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The novel anticonvulsant SB 204269 binds to a stereospecific site in the mouse brain

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Abstract

The novel compound SB 204269 (*trans*-(+)-6-acetyl-4*S*-(4-fluorobenzoylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzo[*b*]pyran-3*R*-ol, hemihydrate) shows potent anticonvulsant activity in the mouse maximal electroshock seizure threshold test. The binding of [^{3}H]SB 204269 to mouse forebrain membranes is saturable (B_{max} 217 fmol/mg protein, K_d 32 nM) and stereospecific. The excellent anticonvulsant profile of SB 204269, combined with the identification of a unique central binding site for the compound, suggest that it has potential clinical utility as a novel treatment for epilepsy.

Keywords: Anticonvulsant; Brain binding site; SB 204269

We have reported previously that the 3*R*,4*S* enantiomers of a series of 4-(benzoylamino)-benzopyran compounds related to the ATP-sensitive K^+ channel opener cromakalim show anticonvulsant activity after oral administration. Unlike cromakalim, such compounds do not show hypotensive activity, suggesting they are not ATP-sensitive K^+ channel openers, whereas their corresponding 3*S*,4*R* enantiomers are hypotensive but only slightly anticonvulsant (Blackburn et al., 1995). We now report that subsequent optimisation of structure-activity relationships has 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 test. In addition, we have identified a stereospecific binding site for [^{3}H]SB 204269 in mouse forebrain membranes which may mediate the anticonvulsant effect of the compound.

The mouse maximal electroshock seizure threshold test was performed as described previously (see Blackburn et al., 1995), except that corneal electrodes were used to administer the electroshock. [^{3}H]SB 204269 (specific activity 21.5 Ci/mmol) was synthesised by SmithKline Beecham Pharmaceuticals. Crude membranes were prepared by homogenisation of mouse forebrain tissue in assay buffer (50 mM Hepes, pH 7.4), followed by centrifugation at 50 000 × *g* and resuspension of the pellet in 30 vols. of assay buffer; all procedures were performed at 4°C. Radioligand binding assays were carried out by incubating [^{3}H]SB 204269 (5–200 nM for saturation studies; 20 nM for competition studies) with brain membranes (1–2 mg protein/ml) in assay buffer for 60 min at room temperature (23°C). Incubation was terminated by rapid filtration through Whatman GF/B filters, followed by five 1 ml washes with ice-cold assay buffer. Radioactivity on the filters was quantified by liquid scintillation counting. Non-specific binding of [^{3}H]SB 204269 was defined using unlabelled SB 204269 (3 μM). Saturation data were analysed using 'Ligand' (McPherson, 1985); competition data were analysed using non-linear least-squares analysis (Bowen and Jerman, 1995) and IC_{50} values converted to K_i values (Cheng and Prussoff, 1973).

The seizure threshold for vehicle-treated (1% methyl cellulose, 10 ml/kg p.o., 1 h pre-test) mice in the maximal electroshock seizure threshold test was typically in the range of 12–16 mA. In this test, SB 204269 produced a dose-dependent increase in seizure threshold (reflecting an anticonvulsant action), with a significant effect at ≥ 0.3 mg/kg p.o. (n = 12–16; $P < 0.05$ compared to controls according to Litchfield and Wilcoxon, 1949) and an elevation of 243% at 100 mg/kg p.o. In marked contrast, SB 204268, the 3*S*,4*R* enantiomer of SB 204269, had no

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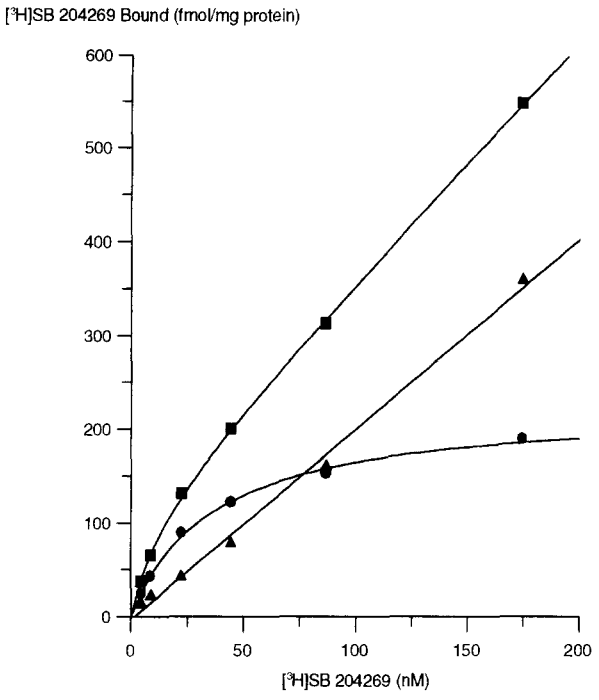

Fig. 1. Total (■), non-specific (▲) and specific (●) binding of [^{3}H]SB 204269 to mouse forebrain membranes with increasing concentrations of [^{3}H]SB 204269. Data shown are from a typical experiment, which was replicated three times with very similar results.

significant effect in the maximal electroshock seizure threshold test at doses as high as 30 mg/kg p.o., indicating the stereospecificity of the anticonvulsant activity. In common with other compounds from this chemical series (Blackburn et al., 1995), SB 204269 did not produce any effects on blood pressure at 100 mg/kg p.o., suggesting a lack of effect at ATP-sensitive K^+ channels; the compound also lacked activity both in the rat rotarod test of motor coordination at 200 mg/kg p.o. and in a wide range of receptor binding assays at 10 μM (Upton et al., in preparation).

Specific binding of [^{3}H]SB 204269 was saturable (Fig. 1). Analysis of binding data gave a B_{max} value of 217 ± 5 fmol/mg protein, a K_d value of 32 ± 2 nM and a Hill coefficient of 0.96 ± 0.03 ($n = 3$). Unlabelled SB 204269 competed for the binding of [^{3}H]SB 204269 with a K_i value of 53 ± 4 nM ($n = 4$) which agrees well with the K_d value derived from saturation analysis. SB 204268 showed no significant competition at up to 10 μM, demonstrating that stereospecificity of binding parallels that of anticonvulsant activity. Other compounds from the same chemical series as SB 204269 (Blackburn et al., 1995) competed to similar maximal extents for binding (data not shown). However, the standard anticonvulsant drugs carbamazepine, diazepam, phenobarbitone and sodium valproate, as well as the newer anticonvulsant drugs lamotrigine, levetiracetam and vigabatrin, showed no activity (IC_{50} > 100 μM).

These experiments show that SB 204269 has potent and stereospecific anticonvulsant properties in a predictive test used to identify anticonvulsant agents (Upton, 1994). The identification of a stereospecific brain binding site for [^{3}H]SB 204269 makes the anticonvulsant action of the compound of particular interest, since it suggests that this activity might be mediated by a unique site not shared by other anticonvulsant drugs. The properties of this site, combined with the lack of hypotensive or neurological side-effects of SB 204269, suggest that this compound has potential clinical utility as a novel treatment for epilepsy.

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