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Effects of intravenous anaesthetic agents on fast inhibitory oscillations in the rat hippocampus *in vitro*

[1]M.A. Whittington, §J.G.R. Jefferys & *†R.D. Traub

Dept. of Physiology & Biophysics, Imperial College School of Medicine at St. Mary's Hospital, London W2 1PG; §Dept. of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TT; *IBM Research Division, T.J. Watson Research Center, Yorktown Heights, NY 10598, U.S.A. and †Dept. of Neurology, Columbia University, New York, NY 10032, U.S.A.

1 General anaesthetic agents prevent awareness of sensory input and subsequent recall of sensory events after administration. The mechanisms involved in higher sensory processing, including awareness and recall, are not fully elucidated. However, fast oscillations in neuronal activity in the 20–80 Hz (gamma) range have been strongly implicated. Here we have investigated the effects of two anaesthetic agents and a sedative/hypnotic drug on these oscillations.

2 Trains of fast oscillations, shown previously to be shaped by γ-aminobutyric acid ($GABA_A$) receptor activation, were evoked by pressure ejection of L-glutamate (10 mM) onto the perisomatic region of hippocampal area CA1 in the presence of 3-((**R**)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (R-CPP), 50 μM, 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX), 50 μM and 2-hydroxysaclofen, 0.2 mM.

3 Thiopentone (10–200 μM) and propofol (0.5–10 μM) dose-dependently decreased both the maximum oscillation frequency, by approx. 90%, and the incidence of evoked rhythmic oscillations by approx. 60%. Diazepam (0.05–1 μM) decreased maximum oscillation frequency by about 40% but did not affect the incidence of evoked oscillations.

4 The similar effects of thiopentone and propofol were mediated by both a large (about 600%) increase in the decay constant (τ_D) of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (i.p.s.cs) and a bicuculline-sensitive leak current. The two drugs had differing effects on i.p.s.c. amplitude. Diazepam caused a small increase in τ_D (about 170%) and did not alter leak currents at the doses used.

5 Effects of the anaesthetic agents were seen on the above measurements at similar concentrations to those estimated in the CNS during clinical and veterinary anaesthesia. We suggest that the effects on fast oscillations associated with cognition may contribute to the mechanism by which these agents produce general anaesthesia.

Keywords: General anaesthesia; thiopentone; propofol; diazepam; fast inhibitory oscillations; hippocampus; $GABA_A$ receptor; inhibition

Introduction

There have been many studies on the cellular mechanisms of general anaesthetics since the original biophysical studies published at the turn of the century (Meyer, 1899). More recently attention has switched to the possibility of actions mediated by specific effects of anaesthesia on protein, as opposed to lipid, targets on neurones (for review, see Franks & Lieb, 1994).

There is now evidence for effects on voltage-gated channels (Haydon & Urban, 1983, 1986) and a number of receptors for neurotransmitters. The most promising putative site of action at this reductionalist level is the $GABA_A$ receptor, or more generally the function of the GABA inhibitory system in the CNS (Tanelian *et al.*, 1993). Most of the currently used anaesthetic agents have direct or indirect effects on particular aspects of $GABA_A$-ergic inhibitory neurotransmission. The barbituates, volatile aneasthetics and ketamine all prolong the decay constant of $GABA_A$ receptor-mediated postsynaptic events, either evoked or spontaneous (Little, 1982; Gage & Robertson, 1985; MacIver *et al.*, 1991). Similar effects are seen with the intravenous agents, propofol and alphaxolone (Harrison *et al.*, 1987; Hales & Lambert, 1991). Agents which are used as adjuvants, such as opiates, benzodiazepines and α_2 adrenoceptor agonists also have demonstrable effects on GABA-ergic inhibition (Mathers & Yoshida, 1987; Doze *et al.*, 1991; Cohen *et al.*, 1992).

At a more holistic level, various sites of action for general anaesthetics have been proposed, ranging from the spinal cord (Kullmann *et al.*, 1989), the reticular nuclei (Frank & Ota, 1979) and particularly the cortex or thalamocortical systems (Angel, 1991). The cortical components of the auditory evoked potential appear to be particularly sensitive to anaesthetic agents (Plourde & Picton, 1990). These evoked potentials can contain a large 'steady state potential' which shows clear rhythmicity at a frequency of ca 40 Hz (Galambos *et al.*, 1981). Simiar rhythms, in the gamma range, can be found in the hippocampus, neocortex, thalamus and associated nuclei (Gray, 1994). These oscillations are seen in a range of species (Engel *et al.*, 1992) and are associated with higher processing of a number of sensory modalities including proprioception, audition and olfaction.

The proposal that these oscillations may be involved in cortical sensory processing stems from the ideas of von der Marlsberg and Singer. These authors suggest that, in the auditory or visual system, the fine detail of the sensory signal can be interpreted via synchronous oscillations produced in populations of neurones (von der Marlsberg, 1986; Singer, 1990). Subpopulations of neurones are driven by their sensory afferents. These discrete populations then can be coupled and rapidly phase-lock with each other thus generating a temporally coded pattern of activity in the neocortex which corresponds to the pertinent aspects of the sensory stimulus or stimuli (Engel *et al.*, 1991; Gray, 1994; Singer & Gray, 1995).

The mechanism behind the generation of fast oscillations in discrete subpopulations has been the subject of much speculation and a number of hypotheses have been proposed (for a

[1] Author for correspondence.

review, see Jefferys *et al.*, 1996). Two of the more promising hypotheses involve the control of principal cell firing patterns by the influence of individual (Llinas *et al.*, 1991), or interconnected networks of inhibitory interneurones (Whittington *et al.*, 1995). The basis of these models invovles the temporal coding of the firing of excitatory neurones by the $GABA_A$ receptor-mediated inhibitory potential (Lytton & Sejnowski, 1991; Cobb *et al.*, 1995; Traub *et al.*, 1996).

Clinical electroencephalographic recording during maintained anaesthesia shows a clear correlation with depth of anaesthesia and the power of the signal in the higher frequency bands (alpha–gamma) (Berezowskyj *et al.*, 1976). This observation, coupled with the actions of anaesthetics on the GABA system and the role of inhibitory interneurones in generating fast oscillations associated with cognition, suggested that disruption of fast oscillations may represent an important mechanism of action for the production of the aneasthetized state. Here we examine the effects of three agents with known primary sites of action on the $GABA_A$-chloride ionophore complex, on fast oscillations generated in pharmacologically isolated populations of inhibitory interneurones in the hippocampus: two general anaesthetics, thiopentone and propofol, and the sedative/hypnotic, diazepam. We compare the effects of the anaesthetic agents with those of diazepam. Some of the following results have appeared in preliminary form (Whittington *et al.*, 1996).

Methods

Transverse, 400 μm thick, dorsal hippocampal slices were prepared from brains of male Sprague-Dawley rats, 250–300 g, after decapitation following cervical dislocation. Slices were maintained at the interface of warm, wet 95% O_2/5% CO_2 and artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 135, $NaHCO_3$ 16, KCl 3, $CaCl_2$ 2, NaH_2PO_4 1.25, $MgCl_2$ 1, D-glucose 10, equilibrated with 95% O_2/5% CO_2, pH 7.4 at 35°C.

$GABA_A$ receptor-mediated postsynaptic events were isolated from ionotropic glutamatergic excitation by the bath application of 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (R-CPP), 50 μM, and 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX), 50 μM, to the aCSF; 2-hydroxysaclofen, 0.2 mM, was also added to block $GABA_B$ receptor-mediated inhibition. I.p.s.cs were recorded by impaling CA1 pyramidal cells at the level of the soma with glass microelectrodes, resistance 30–60 MΩ filled with 2 M potassium methylsulphate and 50 mM lidocaine N-ethyl bromide (QX314). Membrane voltage was controlled by discontinuous single electrode voltage clamp (Axoclamp 2B), switched at 5–10 kHz. Mean cell membrane resistance was 52±7 MΩ (in the presence of QX314), and resting potential −66±3 mV. Using proximal electrical stimulation to evoke maximal i.p.s.cs the whole-cell $GABA_A$ receptor-mediated conductance change in these cells was estimated to be 95±12 nS.

Trains of fast inhibitory oscillations were evoked by pressure ejection, 2 ms pulse width, of 10 mM L-glutamate at the surface of the hippocampal slice in the perisomatic region. The pressure used was modified for each ejection electrode (one per experiment, 0.8–1.2 kg cm^{-2}) in order to maximize the frequency and amplitude of evoked oscillations (see Traub *et al.*, 1996, Figure 1). All currents were recorded with a holding potential of −40 mV.

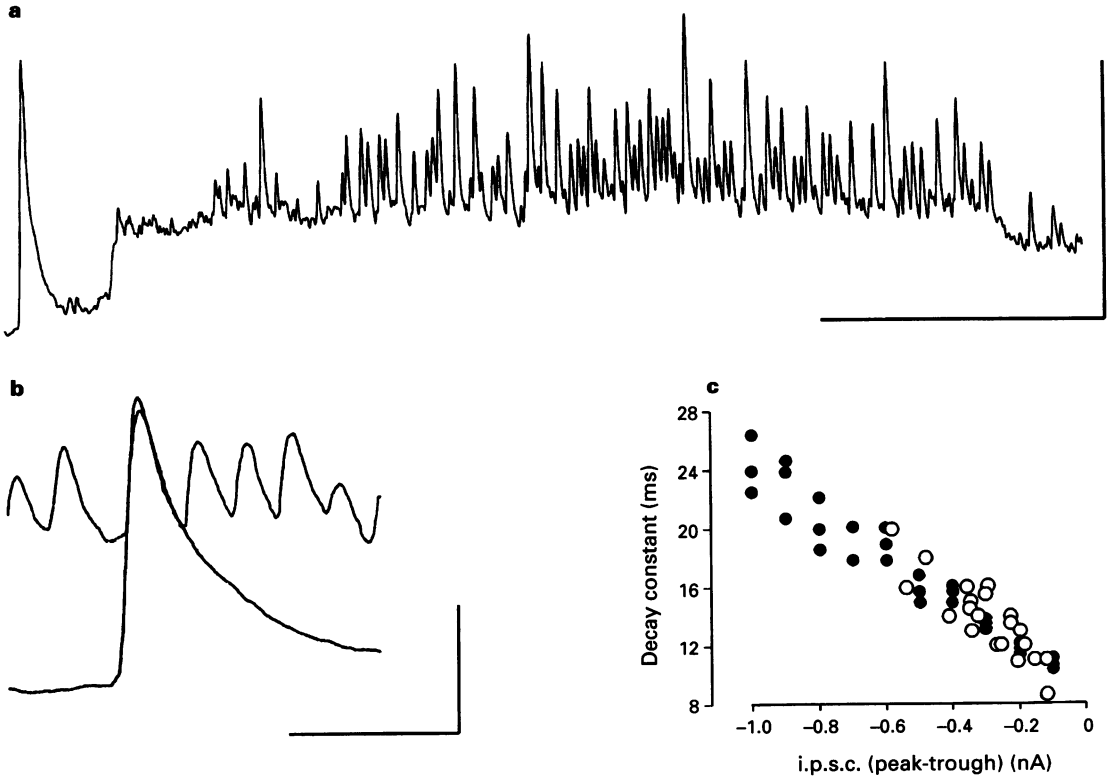

Figure 1 The evoked i.p.s.c. train and measurement of i.p.s.c. τ_D. (a) Example of a train of i.p.s.cs evoked by pressure ejection of 10 mM L-glutamate (2 ms, 0.9 kg cm^{-2}) from a holding potential of −40 mV. Train is preceded by a monosynaptic i.p.s.c. evoked by proximal electrical stimulation adjusted to generate a current with the same amplitude as the maximum current within the train. Maximum i.p.s.c. amplitude in this cell was 2.2 nA from −40 mV. Scale bar = 1 nA, 1 s. (b) Superimposition of the electrically evoked i.p.s.c. and a portion of the i.p.s.c. train. When compared from the peaks the decay phase of the i.p.s.cs are very similar. Scale bar = 0.5 nA, 100 ms. (c) Graph demonstrating the similarity between the electrically evoked single, monosynaptic i.p.s.cs and i.p.s.cs within an evoked train. Points represent distance from peak vs. τ_D for 3 monosynaptic i.p.s.cs (●) compared with the apparent τ_D and visible amplitude for 21 i.p.s.cs within a train (○).

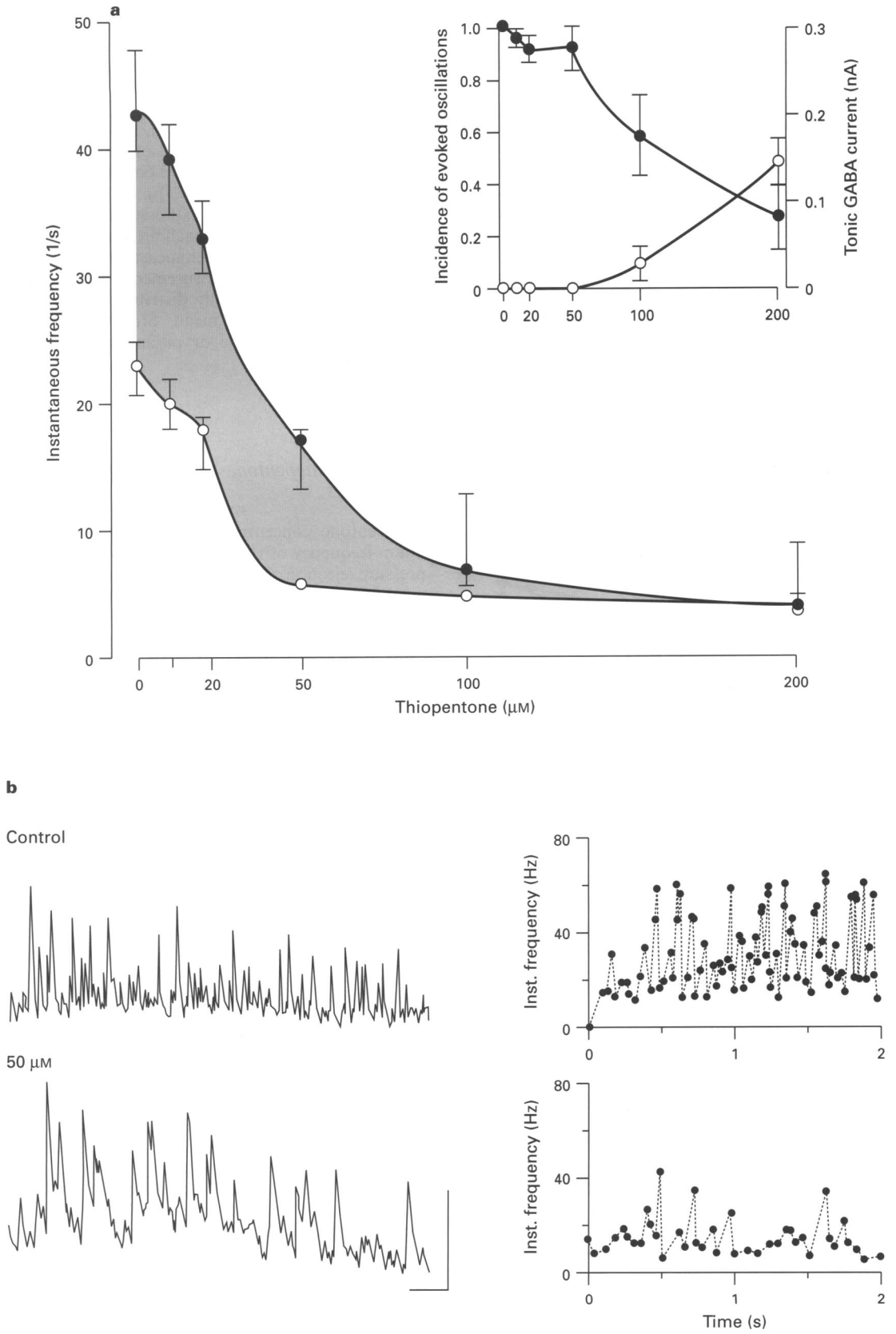

Figure 2 Effects of thiopentone on frequency and incidence of evoked i.p.s.c. trains. (a) Dose-dependence of effects on both maximum (●) and minimum (○) frequency within an evoked train. Data are expressed as median and interquartile range ($n=6-7$, with 3–6 trains analysed per n and concentration). (a) (insert): Graph of incidence of evoked oscillations (●) at each dose of thiopentone plotted alongside the bicuculline-sensitive leak current (○). Data are expressed as mean ± s.e.mean ($n=6-7$). No error bars are shown in leak current measurements unless the mean current was significantly different from control leak. (b) Example traces showing 2 s epochs of evoked trains of i.p.s.cs in controls and in the presence of 50 μM thiopentone. Individual i.p.s.cs were identified by a peak detection programme (CED) and instantaneous frequencies calculated as the reciprocal of the time between adjacent i.p.s.cs. Scale bars = 0.5 nA, 200 ms.

(±)-Thiopentone (Sigma, Poole, Dorset), propofol (RBI, Natick, U.S.A.) and diazepam (Sigma, Poole, Dorset) were prepared as stock solutions in dimethylsulphoxide(DMSO)/distilled water mixtures and kept frozen. Drugs were added to the perfusion solution at the concentrations: thiopentone 10–200 μM, propofol 0.5–10 μM and diazepam 0.05–1 μM. Final DMSO concentration was <0.1%. Slices were allowed to equilibrate for 30 min at each concentration of each drug before recordings were made. Each slice acted as its own control for each drug tested and concentration-response relationships were performed cumulatively.

Recordings were made from 37 CA1 pyramidal cells from slices from 19 rats. Recordings were digitized and analysed with hardware and software from Cambridge Electronic Design (Cambridge, U.K). Power spectra from large epochs (>1 s) within each train of evoked oscillations were used to provide an overview of the type of oscillations elicited. Each train consisted of periods of rhythmic fast, and slower, oscillations interspersed with non-rhythmic trains of i.p.s.cs (Figure 1). Rhythmicity was defined, for the purposes of this study, by measuring 4 consecutive periods of oscillation (5 i.p.s.cs). If the largest and smallest period fell within one standard deviation of the mean period, then the recording was considered to represent a rhythmic oscillation. This procedure was repeated for the slower periods of oscillation within in the train. In this way we could quantify the range of frequencies evoked by L-glutamate and also identify i.p.s.cs within rhythmic periods upon which we performed analysis of i.p.s.cs amplitude and decay constant (fitted to a single exponential function). Decay constants measured from i.p.s.cs within identified rhythmic trains were consistently smaller than those from electrically evoked, monosynaptic i.p.s.cs (Figure 1a). However, when considering the temporal summation of i.p.s.cs within a train, by measuring i.p.s.cs from the peak, good correlation was seen between the two types of data (Figure 1b).

All oscillation data are expressed as median (interquartile range, $n=5-7$ animals for each drug at each concentration) as frequency histograms of each measurement taken showed non-normal distribution. Measurements of anaesthetic-induced leak current were normally distributed and are therefore represented as mean±s.e.mean. Statistical comparisons were made by nonparametric or parametric analysis of variance where appropriate.

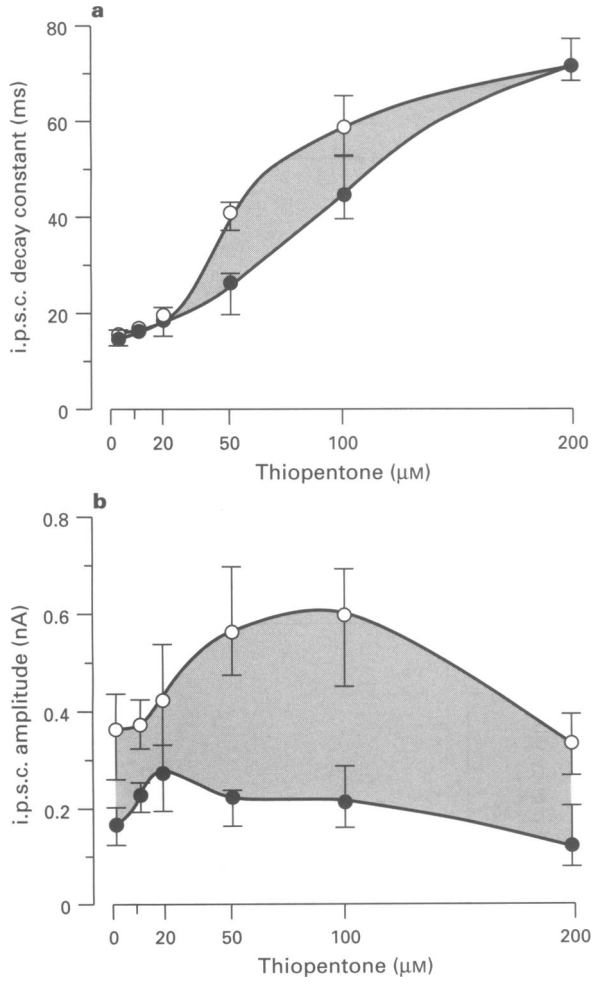

Figure 3 Effects of thiopentone on τ_D and amplitude for i.p.s.cs within evoked trains. (a) Concentration-dependent effect of thiopentone on i.p.s.c. decay constant. Data are expressed as median (interquartile range, $n=6-7$, per concentration) for i.p.s.cs in the faster parts (●), and slower parts (○) of evoked trains (see Figure 2). (b) Effects of thiopentone on the amplitude of the observable portion of individual i.p.s.cs within the fast (●), and slow (○) portions of the evoked trains. Data are expressed as median (interquartile range, $n=6-7$).

Results

Effects of thiopentone on frequency and incidence of oscillations

Thiopentone concentration-dependently decreased the maximum frequency of rhythmic oscillations evoked by glutamate pressure ejection (Figure 2). A small effect on the median maximum frequency were seen with concentrations as low as 20 μM (43 (40–48) Hz reduced to 33 (31–37) Hz). Significant reduction in maximal frequency was seen with bath concentration above 20 μM ($P<0.05$). The effect of the highest concentration of thiopentone used (200 μM) was to reduce the maximum frequency to 5 (5–9) Hz. Qualitative experiments demonstrated that this much slower rhythm was robust at higher concentrations of thiopentone (data not shown) but that the present criteria for rhythmicity were rarely met (see below).

Measurements of the slower frequencies evoked by glutamate revealed a similar pattern. In control slices a slower rhythm of 23 (21–24) Hz was common and was again significantly reduced by concentrations of thiopentone over 20 μM ($P<0.05$). The minimum frequency seen at the highest concentration of thiopentone was 5 (5–6) Hz, indicating that at these higher concentrations (>50 μM) thiopentone not only dramatically decreases oscillation frequency but also removes the intrinsic variability in the frequency of these evoked oscillations.

In control slices, pressure ejection of glutamate to the perisomatic CA1 region almost invariably evoked rhythmic oscillations (incidence = 0.98 (1.00–0.94), pooled control data $n=18$). This was in contrast to the less reliable induction of oscillations by the more physiological method of tetanic stimulation of CA1 afferents (see Discussion). A concentration-dependent decrease in incidence of evoked oscillations was seen throughout the concentration-range used in the present study (Figure 2 insert). At the highest dose, thiopentone significantly decreased oscillation incidence by 70% ($P<0.05$). This decrease in incidence appeared inversely proportional to the emergence of an increase in the tonic leak current required to hold cells at −40 mV. Control tonic leak current was 600±85 pA at −40 mV. This increase in leak was small (140±65 pA) with 200 μM thiopentone and was seen only with concentrations above 50 μM. Bath application of the $GABA_A$ receptor antagonist bicuculline, 30 μM, completely abolished this increase in leak current.

Effects of thiopentone on i.p.s.c. properties

In order to try to elucidate the mechanisms behind the effects of thiopentone on oscillation frequency we measured the decay constant (τ_D) and observable amplitude of i.p.s.cs

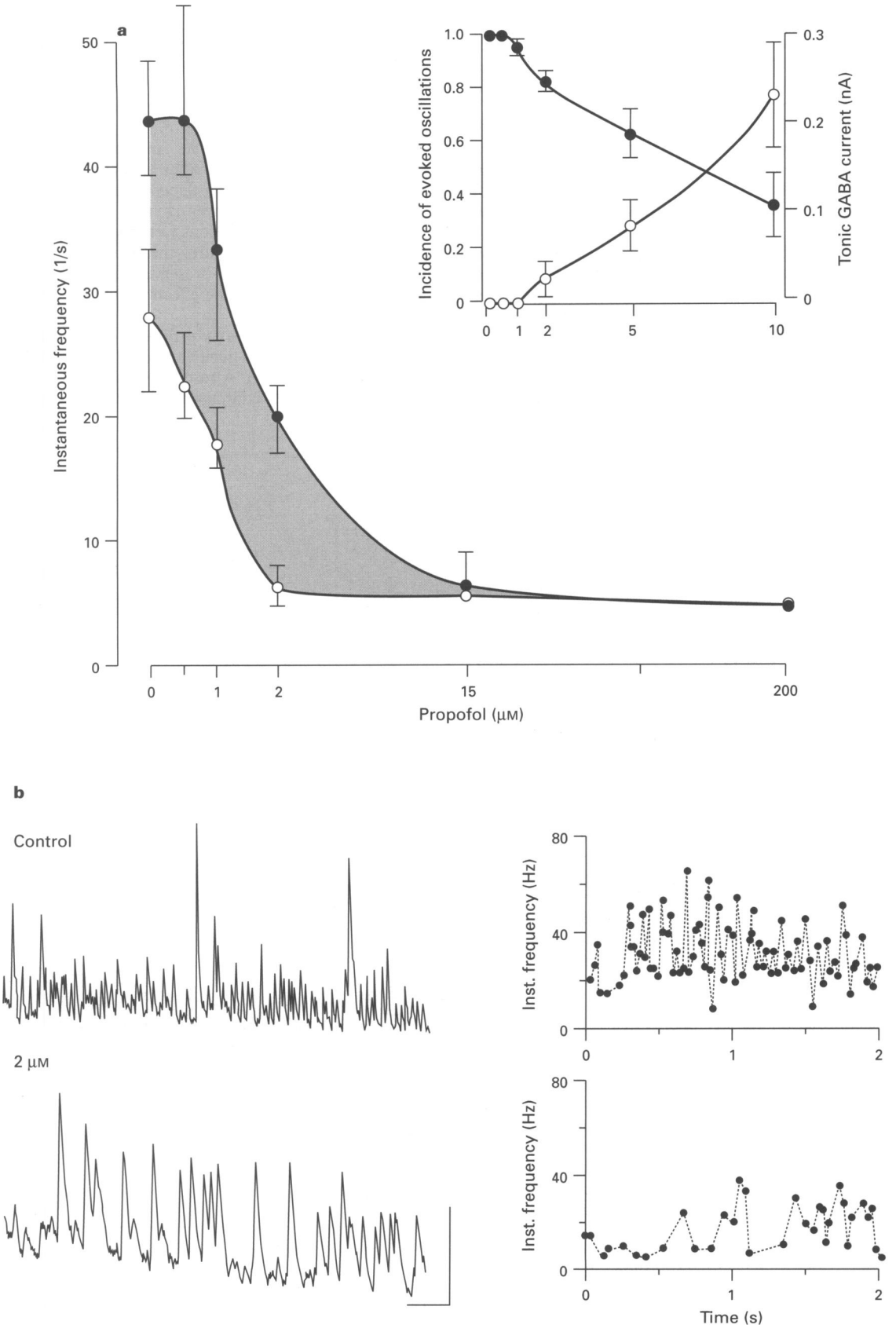

Figure 4 Effects of propofol on frequency and incidence of evoked i.p.s.c. trains. (a) Dose-dependence of effects on both maximum (●) and minimum (○) frequency within an evoked train. Data are expressed as median and interquartile range ($n = 5-7$, with 4–5 trains analysed per n and dose). (a) (insert): Graph of incidence of evoked oscillations (●) at each dose of propofol plotted alongside the bicuculline-sensitive leak current (○). Data are expressed as mean ± s.e.mean ($n = 5-7$). (b) Example traces showing 2 s epochs of evoked trains of i.p.s.cs in controls and in the presence of 2 μM propofol. Instantaneous frequencies were calculated as the reciprocal of the time between adjacent i.p.s.cs. Scale bars = 0.5 nA, 200 ms.

making up the faster and slower rhythmic evoked oscillations. Unlike the frequency measurements, no significant difference was seen in the decay constants for i.p.s.cs making up the faster and slower oscillations (fast = 13 (12–14) ms, slow = 14 (13–15) ms, $P<0.05$). At concentrations above 20 μM thiopentone was seen to have a dramatic, concentration-dependent effect of τ_D (Figure 3a). At the highest concentration a 550% increase was seen for i.p.s.cs within both fast and slower rhythms ($\tau_D = 71$ (68–77) ms). The i.p.s.cs making up the slower rhythms appeared to be relatively more sensitive to thiopentone in the mid-concentration ranges. However, this may only represent the fact that larger i.p.s.cs are seen to make up the slower rhythms. Thus, more of the decay phase of the i.p.s.c is exposed for measurement (see Figure 1b).

There were clear differences in the ability of thiopentone to affect the amplitudes of the i.p.s.cs within the fast and slower rhythms. No significant difference was seen between amplitudes with fast oscillations when comparing any dose of thiopentone with control (Figure 3b, $P<0.05$). In contrast, a significant, 170% increase in i.p.s.c. amplitude within slow rhythms was seen at a concentration of 100 μM ($P<0.05$). This effect was not present at higher concentrations of thiopentone.

Effects of propofol on frequency and incidence of oscillations

Propofol was seen to have effects on measurements of frequency and incidence of evoked oscillations that were qualitatively similar to thiopentone. At concentrations between 0.5 and 5 μM a pronounced decrease in maximum evokable frequency was seen (Figure 4). Significant decreases in the faster rhythm were seen with concentrations above 1 μM ($P<0.05$) with a decrease in frequency down to 4 (3.5–4.5) Hz at the highest concentration used. As with thiopentone, qualitative experiments showed no further decrease in frequency with concentrations up to 50 μM. The slower frequency in the evoked train was also significantly reduced at concentrations above 1 μM ($P<0.05$) with a loss of the intrinsic variability in the frequency of the i.p.s.c. train seen with concentrations above 2 μM.

Over the concentration-range, 1–10 μM, propofol caused a significant, dose-dependent decrease in the incidence of evoked rhythmic oscillations from 1.00 ± 0.02 to 0.37 ± 0.06 ($P<0.05$, Figure 4 insert). This decrease in incidence appeared, as with thiopentone, to be inversely related to the generation of a bicuculline-sensitive tonic leak current. Small fluctuations in leak current were observed with propofol concentrations as low as 2 μM with a significant, maximal effect at 10 μM (increase in leak = 227 ± 68 pA, $P<0.05$).

Effects of propofol on i.p.s.c. properties

Measurement of the effect of propofol on the τ_D of i.p.s.cs within fast and slower rhythms indicated a large increase in decay constant at doses above 1 μM. The maximum effect was seen at the highest concentration of 1 μM, with a significant, 570% increase (τ_D (control) = 13 (12–13), τ_D (fast rhythm) = 73 (67–75) ms, τ_D (slower rhythm = 74 (70–78) ms, $P<0.05$, Figure 5a). An apparent difference in sensitivity to propofol when comparing i.p.s.cs within fast and slower rhythms was again seen (see above).

When compared to thiopentone, propofol had different effects on i.p.s.c. amplitude. The amplitude of i.p.s.cs within both fast and slower rhythms was significantly and sharply increased between 0.5 and 1 μM propofol ($P<0.05$, comparing amplitudes for fast and slow rhythms at 0.5 and 1 μM propofol, Figure 5b). Further increases in i.p.s.c. amplitude were seen with the highest concentration of propofol, with a relatively greater potentiation of i.p.s.cs within the faster rhythms (170% increase within slower rhythms, 311% increase within fast rhythms compared with controls).

Effects of diazepam on frequency and incidence of oscillations

To compare with the effects of the two anaesthetic agents we also examined the effects of the sedative/hypnotic agent, diazepam. Over the concentration-range used (0.05–1 μM) diazepam caused a 46% decrease in the fast evoked rhythms (control 43 (40–47) Hz, 1 μM diazepam 23 (21–27) Hz, $P<0.05$). This lower frequency was not significantly different from the median slower frequency seen in control slices ($P>0.01$, Figure 6). Further increases in diazepam concentration produced to further decrease in fast oscillation frequency. A concentration-dependent effect was also seen on the frequency of the slower rhythm in an evoked train of i.p.s.cs. The slower oscillations were reduced to 7 (5–7) Hz. As can be seen from the figure, the intrinsic variability of the oscillation frequency was preserved, even at 1 μM diazepam. This was in contrast to the effects of the two intravenous anaesthetics.

Unlike thiopentone and propofol, no significant effect of diazepam was seen on incidence of rhythmic evoked oscillations (Figure 6 insert). At a concentration of 1 μM, incidence was 0.82 ($P>0.05$ compared with control incidence). This

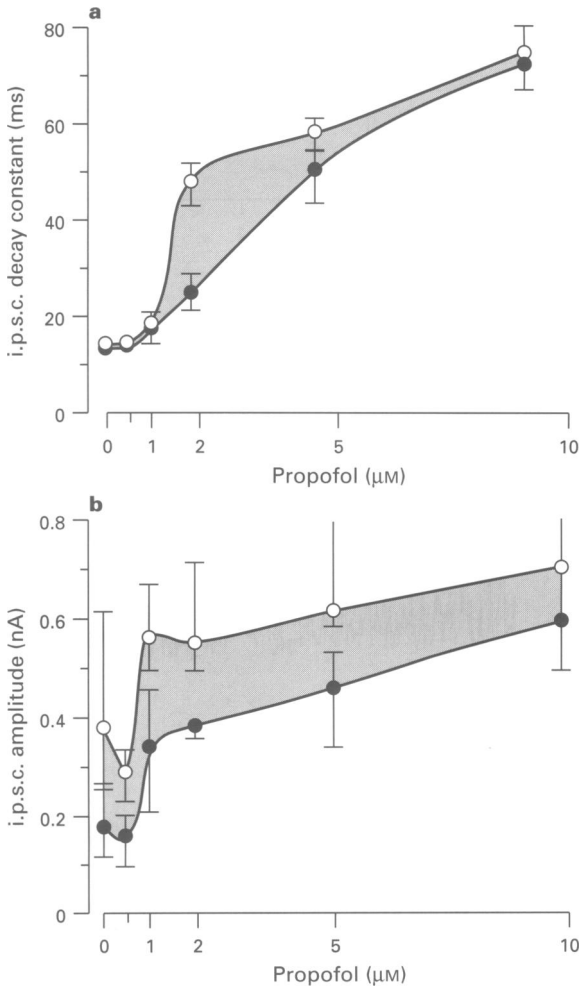

Figure 5 Effects of propofol on τ_D and amplitude for i.p.s.cs within evoked trains. (a) Concentration-dependent effects of propofol on i.p.s.c. decay constant. Data are expressed as median (interquartile range, $n = 5–7$ per concentration) for i.p.s.cs in the faster parts (●), and slower parts (○) of evoked trains (see Figure 4). (b) Effects of propofol on the amplitude of the observable portion of individual i.p.s.cs within the fast (●), and slow (○) portions of the evoked trains. Data are expressed as median (interquartile range, $n = 5–7$).

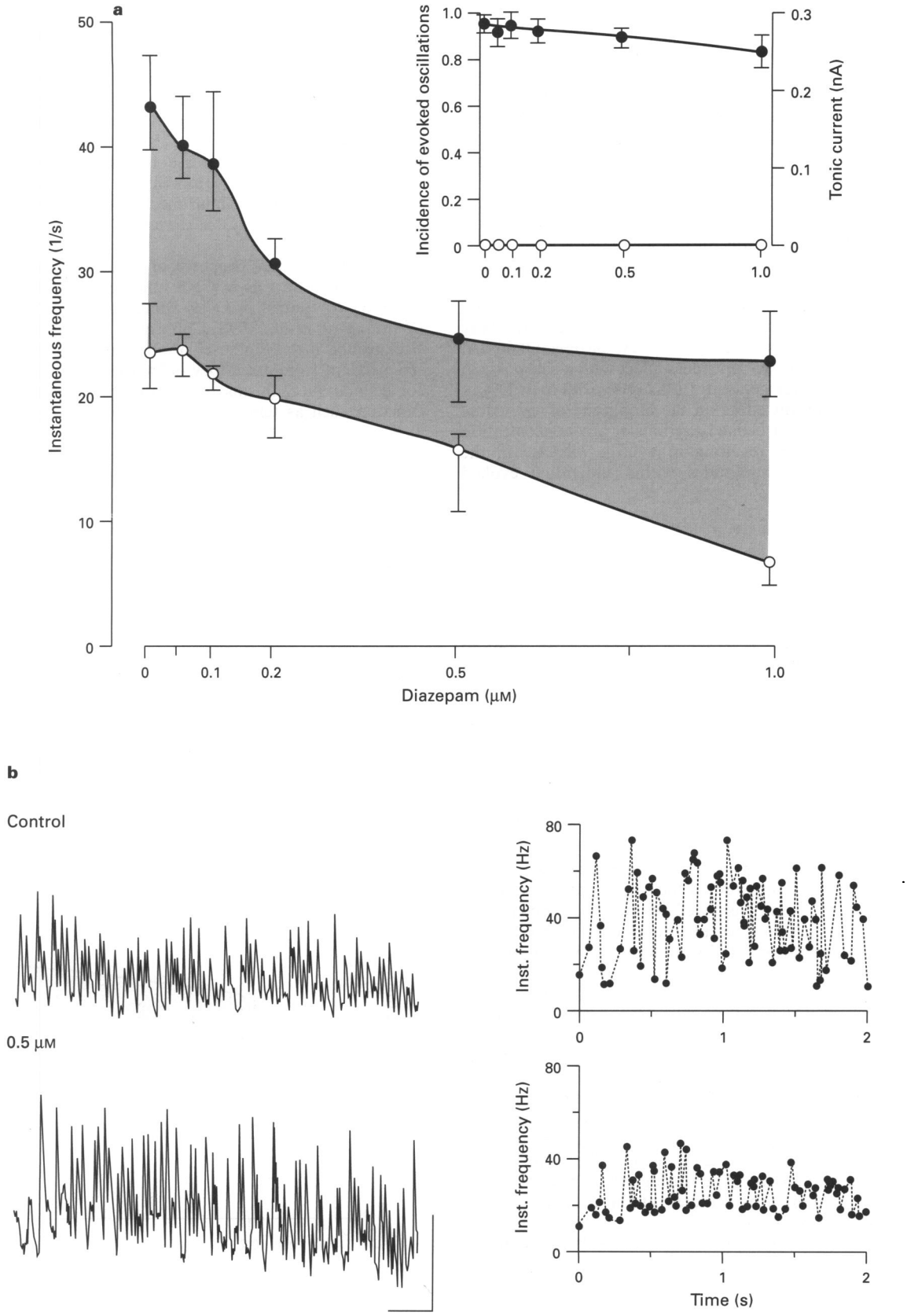

Figure 6 Effects of diazepam on frequency and incidence of evoked i.p.s.c. trains. (a) Dose-dependence of effects on both maximum (●) and minimum (○) frequency within an evoked train. Data are expressed as median and interquartile range ($n=5$, with 4–8 trains analysed per n and dose). (a) (insert): Graph of incidence of evoked oscillations (●) at each dose of diazepam. Diazepam did not generate any measurable increase in leak current (○). Data are expressed as means ± s.e.mean ($n=5$). (b) Example traces showing 2 s epochs of evoked trains of i.p.s.cs in controls and in the presence of 0.5 μM diazepam. Instantaneous frequencies were calculated as the reciprocal of the time between adjacent i.p.s.cs. Scale bars = 0.5 nA, 200 ms.

observation corresponded to the apparent inability of diazepam to generate a bicuculline-sensitive increase in tonic leak current within the concentration-range used.

Effects of diazepam on i.p.s.c. properties

Diazepam had little effect on τ_D for i.p.s.cs measured within fast and slower rhythms at all concentrations investigated (Figure 7a). A maximum median increase of 125% over control values was seen with i.p.s.cs within the faster rhythms (control 12 (11–12) ms, 1 μM diazepam 15 (14–17) ms, $P<0.05$). A larger, 170% increase in median τ_D was seen with i.p.s.c. measurements from the slower rhythms (control 12 (11–12) ms, 1 μM diazepam 20, 17–22) ms, $P<0.05$). These results were in stark contrast to the dramatic effects of propofol and thiopentone in this measurement.

Diazepam did, however, have a large effect on the amplitude of individual i.p.s.cs within slower rhythms in the evoked trains (Figure 7b). A 275% increase in median i.p.s.c. amplitude was seen when comparing control rhythms with those evoked in 1 μM diazepam (control 0.24 nA, diazepam 0.66 nA, $P<0.05$). This was a dose-dependent effect with a sharp rise in amplitude at the lower concentrations (100–200 nM). Diazepam had no significant effect on the amplitude of individual i.p.s.cs within the faster evoked rhythms at any concentration investigated ($P>0.05$), resulting in a large increase in the variability of i.p.s.c. amplitudes within individual evoked trains.

Discussion

Fast oscillations consisting of trains of inhibitory postsynaptic currents could be reliably evoked by pressure ejection of L-glutamate. The oscillations occurred in the 20–50 Hz range in control hippocampal slices and were altered by the three agents tested in a concentration-dependent manner. The two anaesthetic agents, thiopentone and propofol showed qualitatively similar effects, causing a decrease in both the incidence of the evoked trains and the frequencies at which oscillations were seen. These effects were accompanied by concentration-dependent increases in both decay constant and amplitude of i.p.s.cs within evoked trains and the generation of an increase in leak current. Diazepam had a smaller effect on oscillation frequency and did not change the incidence of evoked trains. Large increases of i.p.s.c. amplitude were seen but relatively little effect on decay constant was apparent at the concentrations used.

Each of the drugs used has a well-characterized primary site of action at the $GABA_A$ receptor-ionophore complex (Sieghart, 1992). Generation of fast inhibitory oscillations has been shown to be modulated by the shape of the $GABA_A$ receptor-mediated inhibitory postsynaptic potential, specificaly at the interneurone-interneurone synapse in the hippocampus (Whittington *et al.*, 1995; Traub *et al.*, 1996). It is perhaps not surprising then that the drugs used had adverse effects on these fast oscillations. What is interesting is that the magnitude of the observed effects could be related to their anaesthetic potency. The major effects of the two anaesthetic agents were a large increase in decay constant and an increase in $GABA_A$ receptor-mediated leak current. The increased decay constant prolongs the time during which the inhibitory interneuronal membrane is buffered at the chloride reversal potential. This therefore decreases the rate at which interneurones can fire action potentials as a result of excitation (provided here by L-glutamate activation of presumed metabotropic glutamate receptors, Miles & Poncer, 1993). This sculpting of the interneuronal firing rate would produce the decrease in inhibitory oscillation frequency detected at the principal cell in this study. Tonic inhibition of interneurones with GABA currents of similar magnitude to the leak currents seen in the present study abolished fast oscillations (R.D. Traub, unpublished computer simulations). A decrease in incidence was seen accompanying the increased leak in the present observations suggesting a causative relationship. Similar increases in non-specific membrane leak current with inhalation anaesthetics (MacIver & Kendig, 1991) compared with propofol and barbiturates (Peters *et al.*, 1988; Hara *et al.*, 1993), would have a similar effect on fast oscillations.

The sedative/hypnotic agent, diazepam, had significant effects on fast oscillations but caused considerably less pertubation than the anaesthetic agents. This observation fitted well with the suggestion that the thiopentone and propofol exerted their effects by the above two mechanisms: Diazepam had a much smaller effect on i.p.s.c. decay constant and did not generate a detectable increase in leak current at the concentrations used. These observations bring us closer to an understanding of the cellular mechanisms behind the different pharmacological profiles of diazepam and the general anaesthetics.

The fundamental property of fast inhibitory oscillations which indicates a role in CNS function is the ability of interneurones to control precisely firing patterns in principal cells (Whittington *et al.*, 1995; Cobb *et al.*, 1995). By providing a background membrane oscillation in excitatory cells, partially independent from excitatory influences, a working hypothesis for cellular mechanisms responsible for aspects of cognitive function such as temporal coding (Hopfield, 1995) and neu-

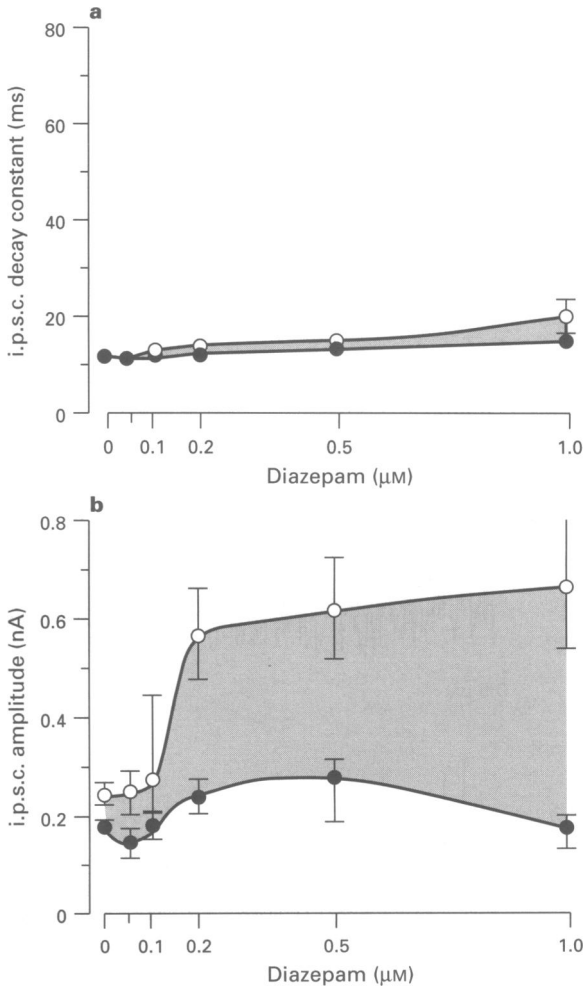

Figure 7 Effects of diazepam on τ_D and amplitude for i.p.s.cs within evoked trains. (a) Concentration-dependent effects of diazepam on i.p.s.c. decay constant. Data are expressed as median (interquartile range, $n=5$ per concentration) for i.p.s.cs in the faster parts (●), and slower parts (○) of evoked trains (see Figure 6). (b) Effects of diazepam on the amplitude of the observable portion of individual i.p.s.cs within the fast (●), and slow (○) portions of the evoked trains. Data are expressed as median (interquartile range, $n=5$).

ronal assembly formation (Hebb, 1958) becomes more tenable. Singer and others have suggested that it is the timing of cell firing that is important for aspects of cognitive function such as figure-ground separation and binding of sensory features into a perceived object. It is likely that the fast inhibitory oscillations described here, contribute to the control of cell firing during the above cognitive tasks (for discussion see Jefferys *et al.*, 1996). However, if we are to link the effects of the general anaesthetics tested to a disruption in these aspects of cognitive function (Flohr, 1995) we have to ask the question, how much fast oscillations need to be disrupted.

The present observations showed differences in the level of disruption of fast oscillations depending on whether the agent used had sedative or anaesthetic effects. Clinically the difference between sedation, sleep and anaesthesia can be very subtle and can be thought of as a sliding scale. From these observations two scenarios are postulated: Firstly that it is the decreased incidence of fast oscillations that corresponds to the anaesthetic effects. Generation of fast oscillations by pressure ejection is very reliable but removed from more physiologically relevant techniques such as tetanic afferent stimulation (Whittington *et al.*, 1995). Preliminary observations show that fast oscillations generated by tetanus are much harder to elicit in the hippocampal slice preparation but their incidence was considerably more sensitive to general anaesthetic agents. There is a body of evidence to show that deep anaesthesia is associated with a lack of higher EEG frequencies (>20 Hz) and that the depth of anaesthesia inversely correlated with the power at higher frequencies (Berezowskyj *et al.*, 1976). Secondly, that it is the decrease in oscillation frequency which corresponds to the anaesthetic action of these drugs. Present theories regarding the binding phenomenon suggest that the setting-up of fast oscillations in a number of brain regions facilitates the generation of synchronous firing between regions and therefore formation of neuronal assemblies. This process takes a finite amount of time, usually a few periods of oscillation (Engel *et al.*, 1991). In the conscious brain this time corresponds to the 'psychological refractory period' and represents the minimum time taken to switch attention from one task to another (Woods *et al.*, 1980).

In conclusion profound effects of the general anaesthetic agents thiopentone and propofol were seen on fast inhibitory oscillations. Effects were seen within the concentration-ranges associated with both clinical and veterinary anaesthesia. We suggest that these effects of general anaesthetic agents may contribute significantly to their ability to disrupt sensory processing.

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