British Journal of Pharmacology (1998) 124, 1633–1642
http://www.stockton-press.co.uk/bjp

Analgesic and sedative concentrations of lignocaine shunt tonic and burst firing in thalamocortical neurones

[1]Stephan K.W. Schwarz & [1,2]Ernest Puil

[1]Department of Pharmacology & Therapeutics and Department of Anaesthesia, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C., V6T 1Z3, Canada

1 The effects of lignocaine [lidocaine] HCl (0.6 μM – 1 mM) on the membrane electrical properties and action potential firing of neurones of the ventral posterolateral (VPL) nucleus of the thalamus were investigated using whole cell recording techniques in rat brain slices *in vitro*.

2 Bath application of lignocaine reversibly decreased the input resistance (R_i) of VPL neurones. This effect was observed at low, clinically sedative and analgesic concentrations (i.e., maximal amplitude at 10 μM) whereas higher concentrations (300 μM – 1 mM) had no effect on R_i.

3 Lignocaine (10 – 100 μM) depolarized VPL neurones up to 14 mV in a reversible manner.

4 Consistent with a decreased R_i, low concentrations of lignocaine shunted the current required for spike generation in the tonic pattern. Lignocaine increased the threshold amplitude of current required for firing and decreased the tonic firing frequency, without concomitant elevation of the voltage threshold for firing or reduction in the maximal rate of rise (dV/dt_{max}) of spikes.

5 Low concentrations of lignocaine shunted low threshold spike (LTS) burst firing evoked either from hyperpolarized potentials or as rebound bursts on depolarization from prepulse-conditioned potentials.

6 Higher concentrations of lignocaine (300 μM – 1 mM), not associated with a decrease in R_i, elevated the voltage threshold for firing and reduced the dV/dt_{max} of spikes in a concentration-dependent fashion.

7 In conclusion, low concentrations of lignocaine shunted tonic and burst firing in VPL neurones by decreasing R_i, a mechanism not previously described for local anaesthetics in the CNS. We suggest that a decreased resistance in thalamocortical neurones contributes to the sedative, analgesic, and anaesthetic properties of systemic lignocaine *in vivo*.

Keywords: Local anaesthetics; lignocaine; CNS actions; thalamocortical neurones; membrane properties; tonic firing; burst firing; low threshold spike; analgesia; anaesthesia

Introduction

Lignocaine is the most frequently employed local anaesthetic in clinical medicine. Its well known peripheral actions to block the propagation of action potentials along nerve fibers are widely exploited for surgical regional anaesthesia. When present in the systemic circulation as a result of local absorption or intravascular injection, however, local anaesthetics exhibit effects that imply an additional central site of action (Garfield & Gugino, 1987). Such effects reflect potentially valuable therapeutic uses as well as central nervous system (CNS) toxicity. The most frequently observed symptoms of lignocaine's CNS toxicity are sedation, drowsiness, and alterations in sensorium (Covino, 1987). These are associated with low, 'subconvulsive' plasma concentrations, typically between one and 5 μg ml^{-1}. The same range of low concentrations produce the therapeutic effects of lignocaine (for review, see de Jong, 1994).

As a systemic analgesic, lignocaine is efficacious in the treatment of a variety of chronic pain syndromes such as neuropathic and central pain (Marchettini *et al*., 1992; Ferrante *et al*., 1996) as well as acute postoperative pain (Bartlett & Hutaserani, 1961). The well known central analgesic properties of local anaesthetics (Peterson, 1955) may contribute to the analgesia resulting from peripheral nerve blockade used for the management of chronic pain (Arnér *et al*., 1990). Lignocaine and other local anaesthetics have been used in the maintenance of general anaesthesia, with analgesic effects comparable to those of nitrous oxide (de Jong, 1994). Somewhat paradoxically, low concentrations of lignocaine also are effective in the treatment of generalized tonic-clonic seizures and status epilepticus (Bernhard & Bohm, 1965; Lemmen *et al*., 1978).

Many observations implicate the thalamus as a crucial site in the CNS where local anaesthetics act to produce these effects. Thalamocortical neurones relay afferent sensory signals, contributing to the generation of conscious states. They are critical for the production of electroencephalographic (EEG) rhythms associated with states of awareness and sleep, e.g., the slow-wave activity and spindles of drowsiness, sedation, and non-REM sleep (Steriade *et al*., 1990). Local anaesthetics infused at subconvulsive doses produce sedation and reduced responsiveness to noxious stimuli, associated with spindling and increased in delta- and theta- activity in the EEG of humans and other mammals (Eriksson & Persson, 1966; Wagman *et al*., 1968; Seo *et al*., 1982).

The ventral posterolateral (VPL) nucleus of the dorsal thalamus is a major relay station for somatosensory signals (Jones, 1985). Neurones in this nucleus have a significant role in the transmission of nociceptive signals and an involvement in the sensory and discriminatory aspects of pain perception (Head & Holmes, 1911; Melzack & Casey, 1968; Albe-Fessard *et al*., 1985). We considered the possibility that low, subconvulsive concentrations of lignocaine could produce central analgesic effects as well as alterations in sensorium and conscious state by actions on VPL neurones. However, the cellular effects of local anaesthetics on thalamocortical

[2]Author for correspondence.

neurones are unknown. Here, we report on the effects of lignocaine on the membrane electrical properties and action potential firing on VPL neurones in brain slices *in vitro* and on a novel mechanism of lignocaine action in the CNS. Parts of the results have appeared in abstract form (Schwarz *et al*., 1997; Schwarz & Puil, 1997a,b, 1998).

Methods

Preparation of brain slices

All animal experiments were approved by the Committee on Animal Care (The University of British Columbia), which issued an appropriate Animal Care Certificate. The results are from experiments with Sprague-Dawley-rats, aged 12–20 days postnatally (P12–P20). The animals were decapitated under deep halothane (Wyeth-Ayerst Canada, Inc., Montréal, Canada) anaesthesia. The cerebrum was rapidly removed and submerged for 1 min in cold (1–4°C) artificial cerebrospinal fluid (ACSF). The ACSF, prepared freshly on each experimental day, contained (in mM): NaCl, 124; KCl, 4; KH_2PO_4, 1.25; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 26; glucose, 10. The measured osmolarity was 310 mOsm (Advanced Digimatic Osmometer 3DII, Advanced Instruments, Inc., Needham Heights, MA, U.S.A.); continuous saturation with 95% O_2/5% CO_2 for >1 h yielded a pH of 7.4 (measured at 20–26°C with a pH meter model 05669-20, Cole-Parmer Instrument Company, Chicago, IL, U.S.A.). The brain was dissected into two blocks, each containing the thalamic tissue of one cerebral hemisphere. After fixation of a tissue block on a Teflon stage with cyanoacrylate adhesive (Accu-Flo™ super glue, Lepage, Boucherville, Québec, Canada), coronal slices of 300–500 μm thickness and containing the VPL were prepared with a Vibroslice (Campden Instruments Ltd., London, England). Prior to recording, the slices were incubated for at least 1 h in ACSF at 20–26°C, continuously aerated with 95% O_2/5% CO_2.

Electrophysiological recordings

Whole cell patch clamp recordings (Hamill *et al*., 1981) were conducted using an Axoclamp 2A amplifier (Axon Instruments, Inc., Foster City, CA, U.S.A.) in the bridge mode. The recording electrodes were prepared from thin-walled borosilicate glass (World Precision Instruments, Inc., Sarasota, FL, U.S.A.) using a PP-83 two-stage electrode puller (Narishige Scientific Instrument Lab., Tokyo, Japan). They were filled with a solution containing (in mM) K-gluconate, 140; ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 10; KCl, 5; NaCl, 4; $MgCl_2$, 3; N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (free acid), 10; $CaCl_2$, 1; adenosine-5′-triphosphate (disodium salt), 3; guanosine-5′-triphosphate (sodium salt), 0.3. The solution was titrated to pH 7.3 with gluconic acid (10%) and KOH. The approximate concentrations of free Ca^{2+} and Mg^{2+} ions, calculated for pH 7.3 and 25°C with the use of WinMAXC Software, version 1.60 (Chris Patton, Stanford University, Hopkins Marine Station, Pacific Grove, CA, U.S.A.), were 11.2 nM and 354 μM, respectively. The estimated electrode resistances were typically ~8 MΩ (range, 6–11 MΩ).

For recording, the slices were transferred into a submersion type chamber with a volume of 1.2 ml, fixed between two pieces of polypropylene mesh, and continuously perfused with aerated ACSF (95% O_2/5% CO_2) at a flow rate of 1.5 ml min^{-1}. The temperature of the perfusing medium in the chamber was 22–27°C. The VPL nucleus was identified by aid of a Wild 5 M5A microscope (Wild, Heerbrugg, Switzerland). The atlas by Palkovits & Brownstein (1988) was used as a reference.

Whole-cell recording was carried out employing the 'blind' technique (Blanton *et al*., 1989). After submersion of the electrode tip in the ACSF, the measured potential difference was set to 0 mV with a DC offset adjustment. Cell membranes were ruptured when seals $\geqslant$ 1 GΩ were achieved. After obtaining the whole cell configuration, access resistance compensation was performed employing bridge balance techniques. A measured liquid junction potential of 11 mV was subtracted from all membrane potentials (*c.f*. Hutcheon *et al*., 1996). The data were filtered at 10 kHz and recorded on chart (Brush Recorder Mark 280, Brush Instruments, Cleveland, Ohio, U.S.A.), and after conversion with a Lab Master DMA 40 kHz analog/digital/analog converter (Scientific Solutions, Inc., Solon, Ohio, U.S.A.) and pCLAMP software, version 5.5 (Axon Instruments, Inc., Foster City, CA, U.S.A.) on the hard disk of an IBM-compatible personal computer. For recording on video cassette (SL-HF 750 super Beta hi-fi Video Cassette Recorder, Sony, Japan), the analog signal was digitally converted by a 44 kHz PCM-1 Digital VCR-Instrumentation Recorder Adaptor (Medical Systems Corp., Greenvale, NY, U.S.A.).

Drugs

Lignocaine [lidocaine] HCl was purchased from Research Biochemicals International (Natick, MA, U.S.A.). The powder was dissolved in fresh ACSF to prepare a concentrated stock solution of 5 mM, stored in aliquots of 2.2 ml at −22°C. Tetrodotoxin (TTX) was obtained from Sigma-Aldrich Canada Ltd. (Mississauga, ON, Canada). From the citrate-buffered TTX, a 300 μM stock solution was prepared with distilled water and stored in 500 μl aliquots at −22°C. Prior to application, required aliquots of the agents were defrosted and dissolved in ACSF to obtain the respective concentrations. Lignocaine applications in the bath were performed by switching from the control perfusate (normal ACSF) to ACSF containing a desired drug concentration. The pH of such solutions did not vary significantly from the pH (7.4) of the buffered normal ACSF (pK_a of lignocaine, 7.8; *c.f*. de Jong, 1994). TTX was applied in an analogous fashion.

Statistical analyses

Statistical analyses were carried out with the use of Student's *t*-tests for comparisons of two groups and testing for differences from a theoretical mean, and one-way analysis of variance (ANOVA) for multisample analyses. The Bonferroni test for pairwise comparisons and the Dunnett test for comparisons to control were employed as *post hoc* tests. Differences were considered significant when $P<0.05$. All data are expressed as mean ± s.e., n = sample size, unless mentioned otherwise. The data were analysed using Prism version 2.0 software (GraphPad, San Diego, U.S.A.), the Clampan and Clampfit components of pCLAMP software version 5.5 and 6.0.2 (Axon Instruments, Inc., Foster City, CA, U.S.A.), and Microsoft Excel version 5.0 software (Microsoft Corporation, Redmond, WA, U.S.A.).

Results

We report here on results from 56 neurones in the VPL nucleus. All neurones accepted for analysis had overshooting

action potentials and stable resting membrane potentials (V_r) < -50 mV, lasting for up to 4 h of recording. The neurones had a mean V_r of -70.3 ± 1.4 mV ($n=17$), consistent with the results of previous investigations on VPL neurones (*c.f.* Ries & Puil, 1993). Input resistances (R_i), determined from the steady-state voltage displacements (ΔV_m) of $\leqslant -10$ mV elicited by injection of hyperpolarizing current pulses of 500 ms duration, averaged 209.7 ± 38.8 MΩ ($n=17$). The mean membrane time constant (τ_m), estimated from single exponential fits to the ΔV_m, was 38.9 ± 3.7 ms ($n=17$). All neurones exhibited the voltage-dependent firing patterns characteristic for thalamocortical relay neurones (*c.f.* Deschênes *et al.*, 1984; Jahnsen & Llinás, 1984a). Injection of suprathreshold depolarizing current pulses into neurones near rest elicited repetitive tonic firing (blocked by 600 nM TTX; not illustrated), whereas on hyperpolarizing the membrane potential (V_m, typically to values between -80 and -90 mV) by direct current (DC) injection, depolarization elicited burst firing on top of a low threshold spike (LTS) (*c.f.* Figure 5). Rebound LTS bursts also could be evoked from a V_m near rest by hyperpolarizing current pulses, known to de-inactivate the T-type Ca^{2+} current, I_T (Jahnsen & Llinás, 1984a).

Effects on resting membrane properties

Application of lignocaine decreased the R_i of VPL neurones in a reversible manner (Figure 1A). The time for an application to produce a steady-state response was typically ~ 5 min (range, 2.5–7 min). This effect exhibited a distinct, non-classical concentration dependence. Whereas the maximal decrease in R_i occurred at a low concentration (10 μM), the amplitude of this effect decreased with higher concentrations, and no change in R_i occurred at 300 μM to 1 mM (Figure 1B). These relationships implied the presence of multiple actions of lignocaine that affect R_i in an overlapping fashion. The data did not fit conventional concentration-response-models (e.g., a four-parameter logistic equation) for construction of a meaningful classic sigmoid curve. Concomitant with the effect on R_i, lignocaine administration caused a reduction in τ_m with a similar concentration dependence (Figure 1C). The respective input capacitances, calculated according to $C_i = \tau_m / R$, were not significantly different from the control values over the range from 0.6–600 μM (data not shown), indicating a primary effect of lignocaine on membrane conductance (*c.f.* Figures 1B and 1C). The reversal potentials for the increased conductances ($1/R_i$) were between V_r and ~ -50 mV.

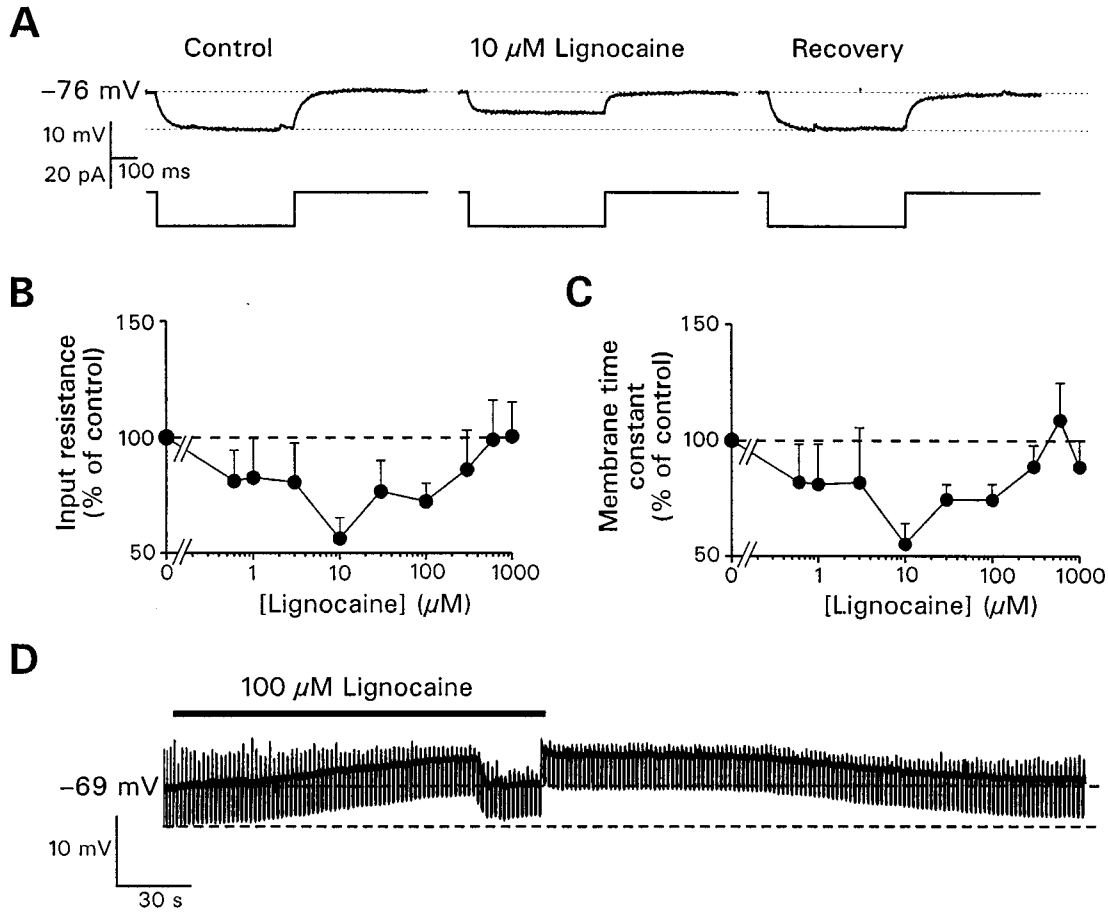

Figure 1 Effects of lignocaine application on input resistance, membrane time constant, and resting membrane potential. (A) A low concentration of lignocaine (10 μM) significantly decreased input resistance, evident as a decreased amplitude of the steady-state voltage response to hyperpolarizing current pulses (duration, 500 ms). The magnitude of the resistance change was 51% (400 MΩ to 196 MΩ). The effects were completely reversed after 10 min of washout. (B) and (C): Concentration-response relationship of the changes in input resistance and membrane time constant induced by lignocaine (for each concentration, $n=4-6$; *cf.* Table 1). (D) Lignocaine application (100 μM) depolarized a VPL neurone from -69 mV to -64 mV. The downward deflections in the voltage trace represent responses to hyperpolarizing current pulses (-40 pA, 500 ms); the positive deflections represent rebound LTS bursts. Near the peak response, V_m was manually clamped at the control level through DC injection for assessment of a change in input resistance. Close to full recovery was reached after 4 min of washout.

At 30 or 100 μM, lignocaine depolarized seven out of nine neurones (Figure 1D). The time required to reach a maximal depolarization was typically 2–3 min. The depolarizing response with 30 μM ranged between two and 14 mV in three out of four neurones, and, with 100 μM, between four and 9 mV in four out of five neurones. Application of 10 μM lignocaine was associated with depolarizations of seven and 9 mV in two out of five neurones. Repolarization to resting values was seen usually after 4–5 min on terminating the application. Higher concentrations (300 μM–1 mM) had no consistent effects on V_r.

In neurones where application of TTX (600 nM) completely abolished action potentials, lignocaine produced small decreases in R_i which were reversible and associated with variable changes in V_r. For example, 10 μM lignocaine reduced R_i to a level of $82.0 \pm 5.8\%$ of control values ($P = 0.04$, $n = 5$).

Figure 2 shows the effects of lignocaine on the *I-V*-relationship and corresponding voltage responses of a representative neurone clamped at a V_m of -66 mV with DC injection. The *I-V*-relationship was typically quasilinear at membrane potentials positive to ~ -90 mV. At more hyperpolarized voltages, neurones exhibited some inward (depolarizing) rectification (*c.f.* Jahnsen & Llinás, 1984b; Hutcheon *et al.*, 1996; Tennigkeit *et al.*, 1996). A low concentration of lignocaine (10 μM), which caused the greatest reduction in R_i, markedly reduced the slope resistance over a wide voltage range (-125 mV to threshold or ~ -50 mV). We observed full recovery from these effects typically ~ 10 min after washout with normal ACSF. Lignocaine had no consistent effect on the inward rectification.

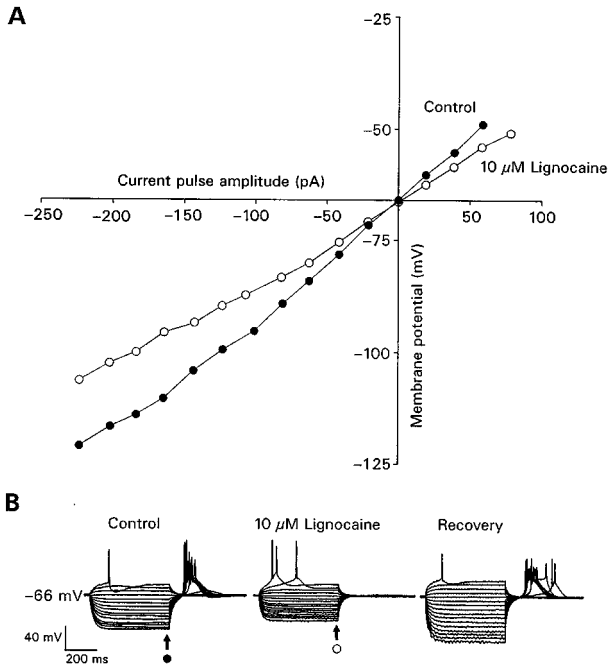

Figure 2 Effects of lignocaine application on current-voltage relationships in a VPL neurone. (A) *I-V*-curves under control conditions (superfusion with normal ACSF) and following application of 10 μM lignocaine for 7 min. Potentials were measured at the end of current pulses (500 ms) injected in 20 pA-steps (see arrows in B). Lignocaine reduced the slope resistance over a wide voltage range (~ -100 to -50 mV). (B) Voltage responses corresponding to A. Recovery was observed after 11 min of washout.

Effects on tonic repetitive firing

Lignocaine, applied in low concentrations associated with a decrease in R_i, suppressed tonic repetitive firing of action potentials elicited by depolarizing current pulses (Figure 3A). The firing frequency, expressed as the number of action potentials per 500 ms-current pulse, decreased in a reversible manner. There was no concomitant elevation of the threshold potential for firing or significant change in the maximal rate of rise (dV/dt_{max}) of spikes in a train. For example, the 5th spike in a train, very sensitive to high lignocaine concentrations (*c.f.* Figure 4), had a dV/dt_{max} of 52.7 ± 5.0 mV ms^{-1} in the control whereas during application of 10 μM, the rate of rise was 51.0 ± 10.8 mV ms^{-1} ($P = 0.84$, $n = 4$). In current-frequency-relationships, lignocaine caused a parallel shift of the curve to the right, indicating a relatively unimpaired distribution of activatable Na^+ channels at the spike generator (Figure 3B). Lignocaine markedly increased the amplitude of a current pulse required for spike generation, consistent with a shunt (Figure 3D). Such applications also diminished a slow component of the spike-after hyperpolarizations (AHPs), which in isolation would be expected to increase firing frequency (Figure 3C; *c.f.* Foehring *et al.*, 1989). These findings illustrated the predominance of a shunt as the primary effect at these concentrations.

In contrast, higher concentrations affected parameters of Na^+ permeability. Concentrations of lignocaine ranging from 300 μM to 1 mM decreased the rate of rise of action potentials and produced a pronounced or complete suppression of tonic repetitive firing (Figure 4). In a concentration-dependent fashion, lignocaine application elevated the threshold potential for firing. These concentrations did not significantly shunt the current required for spike generation (*c.f.* Figure 1 and Table 1). Consistent with previous observations (*c.f.* Butterworth *et al.*, 1993), recovery was significantly delayed on terminating an application at a high concentration, usually requiring washout times that exceeded 45 min.

Effects on burst firing

Low concentrations of lignocaine associated with a decrease in R_i had a depressant influence on the burst firing mode of VPL neurones. We observed this on depolarization from prepulse-conditioned (Figure 5A) or tonically DC-maintained (Figure 5B), hyperpolarized potentials. Lignocaine (10 μM) suppressed the rebound bursts elicited by hyperpolarizing current pulses from potentials near rest (Figure 5C). When hyperpolarization was maintained by DC injection at potentials from which depolarizing pulses elicited LTS burst firing, a subsequent application of lignocaine (10 μM) resulted in a marked elevation in the amount of current required to evoke a spike burst. This was reversible and similar to its effect on the tonic firing mode (Figure 5D). The effects were associated with a marked decrease in R_i, implicating a shunt of input current as a major mechanism for the depression of the burst activity.

Discussion

Here, we have demonstrated that lignocaine suppressed the tonic and burst firing in thalamocortical relay neurones of the VPL nucleus, known to participate in the transfer of somatosensory signals, pain perception, and in general, the generation of conscious states. The most intriguing finding was an effect distinct from Na^+ channel blockade; lignocaine reduced R_i, shunting the current needed for spike generation. This effect predominated at lower concentrations (maximal amplitude at 10 μM) and not at high concentrations.

For terms of clinical relevance of such low concentrations, 10 μM lignocaine HCl converts to approximately 2.7 μg ml^{-1}. Following intravenous injection, concentrations of lignocaine in the cerebrospinal fluid (CSF) correlate to arterial concentrations with a factor between 0.73 and 0.83 (Usubiaga *et al*., 1967). This implies that a CSF concentration of 10 μM corresponds to arterial concentrations near 2 μg ml^{-1} *in vivo*, which is relevant to the preconvulsive CNS effects of lignocaine (*c.f.* Introduction).

To our knowledge, this is the first report that local anaesthetics decrease input resistance in neurones of the CNS. Although previous investigations demonstrated that local anaesthetics reduce neuronal excitability, they focused on concentrations in the high micromolar to millimolar range. In studies of hippocampal CA1 pyramidal cells, extracellular lignocaine (50 μM to 3 mM) does not reduce or increase input resistance (Butterworth *et al*., 1993). In contrast, the quaternary lignocaine analogs QX-222 (Puil & Carlen, 1984), QX-572 (Segal, 1988), and QX-314 (Xie & Sastry, 1992), applied intracellularly in high concentrations, increase input resistance in hippocampal neurones. The observations of an increased conductance during application of 400 nM lignocaine in isolated neuronal somata of rat superior cervical ganglia (Tabatabai & Booth, 1990) are consistent with our

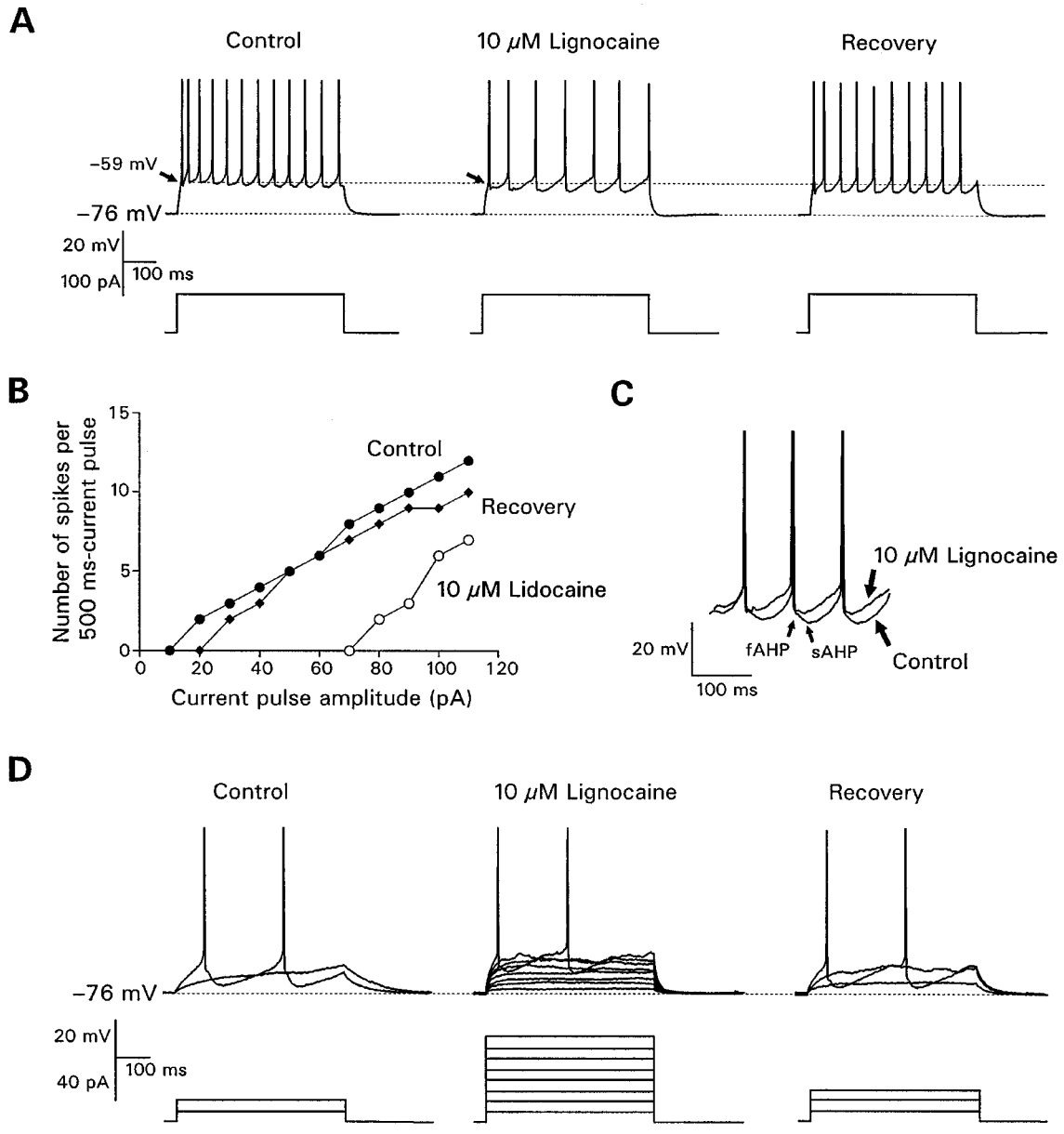

Figure 3 Effects of low concentrations of lignocaine on tonic repetitive firing. (A) Lignocaine reduced, in a reversible manner, the spike frequency and inhibited spike adaptation of neurones firing in the tonic pattern. Tonic firing in the neurone was evoked from a V_r of -76 mV by injecting a 100 pA-current pulse of 500 ms duration (lower traces). The suppression of repetitive firing was not associated with an increase in the threshold potential for firing (arrows) or a decrease in the maximal rate of rise of the spikes (dV/dt_{max}, not illustrated), consistent with an effect secondary to a shunt, rather than Na^+ channel blockade. (B) The relationship between the amplitude of injected current and the firing frequency was quasilinear. The suppression of repetitive firing by lignocaine was characterized by a shift in the current-frequency curve to the right, leaving the slope of the curve unchanged. (C) Lignocaine reduced a slow component of the spike afterhyperpolarizations (sAHP; fAHP denotes fast afterhyperpolarization). The two superimposed traces are matched for spike frequency and were obtained with 60 pA (control) and 110 pA (lignocaine) pulses. (D) In association with the decreased R_i, lignocaine markedly elevated the threshold amplitude of current required for firing in the tonic pattern. The current pulse amplitude required for spike generation reversibly increased to a level of 400% of control (control, 20 pA; 10 μM lignocaine, 80 pA).

findings that the decreased R_i occurred only with low concentrations of lignocaine.

The shunting of inputs and action potentials represents a powerful mechanism for inhibition in the CNS. As a result of the reduction in τ_m, the neuron's capability of temporal summation of synaptic inputs potentially leading to threshold responses would be diminished – effects representing a form of 'temporal shunting'. The observed consequences of the shunt on neuronal excitability were a marked increase in current threshold and a suppression of repetitive firing. Normally, thalamocortical neurones linearly encode the intensity of incoming somatosensory signals into firing frequency for 'faithful' signal transmission to cortical centers (Mountcastle *et al*., 1963; Jahnsen & Llinás, 1984a; Steriade *et al*., 1990). Our findings that lignocaine shunts their tonic firing are consistent with the clinical observations of sensory disturbances and sedation at preconvulsive concentrations.

The principle of encoding stimulus intensity into firing frequency and spike patterns also is valid for nociceptive signals transmitted by the spinothalamic tract (Simone *et al*., 1991). Nociceptive activity in neurones of the ventrobasal complex of the thalamus is depressed by various analgesics

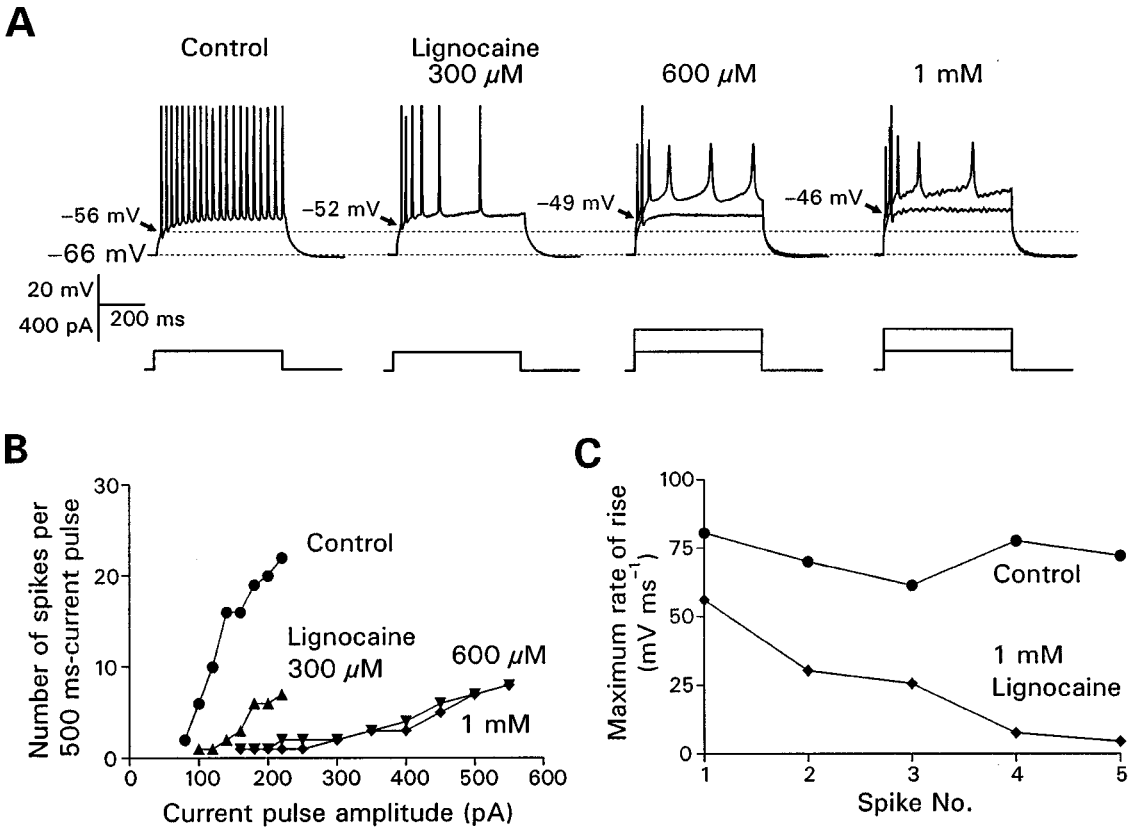

Figure 4 Effects of high concentrations of lignocaine on tonic repetitive firing. (A) High lignocaine concentrations produced a concentration-dependent elevation in voltage threshold for tonic firing (arrows) and changes in spike configuration without significant changes in R_i. Superfusion with 300 μM, 600 μM, or 1 mM lignocaine led to a pronounced reduction in firing produced by injection of depolarizing 500 ms current pulses (200 pA). At 600 μM and higher, repetitive firing was completely suppressed but could be elicited by current pulses of a markedly increased amplitude. Note the refractoriness of the first spike in a train of action potentials to lignocaine. (B) Concentration-dependent effects on the relationship between the amplitude of injected current and firing frequency. The slope of the current-frequency curve was increasingly reduced by increasing lignocaine concentrations, consistent with a successive increase in the ratio of blocked versus unblocked Na^+ channels at the axon hillock. A saturation effect became evident at 600 μM. (C) High lignocaine concentrations successively reduced dV/dt_{max} of spikes in a train ($P<0.001$), with a maximal effect typically seen after the fifth spike. The first spike remained relatively refractory to lignocaine.

Table 1 Effects of lignocaine on input resistance (R_i) and membrane time constant (τ_m)

[Lignocaine] (μM)	R_i *(% of control)*	P *value*	τ_m *(% of control)*	P *value*	n
0.6	81.1±13.3	0.21	81.9±16.4	0.32	6
1	82.6±17.0	0.35	81.0±17.4	0.32	6
3	80.6±16.9	0.30	81.8±23.7	0.48	6
10	56.3±9.1	0.009	55.2±9.2	0.008	5
30	76.6±13.4	0.14	74.5±6.5	0.01	6
100	72.4±7.8	0.02	81.1±6.9	0.04	6
300	86.1±17.0	0.47	88.7±9.2	0.31	4
600	98.9±17.1	0.95	108.9±16.0	0.61	5
1000	100.4±14.8	0.98	88.7±11.4	0.39	4

(Carlsson *et al*., 1988). Abnormal thalamic burst firing occurs in patients suffering from chronic pain syndromes (Tsoukatos *et al*., 1997), in whom lignocaine is particularly efficacious as a systemic analgesic. Our findings that concentrations of lignocaine that produce clinical analgesia effectively suppressed tonic and burst firing in thalamocortical neurones represent an attractive and plausible mechanism contributing to the systemic analgesic properties of lignocaine *in vivo*.

Suppression of repetitive firing also may be a major mechanism by which a variety of antiepileptic agents exert their therapeutic effects, including phenytoin, sodium valproate, and carbamazepine (for review, see Macdonald & Kelly, 1995). Although none of these agents are known to decrease resistance in central neurones, suppression of repetitive firing due to a shunt may well be a mechanism critical to the antiepileptic properties of lignocaine at low concentrations. The volatile general anaesthetic, isoflurane, applied in clinically relevant concentrations, also decreases resistance and shunts tonic and burst firing in neurones of the ventrobasal thalamic complex (Ries & Puil, 1993). This observation is most striking in view of the clinical similarities between general and local anaesthetics. Both lignocaine and isoflurane have sedative, analgesic, and anaesthetic properties, and, like other general anaesthetics, also have antiepileptic

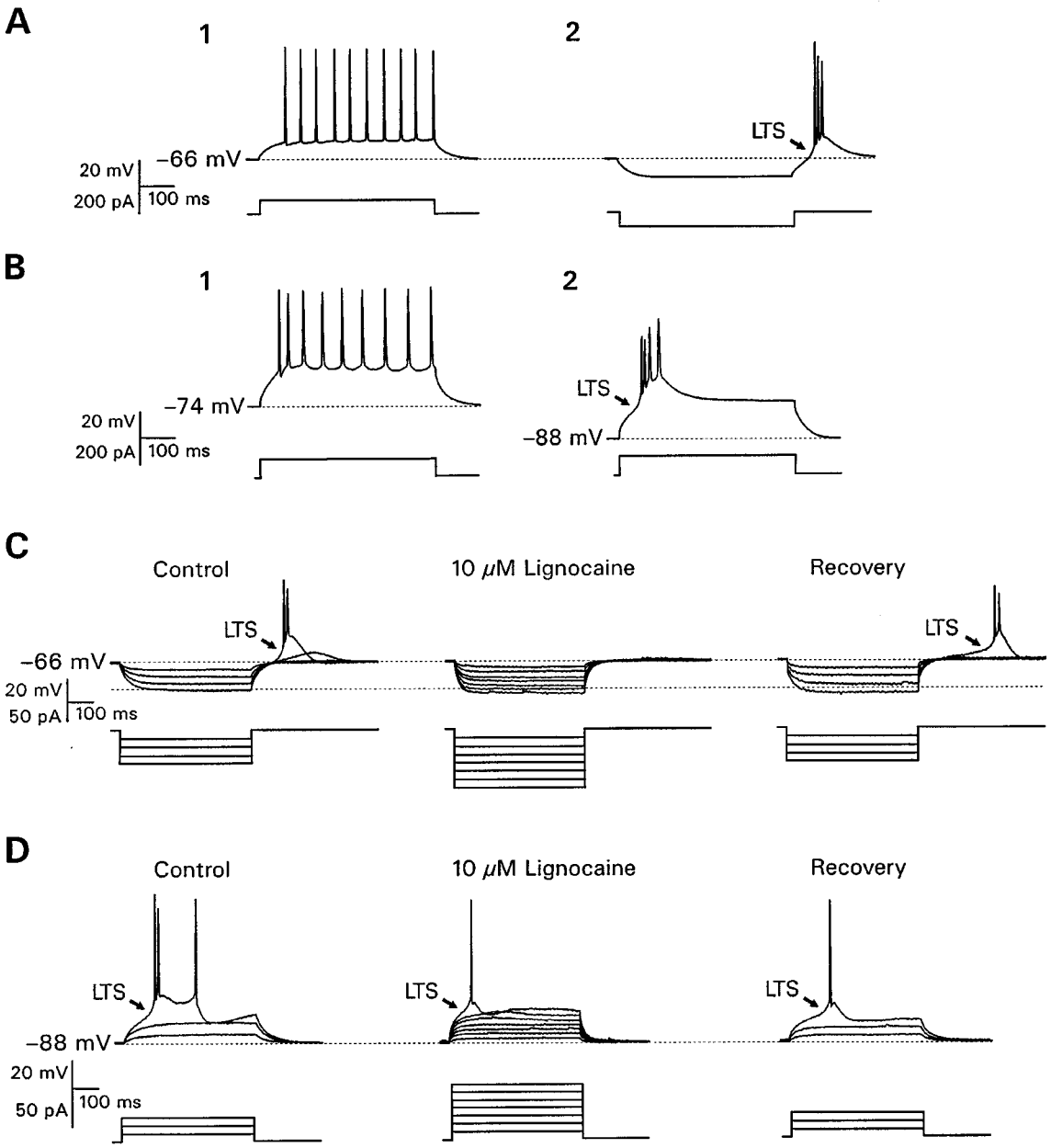

Figure 5 Low threshold spikes (LTSs) and the effects of lignocaine on burst firing. (A) and (B) Voltage dependent spike patterns evoked by different procedures in two VPL neurones. Injection with depolarizing current pulses from near V_r evoked repetitive tonic firing (A1, B1). At the same V_m, hyperpolarizing current pulses of sufficient amplitude to de-inactivate a T-type Ca^{2+} current resulted in a rebound LTS after termination of the pulse. One to seven action potentials fired on top of the bursts (A2, arrow). When V_m was hyperpolarized by DC injection, a depolarizing current pulse evoked LTS burst firing (B2, arrow). (C) In a reversible manner, lignocaine (10 μM) eliminated rebound LTS bursts evoked from potentials near rest. (D) In neurones that were hyperpolarized to elicit LTS burst firing, lignocaine application (10 μM) caused a reversible increase in current threshold for firing. The current pulse magnitude required for firing in the burst mode was increased to a level of 233% of control (control, 30 pA; 10 μM lignocaine, 70 pA). In C and D, note the decreased R_i.

effects (Kofke *et al*., 1985). However, it seems unlikely that the molecular mechanism of action for generating the shunt is identical for isoflurane and lignocaine. Isoflurane hyperpolarizes thalamocortical neurones by increasing a leak K^+ conductance (Ries & Puil, 1997) whereas lignocaine depolarized neurones or had no effects on V_r.

Lignocaine exerted multiple molecular actions that overlapped and affected resistance. This was evident by the distinct, non-classical concentration-response relationship (*c.f.* Results), indicating that the local anaesthetic had at least one action that decreased R_i at lower concentrations, and at least one action that caused R_i to 'return' to control values at high concentrations. The well known property of lignocaine to cause an 'open channel block' of Na^+ channels (for review, see Catterall, 1987) is not compatible with the observed decrease in resistance (*c.f.* Figure 1). A recent study demonstrated that local anaesthetics enhance the increase in conductance induced by GABA in stretch receptor neurones of crayfish (Nordmark & Rydqvist, 1997). In thalamocortical neurones, application of GABA mediates a rapid increase in conductance which is carried by Cl^- (Crunelli *et al*., 1988; Thomson, 1988). In the present study, an increase in Cl^- conductance (E_{Cl} at 25°C = −54 mV) due to lignocaine application would be consistent with the observed decreases in R_i and depolarizations. The increased conductance produced by lignocaine would likely represent a major mechanism for inhibition of firing because the driving potential ($V_m - E_{Cl}$) for a GABA-induced hyperpolarization would be rather small. For example, GABA still produces inhibition of firing while depolarizing cortical neurones (El-Beheiry & Puil, 1990). The depolarizations seen in this study were similarly associated with neuronal inhibition rather than excitation, as the inhibitory effects of the shunt predominated. Whereas we cannot not exclude the possibility that lignocaine may act on nerve terminals or neurons presynaptic to the recorded electrode to produce the changes in membrane properties, the observation that lignocaine still produced decreases in resistance during TTX application implies that a direct postsynaptic action contributes to the shunt.

The reduction of sAHPs and the suppression of LTS bursts also may be interpreted as effects that are secondary to a shunt. However, an isolated reduction of sAHPs, likely produced by small conductance Ca^+ activated K^+ channels (*c.f.* Jahnsen & Llinás, 1984a,b), is not compatible with a decrease in firing frequency (*c.f.* Foehring *et al*., 1989). Secondly, lignocaine (1 mM) blocks Ca^+ activated K^+ channels in hippocampal neurones (Oda *et al*., 1992), but these channels are of the high conductance type. There is uncertainty about the subcellular distribution of the T-type Ca^{2+} current in VPL neurones and the precise subtype of Ca^{2+} channel mediating the LTS. Assuming a primarily dendritic distribution of I_T (for review, see Huguenard, 1996), a pronounced shunting effect may result from a significantly decreased R_i. On the other hand, our findings that lignocaine suppressed LTS bursts even after hyperpolarizing thalamocortical neurones to a voltage range where we would anticipate a maximal de-inactivation of I_T raise the possibility that lignocaine may decrease I_T (*c.f.* Akaike & Takahashi, 1992).

Lignocaine, in low concentrations may have affected the persistent Na^+ current, I_{NaP}. In thalamocortical neurones (Jahnsen & Llinás, 1984b), I_{NaP} amplifies depolarizations in the perithreshold range (for review, see Crill, 1996). Lignocaine (12.5–25 μM) selectively blocks this current in cardiac myocytes (Ju *et al*., 1992). The lower slope of the *I-V*-curve between V_r and ∼−50 mV (Figure 2; *c.f.* Stafstrom *et al*., 1985), decreased amplitudes of voltage responses to depolarizing current and reduction in tonic firing frequency during application of lignocaine in this concentration range are consistent with a blockade of I_{NaP}.

At high concentrations (300 μM–1 mM), lignocaine produced the expected signs of Na^+ channel blockade. These concentrations correspond to those in *in vitro* studies on peripheral nerve preparations (for review, see Strichartz, 1976) and are associated with generalized CNS and cardiovascular depression and death when present in the systemic circulation *in vivo*. In our studies, the refractoriness of the first spike in a train to lignocaine and the increasing depression of subsequent spikes were similar to findings in hippocampal CA1 neurones (Capek & Esplin, 1994). The resistance of the first spike may result from the high density of Na^+ channels in the area of the axon hillock near the electrode, compared to more distal regions.

The major implication of this study is that lignocaine can inhibit thalamocortical signal transmission in clinically relevant, preconvulsive concentrations by a shunting mechanism not previously described for local anaesthetics in the CNS. Our findings also provide further support for the hypothesis (*c.f.* Sugiyama *et al*., 1992; Ries & Puil, 1993, 1997; Tennigkeit *et al*., 1997) that thalamocortical neurones may be a crucial site of anaesthetic and analgesic action.

We are indebted to Lance Corey, Christian Caritey, and Ciprian Jauca for their excellent technical assistance, to Dr. Richard Neuman for thoughtful comments, and to Prof. Walter Stühmer for his thorough review of the manuscript. This work was supported in part by the 1995/96 Pharmacia-Upjohn Award and a 1996/97 British Columbia Medical Services Foundation (B.C.M.S.F.) Fellowship (S. Schwarz), and a grant from The Medical Research Council of Canada (E. Puil).

References

AKAIKE, N. & TAKAHASHI, K. (1992). Tetrodotoxin-sensitive calcium-conducting channels in the rat hippocampal CA1-region. *J. Physiol. (Lond.),* **450,** 529–546.

ALBE-FESSARD, D., BERKLEY, K.J., KRUGER, L., RALSTON, H.J. III. & WILLIS, W.D. JR. (1985). Diencephalic mechanisms of pain sensation. *Brain. Res. Rev.,* **9,** 217–296.

ARNÉR, S., LINDBLOM, U., MEYERSON, B.A., MOLANDER, C. (1990). Prolonged relief of neuralgia after regional anesthetic blocks. A call for further experimental and systemic clinical studies. *Pain,* **43,** 287–297.

BARTLETT, E.E. & HUTASERANI, O. (1961). Xylocaine for the relief of postoperative pain. *Anesth. Analg.,* **40,** 296–304.

BERNHARD, C.G. & BOHM, E. (1965). *Local Anaesthetics as Anticonvulsants: A Study on Experimental and Clinical Epilepsy*. Stockholm: Almqvist & Wiksell.

BLANTON, M.G., LO TURCO, J.J. & KRIEGSTEIN, A.R. (1989). Whole cell recordings from neurons in slices of reptilian and mammalian cerebral cortex. *J. Neurosci. Methods,* **30,** 203–210.

BUTTERWORTH, J., COLE, L. & MARLOW, G. (1993). Inhibition of brain cell excitability by lidocaine, QX 314, and tetrodotoxin: a mechanism for analgesia from infused local anesthetics? *Acta Anaesthesiol. Scand.,* **37,** 516–523.

CAPEK, R. & ESPLIN, B. (1994). Effects of lidocaine on hippocampal cells: depression of repetitive firing. *NeuroReport,* **5,** 681–684.

CARLSSON, K.-H., MONZEL, W. & JURNA, I. (1988). Depression by morphine and the non-opioid analgesic agents, metamizol (dipyrone), lysine acetylsalicylate, and paracetamol, of activity in rat thalamus neurones evoked by electrical stimulation of nociceptive afferents. *Pain,* **32,** 313–326.

CATTERALL, W.A. (1987). Common modes of drug action on Na^+ channels: local anesthetics, antiarrhythmics and anticonvulsants. *Trends Pharmacol. Sci.,* **8,** 57–65.

COVINO, B.G. (1987). Toxicity and systemic effects of local anaesthetic agents. In *Handbook of Experimental Pharmacology, Vol. 81: Local Anesthetics*. ed. Strichartz, G.A. pp. 187–212, Berlin: Springer-Verlag.

CRILL, W.E. (1996). Persistent sodium current in mammalian central neurons. *Annu. Rev. Physiol.,* **58,** 349–362.

CRUNELLI, V., HABY, M., JASSIK-GERSCHENFELD, D., LERESCHE, N. & PIRICHO, M. (1988). Cl^- and K^+-dependent inhibitory postsynaptic potentials evoked by interneurons of the rat lateral geniculate nucleus. *J. Physiol. (Lond.),* **399,** 153–176.

DE JONG, R.H. (1994). *Local Anesthetics*. St. Louis: Mosby.

DESCHÊNES, M., PARADIS, M., ROY, J.P. & STERIADE, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: resting properties and burst discharges. *J. Neurophysiol.,* **51,** 1196–1219.

EL-BEHEIRY, H. & PUIL, E. (1990). Unusual features of GABA responses in layers IV-V neurons of neocortex. *Neurosci. Lett.,* **119,** 83–85.

ERIKSSON, E. & PERSSON, A. (1966). The effect of intravenously administered prilocaine and lidocaine on the human electroencephalogram studied by automatic frequency analysis. *Acta Chir. Scand.* Suppl. **358,** 37–46.

FERRANTE, F.M., PAGGIOLI, J., CHERUKURI, S. & ARTHUR, G.R. (1996). The analgesic response to intravenous lidocaine in the treatment of neuropathic pain. *Anesth. Analg.,* **82,** 91–97.

FOEHRING, R.C., SCHWINDT, P.C. & CRILL, W.E. (1989). Norepinephrine selectively reduces slow Ca^{2+}- and Na^+-mediated K^+-currents in cat neocortical neurons. *J. Neurophysiol.,* **61,** 245–256.

GARFIELD, J.M. & GUGINO, L. (1987). Central effects of local anesthetic actions. In *Handbook of Experimental Pharmacology, Vol. 81: Local Anesthetics*. ed. Strichartz, G.A. pp. 253–284. Berlin: Springer-Verlag.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.,* **391,** 85–100.

HEAD, H. & HOLMES, G. (1911). Sensory disturbances from cerebral lesions. *Brain,* **34,** 102–254.

HUGUENARD, J.R. (1996). Low-threshold calcium currents in central nervous system neurons. *Annu. Rev. Physiol.,* **58,** 329–348.

HUTCHEON, B., MIURA, R.M. & PUIL, E. (1996). Subthreshold membrane resonance in neocortical neurons. *J. Neurophysiol.,* **76,** 683–697.

JAHNSEN, H. & LLINÁS, R. (1984a). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *J. Physiol. (Lond.),* **349,** 205–226.

JAHNSEN, H. & LLINÁS, R. (1984b). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *J. Physiol. (Lond.),* **349,** 227–247.

JONES, E.G. (1985). *The Thalamus*. New York: Plenum Press.

JU, Y.K., SAINT, D.A. & GAGE, P.W. (1992). Effects of lignocaine and quinidine on the persistent sodium current in rat ventricular myocytes. *Br. J. Pharmacol.,* **107,** 311–316.

KOFKE, W.A., SNIDER, M., YOUNG, R.S.K. & RAMER, J.C. (1985). Prolonged low flow isoflurane anesthesia for status epilepticus. *Anesthesiology,* **62,** 653–656.

LEMMEN, L.J., KLASSEN, M. & DUISER, B. (1978). Intravenous lidocaine in the treatment of convulsions. *JAMA,* **239,** 2025.

MARCHETTINI, P., LACERENCA, M., MARANGONI, C., PELLEGATA, G., SOTGIU, M.L. & SMIRNE, S. (1992). Lidocaine test in neuralgia. *Pain,* **48,** 377–382.

MACDONALD, R.L. & KELLY, K.M. (1995) Antiepileptic drug mechanisms of action. *Epilepsia,* **36,** suppl. 2, S2–S12.

MELZACK, R. & CASEY, K.L. (1968). Sensory, motivational, and central control determinants of pain: A new conceptual model. In *The Skin Senses*. ed. Kenshalo, D. pp. 423–443. Springfield: Thomas.

MOUNTCASTLE, V.B., POGGIO, G.F. & WERNER, G. (1963). The relation of thalamic cell response to peripheral stimuli varied over an intensive continuum. *J. Neurophysiol.,* **26,** 807–834.

NORDMARK, J. & RYDQVIST, B. (1997). Local anaesthetics potentiate GABA-mediated Cl^- currents by inhibiting GABA uptake. *NeuroReport,* **8,** 465–468.

ODA, M., YOSHIDA, A. & IKEMOTO, Y. (1992). Blockade by local anaesthetics of the single Ca^{2+}-activated K^+ channel in rat hippocampal neurones. *Br. J. Pharmacol.,* **105,** 63–70.

PALKOVITS, M. & BROWNSTEIN, M.J. (1988). *Maps and Guide to Microdissection of the Rat Brain*. New York: Elsevier.

PETERSON, C.G. (1955). Neuropharmacology of procaine. II. Central nervous actions. *Anesthesiology,* **16,** 976–993.

PUIL, E. & CARLEN, P. (1984). Attenuation of glutamate-action, excitatory postsynaptic potentials, and spikes by intracellular QX 222 in hippocampal neurons. *Neuroscience,* **11,** 389–398.

RIES, C.R. & PUIL, E. (1993). Isoflurane prevents transitions to tonic and burst firing modes in thalamic neurons. *Neurosci. Lett.,* **159,** 91–94.

RIES, C.R. & PUIL, E. (1997). Isoflurane, halothane and enflurane inhibit Na^+ and Ca^{2+} spike potentials of thalamic relay neurons by increasing a leak K^+ conductance (Abstract). *Fifth International Conference on Molecular and Cellular Mechanisms of Anaesthesia,* The University of Calgary, Calgary, Alta., Canada, June 18–20, 1997.

SCHWARZ, S., STÜHMER, W. & PUIL, E. (1997). Lidocaine suppresses repetitive firing in thalamic neurons by inducing a current shunt – a mechanism for sedation and analgesia? (Abstract) *Fifth International Conference on Molecular and Cellular Mechanisms of Anaesthesia, The University of Calgary,* Calgary, Alta., Canada, June 18–20, 1997.

SCHWARZ, S.K.W. & PUIL, E. (1997a). Lidocaine effects on neurons of the ventroposterolateral thalamic nucleus (Abstract). *40th Annual Meeting of the Western Pharmacology Society,* Banff, Alta., Canada, February 8–13, 1997.

SCHWARZ, S.K.W. & PUIL, E. (1997b). Lidocaine alters the membrane properties of neurons in the thalamus – the gateway to sensorium (Abstract). *Can. J. Anaesth.,* **44,** Pt. II, A37.

SCHWARZ, S.K.W. & PUIL, E. (1998). Low, clinically analgesic, sedative and antiepileptic concentrations of lidocaine suppress repetitive firing in thalamic neurons by a mechanism distinct from sodium channel blockade (Abstract). *41st Annual Meeting of the Western Pharmacology Society,* Mazatlán, México, January 25–30, 1998.

SEGAL, M. (1988). Effects of a lidocaine derivate QX-572 on CA1 neuronal responses to electrical and chemical stimuli in a hippocampal slice. *Neuroscience,* **27,** 905–909.

SEO, N., OSHIMA, E., STEVENS, J. & MARC, K. (1982). The tetraphasic action of lidocaine on CNS electrical activity and behaviour in cats. *Anesthesiology,* **57,** 451–457.

SIMONE, D.A., SORKIN, L.S., OH, U., CHUNG, J.M., OWENS, C., LAMOTTE, R.H. & WILLIS, W.D. (1991). Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. *J. Neurophysiol.,* **66,** 228–246.

STAFSTROM, C.E., SCHWINDT, P.C., CUBB, M.C. & CRILL, W.E. (1985). Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex *in vitro*. *J. Neurophysiol.,* **53,** 153–170.

STERIADE, M., JONES, E.G. & LLINÁS, R.R. (1990). *Thalamic Oscillations and Signalling*. New York: Wiley.

STRICHARTZ, G. (1976). Molecular mechanisms of nerve block by local anesthetics. *Anesthesiology,* **45,** 421–441.

SUGIYAMA, K., MUTEKI, T. & SHIMOJI, K. (1992). Halothane-induced hyperpolarization and depression of postsynaptic potentials of guinea-pig thalamic neurons in vitro. *Brain Res.,* **576,** 97–103.

TABATABAI, M. & BOOTH, A.M. (1990). Effects of lidocaine on the excitability and membrane properties of the nerve cell soma. *Clin. Physiol. Biochem.,* **8,** 289–296.

TENNIGKEIT, F., SCHWARZ, D.W.F. & PUIL, E. (1996). Mechanisms for signal transformation in lemniscal auditory thalamus. *J. Neurophysiol.,* **76,** 3597–3608.

TENNIGKEIT, F., RIES, C.R., SCHWARZ, D.W.F. & PUIL, E. (1997). Isoflurane attenuates resonance responses of auditory thalamic neurons. *J. Neurophysiol.,* **78,** 591–596.

THOMSON, A.M. (1988). Biphasic responses of thalamic neurons to GABA in isolated brain slices-II. *Neuroscience,* **25,** 503–512.

TSOUKATOS, J., KISS, Z.H.T., DAVIS, K.D., TASKER, R.R. & DOSTROVSKY, J.O. (1997). Patterns of neuronal firing in the human lateral thalamus during sleep and wakefulness. *Exp. Brain Res.,* **113,** 273–282.

USUBIAGA, J.E., MOYA, F., WIKINSKI, J.A., WIKINSKI, R. & USUBIAGA, L.E. (1967). Relationship between the passage of local anaesthetics across the blood-brain barrier and their effects on the central nervous system. *Br. J. Anaesth.,* **39,** 943–946.

WAGMAN, I.H., DE JONG, R.H. & PRINCE, D.A. (1968). Effects of lidocaine on spontaneous cortical and subcortical activity. *Arch. Neurol.,* **18,** 277–290.

XIE, Z. & SASTRY, B.R. (1992). Actions of somatostatin on GABA-ergic synaptic transmission in the CA1 area of the hippocampus. *Brain Res.,* **591,** 239–247.

(Received November 27, 1997
Revised March 19, 1998
Accepted May 29, 1998)