Striatal output markers do not alter in response to circling behaviour in 6-OHDA lesioned rats produced by acute or chronic administration of the monoamine uptake inhibitor BTS 74 398

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Summary. The monoamine uptake inhibitor BTS 74 398 induces ipsilateral circling in 6-hydroxydopamine (6-OHDA) lesioned rats without induction of abnormal motor behaviours associated with L-dopa administration. We examined whether this was reflected in the expression of peptide mRNA in the direct and indirect striatal output pathways.

6-OHDA lesioning of the nigrostriatal pathway increased striatal expression of PPE-A mRNA and decreased levels of PPT mRNA with PPE-B mRNA expression remaining unchanged. Acute L-dopa administration normalised PPE-A mRNA and elevated PPT mRNA while PPE-B mRNA expression remained unchanged. Acute administration of BTS 74 398 did not alter striatal peptide mRNA levels. Following chronic treatment with L-dopa, PPE-A mRNA expression in the lesioned striatum continued to be normalised and PPT mRNA was increased compared to the intact side. PPE-B mRNA expression was also markedly increased relative to the non-lesioned striatum. Chronic BTS 74 398 administration did not alter mRNA expression in the 6-OHDA lesioned striatum although small increases in PPT mRNA expression in the intact and sham lesioned striatum were observed.

The failure of BTS 74 398 to induce changes in striatal neuropeptide mRNA correlated with its failure to induce abnormal motor behaviours or behavioural sensitisation but does not explain how it produces a reversal of motor deficits. An action in another area of the brain appears likely and may explain the subsequent failure of BTS 74 398 and related compounds to exert anti-parkinsonian actions in man.

Keywords: Monoamine uptake inhibition; motor behaviour; preproenkephalin-A; preproenkephalin-B; preprotachykinin; 6-hydroxydopamine circling in unilateral 6-OHDA lesioned rats and reverses motor deficits in MPTP-treated primates suggesting a potential role in the symptomatic treatment of PD (Hansard et al. 2004; Lane et al. 2005). Indeed BTS 74 398 induces a more normal, less stereotyped improvement of motor function compared to the driven responses observed following L-dopa administration (Hansard et al. 2004). However, in contrast to L-dopa, repeated treatment with BTS 74 398 does not sensitise rotational behaviours or induce abnormal involuntary movements in 6-OHDA lesioned rats. These alterations in behaviour have been proposed to reflect the onset of L-dopa induced motor complications in PD, especially dyskinesia (Cenci et al. 1998; Lane et al. 2005). Similarly, in MPTP-treated common marmosets primed with L-dopa to exhibit dyskinesia, an acute challenge with BTS 74 398 did not elicit involuntary movements despite improving motor deficits. Again, these data point to a potential antiparkinsonian effect of BTS 74 398 but without the problem of dyskinesia induction.

Alterations in motor activity caused by degeneration of the nigrostriatal pathway, increases in motor function by L-dopa and dopamine agonists and the induction of dyskinesia involve alterations in the activity of medium spiny neurons in the direct and indirect striatal output pathways. These are reflected in the expression of neuropeptide mRNAs in these neurons. PPE-A mRNA encodes enkephalin in the indirect pathway while PPE-B and PPT mRNA in the direct pathway encode dynorphin and substance P respectively (Gerfen and Young 1988). In the rat, 6-OHDA lesioning of the nigrostriatal pathway elevates striatal...
PPE-A mRNA expression (Sivam et al. 1987; Voorn et al. 1987; Vernier et al. 1988). Conversely, PPT mRNA expression is reduced while PPE-B mRNA levels are reported to be either unchanged or decreased (Sivam et al. 1987; Voorn et al. 1987; Herrero et al. 1995; Jolkkonen et al. 1995; Zeng et al. 1995; Salin et al. 1997; Carta et al. 2001). Following the acute and long-term administration of L-dopa and D₂ receptor agonists, studies have variously demonstrated both a lack of effect and changes in the mRNA expression of these peptides. One consistent finding is that increased PPE-B mRNA is associated with the onset of abnormal movements in 6-OHDA lesioned rodents and MPTP-treated primates following long-term administration of L-dopa, as well as in PD itself (Anderson et al. 1999; Henry et al. 1999; 2003).

The administration of BTS 74 398 would be expected to alter markers of striatal output to reflect the reversal of motor deficits but not dyskinesia. To determine whether this is the case, we have looked at the effects of acute and chronic administration of BTS 74 398 compared to L-dopa on striatal PPE-A, PPE-B and PPT mRNA expression in unilaterally 6-OHDA lesioned rats at doses producing equivalent behavioural responses.

### Methods

#### Animals

Male Wistar rats (220–250 g, Tucks Ltd., Essex) were housed in pairs under 12-h light-dark cycle with an environment of 50% humidity and temperature of 21 °C in accordance with animals (Scientific Procedures) Act 1996 and under home office regulations and under project licence No. 90/4619. All experiments were carried out following review by the local Ethical Committee at King’s College London. Rats were fed standard Purina rat chow and water ad libitum. Powdered chow mixed with water was supplied for animals following surgery until their body weight stabilised.

#### Drugs

Drugs used in this experiment were BTS 74 398 (1-[1-(3,4-dichlorophenyl)cyclobutyl]-2-(3-diaminomethylaminopropylthio) ethanone monohydrate; shire pharmaceuticals, UK); the methyl ester of L-dihydroxyphenylalanine (L-dopa) 6-hydroxydopamine hydrochloride (6-OHDA), apomorphine hydrochloride and d-amphetamine sulphate (Sigma); carbidopa (Merck Sharp and Dohme, Rahway, NJ). All drug weights refer to base values and were administered post operatively for pain relief in addition to 10 ml/kg of 5% glucose in 0.9% saline to aid hydration administered i.p.

#### Surgery

Rats were anaesthetised in an induction chamber with halothane (3–4% in 95% O₂, 5% CO₂ carrier gas) and placed in a Kopf stereotactic frame where anaesthesia was maintained. An incision was made in the scalp and a hole made in the skull 0.8mm in diameter using a hand drill at co-ordinates (AP: 2.2 mm ML: −1.5 mm, Paxinos and Watson 1987). A 10 µl Hamilton syringe needle was lowered to 8 mm below the dura and 6-OHDA (8 µg in 4 µl of 0.9% saline containing 0.05% ascorbic acid) was injected at a rate of 1 µl/min. The needle remained in place for a further 4 min to ensure diffusion away from the injection site before being removed and the wound was then cleaned and sutured. The same procedure was used for sham lesioned rats with the exception that 4 µl of 0.9% saline containing 0.05% ascorbic acid was injected. Flumixin hydrochloride (2.5 mg/kg s.c.) was administered post operatively for pain relief in addition to 10 ml/kg of 5% glucose in 0.9% saline to aid hydration administered i.p.

The extent of the nigrostriatal lesion was evaluated 3 weeks post-operatively by measuring circling responses to acute challenges of apomorphine (0.05 mg/kg s.c.) and amphetamine (4 mg/kg i.p.). Rats exhibiting rotational responses greater than 6 ipsilateral (amphetamine) or contralateral (apomorphine) turns per minute were used for subsequent drug treatment studies ensuring that all animals used were adequately lesioned.

#### Drug administration

Three groups of sham lesioned rats (n = 5 each) and 3 groups of lesioned rats (n = 10 each) were given a single injection of vehicle (saline 1.0 ml/kg, i.p.), carbidopa (25 mg/kg i.p.) followed 30 min later by L-dopa (12.3 mg/kg, i.p.), or BTS 74 398 (4.7 mg/kg, i.p.). Doses of BTS 74 398 and L-dopa used are ED₅₀ doses for inducing circling behaviour evoked on acute administration as identified in previous experiments in this laboratory (Lane et al. 2005). A further 3 groups of sham lesioned rats and 3 groups of lesioned rats were dosed daily, between 08:30 and 10:00 h for 21 days with either vehicle (saline 1.0 ml/kg, i.p.), carbidopa (25 mg/kg i.p.) followed 30 min later by L-dopa (12.3 mg/kg, i.p.), or BTS 74 398 (4.7 mg/kg, i.p.). Behavioural studies reported previously using the same drug administration paradigm showed chronic administration of L-dopa produced a sensitised circling response and induced abnormal movements in contrast to BTS 74 398 and saline treatment (Lane et al. 2005). Two hours after the final administration of saline, BTS 74 398 or L-dopa, rats were anaesthetised with sodium pentabarbitone (Sagittal; 50 mg/kg i.p.) and perfused transcardially with 100 ml of 0.9% saline. The brains were removed and frozen in isopentane and stored at −70 °C. Serial coronal sections (15 µm) were cut through the striatum on a Bright cryostat at −40 °C and mounted on polylysine coated slides (BDH, Poole, UK). The slide-mounted section were air-dried and stored at −70 °C.

#### In situ hybridisation of PPE-A, PPE-B and PPT mRNA

In situ hybridisation was carried out as described previously by Zeng et al. (1995). Forty-five oligomer DNA probes complementary to bases 388–435 of rat PPE-A cDNA, bases 124–171 of rat PPT cDNA (Zeng et al. 1995) and bases 754–797 of rat PPE-B cDNA (Henry et al. 1999) were labelled at the 3’end with ¹⁵SdATP with terminal deoxynucleotidyl transferase (Promega) at 37 °C and the labelled oligonucleotide was separated from unlabelled probe and excess radiolabel by filtration through probequant G50 columns. Slides were pre-hybridised by incubation in 4% paraformaldehyde (PFA) for 10 min, followed by immersion in 0.1 M triethanolamine containing 0.25% acetic anhydride for 10 min. Sections were then dehydrated through graded ethanol solutions (70, 80, 95 and 100%) followed by delipidation in chloroform for 10 min and partially rehydrated in 100 and 95% ethanol. Sections were then dehydrated and fixed with 100 µl of hybridisation solution (50% deionised formamide, 4 × saline-sodium/citrate solution (SSC); 1 × SSC containing 0.15 M NaCl and 0.15 M sodium citrate), 10% dextran sulphate, 1 × Denhardt’s solution (Sigma) and 0.5 mg/ml boiled salmon sperm ssDNA, 0.25 µg/ml RNA and 1 mM dithiothreitol per slide. Sections were covered with paraffin coverslips and incubated overnight at 37 °C in a humidified box. Following hybridisation, coverslips were removed by a brief wash in 1 × SSC at room temperature and slides were washed 4 × 15 min at 55 °C and 2 × 30 min rinses at room temperature. Slides were then dehydrated in 95 and 100% ethanol solutions and air-dried.