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The γ-aminobutyric acid uptake inhibitor, tiagabine, is anticonvulsant in two animal models of reflex epilepsy

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Abstract

The effects of i.p. administration of the γ-aminobutyric acid (GABA) uptake inhibitors *R*(−)N-(4,4-di(3-methylthien-2-yl)-but-3-enyl) nipecotic acid hydrochloride (tiagabine; molecular weight 412.0), (1-(2-(((diphenylmethylene)-amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride (NNC-711; molecular weight 386.9), and (±)-nipecotic acid (molecular weight 128.2) are compared with those of carbamazepine (molecular weight 236.3) on sound-induced seizures and locomotor performance in genetically epilepsy-prone (GEP) rats. The ED_{50} value against clonic seizures (in μmol kg^{-1} at the time of maximal anticonvulsant effect) for tiagabine was 23 (0.5 h), and for NNC-711 was 72 (1 h), and for carbamazepine was 98 (2 h). (±)-Nipecotic acid (0.4–15.6 mmol kg^{-1}) was not anticonvulsant. High doses of NNC-711 (207–310 μmol kg^{-1}) and of (±)-nipecotic acid (39–78 mmol kg^{-1}) induced ataxia and myoclonic seizures 0.25–1 h. Tiagabine and carbamazepine did not induce myoclonic seizures and had similar therapeutic indices (locomotor deficit ED_{50}/anticonvulsant ED_{50}) ranging from 0.4 to 1.9. In *Papio papio*, we observed a reduction in photically induced myoclonic seizures with tiagabine (2.4 μmol kg^{-1} i.v.) accompanied with neurological impairment. Tiagabine has comparable anticonvulsant action to carbamazepine in rats and has anticonvulsant effects in non-human primates supporting the potential use of inhibitors of GABA uptake as therapy for epilepsy.

Keywords: GABA (γ-aminobutyric acid) uptake inhibitor; Nipecotic acid; Tiagabine; Carbamazepine anticonvulsant; Epilepsy

1. Introduction

Anticonvulsant effects of drugs which inhibit the neuronal and/or glial uptake of γ-aminobutyric acid (GABA) (Horton et al., 1979; Yunger et al., 1984) have been observed in rodent models of epilepsy (Meldrum, 1978, 1981, 1989). These earlier studies were hindered by two problems: intracerebroventricular administration of the GABA uptake inhibitors was necessary to observe anticonvulsant effects and proconvulsant effects of the compounds were common.

An inhibition of [^{3}H]GABA uptake into neuronal and glial cells of the rat brain by (±)-nipecotic acid (128.2) may also be achieved by its more lipophilic derivatives – *R*(−)*N*-(4,4-di(3-methylthien-2-yl)-but-3-enyl) nipecotic acid hydrochloride (tiagabine [formerly NO-05-0328]; molecular weight 412.0) and by (1-(2-(((diphenylmethylene)-amino)oxy)-ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride (NNC-711; molecular weight 386.9) (see Fig. 1) (Krogsgaard-Larsen and Johnston, 1975; Braestrup et al., 1990; Schousboe et al., 1991; Suzdak et al., 1992; Andersen et al., 1993). Tiagabine (0.5–120 μmol kg^{-1} i.p. or i.v.) and NNC-711 (0.3–26 μmol kg^{-1} i.p.) more readily pass the blood-brain barrier than nipecotic acid and have anticonvulsant effects after systemic administration in mice and rats. Tiagabine is also anticonvulsant in baboons (B.S. Meldrum, unpublished observations; see Pierce et al., 1991) and in man (Nielsen et al., 1991; Pierce et al., 1991; Suzdak et al., 1992).

The genetically epilepsy-prone rats (GEP rats) in our colony are a Sprague-Dawley-derived strain from the University of Arizona. Wild running, clonic and tonic seizures can be induced repetitively by exposure of the animals to a sound stimulus (10–12 kHz, 60 s, 100–120 dB). GEP rats remain seizure-susceptible for life, which makes them well-suited for repeated-measure drug studies to demonstrate the anticonvulsant

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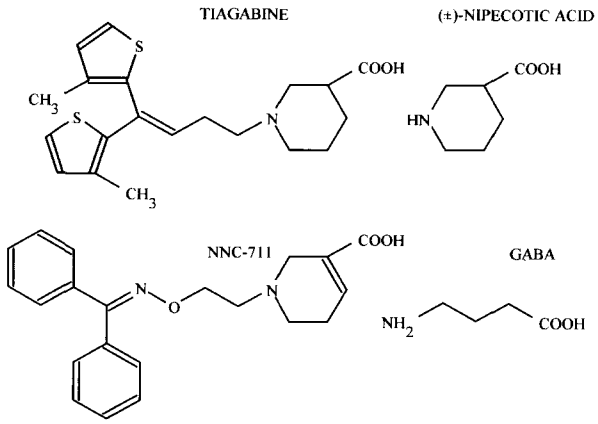

Fig. 1. The chemical structures of the γ-aminobutyric acid (GABA) uptake inhibitors tiagabine: *R*(−)*N*-(4,4-di(3-methyl-thien-2-yl)-but-3-enyl) nipecotic acid hydrochloride (molecular weight 412.0), NNC-711: (1-(2-(((diphenyl-methylene)amino)oxy)-ethyl)-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid hydrochloride (molecular weight 386.9), and (±)-nipecotic acid (molecular weight 128.2) in comparison with γ-aminobutyric acid.

action of novel and familiar compounds (Smith et al., 1993).

Photosensitive *Papio papio* baboons originate in the Casamance region of Senegal and exhibit a genetically-determined epilepsy syndrome amenable to drug study (Meldrum, 1975).

The present study was designed to compare the anticonvulsant and adverse behavioural effects of tiagabine, NNC-711 and (±)-nipecotic acid with carbamazepine in the GEP rat model of reflex epilepsy. We further evaluated tiagabine to determine its anticonvulsant effect in the non-human primate model of reflex epilepsy.

2. Materials and methods

2.1. Sound-induced seizures in GEP rats

GEP rats (Institute of Psychiatry colony) of either sex weighing between 200–400 g were used. The animals were housed in groups of 3–6 in PVC cages (350 × 530 mm long × 180 mm high) in an environment maintained at 19–22°C and a relative humidity of 55 ± 3% respectively with a 14 h/10 h light/dark cycle (light on from 06.00 to 20.00 h). Food and water were available ad libitum. The animals were individually exposed to an audiogenic seizure stimulus (110–120 dB, 12–16 KHz, 60 s) and the resulting behavioural response was scored on a scale of 0–9: 0 = no response; 1 = wild running; 2 = two episodes of wild running followed by mild clonic seizure; 3 = one episode of wild running followed by mild clonic seizure; 4 = two episodes of wild running followed by severe clonic seizure; 5 = one episode of wild running followed by severe clonic seizure; 6 = two episodes of wild running, clonic seizure followed by incomplete tonic seizure; 7 = one episode of wild running, clonic seizure followed by incomplete tonic seizure; 8 = two episodes of wild running, clonic seizure followed by complete tonic seizure; 9 = one episode of wild running, clonic seizure followed by complete tonic seizure. This was repeated once daily for two more days. Only animals which responded consistently (seizure score = 9) for three consecutive days were employed in the study. On the third day the animals received vehicle or drug treatment (i.p.) and were exposed to an audiogenic stimulus at +0.25 h, +0.5 h, +1 h, +2 h, +4 h, and +8 h after vehicle or drug administration.

2.2. Locomotor performance in GEP rats

Groups of 6–8 animals were selected for their ability to remain walking for 60 s on a 9 cm wide rod (with 1.5 mm deep grooves spaced at 10° intervals) revolving at 12 r.p.m. 1 h prior to drug administration. Further testing was performed at times designed to determine the onset, peak and offset times of locomotor deficit for each compound (+0.125 h, +0.25 h, +0.5 h, +1 h, +2 h, +4 h, and +8 h after vehicle or drug administration). Latency to fall (cut-off 60 s) was recorded.

2.3. Drug treatment in GEP rats

Selected GEP rats forming this colony of animals exhibit wild running, clonic seizure and complete tonic seizure (seizure scores = 9) consistently to repeated sound stimulus with an interval of 1 h or more. A shorter interval of 15 min may be employed for studying compounds with short onset and offset of action.

The compounds were tested in each animal with a 2-week interval between each new compound studied to allow time for recruitment of additional consistently responding GEP rats (as detailed above) and to allow time for drug wash-out. Repeated studies showed this to be an effective method of obtaining reliable ED_{50} values with optimal use of animals. The following doses (μmol kg^{-1} i.p.) were studied: tiagabine (3–97), NNC-711 (2–310), (±)-nipecotic acid (0.4–78 × 10^3), carbamazepine (11–296), (n = 6–12 per dose) Figures only show selected doses to improve their clarity.

2.4. Photically induced myoclonus in Papio papio

One male and two female (not in season) adolescent baboons (6–8 kg) with high photosensitivity were selected for study. On the experimental day, each animal, unfed, was placed in a primate chair, usually between 9.00 and 10.00 a.m. After 30 min, each animal was individually subjected under subdued lighting to a control stroboscopic (intermittent light) stimulation (I.L.S.)

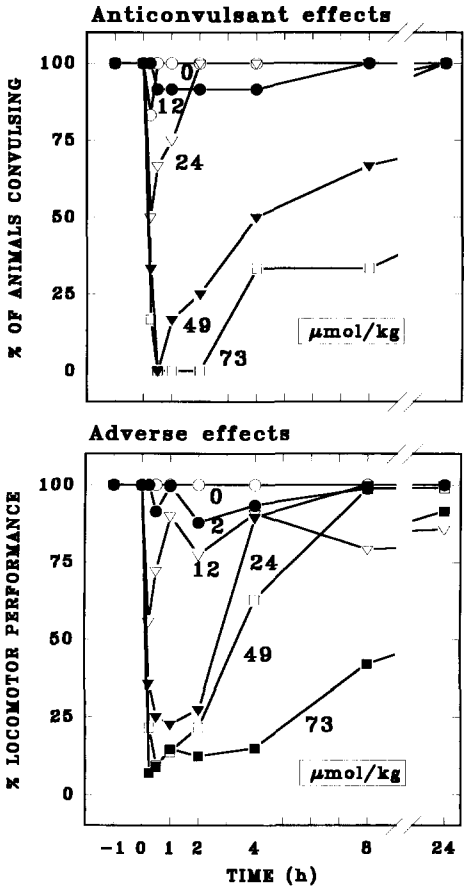

Fig. 2. Effects of tiagabine on sound-induced clonic seizures and locomotor performance in genetically epilepsy prone (GEP) rats. (Top panel) Sound-induced clonic seizure: GEP rats received vehicle (open circles) or tiagabine (12 (closed circles), 24 (open triangles), 49 (closed triangles), 73 (open squares) μmol kg^{-1}) (i.p.) and were exposed to a sound stimulus for seizure at -1 h, $+0.25$ h, $+0.5$ h, $+1$ h, $+2$ h, $+4$ h, $+8$ h, and $+24$ h after vehicle or drug administration. Results are expressed as percentage number of GEP rats responding with clonic seizure ($n = 8$–12 per dose). (Bottom panel) Locomotor performance: Groups of 6–12 rats received vehicle (open circles) or tiagabine (2 (closed circles), 12 (open triangles), 24 (closed triangles), 49 (open squares), 73 (closed squares) μmol kg^{-1}) (i.p.) and were tested for locomotor impairment using a rotarod at -1 h, $+0.125$ h, $+0.25$ h, $+0.5$ h, $+1$ h, $+2$ h, $+4$ h, and $+8$ h after vehicle or drug administration.

(25 Hz) and the resulting myoclonic response scored on a scale of 0–4: 0 = no response; 1 = myoclonus of eyelids; 2 = myoclonus of muscles of face and neck; 3 = myoclonus of muscles of trunk and limbs; 4 = myoclonus continuing beyond the period of photic stimulation. Either 4 ml of vehicle or tiagabine (0.6–2.4 μmol kg^{-1} freshly dissolved in high performance liquid chromatography (HPLC) grade distilled H_2O) was administered intravenously (i.v.) 45 min after the control I.L.S. Each animal was then subjected to I.L.S. at $+15$ min, $+1$ h, $+2$ h, $+3$ h and at $+4$ h after vehicle or drug administration. Adverse behavioural effects of the drug were noted. In each baboon 1 week elapsed between evaluation of different doses of tiagabine.

2.5. Statistical analysis

Numbers of animals responding per seizure type (wild running (score ≥ 1), clonic (score ≥ 2) and tonic (score ≥ 6) seizures) per experimental group were converted to percentages of those animals responding in the respective vehicle-treated control groups. Latencies to fall (s) as a measure of locomotor performance were converted to percentages of respective vehicle-treated groups. ED_{50} values and 95% confidence limits for the anticonvulsant effects and for the impaired rotarod performance were determined according to the method of Litchfield and Wilcoxon (1949).

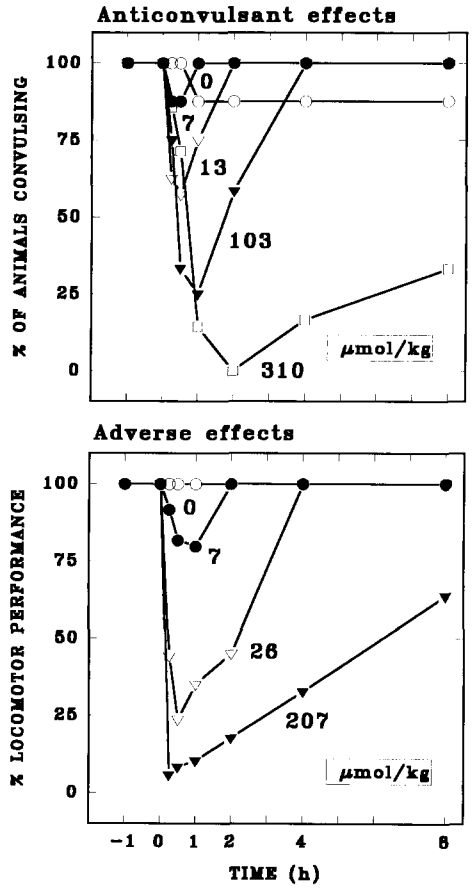

Fig. 3. Effects of NNC-711 on sound-induced clonic seizures and locomotor performance in genetically epilepsy prone (GEP) rats. (Top panel) Sound-induced clonic seizure: GEP rats received vehicle (open circles) or NNC-711 (7 (closed circles), 13 (open triangles), 103 (closed triangles), 310 (open squares) μmol kg^{-1}) (i.p.) and were exposed to a sound stimulus for seizure at $+0.25$ h, $+0.5$ h, $+1$ h, $+2$ h, $+4$ h, and $+8$ h after vehicle or drug administration. Results are expressed as percentage number of GEP rats responding with clonic seizure ($n = 8$–12 per dose). (Bottom panel) Locomotor performance: Groups of 6–12 rats received vehicle (open circles) or NNC-711 (7 (closed circles), 26 (open triangles), 207 (closed triangles) μmol kg^{-1}) (i.p.) and were tested for locomotor impairment using a rotarod at $+0.125$ h, $+0.25$ h, $+0.5$ h, $+1$ h, $+2$ h, and $+4$ h after vehicle or drug administration.

2.6. Drugs

Tiagabine (*R*(−)*N*-(4,4-di(3-methylthien-2-yl)-but-3-enyl) nipecotic acid hydrochloride (tiagabine; molecular weight 412.0) and (1-(2-(((diphenylmethylene)-amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride (NNC-711; molecular weight 386.9) (courtesy Dr. P.D. Suzdak; Novo Nordisk, Denmark) and (±)-nipecotic acid (molecular weight 128.2) (Sigma, Poole, UK) were dissolved in HPLC grade distilled H_2O. Carbamazepine (molecular weight 236.3) (Sigma, Poole, UK) was dissolved in 10% v/v dimethyl sulphoxide in HPLC grade distilled H_2O. Tiagabine and carbamazepine were administered at pH = 4. Dissolution of NNC-711 was achieved by the addition of aliquots of 1 M NaOH to a pH = 6. (±)-Nipecotic acid was administered at pH = 6.5–7.5.

3. Results

3.1. Sound-induced seizures in GEP rats

Tiagabine (6.1–72.8 μmol kg^{-1} i.p.) dose-dependently reduced the number of GEP rats exhibiting sound-induced seizures (see Fig. 2 (top) and Table 1). The ED_{50} values (in μmol kg^{-1}) for suppression of clonic seizures (score ≥ 2; at the time of anticonvulsant effect) for tiagabine were 37 (0.25 h), 23 (0.5 h), 26 (1 h), 39 (2 h), 56 (4 h), 61 (8 h). The time of maximum anticonvulsant effect was at 0.5–1 h after administration of tiagabine.

A maximal anticonvulsant effect was not achieved at earlier time points (0.25–0.5 h) after administration of NNC-711. Low doses of NNC-711 (3–52 μmol kg^{-1} i.p.) reduced the number of GEP rats exhibiting sound-induced seizures to 50%. Higher doses of NNC-711 (103–310 μmol kg^{-1} i.p.) were proconvulsant.

At later time points (1–8 h), NNC-711 (25.9–310.2 μmol kg^{-1} i.p.) dose-dependently reduced the number of GEP rats exhibiting sound-induced seizures (see Fig. 3 (top) and Table 1). The ED_{50} values (in μmol kg^{-1}) for suppression of clonic seizures (score ≥ 2; at the time of anticonvulsant effect) for NNC-711 were 72 (1 h), 131 (2 h), 202 (4 h), 238 (8 h). The time of maximum anticonvulsant without proconvulsant effect was at 1 h after administration of NNC-711.

(±)-Nipecotic acid (0.4–15.6 mmol kg^{-1}) did not reduce the number of GEP rats exhibiting sound-induced seizures (data not shown). Higher doses of (±)-nipecotic acid (39–78 mmol kg^{-1}) induced adverse behavioural effects including ataxia and clonic seizures.

Table 1
Anticonvulsant effects and locomotor deficit

Drug (mol. wt.)	Time (h)	Wild running	Clonic	Tonic	Ataxia	T.I.
Tiagabine	0.125	–	–	–	17 (10–32)	–
(412.0)	0.25	44 (22–87)	37 (23–61)	23 (13–40)	13 (8–20)	0.4
	0.5	26 (17–38)	23 (15–35)	23 (16–36)	16 (9–27)	0.7
	1	36 (27–50)	26 (19–35)	26 (5–137)	16 (8–29)	0.6
	2	44 (29–66)	39 (23–64)	41 (27–63)	41 (29–59)	1.1
	4	56 (36–87)	56 (36–87)	52 (35–79)	66 (47–92)	1.2
	8	61 (39–94)	61 (39–94)	61 (38–98)	NDD	–
NNC-711	0.125	–	–	–	31 (9–109)	–
(386.9)	0.25	NDD	NDD	NDD	20 (5–77)	–
	0.5	NDD	NDD	NDD	21 (6–74)	–
	1	90 (47–171)	72 (43–122)	13 (7–22)	46 (18–118)	0.6
	2	134 (105–170)	131 (101–169)	88 (48–164)	172 (95–311)	1.3
	4	202 (121–335)	202 (120–338)	202 (120–338)	297 (136–651)	1.5
	8	263 (172–407)	238 (130–436)	238 (130–436)	–	–
Carbamazepine	0.125	–	–	–	258 (201–331)	–
(236.3)	0.25	–	–	–	135 (111–164)	–
	0.5	254 (139–463)	254 (139–463)	59 (47–75)	159 (132–193)	0.6
	1	237 (120–466)	168 (86–327)	59 (50–71)	169 (143–200)	1.0
	2	108 (79–148)	98 (69–139)	59 (48–73)	190 (178–204)	1.9
	4	127 (96–168)	132 (88–197)	78 (55–111)	224 (186–271)	1.7
	8	NH	330 (112–970)	106 (72–155)	NDD	–

Anticonvulsant potency and impaired locomotor performance of tiagabine, NNC-711 and carbamazepine in sound-sensitive genetically epilepsy-prone rats. Groups of 6–12 rats received administration of vehicle, tiagabine, NNC-711 or carbamazepine (μmol kg^{-1} i.p.) and were exposed to a sound stimulus at +0.25 h, +0.5 h, +1 h, +2 h, +4 h, +8 h, and +24 h after vehicle or drug administration. Locomotor performance was assessed at similar time points. Results are expressed as ED_{50} values in μmol kg^{-1} (with 95% confidence limits) for antagonism of wild running, clonic or tonic seizure or reduction of locomotor performance. –: Not tested, NDD: not dose-dependent data, NH: not high enough doses tested.

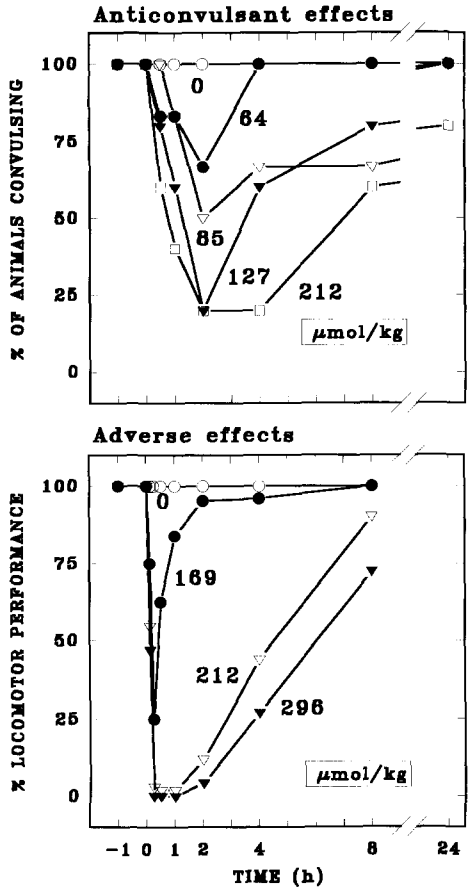

Fig. 4. Effects of carbamazepine on sound-induced clonic seizures and locomotor performance in genetically epilepsy prone (GEP) rats. (Top panel) Sound-induced clonic seizure: GEP rats received vehicle (open circles) or carbamazepine (64 (closed circles), 85 (open triangles), 127 (closed triangles), 212 (open squares) μmol kg^{-1}) (i.p.) and were exposed to a sound stimulus for seizure at +0.5 h, +1 h, +2 h, +4 h, +8 h, and +24 h after vehicle or drug administration. Results are expressed as percentage number of GEP rats responding with clonic seizure (n = 8–12 per dose). (Bottom panel) Locomotor performance: Groups of 6–12 rats received vehicle (open circles) or carbamazepine (169 (closed circles), 212 (open triangles), 296 (closed triangles) μmol kg^{-1}) (i.p.) and were tested for locomotor impairment using a rotarod at +0.125 h, +0.25 h, +0.5 h, +1 h, +2 h, +4 h, and +8 h after vehicle or drug administration.

Carbamazepine (10.6–211.6 μmol kg^{-1} i.p.) dose-dependently reduced the number of GEP rats exhibiting sound-induced seizures (see Fig. 4 (top) and Table 1). The ED_{50} values (in μmol kg^{-1}) for suppression of clonic seizures (score ≥ 2; at the time of anticonvulsant effect) for carbamazepine were 254 (0.5 h), 168 (1 h), 98 (2 h), 132 (4 h), 330 (8 h). The time of maximum anticonvulsant effect was at 2 h after administration of carbamazepine.

3.2. Locomotor performance and adverse behavioural effects in GEP rats

The ED_{50} values at the time of maximum locomotor deficit for tiagabine, NNC-711 and carbamazepine are listed in Table 1. All of the compounds induced a locomotor deficit in a dose-dependent fashion.

The ED_{50} values (in μmol kg^{-1}) for impairment of locomotor performance (at the time of test) for tiagabine were 17 (0.125 h), 13 (0.25 h), 16 (0.5 h), 16 (1 h), 41 (2 h), 66 (4 h). The time of maximum locomotor impairment was at approximately 0.25 h after administration of tiagabine (see Fig. 2 (bottom)). Tiagabine (24.3–97.1 μmol kg^{-1} i.p.) induced locomotor depression and ataxia.

The ED_{50} values (in μmol kg^{-1}) for impairment of locomotor performance (at the time of test) for NNC-711 were 31 (0.125 h), 20 (0.25 h), 21 (0.5 h), 46 (1 h), 172 (2 h), 297 (4 h). The time of maximum locomotor impairment was at approximately 0.25 h after administration of NNC-711 (see Fig. 3 (bottom)). NNC-711 (207–310 μmol kg^{-1} i.p.) induced locomotor depression, ataxia and whole body myoclonic jerks 0.25–0.5 h after administration.

The ED_{50} values [with 95% confidence limits] (in mmol kg^{-1}) for impairment of locomotor performance (at the time of test) for (±)-nipecotic acid were 55 [45–67] (0.25 h), 45 [34–59] (0.5 h), 46 [33–64] (1 h), 46 [36–60] (2 h), 60 [47–78] (4 h), 71 [46–110] (8 h). (±)-Nipecotic acid (39--78 mmol kg^{-1}) induced adverse behavioural effects including: whole body tremor, limbic seizures (forelimb clonus and rearing) and ataxia 0.5–1 h after administration.

The ED_{50} values (in μmol kg^{-1}) for impairment of locomotor performance (at the time of test) for carbamazepine were 258 (0.125 h), 135 (0.25 h), 159 (0.5 h), 169 (1 h), 190 (2 h), 224 (4 h). The time of maximum locomotor impairment was at 0.25 h after administration of carbamazepine (see Fig. 4 (bottom)). Carbamazepine (127–296.2 μmol kg^{-1} i.p.) induced locomotor depression and ataxia.

3.3. Photically induced myoclonus in Papio papio

Tiagabine (0.6–2.4 μmol kg^{-1} i.v.) reduced mean seizure scores for photically induced myoclonus in *Papio papio*. The time of maximum anticonvulsant effect was at 1–2 h after administration. The duration of anticonvulsant effect of tiagabine was dose-dependent; 0.6 and 1.2 μmol kg^{-1} being shorter-acting (0.25–2 h) than 2.4 μmol kg^{-1} (0.25–4 h) (see Fig. 5).

An accurate ED_{50} value for suppression of clonic seizures (score ≥ 3; at (0.25–2 h) after administration cannot be calculated from the data (but is approximately 1.5 μmol kg^{-1}) (see Fig. 5).

3.4. Adverse effects of tiagabine in Papio papio

In *Papio papio*, low doses of tiagabine (0.6–1.2 μmol kg^{-1} i.v.) were without adverse behavioural effects; tiagabine (2.4 μmol kg^{-1}) induced impaired mo-

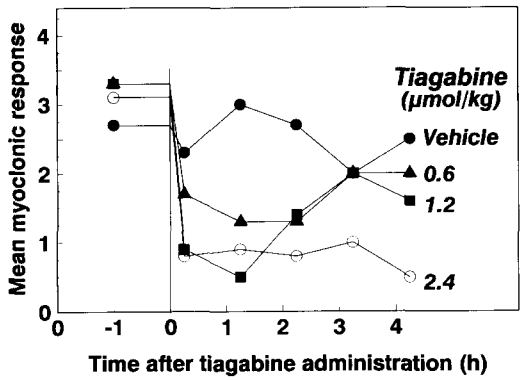

Fig. 5. *Papio papio* baboons were individually exposed to a control intermittent light stimulation (I.L.S.) (25 Hz) and the resulting myoclonic response was scored on a scale of 0–4. 45 min later the animals received 4 ml i.v. of vehicle (closed circles) or tiagabine (0.6 (closed triangles), 1.2 (closed squares), 2.4 (open circles) μmol kg^{-1}) and were exposed to I.L.S. at +0.25 h, +1 h, +2 h, +3 h, and at +4 after vehicle or tiagabine administration. Results are expressed as mean myoclonic response for 3 animals.

tor co-ordination, diffuse tremor, and slow abnormal movements of the limbs of the baboons within 30 min of administration.

4. Discussion

Tiagabine is a more potent anticonvulsant than NNC-711, nipecotic acid or carbamazepine in the GEP rat model of reflex epilepsy reported here. Tiagabine, NNC-711 and carbamazepine impair locomotor performance of GEP rats at doses which are similar to anticonvulsant doses; therapeutic indices for tiagabine are 0.4–1.2, for NNC-711 are 0.6–1.5 and for carbamazepine are 0.6–1.9. (±)-Nipecotic acid is not anticonvulsant (high doses were proconvulsant) after intraperitoneal administration in GEP rats. High doses of NNC-711 were proconvulsant at times early after administration and maximal anticonvulsant effects could not be achieved.

This confirms earlier reports of an anticonvulsant effect of tiagabine (ED_{50} value against tonic seizure = 1 μmol kg^{-1} i.p.), NNC-711 (ED_{50} value against tonic seizure = 0.6 μmol kg^{-1} i.p.), and carbamazepine (ED_{50} value against tonic seizure = 30 μmol kg^{-1} i.p.) but not (±)-nipecotic acid in sound-sensitive DBA/2 mice (Chapman et al., 1984; Suzdak et al., 1992) and for tiagabine in GEP rats (ED_{50} value against tonic seizure 27 μmol kg^{-1}) (Faingold et al., 1990). Systemic administration of high doses of (±)-nipecotic acid (1.2 mmol kg^{-1}) do not have anticonvulsant effects in sound-sensitive epilepsy-prone DBA/2 mice (Horton et al., 1979).

The ED_{50} values for carbamazepine (i.p.) against sound-induced clonic seizure in GEP rats in this study are higher than that reported by Dailey and Jobe (1985) (carbamazepine 12.8 μmol kg^{-1} i.p. 0.5–2 h) but are similar to doses that suppress myoclonic seizure responses in photosensitive *Papio papio* baboons (carbamazepine 170 μmol kg^{-1} i.v.) (Meldrum et al., 1975).

A comparison of the inhibition of [^{3}H]GABA uptake in vitro into forebrain synaptosomes in the rat reveals IC_{50} values of 67 nM for tiagabine, 47 nM for NNC-711, and of 3790 nM for (±)-nipecotic acid. A comparison of IC_{50} values for inhibition of [^{3}H]GABA uptake using cultured cell lines shows that (±)-nipecotic acid is two-fold more potent at inhibiting neuronal than glial uptake of [^{3}H]GABA, whilst tiagabine and NNC-711 are two-fold more effective at inhibiting glial than neuronal uptake of [^{3}H]GABA. The toxicity of GABA uptake inhibitors is thought to result from a neuronal depletion of GABA, which normally maintains a nonepileptogenic state. The finding that NNC-711 but not tiagabine has proconvulsant effects does not accommodate with this reasoning, and suggests that further modes of action of NNC-711 at higher doses are possible.

(±)-Nipecotic acid (100 μM–1 mM) has effects which resemble those of tiagabine in vitro (Roepstroff and Lambert, 1992) but its low efficacy and its limited ability to pass the blood-brain barrier complicate interpretation of its effects in vivo. (±)-Nipecotic acid unlike tiagabine and NNC-711 is a substrate for the GABA carrier. Tiagabine and NNC-711 are thought to use their lipophilic anchors to bind to a site near the carrier to noncompetitively inhibit GABA uptake. Systemic administration of anticonvulsant doses of tiagabine (28 or 51 μmol kg^{-1} i.p.) increase the extracellular concentration of GABA by 200–350% in the globus pallidus, ventral pallidum, and substantia nigra in conscious rats (Fink-Jensen et al., 1992).

In *Papio papio*, we observed a reduction in photically induced myoclonic seizures without neurological impairment after intravenous administration of tiagabine (0.6–1.2 μmol kg^{-1}). Oral administration of tiagabine in healthy human volunteers is well-tolerated in doses up to 10 mg [24 μmol] (p.o.) daily for 5 days (Leppick et al., 1993; Dr H. Mengel, personal communication, 1994). A preliminary study has shown a moderate reduction in seizure frequency in patients with intractable complex partial seizures treated with tiagabine (30 mg [73 μmol] day^{-1} p.o. for 4 weeks) (Sveinbjornsdottir et al., 1992; also Richens et al., submitted).

Adverse effects attributed to tiagabine (at anticonvulsant doses) in humans are mild to moderate in severity and include asthenia (1/24), ataxia (1/24), dizziness (2/24), insomnia (1/24) (patients with adverse effect/patients studied) (Leppick et al., 1993; Dr, H. Mengel, personal communication, 1994; Richens et

al., submitted). Our study in *Papio papio* shows slight ataxia with tiagabine at the highest anticonvulsant dose (2.4 μmol kg^{-1} i.v.) studied, although a similar anticonvulsant effect was observed with a lower dose of tiagabine (1.2 μmol kg^{-1} i.v.) which induced no observable adverse effect. Thus the more severe adverse effects of tiagabine which we observe in rodents are predictive of adverse effect of tiagabine in non-human primates (this study) and in humans and are presumably related to increased availability of GABA to depress motor function.

In summary, the systemic administration of compounds which potently increase extracellular GABA concentration in the brain results in an anticonvulsant effect, supporting a role for the inhibition of GABA uptake as a therapeutic target in epilepsy.

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