



SPECIAL REPORT

Pregabalin may represent a novel class of anxiolytic agents with a broad spectrum of activity

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The present study examines the effect of pregabalin (previously S-Isobutylgaba and CI-1008) in two distinct rat models of anxiety. Pregabalin binds with high affinity and selectivity to the $\alpha_2\delta$ subunit of voltage dependent calcium channels (VDCC). Its corresponding R-enantiomer (R-isobutylgaba) is approximately 10 fold weaker. Pregabalin dose-dependently induced anxiolytic-like effects in both the rat conflict test and elevated X-maze with respective minimum effective doses (MED) of 3 and 10 mg kg⁻¹. In contrast, R-isobutylgaba only showed activity at the highest dose of 100 mg kg⁻¹ in the conflict test. These data indicate that pregabalin may possess clinical utility as a novel anxiolytic agent and demonstrates the importance of the $\alpha_2\delta$ subunit of VDCC in the mediation of anxiety related behaviours.

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Keywords: Anxiety; conflict test; elevated X-maze; rat; R-isobutylgaba; $\alpha_2\delta$ subunit of voltage-dependent calcium channels
Abbreviations: CCK, cholecystokinin; CDP, chlordiazepoxide; CNS, central nervous system; MED, minimum effective dose; TOA, time on open arms; TEOA, time on ends of open arms; TE, total entries; VDCC, voltage-dependent calcium channels

Introduction For the last four decades the pharmacological treatment of generalized anxiety has been dominated by benzodiazepines. However, the use of these compounds is limited due to their side-effects. They induce sedation and interact with other CNS depressants (Woods *et al.*, 1992). Major problems with benzodiazepines are dependence and withdrawal symptoms which have hindered their long term use (Lader, 1999; Woods *et al.*, 1992). Thus, a great deal of research has been dedicated towards the discovery of novel anxiolytic agents with an improved side-effect profile.

Pregabalin (previously S-isobutylgaba and CI-1008) is structurally related to the inhibitory neurotransmitter GABA. However, radioligand binding studies have shown that pregabalin has negligible affinity for GABA and many other receptors but binds with high affinity and selectivity to a novel binding site in the CNS, recently identified as the $\alpha_2\delta$ subunit of voltage-dependent calcium channels (VDCC) (Gee *et al.*, 1996). The corresponding R-enantiomer (R-isobutylgaba) of pregabalin has a 10 fold lower affinity for the $\alpha_2\delta$ site (Taylor *et al.*, 1993). The present study examines and compares the effects of pregabalin and R-isobutylgaba in animal models of anxiety.

Methods *Animals* Male Hooded Lister rats (200–250 g) were obtained from Interfauna Universal (Huntingdon, U.K.). Animals were housed in groups of six under a 12 h light/dark cycle (lights on at 0700 h) with food and water *ad libitum*, except rats used in the conflict procedure were maintained at 80% of their free feeding body weight.

X-maze A standard elevated X-maze apparatus was used in these experiments (Singh *et al.*, 1991). Animals were placed onto the centre of the X-maze facing one of the open arms. The time (TOA) and % time (%TOA) spent on open arms and time spent in the end half sections of the open arms (TEOA) together with total arm entries (TE) were measured in a 5 min test period.

Rat conflict test These methods have been described before (Singh *et al.*, 1991). Briefly, rats were trained to press levers for food reward in operant chambers. The schedule consisted of alternations of four 4-min unpunished periods on variable interval of 30 s signalled by chamber lights on and three 3-min punished periods on fixed ratio 5 (by foot shock concomitant to food delivery) signalled by chamber lights off. The degree of foot shock was adjusted for each rat to obtain approximately 80–90% suppression of responding in comparison with unpunished responding. Rats received saline vehicle on training days.

Drugs Drugs used were pregabalin and R-isobutylgaba (Pfizer Global Research and Development, Ann Arbor Laboratories, MI, U.S.A.) and chlordiazepoxide (Sigma, Poole, U.K.). All compounds were dissolved in 0.9% w/v⁻¹ NaCl (normal saline) and administered s.c. in a volume of 1 ml kg⁻¹, except the top dose of 100 mg kg⁻¹ pregabalin and R-isobutylgaba which were administered in a volume of 3 ml kg⁻¹. The pretreatment times, route of administration and doses were taken from previous studies (Taylor *et al.*, 1993; Singh *et al.*, 1996).

Results *Rat elevated X-maze* The s.c. administration of pregabalin (3–30 mg kg⁻¹) 40 min before test, showed an anxiolytic-like action in the elevated X-maze as indicated by

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an increase in time and per cent time spent on the open arms and time spent in the end half sections of the open arms with a MED of 10 mg kg⁻¹ (Figure 1a–c). The administration of chlordiazepoxide (3 mg kg⁻¹) produced a similar anxiolytic action on the X-maze to that of pregabalin (Figure 1a–c). Neither compound had any adverse effect on the total number of arm entries indicating a lack of sedative action at the doses used (Figure 1d).

Rat conflict test The s.c. administration of pregabalin 40 min before test, dose-dependently (1–100 mg kg⁻¹) increased lever pressing in the punished period with a MED of 3 mg kg⁻¹ which produced a 48.1% increase (Figure 2a). A maximal effect (304.9% increase) was observed with 30 mg kg⁻¹ (Figure 2a). Unpunished responding was only significantly reduced at the highest dose of 100 mg kg⁻¹ (Figure 2a). R-isobutylgaba failed to affect punished responding except at the highest dose of 100 mg kg⁻¹ which produced a 76.4% increase (Figure 2b).

Discussion The results presented here show that pregabalin possesses anxiolytic-like activity in both ethological and conflict models of anxiety. Thus, it showed good anxiolytic-like effects in the rat elevated X-maze and the rat conflict test. The R-isomer of pregabalin, which

possesses weaker affinity for the $\alpha_2\delta$ site only had a modest effect in the conflict model at the highest dose examined. These results indicate the importance of the $\alpha_2\delta$ subunit of VDCC in mediating the anxiolytic-like action of pregabalin. This is consistent with previous studies demonstrating activity for pregabalin and limited activity of R-isobutylgaba in both epilepsy and pain models (Field *et al.*, 1997; 1999; Partridge *et al.*, 1998; Taylor *et al.*, 1993). It has been shown that $\alpha_2\delta$ binding sites are present throughout the CNS, including the limbic system (Hill *et al.*, 1993). This area has high concentrations of benzodiazepine receptors and is known to play a major role in the control of emotional behaviours.

It is known that the $\alpha_2\delta$ subunit is common to all VDCC (Hofmann *et al.*, 1994; Isom *et al.*, 1994) and it is also clear that these channels play an important role in neurotransmission (e.g. control of neurotransmitter release). It seems unlikely that pregabalin interacts with all VDCC as an unselective blockade of Ca²⁺ channels would lead to severe side effects. Animal studies examining pregabalin in models of nociception indicate that it has a good side effect profile (Field *et al.*, 1997). Recently, it has been reported there are at least three subtypes of $\alpha_2\delta$ subunits (Klugbauer *et al.*, 1999) and pregabalin may interact specifically with one of these.

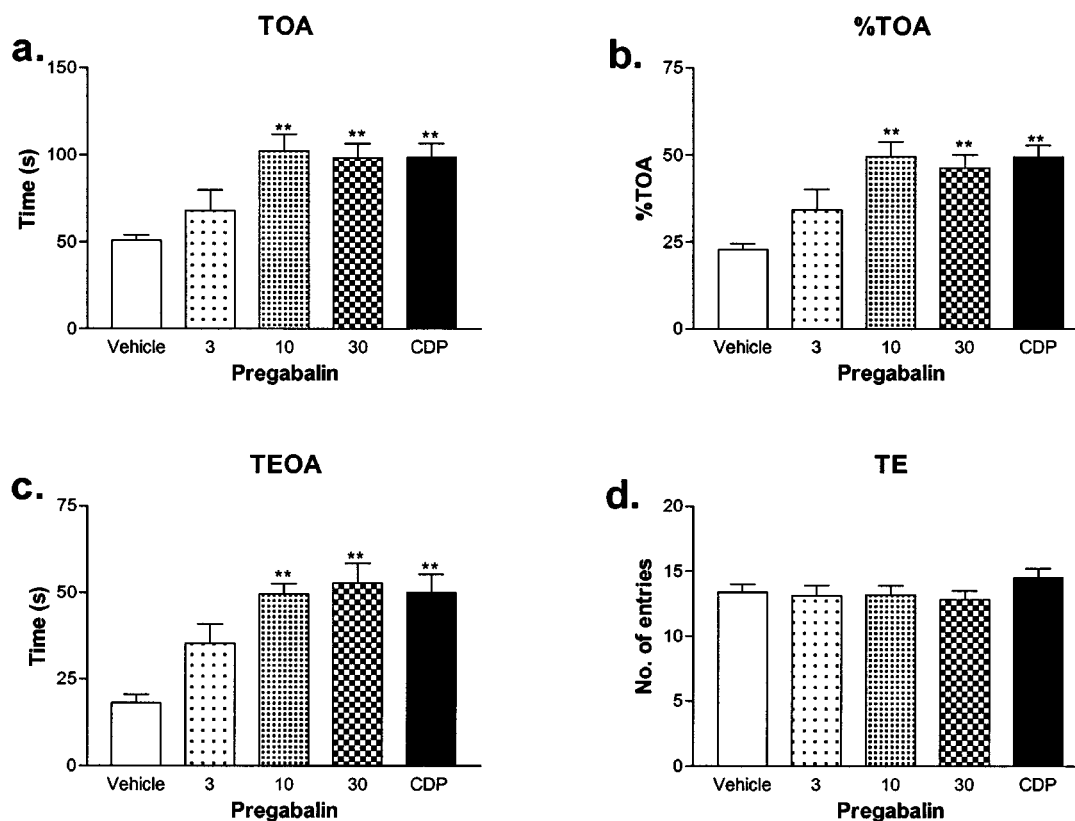


Figure 1 Effect of pregabalin in the rat elevated X-maze. Pregabalin (3.30 mg kg⁻²) or CDP (chlordiazepoxide, 3 mg kg⁻¹ s.c.) was administered s.c. 40 min before test. The time (a: TOA) and per cent time spent (b: % TOA) on open arms and time spent in the end half sections of the open arms (c: TEOA) together with total arm entries (d: TE) were measured. The duration of each test was 5 min. Results are shown as the mean (vertical bars represent \pm s.e.mean) of 7–10 animals per group. **Significantly different from vehicle treated controls $P < 0.01$ (ANOVA followed by Dunnett's *t*-test).

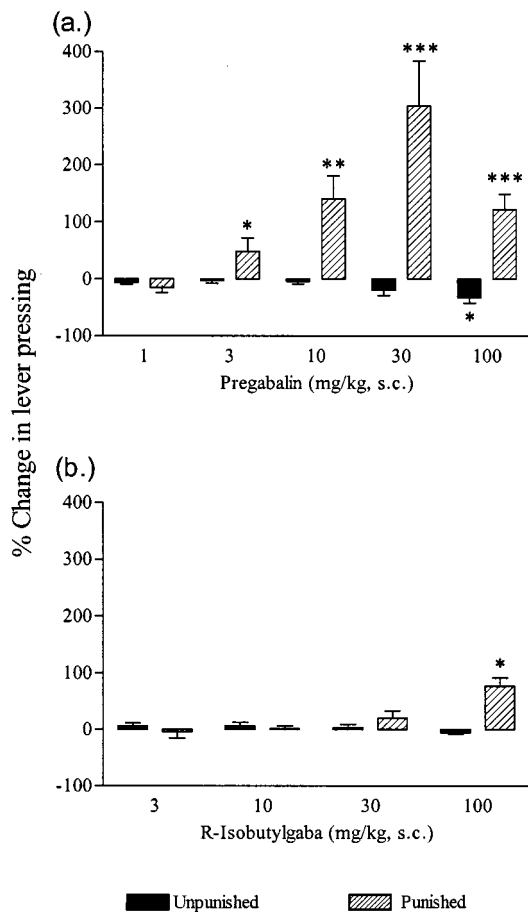


Figure 2 Effect of (a) pregabalin and (b) R-isobutylgaba in the rat conflict test. Pregabalin or R-isobutylgaba was administered s.c. 40 min before test. The results are expressed as mean per cent increase or decrease of lever pressing of at least six animals per group on test day compared with mean performances obtained the two previous days following vehicle administration. Significantly different from previous control days * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (paired student t -test).

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