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RESEARCH PAPER

# Effects of pregabalin on visceral pain responses and colonic compliance in rats

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**Background and purpose:** Pregabalin, which binds to the $\alpha_2$-δ subunit of voltage-gated calcium channels, increased the threshold for pain during colorectal distension (CRD) in irritable bowel syndrome (IBS) patients. We tested the effects of oral pregabalin on the visceral pain-related viscerosomatic and autonomic cardiovascular responses to CRD and colonic compliance in rats.
**Experimental approach:** The activity of the abdominal musculature (viscerosomatic response), monitored by electromyography and intracolonic manometry, and changes in blood pressure and heart rate, monitored by telemetry, were assessed simultaneously in conscious rats during CRD.
**Key results:** Pregabalin (10–200 μmol kg$^{-1}$, p.o.) inhibited dose dependently the viscerosomatic response to phasic, noxious CRD (12 distensions at 80 mm Hg). At 200 μmol kg$^{-1}$, pregabalin also reduced the increase in blood pressure and heart rate associated with noxious CRD. Moreover, pregabalin (200 μmol kg$^{-1}$, p.o.) reduced the visceromotor response to ascending phasic CRD (10–80 mm Hg) and significantly increased the threshold pressure for response. During phasic CRD (2–20 mm Hg), pregabalin (200 μmol kg$^{-1}$, p.o.) increased intracolonic volume, resulting in a shift to the left of the pressure–volume relationship curve, indicative of an increase of compliance.
**Conclusions and implications:** Pregabalin reduced the viscerosomatic and autonomic responses associated with CRD-induced visceral pain and increased colonic compliance in rats. These observations confirm the analgesic activity of pregabalin on visceral pain and support the translational value of the CRD model to humans. Ligands for the $\alpha_2$-δ subunit might represent interesting compounds for the treatment of visceral pain disorders, such as IBS.
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**Keywords:** autonomic responses; cardiovascular responses; colonic compliance; colorectal distension; pregabalin; viscerosomatic responses; visceral pain

**Abbreviations:** b.p.m., beats per min; CRD, colorectal distension; IBS, irritable bowel syndrome

## Introduction

Altered colonic sensitivity with increased perception of balloon distension of the colorectal area is one of the manifestations of the functional bowel disorder, irritable bowel syndrome (IBS) (Ritchie, 1973; Stacher and Christensen, 2006; Azpiroz *et al*., 2007). These observations have led to the consideration that visceral sensory hypersensitivity should be regarded as a significant component of the pathophysiological basis of IBS. Therefore, the modulation of sensory neurotransmission from the intestine could be an effective approach for the therapeutic treatment of IBS. Accordingly, numerous potential targets expressed in sensory afferents from the intestine have been tested in several preclinical models and clinical conditions for their effectiveness to modulate visceral pain (Kirkup *et al*., 2001; Hicks, 2006).

Despite its relation to GABA, there is little evidence to indicate that pregabalin exerts its pharmacological activity through direct interaction with GABA receptors. Although the mechanism of action is not completely clear, it has been shown that pregabalin binds with high affinity to the $\alpha_2$-δ subunit of voltage-gated calcium channels (Bryans and Wustrow, 1999; Belliotti *et al*., 2005; Bian *et al*., 2006). Thus, the compound reduces depolarization-induced calcium influx at nerve terminals, and thereby reduces the release of several excitatory neurotransmitters, such as noradrenaline, glutamate, substance P and calcitonin gene-related peptide, which have been involved in pain mechanisms (Ben-Menachem, 2004; Huckle, 2004; Belliotti *et al*., 2005; Joshi and Taylor, 2006; Dooley *et al*., 2007). Consistent with this hypothesis, recent studies in mice with a mutation in the $\alpha_2$-δ1 calcium channel subunit preventing the binding of pregabalin, suggest that the analgesic effects of the

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compound in mouse pain models are mediated by binding to this subunit (Bian *et al*., 2006; Field *et al*., 2006). Pregabalin has been evaluated in multiple clinical trials involving diabetic peripheral neuropathy, post-herpetic neuralgia and epilepsy, and has also been shown to be effective in several animal models of inflammatory and neuropathic pain (Hunter *et al*., 1997; Dworkin and Kirkpatrick, 2005; Blommel and Blommel, 2007; Field *et al*., 2007). Furthermore, in animal models of visceral pain, pregabalin has been shown to dose dependently reduce both normal colonic pain responses and colonic hyperalgesia (Eutamene *et al*., 2000; Diop *et al*., 2002; Million *et al*., 2007). A recent report also showed that pregabalin restored sensory thresholds to normal levels in IBS patients with rectal hypersensitivity during rectal balloon distension (Houghton *et al*., 2007). In the same study, a concomitant increase in rectal compliance was also observed, although its relation to the reduction in sensitivity was unclear.

Several means of assessing anti-nociceptive-like effects of potential visceral analgesics have been developed using *in vivo* preclinical models. Monitoring the electrical activity of the abdominal muscle in response to colorectal distension (CRD) (the so-called visceromotor response) has been the most common method of choice (Ness and Gebhart, 1988). Indeed, most of the aforementioned effects of pregabalin in animal models were characterized using this parameter. However, we have recently described an alternative readout, termed the mechanical visceral motor response to CRD, which appears to be more sensitive at detecting analgesic-like effects of compounds than electrical recordings and is independent of changes in colonic motility (Tammpere *et al*., 2005; Arvidsson *et al*., 2006). In addition, cardiovascular responses have also been demonstrated as valid pseudo-affective responses to noxious visceral stimuli in animals (Ness and Gebhart, 1988). Thus, monitoring these three pseudo-affective reflexes in parallel is expected to provide more confidence in the possible preclinical anti-nociceptive effects of compounds, reinforcing the translational value of results in animals to human conditions.

Although pregabalin has previously been shown to inhibit the visceral motor response using electromyographical (EMG) recordings (Eutamene *et al*., 2000; Million *et al*., 2007), plasma levels of pregabalin required for efficacy have not been reported. Thus, if the plasma exposure of pregabalin required for efficacy in preclinical models of visceral pain is relevant to the plasma exposure reached with clinical doses is unknown.

The aim of the present study was therefore to extend previous findings by further assessing the effects of pregabalin on three independent visceral pain-like responses, namely electrical and mechanical visceromotor responses and cardiovascular reflexes, elicited by mechanical stimulation of the colon in conscious rats using distension protocols equivalent to those used in clinical studies. In addition, taking into account the potential effects in colonic compliance observed clinically (Houghton *et al*., 2007), we assessed, for the first time, the effects of pregabalin in colonic compliance during CRD in animals. Finally, we measured plasma levels of pregabalin to examine how different exposures of pregabalin affected the outcomes with the intention of providing translational relevance to these findings in man.

## Methods

*Animals*

All animal procedures and experiments were approved by the Local Animal Ethics Review Committee in Göteborg, Sweden. Adult female Sprague–Dawley rats (Harlan, The Netherlands; 250–300 g) were used. The rats were allowed to acclimatize to the animal facility for at least 1 week after arrival. Rats were housed in groups of five in an enriched environment with free access to food (Standard pellets, R3, Lactamin AB, Kimstad, Sweden) and water under controlled conditions of temperature (21 °C) and humidity (50%) on a 12:12-h light–dark cycle. The phase of the oestrous cycle was not taken into consideration in the current study.

*Surgical preparation*

*Implantation of EMG electrodes*. For the implantation of EMG electrodes, rats were anaesthetized with an i.p. injection of $2\,mL\,kg^{-1}$ of combined ketamine ($88\,mg\,kg^{-1}$; Ketalar vet, Pfizer AB, Täby, Sweden) and xylazine ($5\,mg\,kg^{-1}$; Rompun vet, Bayer AG, Leverkusen, Germany) and were kept on a heating pad during surgery to maintain body temperature. The peritoneal cavity was opened through a midline incision and a pair of Teflon-coated stainless steel wire electrodes (Cooner Wire Co., Chatsworth, CA, USA) was implanted in the left internal oblique muscle of the abdomen. The wires were exteriorized for future access through a plastic fistula (AstraZeneca, Mölndal, Sweden) attached to the opposite side of the abdominal wall. The animals recovered from surgery in a quiet and dim room for 24 h post-operatively and were used in experiments, at the earliest, 10 days after surgery.

*Implantation of radio transmitters*. When assessing cardiovascular responses, a telemetric system was used. Rats were anaesthetized with a mixture ($2\,mL\,kg^{-1}$, i.p.) of ketamine ($88\,mg\,kg^{-1}$; Ketalar vet; Pfizer AB) and xylazine ($5\,mg\,kg^{-1}$; Rompun vet; Bayer AG) and were surgically equipped with i.p. radio transmitters with two electrodes to record biopotentials and one catheter to record blood pressure (PhysioTel C50-PXT, DSI, St Paul, MN, USA). The two electrodes were implanted in the left internal oblique muscle of the abdomen, as described above, for EMG measurements. The catheter was inserted into the abdominal aorta and fixed with tissue adhesive (Vetbond, 3M, St Paul, MN, USA) for blood pressure measurements. The animals recovered from surgery in a quiet and dim room for 24 h post-operatively and also received antibiotic (Bactrim, Roche, Basel, Switzerland) and analgesic (Finadyne, Schering-Plough, Kenilworth, NJ, USA) treatment. Thereafter, a 7- to 10-day recovery period was allowed before starting any experimental procedures.

*Colorectal distension*

Rats were habituated to Bollmann cages (Plexiglass tubes, length 18 cm, diameter 6 cm, AstraZeneca) 30 min per day

for 3 consecutive days prior to experiments to reduce motion artefacts and confounding effects due to stress-related responses.

A 3-cm polyethylene balloon (made in-house) with connecting catheter (PE-50) was inserted in the distal colon, 2 cm from the base of the balloon to the anus, during light isoflurane anaesthesia (Forene, Abbott Scandinavia AB, Solna, Sweden). The catheter was fixed to the tail with tape. The balloons were connected to pressure transducers (P-602, CFM-k33, 100 mm Hg, Bronkhorst HI-TEC, Veenendaal, The Netherlands), and the fistula was connected to the amplifier for EMG recordings. Rats were allowed to recover from sedation in the Bollmann cages for at least 15 min before the start of experiments.

A customized barostat (AstraZeneca) was used to manage air inflation and balloon pressure control. A customized computer software (PharmLab on-line 5.0) running on a standard computer was used to control the barostat and to perform data collection. A multifunction board from National Instruments (PCI-MIO-16E-4, Solna, Sweden) was used. The distension paradigms generated by the barostat were achieved by generating pulse patterns on an analogue output channel. For the assessment of visceral pain responses, two CRD paradigms were used: (1) repeated phasic distensions, 12 times at 80 mm Hg, with a pulse duration of 30 s at 5 min intervals; and (2) increasing phasic distensions from 10 to 80 mm Hg with a pulse duration of 30 s at 2.5 min intervals. For the assessment of compliance, increasing phasic distensions from 2 to 20 mm Hg, at 2 mm Hg increasing steps, with a pulse duration of 1 min at 5 min intervals were used. In this case, low distension pressures (within a range considered non-noxious) were chosen to minimize pain-related visceromotor responses that might interfere with the measurements of the intraballoon volume. Similar protocols have been used before to assess responses to CRD in rats (Tammpere *et al*., 2005; Käll *et al*., 2007; Martínez *et al*., 2007; Lindström *et al*., 2008). The electrical (that is, EMG recording) and mechanical responses to CRD were monitored simultaneously in the same rats at all times.

*Data collection and analysis*

The analogue input channels were sampled with individual sampling rates, and digital filtering was performed on the signals. The balloon pressure signals were sampled at 50 samples per s. A high-pass filter at 1 Hz was used to separate the contraction-induced pressure changes from the slow varying pressure generated by the barostat. A resistance in the airflow between the pressure generator and the pressure transducer further enhanced the pressure variations induced by abdominal contractions of the animal. The EMG signals were sampled at 1000 samples per s and high pass filter at 2 Hz. In addition, a band-stop filter at 49–51 Hz was used to remove line frequency interference. A customized computer software (PharmLab off-line 5.0) was used to quantify the magnitude of EMG signals and high-pass-filtered balloon pressure signals. Data analysis was performed using pre-designed automatic analysis paradigms. Hence, manual analysis and potential bias by the investigator were avoided. The average rectified value of the EMG and the high-pass-filtered balloon pressure signals was calculated for 30 s before the pulse (that is, baseline response) and for the duration of the pulse. When calculating the magnitude of the high-pass-filtered balloon pressure signals, the first and last seconds of each pulse were excluded, as these reflect artefact signals produced by the barostat during inflation and deflation and do not originate from the animal.

Threshold pressures for response to CRD were determined using the phasic ascending paradigm (10–80 mm Hg). For every animal, the threshold pressure for response was defined as the pressure of the distending pulse at which the response evoked exceeded the mean baseline activity plus 2 times the standard deviation (Tammpere *et al*., 2005; Martínez *et al*., 2007).

For the determination of compliance, the maximal intracolonic volume achieved during each distension (2–20 mm Hg) was determined and pressure–volume curves were constructed. Experimental data were also fitted to a nonlinear power exponential model in which the volume ($V$, ml) at any given distension pressure ($P$, mm Hg) is defined as

$$V = V_{max} \times \exp[-(\kappa \times \mathrm{RelP})^{\beta}] \quad (1)$$

In equation (1), $P$ is the relative pressure, defined as $\mathrm{RelP} = (1/P - 1/P_{max})$, $V_{max}$ the maximal volume achieved during the distension procedure and $P_{max}$ the maximal pressure (in the current experimental conditions fixed to 20 mm Hg). The parameter $\beta$ reflects the overall shape of the curve, and $\kappa$ is the change in volume as a function of $1/P$ at any given point, and is basically a measure of the slope of the curve. The parameters $\beta$ and $\kappa$ were estimated by fitting the experimental data to equation (1) using the R software application (Version 2.2.0; The R Foundation for Statistical Computing; Vienna University of Technology, Vienna, Austria). From the fitting process, estimated values for $\beta$ and $\kappa$ parameters and for $V_{max}$ were obtained. Thereafter, from equation (1), the distending pressures necessary to increase the colonic volume by 10% ($P_{10}$) and by half of the maximal volume ($P_{50}$) were calculated for the different experimental conditions. This mathematical model has been used previously when assessing pressure–volume responses during CRD in humans and rats (Bharucha *et al*., 1997; Käll *et al*., 2007; Martínez *et al*., 2007).

*Experimental protocols*

Pregabalin (10, 50 or 200 μmol kg$^{-1}$; equivalent to 1.6, 8 and 32 mg kg$^{-1}$) or vehicle (0.9% saline solution, 5 mL kg$^{-1}$) was administered orally (p.o.) 1 h before starting the CRD procedure. In all experiments, each rat received both vehicle and a dose of compound on different occasions, with at least 4 days between experiments. Hence, each rat served as its own vehicle control. Experiments were performed in a counterbalanced crossover fashion in which vehicle and different doses of pregabalin were tested during the same experiment, and repeated in several occasions.

*Plasma levels of pregabalin*

A separate group of animals was used for the determination of plasma levels of pregabalin after oral dosing. Animals were dosed orally with pregabalin at 50 and 200 μmol kg$^{-1}$

($5\,mL\,kg^{-1}$; $n=4$ and 5, respectively). Blood samples were obtained at 60, 90 and 120 min after dosing, and plasma levels of pregabalin were determined using standard HPLC combined with mass spectroscopy procedures (limit of quantification: $0.1\,\mu mol\,L^{-1}$).

*Statistical analysis*

Data are expressed as mean ± s.e.mean. Differences between two groups were assessed by a paired or unpaired Student's *t*-test, as appropriate. Differences between multiple groups were determined by a repeated or non-repeated measure one-way ANOVA, as appropriate, followed, when necessary, by a Student–Newman–Keuls multiple comparisons test. Data were considered statistically significant when $P<0.05$.

*Drugs*

Pregabalin ((*S*)-(+)-3-(aminomethyl)-5-methylhexanoic acid) (AstraZeneca R&D) was dissolved in 0.9% saline solution at the appropriate concentration. Saline solution was used as vehicle control.

## Results

*Effects of pregabalin on visceromotor responses to repetitive noxious CRD*

In vehicle-treated animals, noxious CRD (80 mm Hg) evoked a visceromotor response observed as simultaneous changes in the EMG activity of the abdominal musculature and in the intraballoon manometric recordings, with a significant increase in both parameters when compared with their respective basal activities (Figures 1a and b). Moreover, intraballoon manometric recordings showed an increase over the CRD protocol: from the first to the 12th pulse, the response to CRD increased by 47 ± 29% ($n=12$) (Figure 1b). On the other hand, the response to EMG was stable over the experimental time without changes in magnitude throughout the protocol (Figure 1a).

Pregabalin inhibited in a dose-related manner the responses to CRD, either assessed through EMG or through intraballoon manometric recordings. At $50\,\mu mol\,kg^{-1}$, pregabalin reduced the overall response to CRD by 10 ± 13% ($P>0.05$ vs vehicle) and 32 ± 9% ($P<0.05$ vs vehicle) when assessing EMG and balloon pressure recordings, respectively. A further increase in the dose ($200\,\mu mol\,kg^{-1}$) resulted in a significant inhibition of both measurements compared with the response in vehicle-treated animals (EMG: 26 ± 10%; intraballoon pressure: 50 ± 5%; both $P<0.05$) (Figures 1c and d). A lower dose of pregabalin ($10\,\mu mol\,kg^{-1}$, p.o.) did not affect the responses to distension, although a tendency was observed when assessing intraballoon pressure recordings (16 ± 7% inhibition, $P=0.079$ vs vehicle) (Figures 1c and d).

*Effects of pregabalin on visceromotor responses to ascending phasic CRD*

During ascending phasic CRD (10–80 mm Hg), vehicle-treated animals showed a simultaneous pressure-related increase in EMG activity of the abdominal musculature and in the intraballoon manometric recordings. Pregabalin ($200\,\mu mol\,kg^{-1}$, p.o., $n=9$) significantly reduced the overall response to distension, either when assessing EMG or intraballoon pressure changes (Figures 2a–d).

Thresholds for response to CRD in the vehicle-treated group were similar when assessing EMG activity or intraballoon pressure changes (Figures 2e and f). Pregabalin increased the threshold for responses of EMG ($P=0.002$ vs vehicle) or intraballoon pressure changes ($P=0.005$ vs vehicle) (Figures 2e and f).

*Effects of pregabalin on repetitive noxious CRD-induced cardiovascular responses*

In vehicle-treated animals ($n=6$), noxious CRD (80 mm Hg × 12 pulses) elicited a visceromotor response similar to that described above (Figures 3a and b), and characterized by an increase in the EMG activity and the balloon pressure fluctuations during the duration of the distension pulses. In addition, CRD evoked significant rises in blood pressure and heart rate (Figures 3c and d). When recording the EMG activity, the visceromotor response was similar in magnitude over the complete CRD protocol. However, when assessing the changes in intracolonic balloon pressure, the responses increased in magnitude by 85 ± 33% from the first to the last pulse ($F(5, 11)=6.076$; $P<0.001$; $P<0.05$ for distensions 8–12 vs distension 1). Similarly, the change in blood pressure also increased throughout the distension protocol by 63 ± 27% ($F(5, 11)=4.819$, $P<0.001$; $P<0.05$ for distensions 7–12 vs distension 1). The heart rate also increased during the distension time compared with the baseline activity. However, the heart rate increased over time in only four out of six animals tested, therefore the presence of sensitization was not as clear (Figure 3c). After each pulse, blood pressure and heart rate returned to their baseline control values, which were stable over the experiment (−1 ± 2 and −6 ± 1% change over the complete CRD protocol, respectively).

Pregabalin ($200\,\mu mol\,kg^{-1}$, p.o., $n=6$) attenuated the overall visceromotor response to CRD by 16 ± 8 and 47 ± 6%, as determined by EMG or colonic manometry, respectively (Figures 3a and b, both $P<0.05$ vs respective response in the vehicle-treated group). A similar effect of pregabalin was also observed for CRD-induced blood pressure and heart rate changes, which were attenuated, overall, by 28 ± 12% ($P=0.018$ vs vehicle; Figure 3c) and 25 ± 8% ($P=0.026$ vs. vehicle; Figure 3d), respectively. Pregabalin, *per se*, had no effects on basal blood pressure (mean basal blood pressure at the beginning of the CRD procedure: vehicle, 118.3 ± 5.1 mm Hg; pregabalin, 113.4 ± 8.8 mm Hg; $P>0.05$) or heart rate (mean heart rate at the beginning of the CRD procedure: vehicle, 424 ± 9 b.p.m. (beats per min); pregabalin, 394 ± 8 b.p.m.; $P>0.05$). As described above, inhibitory effects of pregabalin on pain-related visceromotor and cardiovascular responses were observed throughout the distension procedure (Figure 3).

*Effects of pregabalin on colonic compliance*

In vehicle-treated animals, a positive pressure–volume relationship was observed during increasing phasic CRD

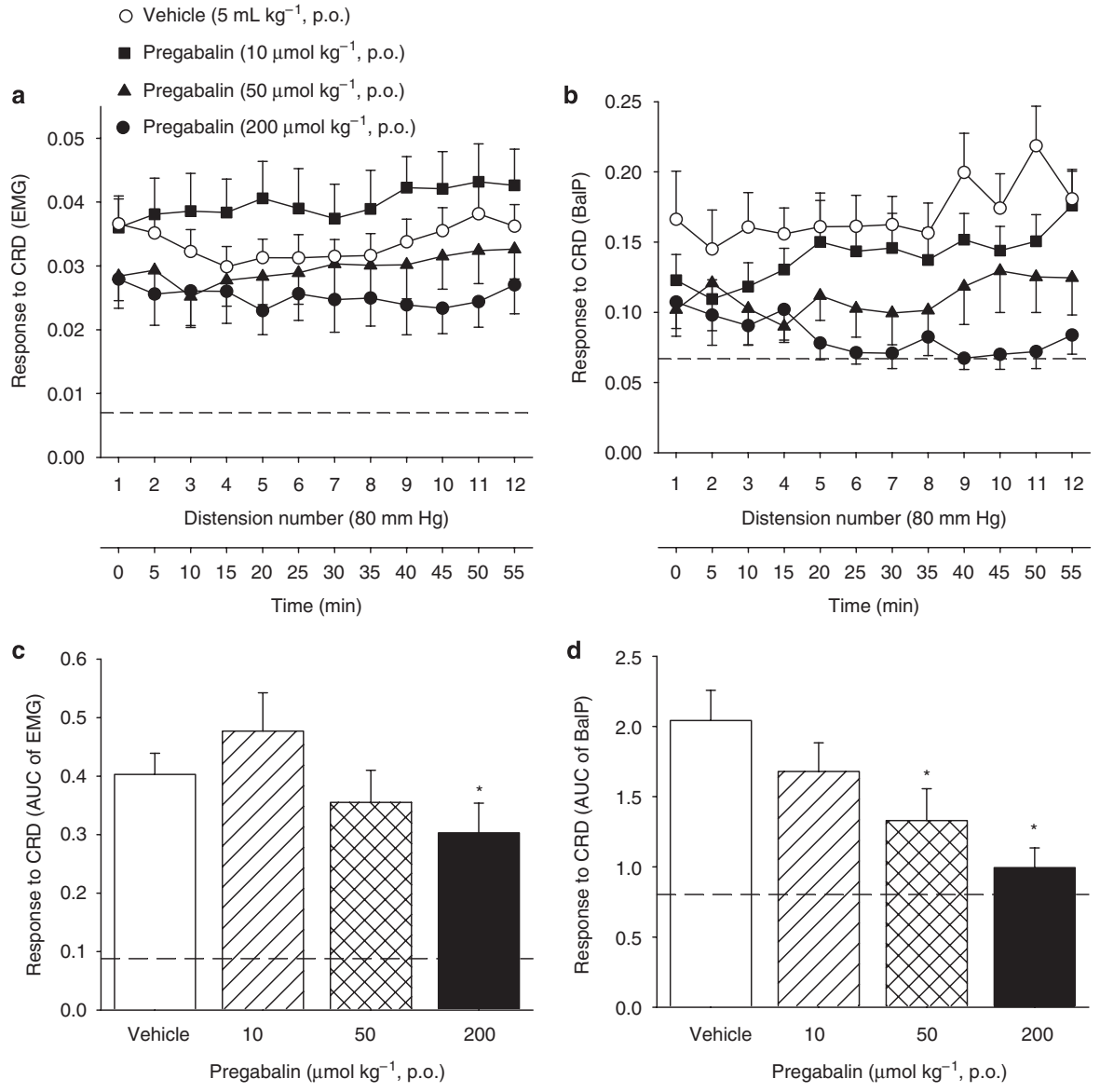


**Figure 1** Effects of oral pregabalin on the visceromotor responses to repetitive noxious colorectal distension (CRD; 80 mm Hg) in rats. Responses to CRD (12 distensions in 60 min) were determined simultaneously in the same animals by electromyographical (EMG) recording of the activity of the abdominal musculature (**a**) and by colonic manometry assessing changes in the intracolonic balloon pressure (BalP) (**b**). (**c** and **d**) Overall response to CRD assessed by EMG recordings (**c**) or colonic manometry (**d**). In all cases, the dashed line represents the mean basal activity. Data are mean ± s.e.mean. of 12 animals per group. *$P<0.05$ vs vehicle-treated group (ANOVA).

(2–20 mm Hg × 1 min) (Figure 4a). At low distension pressures (below 10 mm Hg), the pressure–volume curves were essentially identical in pregabalin-treated (200 μmol kg$^{-1}$, p.o.) or vehicle-treated animals. However, at higher pressures, the pressure–volume curve in pregabalin-treated animals was displaced to the left, indicating an increase in compliance (Figure 4a). Accordingly, in five out of six animals, the maximum volume increased and the $P_{50}$ and $P_{10}$ values decreased after pregabalin treatment (Figure 4b). Experimental data, both in vehicle-treated and in pregabalin-treated animals, fitted well to the mathematical model, and values for the parameters $\beta$ and $\kappa$ and the estimated $V_{max}$, $P_{10}$ and $P_{50}$ values are given in Table 1.

*Plasma levels of pregabalin after oral dosing*

Pregabalin was detectable in plasma 60–120 min post-oral administration. Plasma levels were dose related (levels were about 3.5- to 4-fold higher at the dose of 200 μmol kg$^{-1}$ compared with 50 μmol kg$^{-1}$) and stable during the 60–120 min post-administration (Figure 5).

## Discussion

The present results show that pregabalin reduced the pain-related visceromotor and autonomic responses associated with mechanical stimulation of the colon in rats,

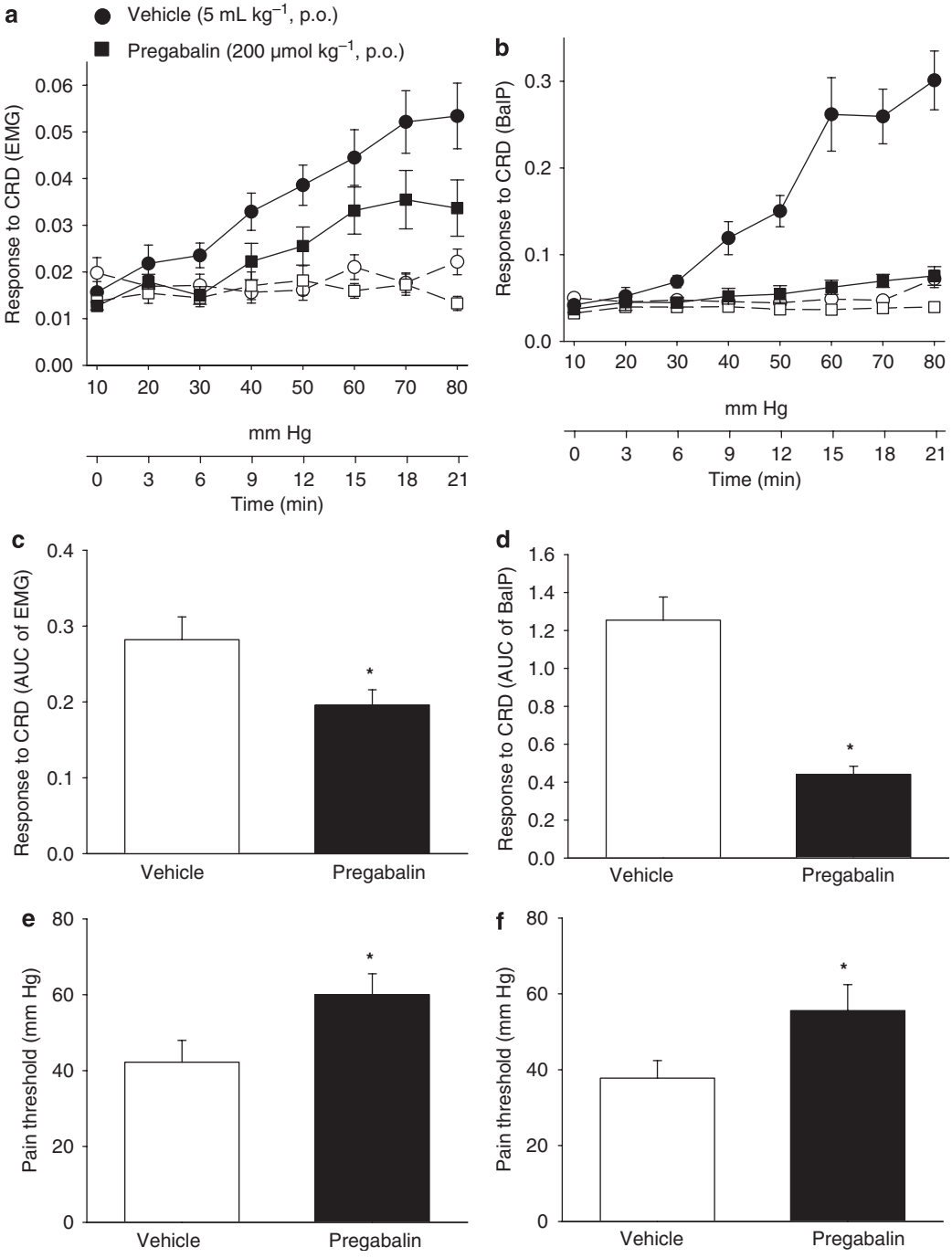


**Figure 2** Effects of oral pregabalin on the visceromotor responses to phasic ascending colorectal distension (CDR; 10–80 mm Hg over 20 min) in rats. Responses to CRD were determined simultaneously in the same animals by electromyographical (EMG) recording of the activity of the abdominal musculature (**a**) and by colonic manometry assessing changes in the intracolonic balloon pressure (BalP) (**b**). In both cases, the dashed lines with open symbols represent the basal electrical or mechanical activity for each experimental group. (**c** and **d**) Overall response to CRD assessed by EMG recordings (**c**) or colonic manometry (**d**). (**e** and **f**) Threshold for response during CRD determined from the EMG (**e**) or the manometric recording (**f**). Data are mean ± s.e.mean. of nine animals per group. *$P<0.05$ vs vehicle-treated group.

confirming its anti-nociceptive properties. In addition, pregabalin also increased the pressure–volume relationship during distension, suggesting that at least part of the analgesic effects of the compound might be associated with the modulation of colonic compliance. The effects of pregabalin on colonic pain and compliance were achieved at doses giving rise to clinically relevant plasma exposures.

Pregabalin significantly increases rectal sensory thresholds to distension in hypersensitive IBS patients and normalizes, rather than desensitizes (that is, make hyposensitive), the perception of rectal distension (Houghton *et al.*, 2007). These observations in humans support findings in animals showing that pregabalin reduced the normal pain response to CRD and also modulated hypersensitivity states (Eutamene

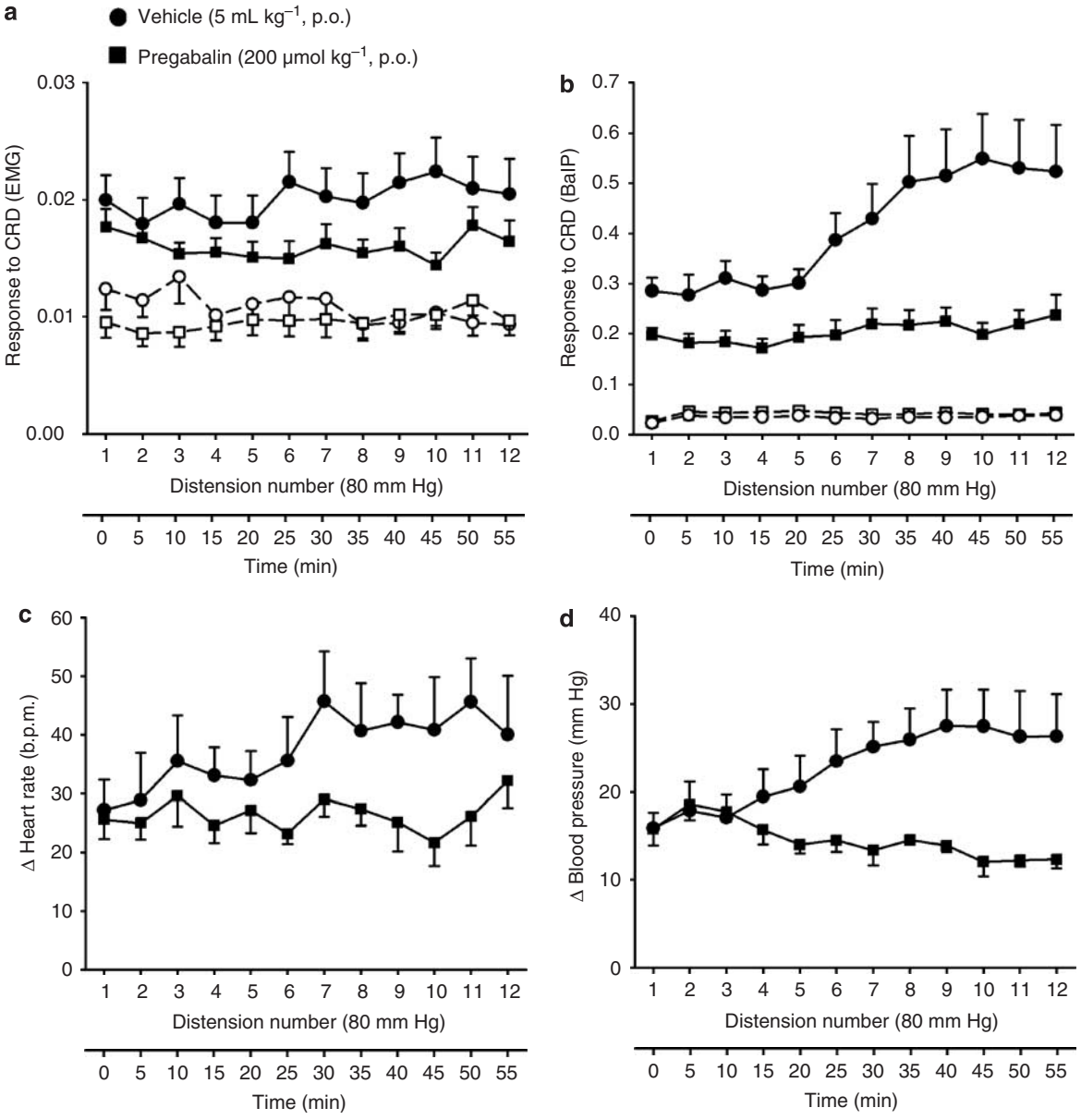


**Figure 3** Effects of oral pregabalin on the visceromotor and autonomic cardiovascular responses to repetitive noxious colorectal distension (CRD; 80 mm Hg) in rats. Visceromotor responses to CRD were determined simultaneously in the same animals by electromyographical (EMG) recording of the activity of the abdominal musculature (**a**) and by colonic manometry assessing changes in the intracolonic balloon pressure (BalP) (**b**). In panels a and b, the dashed lines with open symbols represent the basal electrical (**a**) or mechanical (**b**) activity of each experimental group. Cardiovascular responses were determined by assessing changes in heart rate (**c**) and blood pressure (**d**). Data are mean ± s.e.mean. of six animals per group.

*et al*., 2000; Diop *et al*., 2002; Million *et al*., 2007). In the present study, we tested the effects of pregabalin on pain-related visceromotor and autonomic responses to repetitive noxious CRD-induced acute sensitization in normal animals. As previously shown, the CRD at 80 mm Hg induced a viscerosomatic response, indicative of pain, in conscious rats, resulting in augmented activity of the abdominal musculature, as determined measuring its electrical or mechanical activity (Tammpere *et al*., 2005). Moreover, the magnitude of the response to repetitive distensions tended to increase over time, an effect particularly evident when assessing the mechanical responses to distension (Tammpere *et al*., 2005), and indicative of acute mechanical sensitization. Oral pregabalin reduced the normal viscerosomatic response to CRD and prevented in a dose-related manner repetitive CRD-induced acute hypersensitivity. The analgesic effects of pregabalin were clearer when assessing the mechanical than the electrical response to CRD of the abdominal musculature. This further confirms our previous results indicating that monitoring the mechanical activity of the abdominal musculature might be more sensitive than the standard electromyographic procedures as a readout for visceral pain-related responses (Tammpere *et al*., 2005; Arvidsson *et al*., 2006). Although not determined simultaneously in the same animals, the overall dose-related effects of pregabalin on CRD-induced visceromotor responses show

a good correspondence with the mean plasma levels reached after oral dosing. Moreover, the analgesic effects of pregabalin lasted throughout the experimental time (between 21 and 55 min depending on the CRD protocol used, with pregabalin dosed 60 min before), reflecting also the relatively stable plasma levels detected between 60 and 120 min after oral dosing. Effective plasma levels were consistent with those showing efficacy in rodent models of epilepsy and ataxia (Vartanian *et al*., 2006) or those reported in humans within a therapeutic range (Randinitis *et al*., 2003; Brodie *et al*., 2005; Zareba, 2005). In addition, the plasma levels achieved in the current study are likely to be in a similar range to those that can be expected from Houghton *et al*. (2007) using doses between 50 and 200 mg (Zareba, 2005). Thus, the doses used here and the effects observed might be of therapeutic relevance for IBS patients.

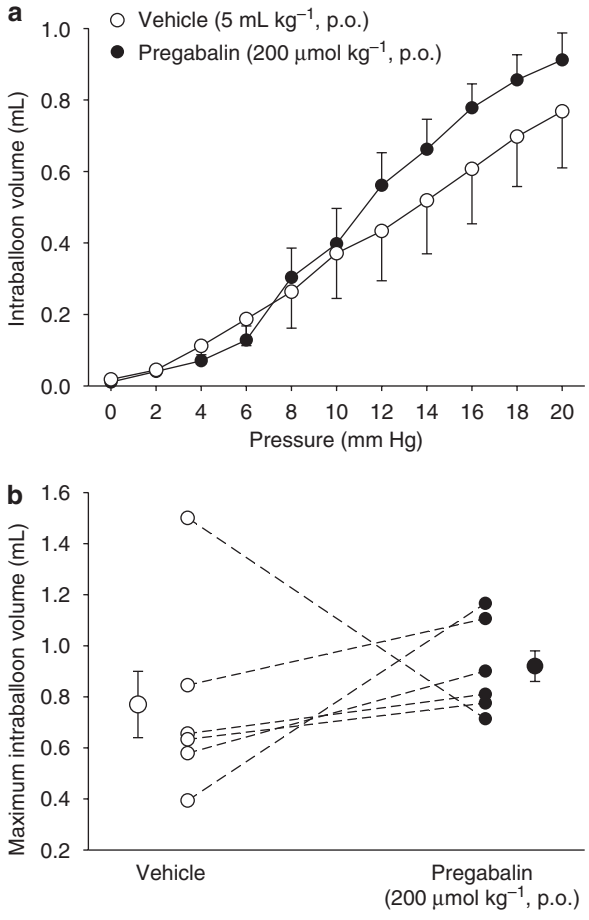


**Figure 4** Effects of pregabalin on colorectal compliance during colorectal distension in rats. (**a**) Pressure–volume curves during phasic colorectal distension (2–20 mm Hg) in animals treated orally with pregabalin (200 μmol kg$^{-1}$) or vehicle (5 mL kg$^{-1}$). Data are mean ± s.e.mean. of six animals and represent the maximal intraballoon volume (mL) for each pressure level. (**b**) Individual maximal intracolonic volumes at a distension pressure of 20 mm Hg after vehicle or pregabalin treatment in the same animal. The symbols with error bars represent the mean ± s.e.mean. for both experimental groups. Notice that pregabalin increased the maximal intracolonic volume in all animals except one.

In the present experimental conditions, noxious CRD (80 mm Hg) resulted, in addition to the visceromotor responses, in an autonomic cardiovascular response characterized by an increase in heart rate and arterial blood pressure. Moreover, repetitive CRD resulted also in an increase in these cardiovascular responses, indicative of acute sensitization. These cardiovascular responses are similar to those described previously in rats (Ness and Gebhart, 1988, 2001; Lindström *et al*., 2008). Pregabalin, at the highest dose tested, also reduced the cardiovascular autonomic responses associated with noxious CRD. Interestingly, pregabalin only prevented the acute sensitization during repetitive CRD, but did not affect the rise in blood pressure or heart rate associated with a normal pain response, as cardiovascular responses at the initiation of the CRD protocol, before the presence of mechanical sensitization, were similar in vehicle- and pregabalin-treated animals. Moreover, at the same dose, pregabalin significantly inhibited the visceromotor responses to phasic ascending

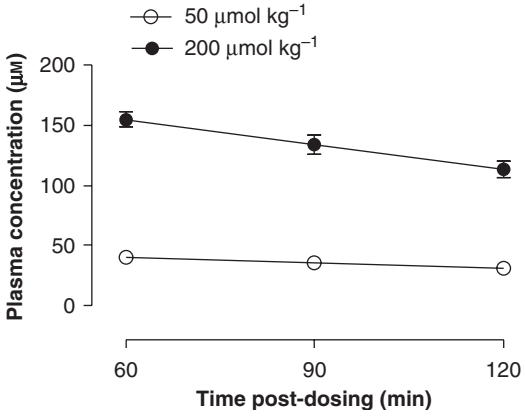


**Figure 5** Plasma levels of pregabalin in the 60–120-min period after oral dosing. Data are mean ± s.e.mean. of 4–5 animals per group.

**Table 1** Effects of pregabalin on the parameters characterizing the pressure–volume relationship during CRD

| | κ | β | $V_{max}$ *(mL)* | $P_{50}$ *(mm Hg)* | $P_{10}$ *(mm Hg)* |
|---|---|---|---|---|---|
| Vehicle | 19.86 ± 3.67 | 0.84 ± 0.04 | 0.78 ± 0.13 | 13.63 ± 1.45 | 7.80 ± 1.40 |
| Pregabalin | 17.59 ± 2.26 | 1.15 ± 0.06 | 0.92 ± 0.06 | 9.10 ± 0.82 | 4.55 ± 0.44 |
| | | $P = 0.0152$ | | $P = 0.0401$ | $P = 0.0423$ |

CRD, colorectal distension.
Data shown are mean ± s.e.mean., $n = 6$.
$\kappa$, $\beta$, $P_{50}$ and $P_{10}$ were calculated as described in the Methods section. The statistical difference between values from vehicle and pregabalin treatments is shown by the *P*-values in the last row of the Table (Student's *t*-test).

CRD and increased the threshold pressure for response during the same CRD protocol or during continuous ramp (0–80 mm Hg) CRD (data not shown).

Altered colonic tone and accommodation to distension have been suggested as contributing mechanisms to the altered colonic sensitivity observed in functional gastrointestinal disorders (Delgado-Aros and Camilleri, 2005). Therefore, an improvement in the pressure–volume relationship during distension, reflected as shift to the left in the pressure–volume relationship curves (that is, greater compliance and improved accommodation), might result in reduced pain sensitivity. Nevertheless, there is no clear correlation between compliance and pain perception. For instance, intrarectal lidocaine reduced compliance in rats but had analgesic effects during CRD (Käll *et al*., 2007), and the metabotropic glutamate 5 receptor ($mGluR_5$) antagonist MPEP had analgesic effect during CRD without affecting colonic compliance (Lindström *et al*., 2008). On the other hand, clonidine increased gastric and colonic compliance and also reduced pain perception (Thumshirn *et al*., 1999; Malcolm *et al*., 2000), and Wistar Kyoto rats with reduced colonic accommodation responses are also hypersensitive during CRD (Martínez *et al*., 2007). A recent report showed that pregabalin, in addition to increasing pain thresholds during CRD, also increased colonic compliance in IBS patients with colonic hypersensitivity (Houghton *et al*., 2007). Similarly, in the present study, pregabalin, at a dose inducing clear analgesia, also modulated colonic compliance, with an increase in the volume response during CRD observed in five out of six animals tested. Altogether, these observations indicate that pregabalin increases colonic compliance in humans and animals. The potential relationship between the analgesic properties of pregabalin and its effects on compliance needs to be further characterized.

In addition to its effects on pain, pregabalin has anxiolytic properties in both animals and humans (Huckle, 2004; Pohl *et al*., 2005; Rickels *et al*., 2005; Bandelow *et al*., 2007). Anxiety might be a contributing factor to the viscerosomatic and autonomic responses elicited by CRD. Therefore, anxiolytic effects of compounds might result in the reduction of pain-related readouts and be misinterpreted as analgesic activity. In this sense, other compounds (such as buspirone or $mGluR_5$ antagonists) with potential anxiolytic activity also had analgesic effects in the CRD model in rats (Sivarao *et al*., 2004; Lindström *et al*., 2008). In humans, modulation of anxiety or sedation-like effects was discarded as a contributing factor to the analgesic effects of pregabalin (Houghton *et al*., 2007). In the present study, anxiety-like behaviours were not directly assessed, although the generation of anxiety and stress during the experimental procedures was minimized as far as possible. In addition, no gross side effects consistent with sedation-like effects, which might interfere with the manifestation of visceromotor responses to pain, were observed at any of the doses tested. This is consistent with data showing that pregabalin caused ataxia and decreased spontaneous locomotor activity at dosages 10–30-fold higher than those active to prevent seizures ($ED_{50}$ $\sim 130\,\mu mol\,kg^{-1}$, p.o.) in rodent models of epilepsy (Vartanian *et al*., 2006). This also agrees with observations with gabapentin in somatic pain models, in which sedation was only observed at a dose of $300\,mg\,kg^{-1}$ (approximately 10-fold higher than the maximal dose used in the present study) (Jones and Sorkin, 1998). Thus, although potential central anxiolytic effects of pregabalin cannot be completely ruled out, sedation-like effects can be excluded as a confounding analgesic factor.

In summary, we show that the $\alpha_2$-$\delta$ ligand pregabalin has analgesic properties in the CRD model in rats, with consistent effects in several independent visceral pain-related parameters. Pregabalin was effective in attenuating both the viscerosomatic (contractions of the abdominal musculature) and the cardiovascular autonomic responses (hypertension and tachycardia) associated with the noxious mechanical distension of the colon in rats. In addition, pregabalin also modulated colonic tone, resulting in an increase in compliance. These observations support the preliminary clinical data obtained in humans supporting a potential therapeutic use of pregabalin, or other $\alpha_2$-$\delta$ ligands, for the treatment of visceral hypersensitivity (Lee *et al*., 2005; Houghton *et al*., 2007). Moreover, the CRD protocols used here are closer to those usually applied in clinical conditions than those previously used in similar animal studies (isovolumetric distensions or tonic distensions) (Eutamene *et al*., 2000; Million *et al*., 2007), which contributes to the translational value of the present studies. The exact mechanism for the visceral analgesic effects of pregabalin and the potential role of colonic tone warrant further studies. Finally, the present observations, together with the data recently published in humans (Houghton *et al*., 2007), support the translational value of the CRD model of visceral pain, combined with the simultaneous assessment of multiple surrogate markers of visceral pain, as a predictor for clinical efficacy in humans.

## Conflict of interest

The authors state no conflict of interest.

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