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Inhibition of [^{3}H]D-aspartate release by deramciclane

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Abstract

The effects of the 5-HT$_{2C}$ receptor inverse agonist deramciclane on the γ-aminobutyric acid (GABA) uptake and excitatory amino acid release processes were compared in rat cerebrocortical homogenates containing resealed plasmalemma fragments and nerve endings. Deramciclane non-competitively inhibited the uptake of [^{3}H]GABA with a K_i value of 13.7 ± 0.5 μM and partially displaced specifically bound [^{3}H](*R,S*)-*N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]nipecotic acid ([^{3}H]NNC-328) with high affinity (IC_{50} = 2.0 ± 0.7 nM). Depolarization by 4-aminopyridine or by 4-aminopyridine with (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionate [(*S*)-AMPA] induced the release of [^{3}H]D-aspartate. Deramciclane (10 μM) partially (~ 50%) inhibited the release of [^{3}H]D-aspartate without affecting [^{3}H]D-aspartate uptake. These results suggest a role for presynaptic inhibition of excitatory amino acid release and GABA uptake in the anxiolytic properties of deramciclane.

Keywords: Deramciclane; [^{3}H]D-aspartic acid, release, uptake; [^{3}H]GABA ([^{3}H]γ-aminobutyric acid), uptake; Quench flow; [^{3}H]NNC-328 {[^{3}H](*R,S*)-*N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]nipecotic acid} binding; Cerebrocortical homogenate; (Rat)

1. Introduction

Deramciclane fumarate, (1*R*,2*S*,4*R*)-(−)-*N*,*N*-dimethyl-2-{(1,7,7-trimethyl-2-phenylbicyclo[2,2,1]hept-2-yl)oxy}ethane amine-2-(*E*)-butenedionate is a novel anxiolytic agent lacking muscle relaxant and sedative effects (Gacsályi et al., 1988, 1996). Although there is evidence that deramciclane inhibits the γ-aminobutyric acid (GABA) transporter (Kovács et al., 1989; Borden et al., 1994; Dhar et al., 1994), and is also an inverse agonist at the 5-HT$_{2C}$ receptor (Pälvimäki et al., 1998), the effects of deramciclane on the release and uptake on excitatory amino acids have so far not been investigated. Since [^{3}H]D-aspartate is released, at least in part, from synaptic vesicle via exocytosis (Gallo et al., 1992; Cousin et al., 1996), the evoked release of [^{3}H]D-aspartate can be considered as a valid, non-metabolized marker of synaptic excitatory amino acid release in studies of combined exocytotic and non-exocytotic events (Patel and Croucher, 1997 and references cited therein; Nyitrai et al., 1999a). Here, we describe the effects of deramciclane on evoked [^{3}H]D-aspartate release in cerebrocortical homogenates from the rat. These homogenates of resealed nerve endings and plasmalemma fragments contain functional GABA$_A$ receptors (Cash and Subbarao, 1987a,b; Kardos, 1992, 1993; Kardos and Blandl, 1994; Kardos et al., 1991, 1996), P2 pyrimidoceptors (Kardos et al., 1999) as well as GABA (Kardos et al., 1994, 1997), glycine, glutamate (Kovács et al., 1998) transporters and GABA release sites (Kardos and Blandl, 1994; Kardos et al., 1994). The effects of different GABA uptake inhibitors and deramciclane on [^{3}H](*R,S*)-*N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]nipecotic acid ([^{3}H]NNC-328) binding and [^{3}H]GABA and [^{3}H]D-aspartate uptake were compared, too.

2. Materials and methods

2.1. Materials

Male Wistar rats (4–6 weeks old) were from LATI (Gödöllõ, Hungary). Deramciclane was obtained from EGIS Pharmaceuticals. [^{3}H]D-aspartate (24 Ci/mmol), [^{3}H]GABA (61 Ci/mmol), and [^{3}H]NNC-328 (45 Ci/mmol) were from Amersham. 1-{2-[((Diphenymethylene)-

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imino)oxy]ethyl}-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid hydrochloride (NO-711), guvacine, and nipecotic acid were purchased from RBI. Aprotinin, leupeptin, antipain, pepstatin A, butylated hydroxy-toluene, phenyl-methanesulfonyl fluoride, GABA, (L)-2,4-diaminobutyric acid {(L)-DABA} and 4-aminopyridine were from Sigma. (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionate {(*S*)-AMPA} and (L)-*t*-pyrrolidine-2,4-dicarboxylic acid (*t*-PDC) were purchased from Tocris. HEPES was obtained from Calbiochem. $CaCl_2$, $MgCl_2$ and orto-phthalaldehyde were from Merck. Dibutylphthalate and dinonylphthalate were provided by BASF. Other chemicals were from Reanal.

Buffer A had the following composition: 0.32 M sucrose, leupeptin, antipain, pepstatin A (0.005 mg/ml each), 0.01 mg/ml aprotinin, 1 mM phenyl-methanesulfonyl fluoride, 0.02 mM butylated hydroxy-toluene and 10 mM HEPES adjusted to pH 7.5 with NaOH. Buffer B had the following composition: 135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 20 mM HEPES adjusted to pH 7.5 with NaOH. Buffer C had the following composition: 1 M NaCl and 5 mM Tris–HCl adjusted to pH 7.2 with NaOH.

The stock solution (10 mM) of deramciclane was made in propylene glycol. One percent propylene glycol, which was present throughout the measurements, did not interfere with the results obtained.

2.2. Preparation of cerebrocortical homogenates

Native plasma membrane homogenates from the rat cerebral cortex were prepared as described previously (Kovács et al., 1998). The cortex was dissected and homogenized by means of an Ultraturrax in 30 ml buffer A. Thereafter 30 ml buffer B was added and the suspension was centrifuged at $270 \times g$ for 4 min at 4°C. The supernatant was centrifuged at $6500 \times g$ for 20 min at 4°C followed by resuspension of the pellet in a Potter–Elvehjem glass-Teflon homogenizer by hand. Thereafter, aliquots of the suspension were centrifuged at $3500 \times g$ for 15 min at 4°C. The resulting pellets kept on ice were resuspended as above in buffer B to give a protein concentration of 0.6 mg/ml (Lowry et al., 1951).

2.3. [^{3}H]GABA uptake

Samples for determination of endogenous GABA in cerebrocortical homogenates were prepared and analysed as described previously (Nyitrai et al., 1999b). Briefly, aliquots from five preparations were centrifuged at 10,000 $\times g$ for 5 min and the supernatants were filtered through Millipore 0.22-μm pore size sterile syringe filters. Samples of buffer B, used for making up solutions for [^{3}H]GABA uptake measurements, were treated in the same way. The samples were stored at −80°C. Quantitative analysis of the orto-phthalaldehyde-derivatized GABA was performed with an automated high performance liquid chromatograph-combined fluorescence detection system (Pharmacia AminoSys) using 305–395 nm excitation and 430–470 nm emission filter and an HP Hypersyl ODS reversed-phase column.

The progress of [^{3}H]GABA uptake was followed over 1–50 s at 30°C using a rapid quenched-incubation technique with a mixing time of < 3 ms (Cash and Hess, 1981; Cash and Subbarao, 1987a,b; Kardos, 1992; Kardos and Blandl, 1994; Kardos et al., 1994, 1997). Equal amounts of 200 nM [^{3}H]GABA in buffer B containing 0–30 μM deramciclane and cerebrocortical homogenates, preincubated for 10 min at 30°C in the presence and absence of various concentrations of deramciclane in buffer B, were mixed. The mixture was incubated for different periods of time. The influx of [^{3}H]GABA was rapidly quenched (< 3 ms) by mixing the reaction mixture with an equal amount of buffer B containing the specific GABA uptake inhibitor NO-711 (20 μM after mixing). The suspension was passed through a glass fibre disk (Whatman GF/B) and washed (2×5 s) with 10 ml ice-cold buffer B.

To obtain K_i values for different GABA uptake inhibitors, aliquots of the cerebrocortical homogenates (300 μl) were preincubated with 300 μl buffer B with or without the test compounds in different concentrations for 10 min at 30°C. Then 500 μl of the above suspension and 500 μl [^{3}H]GABA solution containing 20 nM [^{3}H]GABA (final concentration: 0.61 μCi/ml) and 180 nM GABA in the presence or absence of test compounds were vortexed. After a 1-min incubation at 30°C, the suspension was immediately filtered through glass fibre filters (Whatman GF/B) and washed (2×4 s) with 6 ml of ice-cold buffer B. The radioactivity of samples was counted in ''HiSafe'' II (LKB) scintillation mixture with an efficiency of 25%. Non-specific uptake was determined in the presence of 20 μM NO-711.

2.4. [^{3}H]NNC 328 binding

An aliquot of cerebrocortical homogenates was preincubated in buffer B for 40 min at 30°C, centrifuged at $6500 \times g$ for 20 min and resuspended in buffer C (Braestrup et al., 1990). For [^{3}H]NCC 328 binding, aliquots (500 μl) of the cerebrocortical homogenate in buffer C were incubated with equal amounts of [^{3}H]NNC-328 (0.8 nM, 0.036 μCi/ml) in the absence or (to define non-specific binding) the presence of 100 mM GABA in buffer C for 40 min at 30°C. Samples of 1000 μl suspension were filtered (Whatman GF/B) and immediately washed (2×4 s) with 6 ml ice-cold buffer C. The radioactivity of the samples was counted in ''HiSafe'' II (LKB) scintillation mixture with an efficiency of 25%.

2.5. [^{3}H]D-aspartate uptake

Aliquots (300 μl) of the cerebrocortical homogenates were preincubated with 300 μl buffer B with or without

the test compounds for 10 min at 30°C. Then 500 μl of the above suspension and 500 μl [^{3}H]D-aspartate (final concentration 9 nM, 0.23 μCi/ml) solution made with or without the test compounds were mixed. After a 1-min incubation at 30°C, the suspension was immediately filtered through glass fibre filters (Whatman GF/B) and washed (2 × 4 s) with 10 ml of ice-cold buffer B. Non-specific uptake was determined in the presence of 200 μM *t*-PDC (Mitrovic and Johnston, 1994). The radioactivity of samples was counted in ''HiSafe'' II (LKB) scintillation mixture with an efficiency of 25%.

2.6. [^{3}H]D-aspartate release

Equal volumes of cerebrocortical homogenates and [^{3}H]D-aspartate solution (final concentration: 10 nM, 0.24 μCi/ml) in buffer B were mixed at 30°C and incubated. After 55 min the mixture was centrifuged at 10,000 × *g* for 3 min at room temperature. The pellet was rinsed (twice) and resuspended in buffer B followed by incubation for 5 min at 30°C. The above centrifugation–resuspension–incubation cycle was repeated once again. Then 320 μl of [^{3}H]D-aspartate-loaded cerebrocortical homogenate was added to Eppendorf tubes containing 80 μl of buffer B, 4-aminopyridine, (*S*)-AMPA (final concentration: 20 μM and 100 μM, respectively) with or without 10 μM deramciclane and incubated at 30°C. After 7 min, 100 μl of dibutylphthalate:dinonylphthalate mixture (35:19) was added, and the tubes were vortexed and centrifuged at 10,000 × *g* for 3 min at room temperature (Nyitrai et al., 1999a). Samples (2 × 150 μl) were taken out from the supernatant above the phthalate ester layer and the radioactivity was counted in ''HiSafe'' II (LKB) scintillation mixture with an efficiency of 25%.

2.7. Data analysis

Experiments carried out in duplicate were repeated three times. Data are expressed as means ± S.E. and were analysed using one-way analysis of variances (ANOVAs, Origin ver. 3.5) followed by Tukey's test for the post hoc comparisons. A value of $P < 0.05$ was considered significant. Displacement data were fitted to a logistic function (Origin ver 3.5). Data from GABA uptake rate measurements were analysed (MicroMath Scientist Microsoft Windows ver. 2.03) using the first-order rate equation:

$$M = M_\infty[1 - \exp(-kt)] \quad (1)$$

where M_∞ is the final influx value, and k is the overall rate constant (Kardos et al., 1994, 1997). The relative dissociation equilibrium constant for inhibition were determined by fitting the rate constants measured at different inhibitor concentration to equations (Kardos et al., 1997) for non-competitive (Eq. 2) and competitive (Eq. 3) inhibition:

$$k = k_{max}[U][G]/(K_d + [G])(1 + [I]/K_i), \quad (2)$$

$$k = k_{max}[U][G]/K_d(1 + [I]/K_i) + [G], \quad (3)$$

where k_{max} is the saturation rate constant, $[G]$ the experimental concentration of GABA, U represents the uptake sites per internal volume ($[U] = 1$); K_d is the equilibrium dissociation constant for GABA and was taken to be 4.3 μM (Kardos et al., 1994).

3. Results

3.1. Effects of deramciclane on GABA uptake processes

In measurements of rates, application of 4.5, 6.6 and 26.4 μM deramciclane decreased the first-order overall rate constant for GABA uptake obtained in its absence $k = 5.30(\pm 0.04) \times 10^{-3}$ s^{-1} (Eq. (1), cf. also Kardos et

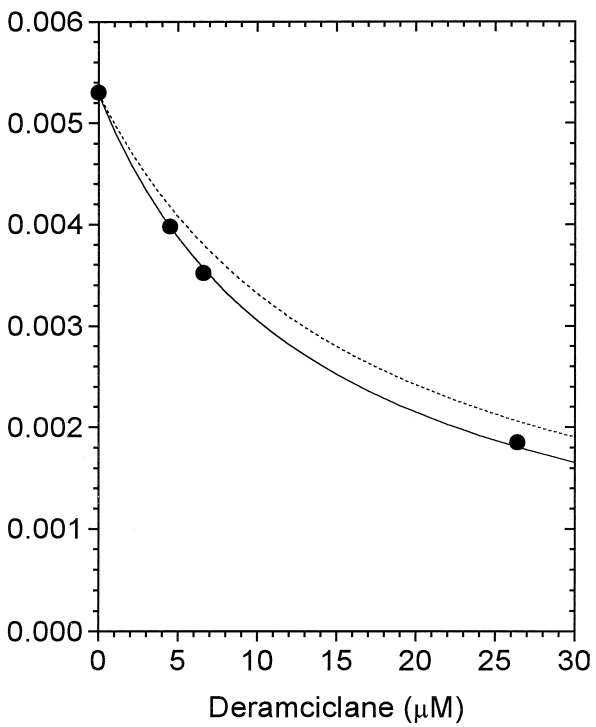

Fig. 1. Dependence of GABA uptake rate on deramciclane concentration. First-order overall rate constants (●) describing the progress of the specific uptake of 1 μM GABA into rat cerebrocortical homogenates at 30°C in the absence and presence of 4.5, 6.6 and 26.4 μM deramciclane ($k = 5.30(\pm 0.04) \times 10^{-3}$ s^{-1}, $3.98(\pm 0.08) \times 10^{-3}$ s^{-1}, $3.52(\pm 0.07) \times 10^{-3}$ s^{-1} and $1.85(\pm 0.05) \times 10^{-3}$ s^{-1}, respectively) were fitted to models of non-competitive (Eq. (2), solid line) and competititive (Eq. (3), dashed line) reversible inhibition. The precision of determinations, expressed as standard deviation for each k value, was less than the size of the symbol ●. The values for the final influx and saturation rate constant were $M_\infty = 1105 \pm 28$ pmol GABA/mg of protein and $k_{max} = 0.0280 \pm 0.0002$ s^{-1}, respectively, in these calculations.

al., 1994, 1997) in a concentration-dependent manner, giving $k = 3.98(\pm 0.08) \times 10^{-3}$ s^{-1}, $k = 3.52(\pm 0.07) \times 10^{-3}$ s^{-1} and $k = 1.85(\pm 0.05) \times 10^{-3}$ s^{-1}, respectively. The concentration of endogenous GABA in cerebrocortical homogenates was determined and found to be 1.80 ± 0.12 μM. Using the experimental concentration of GABA (1 μM) as derived for a 1:1 mixing of the [^{3}H]GABA solution (200 nM) and the cerebrocortical homogenate ([GABA] = 1.8 μM) in the quenched-flow experiments, the value for the dissociation constant of the reversible inhibition by deramciclane (K_i) was calculated by fitting the rate constants measured at different deramciclane concentrations according to the non-competitive model of reversible inhibition (Eq. (2), Fig. 1, solid line). The K_i value for deramciclane thus obtained was equal to 13.7 ± 0.5 μM. This K_i value was less accurate in predicting the measured rate constant than that obtained by applying the competitive model of reversible inhibition (Eq. (3), Fig. 1, dashed line). The K_i values for inhibition of GABA uptake in 1 min at 30°C for NO-711, GABA, guvacine, (L)-DABA and β-alanine were 0.045 ± 0.004 μM, 3.9 ± 0.3 μM, 6.6 ± 0.2 μM, 27 ± 2 μM and 2.0 ± 0.3 mM, respectively (Fig. 2), and were similar to IC_{50} values obtained previously with rat cerebrocortical homogenates (Kardos et al., 1994) and forebrain synaptosomes (Suzdak et al., 1992) as well as with HeLa cells expressing the GAT-A transporter (Clark et al., 1992). Under our conditions, the K_i for the inhibitory effects of deramciclane was found to be 13.2 ± 0.8 μM (Fig. 2). This value is in agreement with the K_i value of 13.7 ± 0.5 μM derived from the rate measurements above and compares with the reported value of $IC_{50} = 19 \pm 7$ μM for deramciclane obtained by measuring the uptake of 50 nM [^{3}H]GABA in 10 min, at 25°C, in crude synaptosomal fractions (Kovács et al., 1989). The IC_{50} value for inhibition of [^{3}H]GABA uptake in stable cell lines by the human GAT-1 transporter was found to be 26 ± 5 μM (Borden et al., 1994; Dhar et al., 1994).

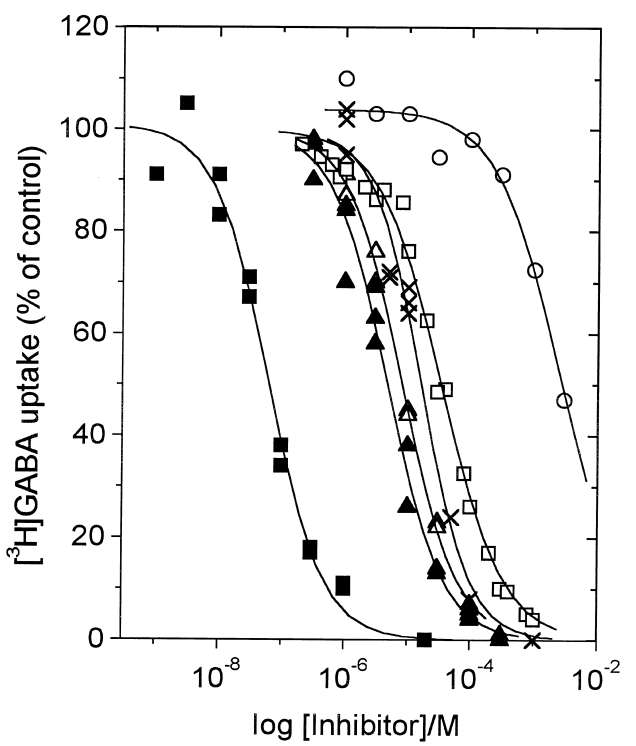

Fig. 2. The effect of different inhibitors on specific [^{3}H]GABA uptake in 1 min at 30°C in rat cerebrocortical homogenates. The precision of determinations, expressed as standard deviation for each displacement value, was less than the size of the symbol. Symbols: NO-711 (■), GABA (▲), guvacine (△), deramciclane (×), (L)-DABA (□), β-alanine (○).

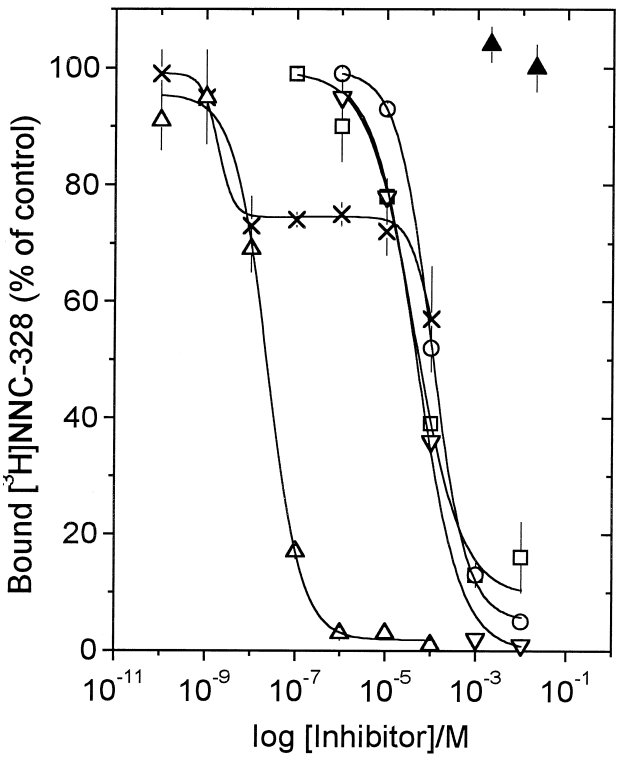

Fig. 3. Comparison of the effects of NO-711 (△), guvacine (□), nipecotic acid (▽), GABA (○), deramciclane (×) and β-alanine (▲) on the specific binding of [^{3}H]NNC-328 in rat cerebrocortical homogenates as measured at 30°C. If otherwise not indicated, the precision of determinations, expressed as standard deviation for each displacement value, was less than the size of the symbol.

NO-711, nipecotic acid, guvacine and GABA displaced specifically bound [^{3}H]NNC-328 (0.8 nM) in cerebrocortical homogenates at 30°C, giving IC_{50} values of 26 ± 4 nM, 52 ± 2 μM, 58 ± 4 μM and 100 ± 13 μM, respectively, with Hill coefficients near to one, whereas β-alanine (2–20 mM) was inactive (Fig. 3). Similar inhibitor sensitivities were obtained for rat forebrain membrane suspensions with the use of [^{3}H]NNC-328 ([^{3}H]tiagabine, Braestrup et al., 1990). Deramciclane partially displaced specifically bound [^{3}H]NNC-328 with high affinity ($IC_{50} = 2.0 \pm 0.7$ nM, Fig. 3). Some low-affinity binding of deramciclane to [^{3}H]NNC-328-labelled sites was also seen; however, due to the low aqueous solubility of deramci-

Table 1
Effect of deramciclane on [^{3}H]D-aspartate release in native plasma membrane vesicle suspensions at 30°C

Deramciclane (μM)	4-aminopyridine (μM)	(*S*)-AMPA (μM)	[^{3}H]D-aspartate release over basal (%)
0	0	0	0.0 ± 0.3
10	0	0	0.2 ± 0.2
0	20	0	1.7 ± 0.1
1	20	0	1.4 ± 0.3
3	20	0	1.1 ± 0.4
10	20	0	0.8 ± 0.2[a]
0	20	100	5.0 ± 0.7
0.3	20	100	4.0 ± 0.3
1	20	100	3.0 ± 0.1[a]
3	20	100	2.6 ± 0.1[b]
10	20	100	2.7 ± 0.2[b]

[a] $P < 0.05$ compared to the release of [^{3}H]D-aspartate measured in the presence of 20 μM 4-aminopyridine.
[b] $P < 0.01$ compared to the release of [^{3}H]D-aspartate measured in the presence of 20 μM 4-aminopyridine and 100 μM (*S*)-AMPA.

clane, this binding could not be characterized further (Fig. 3).

3.2. Effects of deramciclane on [^{3}H]D-aspartate uptake and release processes

The effect of NO-711, guvacine and deramciclane (3–100 μM) on [^{3}H]D-aspartate uptake in 1 min was measured in cerebrocortical homogenates at 30°C. A high concentration of deramciclane (100 μM) inhibited the uptake of [^{3}H]D-aspartate by 27 ± 8% ($P < 0.05$), whereas the GABA uptake inhibitors, NO-711 (1–100 μM) and guvacine (30–1000 μM), showed no effects.

The basal [^{3}H]D-aspartate release was 3.0 ± 0.4% of the amount of radioactivity present in cerebrocortical homogenates. 4-Aminopyridine (20 μM) increased the release of [^{3}H]D-aspartate by 1.7 ± 0.1%. Addition of both 20 μM 4-aminopyridine and 100 μM (*S*)-AMPA to the [^{3}H]D-aspartate-loaded cerebrocortical homogenates evoked a greater increase of [^{3}H]D-aspartate release (5.0 ± 0.7%, Table 1). Deramciclane (10 μM) did not change basal [^{3}H]D-aspartate release. Inhibition of the release of [^{3}H]D-aspartate induced by either 4-aminopyridine or 4-aminopyridine with (*S*)-AMPA was observed in the presence of deramciclane (0.3–10 μM, Table 1). The inhibitory effect of deramciclane on the release of [^{3}H]D-aspartate induced by 4-aminopyridine with (*S*)-AMPA was significant in the 1–10 μM range of deramciclane concentration (Table 1).

4. Discussion

In the present study, 10 μM deramciclane caused an about 50% inhibition of the evoked release of [^{3}H]D-aspartate in the absence of effects on basal efflux and without affecting high-affinity [^{3}H]D-aspartate uptake in cerebrocortical homogenates. The concentration of deramciclane that can be estimated to cause 50% inhibition of evoked [^{3}H]D-aspartate release is in the range of 0.3–1.0 μM. The lack of effect on basal efflux and high-affinity uptake of [^{3}H]D-aspartate suggests that this action is mediated by presynaptic receptors and not via inhibition of [^{3}H]D-aspartate reuptake. At this concentration (10 μM) deramciclane inhibited the uptake of [^{3}H]GABA and displaced high-affinity binding of [^{3}H]NNC-328. In accordance with earlier suggestions (Kovács et al., 1989), the mechanism for inhibition of GABA uptake was found to be non-competitive with a K_i value of 13.7 ± 0.5 μM. It is conceivable therefore that, like tiagabine {(*R*)-NNC-328} and NO-711 (Smith et al., 1995), deramciclane binds to the [^{3}H]NNC-328-labelled site near the transporter with high affinity, causing the non-competitive inhibition of GABA uptake. The relative inhibitor sensitivity was NO-711 = 87 ≫ GABA = 1 > guvacine = 0.59 > (L)-DABA = 0.14 ≫ β-alanine = 0.002, which is typical for the GAT-1 type transporter of the central nervous system (Guastella et al., 1990; Borden et al., 1992, 1994; Clark et al., 1992; Dhar et al., 1994; Kardos et al., 1994). This is the most abundant transporter in rat cerebrocortical homogenates. Based on the deramciclane sensitivity (Borden et al., 1994; Dhar et al., 1994) and the expression pattern of GABA transporters (Guastella et al., 1990; Clark et al., 1992), the quantitatively most important transporter affected by deramciclane in the brain is likely a GAT-1 type transporter.

Deramciclane antagonized serotonin-stimulated phosphoinositide hydrolysis in the rat choroid plexus with an IC_{50} of ~0.2 μM. Deramciclane also decreased basal phosphoinositide hydrolysis by up to ~30% (Pälvimäki et al., 1998). Single deramciclane doses of 1.2 and 23.9 μmol/kg resulted in 45% and 79% 5-HT_{2C} receptor occupancy in the rat choroid plexus, respectively (Pälvimäki et al., 1998). The plasma concentration of deramciclane after absorption from the gastrointestinal tract, expressed as percentage of the dose, is ~65% (Lengyel et al., 1998). By using these figures, the estimated concentration of deramciclane leading to 45%–79% 5-HT_{2C} receptor occupancy in the brain can be calculated to range from 0.8 to 15.5 μM. Collectively, these results suggest that a dose effective at 5-HT_{2C} receptors can also inhibit the release of excitatory amino acids and GABA uptake in the brain. The in vitro potency of deramciclane to inhibit GABA uptake may be relevant for anticonvulsive therapy (Frey et al., 1979; Krogsgaard-Larsen et al., 1984) as well. It shoud be noted, however that correlations between the inhibitor sensitivity found in vitro for GABA uptake inhibitors capable of penetrating the blood–brain-barrier and the effective doses seem to depend on the anticonvulsive model applied (cf. Frey et al., 1979; Andersen et al., 1993 and Smith et al., 1995).

Our data imply that in addition to 5-HT_{2C} receptor inverse agonism and GABA uptake inhibition, deramci-

clane may modulate excitatory pathways specifically via inhibition of the presynaptic release of excitatory amino acids. The precise mechanism by which deramciclane elicits its inhibition of [^{3}H]D-aspartate release is unknown at present; however, inhibition of Ca^{2+} mobilization induced by activation of excitatory amino acid receptors is also possible. These effects of deramciclane on the various processes of seroton*ergic*, GABA*ergic* and excitatory amino acid signalling may be convergent in the central nervous system and underlie the unique anxiolytic properties of deramciclane.

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