BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 160, 1316–1325

0007-1188/10
www.brjpharmacol.org

RESEARCH PAPER

# Effects of ionotropic glutamate receptor antagonists on rat dural artery diameter in an intravital microscopy model

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**Background and purpose:** During migraine, trigeminal nerves may release calcitonin gene-related peptide (CGRP), inducing cranial vasodilatation and central nociception; hence, trigeminal inhibition or blockade of craniovascular CGRP receptors may prevent this vasodilatation and abort migraine headache. Several preclinical studies have shown that glutamate receptor antagonists affect the pathophysiology of migraine. This study investigated whether antagonists of NMDA (ketamine and MK801), AMPA (GYKI52466) and kainate (LY466195) glutamate receptors affected dural vasodilatation induced by α-CGRP, capsaicin and periarterial electrical stimulation in rats, using intravital microscopy.

**Experimental approach:** Male Sprague-Dawley rats were anaesthetized and the overlying bone was thinned to visualize the dural artery. Then, vasodilator responses to exogenous (i.v. α-CGRP) and endogenous (released by i.v. capsaicin and periarterial electrical stimulation) CGRP were elicited in the absence or presence of the above antagonists.

**Key results:** α-CGRP, capsaicin and periarterial electrical stimulation increased dural artery diameter. Ketamine and MK801 inhibited the vasodilator responses to capsaicin and electrical stimulation, while only ketamine attenuated those to α-CGRP. In contrast, GYKI52466 only attenuated the vasodilatation to exogenous α-CGRP, while LY466195 did not affect the vasodilator responses to endogenous or exogenous CGRP.

**Conclusions and implications:** Although GYKI52466 has not been tested clinically, our data suggest that it would not inhibit migraine via vascular mechanisms. Similarly, the antimigraine efficacy of LY466195 seems unrelated to vascular CGRP-mediated pathways and/or receptors. In contrast, the cranial vascular effects of ketamine and MK801 may represent a therapeutic mechanism, although the same mechanism might contribute, peripherally, to cardiovascular side effects.
*British Journal of Pharmacology* (2010) **160,** 1316–1325; doi:10.1111/j.1476-5381.2010.00733.x

**Keywords:** capsaicin; α-CGRP; electrical stimulation; GYKI52466; intravital microscopy; ketamine; LY466195; migraine; MK801; vasodilatation

**Abbreviations:** AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CGRP, calcitonin gene-related peptide; GYKI52466, 1-(4-Aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride; HBC, 2-hydroxypropyl-β-cyclodextrin 45%; LY466195 (3S,4aR,6S,8aR)-6-[[(2S)-2-carboxy-4,4-difluoro-1-pyrrolidinyl]-methyl]decahydro-3-isoquinolinecarboxylic acid); MAP, mean arterial blood pressure; MK801 (5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate; NMDA, N-methyl-D-aspartate

## Introduction

Migraine is a neurovascular disorder that has been associated with cranial vasodilatation and release of calcitonin gene-related peptide (CGRP) resulting from activation of perivascular trigeminal sensory nerves that originate in the trigeminal ganglion and the trigeminocervical complex (Goadsby and Edvinsson, 1993; Villalón and Olesen, 2009). Interestingly, the excitatory neurotransmitter, glutamate, has

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Received 4 November 2009; revised 23 December 2009; accepted 3 January 2010

also been involved in migraine (Pollack and French, 1975) as it is found in neurons of structures related to migraine pathophysiology, including the trigeminal ganglion, trigeminocervical complex and the thalamus (Kai-Kai and Howe, 1991). Indeed, glutamate and CGRP are released from trigeminal ganglion neurons by calcium channel-dependent mechanisms (Xiao *et al*., 2008), and increased levels of glutamate have been found in the trigeminocervial complex after stimulation of dural structures (Oshinsky and Luo, 2006), and in the cerebrospinal fluid of migraine patients (Rothrock *et al*., 1995).

Glutamate exerts its effects by activating ionotropic (ligand-gated ion channels) and metabotropic (G-protein coupled) receptors. Ionotropic glutamate receptors are divided into N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (Monaghan *et al*., 1989; receptor nomenclature follows Alexander *et al*., 2009). Activation of ionotropic glutamate receptors on neurons leads to calcium influx, activation of intracellular signalling pathways and production and release of vasoactive agents like nitric oxide (NO) (Bhardwaj *et al*., 1997). Moreover, the ionotropic glutamate receptors are activated during cortical spreading depression, which is considered to be involved in the migraine aura (Gorji *et al*., 2001).

Several preclinical studies have suggested that ionotropic glutamate receptor antagonists affect the pathophysiology of migraine. The NMDA receptor antagonist MK801 and the AMPA receptor antagonist GYKI52466 blocked trigeminovascular nociception in the trigeminocervical nucleus (Storer and Goadsby, 1999; Goadsby and Classey, 2000; Classey *et al*., 2001), while MK801 reduced capsaicin-evoked CGRP release (Garry *et al*., 2000); the latter finding points to potential vascular effects of glutamate receptor antagonists. In addition, blockade of both NMDA and non-NMDA ionotropic receptors reduces the expression of c-fos protein in the trigeminal nucleus caudalis after intracisternal capsaicin injection (Mitsikostas *et al*., 1998; 1999). Most notably, several glutamate receptor antagonists are effective in the acute treatment of migraine, including the mixed AMPA/kainate receptor antagonist LY293558 (Sang *et al*., 2004) and the kainate receptor antagonist LY466195 (Johnson *et al*., 2008). Likewise, the anticonvulsant topiramate is effective in migraine prophylaxis (Silberstein *et al*., 2004), probably, at least partly, due to blockade of AMPA and kainate receptors. In patients taking nitroglycerin for reducing the risk of cardiac ischemia, infusion of ketamine, an NMDA receptor antagonist, was proposed to be effective against this NO-induced headache (Roffey *et al*., 2001). Moreover, in a small open-label study, intranasal ketamine reduced the severity and duration of the neurological deficits due to the aura in five out of 11 patients with familial hemiplegic migraine (Kaube *et al*., 2000).

On this basis, the present study set out to analyse the effects of several ionotropic glutamate receptor antagonists on an experimental neurovascular model of migraine, using intravital microscopy. Accordingly, the NMDA receptor antagonists ketamine and MK801, the AMPA receptor antagonist GYKI52466, and the kainate receptor antagonist LY466195 were tested on the vasodilatation of the rat dural artery induced by exogenous and endogenous (released by i.v. capsaicin or periarterial electrical stimulation) CGRP.

## Methods

*Animals*

All animal care and experimental protocols were approved by the Ethical Committee of our institution, dealing with the use of animals in scientific experiments. Male Sprague-Dawley normotensive rats (300–400 g), purchased from Harlan Netherlands (Horst, the Netherlands), were maintained at a 12/12-h light–dark cycle (with light beginning at 7 am) and housed in a special room at constant temperature (22 ± 2°C) and humidity (50%), with food and water freely available in their home cages.

*General methods*

Experiments were carried out in a total of 69 rats. During the experiments the rats were anaesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (Nembutal, 60 mg·kg$^{-1}$, followed by 18 mg·kg$^{-1}$ per hour when necessary). The trachea was cannulated and connected to a pressure ventilator (small animal ventilator SAR-830 series, CWE Inc., Ardmore, PA, USA). End-tidal $pCO_2$ was monitored (Capstar-100 CWE Inc., PA, USA) and kept between 35 and 48 mmHg. The left femoral vein and artery were cannulated for intravenous (i.v.) administration of drugs and continuous monitoring of blood pressure respectively. Two or three samples of blood (at the beginning and at the end of the experiment) were withdrawn via the femoral artery to monitor blood gases, which were kept between normal values (pH: 7.35–7.48; $pCO_2$: 35–48 mmHg; $pO_2$: 100–120 mmHg). These values and therefore normal physiological conditions were kept stable throughout the experiment. The body temperature of animals was monitored via a rectal thermometer and maintained throughout the experiment between 36.5°C and 37.5°C by a homeothermic blanket system for rodents (Harvard Instruments, Edenbridge, Kent, UK). The rats were placed in a stereotaxic frame and the bone overlying a segment of the dural meningeal artery was carefully drilled thin, applying cold saline (4°C) during the drilling until the artery was clearly visible. As drilling of the skull induces vasodilatation, we allowed the animal to recover for 1 h before proceeding with the experimental protocol. The drilled area was covered with mineral oil to prevent drying and to facilitate visualization of the meningeal artery. Images of the artery were captured with an intravital microscope (model MZ 16; Leica microsystem Ltd, Heerbrugg, Switzerland) using a cyan blue filter on a cold source of light. A zoom lens (80–450× magnification) and a camera were used to display the images on a standard television monitor. The blood vessel diameter (30–40 μm at baseline) was continuously monitored and measured with a video dimension analyser (Living Systems Instrumentation Inc., Burlington, VT, USA) and the effects of α-CGRP, capsaicin and periarterial electrical stimulation were studied as specified below. In rats where periarterial electrical stimulation was used to evoke dilatation of the dural blood vessels, a bipolar stimulating electrode (NE 200X, Clark Electromedical, Edenbridge, Kent, UK) was placed on the surface of the cranial window approximately within 200 μm from the vessel of interest. The surface of the cranial window was stimulated at 5 Hz, 1 ms for 10 s (Stimulator model S88, Grass Instruments, West Warwick,

RI, USA). For neurogenic dural vasodilatation, we initially started with a current intensity (monitored on an oscilloscope, model 54601A, Hewlett Packard, Palo Alto, CA, USA) of 100 μA and increased with 50 μA steps until a maximal level of dilatation was achieved, usually at 200 μA. The resulting data were displayed and recorded using a WINDAQ data acquisition system (Version 2.54; DataQ Instruments Inc., Akron, OH, USA).

*Experimental protocol*

After a stable haemodynamic condition for at least 60 min, baseline values of mean arterial blood pressure (MAP) and heart rate were determined. Then, the animals were divided into three groups ($n$ = 25, 24 and 20) which received α-CGRP (1 μg·kg$^{-1}$, i.v.), capsaicin (10 μg·kg$^{-1}$, i.v.) and periarterial electrical stimulation (150–250 μA) respectively. 30 min were allowed to elapse after each of these treatments for the recovery of baseline diameter. Each of these groups was subsequently subdivided into four subgroups ($n$ = 5–7) which were given (after 30 min) i.v. cumulative doses of, respectively, ketamine (10, 18 and 30 mg·kg$^{-1}$), MK801 (0.2, 0.5, 1 and 3 mg·kg$^{-1}$), GYKI52466 (0.5, 2 and 5 mg·kg$^{-1}$) and LY466195 (0.03, 0.1 and 0.3 mg·kg$^{-1}$). Each dose of antagonist was administered 5 min before a subsequent treatment with α-CGRP, capsaicin or periarterial electrical stimulation. The selected doses of ketamine (Castroman and Ness, 2002), MK801 (Goadsby and Classey, 2000), GYKI52466 (Storer and Goadsby, 1999) and LY466195 (Weiss *et al*., 2006) have previously been shown to be effective in blocking their respective receptors. The duration of each experiment was approximately 2.5 h after stabilization.

*Data presentation and statistical evaluation*

All data are presented as mean ± SEM The peak increases in diameter of the dural meningeal artery are expressed as percent change from baseline. The changes in MAP and heart rate are expressed as, respectively, absolute values (mmHg) and percentage change from baseline. The difference between the variables within one group of animals was compared by using a one-way repeated measures analysis of variance followed by the Dunnet's test (Steel and Torrie, 1980). Statistical significance was accepted at $P < 0.05$ (two-tailed).

*Materials*

The materials used in the present study were obtained from the sources indicated: capsaicin, MK801 hydrogen maleate, GYKI52466 hydrochloride, 2-hydroxypropyl-β-cyclodextrin 45% (HBC) (Sigma Chemicals Co., Steinheim, Germany); rat α-CGRP (NeoMPS S.A., Strasbourg, France); nembutal (Ceva Sante Animale B.V., Maassluis, the Netherlands); ketamine hydrochloride (Alfasan, Woerden, the Netherlands); LY466195 (Eli Lilly and Company, Indianapolis, IN, USA). Capsaicin (1 mg·mL$^{-1}$) was dissolved in a mixture of tween 80, ethanol 70% and water (1:1:8); GYKI52466 (20 mg·mL$^{-1}$) was dissolved in 45% HBC, whereas the other compounds were dissolved in isotonic saline. All compounds were stored in aliquots at –80°C, until required. Just before use, the stock solutions were further diluted to the appropriate concentration in isotonic saline for injection. The doses of all compounds refer to their respective salts.

## Results

*Effect of α-CGRP, capsaicin and periarterial electrical stimulation on dural diameter, MAP and heart rate*

I.v. administration of 1 μg·kg$^{-1}$ α-CGRP or 10 μg·kg$^{-1}$ capsaicin increased dural artery diameter by, respectively, 103 ± 7% ($n$ = 25) and 77 ± 6% ($n$ = 24), whereas periarterial electrical stimulation (150 μA–250 μA) increased dural artery diameter by 78 ± 5% ($n$ = 20). Repeated treatment (up to four times) with α-CGRP, capsaicin or periarterial electrical stimulation produced reproducible increases in the dural artery diameter (data not shown).

At the beginning of the experiments, the average baseline MAP from all animals was 96 ± 2 mmHg. There were no significant differences between the baseline values before and after the experiments in most groups ($P > 0.1$), except in those given capsaicin with ketamine (Figure 1; right middle panel) or electrical stimulation with MK801 (Figure 2; right lower panel).

The MAP was decreased after infusion of α-CGRP, but not after infusion of saline when the dilatation of the dural artery was maximal in the MK801, GYKI52466 and LY466195 treated groups; these decreases were similar after giving these antagonists (Figures 2–4; right upper panels). α-CGRP significantly decreased MAP in the ketamine group at the highest dose (Figure 1; right upper panels). In general, capsaicin and periarterial electrical stimulation did not significantly affect MAP in most groups, except in that of capsaicin with GYKI52466 (Figures 1–4; right middle and lower panels). Heart rate was not affected by any of the above treatments (data not shown), except for the ketamine-treated group (see below).

*Effect of the NMDA receptor antagonists ketamine and MK801 on dural artery vasodilatation, MAP and heart rate after treatment with α-CGRP, capsaicin and periarterial electrical stimulation*

Ketamine induced a dose-dependent attenuation of the vasodilator responses to α-CGRP (Figure 1; left upper panel) and capsaicin (10 mg·kg$^{-1}$: 42 ± 11%; 30 mg·kg$^{-1}$: 28 ± 10%, Figure 1; left middle panel) compared with the control groups for α-CGRP and capsaicin respectively. Ketamine also produced a significant attenuation of the vasodilatation in the electrical stimulation group at the doses of 18 mg·kg$^{-1}$ and 30 mg·kg$^{-1}$ compared with control (Figure 1; left lower panel).

In contrast, the doses used of MK801 did not block the vasodilator responses to α-CGRP (Figure 2; left upper panel), although they significantly attenuated the vasodilator responses to both capsaicin (at 1 mg·kg$^{-1}$; Figure 2; left middle panel) and electrical stimulation (at 3 mg·kg$^{-1}$; Figure 2; left lower panel), compared with the corresponding controls. The NMDA receptor antagonists did not affect dural artery diameter *per se* (data not shown).

Ketamine significantly decreased MAP at the highest doses used in the α-CGRP group and at all doses in the capsaicin-treated groups (Figure 1; right upper and middle panel). In the electrical stimulation group, ketamine did not decrease MAP

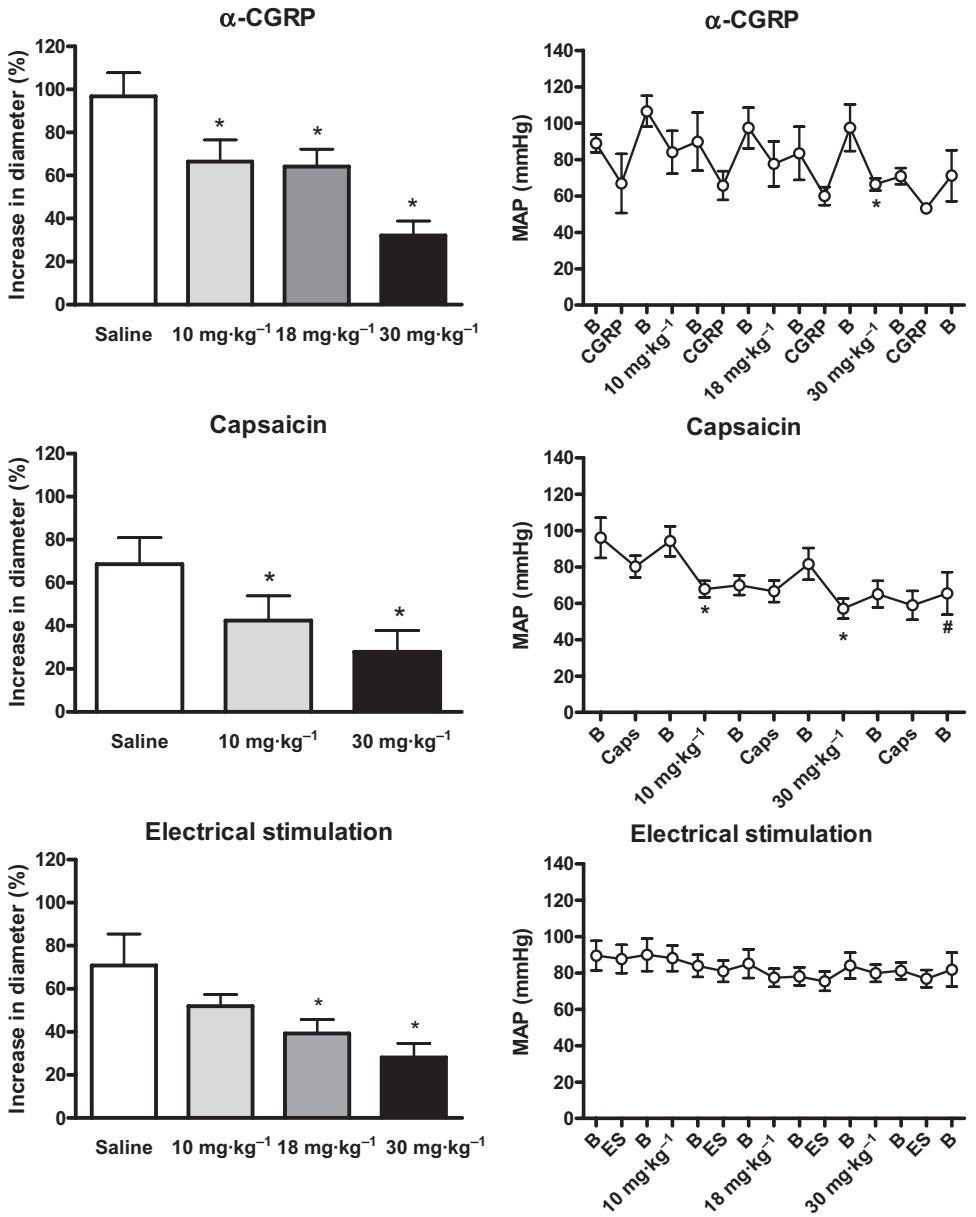


**Figure 1** Effect of increasing doses of ketamine on vasodilatation of the dural artery (percentage of increase in diameter, left panels) and mean arterial blood pressure (MAP) (mmHg, right panels) induced by α-CGRP (upper panels, $n = 6$); capsaicin (middle panels, $n = 6$) and periarterial electrical stimulation (lower panel, $n = 6$). B, baseline; Caps, 10 μg·kg$^{-1}$ capsaicin i.v.; CGRP 1 μg·kg$^{-1}$, calcitonin gene-related peptide i.v.; ES, periarterial electrical stimulation 150–250 μA. *$P < 0.05$ compared with the control or the corresponding baseline; $^{\#}P < 0.05$ compared with the baseline at the beginning of the experiment.

at any of the doses tested. Moreover, MK801 did not change MAP in the α-CGRP and capsaicin treated groups, while it increased the MAP in the electrical stimulation treated group (Figure 2, right lower panel). Ketamine significantly decreased heart rate at all doses tested (10 mg·kg$^{-1}$: –10 ± 3%; 18 mg·kg$^{-1}$: –14 ± 4%; 30 mg·kg$^{-1}$: –16 ± 4%), while MK801 did not affect heart rate (data not shown).

*Effect of the AMPA receptor antagonist GYKI52644 on dural artery vasodilatation, MAP and heart rate after treatment with α-CGRP, capsaicin and periarterial electrical stimulation*

GYKI52466, at all doses tested, did not significantly modify the vasodilatation to capsaicin or periarterial electrical stimulation (Figure 3; left middle panel and lower panel) but, at 5 mg·kg$^{-1}$, attenuated the vasodilatation to exogenous α-CGRP, compared with the control group (Figure 3; left upper panel). The effects of GYKI52466 on dural artery diameter were not different from that of its vehicle (HBC; concentration corresponding to that used at 5 mg·kg$^{-1}$ GYKI52466) and did not affect the dural diameter *per se* (data not shown).

Interestingly, the vehicle of GYKI52466 (HBC) significantly increased MAP in the α-CGRP, capsaicin and electrical stimulation groups (Figure 3; right panels). This increase is also present at the highest doses of GYKI52466 in the capsaicin group (Figure 3; right middle panel) and at all doses of GYKI52466 in the electrical stimulation group (Figure 3; right

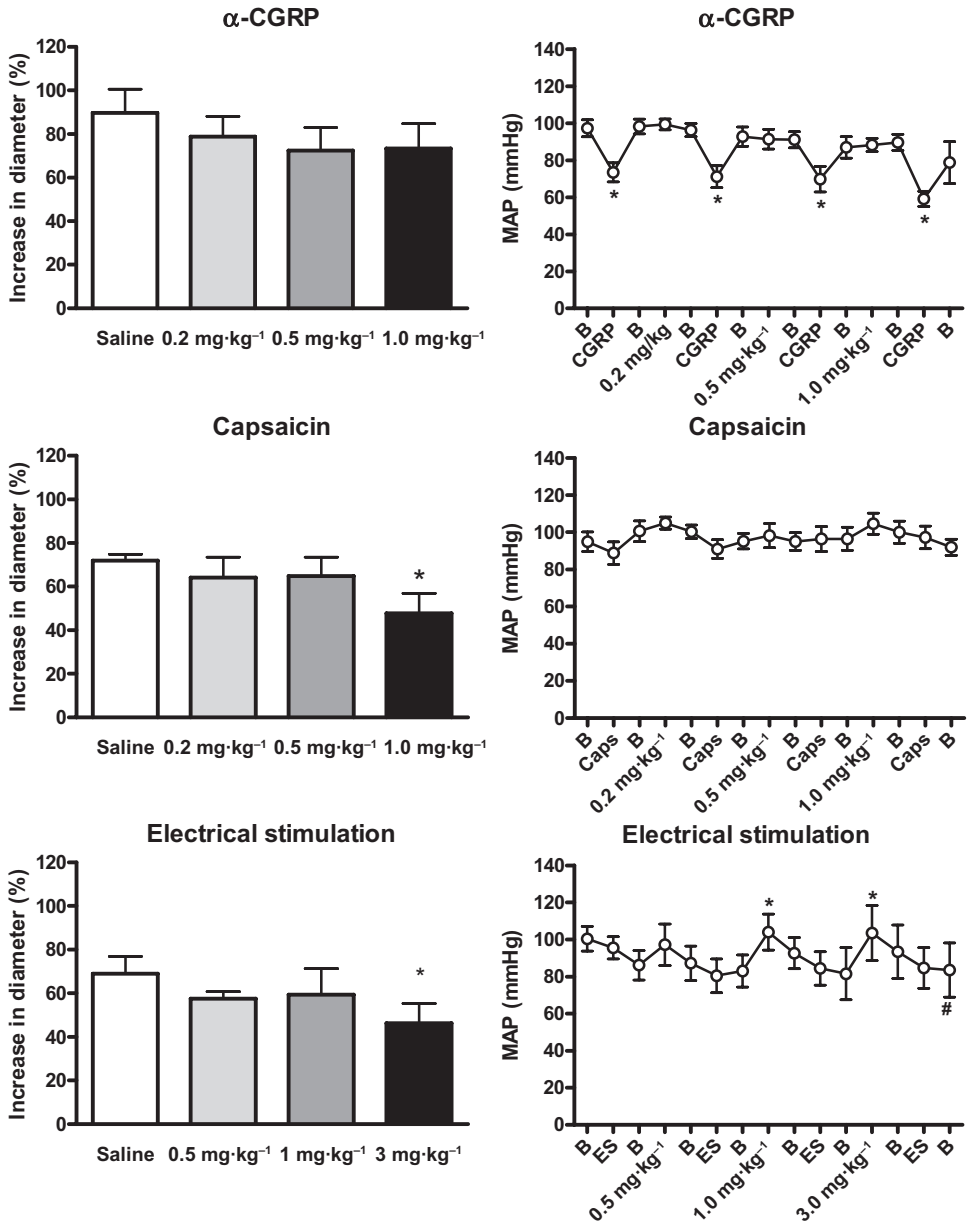


**Figure 2** Effect of increasing doses of MK801 on vasodilatation of the dural artery (percentage of increase in diameter, left panels) and mean arterial blood pressure (MAP) (mmHg, right panels) induced by α-CGRP (upper panels, $n = 8$), capsaicin (middle panels, $n = 7$) and periarterial electrical stimulation (lower panels, $n = 5$). B, baseline; Caps, 10 μg·kg$^{-1}$ capsaicin i.v.; CGRP 1 μg·kg$^{-1}$, calcitonin gene-related peptide i.v.; ES, periarterial electrical stimulation 150–250 μA. *$P < 0.05$ compared with the control or the corresponding baseline; $^{\#}P < 0.05$ compared with the baseline at the beginning of the experiment.

lower panel). Heart rate was not attenuated by this compound (data not shown).

*Effect of the kainate receptor antagonist LY466195 on dural artery vasodilatation, MAP and heart rate after treatment with α-CGRP, capsaicin and periarterial electrical stimulation*

At all doses used, LY466195 did not affect the vasodilatation induced by α-CGRP, capsaicin or electrical stimulation (Figure 4; left panels). Moreover, LY466195 did not affect dural artery diameter per se (data not shown). MAP (Figure 4; right panels) and heart rate (data not shown) were not changed in the presence of LY466195.

## Discussion and conclusions

The present study investigated the effects of the NMDA receptor antagonists ketamine and MK801, the AMPA receptor antagonist GYKI52466, and the kainate receptor antagonist LY466195 on vasodilatation of the rat dural artery induced by endogenous and exogenous α-CGRP, using intravital microscopy (Williamson *et al*., 1997). The release of endogenous CGRP was induced by either chemical stimulation with capsaicin or periarterial electrical stimulation. It should be noted that normal physiological conditions were kept stable throughout the experiment.

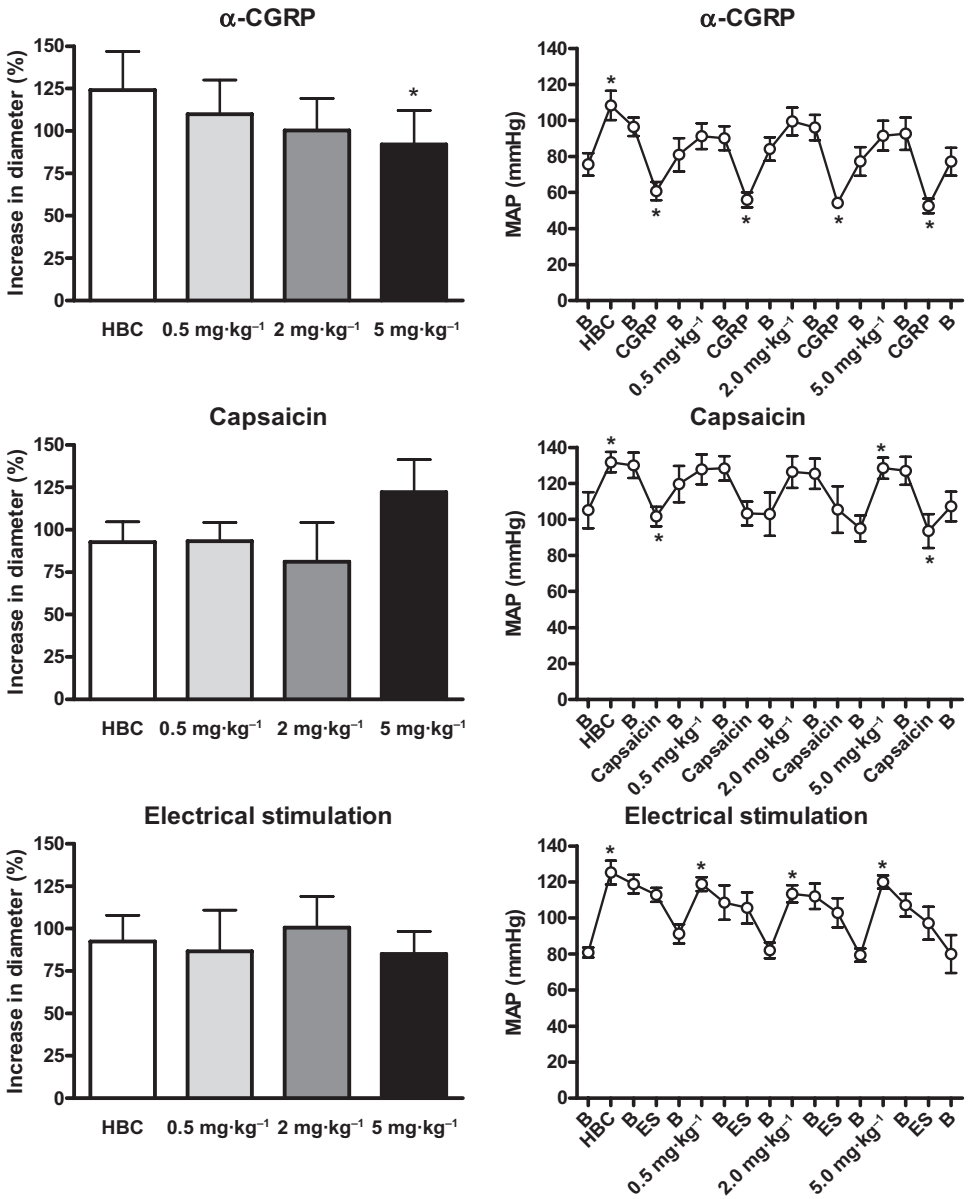


**Figure 3** Effect of increasing doses of GYKI52466 on vasodilatation of the dural artery (percentage of increase in diameter, left panels) and mean arterial blood pressure (MAP) (mmHg, right panels) induced by α-CGRP (upper panels, $n = 5$), capsaicin (middle panels, $n = 5$) and periarterial electrical stimulation (lower panels, $n = 4$). 2-hydroxypropyl-β-cyclodextrin (HBC) is the vehicle control of GYKI52466. B, baseline; Caps, 10 μg·kg$^{-1}$ capsaicin i.v.; CGRP 1 μg·kg$^{-1}$, calcitonin gene-related peptide i.v.; ES, periarterial electrical stimulation 150–250 μA. *$P < 0.05$ compared with the control or the corresponding baseline; $^{\#}P < 0.05$ compared with the baseline at the beginning of the experiment.

Apart from the implications discussed below, our results confirm that 1 μg·kg$^{-1}$ α-CGRP, 10 μg·kg$^{-1}$ capsaicin or 150 μA–250 μA periarterial electrical stimulation induced dural vasodilator responses in the rat, as previously reported by others (Petersen *et al*., 2004a; Juhl *et al*., 2007). Moreover, the maximal responses on the vasodilatation induced by α-CGRP and capsaicin were not different from those in our previous study (Gupta *et al*., 2007). However, the vasodilatation induced by electrical stimulation needed a higher voltage to cause the maximal vasodilatation. This difference may well be due to the difference in sex of the animals between both studies, which is in accordance with the higher density of CGRP-containing fibres in female than in male rats (Aukes *et al*., 2008). As expected, α-CGRP decreased the MAP due to stimulation of vascular CGRP receptors resulting in systemic vasodilatation (Arulmani *et al*., 2004). This vasodepressor response is unrelated to cardiac effects, as our results did not show any significant change on heart rate as compared with the saline control. In contrast, electrical stimulation was given locally and, consequently, did not affect the peripheral (systemic) vascular tone.

Activation of the ionotropic glutamate receptors leads to calcium influx in neurons and induces the release of vasoactive agents (Bhardwaj *et al*., 1997). Accordingly, we hypothesized that ionotropic glutamate receptor antagonists would block the influx of calcium, preventing CGRP release from

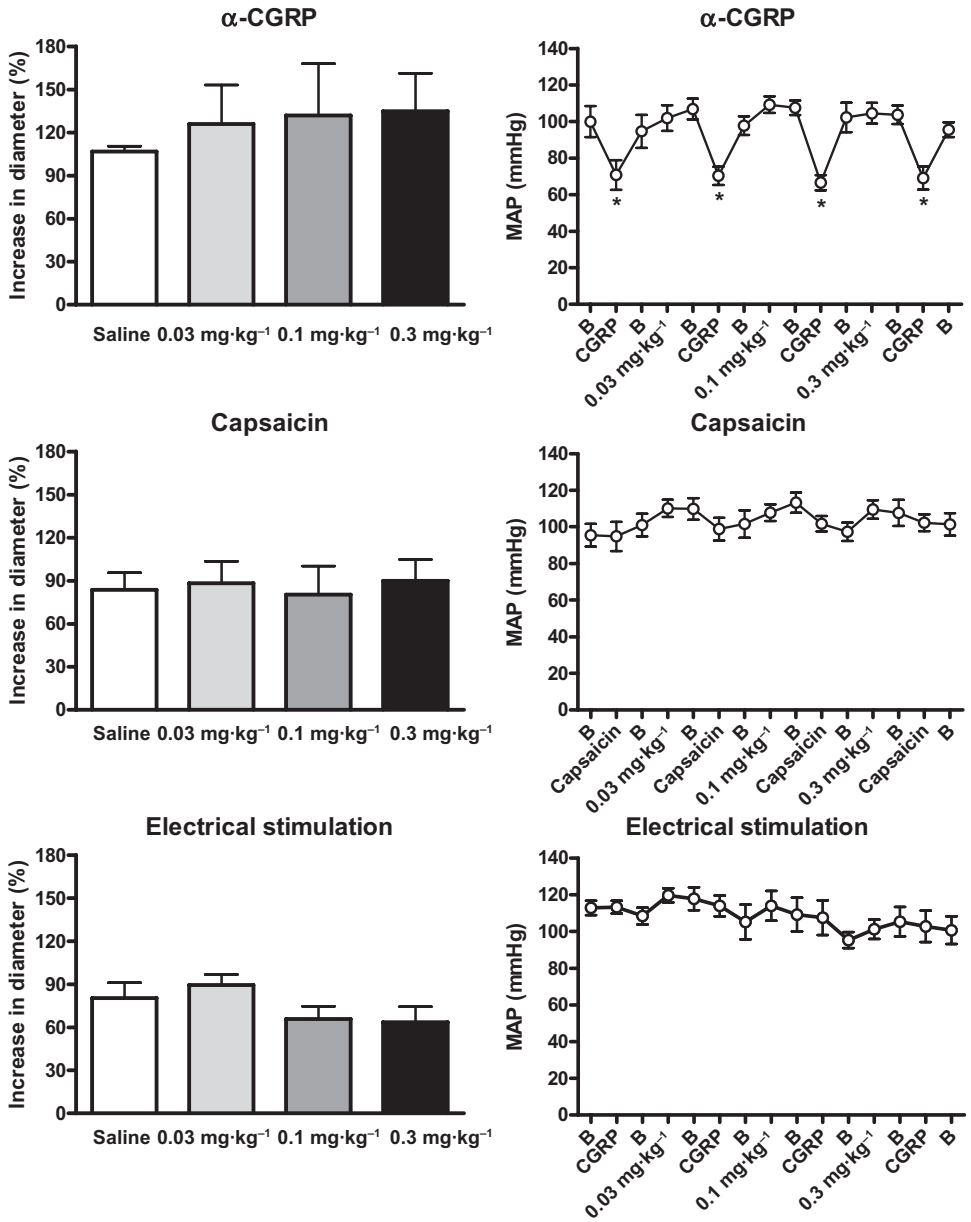


**Figure 4** Effect of increasing doses of LY466195 on vasodilatation of the dural artery (percentage of increase in diameter, left panels) and mean arterial blood pressure (MAP) (mmHg, right panels) induced by α-CGRP (upper panels, $n = 6$), capsaicin (middle panels, $n = 6$) and periarterial electrical stimulation (lower panels, $n = 5$). B, baseline; Caps, 10 μg·kg$^{-1}$ capsaicin i.v.; CGRP 1 μg·kg$^{-1}$, calcitonin gene-related peptide i.v.; ES, periarterial electrical stimulation 150–250 μA. *$P < 0.05$ compared with the control or the corresponding baseline; $^{\#}P < 0.05$ compared with the baseline at the beginning of the experiment.

periarterial nerve endings and would thus attenuate endogenous CGRP release, but not the response to exogenous α-CGRP. However, our results showed that the NMDA receptor antagonist ketamine not only attenuated the vasodilatation to endogenous CGRP (i.e. in response to capsaicin and perivascular electrical stimulation), but also that to exogenous α-CGRP. Moreover, the attenuation induced by increasing doses of ketamine on the vasodilatation to α-CGRP, capsaicin or periarterial electrical stimulation is not due to tachyphylaxis, as repeated treatment (up to four times) with α-CGRP, capsaicin or periarterial electrical stimulation in control experiments (without antagonists) was highly reproducible; in addition, in most cases there was no difference between the MAP value at the beginning and the end of the experiments, indicating that our model was stable during the whole duration of the experiments. However, the NMDA receptor antagonist ketamine induced a vasodepressor response in the α-CGRP and capsaicin groups, which is most likely due to the inhibitory effect of ketamine on heart rate (McGrath *et al.*, 1975). This finding is in accordance with our observations.

It could be argued that the effect of ketamine on the vasodilatation to α-CGRP and capsaicin may be an artefact if this high vasodepressor response might have decreased dural artery diameter. However, this possibility is highly unlikely

because ketamine, as the other antagonists, did not affect dural artery diameter *per se*. Furthermore, it has previously been shown that dural artery diameter in this model is not affected by a decrease in blood pressure up to 60 mmHg (Petersen *et al*., 2004b); in our experiments, this decrease did not reach 60 mmHg. Thus, the changes on the dural artery diameter induced by ketamine are most likely to be caused by a direct pharmacological effect. As ketamine also reduced the dural artery vasodilatation to exogenous α-CGRP, such an effect cannot be explained in terms of an interaction with the NMDA receptor. Although ketamine has been reported to be a selective antagonist for NMDA receptors (Anis *et al*., 1983), Ho and Su (2006) have shown that ketamine also attenuates sympathetic activity, which is not mediated by NMDA receptors. Further, ketamine has an effect on μ-opioid receptors (Mao, 1999), which has been shown to inhibit neurogenic dural vasodilation in this experimental model (Williamson *et al*., 2001). Therefore, the effect of ketamine on neurogenic dural vasodilation in this study might be also mediated via μ-opioid receptors. Finally, the possibility that ketamine might also have affinity for CGRP receptors, which could then contribute to the potential antimigraine efficacy of ketamine, cannot be excluded, although no receptor binding data are available to confirm or reject this possibility. Another possibility is that ketamine might reduce cranial vasodilatation via a still unknown mechanism, as ketamine has also been shown to reduce cerebral vasodilatation induced by isoflurane (Nagase *et al*., 2003).

Because of the limited pharmacological specificity of ketamine, we also studied the NMDA receptor antagonist, MK801. This antagonist did not affect the vasodilatation to exogenous α-CGRP, but it attenuated the vasodilatation to endogenous CGRP. This may suggest that such attenuation is via blockade of NMDA receptors which, in turn, results in less release of CGRP. The effect of MK801 on the vasodilatation to capsaicin and electrical stimulation is not an effect of MK801 *per se*, nor is it an artefact effect due to the changes in MAP, as MK801 only affected the MAP in the electrical stimulation group and not in the capsaicin group. As the vasodilatation to capsaicin is blocked by ketamine and MK801, and capsaicin stimulates neuronal vanilloid receptors, we cannot rule out that these antagonists also interact with vanilloid receptors. Clearly, further experiments which fall beyond the scope of the current study are required to investigate this possibility.

Interestingly, the AMPA receptor antagonist GYKI52466 only attenuated the vasodilatation induced by exogenous α-CGRP. Although the MAP values were increased in this group, the attenuation of the vasodilatation was not due to this effect, as MAP was also affected in the capsaicin and electrical stimulation group. This apparent increase in MAP by GYKI52466 could be rather attributed to its vehicle, namely HBC, because HBC alone also increased MAP. As GYKI52466 only attenuated α-CGRP-induced vasodilatation, but not the vasodilatation to endogenous CGRP, the possible anti-migraine effect of AMPA receptor antagonists may be unrelated to an interaction with the AMPA receptor. A possibility would be that GYKI52466 might behave like a CGRP scavenger. In this respect, Juhl *et al*. (2007) have recently shown that CGRP scavengers inhibit the vasodilatation induced by exogenous CGRP, but not the endogenously released CGRP in the rat closed cranial window model.

The kainate receptor antagonist, LY466195, did not affect the vasodilatation in the rat dural arteries induced by either endogenous or exogenous CGRP. This suggests that LY466195 does not attenuate the release of CGRP induced by capsaicin and periarterial electrical stimulation, nor does it affect the binding of CGRP to its receptor. This is in line with an earlier finding, where the kainate receptor antagonist UBP302 did not affect the dural vasodilatation induced by electrical stimulation or exogenous CGRP in a neurogenic dural vasodilatation model (Andreou *et al*., 2009). In addition, LY466195 blocked the calcium influx evoked by glutamate and attenuated the amount of c-fos positive cells after trigeminal neurone stimulation (Weiss *et al*., 2006). Moreover, LY466195 does not induce vasoconstriction *per se* nor does it affect the vasoconstrictor properties of sumatriptan in the rabbit saphenous vein (Weiss *et al*., 2006). In contrast, the anti-epileptic drug topiramate, which has affinity for the kainate receptor, attenuated the vasodilatation induced by electrical stimulation and NO infusion after 15 min, but not the CGRP-induced vasodilatation in the same intravital microscopy model (Akerman and Goadsby, 2005). Interestingly, LY466195 is effective in the treatment of migraine, but it also causes mild reversible visual distortions (Johnson *et al*., 2008). Hence, we cannot exclude the possibility that the antimigraine efficacy of LY466195 could involve a central effect unrelated to vascular CGRP-mediated pathways and/or its receptors. This possibility is reinforced by other findings showing that the trigeminocervical complex and the ventroposteromedial thalamic nucleus are important sites of action for the antimigraine effect of LY466195 (Andreou and Goadsby, 2009).

From our data, we suggest that NMDA receptor antagonists could be candidates for the treatment of migraine, because of blockade of vasodilatation in response to endogenously released CGRP in the dural artery. However, blockade of NMDA receptors, the activation of which mediates coronary vasodilatation (Nguyen-Duong, 2001), might also negatively affect cardiovascular protection by CGRP (Li and Peng, 2002; Chai *et al*., 2006; Li *et al*., 2008).

In addition, frequent and recreational ketamine used is known to be associated with cognitive impairments and elevated psychopathological symptoms (Morgan *et al*., 2009). However, another NMDA receptor antagonist, memantine, reduces headache frequency with uncommon and generally mild side effects (Bigal *et al*., 2008). The effects of AMPA receptor antagonists in migraine are still unknown. The AMPA/kainate receptor antagonist tezampanel is well tolerated and has no vasoconstrictor liability in clinical trials (Murphy *et al*., 2008). This finding supports our data that GYKI52466 did not affect vasodilatation induced by endogenous CGRP. However, due to the mixed AMPA and kainate receptor action of tezampanel, it is not clear which receptor is responsible for its anti-migraine effect (Murphy *et al*., 2008). Although GYKI52466 has not been tested clinically, our data suggest that it would not inhibit migraine via vascular mechanisms. Moreover, given the physicochemical properties of the glutamate receptor antagonists used in this study, we cannot rule out that these compounds are capable of crossing the blood brain barrier; therefore, they may have effects in the

central nervous system which could also contribute to the pharmacological profile of the mechanisms characterized in this study.

On the basis of our results, it is tempting to suggest that the kainate receptor antagonist, LY466195, may have antimigraine properties without cardiovascular side effects. Evidently, further studies on its cardiovascular safety, which fall beyond the scope of the present investigation, are warranted, as exemplified by the small increase in MAP after its high dose. This small vasopressor effect may be due to the moderate affinity of LY466195 for the NMDA receptor (Andreou and Goadsby, 2009). More specific kainate receptor antagonists may provide a neuronal and non-vascular migraine treatment. Obviously, it has to be kept in mind that ionotropic glutamate receptors are involved in several mechanisms in the brain and spinal cord; thus, blockade of these receptors may induce neurological side effects.

In conclusion, this study demonstrates that the different ionotropic glutamate receptor antagonists affect in a differential manner the vasodilatation induced by endogenous and exogenous CGRP. The NMDA receptor antagonists ketamine and MK801 are capable of inhibiting neurovascular CGRP release. This property may represent a therapeutic mechanism of action in the treatment of migraine, but might also result in cardiovascular side effects. As the AMPA receptor antagonist GYKI52466 did not affect CGRP release, potential antimigraine efficacy of AMPA receptor antagonists is unlikely to be related to a vascular mode of action. Similarly, the kainate receptor antagonist LY466195, which has demonstrated antimigraine efficacy, did not affect CGRP release and/or its vasodilator effects. Thus, its antimigraine action is most likely mediated via a central mechanism, not involving vascular CGRP-mediated pathways and/or receptors. This study extends the knowledge of ionotropic glutamate receptors in migraine, although further studies are required to explore the effect of glutamate and glutamate receptors in the pathophysiology of migraine.

## Acknowledgements

The authors would like to thank Dr Kirk Johnson (Lilly Research Laboratories, Eli Lilly and Company, IN, USA) for providing LY466195.

## Conflicts of interest

None to declare.

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