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Propranolol modulates trigeminovascular responses in thalamic ventroposteromedial nucleus: a role in migraine?

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Summary

Migraine is a common, debilitating condition affecting up to 15% of the population. The ventroposteromedial nucleus of the thalamus relays trigeminal sensory input to the primary somatosensory cortex. *In vivo* electrophysiological recordings were made from the cell bodies of thalamocortical relay neurons in rats. We investigated whether microiontophoretic ejection of β antagonists could inhibit thalamocortical activity in response to superior sagittal sinus (SSS) stimulation. We also studied 'postsynaptic' actions of these drugs through their modulatory actions on L-glutamate-evoked third order neuronal firing. Propranolol inhibited responses to SSS stimulation ($P < 0.001$) and L-glutamate ejection ($P < 0.001$). This was due to an action on β receptors as it could be partially reversed by co-ejection of isoproterenol (SSS, $P = 0.02$; L-glutamate, $P = 0.006$). Serotonin (5-HT) receptor antagonism did not contribute to propranolol's action since the 5-HT_{1A} receptor antagonist, (S)-WAY 100135 ($P = 0.2$), and the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 ($P = 0.6$), did not affect L-glutamate-evoked neuronal firing. Atenolol inhibited both responses (SSS, $P = 0.003$; L-glutamate, $P < 0.001$). The β_2 antagonist ICI 118,551 had no effect (SSS, $P = 0.9$; L-glutamate, $P = 0.4$), nor did the β_2 agonist procaterol (SSS, $P = 0.6$; L-glutamate, $P = 0.9$). SR 59230A (β_3 antagonist) also produced no significant inhibition (SSS, $P = 0.7$; L-glutamate, $P = 0.2$), indicating an inhibitory role for β_1 antagonists only. β Blockers therefore may exert some of their therapeutic effects in migraine through β_1 adrenoceptor antagonist actions in the thalamus. Thalamic involvement in migraine is attractive given the complex and widespread nature of the sensory disturbance.

Keywords: thalamus; migraine; β blockers; trigeminovascular

Abbreviations: SSS = superior sagittal sinus; VPM = ventroposteromedial

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Introduction

Clinical observations and electrophysiological studies suggest that migraine is a brain disorder (Goadsby *et al*., 2002). Abnormalities of sensory evoked potentials, including visual evoked potentials and auditory evoked cortical responses, in migraine both with and without aura suggest a dysfunction in sensory processing expressed, at least, at the cortical level (Ambrosini and Schoenen, 2003; Ambrosini *et al*., 2003). One potential explanation for these findings is that there is an underlying abnormality of activity in thalamocortical neurons (Vandenheede *et al*., 2003). Interestingly, it appears that these interictal electrophysiological abnormalities normalize after treatment with propranolol and this may be associated with a decrease in headache frequency (Sandor *et al*., 2000).

The ventroposteromedial (VPM) nucleus of the thalamus is responsible for relaying trigeminal sensory input to the primary somatosensory cortex (Steriade *et al*., 1997). The VPM is part of the ventrobasal somatosensory complex. Functional imaging studies have consistently shown activation of this region during spontaneous and evoked primary headache disorders including migraine (Kobari *et al*., 1989; Bahra *et al*., 2001; Matharu *et al*., 2004) and cluster headache (May *et al*., 1998). It is also activated in human models of trigeminal activation (DaSilva *et al*., 2002). Given that activation, or perceived activation, of the trigeminal system is a necessary component of typical migraine (Headache Classification Committee of the International Headache Society, 2004),

we have used electrical stimulation of the superior sagittal sinus (SSS), a known pain-producing intracranial structure (Feindel *et al*., 1960), to identify thalamocortical neurons. In this study, we have performed *in vivo* electrophysiological recordings from the cell bodies of thalamocortical relay neurons. Using electrical stimulation of the SSS as a search stimulus, neuronal cell bodies were identified by their responses to microiontophoretic pulses of L-glutamate. Microiontophoresis, with its technical advantage of precise anatomical localization at the level of individual neurons (Bloom, 1974), was used to study the effects of propranolol on these third order cells. Propranolol is a widely used drug for the prophylactic treatment of migraine (Welch, 1993; Silberstein and Goadsby, 2002); however, little is known of its mechanism or site of action. Given that microiontophoresis of β blockers was reported to have an inhibitory action on thalamic neurons (Phillis and Tebecis, 1967), we investigated whether propranolol may have an action on thalamocortical relay cells responding to SSS stimulation. In addition, we looked more specifically at 'postsynaptic' actions, which in practice refer to neuronal elements including dendrites and cell bodies distal to the synaptic cleft, of these drugs through their modulatory actions on L-glutamate-evoked third order neuronal firing. We report a robust β_1 adrenoceptor inhibition of activation of third order trigeminovascular nociceptive neurons that may in part explain the preventive action of propranolol in migraine.

Materials and methods

General procedure and surgery

All studies were conducted and terminated under general anaesthesia in accordance with a project licence issued by the Home Office of the UK under the Animals (Scientific Procedures) Act 1986. Male Sprague–Dawley rats (250–400 g) were anaesthetized with pentobarbitone sodium ('Sagatal', 60 mg/kg i.p., Rhone Merieux, Harlow, Essex, UK) and were prepared for physiological monitoring. The right femoral vein and artery were cannulated for administration of drugs and monitoring of blood pressure, respectively. The trachea was intubated before the head of the animal was fixed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Core temperature was monitored and maintained using a rectal thermistor probe linked to a homeothermic heater blanket system (TC-1000, CWE Inc., Ardmore, PA). A midline incision was made and a small craniotomy was performed to expose the SSS and the right parietal region above the VPM. The dura mater was incised and reflected to expose the cortex before the area was covered in mineral oil to prevent desiccation of the cortex and provide electrical insulation. Anaesthesia was maintained with supplementary doses of pentobarbitone sodium (30 mg/kg). The depth of anaesthesia was not monitored by EEG in these experiments. We did, however, use physical signs and receptive field properties to estimate the stages of anaesthesia as described by Guedel. Though physical signs are less discriminating than the EEG for determining the stage of anaesthesia, most of our recording probably took place within stages III-3 and III-4 (Friedberg *et al*., 1999). The animals were paralysed with pancuronium bromide (Pavulon, Organon, Cambridge, UK, 1 mg/kg initially, maintenance with 0.4 mg/kg) and ventilated (7025 Rodent Ventilator, Ugo Basile, Varese, Italy) artificially with oxygen-enriched room air. End-tidal CO_2 was continuously monitored and maintained between 3.5 and 4.5%. A sufficient depth of anaesthesia was judged from the absence of withdrawal reflexes, when the blocking agent was periodically allowed to wear off, and gross fluctuations in blood pressure during neuromuscular blockade. At the end of each experiment, animals were given a lethal dose of pentobarbital sodium ('Lethobarb', 200 mg/ml; 1 ml, Fort Dodge Animal Health, Southampton, UK).

Stimulation and recording

Two platinum wire stimulating electrodes were placed onto the SSS taking care not to make contact with the cortex. Trigeminal afferents were activated by stimulating the SSS with square-wave pulses using the lowest possible stimulus intensity to active trigeminovascular afferents (Grass Instruments S88 Stimulator, West Warwick, RI; 6–30 V, 250 μs, 0.5 Hz). This was done to reduce the risk of current spread to the cortex and corticothalamic activation (which may have modulatory actions on thalamocortical neurotransmission) that may occur at higher stimulus intensities. The voltage necessary to activate sensory afferents varied between experiments, and we attributed this to differences in depth of anaesthesia and electrical contact. Extracellular recordings were made from neurons in the region of the VPM based on the stereotaxic coordinates derived from the atlas of Paxinos and Watson (1998), using microiontophoretic combination electrodes (Carbostar 7S, Kation Scientific, Minneapolis, MN) consisting of a seven-barrelled glass pipette incorporating a carbon fibre recording electrode (impedance at 1 kHz = 0.4–0.8 MΩ), with a tip length of ∼10 μm. The electrode was advanced into the VPM in 5 μm steps using a hydraulic microdrive (Kopf Instruments, Tujunga, CA). The signal from the recording electrode was fed via a headstage amplifier (NL100AK, Neurolog, Digitimer, Herts, UK), AC pre-amplifier (Neurolog NL104A, gain ×1000) and noise eliminator (Humbug, Quest Scientific, North Vancouver, BC, Canada) to Neurolog filters (NL125, bandwidth ∼700–10 kHz) and thence to a second stage variable amplifier (Neurolog NL106, gain ∼×50–90). This signal was also fed to a gated amplitude discriminator (Neurolog NL201). The filtered and amplified signal was displayed on an oscilloscope (OS 7020A, Goldstar Precision Co., Korea) and also fed to an audio amplifier (Neurolog NL120) to assist with the discrimination of single unit activity from background noise. A personal computer (Dell Computer Corporation, Berks, UK) running Spike 4.15 software (Cambridge Electronic Design, Cambridge, UK) was used to collect and analyse data.

To record the response of units to electrical stimulation of the SSS, post-stimulation histograms were constructed on-line and saved to disc. Each histogram was constructed from a series of 1 ms bins. Neuronal action potential firing in response to microiontophoresis of L-glutamate was analysed as cumulative rate histograms, the data being collected into successive 1 s bins. Physiological parameters (blood pressure, end-expired pCO_2 and core temperature) were monitored continuously during the experiments and recorded onto tape (PCM-R300, Bio-Logic, Claix, France).

Receptive fields

Neurons activated by electrical stimulation of the SSS were identified as the electrode was slowly advanced through the VPM. Once a cell

was located, receptive fields were sought on the contralateral craniofacial region. For those cells with a receptive field not involving the vibrissae, non-noxious stimuli were provided by gentle brushing with a blunt probe, while noxious stimuli were produced by pinching with toothed forceps. Cells were classified as low threshold mechanoreceptive (LTM) if they responded only to non-noxious stimuli, as nociceptive specific (NS) if they responded to noxious stimulation only, and as wide dynamic range (WDR) if they responded to both. WDR cells generally had an increase in the rate of firing in response to noxious stimuli (Hu *et al*., 1981). The majority of cells, however, had receptive fields limited to the vibrissae and furry buccal pad, which is not surprising given the large volume dedicated to the representation of vibrissae within the rat VPM (Vahle-Hinz and Gottschaldt, 1983). With such cells, each vibrissa was manipulated with a fine needle mounted on a probe, taking care not to deflect surrounding vibrissae, to find the whisker(s) that triggered a response upon deflection.

Drugs and microiontophoresis

Micropipette barrels were filled with: L-glutamate monosodium 0.2 M, pH 8.0 (Sigma, St Louis, MO); (*S*)-(−)-propranolol 0.1 M, pH 4.5 (Tocris Cookson, Avonmouth, UK); (*S*)-(−)-atenolol 0.025 M, pH 4.5 (Tocris Cookson); ICI 118,551 hydrochloride 0.01 M, pH 4.5 (Tocris Cookson); SR 59230A hydrochloride 0.05 M, pH 4.5 (Tocris Cookson); (−)-isoproterenol hydrochloride 0.2 M, pH 4.5 (Sigma); procaterol hydrochloride 0.1 M, pH 4.5 (Tocris Cookson); GR 127935 hydrochloride 0.02 M, pH4 (Tocris Cookson); (S)-WAY 100135 0.01 M, pH 4.5 (Tocris Cookson); pontamine sky blue [Gurr 6BX, BDH Laboratory Supplies, Poole, UK; 2.5% (w/v) in 100 mM sodium acetate, pH 6.5]; NaCl 0.2 M, pH 4.5 (for controls), and 1.0 M NaCl for automated current balancing. Propranolol, atenolol, ICI 118,551, SR 59230A, isoproterenol, (S)-WAY 100135, GR 127935 and procaterol were ionized as cations and retained in the iontophoretic barrels with small negative currents (5 nA). L-Glutamate and pontamine sky blue were ionized as anions and retained with small positive currents (5 nA). Ejection currents in directions opposite to the retaining currents were used (10–90 nA). Sodium ions were ejected as the control. Na^+ referred to as the control represents the ejection of both sodium and H^+ ions, since the pH of the saline was adjusted by the addition of 0.01 M HCl, and therefore H^+ will be ejected as well. Microiontophoretic barrels had resistances of 20–150 MΩ. A microiontophoresis current generator (Dagan 6400, Dagan Corporation, MN) provided ejecting and retaining currents for each test substance. The ejection current of L-glutamate was titrated for each cell to produce a sustainable firing rate that was comparable with the response elicited by stimulation of the receptive field, before it was intermittently ejected in trains of 5 s pulses on a 50% eject/retain cycle. To test the effect of β adrenergic antagonists, each drug was co-ejected with L-glutamate, as was the control at the same current and duration. In studies where we wished to investigate the ability of isoproterenol to block the effect of propranolol, the action of propranolol on both responses was studied. The cell was allowed to recover before both propranolol and isoproterenol were ejected simultaneously with L-glutamate. The inhibition produced by agonist ± antagonist over the same time scale was then compared though not necessarily in this order.

The location of the recording site was either obtained by direct marking of the site by ejection of pontamine sky blue (2 μA, 10 min), or estimated from marked reference points and microdrive readings. They are reconstructed, approximating their original positions into two suitable coronal planes for diagrammatic purposes (Fig. 1A). Upon termination of the experiment, the tissue was fixed in neutral buffered 10% formalin, sectioned (40 μm sections) and stained with neutral red (Fig. 1B).

Statistical analysis

The effect of a given compound on the neuronal response to microiontophoretic ejection of L-glutamate was studied using cohorts of five successive pulses of L-glutamate. The mean firing rate (Hz) during each of the 5 s ejection periods was calculated using the 'Spike' software package. The response of each cell under three test conditions was examined: (i) L-glutamate (baseline); (ii) L-glutamate co-ejected with the control (Na^+); and (iii) L-glutamate co-ejected with β antagonist. In the case of studies using the agonist isoproterenol, four test conditions existed: (i) baseline; (ii) isoproterenol; (iii) propranolol; and (iv) propranolol + isoproterenol. Five pulses of L-glutamate were analysed as the response of a cell could vary between individual pulses. As a result, a repeated measures analysis of variance (ANOVA; SPSS v 11.5, Chicago, IL) was computed with two factors: drugs and repeats, to determine within- and between-drug effects and interactions. Where the assumption of sphericity with regards to the factor of repeats was violated, we made corrections for the degrees of freedom according to the Greenhouse–Geisser calculation. *F* and *P* values are cited. Results were pooled and the mean response during each test condition was calculated (±SEM) for each of the treatment groups.

In the case of the response to SSS stimulation, the baseline response probability was obtained following 50 stimuli. We calculated the response probability following SSS stimulation by constructing post-stimulus histograms displaying the number of spikes per 1 ms bin. The main body of the response was found to have a latency of 10–20 ms following the stimulus. The mean number of spikes per bin from the main body of the response was calculated and converted to a percentage by multiplying by 2. The baseline response probability was calculated from up to three trials and repeated following treatment with drug and then control. The results were compared with a paired value *t* test. *P* values were assessed at the 0.05 level of significance.

Results

A total of 76 cells were studied (receptive fields, 11 WDR, nine LTM, one NS, 55 vibrissae; number of vibrissae, 2.8 ± 0.2, mean ± SEM). Cells responded with an increased probability of firing to electrical SSS stimulation with an average latency of 10 ms to the onset and 15 ms to the peak of the response (see Fig. 2A and B for representative examples). An additional shorter latency peak was occasionally observed that may represent corticothalamic afferents. To be included in the analysis, the following requirements had to be met: a probability of firing of >35% (77 ± 2%, mean ± SEM) (Nagler *et al*., 1973); a craniofacial RF; and a stable sustained response to pulsed ejection of L-glutamate. Only cell bodies were recorded, which were characterized by their biphasic unfiltered action potential morphology and increased firing response to L-glutamate (Fries and Zieglgansberger, 1974). This study concentrated on cells in the vicinity of VPM,

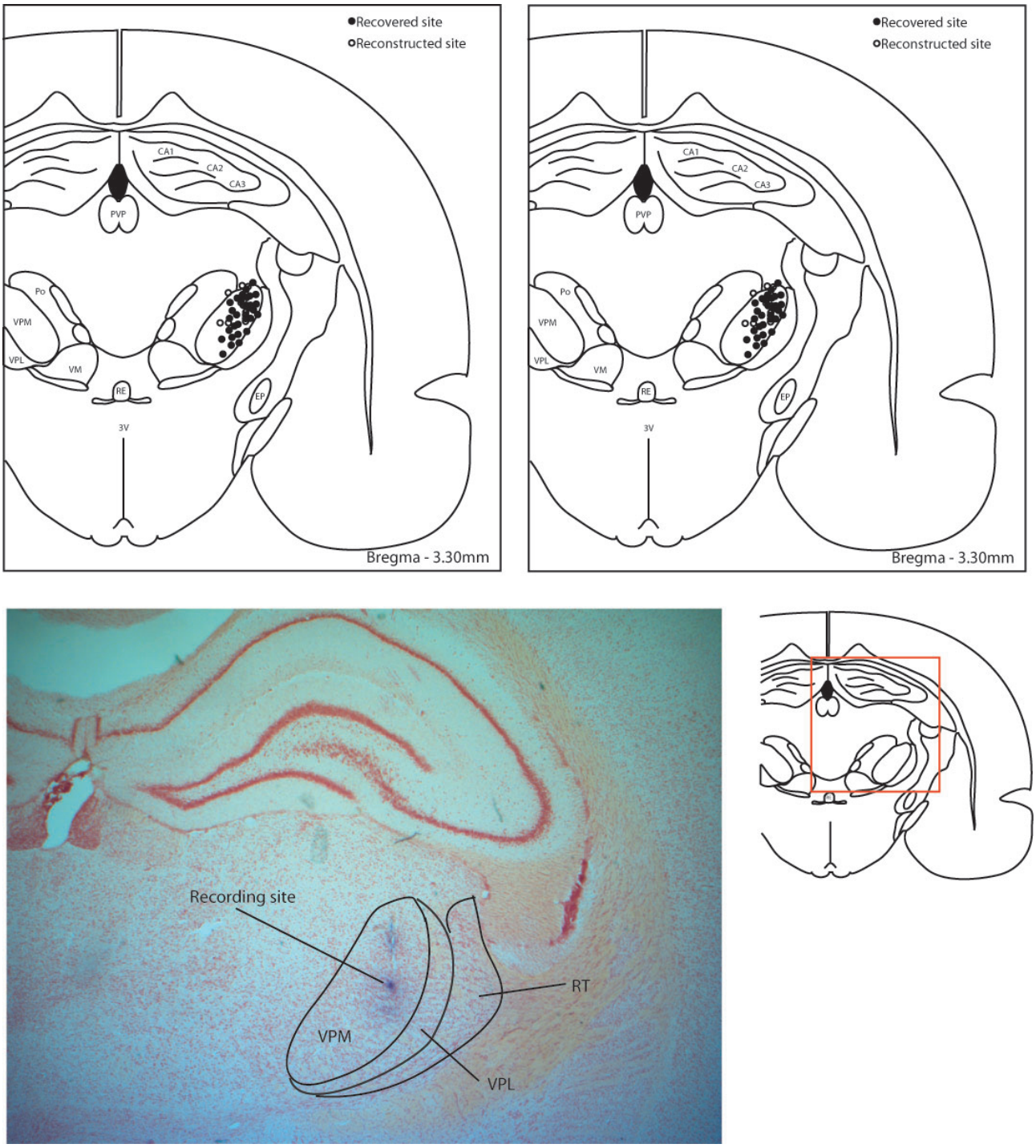

Fig. 1 Reconstruction of recording sites from the region of the ventroposteromedial thalamus (VPM) represented in two coronal planes (**A**). Sites found within these planes are reconstructed to approximate their locations (Paxinos and Watson, 1998). A recording site is shown marked by ejection of pontamine sky blue (**B**). The tissue was counter-stained with neutral red (taken with an AxioCam MRc5 camera mounted on an Axioplan microscope (Zeiss, Germany) at 2.5 × 10 magnification). CA1-3 = fields CA1-3 of Ammon's horn; EP = entopeduncular nucleus; Po = posterior complex; PVP = paraventricular nucleus; RE = reuniens nucleus; RT = reticular nucleus; VM = ventromedial nucleus; VPL = ventroposterolateral nucleus; VPM = ventroposteromedial nucleus.

where histological and electrophysiological (such as RF characteristics) evidence suggested, otherwise cells were not included in the analysis. The results are presented in detail in Tables 1–3.

Effects of propranolol

Propranolol was able to reduce reversibly the probability of thalamocortical neuron firing in response to electrical stimulation of the SSS (n = 13, $P < 0.001$) in comparison with

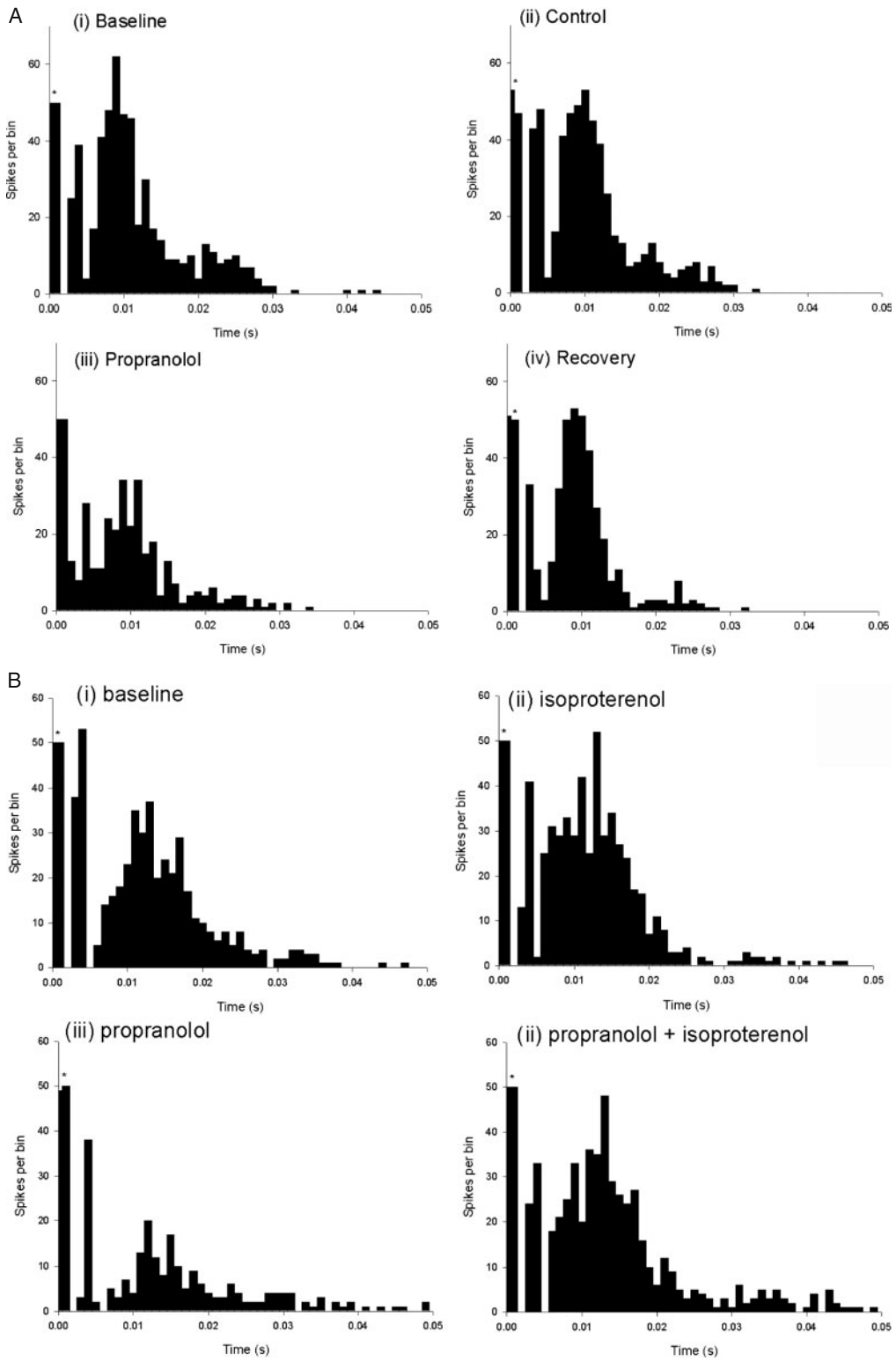

Fig. 2 Post-stimulus histograms demonstrating the response of a representative thalamocortical neuron to electrical stimulation of the superior sagittal sinus (SSS). The number of spikes per 1 ms bin was recorded (sweep length 50 ms) over 50 stimuli for the same neuron under the four test conditions (**A**): (i) baseline; (ii) control (ejection of Na^+ and H^+ ions at the same current as propranolol and for the same duration); (iii) propranolol (the period of ejection varied from 1 to 4 min); and (iv) recovery. The inhibitory action of propranolol could be antagonized by co-ejection of the non-selective β-agonist isoproterenol on every cell tested (**B**). In some animals, isoproterenol appeared to have a facilitatory effect on the response of the thalamocortical cell to SSS stimulation that was not significant for the cohort as a whole. In general the latency to the first response was ~10 ms, with the peak of the response at 15 ms. We often observed a peak at 5 ms which may represent activation of corticothalamic afferents; this was not affected by drug ejection (*stimulus artefact).

Table 1 *Effects of β adrenergic antagonists on the L-glutamate-evoked neuronal response*

β Adrenoceptor antagonist	Baseline	Control	Drug
Propranolol	45.7 ± 6.4	45.5 ± 9.5	12.7 ± 4.5*
Atenolol	98 ± 12.4	81 ± 12.5	29.8 ± 7.8*
ICI 118,551	61.5 ± 13	39.8 ± 9.1	41.5 ± 8.8
SR 59230A	84.5 ± 20	68.6 ± 15.1	82.7 ± 18.4

Action potential firing rates (Hz) of third order neurons in response to ejection of 5 s pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result ± SEM (*P was calculated < 0.05).

β Adrenoceptor antagonist	Drug versus control	Drug versus baseline	Baseline versus control
Propranolol	$F(1,4) = 37.8$, $P < 0.001$	$F(1,4) = 114.9$, $P < 0.001$	$F(1,2.5) = 0.05$, $P = 0.95$
Atenolol	$F(1,4) = 45.8$, $P < 0.001$	$F(1,4) = 83.3$, $P < 0.001$	$F(1,1.8) = 3.2$, $P = 0.1$
ICI 118,551	$F(1,4) = 0.8$, $P = 0.4$	$F(1,2.1) = 3.9$, $P = 0.1$	$F(1,4) = 5$, $P = 0.06$
SR 59230A	$F(1,1.2) = 0.2$, $P = 0.2$	$F(1,1.5) = 0.003$, $P = 0.9$	$F(1,1.3) = 8$, $P = 0.07$

F and P values calculated for each treatment group comparing the effects of β adrenergic antagonists and control during co-ejection with L-glutamate. The effect of control ejection on the baseline response was also studied.

Table 2 *Effects of β adrenergic antagonists on the response to SSS stimulation*

β Adrenoceptor antagonist	Baseline	Control	Drug
Propranolol	69 ± 3.8%	76.4 ± 5.6%	39 ± 3.6%*
Atenolol	84.4 ± 9%	80.2 ± 10.2%	42.4 ± 7.2%*
ICI 11,8551	94.4 ± 5%	93.8 ± 6.8%	90.6 ± 5.2%
SR 59230A	90.8 ± 7.8%	87.6 ± 8.4%	86.4 ± 9.8%

Response probabilities (%) of thalamocortical neurons following electrical stimulation of the SSS. When a neuron was located, a series of 50 stimuli were delivered to the SSS and the response probability (as a percentage) calculated. Results under the three test conditions were pooled and are presented as the mean ± SEM (*P was calculated < 0.05).

β Adrenoceptor antagonist	Drug versus control	Drug versus baseline	Baseline versus control
Propranolol	$t(12) = 9$, $P < 0.001$	$t(12) = 9.8$, $P < 0.001$	$t(12) = 1.7$, $P = 0.1$
Atenolol	$t(5) = 5.3$, $P = 0.003$	$t(5) = 7.9$, $P = 0.001$	$t(5) = 2.1$, $P = 0.09$
ICI 118,551	$t(5) = 0.2$, $P = 0.9$	$t(5) = 2.1$, $P = 0.09$	$t(5) = 0.8$, $P = 0.4$
SR 59230A	$t(5) = 0.4$, $P = 0.7$	$t(5) = 1.3$, $P = 0.3$	$t(5) = 0.8$, $P = 0.5$

Paired sample t-test analysis of the effects of serotonergic agonists on the response probabilities of thalamocortical neurons following electrical stimulation of the SSS.

control ejection (Figs 2A and 3A). Similarly, it was able to inhibit the response of relay neurons to ejection of L-glutamate ($n = 12$, $P < 0.001$). The inhibition was often prolonged (6–10 min) but reversible (Figs 4A and 5A). We did not examine specifically the effects of propranolol on spontaneous neuronal activity.

This response appeared to be due largely to β adrenoceptor antagonist properties of propranolol because the inhibition could be reduced by concurrent ejection of the β agonist isoproterenol. The reduction in firing probability in response to SSS stimulation was significantly attenuated ($n = 7$, $P = 0.02$) in comparison with propranolol alone (Figs 2B and 3B). The neuronal firing rate in response to L-glutamate was also increased by co-ejection of isoproterenol ($n = 5$, $P = 0.006$) over that of propranolol itself (Figs 4B and 5B). Isoproterenol did not produce a significant increase in neuronal firing in each of the two types of experiment ($P = 0.4$, $P = 0.6$). Antagonism of 5-HT_{1A} receptors with (S)-WAY 100135 [$n = 7$, $F(1,4) = 2.6$, $P = 0.2$] and of 5-$HT_{1B/1D}$ receptors using GR 127935 [$n = 5$, $F(1,1.2) = 0.3$, $P = 0.6$] did not significantly inhibit the response to L-glutamate.

β Receptor selectivity of inhibition produced by antagonists

The β_1 receptor antagonist atenolol was able to produce inhibition on both responses in a similar fashion to propranolol (SSS, $n = 6$, $P = 0.003$; L-glutamate, $n = 9$, $P < 0.001$) (Figs 4A, and 6A and B). The selective β_2 antagonist ICI 118,551, however, was not able to produce any inhibition in comparison with ejection of Na^+ (SSS, $n = 6$, $P = 0.9$; L-glutamate, $n = 7$, $P = 0.4$) (Fig. 6A and B). Given the low solubility of ICI 118,551, to ensure that a possible action of β_2 receptors was not missed, we studied the effects of microiontophoresis of the potent β_2 agonist procaterol. This again had no effect on the response to SSS stimulation [$t(6) = 0.5$, $P = 0.9$] or L-glutamate ejection [$n = 6$, $F(1,4) = 0.01$, $P = 0.9$]. SR 59230A, a selective β_3 receptor antagonist, also had no demonstrable effect on the response probability to SSS stimulation ($n = 6$, $P = 0.7$) or firing rate resulting from L-glutamate ejection ($n = 4$, $P = 0.2$) (Fig. 6A and B).

Discussion

The data demonstrate a robust and reproducible inhibitory effect for β_1 adrenoceptor antagonist activity in VPM thalamic neurons responding to nociceptive trigeminovascular input. There is no effect of β_2 or β_3 adrenoceptor antagonists in our model. These data offer a plausible locus of activity for propranolol via β_1 receptor antagonism in the trigeminovascular pain pathway, and a reasonable basis for considering the thalamus as a possible target for preventive treatments in migraine. In this context, it is noteworthy that models of neurogenic dural vasodilation (Akerman *et al*., 2001) and plasma protein extravasation (Markowitz *et al*.,

Table 3 *Antagonism of the actions of propranolol on the L-glutamate and SSS stimulation response*

Neuronal response	Baseline	Isoproterenol	Propranolol	Propranolol + isoproterenol
(i) L-Glutamate microiontophoresis	93.7 ± 16.4	87.2 ± 8.4	21.9 ± 2.4*	57.4 ± 5.5†
(ii) Electrical SSS stimulation	77.8 ± 5.2%	69.6 ± 4.1%	40.2 ± 6.6%*	67.8 ± 6.2%†

(i) Action potential firing rates (Hz) of third order neurons in response to ejection of 5 s pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result ± SEM. (ii) Response probabilities (%) of thalamocortical neurons following electrical stimulation of the SSS. When a neuron was located, a series of 50 stimuli were delivered to the SSS and the response probability (as a percentage) calculated. Results under the three test conditions were pooled and are presented as the mean ± SEM (*†P was calculated < 0.05, *relative to the baseline response, †relative to the propranolol response).

Neuronal response	Propranolol versus baseline	Isoproterenol versus baseline	Propranolol + isoproterenol versus propranolol	Propranolol + isoproterenol versus baseline
(i) L-Glutamate microiontophoresis	$F(1,4) = 21.3$, $P = 0.01$	$F(1,4) = 0.4$, $P = 0.6$	$F(1,4) = 28.4$, $P = 0.006$	$F(1,4) = 7.8$, $P = 0.05$
(ii) Electrical SSS stimulation	$t(6) = 8.0$, $P < 0.001$	$t(6) = 0.8$, $P = 0.4$	$t(6) = 3.0$, $P = 0.02$	$t(6) = 1.5$, $P = 0.2$

(i) F and P values calculated for each treatment group comparing the effects of β adrenergic antagonists and control during co-ejection with L-glutamate. The effect of control ejection on the baseline response was also studied.
(ii) Paired sample t-test analysis of the effects of serotonergic agonists on the response probabilities of thalamocortical neurons following electrical stimulation of the SSS.

1988) failed to demonstrate a peripheral action for propranolol, which makes a central action even more attractive.

The SSS is densely innervated by A and C fibres (Andres *et al*., 1987) and SSS stimulation has been used extensively as a model for the study of trigeminal nociception (Goadsby, 1999). Stimulation of the SSS in humans causes pain that is referred to the territory of the ophthalmic (first, V_1) division of the trigeminal nerve, thus mimicking the distribution of pain most often experienced in migraine (Penfield and McNaughton, 1940; Ray and Wolff, 1940). Mechanical stimulation of the dura mater produces inconsistent effects in humans (Wolff, 1948), and localized chemical stimulation is essentially untested. Thus, from a translational neuroscience viewpoint, there is clear evidence in humans that electrical stimulation reproduces at least the sensory modality of interest in migraine, pain. SSS stimulation in the cat has been a good model for predicting the efficacy of drugs as therapeutic interventions against migraine in humans (De Vries *et al*., 1999; Goadsby, 1999). SSS stimulation does activate cells in several thalamic nuclei in addition to the VPM, including the posterior complex, intralaminar complex, zona incerta and nucleus submedius (Davis and Dostrovsky, 1988; Zagami and Lambert, 1990; Angus-Leppan *et al*., 1995), although the VPM appears to convey the sensory discriminative aspects of trigeminal sensation (Steriade *et al*., 1997).

Local ejection of propranolol by microiontophoresis was able to inhibit the response to this stimulus (which is nociceptive in humans). Furthermore, propranolol was also able to modulate the response of third order neurons to L-glutamate, indicating a probable postsynaptic site of action. Unfortunately, we did not examine the effect of propranolol on spontaneous neuronal firing so no conclusions can be drawn regarding tonic catecholaminergic influences on VPM neurons. Propranolol, however, in addition to its actions on β adrenoceptors, acts as an antagonist at rat 5-$HT_{1A/1B/1D}$ receptors (Nishio *et al*., 1989). It may also have membrane-stabilizing properties. Concurrent ejection of isoproterenol, a non-specific β agonist, partially antagonized the inhibitory actions of propranolol on both the response to SSS stimulation and L-glutamate ejection. Ejection of the selective 5-HT_{1A} and $5HT_{1B/1D}$ antagonists, however, had no appreciable effects. These findings would suggest that the inhibitory action of propranolol was mediated by its antagonism of β adrenoceptors, specifically the β_1 type. This is supported by the actions of the selective β_1 antagonist atenolol, which had similar effects to propranolol, while the β_2 receptor antagonist (ICI 118,551) and β_3 receptor antagonist (SR 59230A) produced no appreciable inhibition.

The ventrobasal complex is densely innervated by noradrenergic fibres arising largely from the ipsilateral locus coeruleus (Kobayashi *et al*., 1975; Ishikawa and Tanaka, 1977; Westlund *et al*., 1990). Noradrenaline is found in the thalamus and its release can be triggered by activation of locus coeruleus neurons (Enna *et al*., 1977; Brun *et al*., 1993). Both α and β adrenoceptors are found in the somatosensory thalamus. β Receptors have been demonstrated in rat and human tissue, though it would appear that β_1 receptors are the predominant β receptor subtype in the ventrobasal complex (Rainbow *et al*., 1984; Pazos *et al*., 1985; Reznikoff *et al*., 1986; van Waarde *et al*., 1997). This would be consistent with our electrophysiological findings. Microiontophoresis of noradrenaline in the feline ventrobasal complex produced both facilitatory and inhibitory responses (Phillis and Tebecis, 1967), although it is not clear if this reflected a differential

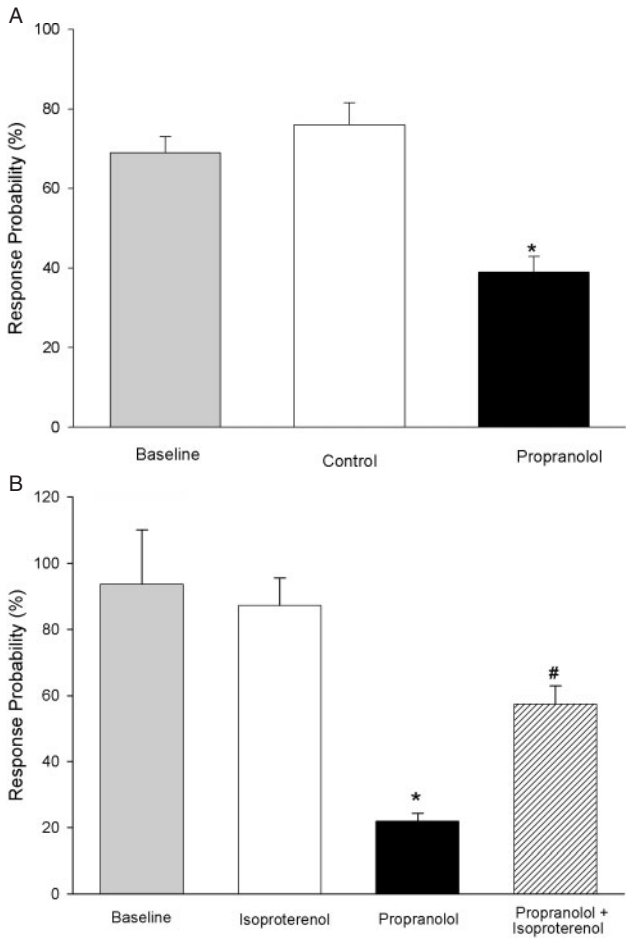

Fig. 3 The overall effect of propranolol ejection on the mean (±SEM) response probabilities of thalamocortical neurons following electrical stimulation of the superior sagittal sinus is shown (**A**). The response probabilities of neurons following ejection of propranolol compared with that following propranolol (at the same ejection current and duration) co-ejected with isoproterenol are shown (**B**). Propranolol's inhibitory effect was significantly antagonized by co-ejection of isoproterenol (**B**). Isoproterenol itself did not have a significant effect in comparison with the baseline (*$^{\#}P < 0.05$; *relative to the baseline and control, $^{\#}$relative to propranolol alone).

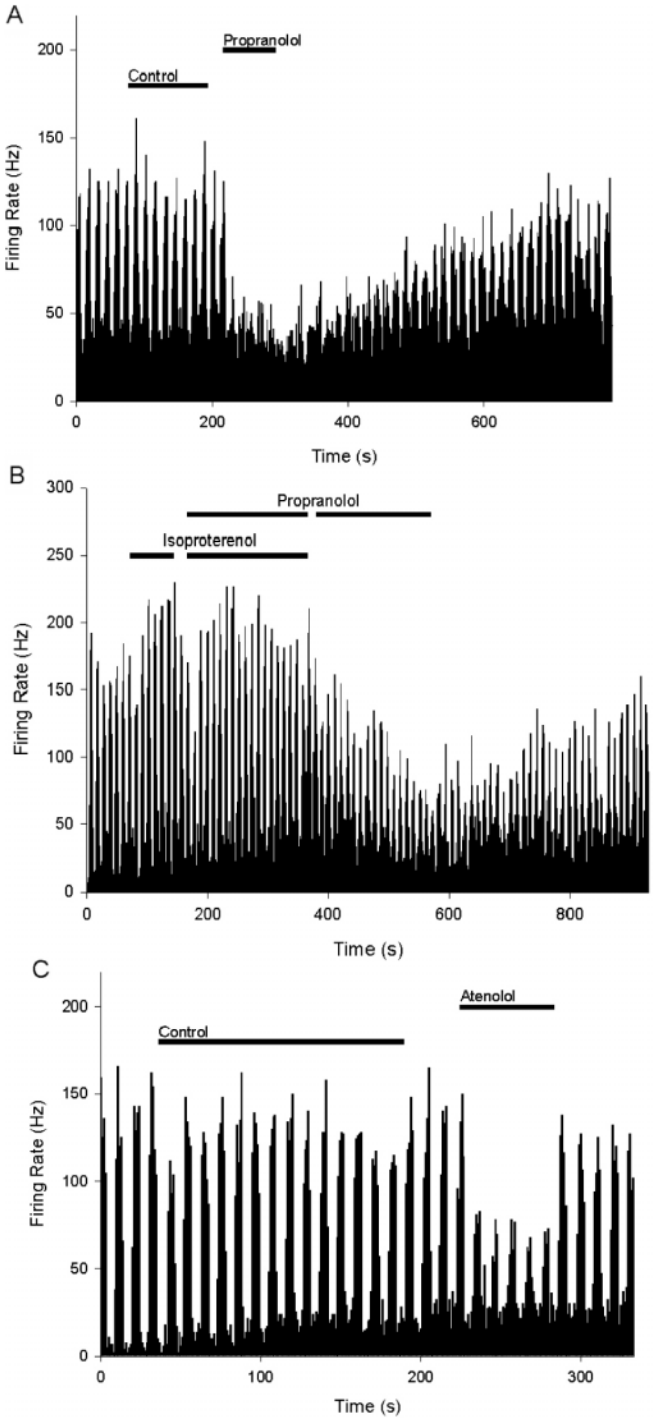

Fig. 4 Effects of β-antagonists on the firing rates of a third order relay neuron to pulsed ejection of L-glutamate. Diagrams are constructed from cumulative rate histograms of neuronal firing (1 s bin width) as L-glutamate was ejected in 5 s pulses on a 50% ejection/retention cycle. After a stable baseline firing rate response was established, comparable with that following stimulation of the receptive field, drugs were also co-ejected. The effects of propranolol and control (Na^+ and H^+ ions)—both ejected at the same ejection current (55 nA) and for approximately the same duration (70 and 100 s, respectively)—were compared (**A**), also antagonism of the effects of propranolol by co-ejection of isoproterenol (**B**) and the inhibitory action of the selective β_1 antagonist atenolol (**C**).

action on thalamocortical cells and intrinsic interneurons. It has a purely facilitatory action on afferent excitation of relay neurons in the lateral geniculate nucleus (Rogawski and Aghajanian, 1980). A potential advantage of utilizing a rat model is that its VPM lacks interneurons in significant numbers (Barbaresi *et al*., 1986). In regard to possible central noradrenergic involvement in the therapeutic effects of propranolol, it has been shown that locus coeruleus-induced trigeminal neuronal inhibition has a β adrenoceptor influence (Sasa *et al*., 1976, 1986), so the use of microiontophoresis in our studies was particularly important in localizing a possible effect of β adrenoceptor antagonism to the thalamus. Our results do not exclude an important effect for propranolol in the locus coeruleus or at other sites, such as the medullary dorsal horn.

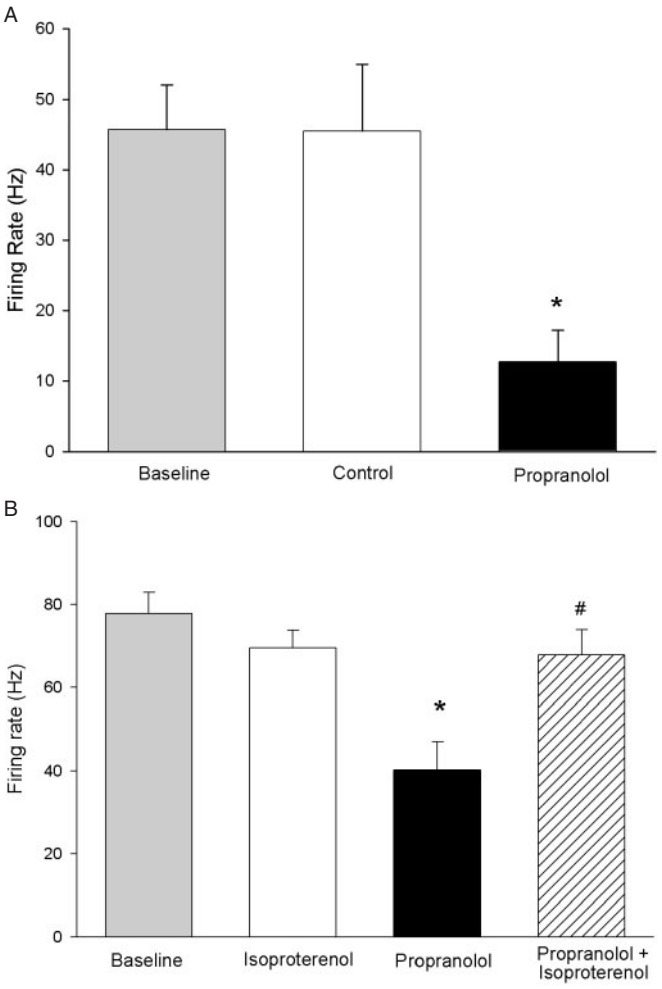

Fig. 5 The effects of drug ejection on the mean (±SEM) firing rates (Hz) of thalamocortical neurons during a 5 s ejection pulse of L-glutamate are shown. The mean firing rate was calculated from five successive pulses of L-glutamate for each of the test conditions. These were pooled for all cells to calculate the mean (±SEM) firing rate for (see text for further details) propranolol versus control (**A**) and propranolol versus propranolol and isoproterenol (**B**). Co-ejection of isoproterenol significantly antagonized the inhibition produced by propranolol, but did not have a significant effect by itself relative to the baseline ($*^{\#}P < 0.05$; *relative to the baseline and control, #relative to propranolol alone).

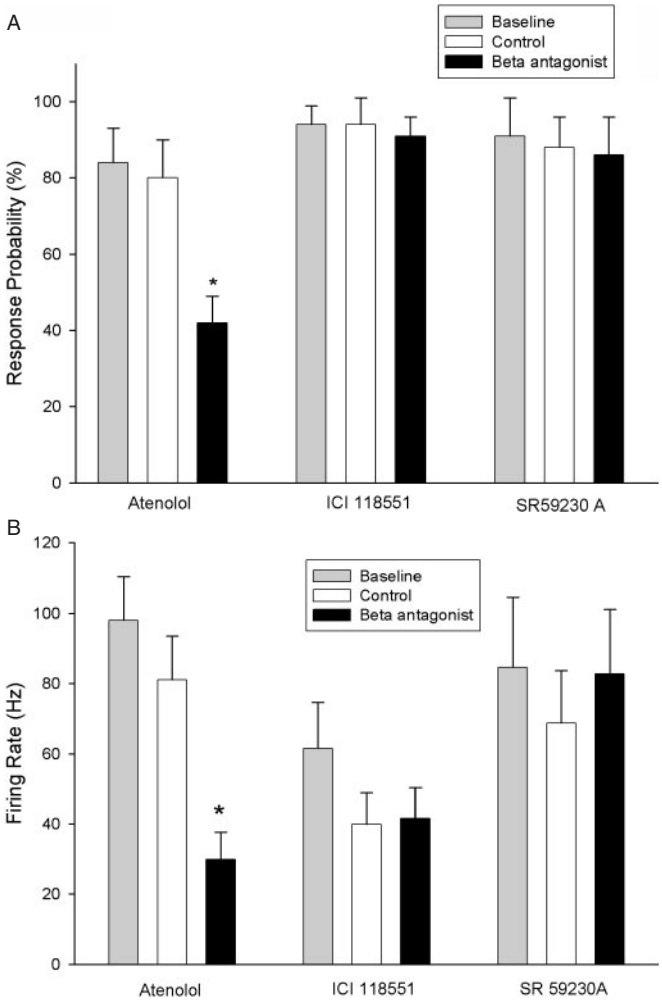

Fig. 6 Comparison of the effects of ejection of selective β-adrenoceptor antagonists (β_1, atenolol; β_2, ICI 118,551; β_3, SR 59230A) on the response of thalamocortical cells to electrical stimulation of the SSS (**A**) and ejection of L-glutamate (**B**) relative to control and baseline responses ($*P < 0.05$).

Microiontophoresis, however, has technical limitations. It is not possible to know the concentration of drug each cell is exposed to, an obvious disadvantage when trying to establish if the observed effects occur at physiological doses. A possible solution would involve comparing the effect following intravenous and microiontophoretic administration. This approach is open to criticism as it cannot exclude an action in extrathalamic sites and also adversely affects the haemodynamic status of the subject.

Activation of α_1 receptors in the thalamus results in a slow depolarization. β Adrenoceptor activation, however, has a complex action, with the enhancement of the inward rectifying current I_h (McCormick and Pape, 1990; McCormick *et al*., 1991). This current has several functions. It is responsible for determining the resting membrane potential of the neuron. It also decreases the response of the thalamocortical neurons to hyperpolarization, as might occur following activation of GABAergic reticular neurons or interneurons, and is responsible for generating 'pacemaker' potentials (Luthi and McCormick, 1998). Antagonism by propranolol of this β adrenergic response could lead to inhibition of thalamocortical neurons. Reduction of I_h may directly inhibit the neuron, as blocking it results in hyperpolarization of the cell's resting membrane potential. It may also prevent the enhancement of the 'anomalous rectification' of I_h that counters the hyperpolarization of the neuron in response to inhibitory inputs.

Each cell studied received convergent inputs from both visceral and cutaneous structures. This is well described for venterobasal complex neurons (Berkley *et al*., 1993; Guilbaud *et al*., 1993; Al-Chaer *et al*., 1996; Zhang *et al*., 2003). Like many other visceral structures, the only sensation generated

by the SSS appears to be pain (Penfield and McNaughton, 1940; Ray and Wolff, 1940; Cervero and Laird, 1999). The cutaneous receptive fields of the cells in this study were mostly restricted to the vibrissae, fewer being nociceptive. This is not surprising given the large volume of the VPM given over to the barreloids (Vahle-Hinz and Gottschaldt, 1983). The only previous studies to have examined this found somewhat similar findings, most receptive fields being either WDR or LTM and restricted to the V_I or V_{II} territories (Davis and Dostrovsky, 1988; Zagami and Lambert, 1990; Angus-Leppan *et al*., 1995). These studies were, however, performed in cats. The nature of the convergent cutaneous input may be important in determining the location of referred pain. It may not necessarily have significant implications for the processing of the nociceptive visceral input. This study, however, did not address the differential effect of β antagonists on processing of cutaneous versus visceral neurotransmission. This limitation means that it is not possible for us to make general observations on the thalamic processing of cutaneous information.

Functional imaging studies of patients with angina versus silent ischaemia provide a possible insight into a 'gating' role for the thalamus in visceral-type pain (Rosen *et al*., 1994, 1996). The thalamus may be responsible for regulating the flow of visceral sensory information to the cortex. This makes it a potentially fruitful target in migraine research. Virtually all afferent sensory information to the cerebral cortex is relayed through the thalamus. It receives large modulatory inputs from brainstem monoaminergic centres. These areas may be the site of a proposed 'migraine generator', suggested by changes demonstrated with functional imaging techniques during migraine (Weiller *et al*., 1995; Bahra *et al*., 2001; Matharu *et al*., 2004). One potential explanation is that abnormal ictal activity in these regions may directly alter sensory processing in the thalamus. Facilitation of sensory neurotransmission could possibly explain the sensory hypersensitivity experienced by migraineurs. If this was the case, we might have expected to observe an increase in both the responses to SSS stimulation and L-glutamate ejection following ejection of isoproterenol. This was not so, suggesting that this hypothesis is an overly simplistic view of a complex condition. We cannot, for example, replicate the interictal abnormalities that may indicate dysfunctional activity in the cortex and thalamocortical afferents (Sandor *et al*., 2000; Ambrosini *et al*., 2003; Vandenheede *et al*., 2003) with this model. These abnormalities, detected on electrophysiological testing, normalize before the onset of a migraine and following treatment with both acute (Proletti-Cecchini *et al*., 1997) and especially prophylactic migraine agents (Sandor *et al*., 2000). Though this appears at first counter-intuitive, and it must be recognized that the significance of these findings is not fully understood, it may suggest that prophylactics and acute treatment agents have a similar mechanism of action at some level. Indeed clinical evidence suggests a blurring of the distinction between acute and preventative treatments, sodium valproate being an example. Preliminary data derived from our model suggest that triptans may also be able to modulate thalamic trigeminovascular nociceptive neurotransmission (Shields and Goadsby, 2004). In this model, it therefore appears that modulation of rostral brainstem monoaminergic inputs is a common mechanism of action for both drug classes.

Propranolol is only recognized as a prophylactic treatment of migraine. Although some patients experience an immediate response to propranolol, the number of patients responding generally increases with time (Rosen, 1983). It is also complicated by the wide divergence in sensitivity to β blockers, based on inter-individual pharmokinetic and pharmacodynamic differences, as well as perhaps in disease differences observed in migraineurs. While higher oral doses are generally more effective than lower, there does not appear to be a strong correlation between plasma levels of β blockers and their clinical effect (Cortelli *et al*., 1985; Ziegler *et al*., 1993). It is difficult to assess the effects of chronic exposure to propranolol on β adrenoceptor dynamics in the thalamus. Whether longer exposure results in quantitative or qualitative changes is purely speculative, and further investigations will be required. This will obviously require an entirely different experimental design.

Migraine remains a complex neurobiological condition, and head pain is only one aspect of it. From a translational neurosciences perspective, this model of trigeminovascular nociception is, however, a useful surrogate for investigating the pain component of migraine. It is possible to draw the following conclusions. The VPM is a sensory processing centre for trigeminovascular nociception that is likely to be involved in migraine. Propranolol is able to negatively modulate trigeminal nociception through antagonism of β_1 receptors on thalamocortical neurons. β Blockers may therefore have a therapeutic action on thalamic neurotransmission in migraine. Abnormalities in thalamic and cortical sensory processing may explain some of the other cardinal symptoms of migraine, such as photophobia and phonophobia, which do not readily conform to traditional vascular models. Thalamic neurons may be an attractive target for understanding the mode of action of acute and preventive treatments in migraine and for the development of novel new medicines.

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