective dose of baclofen (0.8 μg). In these experiments, 6 h following 90 min of monitoring the effects of an initial injection of baclofen (0.8 μg in 0.5 μl), rats were injected with either CGP 46381 (2.4 μg) or vehicle (0.5 μl PBS) into the same site. Rotational behaviour was monitored throughout the 30-min equilibration period for CGP 46381 and for a further 60 min following a repeat dose of baclofen (0.8 μg in 0.5 μl). At the end of each experiment, fast blue dye (0.2 μl of 1%, w v^{-1}) was injected *via* the guide cannula to allow histological verification of injection sites. Approximately 5 min after dye injection, animals were killed by enflurane overdose and cervical dislocation. The brains were snap frozen in isopentane (cooled to −45°C with solid CO_2) and stored desiccated at −70°C until subsequent cryostat sectioning (20 μm) and cresyl violet (0.1% w v^{-1}) staining.

Assessment of locomotor activity following intracerebroventricular drug administration

For intracerebroventricular (i.c.v.) studies, visual locomotor assessments were performed in rectangular cages with 5 cm square grid lines covering the base. After a 15-min acclimatisation period, a 30-min baseline period was first recorded. Animals ($n = 6–7$ per dose) then received a single i.c.v. injection of baclofen (0.8–6 μg in 2 μl PBS) or vehicle (2 μl PBS, pH 7.4) over a 2-min period and were videotaped for a further 80 min. Locomotor activity was measured manually in 5-min timebins over an 80-min period in arbitrary locomotor units (ALUs), where one ALU refers to both front paws crossing a grid line. In preliminary studies, i.c.v. injection of the selective $GABA_B$ receptor antagonists CGP 46381 (0.6–6 μg) or CGP 35348 (0.1–10 μg) was found to induce convulsions and so could not be used to confirm receptor specificity of the response to baclofen. Therefore, the effects of a second $GABA_B$ receptor agonist, SKF 97541 (3-aminopropyl (methyl) phosphinic acid; Seabrook *et al*., 1990) were examined instead. The effects of i.c.v. injection of SKF 97541 (4–64 μg in 2 μl PBS) were examined in an identical manner to that outlined above for baclofen. The positioning of cannulae was checked in all cases by examination of needle tracts in freshly dissected brain.

Data analysis

In all cases, measurements were taken from video recordings using a single blinded approach to avoid experimenter bias. Differences between the various doses of baclofen, SKF 97451 or vehicle were compared using a one-way analysis of variance with a Student–Newman–Keuls *post hoc* analysis. For SNr data, the effects of baclofen prior to and following CGP 46381 or vehicle treatment were compared using a two-tailed paired *t*-test. In all cases, $P<0.05$ was taken to represent a significant difference.

Drugs

Baclofen, 3-aminopropyl (cyclohexylmethyl) phosphinic acid (CGP 46381) and 3-aminopropyl (methyl) phosphinic acid (SKF 97541) were obtained from Tocris Cookson Ltd U.K. Reserpine and PBS reagents were obtained from Sigma, U.K.

Results

Throughout baseline recordings, all reserpine-treated rats displayed negligible circling behaviour (0–3 rotations $30\,min^{-1}$) or locomotor activity and were thereby deemed fully akinetic. Only those animals with correctly placed cannulae, as shown on histological examination, were included in the data analyses. This excluded less than 10% of animals from the final analysis.

Locomotor effects following intrapallidal injection of $GABA_B$ receptor agonist

Unilateral injection of the $GABA_B$ receptor agonist, baclofen, into the GP of reserpine-treated rats did not produce any significant increase in net contraversive turning compared to vehicle (PBS) for the entire dose range tested (1–5 μg) over a 100-min recording period (data not shown, $n=6$ animals per dose). In addition, the highest dose administered (6 μg) caused central excitation manifest as fitting, loss of balance and rigid paralysis in all animals tested.

Locomotor effects following intranigral injection of $GABA_B$ receptor agonist and antagonist

Unilateral injection of the $GABA_B$ receptor agonist baclofen into the SNr of reserpine-treated rats caused a significant increase in net contraversive rotations. The time course for this effect, as shown for the maximally effective dose (1.6 μg) in Figure 1a, revealed a peak response within the first 5 min after injection then a plateau that was maintained for up to 90 min. Accordingly, quantification of circling behaviour produced by the full range of baclofen doses was conducted over a 90-min period. Neither vehicle (PBS) nor the lowest baclofen dose (0.08 μg) produced any significant net contraversive rotations over this period. In contrast, baclofen (0.2–1.6 μg) produced a significant and dose-dependent increase in net contraversive rotations $90\,min^{-1}$ compared to vehicle ($n=6$–8 rats per dose; Figure 1b). As a true maximum response was not obtained, calculation of the ED_{50} for baclofen was not possible.

Pretreatment with the selective $GABA_B$ receptor antagonist, CGP 46381 (2.5 μg) significantly inhibited the submaximal baclofen (0.8 μg)-induced net contraversive rotations $90\,min^{-1}$ by 68±9% (mean±s.e.m., $n=6$). In contrast, pretreatment with vehicle for CGP 46381 (PBS) did not affect the subsequent response to baclofen ($n=6$) (Figure 2). No rotational behaviour was observed during the equilibration period with CGP 46381 alone.

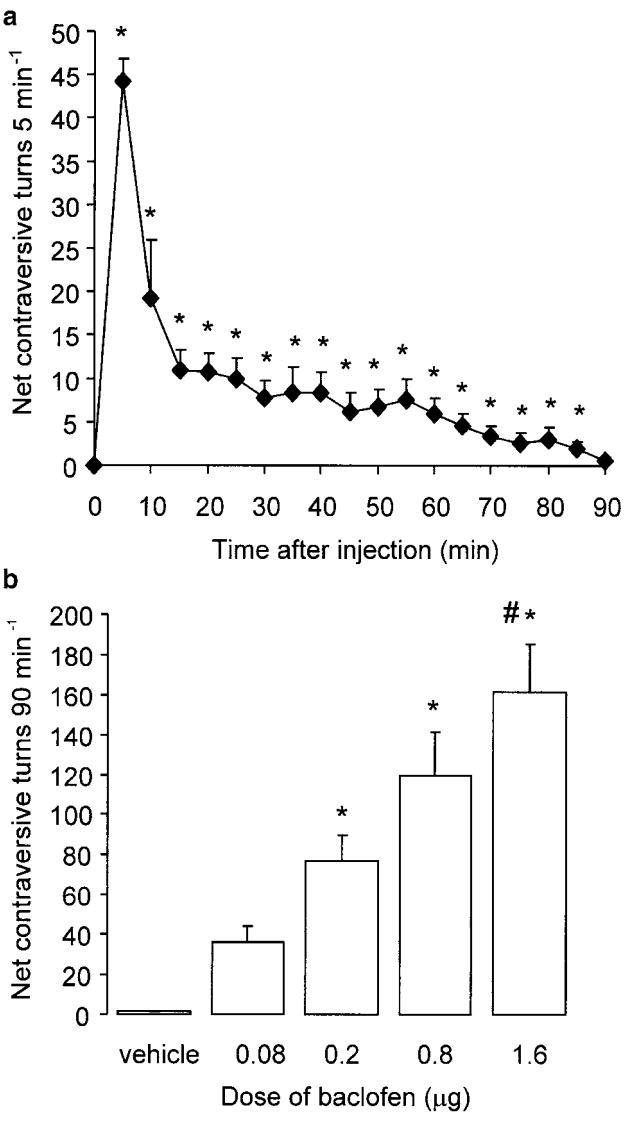

Figure 1 (a) Time course of locomotor activity induced by a maximally effective dose of the $GABA_B$ receptor agonist, baclofen (1.6 μg in 0.5 μl) and (b) dose-dependent locomotor effects of baclofen (0.08–1.6 μg in 0.5 μl) or vehicle (0.5 μl PBS), following unilateral injection into the SNr of the reserpine-treated rat. Values represent mean±s.e.m. ($n=6$–8 animals per dose). *Indicates a significant difference compared to vehicle; # indicates a significant difference to an 0.08 μg dose (one-way ANOVA, $P<0.05$).

Locomotor effects following i.c.v. injection of $GABA_B$ receptor agonist

Reversal of reserpine-induced akinesia was also observed following i.c.v. injection of baclofen. The time course of a maximally effective dose of baclofen showed a time to onset and peak locomotor activity within the first 5 min following injection (Figure 3a). The duration of this effect, before a

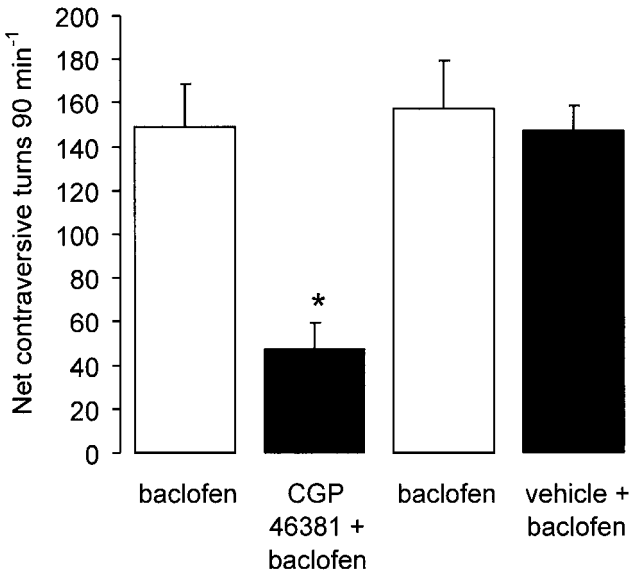

Figure 2 Effect of intranigral injection of the GABA$_B$ receptor agonist, baclofen (0.8 μg in 0.5 μl) on locomotor activity prior to (open bars) and 30 min following (closed bars) treatment with the GABA$_B$ receptor antagonist, CGP 46381 (2.4 μg in 0.5 μl) or vehicle (0.5 μl PBS). Data represent mean $\pm$ s.e.m. ($n = 6$ animals per group). *Indicates a significant difference between pretreatment and post-treatment responses to baclofen (paired t-test, $P < 0.05$).

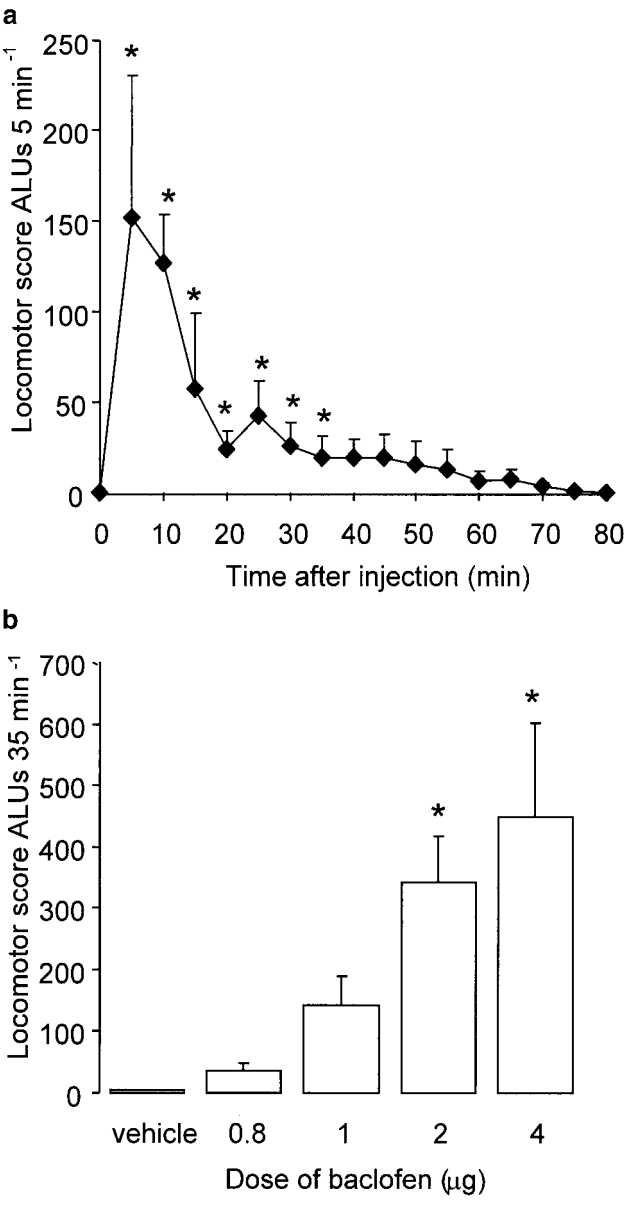

Figure 3 (a) Time course of locomotor activity induced by a maximally effective dose of the GABA$_B$ receptor agonist, baclofen (4 μg in 2 μl) and (b) dose-dependent locomotor effects of baclofen (0.8–4 μg in 2 μl) or vehicle (2 μl PBS), following injection into the third ventricle of the reserpine-treated rat. Values represent mean ALUs $\pm$ s.e.m. ($n = 6$–7 animals per dose). *Indicates a significant difference compared to vehicle (one-way ANOVA, $P < 0.05$).

return to baseline levels, led to the subsequent quantification of locomotor activity over a 35-min period for the entire dose range of baclofen examined. Neither vehicle nor the two lowest doses (0.8 and 1 μg) of baclofen caused a significant change in locomotor activity compared to vehicle. In contrast, baclofen (2 and 4 μg) produced a significant, dose-dependent increase in net ALUs 35 min^{-1} compared to vehicle ($n = 6$–7) (Figure 3b).

SKF 97541 (4–32 ng), produced a similar dose-dependent induction of locomotor activity, albeit with a shorter duration of action and reduced efficacy compared to baclofen (Figure 4a). Full quantification of locomotor activity over a 15-min period revealed this response to be dose-dependent, with a significant increase in locomotor activity compared to vehicle achieved at the highest dose tested (32 ng; $n = 6$; Figure 4b). Since neither baclofen nor SKF-induced responses reached saturation, calculation of ED$_{50}$ values was again not possible.

Discussion

This study aimed to investigate the hypothesis that central GABA$_B$ receptor activation, specifically within either the GP or SNr, would promote locomotor behaviour in akinetic rodents. The results presented here show that GABA$_B$ receptor agonists administered *via* i.c.v. and intranigral, but not intrapallidal injection, alleviate akinesia in the reserpine-treated rat model of PD.

Potential reasons underlying the lack of effect of intrapallidal injection of baclofen in reserpine-treated rats

GABA containing striatopallidal projections are overactive under parkinsonian conditions of dopamine depletion, releasing an excess of GABA in the GP (Levy *et al*., 1997). Pharmacological inhibition of the excess GABA release by, for example, activation of 5-HT$_{1B}$ heteroreceptors on the pre-terminal elements of striatopallidal neurones has been shown to bring about relief of reserpine-induced akinesia (Chadha *et al*., 2000a). Immunocytochemical studies have confirmed the presence of GABA$_B$ receptor protein on presumed striatopallidal terminals at symmetrical synapses within the GP (Charara *et al*., 2000) and a preliminary microdialysis study in normal rodents suggested that activation of presynaptic GABA$_B$ receptors in the GP may reduce GABA release (Singh, 1990). Taken together, these previous data support the

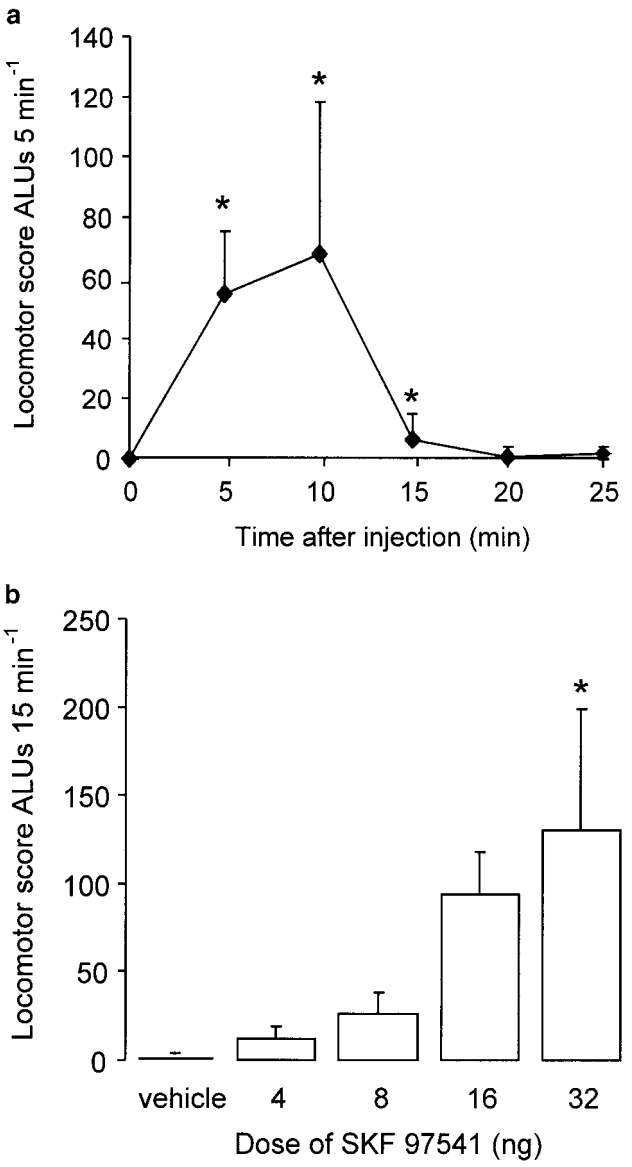

Figure 4 (a) Time course of locomotor activity induced by a maximally effective dose of the $GABA_B$ receptor agonist SKF 97541 (32 μg in 2 μl) and (b) dose-dependent locomotor effects of SKF 97541 (4–32 μg in 2 μl) or vehicle (2 μl PBS), following injection into the third ventricle of the reserpine-treated rat. Values represent mean ALUs $\pm$s.e.m. ($n=6$ animals per dose). *Indicates a significant difference compared to vehicle (one-way ANOVA, $P<0.05$).

potential for $GABA_B$ receptor activation to provide relief of akinesia in the reserpine-treated rat. Therefore, the lack of antiakinetic effect of baclofen in the present study is initially surprising. However, $GABA_B$ receptors have also been located on presumed STN efferent terminals at asymmetrical (glutamatergic) synapses within the GP (Charara *et al*., 2000), where their activation may lead to inhibition of glutamate release, as evidenced from microdialysis and electrophysiological studies (Singh, 1990; Chen *et al*., 2002). Therefore, it is highly likely that $GABA_B$ receptor activation in the GP produces a simultaneous reduction in both excitatory and inhibitory inputs to this region, thereby exacting negligible net locomotor effects.

Reversal of akinesia following intranigral $GABA_B$ receptor agonist injection

In contrast to the lack of effect seen with baclofen in the GP, injection of baclofen into the SNr produced a significant dose-dependent reversal of akinesia in the reserpine-treated rat. The response was of rapid onset, peaking in the first 5 min of application, with a lower level of significant activity being maintained for over an hour. The specific $GABA_B$ receptor antagonist, CGP 46381, inhibited the effect of a submaximal dose of baclofen by approximately two-thirds, confirming that this effect is mediated primarily *via* activation of $GABA_B$ receptors within the SNr.

The population of $GABA_B$ receptors mediating this response remains to be established, but at least three putative candidates exist. Of these, $GABA_B$ receptors present on STN-like terminal boutons within the SNr (Charara *et al*., 2000) are the most likely target. A functional heteroreceptor capacity of these receptors has been demonstrated by electrophysiological studies, showing that baclofen inhibited glutamate-mediated EPSPs in the SNr (Shen & Johnson, 1997). As noted above, overactivity of glutamatergic inputs from the STN account for the increased activity of the SNr and downstream inhibition of thalamocortical feedback that contributes to the akinetic symptoms of PD (Vila *et al*., 1997; Obeso *et al*., 2000). Therefore, it is highly likely that $GABA_B$ receptor-mediated inhibition of glutamate release from STN efferent projections may account for the anti-akinetic efficacy of baclofen. A role for the $GABA_B$ receptors known to be present on terminals of the GABAergic striatonigral pathway (Charara *et al*., 2000) seems unlikely. These receptors appear to function as autoreceptors since their activation has been shown electrophysiologically to inhibit GABA-mediated IPSPs in the SNr (Shen & Johnson, 1997). However, inhibiting GABA release in the SNr would not lead to reversal of akinesia. The activity of this so-called 'direct' pathway is reduced in the parkinsonian state, leading to lower levels of GABA release in the SNr as shown, for example, by the upregulation of $GABA_A$ receptor binding in the SNr in animal models of PD (e.g. Pan *et al*., 1985; Chadha *et al*., 2000b). Restricting GABA release from an already underactive pathway *via* $GABA_B$ receptor activation would, therefore, be predicted to have little net functional outcome. In any event, reducing GABA release in the SNr would not provide relief of akinesia but would, on the contrary, disinhibit an already overactive SNr and thus serve to exacerbate the existing parkinsonian pathophysiology. Whether such an action actually restricts the degree of relief of akinesia afforded by the favoured action of baclofen on $GABA_B$ receptors on the STN efferent terminals remains to be seen. Finally, while conceivable that baclofen may cause a decrease in SNr excitability and alleviation of akinesia directly, *via* activation of postsynaptic $GABA_B$ receptors in the SNr, *in vitro* electrophysiological studies cast doubt upon the functional significance of these receptors (Chan *et al*., 1998). In addition, in contrast to the high levels noted in the STN, mRNA species encoding $GABA_B$ receptor variants are low in the SNr (Bischoff *et al*., 1999; Durkin *et al*., 1999), suggesting that little functional $GABA_B$ receptor protein might be present within SNr cell bodies. These findings further support the notion that activation of $GABA_B$ receptors located on the STN efferent terminals in the SNr underlies the antiakinetic

actions of baclofen seen here in the reserpine-treated rat model of PD.

Reversal of akinesia following i.c.v. GABA$_B$ receptor agonist injection

The reversal of akinesia with baclofen was not restricted to site-directed injections, but was also demonstrated following injection into the third ventricle. In this case, the locomotor response showed a similar time to onset, suggesting a rapid diffusion of baclofen to its proposed site of action in the SNr. However, the response was more short-lived than that following intranigral injection, being over within 40 min of injection. The reason for this is not known, but probably does not reflect metabolism of baclofen within the cerebral ventricular fluid, since baclofen is excreted largely ($\sim$80%) unchanged (Faigle & Kerberle, 1972).

Unlike the situation in the SNr, problems were encountered when demonstrating that the locomotor effects of i.c.v. baclofen were specific to GABA$_B$ receptor activation. Injection of two different GABA$_B$ receptor antagonists, CGP 46381 (0.6–6 μg) and CGP 35348 (0.1–10 μg), both incurred fitting, thus preventing their use for this purpose. The concentrations of GABA$_B$ receptor antagonist employed were not particularly high, especially given the unhindered use of similar concentrations in other studies. For example, in normal rats, CGP 35348 injected intracerebroventricularly at a dose of 25 μg was effective at inhibiting tiagabine-induced antinociception, without causing convulsions (Ipponi *et al*., 1999). However, convulsant actions of GABA$_B$ receptor antagonists have been observed in other studies following intrathalamic and systemic administration (Vergnes *et al*., 1997). Moreover, it has long been known that reserpine treatment can increase seizure sensitivity. This was first demonstrated by Chen *et al*. (1954), who showed that reserpine-treated mice had a lowered convulsive threshold to pentylenetetrazol, caffeine and electrical stimuli (cited in Weinshenker & Szot, 2002). Whatever the exact cause of seizures in the present study, this activity prevented receptor specificity being confirmed for the intraventricular effects of baclofen. However, that a second GABA$_B$ receptor agonist, SKF 97541, elicited a similar profile of reversal of reserpine-induced akinesia following i.c.v. injection supports the involvement of GABA$_B$ receptors in this response.

In conclusion, these data indicate that GABA$_B$ receptor agonists such as baclofen display antiakinetic behaviour in the reserpine-treated rat model of PD. This effect is mediated *via* the activation of GABA$_B$ receptors within the SNr, although actions outside the SNr cannot be excluded at this stage, aside from the GP. The mechanism underlying this response may be activation of GABA$_B$ heteroreceptors present on STN efferent terminals although this proposal is the subject of further investigations. That successful alleviation of reserpine-induced akinesia is also achieved following i.c.v. injection of baclofen indicates that drug targeting of desired brain regions can occur without the need for direct, site-specific injection. Thus, activation of GABA$_B$ receptors may be a useful pharmacological approach in the symptomatic treatment of akinesia in PD.

T.J. was funded by an A.J. Clark Studentship from the British Pharmacological Society.

References

AMBARDEKAR, A.V., ILINSKY, I.A., FORESTL, W., BOWERY, N.G. & KULTAS-ILINSKY, K. (1999). Distribution and properties of GABA$_B$ antagonist [^{3}H]CGP 62349 binding in the rhesus monkey thalamus and basal ganglia and the influence of lesions in the reticular thalamic nucleus. *Neuroscience,* **93,** 1339–1347.

BEZARD, E., BROTCHIE, J.M. & GROSS, C.E. (2001). Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. *Nat. Rev. Neurosci.,* **2,** 577–588.

BISCHOFF, S., LEONHARD, S., REYMANN, N., SCHULER, V., SHIGEMOTO, R., KAUPMANN, K. & SETTLER, B. (1999). Spatial distribution of GABA$_B$R1 receptor mRNA and binding sites in the rat brain. *J. Comp. Neurol.,* **412,** 1–16.

CHADHA, A., SUR, C., ATACK, J. & DUTY, S. (2000a). The 5HT$_{1B}$ receptor agonist, CP-93129, inhibits [^{3}H]-GABA release from rat globus pallidus slices and reverses akinesia following intrapallidal injection in the reserpine-treated rat. *Br. J. Pharmacol.,* **130,** 1927–1932.

CHADHA, A., HOWELL, O., ATACK, J., SUR, C. & DUTY, S. (2000b). Changes in [^{3}H]zolpidem and [^{3}H]Ro 15-788 binding in rat globus pallidus and substantia nigra pars reticulata following a nigrostriatal tract lesion. *Brain Res.,* **862,** 280–283.

CHAN, P.K., LEUNG, C.K. & YUNG, W.H. (1998). Differential expression of pre- and postsynaptic GABA$_B$ receptors in rat substantia nigra pars reticulata neurones. *Eur. J. Pharmacol.,* **349,** 187–197.

CHARARA, A., HEILMAN, T.C., LEVEY, A.I. & SMITH, Y. (2000). Pre- and postsynaptic localization of GABA$_B$ receptors in the basal ganglia in monkeys. *Neuroscience,* **95,** 127–140.

CHEN, G., ENSOR, G.F. & BOHNER, B. (1954). A facilitation of reserpine on the central nervous system. *Proc. Soc. Exp. Biol. Med.,* **86,** 507–510.

CHEN, L, CHAN, S. & YUNG, W. (2002). Rotational behavior and electrophysiological effects induced by GABA$_B$ receptor activation in rat globus pallidus. *Neuroscience,* **114,** 417.

DURKIN, M.M., GUNWALDSEN, C.A., BOROWSKY, B., JONES, K.A. & BRANCHEK, T.A. (1999). An *in situ* hybridization study of the distribution of the GABA$_{B2}$ protein mRNA in the rat CNS. *Brain Res. Mol. Brain Res.,* **71,** 185–200.

FAIGLE, J.W. & KEBERLE, H. (1972). The chemistry and kinetics of Lioresal. *Postgrad. Med. J.,* **48,** S13.

FRITSCHY, J.M., MESKENAITE, V., WEINMANN, O., HONER, M., BENKE, D. & MOHLER, H. (1999). GABA$_B$-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. *Eur. J. Neurosci.,* **11,** 761–768.

IPPONI, A., LAMBERTI, C., MEDICA, A., BARTOLINI, A. & MALMBERG-AIELLO, P. (1999). Tiagabine antinociception in rodents depends on GABA$_B$ receptor activation: parallel antinociception testing and medial thalamus GABA microdialysis. *Eur. J. Pharmacol.,* **368,** 205–211.

JOHNSTON, T., JENNER, P. & DUTY, S. (2001). Intranigral but not intrapallidal baclofen alleviates akinesia in the reserpine treated rat. *Proceedings ASCEPT, BPS, CSPC, PSC, WPS*, Vancouver, Canada. 116 p.

JOHNSTON, T. & DUTY, S. (2002). Baclofen alleviates akinesia following intranigral and intraventricular injection in the reserpine-treated rat but fails to inhibit glutamate release from rat nigral slices. *Br. J. Pharmacol.,* **136,** 23.

KAUPMANN, K., HUGGEL, K., HEID, J., FLOR, P.J., BISCHOFF, S., MICKEL, S.J., MCMASTER, G., ANGST, C., BITTIGER, H., FROESTL, W. & BETTLER, B. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature,* **386,** 239–246.

LEVY, R., HAZRATI, L.N., HERRERO, M.T., VILA, M., HASSANI, O.K., MOUROUX, M., RUBERG, M., ASENSI, H., AGID, Y., FEGER, J., OBESO, J.A., PARENT, A. & HIRSCH, E.G. (1997). Re-evaluation of the functional anatomy of the basal ganglia in normal and Parkinsonian states. *Neuroscience,* **76,** 335–343.

LOZANO, A.M. (2003). Surgery for Parkinson's disease, the five W's: why, who, what, where, and when. *Adv. Neurol.,* **91,** 303–307.

MARGETA-MITROVIC, M., MITROVIC, I., RILEY, R.C., JAN, L.Y. & BASBAUM, A.I. (1999). Immunohistochemical localization of $GABA_B$ receptors in the rat central nervous system. *J. Comp. Neurol.,* **405,** 299–321.

OBESO, J.A., RODRIGUEZ-OROZ, M.C., RODRIGUEZ, M., LANCIEGO, J.L., ARTIEDA, J., GONZALO, N. & OLANOW, C.W. (2000). Pathophysiology of the basal ganglia in Parkinson's disease. *Trends Neurosci.,* **23,** S8–S19.

OLPE, H.R., STEINMANN, M.W., FERRAT, T., POZZA, M.F., GREINER, K., BRUGGER, F., FROESTL, W., MICKEL, S.J. & BITTIGER, H. (1993). The actions of orally active $GABA_B$ receptor antagonists on GABAergic transmission *in vivo* and *in vitro*. *Eur. J. Pharmacol.,* **233,** 179–186.

PAN, H.S., PENNEY, J.B. & YOUNG, A.B. (1985). γ-aminobutyric acid and benzodiazepine receptor changes induced by unilateral 6-hydroxydopamine lesions of the median forebrain bundle. *J. Neurochem.,* **45,** 1396–1404.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*, U.K.: Academic Press.

SEABROOK, G.R., HOWSON, W. & LACEY, M.G. (1990). Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic $GABA_B$ receptors on neurones in rat brain slices. *Br. J. Pharmacol.,* **101,** 949–957.

SEGOVIA, J., TOSSMAN, U., HERRERA-MARSCHITZ, M., GARCIA-MUNOZ, M. & UNGERSTEDT, U. (1986). Gamma-aminobutyric acid release in the globus pallidus *in vivo* after a 6-hydroxydopamine lesion in the substantia nigra of the rat. *Neurosci. Lett.,* **70,** 364–368.

SHEN, K.Z. & JOHNSON, S.W. (1997). Presynaptic $GABA_B$ and adenosine A1 receptors regulate synaptic transmission to rat substantia nigra reticulata neurones. *J. Physiol. (Lond.),* **505** (Part 1), 153–163.

SINGH, R. (1990). $GABA_B$ receptors mediate glutamate release in the rat caudate and globus pallidus. *Soc. Neurosci. Abstr.,* **16,** 1041.

VERGNES, M., BOEHRER, A., SIMLER, S., BERNASCONI, R. & MARESCAUX, C. (1997). Opposite effects of $GABA_B$ receptor antagonists on absences and convulsive seizures. *Eur. J. Pharmacol.,* **332,** 245–255.

VILA, M., LEVY, R., HERRERO, M.T., RUBERG, M., FAUCHEUX, B., OBESO, J.A., AGID, Y. & HIRSCH, E.G. (1997). Consequences of nigrostriatal denervation on the functioning of the basal ganglia in human and nonhuman primates: an *in situ* hybridization study of cytochrome oxidase subunit I mRNA. *J. Neurosci.,* **17,** 765–773.

WEINSHENKER, D. & SZOT, P. (2002). The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. *Pharmacol. Ther.,* **94,** 213–233.

(Received April 7, 2003
Revised May 7, 2003
Accepted May 13, 2003)