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# GABA transporter type 1 (GAT-1) uptake inhibition reduces stimulated aspartate and glutamate release in the dorsal spinal cord in vivo via different GABAergic mechanisms

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**Abstract**

Mechanisms through which the reported antinociceptive activity of GABA re-uptake inhibitors is mediated (and where on the sensory neuraxis) have not been defined. Here, microdialysis in the anaesthetised rat was used to examine the effect of selective GABA transporter type 1 (GAT-1) inhibition on basal and evoked amino acid release in the dorsal spinal cord. Reverse dialysis of the selective GAT-1 inhibitor NO-711 (10–300 μM) induced a concentration-related increase in extracellular GABA (maximal ~threefold of basal levels) without affecting other amino acids. Employing an S2/S1 paradigm, release evoked by brief high (45 mM) $K^+$-induced depolarisation of aspartate and glutamate, but not GABA or glycine, was found to be significantly reduced by reverse dialysis of NO-711 (300 μM). Co-administration of selective antagonists for $GABA_A$ or $GABA_B$ receptors ((+)-bicuculline (100 μM) or SCH 50911 (100 μM), respectively) prevented the GAT-1 inhibition-induced reduction of evoked aspartate. In contrast, while (+)-bicuculline also antagonised the reduction of evoked glutamate, SCH 50911 (up to 1 mM) was without effect. Inhibition of GAT-1 re-uptake was further found to play a permissive role in autoinhibitory effects on GABA release mediated through $GABA_A$ and $GABA_B$ receptors. These data demonstrate that augmentation of GABAergic neurotransmission by re-uptake inhibition activates pharmacologically distinguishable inhibitory influences on aspartate and glutamate release in the dorsal spinal cord. Thus, inhibition of spinal pro-nociceptive neurotransmitter release may contribute to the analgesic action of this drug class.


*Keywords:* GAT-1; GABA re-uptake; Spinal cord; Excitatory amino acid; Microdialysis; Analgesia

## 1. Introduction

The presence of γ-aminobutyric acid (GABA) containing synaptic contacts with spinothalamic output neurones, populations of intrinsic neurones and with primary afferent fibres terminating in the dorsal horn of the spinal cord (Todd et al., 1996) indicates an important role for GABAergic transmission in the inhibitory regulation of nociceptive traffic at this level. Indeed, stimulation of both $GABA_A$ and $GABA_B$ receptors in the dorsal spinal cord has been shown to produce analgesia in rodent and primate models of acute nociception (see Jasmin et al., 2004). Modulation of pro-nociceptive transmitter release could make a significant contribution to these effects. Studies in animals have confirmed the important role in spinal nociceptive neurotransmission of the excitatory amino acids (EAA) aspartate (ASP) and glutamate (GLU). Activation of spinal EAA receptor populations evokes heightened pain behaviours (Meller et al., 1996a,b) while receptor blockade reduces such behaviours when evoked by noxious stimulation (Fundytus, 2001). Further, spinal cord microdialysis studies have demonstrated peripheral noxious stimulation to evoke release of ASP and GLU (Skilling et al., 1988; Dmitrieva et al., 2004). The release of EAA in the spinal cord is under inhibitory control and has been shown to be reduced by opioid analgesics in vitro (Kangrga and Randic, 1991) and in vivo

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(Malmberg and Yaksh, 1995). However, serious side effects and the unreliable efficacy against chronic pain of this drug class have maintained the drive for novel therapeutics.

Alternative mechanisms to inhibit EAA mediated spinal transmission may have potential as new analgesic strategies. Augmenting the inhibitory actions of GABA in the dorsal spinal cord through inhibiting re-uptake could be expected to promote such an effect, potentially without the use-limiting side effects associated with direct GABA receptor activation (Jasmin et al., 2004).

Four distinct transport proteins for GABA have been cloned, termed (employing nomenclature for expression in the rat) GAT-1, GAT-2, GAT-3 and betaine/GABA transporter (BGT)-1 (for review see Borden, 1996). GAT-1 accounts for the majority of uptake within both neuronal and astrocyte cultures (Borden et al., 1995) and has a distribution that closely matches that of GABAergic neurons, including a high density of sites in superficial laminae of the dorsal spinal cord (Ng and Ong, 1999). Selective GAT-1 inhibitors are becoming available, for example tiagabine (previously known as NO-328), CI-966 and SK&F 89976A (see Borden et al., 1994). Tiagabine HCl (Gabitril®) has been introduced into medicine for the treatment of epilepsy (Leach and Brodie, 1998). As indicated by the reported antinociceptive activity of systemically administered tiagabine in rodents (Giardina et al., 1998; Ipponi et al., 1999), this class of drug may also have clinical usefulness as analgesic agents (Novak et al., 2001; Todorov et al., 2005). Whether inhibitory regulation of neurotransmitter release at the level of the dorsal spinal cord contributes to this activity is not known.

This study was conducted in order to evaluate the possible contribution of inhibition of spinal excitatory neurotransmitter release to the analgesic action of this drug class. We employed the selective GAT-1 inhibitor NO-711 (previously NNC-711; Suzdak et al., 1992) and microdialysis perfusion to determine the effect of local inhibition of spinal GAT-1 mediated GABA uptake on the extracellular concentration of GABA in the rat lumbar dorsal spinal cord in vivo. The ability of this treatment to modulate evoked release of spinal neurotransmitter amino acids and the role of specific GABA receptor subtypes therein was investigated. Some of these data have previously been presented in abstract form (Smith et al., 2002; Smith and Whitehead, 2003).

## 2. Methods

Microdialysis was performed in halothane-anaesthetised rats with a microdialysis probe placed in the dorsal horn of the lumbar spinal cord. Male Wistar rats (250–350 g; Charles Rivers, UK; habituated to a standard 12 h light/dark cycle) were used in this study. Animal usage and suffering were minimised in accordance with the UK Animals (Scientific Procedures) Act, 1986.

The effect of the selective GAT-1 inhibitor NO-711 on basal and high $K^+$-evoked amino acid release in the absence and presence of either the $GABA_A$ antagonist bicuculline or the $GABA_B$ antagonist SCH50911 was determined. Experiments were also performed to assess calcium dependency of evoked amino acid release.

### 2.1. In vivo microdialysis

Dorsal spinal cord microdialysis was performed essentially as previously described (Whitehead et al., 2004). Male Wistar rats were anaesthetised under halothane throughout (induction 4% in $O_2$; maintenance 0.75–1% in $O_2/N_2O$ (1:1)) and core body temperature maintained at around 37 °C by a homeothermic blanket (Harvard Instruments UK Ltd.). Bupivicaine (Sigma; 2.5 mg $ml^{-1}$ in 0.9% saline) was applied to all surgical incisions, until exposure of the spinal meninges. Rats were mounted on a stereotaxic frame (David Kopf, USA) and an incision made along the dorsal midline such that the muscle overlying the 13th thoracic vertebra ($Th_{13}$) and the first lumbar vertebra ($L_1$) could be removed. Vertebra $Th_{13}$ was then held immobilised on the horizontal plane by use of a spinal clamp and a burr hole created in the dorsal surface. The exposed dura was then carefully opened and a microdialysis probe of concentric design inserted into the spinal cord at an angle of 16° from the horizontal and 0.4 mm lateral from, and in parallel to, the midline. This placed a 1.5 mm length of dialysis membrane (Hemophan®, Gambro Hospal Ltd., UK; o.d. 218 μm) unilaterally into the medial dorsal horn of the L4 lumbar region of the spinal cord. This position was fixed by application of dental cement around the probe and two stainless steel anchorage screws (BAS Technicol, UK) located in the $Th_{13}$ and $L_1$ vertebrae and the rat was removed from the frame for continuing maintenance anaesthesia. The microdialysis probe was perfused (CMA/100 syringe pump, CMA/Microdialysis, Sweden) with an artificial extracellular fluid solution (aECF), composition (mM) NaCl 138; KCl 3; $CaCl_2$ 1.2; $MgCl_2$ 0.9; $NaH_2PO_4$ 1; $NaHCO_3$ 10; pH 7.4 at a rate of 2 μl $min^{-1}$ via a CMA/110 liquid switch (CMA/Microdialysis, Sweden) to allow changes in the perfusate. A stabilisation period of 120 min was allowed before consecutive 10 min samples of dialysate were collected. At the end of all experiments, placement within the dorsal horn was confirmed by perfusion of the probe with 0.1% Methylene Blue solution and subsequent viewing under low power microscopy of frozen coronal 30 μm sections through the site of implantation.

### 2.2. Experimental protocols

To investigate the effect of NO-711 on non-stimulated GABA efflux, separate groups received a 60 min period of perfusion with aECF containing 10, 100 or 300 μM NO-711 preceded by a 40 min basal period. Following drug administration, perfusion continued with aECF alone for a further 40 min. A separate control group received aECF alone throughout (0–140 min). The effect of NO-711 on the efflux of other amino acids was determined at the highest concentration of NO-711, employing the same protocol.

Perfusion with aECF containing 45 mM $K^+$ (with appropriate reduction in $Na^+$) for 6 min during the 5th (S1) and 11th (S2) samples was used to evoke depolarisation-induced release in control groups. The dependency of evoked release on $Ca^{2+}$ influx was assessed by perfusion with an aECF solution containing zero $Ca^{2+}$ and a ~13-fold increased $Mg^{2+}$ concentration (12 mM). In a separate study to determine the effect of NO-711, the S2 stimulation was applied in the presence of 300 μM NO-711. The involvement of $GABA_A$ or $GABA_B$ receptors, respectively, in the effect of NO-711 was determined by co-administration with NO-711 of either (+)-bicuculline (100 μM) or SCH 50911 (100 μM and 1 mM). The concentration of bicuculline employed was in range found to be effective from previous studies (for example Whitehead et al., 2001). To our knowledge, the administration of SCH 50911 by reverse dialysis has not been reported previously. Accordingly, this compound was administered at two concentrations predicted to be maximal and supramaximal on the basis of reported relative antagonist potencies (Bolser et al., 1995) and effective concentration for administration by reverse dialysis of the less potent $GABA_B$ receptor antagonist CGP 35348 (Harte and O'Connor, 2005). Separate time matched control groups were employed to determine the effect of either (+)-bicuculline or SCH 50911 alone on evoked release. In all cases the effect of treatment on evoked release was determined by switching perfusion to the modified aECF solution 30 min preceding, 30 min after, and during S2.

### 2.3. Assay of dialysate samples

Dialysate samples were assayed for amino acid content by liquid chromatography coupled to fluorescence detection after derivatisation with *o*-phthaldialdehyde/3-mercaptoproponoic acid. Chromatography was performed on a Hypersil ODS column, 5 μm particle size reverse phase analytical column (0.3 cm i.d. × 15 cm; Chrompack, UK) employing a tertiary gradient between