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Profile of SB-204269, a mechanistically novel anticonvulsant drug, in rat models of focal and generalized epileptic seizures

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1 Earlier optimization of structure-activity relationships in a novel series of 4-(benzoylamino)-benzopyrans, led to the discovery of SB-204269 (*trans*-(+)-6-acetyl-4**S**-(4-fluorobenzoylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzo[*b*]pyran-3**R**-ol, hemihydrate), a potent orally-active anticonvulsant in the mouse maximal electroshock seizure threshold (MEST) test.

2 Studies have now been undertaken to determine the effects of SB-204269 in a range of seizure models and tests of neurological deficits in rats. In addition, the compound has been evaluated in a series of *in vitro* mechanistic assays.

3 SB-204269 proved to be an orally-effective anticonvulsant agent, at doses (0.1–30 mg kg^{-1}) devoid of overt behavioural depressant properties, in models of both electrically (MEST and maximal electroshock (MES)) and chemically (i.v. pentylenetetrazol (PTZ) infusion)-evoked tonic extension seizures. However, the compound did not inhibit PTZ-induced myoclonic seizures at doses up to 30 mg kg^{-1}, p.o.

4 SB-204269 also selectively reduced focal electrographic seizure activity in an *in vitro* elevated K^+ rat hippocampal slice model at concentrations (0.1–10 μM) that had no effect on normal synaptic activity and neuronal excitability.

5 In all of these seizure models, SB-204269 was equivalent or better than the clinically established antiepileptic drugs carbamazepine and lamotrigine, in terms of anticonvulsant potency and efficacy.

6 Unlike SB-204269, the corresponding *trans* 3**S**,4**R** enantiomer, SB-204268, did not produce marked anticonvulsant effects, an observation in accord with previous findings for other related pairs of *trans* enantiomers in the benzopyran series.

7 In the rat accelerating rotarod test, a sensitive paradigm for the detection of neurological deficits such as sedation and motor incoordination, SB-204269 was inactive even at doses as high as 200 mg kg^{-1}, p.o. This was reflected in the excellent therapeutic index (minimum significantly effective dose in the rotarod test/ED_{50} in the MES test) for SB-204269 of >31, as compared to equivalent values of only 7 and 13 for carbamazepine and lamotrigine, respectively.

8 At concentrations ($\geqslant 10$ μM) well above those required to produce anticonvulsant activity *in vivo* (i.e. 0.1 μM in brain), SB-204269 did not interact with many of the well known mechanistic targets for established antiepileptic drugs (e.g. Na^+ channels or GABAergic neurotransmission). Subsequent studies have shown that the anticonvulsant properties of SB-204269 are likely to be mediated by a novel stereospecific binding site present in the CNS.

9 The overall efficacy profile in rodent seizure models, together with a minimal liability for inducing neurological impairment and an apparently unique mechanism of action, highlight the therapeutic potential of SB-204269 for the treatment of refractory partial and generalized tonic-clonic seizures.

Keywords: SB-204269; fluorobenzoylamino benzopyran; anticonvulsant; focal seizures; generalized seizures; novel mechanism; minimal neurotoxic liability; SB-204268

Introduction

Modern epidemiological studies indicate that epilepsy is the most prevalent serious neurological condition (Shorvon, 1990; Senanayake & Roman, 1993). Unfortunately, it is general experience that even if diagnosis and treatment are optimally managed, as many as 20–30% of patients who develop partial and secondarily generalized seizures are refractory to treatment with the current antiepileptic armamentarium which is headed by carbamazepine, phenytoin and valproate (Juul-Jenson, 1986). Moreover, dose-limiting side effects are common with these drugs (Brodie, 1990; Kälviäinen *et al*., 1993). Recently, a new generation of antiepileptic drugs has reached the market including lamotrigine, vigabatrin and gabapentin. In early clinical trials, these agents have shown the promise of increased efficacy with decreased toxicity relative to traditional antiepileptic drugs, but it is clear that they are neither magic bullets nor panaceas for epilepsy (Upton, 1994). Thus, when used as add-on therapy in refractory patients, only 7% become totally seizure free (Fisher, 1993). As a consequence, there still remains an urgent need for efficacious and safe drugs for refractory epilepsy.

We have shown previously that the 3**R**,4**S** enantiomers of a series of 4-(benzoylamino)-benzopyran compounds related to the adenosine 5′-triphosphate (ATP)-sensitive potassium channel opener cromakalim show anticonvulsant activity after oral administration. Unlike cromakalim, such compounds do not show hypotensive activity, whereas their corresponding 3**S**,4**R** enantiomers are hypotensive but only slightly anticonvulsant (Blackburn *et al*., 1995). Subsequent optimization of structure-activity relationships led to the discovery of SB-204269 (*trans*-(+)-6-acetyl-4**S**-(-4-fluorobenzoylamino)-3, 4-dihydro-2,2-dimethyl-2*H*-benzo[*b*]pyran-3**R**-ol, hemihydrate;

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Figure 1), a potent orally-active anticonvulsant in the mouse maximal electroshock seizure threshold (MEST) test (Chan *et al*., 1996). We now describe the effects of this compound in a range of seizure models and tests of neurological deficits in rats. The seizure tests included paradigms with threshold (i.v. pentylenetetrazol infusion and MEST) and suprathreshold (traditional maximal electroshock) induction of seizures in order to ensure that effects of SB-204269 against specific seizure types were not missed and also to determine whether anticonvulsant activity arose from elevation of seizure threshold and/or prevention of seizure spread (Löscher & Schmidt, 1988). Carbamazepine and lamotrigine, representatives of the older and newer generations of clinically used antiepileptic drugs, respectively, were tested in parallel for comparative purposes. In addition, the corresponding 3**S**,4**R** enantiomer, SB-204268, was assessed in several of the seizure models. Finally, a preliminary series of experiments was undertaken, by use of a diverse range of *in vitro* radioligand binding and functional assays, in an attempt to determine the mechanism of action of SB-204269. In the accompanying paper (Herdon *et al*., 1997), we describe the subsequent discovery of a unique stereospecific binding site in rat brain for this compound.

Methods

Animals

Animal husbandry and experimentation 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 SmithKline Beecham Procedures Review Panel.

Sprague Dawley rats of either sex and male Hooded Lister rats were obtained from Charles River, U.K. The sex, age and body weight of the animals are given in the appropriate sections below. Animals were housed in groups of 6–10 (except following surgery where they were housed singly) under a 12 h light/dark cycle (lights on at 07h 00min) with standard diet and water *ad libitum*. All procedures were carried out between 10h 00min and 17h 00min and animals were used on only one occasion.

Maximal electroshock seizure threshold (MEST) test

The threshold for maximal (tonic hindlimb extension) electroshock seizures in male rats (Sprague Dawley, 80–150 g, 6 weeks old) was determined by a Hugo Sachs Elektronic stimulator which delivered a constant current (0.3 s duration, 50 Hz, sinewave form, fully adjustable between 1–300 mA) *via* corneal electrodes. The stimulus intensity was varied (from a baseline current of 25 mA) by an 'up-and-down' method of shock titration (see Löscher & Schmidt, 1988) whereby the current was lowered or raised (in 5–20 mA steps) if the preceding animal did or did not show hindlimb extension, respectively. The data thus generated in treatment groups of 12–15 rats were used to calculate the CC_{50} value (current producing maximal seizures in 50% of animals) ±s.e. according to the method of Kimball *et al*. (1957).

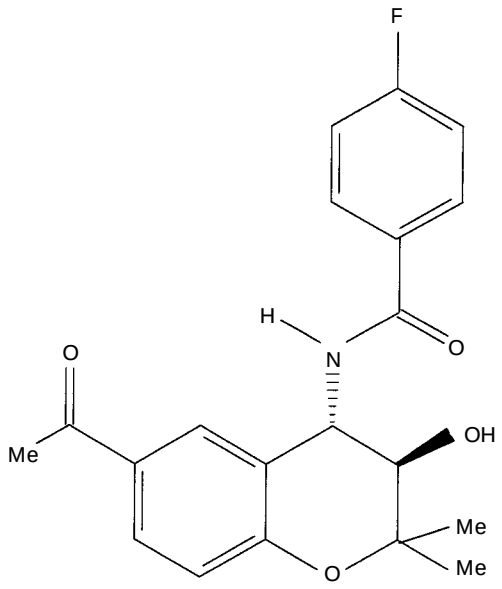

Figure 1 Structure of SB-204269.

The effects of SB-204269 (0.1–30 mg kg^{-1}, p.o., 4 h pretest) on seizure threshold were compared to those of carbamazepine (2.5–20 mg kg^{-1}, p.o., 1 h pretest), lamotrigine (0.5–7.5 mg kg^{-1}, p.o., 6 h pretest) and SB-204268 (10 mg kg^{-1}, p.o., 4 h pretest). These pretest times were chosen to reflect the time of peak effect for the respective agents, determined previously in a series of preliminary time-course experiments, and were used in all the seizure tests. Immediately following the conclusion of the MEST studies, whole brain samples ($n=6$ per group) from SB-204269-treated rats were collected and subsequently assayed for the presence of SB-204269. Briefly, this involved liquid/liquid extraction of SB-204269 from brain homogenates with diethylether followed by high performance liquid chromatography (h.p.l.c.) with u.v. detection (column; Supelcosil ABZ: mobile phase; 32% acetonitrile/68% 0.05 M ammonium acetate). The lower limit of quantification was 0.05 μmol kg^{-1}. Results are expressed as mean±s.e.mean brain concentration of SB-204269 for each treatment group.

Maximal electroshock seizure (MES) test

Male rats (Sprague-Dawley, 80–150 g, 6 weeks old) in treatment groups of 10–11 were assessed for production of maximal seizures following application (*via* corneal electrodes) of a fixed high-intensity supramaximal current of 120 mA (approximately 5 × basal threshold current; 0.3 s duration, 50 Hz, sinewave form). In vehicle-treated control groups, all animals exhibited tonic extension of hindlimbs. For SB-204269 (1–30 mg kg^{-1}, p.o., 4 h pretest), carbamazepine (5–20 mg kg^{-1}, p.o., 1 h pretest) and lamotrigine (2.5–20 mg kg^{-1}, p.o., 6 h pretest), the degree of protection from electroshock was determined at each dose level and used to calculate an ED_{50} value (the dose required to inhibit tonic hindlimb extension seizures in 50% of treated animals) ±s.e. by the iterative curve fitting programme ALLFIT (DeLean *et al*., 1978).

Intravenous pentylenetetrazol (PTZ) seizure threshold test

Male rats (Sprague-Dawley, 175–225 g, 7 weeks old) were lightly restrained in a ventilated plastic tube and infused with PTZ (20 mg ml^{-1}, 1 ml min^{-1}) *via* a butterfly cannula placed in a superficial tail vein. Latencies (t s) to onset of myoclonic (defined as one or more isolated whole body jerks) and tonic forelimb extension seizures (which proved to be a more reliable end-point than the tonic hindlimb extension seizures used in the electroshock models) were then recorded up to a cut-off point of 2 min. On occasions where death ensued before the occurrence of tonic forelimb extension, the former was taken as the end-point. The median (and upper quartile) threshold doses of PTZ required to produce the different seizure types were determined for treatment groups of 11–12 rats following administration of SB-204269 (1–30 mg kg^{-1}, p.o., 4 h pretest) or SB-204268 (10 mg kg^{-1}, p.o., 4 h pretest). Threshold doses (mg kg^{-1}) were calculated according to the following formula: conc. of PTZ (mg ml^{-1}) × t (s) × flow rate (ml s^{-1})/body weight (kg).

In vitro *hippocampal slice studies*

The high K^+ afterdischarge model was modified from that originally described by Traynelis and Dingledine (1988). In

brief, Sprague Dawley rats (of either sex, 40–50 g, 3–4 weeks old) were anaesthetized with halothane, the heart stopped by administering a blow to the back of the thorax, and decapitated. Hippocampal brain slices (400–500 μm thick) were prepared and placed in a recording chamber perfused (at 4 ml min^{-1}) with standard artificial cerebrospinal fluid (ACSF) maintained at 32°C. An extracellular recording microelectrode was positioned in the stratum pyrimidale of region CA1 and a stimulating electrode in stratum radiatum.

Following a period for equilibration, the ACSF was changed to contain elevated levels of KCl (8.5 mM as opposed to the normal 2.5 mM), whereupon application of a brief train (10–20 pulses, 25 ms intervals) of suprathreshold stimuli resulted in normal population spikes to each stimulus followed by spontaneous electrographic seizures (EGS). These afterdischarges, which typically lasted for 20–30 s and were analogous to the ictal phase of seizure EEG, were analysed off-line by a programme written in Spike2 (CED, Cambridge). The programme counted the number of spikes that exceeded a threshold level derived to exclude ongoing activity unrelated to the stimulus train.

The number of afterdischarges was determined following the addition (at 10 min intervals) of cumulative concentrations of SB-204269 (0.1–10 μM), carbamazepine (0.1–30 μM), lamotrigine (0.1–30 μM) or SB-204268 (0.1–3 μM) to the perfusing ACSF. The data thus generated in 3–6 experiments were then used to calculate (ORIGIN analysis; Microcal software) the concentration of test drug required to produce 50% of the maximum inhibition of afterdischarges.

Accelerating rotarod test

Minimal neurological deficits, such as sedation and impaired motor function, were quantified by the accelerating rotarod test (Jones & Roberts, 1968). Male rats (Sprague Dawley, 200–250 g, 8 weeks old) were pretrained to balance on a fixed slow speed (2.5 r.p.m.) rotating treadmill (Ugo-Basile, Italy) 2–3 h before the experiment began. The ability of animals to remain on the rotarod in acceleration mode (2.5–20 r.p.m.) was then assessed on several separate occasions at intervals between 15–360 min after administration of SB-204269 (50–200 mg kg^{-1}, p.o.), carbamazepine (60–100 mg kg^{-1}, p.o.) or lamotrigine (50–200 mg kg^{-1}, p.o.). Median rotarod performance (measured as the time in s, up to a cut-off point of 300 s, spent on the treadmill) at the various test intervals was calculated for each drug treatment group ($n=11-12$) and expressed as percentage change from vehicle-treated control values. In order to assess rigorously the degree of separation between doses that impair motor coordination and those which produce anticonvulsant activity, therapeutic indices (minimum significantly effective dose (MED) in the rotarod test/ED_{50} in the MES test) were determined for the three test drugs (see Swinyard *et al*., 1989).

In addition to this quantitative measurement of neurological deficit, animals were assessed in their home cages and upon handling for overt signs of behavioural depression (e.g. reduced locomotor activity).

Cardiovascular studies

Male rats (Hooded Lister, 300–400 g, 10 weeks old at the start of the study) were anaesthetized (Domitor 0.4 ml kg^{-1}, i.m. and Sublimaze 9 ml kg^{-1}, i.p.) and surgically implanted with an indwelling cannula positioned in the femoral artery (accessed *via* the left hindquarter) and exteriorized between the scapulae. Anaesthesia was reversed by Antisedan/Nubain (50% v/v, 0.2 ml kg^{-1}, i.p.) and the animals allowed a minimum recovery period of 7 days before commencement of haemodynamic studies.

On study days, the rats were placed in appropriate restraining cages. Arterial blood pressure (systolic and diastolic) and heart rate were then recorded electronically from the indwelling cannula at 15 min intervals for approximately 60 min before dosing (to allow for stable baselines to be attained) and for a further 240 min post dosing. Mean (±s.e.mean) values for these parameters were calculated at each time-point for the various treatment groups ($n=5-8$). Where patency of the cannula allowed, animals received vehicle or SB-204269 (30 or 100 mg kg^{-1}, p.o.) in a random cross-over design with at least 2 days between studies.

Mechanism of action studies

SB-204269 was evaluated in a diverse range of *in vitro* radioligand binding assays (for both receptors and ion channels) and functional assays with a variety of tissue preparations. The studies were performed in the laboratories of SmithKline Beecham Pharmaceuticals (U.K., U.S.A. and Italy), NovaScreen (Maryland, U.S.A.) or Tocris Neuramin (Bristol, U.K.), by use of published protocols.

Drugs and administration

SB-204269 (*trans*)-(+)-6-acetyl-4**S**-(4-fluorobenzoylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzo[*b*]pyran-3**R**-ol, hemihydrate) and SB-204268 (*trans*-(−)-6-acetyl-4**R**-(4-fluorobenzoylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzol[*b*]pyran-3**S**-ol, hemihydrate) were synthesized in the Medicinal Chemistry Department at SmithKline Beecham Pharmaceuticals, Harlow. Carbamazepine and PTZ were obtained from Sigma (Poole, U.K.) and lamotrigine kindly provided by The Wellcome Foundation Ltd. (Beckenham, U.K.). Drugs used for surgery and recovery were halothane (Mallinckrodt Veterinary Ltd., U.K.), meditomidine hydrochloride (Domitor), atipamezole hydrochloride (Antisedan) (SmithKline Beecham, U.K.), fentanyl (Sublimaze; Janssen, Belgium) and nalbuphine (Nubain; DuPont, U.S.A.). All other chemicals used were of reagent grade or of the purest commercially available grade.

For the behavioural studies, test drugs were administered orally by gavage as a fine suspension in 1% methylcellulose (Fisons) in water in a dose volume of 1 ml kg^{-1}. All experiments included appropriate vehicle-treated control groups. The convulsant agent PTZ was dissolved in 0.9% saline and infused intravenously. For the *in vitro* hippocampal slice studies, test drugs were prepared fresh as a 100 mM stock solution in dimethyl sulphoxide (DMSO) and then added to the ACSF perfusate to give a maximal final DMSO dilution of 1 : 500.

Statistical analysis

Significant effects ($P<0.05$) of drugs on the threshold for maximal electroshock seizures were determined by comparison of the potency ratios of the CC_{50} values of the drug and vehicle-treated groups by the method of Litchfield & Wilcoxan (1949). For the MES test, statistical comparisons of the number of animals displaying tonic hindlimb extension in the individual treatment groups and vehicle-treated controls were made by the (two-tail) Fisher exact probability test. The statistical significance of drug-induced changes in PTZ seizure thresholds and rotarod performance relative to vehicle-treated controls, was assessed by Kruskal-Wallis one-way analysis of variance and the (two-tail) Mann Whitney U test. Statistical comparisons of the blood pressure and heart rate of drug and vehicle-treated groups were made by one-way analysis of variance.

Results

Effects of SB-204269 in seizure models

MEST test Following oral administration, SB-204269 (0.1–30 mg kg^{-1}) produced dose-related increases in the threshold for tonic hindlimb extension seizures in rats over a wide range of doses (Table 1). The level of anticonvulsant efficacy attained by SB-204269 in the MEST test was comparable to that evoked

Table 1 Effects of SB-204269 and SB-204268 on the threshold for maximal (tonic hindlimb extension) electroshock seizures in rats

Treatment	*Dose* (mg kg^{-1} p.o.)	*Seizure threshold* (mA)
Vehicle	–	25.0±1.1
SB-204269	0.1	34.5±1.1***
	0.3	38.3±1.7***
	1	53.3±1.7***
	3	97.9±4.3***
	10	163.3±4.0***
	30	266.7±6.8***
Vehicle	–	20.0±1.1
SB-204269	10	133.0±4.3***
SB-204268	10	16.8±1.4
Vehicle	–	22.5±1.3
Carbamazepine	2.5	25.0±1.1
	5	32.5±2.3***
	10	80.0±6.0***
	15	168.3±2.8***
	20	300.0±9.9***
Vehicle	–	21.7±1.7
Lamotrigine	0.5	22.5±1.1
	1	25.4±1.0*
	2.5	31.1±3.1**
	5	96.7±5.3***
	7.5	270.0±6.5***

Seizure thresholds were evaluated as CC_{50} (±s.e.) values in groups of 12–15 rats. Determinations were made at the times of peak drug effects, i.e. 60 min (carbamazepine), 240 min (SB-204269) and 360 min (lamotrigine). SB-204268 was assessed with the same pre-test period as SB-204269. *$P<0.05$, **$P<0.01$, ***$P<0.001$, compared to vehicle-treated controls.

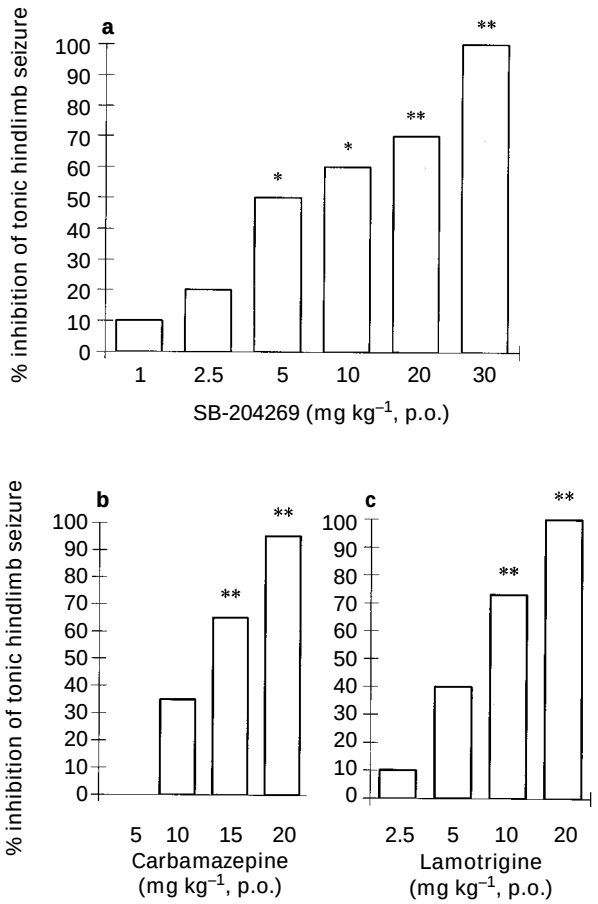

Figure 2 Anticonvulsant effects of (a) SB-204269, (b) carbamazepine and (c) lamotrigine in the rat MES test. Drugs were administered to groups of 10–11 rats 60 min (carbamazepine), 240 min (SB-204269) or 360 min (lamotrigine) before testing. The percentage of animals protected from tonic hindlimb extension seizures was then determined at 4–6 dose levels per drug and used to calculate ED_{50} values. For the data illustrated, these values (±s.e.) were 6.3±1.1, 12.0±0.6 and 6.1±0.3 mg kg^{-1}, p.o., for SB-204269, carbamazepine and lamotrigine, respectively. *$P<0.05$, **$P<0.01$, for individual dose groups compared to vehicle-treated controls.

by the standards carbamazepine (2.5–20 mg kg^{-1}, p.o.) and lamotrigine (0.5–7.5 mg kg^{-1}, p.o.), at the doses tested (Table 1). Moreover, in terms of MED, SB-204269 ($\leqslant$0.1 mg kg^{-1}) was clearly more potent than both lamotrigine (1 mg kg^{-1}) and carbamazepine (5 mg kg^{-1}).

Evaluation of the brain levels of SB-204269 present at the conclusion of the MEST study indicated that there was a linear relationship between the brain concentration achieved and the percentage increase in seizure threshold (data not shown). A concentration of 0.12±0.03 μmol kg^{-1} (which equates to approximately 0.1 μM) was observed at the MED level.

MES test In the MES test with high intensity suprathreshold stimulation, SB-204269 (1–30 mg kg^{-1}, p.o.) again exhibited dose-related anticonvulsant activity (Figure 2). As is reflected by the ED_{50} values (shown in parentheses) determined in this model, SB-204269 (6.3±1.1 mg kg^{-1}, p.o.) showed an equivalent or greater level of potency than lamotrigine (6.1±0.3 mg kg^{-1}, p.o.) and carbamazepine (12.0±0.6 mg kg^{-1}, p.o.). SB-204269 also produced a high level of efficacy as indicated by its ability to inhibit completely tonic hindlimb extension seizures at a dose of 30 mg kg^{-1}, p.o. (Figure 2).

Intravenous PTZ infusion test SB-204269 was found to elevate markedly the threshold for PTZ-induced tonic forelimb extension seizures by over 200% (relative to vehicle-treated controls) at doses $\geqslant$10 mg kg^{-1}, p.o., but even at the high dose of 30 mg kg^{-1}, p.o., had no effect on myoclonic seizures induced by this chemical convulsant (Figure 3).

In vitro *hippocampal slice studies* In control hippocampal slice preparations perfused with standard ACSF, SB-204269 (10 and 50 μM) had no effect on normal excitatory synaptic activity (as measured by the input-output relationship of the excitatory post synaptic potential) and neuronal excitability (as measured by the population spike (p.s.) amplitude). The absence of extra p.s. after administration of SB-204269 also indicated that there was no effect on inhibitory synaptic transmission (data not shown).

When introduced to the slices in the high K^+ afterdischarge model, SB-204269 (0.1–10 μM) showed a concentration-dependent reduction of the EGS activity in the CA1 region (Figure 4) without affecting the initial trigger stimulus. The threshold concentration for activity was 0.1 μM and 80% inhibition was observed at the maximum concentration of 10 μM. SB-204269 achieved 50% of maximum effect at a concentration of 0.2 μM. The equivalent values for carbamazepine and lamotrigine were 3.1 and 3.4 μM, respectively (Figure 4), thus highlighting the greater potency of SB-204269 in this model.

Effects of SB-204268 in seizure models

In marked contrast to SB-204269, the corresponding 3**S**,4**R** enantiomer, SB-204268, did not produce significant ($P>0.05$) anticonvulsant activity in the MEST (10 mg kg^{-1}, p.o., Table 1), intravenous PTZ infusion (10 mg kg^{-1}, p.o., Figure 3) or high K^+ afterdischarge (up to 3 μM, Figure 4) models at the doses tested.

Behavioural and neurological assessment

For animals treated with high doses of SB-204269 (50–200 mg kg^{-1}, p.o.), no overt behavioural changes were ob-

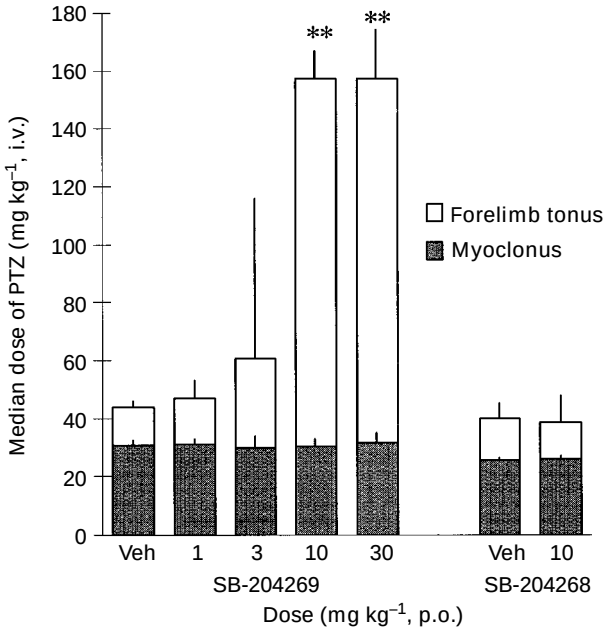

Figure 3 Effects of SB-204269 and SB-204268 in the rat i.v. pentylenetetrazol (PTZ) infusion test. Data are the median (and upper quartile) doses of PTZ required to induce myoclonic and tonic forelimb extension seizures in groups of 11–12 rats determined 240 min after administration of the test drugs. $^{**}P<0.01$, compared to corresponding vehicle-treated controls (Veh) by (two-tail) Mann Whitney U test following significant Kruskal-Wallis one-way analysis of variance. SB-204269: myoclonic seizures $H=2.162$, d.f. $=4$ – not significant; tonic seizures $H=24.413$, d.f. $=4$ – $P<0.001$. SB-204268 was not significantly different from controls according to (two-tail) Mann Whitney U test.

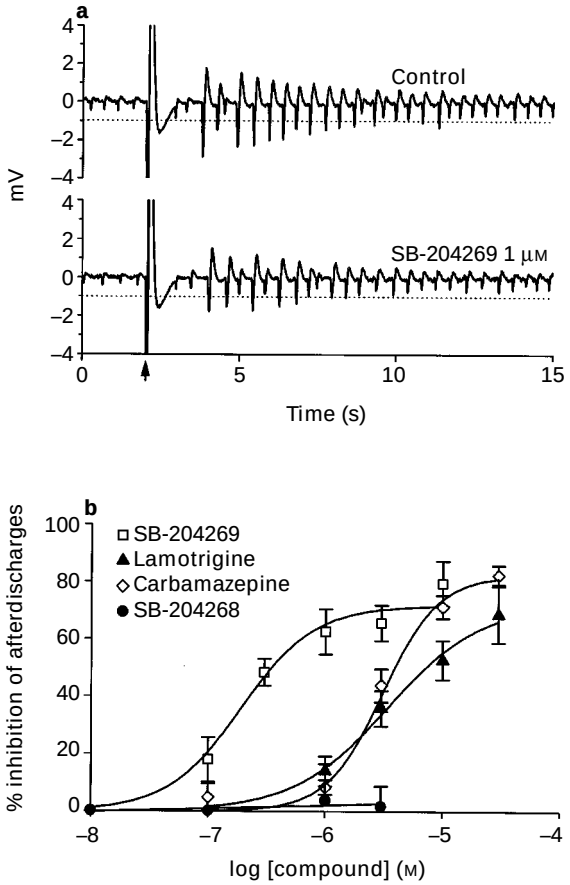

Figure 4 (a) Representative data illustrating the spontaneous afterdischarges observed following application of a brief train (10–20 pulses, 25 ms intervals) of suprathreshold stimuli (↑) to a rat hippocampal slice perfused with ACSF containing elevated levels of KCl (8.5 mM). Under control conditions 14 spikes were counted above the threshold level (dotted line) derived to exclude ongoing activity unrelated to the stimulus train, as compared to only 5 spikes following addition of SB-204269 (1 μM) to the perfusing ACSF. (b) Concentration-effect curves for SB-204269, carbamazepine, lamotrigine and SB-204268 in the high K^+ afterdischarge model. Data represent the mean ($\pm$s.e.mean, $n=3$–6 experiments) % inhibition of afterdischarges for a range of concentrations of each test drug; 50% of maximum antiseizure effect was achieved at concentrations of 0.2, 3.1 and 3.4 μM for SB-204269, carbamazepine and lamotrigine, respectively, whereas SB-240268 was inactive in this model at concentrations up to 3 μM.

served (either when in their home cages or upon handling) before the rotarod testing. In the accelerating rotarod test itself, SB-204269 had no significant effect on performance over a 4 h evaluation period (Table 2). In marked contrast, carbamazepine and lamotrigine produced largely dose-related impairments of rotarod performance, both with a MED of 80 mg kg^{-1}, p.o. (Table 2).

This apparent lack of behavioural or neurological impairment for SB-204269 is reflected in the compound's superior therapeutic index (MED in rotarod test (Table 2)/ED_{50} in MES test (Figure 2)) of >31 as compared to equivalent ratios of only 7 and 13 for carbamazepine and lamotrigine, respectively.

Cardiovascular studies

In conscious rats, SB-204269 (30 and 100 mg kg^{-1}, p.o.) did not induce any significant ($P>0.05$) changes in arterial blood pressure (systolic and diastolic) or heart rate for up to 4 h after dosing, relative to vehicle-treated control values (data not shown).

Mechanism of action studies

SB-204269 was inactive in a wide range of radioligand binding (pK_i values <5; Table 3) and functional assays (no effect at concentrations $\geqslant 10$ μM; Table 4) chosen to reflect known target sites (e.g. amino acid-related receptors; ion channels) for established anticonvulsant agents and also sites at which potential side effects might be indicated.

Discussion

The present studies show that in a range of rat models of electrically and chemically-evoked seizures, SB-204269 is an orally-effective anticonvulsant agent at doses that do not cause overt behavioural depression. Thus, the compound markedly elevated the threshold to tonic extension seizures following i.v. infusion of PTZ or application of corneal electroshock (MEST test) and completely inhibited suprathreshold electroshock seizures (MES test). In addition, SB-204269 reduced EGS activity in an *in vitro* elevated K^+ rat hippocampal slice afterdischarge model at concentrations that had no effect on normal synaptic activity and neuronal excitability, thereby further highlighting the specificity of the anticonvulsant properties of this agent. In all of these models, SB-204269 was equivalent to or better than the clinically established anticonvulsants carbamazepine and lamotrigine in terms of potency and efficacy. Unlike SB-204269 (which exhibits *trans* 3**R**,4**S** stereochemistry), the corresponding *trans* 3**S**,4**R** enantiomer, SB-204268, did not produce marked anticonvulsant effects, an observation in keeping with previous findings for other related pairs of *trans* enantiomers (Blackburn *et al*., 1995).

The selection and design of the seizure models used here was based on well validated approaches to the evaluation of potential new antiepileptic drugs (Löscher & Schmidt, 1988; Swinyard *et al*., 1989). The traditional MES test is widely used

and is considered to be a good predictor of likely therapeutic efficacy against generalized tonic-clonic seizures with some predictive value for partial seizures. By contrast, the i.v. PTZ threshold test for myoclonic/clonic (so-called minimal) seizures is thought to be a sensitive model for not only human generalized myoclonic seizures, but also non-convulsive absence seizures. The validity of these claims is supported by comparisons between the relative efficacies of a range of antiepileptic drugs in experimental models and human epilepsy disorders (Löscher & Schmidt, 1988; Swinyard *et al*., 1989; Upton, 1994). In the elevated K^+ rat hippocampal slice afterdischarge model, perfusing concentrations of K^+ are increased to reflect the ionic constitution of the extracellular microenvironment observed in animals during focal and gen-

Table 2 Effects of SB-204269 and standards in the rat accelerating rotarod test

		% change in time spent on rotarod					
	Dose	*Post-dose time* (min)					
Treatment	(mg kg^{-1} p.o.)	*15*	*30*	*60*	*120*	*240*	*360*
SB-204269	50	+7	+5	−11	+3	−1	ND
	100	+33	+19	−13	−9	−8	ND
	200	−22	−8	−17	−16	−3	ND
H-values		4.078	3.740	1.381	1.523	0.490	
d.f. = 3		NS	NS	NS	NS	NS	
Carbamazepine	60	−6	−8	−6	−25	+20	ND
	80	−30*	−45**	−28	−36*	−28	ND
	100	−31**	−29**	−24	−43**	−34**	ND
H-values		13.966	16.037	6.366	10.388	16.275	
d.f. = 3		$P<0.01$	$P<0.01$	NS	$P<0.02$	$P<0.001$	
Lamotrigine	50	ND	ND	−8	−31	+17	+1
	80	ND	ND	0	−24*	−36**	−38**
	100	ND	ND	−32	−35	−25	−39*
	200	ND	ND	−59**	−65**	−60**	−52**
H-values				15.672	14.296	19.430	18.150
d.f. = 4				$P<0.01$	$P<0.01$	$P<0.001$	$P<0.01$

Rotarod performance was determined in groups of 11–12 rats and expressed as a percentage change from control values. The absolute time spent on the rotarod by control rats was typically within the range of 180–260 s. MED values were >200, 80 and 80 mg kg^{-1}, p.o., for SB-204269, carbamazepine and lamotrigine, respectively. *$P<0.05$, **$P<0.01$, compared to vehicle-treated controls by (two-tail) Mann Whitney U test following significant Kruskal-Wallis one-way analysis of variance (*H*-values shown; NS: not significant). ND: not determined.

Table 3 Evaluation of SB-204269 in radioligand binding assays

Amino-acid related binding sites	*Radioligand*	*Miscellaneous binding sites*	*Radioligand*
Benzodiazepine (central)	[^{3}H]-flunitrazepam (1)	Adenosine A_1	[^{3}H]-PIA (1)
$GABA_A$	[^{3}H]-muscimol (1)	Adenosine uptake	[^{3}H]-NBTI (2)
$GABA_A/C1^-$channel	[^{35}S]-TBPS (1)	α_1-Adrenoceptor	[^{3}H]-prazosin (1)
NMDA channel	[^{3}H]-MK 801 (1)	α_2-Adrenoceptor	[^{3}H]-rauwolscine (1)
Glycine (strychnine-insensitive)	[^{3}H]-glycine (1)	β_1-Adrenoceptor	[^{125}I]-iodocyanopindolol (3)
Sigma	[^{3}H]-DTG (1)	β_2-Adrenoceptor	[^{125}I]-iodocyanopindolol (3)
Phencyclidine	[^{3}H]-TCP (2)	Histamine H_1	[^{3}H]-pyrilamine (4)
		Muscarinic (central)	[^{3}H]-QNB (1)
		Nicotinic	[^{3}H]-nicotine (2)
Na^+/K^+ channel-related			
Na^+ channel (site 1)	[^{3}H]-saxitoxin (2)	κ-Opioid	[^{3}H]-U-69593 (4)
Na^+ channel (site 2)	[^{3}H]-batrachotoxin (1)	CRF	[^{125}I]-CRF (1)
K^+ channel (Ca^{2+}-activated)	[^{125}I]-apamin (2)	NPY	[^{125}I]-NPY (2)
K^+ channel (voltage-dependent)	[^{125}I]-charybdotoxin (2)	CCK_B	[^{125}I]-CCK (5)
Sulphonylurea	[^{3}H]-glibenclamide (1)	Somatostatin	[^{125}I]-somatostatin (2)

SB-204269 was inactive in the above radioligand binding assays with pK_1 values <5. Origin of membranes as follows: (1) rat cortex; (2) rat forebrain; (3) CHO cells stably transfected with human β_1- or β_2-adrenoceptors; (4) guinea-pig cerebellum; (5) mouse forebrain. Abbreviations: TBPS, *t*-butyl-bicyclophosphorothionate; DTG, 1,3-di(2-tolyl) guanidine; TCP, tenocyclidine; NBTI, nitrobenzylthioinosine; QNB, quinuclidinylbenzilate.

Table 4 Effects of SB-204269 in functional assays

SB-204269 had no effect in the following functional assays at concentrations ⩾10 μM:

[^{3}H]-GABA release (K^+-stimulated) from rat hippocampal slices (1)
[^{3}H]-GABA uptake into rat hippocampal slices (2)
Glutamate release (3,4-diaminopyridine-stimulated) from rat hippocampal slices (3)
[^{3}H]-aspartate release (veratridine-stimulated) from rat cultured cerebellar neurones (4)
Ca^{2+} influx (K^+-stimulated) into synaptosomes prepared from rat cortex (5)
Na^+ currents in cultured rat hippocampal neurones (6)
Excitatory amino-acid transmission in rat hemisected spinal cord (7)

Assays were based on the published methods indicated: (1) Raiteri *et al*., 1989; (2) Iversen & Kelly, 1975; (3) Burke & Nadler, 1988; (4) Lysko *et al*., 1994; (5) Meakin & Smith, 1993; (6) Hamill *et al*., 1981; (7) Otsuka & Konishi, 1974.

eralized tonic-clonic seizures *in vivo* (see Leschinger *et al*., 1993). This manipulation, paired with brief trains of suprathreshold stimuli, results in the production of localized EGS (tonic and clonic ictaform events) in CA1 region and as such may provide an *in vitro* model of focal (or partial) epilepsy (Traynelis & Dingeldine, 1988; Leschinger *et al*., 1993). In view of the overall anticonvulsant profile of SB-204269 in the models described above, it can be predicted that the compound will be an effective treatment for partial and generalized tonic-clonic seizures. Studies are underway to evaluate further the spectrum of activity of the drug in terms of potential antiepileptogenic properties by determining the effects of chronic administration of SB-204269 on the rate of acquisition of amygdala-kindled partial seizures (Löscher & Schmidt, 1988).

By comparison of drug effects in threshold (i.v. PTZ infusion and MEST) and suprathreshold (MES) seizure models it can be determined whether anticonvulsant activity arises from elevation of seizure threshold and/or from prevention of seizure spread. The ability of SB-204269 to inhibit tonic extension seizures but not myoclonus, strongly suggests that the compound produces anticonvulsant activity primarily by inhibiting the spread of seizures (Piredda *et al*., 1985; Löscher & Schmidt, 1988; Swinyard *et al*., 1989), as is also the case for a number of antiepileptic drugs used clinically for partial and generalized tonic-clonic seizures, including carbamazepine and lamotrigine (Miller *et al*., 1986; Löscher & Schmidt, 1988). This conclusion is consistent with the observation that SB-204269 effectively attenuated EGS in the elevated K^+ hippocampal slice model without affecting the initial trigger stimulus. Browning (1992) has proposed an alternative mode of action for abolition of tonic hindlimb extension seizures, speculating that such an effect is more indicative of an ability of anticonvulsants to reduce seizure discharge within the brainstem rather than an ability to block seizure spread.

Dose-related CNS side-effects limit the use of all currently available antiepileptic drugs (Brodie, 1990; Kälviäinen *et al*., 1993). Acute toxicity from antiepileptic drugs in rodents is manifested invariably by neurological deficits encompassing sedation, muscle relaxation and motor incoordination. The rat accelerating rotarod test is a very sensitive procedure for detecting such properties (Jones & Roberts, 1968). SB-204269 did not impair performance in this test (or induce any gross behavioural changes) even at very high doses relative to its anticonvulsant potency. This is reflected in the excellent therapeutic index for SB-204269 of >31 as compared to equivalent values of only 7 and 13 for carbamazepine and lamotrigine, respectively. The lack of adverse effects for SB-204269 cannot be ascribed to a plateau in absorption since plasma concentrations of this agent are still increasing at doses up to 2 g kg^{-1}, p.o. (J. Connelly, personal communication).

Although the precise molecular mechanism of SB-204269 remains to be determined, the compound clearly differed from established anticonvulsants regarding its profile of effects in a wide range of *in vitro* mechanistic assays. For example, SB-204269 did not block Na^+ currents, displace [^{3}H]-batrachotoxin binding or inhibit glutamate release (cf carbamazepine, lamotrigine, phenytoin), nor did it modulate γ-aminobutyric acid (GABA)ergic neurotransmission (cf diazepam, phenobarbitone, valproate) (Upton, 1994). Overall, these studies illustrate that at concentrations well above those required to produce anticonvulsant activity *in vivo* (i.e. 0.1 μM in brain), SB-204269 does not interact with many of the well known mechanistic targets for antiepileptic drugs (Upton, 1994). This finding may correlate with the apparent lack of behavioural and neurological impairment seen *in vivo*.

Despite the fact that SB-204269 is derived from a series of hypotensive agents related to cromakalim which exert their actions by opening ATP-regulated K^+ channels (see Introduction), there is no evidence to suggest that this compound acts in a similar manner, as indicated by its inability to lower blood pressure in conscious rats at very high doses. However, the observation that the 4**S** stereochemistry is an absolute requirement for the anticonvulsant efficacy of SB-204269 and related compounds, and that the 4**R** configuration (as in SB-204268) is associated with predominantly hypotensive properties (Blackburn *et al*., 1995; Brown *et al*., 1995), provides some indication that the former agents may interact with a specific site with stringent conformational requirements. This has indeed proved to be the case and the accompanying paper describes the discovery of a unique stereospecific binding site for SB-204269 in rat brain (Herdon *et al*., 1997).

In conclusion, SB-204269 is a chemically novel anticonvulsant with potent oral activity in a range of rat seizure models and an overall efficacy profile indicative of potential utility for the treatment of partial (currently the most refractory type of epilepsy in adults) and generalized tonic-clonic seizures. These features, together with a unique mechanism of action and a markedly reduced potential for inducing neurological deficits relative to existing antiepileptic drugs, suggest that SB-204269 will provide improved therapy against refractory epilepsy in the future.

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