British Journal of Pharmacology (2005) 144, 628–635
www.nature.com/bjp

Postsynaptic 5-HT_{1B} receptors modulate electroshock-induced generalised seizures in rats

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1 Although an important regulatory role for serotonin (5-HT) in seizure activation and propagation is well established, relatively little is known of the function of specific 5-HT receptor subtypes on seizure modulation.

2 The aim of the present study was to investigate the role of 5-$HT_{1A,\ 1B\ and\ 1D}$ receptors in modulating generalised seizures in the rat maximal electroshock seizure threshold (MEST) test.

3 The mixed 5-HT receptor agonists SKF 99101 (5–20 mg kg^{-1} i.p.) and RU 24969 (1–5 mg kg^{-1} i.p.), 0.5 h pretest, both produced marked dose-related increases in seizure threshold. These agents share high affinity for 5-$HT_{1A,\ 1B\ and\ 1D}$ receptors.

4 Antiseizure effects induced by submaximal doses of these agonists were maintained following p-chlorophenylalanine (150 mg kg^{-1} i.p. $\times$ 3 days)-induced 5-HT depletion.

5 The anticonvulsant action of both SKF 99101 (15 mg kg^{-1} i.p.) and RU 24969 (2.5 mg kg^{-1} i.p.) was dose-dependently abolished by the selective 5-HT_{1B} receptor antagonist SB-224289 (0.1–3 mg kg^{-1} p.o., 3 h pretest) but was unaffected by the selective 5-HT_{1A} receptor antagonist WAY 100635 (0.01–0.3 mg kg^{-1} s.c., 1 h pretest). This indicates that 5-HT_{1B} receptors are primarily involved in mediating the anticonvulsant properties of these agents.

6 In addition, the ability of the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (0.3–3 mg kg^{-1} s.c., 60 min pretest) to dose-dependently inhibit SKF 99101-induced elevation of seizure threshold also suggests possible downstream involvement of 5-HT_{1D} receptors in the action of this agonist, although confirmation awaits the identification of a selective 5-HT_{1D} receptor antagonist.

7 Overall, these data demonstrate that stimulation of postsynaptic 5-HT_{1B} receptors inhibits electroshock-induced seizure spread in rats.
British Journal of Pharmacology (2005) **144,** 628–635. doi:10.1038/sj.bjp.0706027
Published online 24 January 2005

Keywords: Postsynaptic 5-HT_{1B} receptors; electroshock; generalised seizures; rat; SKF 99101; RU 24969; SB-224289

Abbreviations: GEPR, genetically-epilepsy-prone rat; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; mCPP, m-chlorophenylpiperazine; MEST, maximal electroshock seizure threshold; PCPA, p-chlorophenylalanine; TFMPP, trifluoromethyl-phenylpiperazine

Introduction

Advances in our understanding of brain serotonergic transmission have established an important regulatory role for this centrally abundant neurotransmitter within the complex mechanisms underlying seizure activation and propagation. Considerable focus has been placed on the modulation of audiogenic seizures in the genetically-epilepsy-prone rat (GEPR), in which there is growing evidence for an inhibitory action of brain 5-HT (e.g. Statnick *et al*., 1996). Moreover, the antiepileptic drug carbamazepine has been shown to increase extracellular 5-HT at anticonvulsant doses in GEPRs, an effect thought to be mediated by a direct action of the compound on serotonergic nerve terminals (Dailey *et al*., 1997). It has also been shown that the anticonvulsant action of the 5-HT reuptake inhibitor fluoxetine in the substantia nigra is dependent upon endogenous serotonin (Pasini *et al*., 1996). Conversely, early reports suggested that alpha-guanidinoglutaric acid-induced seizures in rats are initiated by a decrease in brain 5-HT levels (Shiraga *et al*., 1986). Furthermore, dysfunction of serotonergic neurons is thought to be involved in the seizure susceptibility of inbred mutant El mice (Hiramatsu *et al*., 1987). Overall, the consensus of opinion is that elevation of extracellular 5-HT has an inhibitory effect on seizures, whereas depletion of 5-HT facilitates seizure activity (see Upton *et al*., 1998).

Several 5-HT receptor subtypes have now been implicated in seizure modulation. For example, activation of presynaptic 5-HT_{1B} receptors inhibits the action-dependent 5-HT release in the anaesthetised rat amygdalopiriform cortex (a transition zone between the amygdala and piriform cortex) (Kikvadze & Foster, 1995) and may also cause inhibition of low Mg^{2+}-induced late recurrent discharges generated in the rat

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Published online 24 January 2005

Table 1 Receptor binding affinities for selected 5-HT receptor agonists/antagonists

	5-HT receptor subtype (pK_i# values)									
	1A	*1B*	*1D*	*1E*	*1F*	*2A*	*2B*	*2C*	*6*	*7*
SKF 99101[a]	7.43	8.70	8.72	6.84	6.96	5.79	6.40	6.38	6.89	7.29
RU 24969[a]	8.13	7.57	7.76	6.54	6.52	<5.9	6.91	6.23	<5.00	<5.00
SB-224289[b]	<6	8.16	6.27	<5	<5	5.92	<5.5	6.2	<5.67	<6
WAY 100635[a]	8.87	<5.5	6.67	<5.3	<5.3	6.2	7.3	6.17	<5.07	6.93
GR 127935[c]	7.2	9.0	8.6	5.4	6.4	7.8	6.2	7.0	5.8	6.2
(±)pindolol[d]	6.5–34	17.8–34	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Metergoline[e]	8.1	7.6	9.1	ND	ND		9.0	9.2	ND	ND

#: with the exception of pindolol, for which binding affinities are expressed as K_i (nM) values.
[a]Values represent means from three to nine separate evaluations, determined according to the methods described in Kennett *et al*. (1997).
[b]Data taken from Selkirk *et al*. (1998).
[c]Data taken from Price *et al*. (1997).
[d]Data taken from Artigas *et al*. (2001).
[e]Data taken from Chopin *et al*. (1994); ND: not determined.

subiculum (Behr & Heinemann, 1996). Furthermore, intrahippocampal injection of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT raises the seizure threshold for eliciting generalised seizures in hippocampal-kindled cats (Wada *et al*., 1993). Activation of 5-HT$_{1A}$ receptors also retards the development of amygdala kindling in rats, whereas 5-HT$_2$ receptor stimulation facilitates this process (Wada *et al*., 1997). In contrast, stimulation of 5-HT$_2$ receptors has been linked to the anticonvulsant action of trifluoromethyl-phenylpiperazine (TFMPP) (5-HT$_{2A/2C}$) (Przegalinski *et al*., 1994) and m-chlorophenylpiperazine (mCPP) (5-HT$_{2B/2C}$) (Upton *et al*., 1997) on rodent maximal electroshock convulsions.

To date, little is known of any function for 5-HT$_{1A,1B \text{ and } 1D}$ receptors in mediating electroshock-induced seizures in rodents. The aim of the present study, therefore, was to investigate the role of these receptors in modulating generalised tonic hindlimb seizures in the rat maximal electroshock seizure threshold (MEST) test (Löscher & Schmidt, 1988). The mixed 5-HT agonists SKF 99101 (Hatcher *et al*., 1995) and RU 24969 (Tricklebank *et al*., 1986), which although nonselective share high affinities only for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Table 1), were chosen as tool compounds for these investigations and were evaluated in the presence and absence of a range of antagonists with varying selectivities for these receptor subtypes. Both SKF 99101 and RU 24969 were found to produce anticonvulsant effects and studies were subsequently undertaken to determine whether their activity was attributable to pre- and/or post-synaptic 5-HT receptors using the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA).

Methods

Animals

All experimental work was conducted in compliance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986, and was reviewed and approved by the GlaxoSmithKline Procedures Review Panel.

Male Sprague–Dawley rats (80–150 g), supplied by Charles River, U.K., were housed in groups of 10 at a room temperature of 20–22°C. Animals were maintained on a 12 h light/dark cycle with lights on between 06:00 and 18:00 hours. Food (combined rat and mouse diet, Special Diet Services, Witham, U.K.) and water were available *ad libitum*. Drug treatments were evaluated between 14:00 and 18:00 hours alongside time-matched vehicle-treated controls.

Seizure threshold determination

In rats, the threshold current for electroshock-induced tonic hindlimb extensor seizure was determined using a Hugo Sachs Electronik stimulator, which delivered an adjustable constant current (1–300 mA) of 0.3 s duration, 50 Hz, sinewave form, *via* corneal electrodes. The stimulus intensity was varied, from a typical baseline of 25 mA, by an 'up and down' method of shock titration (Kimball *et al*., 1957). Thus, the first rat within a treatment group was given a shock at the expected or estimated seizure threshold (CC_{50}) current, that is, the current producing tonic hindlimb extensor seizure in 50% of animals. For subsequent animals, the stimulus intensity was lowered or raised (in 5 or 10 mA intervals) if the preceding rat did or did not show tonic hindlimb extension, respectively. This procedure continued for all rats within a treatment group. Data generated from treatment groups of $n = 12$–15 were used to calculate the $CC_{50} \pm$ s.e. values according to the method of Kimball *et al*. (1957). Elevation of seizure threshold is indicative of anticonvulsant activity, whereas a reduction in seizure threshold is indicative of a proconvulsant action.

Studies were conducted to determine the effects of the mixed 5-HT receptor agonists SKF 99101 (5–20 mg kg^{-1} i.p., 30 min pretest) and RU 24969 (1–5 mg kg^{-1} i.p., 30 min pretest) on the threshold to generalised seizures, in both drug-naïve and PCPA (150 mg kg^{-1} i.p., u.i.d. × 3 days)-pretreated rats. In the PCPA studies, the agonists were evaluated 24 h after the last administration of the 5-HT synthesis inhibitor. Immediately after application of the electroshock, animals were killed and brains removed and assayed for 5-HT content as described below. In addition, the effects of the selective 5-HT$_{1B}$ receptor antagonist SB-224289 (0.1–3 mg kg^{-1} p.o., 180 min pretest) (Gaster *et al*., 1998; Selkirk *et al*., 1998), the 5-HT$_{1A}$ receptor antagonist WAY 100635 (0.01–0.3 mg kg^{-1} s.c., 60 min pretest) (Fletcher *et al*., 1995) and the 5-HT$_{1A/1B}$ receptor antagonist pindolol (2–8 mg kg^{-1} s.c., 60 min pretest) (Hjorth & Carlsson, 1986) were evaluated on both SKF 99101 (15 mg kg^{-1} i.p.) and RU 24969 (2.5 mg kg^{-1} i.p.)-induced elevation of electroshock-evoked seizure threshold. The action of the mixed 5-HT receptor antagonist metergoline (0.3–3 mg kg^{-1} s.c., 60 min pretest) (Hoyer, 1989) and the 5-HT$_{1B/1D}$ receptor antagonist

GR 127935 (0.3–3 mg kg^{-1} s.c., 60 min pretest) (Skingle *et al.*, 1993) were also tested on the anticonvulsant properties of SKF 99101.

Measurement of brain 5-HT levels following PCPA pretreatment using high-performance liquid chromatography (HPLC)

In order to measure the extent of 5-HT depletion following administration of PCPA, groups of animals ($n = 11$–12) were treated with vehicle (saline i.p. u.i.d. × 3 days) or PCPA (150 mg kg^{-1} i.p. × 3 days). At 24 h after the last dose, animals were killed and brains were frozen and stored at −80°C until assay. While frozen, whole brains were dissected into left and right hemispheres, and cerebellum. Each region was weighed, placed into Eppendorf tubes and 10 μl of homogenising buffer (0.1% w v^{-1} Na metabisulphite, 0.01% w v^{-1} EDTA, 0.1% w v^{-1} L-cysteine, 0.4 M perchloric acid) was added, per mg of tissue. Samples were subsequently homogenised using an electric homogeniser (Ultra Turrax T25, Janke and Kunkel, Germany). Homogenised samples were spun on a centrifuge (Labofuge 400R, Heraeus Instruments, Germany) at 10,000 r.p.m., 4°C for 10 min and the resulting supernatant was injected into the HPLC system (Table 2).

The HPLC system used consisted of a solvent delivery pump (PU-980, Jasco, Japan), an electrochemical amperometric detector (Decade, Antec-Leyden, Netherlands) with a 3 mm glassy carbon electrode and a working electrode set at +800 mV vs Ag/AgCl (VT-03, Antec-Leyden, Netherlands), a Low pass In-Line Noise Killer (Antec-Leyden, Netherlands) set at 26 s peak width before data capture using Waters Millennium32 and a 234 autosampler (Gilson, France) with six-port rotary valve (Model 7125, Rheodyne, Berkley, CA, U.S.A.). Chromatographic separations were performed using a Waters Symmetry (C18 3.5 μm, 4.6 × 75 mm) column with a 20 mm C18 Waters Sentry guard. The mobile phase consisted of 0.07 M KH_2PO_4 (Fisher, U.K., HPLC grade), 1 mM octane sulphonic acid (Fisher, U.K., HPLC grade) and 0.1 mM Na_2 EDTA (Fisher, U.K., 0.1 M solution, HPLC grade) made up in 10% methanol 205 (Super Purity, Romil, Cambridge, U.K.), 0.5% propan-2-ol (Super Purity, Romil, Cambridge, U.K.), buffered to pH = 2.85 with orthophosphoric acid (85%, Fisher, U.K., HPLC grade). The mobile phase was filtered through a 0.22 μm filter (Millipore, Bedford, MA, U.S.A.), degassed online (DEG-103, Kontron, Japan) and delivered at 1.50 ml min^{-1} at 30°C.

Serotonin peaks were identified and levels determined by comparison to external standards (Sigma, St Louis, U.S.A.). The minimum level of detection for this system was 50 pg mg^{-1}. Only animals showing >90% depletion of 5-HT were included in determination of electroshock seizure threshold.

Drugs

SKF 99101, 3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate; SB-224289, tetrahydro-1′-methyl-5-[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo[2,3-f]indole-3-spiro-4′-piperidine hydrochloride; WAY 100635, *N*-(2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl)-*N*-(2-pyridyl) cyclohexanecarboxamide oxalate and GR 127935, *N*-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide were all synthesised by the Medicinal Chemistry Department at GlaxoSmithKline Pharmaceuticals, U.K. RU 24969, 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridyl)-1H-indole hemisuccinate and metergoline were purchased from Tocris, Bristol, U.K. and Research Biochemicals International, Natick, U.S.A., respectively. (±)Pindolol and PCPA methyl ester were obtained from Sigma, Poole, U.K.

With the exception of SB-224289, which was suspended in 1% methyl cellulose (Sigma, Poole, U.K.) in water, drugs were dissolved in appropriate solvents, shown in brackets: SKF 99101, RU 24969 and PCPA (0.9% saline); metergoline and pindolol (1% glacial acetic acid, neutralised with sodium hydroxide); WAY 100 635 (water) and GR 127935 (water + a few drops of Brij 35® (Sigma, Poole, U.K.)). A 1 ml kg^{-1} dose volume was used for all treatments and doses are expressed as free base.

Statistical analysis

Statistical comparisons between appropriate groups on seizure threshold were made according to Litchfield & Wilcoxon (1949). ID_{50} ± s.e. (dose producing 50% inhibition of agonist-induced response) values were determined, where applicable, for antagonist activities, using iterative 'ALLFIT' analysis (DeLean *et al.*, 1978).

Results

Throughout the present studies there was low variability in the control threshold to electroshock-induced generalised seizures in rats, as shown in Figures 1–3 and Tables 3–5. The mixed 5-HT receptor agonists SKF 99101 (5–20 mg kg^{-1} i.p.) and RU 24969 (1–5 mg kg^{-1} i.p.) both dose-dependently increased seizure threshold by up to 1141 and 768%, respectively (Figure 1a and b). In addition to the anticonvulsant effects observed, SKF 99101 and RU 24969 induced hyper-reactivity,

Table 2 Steady-state levels of 5-HT in rat brain in the presence and absence of PCPA

Treatment	N	*Subregion 5-HT concentration* (ng mg^{-1} of tissue)		
		Cerebellum	*Left hemisphere*	*Right hemisphere*
Vehicle alone	24	32.9 ± 3.0	51.2 ± 5.2	49.2 ± 4.8
PCPA alone	22	2.7 ± 0.4	2.4 ± 0.5	2.2 ± 0.4
PCPA + SKF 99101	24	2.8 ± 0.5	3.3 ± 0.7	3.3 ± 0.6
PCPA + RU 24939	24	3.2 ± 0.5	2.4 ± 0.4	2.6 ± 0.5

Data represents mean ± s.e. values for each subregion ($n = 2$ per animal).

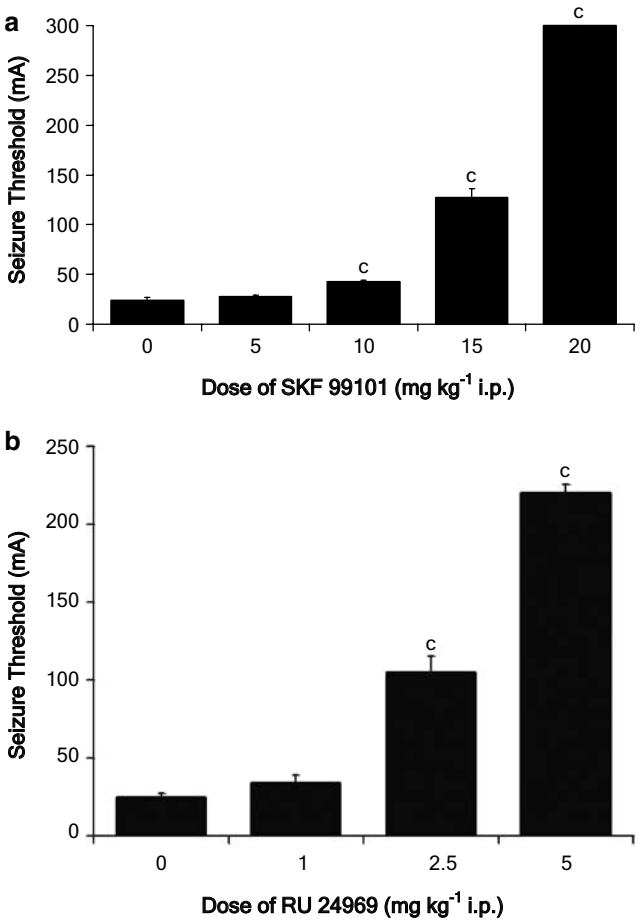

Figure 1 Effect of SKF 99101 (a) and RU 24969 (b) on electroshock-induced generalised seizures in rats; data represent $CC_{50} \pm$s.e. values for groups of 13 (a) and 14 (b) rats; SKF 99101 and RU 24969 were administered 30 min pretest; c: $P<0.001$ compared to the corresponding vehicle-treated control groups.

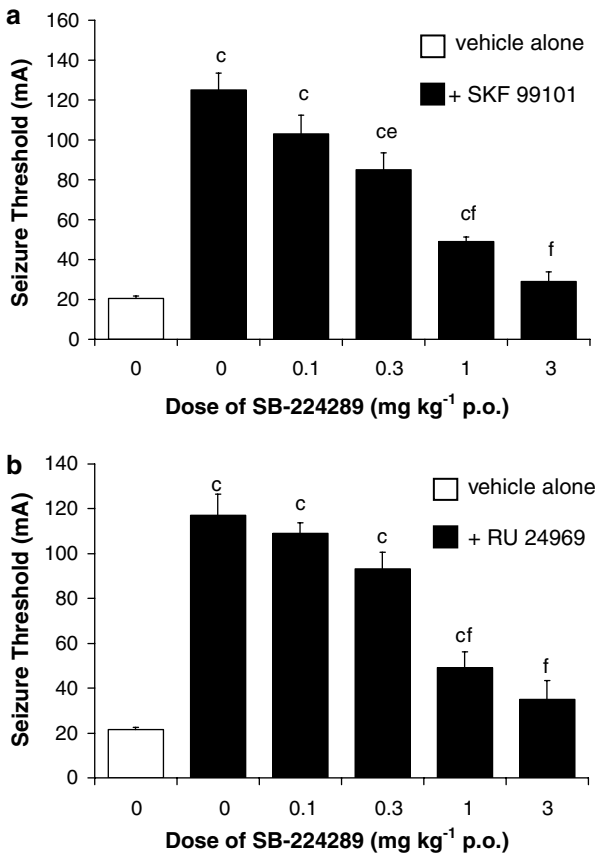

Figure 2 Effect of SB-224289 on SKF 99101 (a) and RU 24969 (b)-induced elevation of seizure threshold in the rat MEST test; data represent $CC_{50} \pm$s.e. values for groups of 12 rats; SB-224289 was administered 3h pretest; SKF 99101 and RU 24969 were administered at 15 and 2.5 mg kg^{-1} i.p., respectively, 30 min pretest; zero dose groups received pretreatment with vehicle; c: $P<0.001$ compared to corresponding vehicle-treated group; e: $P<0.01$ and f: $P<0.001$ compared to the corresponding SKF 99101 or RU 24969 alone group.

flushing and classical '5-HT syndrome' (Hole *et al*., 1976) behaviours, such as flat body posture, at the higher doses evaluated.

Pretreatment with the selective 5-HT$_{1B}$ receptor antagonist SB-224289 (0.1–3 mg kg^{-1} p.o.) produced potent dose-related blockade of both SKF 99101 (15 mg kg^{-1} i.p.) (Figure 2a) and RU 24969 (2.5 mg kg^{-1} i.p.) (Figure 2b)-induced anticonvulsant activity, with comparable ID_{50} values of 0.41 ± 0.03 and 0.51 ± 0.03 mg kg^{-1} p.o., respectively, and with no significant ($P>0.05$) effect on seizure threshold in its own right (0.3–3 mg kg^{-1} p.o.), (Table 3). In fact, the seizure threshold of animals treated with either SKF 99101 or RU 24969 in the presence of SB-224289 at 3 mg kg^{-1} was not significantly different from that of the corresponding vehicle control group. This dose of SB 224289 has previously been shown to partially inhibit 5-HT$_{1B}$ receptor-mediated SKF 99101-induced hypothermia in guinea-pigs (Hagan *et al*., 1997).

The effects of various other 5-HT receptor antagonists alone on the threshold to electroshock-induced generalised seizures and on SKF 99101 and RU 24969-induced anticonvulsant activity are shown in Tables 3, 4 and 5, respectively. The 5-HT$_{1A/1B}$ receptor antagonist ($\pm$)pindolol (2–8 mg kg^{-1} s.c.) inhibited the anticonvulsant activity produced by both agonists, in a largely dose-related manner, despite weakly elevating seizure threshold in its own right. In addition, the 5-HT$_{1B/1D}$ receptor antagonist GR 127935 (0.3–3 mg kg^{-1} s.c.), which had no significant ($P>0.05$) effect alone in the MEST model, dose-dependently reversed SKF 99101-induced elevation of seizure threshold. This inhibitory effect was also observed, albeit to a lesser extent, with the mixed 5-HT antagonist metergoline (0.3–3 mg kg^{-1} s.c.), which produced a weak proconvulsant effect in its own right at 0.3 mg kg^{-1} s.c. In marked contrast, the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (0.01–0.3 mg kg^{-1} s.c.) did not antagonise the effects of either SKF 99101 or RU 24969 in the MEST test, at doses previously shown to reverse the 5-HT$_{1A}$ receptor-mediated anxiolytic effect of 8-OH DPAT (Collinson & Dawson, 1997).

Figure 3 illustrates the effect of submaximal doses of SKF 99101 (15 mg kg^{-1} i.p.) and RU 24969 (2.5 mg kg^{-1} i.p.) on electroshock-induced generalised seizures in both PCPA (150 mg kg^{-1} i.p., u.i.d $\times$ 3 days)-pretreated and drug-naïve rats. PCPA significantly lowered seizure threshold by 50%. In untreated rats, SKF 99101 and RU 24969 produced a high

level of anticonvulsant activity, achieving 520±23 and 480±18% increases in seizure threshold, respectively. Moreover, the antiseizure activity observed with SKF 99101 and RU 24969 in this model was retained following PCPA administration, with increases in seizure threshold values from PCPA-alone treated animals of 840±19 and 720±19%, respectively. Furthermore, steady-state levels of 5-HT in all the above PCPA-treated animals were depleted by >90%, with no differences between subregions. In addition, levels of depletion were highly consistent across groups.

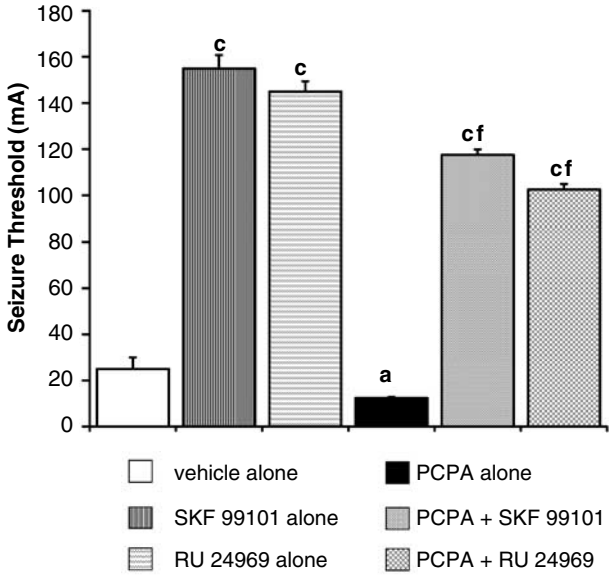

Figure 3 Effect of SKF 99101 and RU 24969 on electroshock-induced generalised seizures in drug-naïve and PCPA-pretreated rats; data represent CC_{50}±s.e. values for groups of 11–12 rats; PCPA was administered at 150 mg kg^{-1} i.p., at 24 h intervals for 3 days prior to testing. At 24 h after the last dose, SKF 99101 and RU 24969 were administered at 15 and 2.5 mg kg^{-1} i.p., respectively, 30 min pretest; a: $P<0.05$, c: $P<0.001$ compared to the vehicle alone group, f: $P<0.001$ compared to the PCPA alone group; all PCPA-pretreated rats exhibited >90% depletion of 5-HT.

Discussion

This study has demonstrated that stimulation of postsynaptic 5-HT$_{1B}$ receptors causes inhibition of electroshock-induced seizure spread in rats. This finding emerged from the initial observation that the nonselective 5-HT agonists SKF 99101 and RU 24969 both produce a high level of protection from electroshock-induced generalised tonic hindlimb seizures in rats. The anticonvulsant profile of SKF 99101 has been confirmed by its ability to significantly inhibit forelimb tonus at 30 mg kg^{-1} i.p. in the rat intravenous metrazol infusion test (data not shown). In fact, the magnitude of anticonvulsant activity achieved in the MEST test is comparable to that of established antiepileptic drugs, such as carbamazepine (Upton *et al*., 1997) in this rodent model.

The receptor affinity profiles in Table 1 show that SKF 99101 and RU 24969 only share a high affinity (pK_i ⩾7.4) for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. The various 5-HT antagonists, which inhibited SKF 99101 and/or RU 24969-induced elevation of seizure threshold in rats in this study, all share in common a high affinity for the 5-HT$_{1B}$ receptor. In accord with early reports (Khanna *et al*., 1989), the weak anticonvulsant properties observed with pindolol alone are unlikely to be related to a serotonergic mechanism, particularly as this effect did not hamper the compound's inhibitory

Table 3 Effect of SB-224289, pindolol, GR 127935, metergoline and WAY 100635 on electroshock-induced generalised seizure threshold in rats

Treatment	*Dose* (mg kg^{-1})	Route	*Pretest time* (min)	N	CC_{50}±*s.e.* (mA)
SB-224289	0	p.o.	180	14	30.4±1.0
	0.3	p.o.	180	14	30.5±3.3
	0	p.o.	180	14	21.8±0.6
	1	p.o.	180	14	21.1±0.9
	3	p.o.	180	14	23.2±1.4
Pindolol	0	s.c.	60	14	18.9±1.6
	2	s.c.	60	14	24.2±1.0^b
	4	s.c.	60	14	24.2±1.0^b
	8	s.c.	60	14	26.7±7.7
GR 127935	0	s.c.	60	12	27.5±6.9
	0.3	s.c.	60	12	19.5±1.2
	1	s.c.	60	12	22.5±1.3
	3	s.c.	60	12	22.5±1.3
Metergoline	0	s.c.	60	14	22.5±1.3
	0.3	s.c.	60	14	18.9±0.9^a
	1	s.c.	60	14	21.7±2.7
	3	s.c.	60	14	23.9±2.4
WAY 100635	0	s.c.	60	15	23.9±0.9
	0.03	s.c.	60	15	23.9±0.9
	0.1	s.c.	60	15	23.2±0.6
	0.3	s.c.	60	15	21.8±1.4

Zero dose groups were administered with corresponding vehicle treatment; $^aP<0.05$ and $^bP<0.01$ compared to the corresponding vehicle-treated control group.

Table 4 Effect of various 5-HT receptor antagonists on SKF 99101-induced elevation of seizure threshold in the rat MEST test

Treatment 1	*Dose (*mg kg^{-1} s.c *60* min *pretest)*	*Dose of SKF 99101 (*mg kg^{-1} *i.p. 30* min *pretest)*	N	$CC_{50}\pm s.e.$ *(*mA*)*	$ID_{50}\pm s.e.$ (mg kg^{-1}*)*
Pindolol	0	0	14	18.9±1.6	
	0	15	14	161.0±4.8[c]	
	2	15	14	75.0±0.6[c,f]	2.8±0.5
	4	15	14	55.0±19.1[f]	
	8	15	14	46.3±12.1[a,f]	
GR 127935	0	0	12	27.5±6.9	
	0	15	12	116.7±3.4[c]	
	0.3	15	12	122.5±20.5[c]	0.9±0.2
	1	15	12	60.0±2.6[c,f]	
	3	15	12	43.3±11.3[f]	
Metergoline	0	0	14	22.5±1.3	
	0	15	16	165.0±16.4[c]	
	0.3	15	15	170.0±6.0[c]	2.2±0.7
	1	15	15	117.0±22.8[c]	
	3	15	15	85.0±8.5[c,f]	
WAY 100635	0	0	13	29.2±2.9	
	0	15	14	189.0±12.6[c]	
	0.01	15	14	181.7±13.8[c]	
	0.03	15	14	183.3±3.4[c]	
	0.1	15	14	180.0±12.0[c]	>0.3
	0	0	12	22.5±1.3	
	0	15	12	130.0±6.0[c]	
	0.3	15	12	121.7±3.9[c]	

Data represents seizure threshold values for groups of 12–15 rats; zero dose groups were administered with corresponding vehicle treatment; [a]$P<0.05$ and [c]$P<0.001$ compared to the corresponding vehicle-alone treated group; [f]$P<0.001$ compared to the corresponding SKF 99101-alone treated group.

Table 5 Effect of pindolol and WAY 100635 on RU 24969-induced elevation of seizure threshold in the rat MEST test

Treatment 1	*Dose (*mg kg^{-1} *s.c 60* min *pretest)*	*Dose of RU 24969(*mg kg^{-1} *i.p. 30* min *pretest)*	n	$CC_{50}\pm s.e.$ *(*mA*)*	$ID_{50}\pm s.e.$ *(*mg kg^{-1}*)*
Pindolol	0	0	14	20.0±1.1	
	0	2.5	14	96.7±7.3[c]	
	2	2.5	14	76.7±3.4[c,d]	3.6±1.4
	4	2.5	14	45.5±2.4[c,f]	
	8	2.5	14	47.5±4.2[c,f]	
WAY 100635	0	0	14	23.9±0.9	
	0	2.5	14	76.7±19.5[b]	
	0.03	2.5	14	115.0±6.7[c]	>0.3
	0.1	2.5	14	79.0±7.3[c]	
	0.3	2.5	14	108.3±5.8[c]	

Zero dose groups were administered with corresponding vehicle treatment; [b]$P<0.01$ and [c]$P<0.001$ compared to the corresponding vehicle-alone-treated group; [d]$P<0.05$ and [f]$P<0.001$ compared to the corresponding RU 24969-alone-treated group.

actions on the SKF 99101 and RU 24969-induced responses. The ability of the selective 5-HT$_{1B}$ receptor antagonist SB-224289 to abolish the anticonvulsant action of SKF 99101 and RU 24969 also strongly suggests that the anticonvulsant effect observed is primarily mediated by 5-HT$_{1B}$ receptor activation. However, the 5HT$_{1B/1D}$ receptor antagonist GR 127935 also potently blocked the increase in seizure threshold produced by SKF 99101, raising the possibility that 5-HT$_{1D}$ receptors, downstream of 5-HT$_{1B}$ pathways may play a role in the anticonvulsant action of this agent. Definitive confirmation of the involvement of 5-HT$_{1D}$ receptors in regulating seizure threshold awaits the identification of a selective antagonist for this receptor subtype. The inability of the potent and selective 5-HT$_{1A}$ receptor antagonist WAY 100635 to prevent either SKF 99101 or RU 24969-induced elevation of seizure threshold indicates that the antiseizure activity seen with both agonists is not mediated by stimulation of 5-HT$_{1A}$ receptors.

Administration of PCPA at 150 mg kg^{-1} i.p. over 3 successive days markedly depleted brain 5-HT levels by 93–95%, as shown in Table 2 and led to an increase in seizure susceptibility, a finding consistent with the consensus that depletion of 5-HT induces proconvulsant activity (see Upton *et al*., 1998). The inability of PCPA to prevent the anticonvulsant response of either SKF 99101 or RU 24969 indicates

that the anticonvulsant activity seen with these agonists was mediated by postsynaptic 5-HT receptors.

Importantly, blockade of 5-HT$_{1B}$ receptors in rats with SB-224289 did not enhance susceptibility to generalised seizures, indicating a low basal 5-HT tone at this receptor subtype. This finding is in accord with the present lack of any evidence for a seizure phenotype in 5-HT$_{1B}$ receptor knockout mice (Zhuang *et al*., 1999) and suggests that the proconvulsant effects observed following PCPA treatment are unlikely to be mediated by a lack of tone at 5-HT$_{1B}$ receptors.

Several investigations have focused on determining the mechanism underlying the production of generalised seizures following electroshock stimulation. Of the many neurotransmitters thought to be involved in mediating electroshock-induced seizures, 5-HT has long been known to inhibit this type of convulsion (Burley & Ferrendelli, 1984). Early reports showed that electroconvulsive shock in rats involves activation of the striatum and cerebellum (Blackwood *et al*., 1981), both of which are areas of the brain in which postsynaptic 5-HT$_{1B}$ receptors are thought to be located, based on the assumption that receptor distribution matches mRNA levels (Voigt *et al*., 1991; Neumaier *et al*., 1996). 5-HT$_{1B}$ receptors are also densely populated in the subiculum (Pazos & Palacios, 1985), an area of the hippocampal complex, in which it has been suggested that complex serotonergic innervation may influence the spread of epileptic seizures (Behr & Heinemann, 1996).

Although further work is required to ascertain the pathways involved in generating the anticonvulsant activity observed in the present study, this is the first report demonstrating an unequivocal role for postsynaptic 5-HT$_{1B}$ receptors in modulating electroshock-induced generalised seizures.

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(Received June 16, 2004
Revised August 24, 2004
Accepted September 23, 2004)