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FREQUENCY-DEPENDENT DEPRESSION OF INHIBITION IN GUINEA-PIG NEOCORTEX *IN VITRO* BY $GABA_B$ RECEPTOR FEED-BACK ON GABA RELEASE

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SUMMARY

1. The mechanisms involved in the lability of inhibition at higher frequencies of stimulation were investigated in the guinea-pig *in vitro* neocortical slice preparation by intracellular recording techniques. We attempted to test the possibility of a feed-back depression of GABA on subsequent release.

2. At resting membrane potential (E_m, $-75{\cdot}8 \pm 5{\cdot}2$ mV) stimulation of either the pial surface or subcortical white matter evoked a sequence of depolarizing and hyperpolarizing synaptic components in most neurones. An early hyperpolarizing component ($IPSP_A$) was usually only obvious as a pronounced termination of the EPSP, followed by a later hyperpolarizing event ($IPSP_B$). Current–voltage relationships revealed two different conductances of about 200 and 20 nS and reversal potentials of $-73{\cdot}0 \pm 4{\cdot}4$ and $-88{\cdot}6 \pm 6{\cdot}1$ mV for the early and late component, respectively.

3. The conductances of $IPSP_A$ and $IPSP_B$ were fairly stable at a stimulus frequency of 0·1 Hz. At frequencies between 0·5 and 2 Hz both IPSPs were attenuated with the second stimulus and after about five stimuli a steady state was reached. Concomitantly IPSPs were shortened. The average decrease in synaptic conductance between 0·1 and 1 Hz was 80% for the $IPSP_A$ and 60% for the $IPSP_B$. At these frequencies the reversal potentials decreased by 5 and 2 mV, respectively; E_m and input resistance (R_{in}) were not consistently affected.

4. The amplitudes of field potentials, action potentials and EPSPs of pyramidal cells were attenuated less than 10% at stimulus frequencies up to 1 Hz, suggesting that alterations in local circuits between the stimulation site and excitatory input onto inhibitory interneurones may play only a minor role in the frequency-dependent decay of IPSPs.

5. Localized application of GABA produced multiphasic responses. With low concentrations and application near the soma an early hyperpolarization prevailed followed by a depolarizing late component. Brief application of GABA at low frequencies induced constant responses; at higher frequencies, the responses sometimes declined. The current–voltage relationships of the two GABA responses

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were similar to each other and to the early IPSP. An apparently fivefold higher conductance was estimated at lower E_ms, suggesting that the GABA response had a voltage sensitivity. The slope conductance of IPSPs was decreased by up to 50% for tens of seconds after postsynaptically detectable effects of GABA had dissipated.

6. Application of the GABA uptake inhibitor nipecotic acid (50–500 μM) reduced the conductance of both components of orthodromically evoked inhibition and shortened the IPSP at low frequencies, but had no additional effects at higher stimulation rates. E_m and R_{in} were not consistently affected. Application of nipecotic acid usually decreased the latency and increased the slope conductance of GABA responses. Depression of IPSPs by nipecotic acid at low frequencies, in the face of enhanced GABA responses, is probably presynaptically mediated. The prolonged time course of synaptically liberated GABA by nipecotic acid may cause extended presynaptic effects.

7. Application of baclofen elicited a dose-dependent hyperpolarization of increasing amplitude (up to 4 mV) and duration (up to 1 min). The postsynaptic conductance increase was small. Repetitive application of baclofen elicited responses of constant amplitude, suggesting that the postsynaptic baclofen response exhibits little if any desensitization. Baclofen markedly and reversibly reduced both components of the IPSP. The attenuation of IPSPs outlasted the postsynaptically detectable effects of baclofen.

8. The alteration of IPSP frequency dependence by reduction of GABA uptake suggests that the GABA transient in the synapse plays a key role in this phenomenon. The similar depression of $IPSP_A$ and $IPSP_B$, despite their different postsynaptic properties, by GABA and by baclofen implies a common presynaptic mechanism. It is proposed that GABA exerts a negative feed-back on its release through activation of a $GABA_B$ type receptor.

INTRODUCTION

The inhibitory function of GABA (γ-aminobutyric acid) in the central nervous system is well documented (Krnjević, 1974; Nistri & Constanti, 1979) and various drugs impairing GABAergic inhibition invariably induce abnormal neuronal discharges (Kandel & Spencer, 1961). Inhibitory postsynaptic potentials (IPSPs) were also shown to be reduced at higher frequencies of stimulation in motoneurones (Curtis & Eccles, 1960), hippocampal pyramidal cells *in vivo* (Ben-Ari, Krnjević & Reinhardt, 1979) and *in vitro* (McCarren & Alger, 1985) and in neocortical cells (Connors, Gutnick & Prince, 1982). Like drug-induced depression of IPSPs, frequency-dependent attenuation may facilitate the onset of abnormal neuronal discharges. The contribution of various mechanisms such as changes in ionic gradients, desensitization of postsynaptic receptors or frequency dependence of excitatory transmission to the attenuation of IPSPs has not been fully evaluated in neocortical neurones.

For example, the attenuation of EPSPs at higher stimulus frequencies (Curtis & Eccles, 1960; Miles, 1986) may also occur at inhibitory interneurones and result in reduced IPSPs. Alternatively, the ionic gradient underlying postsynaptic inhibition may exhibit a use-dependent decrease. The decline of IPSPs at higher frequencies of

stimulation in hippocampal neurones *in vitro* is associated with changes in conductance and the ionic gradient (McCarren & Alger, 1985) and fading of GABA responses has been attributed to shifts of the Cl^- equilibrium potential (Segal & Barker, 1984; Huguenard & Alger, 1986). The concomitant extracellular K^+ changes reported (McCarren & Alger, 1985) may have also affected the inhibitory gradient. For example, in the crayfish stretch receptor elevation of extracellular K^+ was shown to decrease the driving force for inhibition by causing an increase in intracellular Cl^- activity (Deisz & Lux, 1982), an effect attributed to the decreased net outward transport of Cl^- by a K^+–Cl^- co-transport (Aickin, Deisz & Lux, 1982). The decline of inhibition may also be brought about by a postsynaptic desensitization of GABA receptors as demonstrated in peripheral (Desarmenien, Feltz & Headley, 1980; Gallagher, Nakamura & Shinnick-Gallagher, 1983) and central neurones (Numann & Wong, 1984; Huguenard & Alger, 1986).

Finally, GABA uptake, if once activated by released GABA, may reduce the concentration of available transmitter and hence the efficacy of subsequent IPSPs (Krnjević, 1984). Initially, GABA uptake was proposed to remove synaptically liberated GABA and thereby terminate transmitter action (Iversen & Neal, 1968), analogous to the function of acetylcholinesterase at the end-plate (Katz & Miledi, 1973). Enhancement of GABA responses by nipecotic acid, a specific blocker of GABA uptake (Krogsgaard-Larsen & Johnston, 1975) in peripheral ganglion cells (Brown & Galvan, 1977; Gallagher *et al.* 1983; but see Desarmenien *et al.* 1980), has only limited implications for GABAergic transmission, since GABAergic innervation is lacking in these neurones. Nevertheless, the variability observed (cf. Brown & Galvan, 1977, and Desarmenien *et al.* 1980) might indicate that the effect of uptake reduction may vary also at different GABAergic synapses, perhaps dependent upon geometric factors as shown in glutaminergic synapses (compare Crawford & McBurney, 1977; Clark, Gration & Usherwood, 1980).

Results of extracellular recordings from central neurones indicate that the effects of ionophoretically applied GABA are enhanced and accelerated by nipecotic acid (Curtis, Game & Lodge, 1976). Inhibitory transmission, however, was either little affected (Curtis *et al.* 1976) or prolonged (Matthews, McCafferty & Setler, 1981) by reduction of GABA uptake. Intracellular recordings from hippocampal pyramidal cells confirmed a prolongation of IPSPs by nipecotic acid (Dingledine & Korn, 1985), attributable to enhancement of a late component of the synaptic current (Hablitz & Lebeda, 1985). But, nipecotic acid severely reduced IPSP amplitudes in the majority of neurones (Dingledine & Korn, 1985). The latter observation is remarkably similar to that in the crayfish stretch receptor where synaptic efficacy declined after blockade of GABA uptake by nipecotic acid (Aickin & Deisz, 1981). Yet, neither the time course of inhibitory potentials nor that of currents (Deisz, Dose & Lux, 1984) were affected, despite evidence for a sodium-dependent GABA uptake system sensitive to nipecotic acid (Deisz & Dose, 1983).

We have investigated the properties of postsynaptic inhibition of neocortical neurones in order to elucidate the critical mechanisms involved in the lability of central inhibition. In addition we tried to resolve the role of GABA uptake for inhibitory transmission in the neocortex. The results of these experiments indicate that postsynaptic inhibition is attenuated by frequencies of stimulation above

0·1 Hz. This marked frequency dependence cannot be found in any of the other accessible parameters, suggesting its localization at the inhibitory synapse. We propose that the mechanism accounting for the lability of inhibition at moderate frequencies of stimulation is the modulation of GABA release by a negative feedback mechanism. Some of these results have been reported in preliminary form (Deisz & Prince, 1986).

METHODS

General

The methods employed were similar to those described in previous studies from the same laboratory (Connors *et al.* 1982; McCormick, Connors, Lighthall & Prince, 1985; Deisz & Prince, 1987). Guinea-pigs (200–400 g) were anaesthetized with 5 mg Nembutal/100 g, cooled to 30–35 °C, and decapitated using a small-animal guillotine. A block of cortical tissue corresponding to the sensorimotor area was rapidly removed, immersed in Ringer solution (10–12 °C) for about 1 min and then mounted on a vibratome (Lancer). Typically four slices (approximate thickness, 500 μm) were transferred within 5–10 min into an interface-type experimental chamber. Slices were allowed to recover for at least 1 h before recording commenced. The acrylic stage of the chamber was covered with layers of lens paper. The normal saline solution contained, in mM: NaCl, 124; KCl, 5; NaH_2PO_4, 1·25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26 and dextrose, 10. After equilibration with a mixture of 95% O_2 and 5% CO_2, this solution had a pH of 7·4 at the experimental temperature of 37 °C. The low perfusion rate (about 0·5 ml/min) facilitated prolonged recordings. The atmosphere of the chamber consisted of humidified 95% O_2 and 5% CO_2. In some experiments nipecotic acid was bath-applied at concentrations between 50 and 500 μM. GABA (2 mM) or (±)-baclofen (100 μM) in normal Ringer solution were applied from broken micropipettes (tip diameter, 1 μm) by pressure pulses (10–2000 ms, 8–20 lbf/in^2). The tip diameter and pressure were adjusted so that a 2 s pressure pulse ejected a droplet of about 50 μm in diameter. If a linear relationship between time and ejected volume is assumed for a given electrode, the typical application of GABA of about 20 ms ejected a volume of less than 1 pl. The few femtomoles applied were sufficient to evoke a localized response. Ejection electrodes were positioned about 50 μm from the presumed somatic recording site and advanced into the slice with a manually operated micro-manipulator (Narishige). The position yielding a maximum response was determined by advancing or withdrawing the electrodes by up to 20 μm before starting a series of measurements. The absence of a response at both ends of this traverse verified a localized action. Usually the responses were constant for a given pulse duration.

Recording and stimulation

Intracellular electrodes were pulled from glass capillaries (0·5 mm i.d., 1 mm o.d., Frederick Haer) with a horizontal airjet puller (BV10, Sutter Instruments). After bevelling, the electrodes had resistances between 150 and 180 MΩ when filled with 4 M-potassium acetate titrated to pH 7·4. Electrodes were tested for rectification before and after impalement in the range of currents applied (typically ±0·5 nA) and only recordings with satisfactory electrodes were considered for analysis. Bipolar sharpened tungsten electrodes (tip separation 200 μm) were positioned at the junction of the cortex and subcortical white matter. Stimulus pulses (5–50 V, 100 or 200 μs) were delivered through a stimulus isolation unit (WPI) at the frequency indicated. The stimulus intensity was adjusted to about 60% of the apparent maximal response and maintained constant throughout an experiment. Intracellular electrodes were connected to a high-impedance amplifier (Neurodata Instruments) and the recorded signals were stored on tape (Ampex, bandwidth 0–5 kHz) or on a VCR recording system (Neurocorder) for subsequent analysis with a digital oscilloscope (Nicolet) and a minicomputer (DEC, Minc 23).

Data analysis

We adopted the following protocol to evaluate the efficacy of synaptic inhibition despite the spatial and temporal overlap of the synaptic components. The synaptic potentials were elicited at a given frequency after stimulus-free intervals of up to 2 min. When the depression of IPSPs had attained a steady state, a series of depolarizing and hyperpolarizing current pulses was injected at

the same frequency as the orthodromic stimulus. Each family of synaptic events, consisting of fifteen to twenty records at different membrane potentials, was digitized and measured. Plots of IPSP amplitudes *versus* membrane potential yielded the apparent reversal potential. Membrane conductance was estimated from the slope of membrane potential deflection, measured just before the orthodromic stimulus, *versus* injected current. Similarly, plots of membrane potential at the peak of the IPSPs *versus* injected current were used to determine IPSP conductance. Assuming that the total conductance at the peak of the IPSP is governed by the sum of the 'passive' conductance and the transmitter-induced conductance, the resting membrane conductance was subtracted to give an estimate of the synaptic conductance. This method allowed a reliable estimate of synaptic conductances between 10^{-8} and 2×10^{-6} S. To account for the voltage-dependent rectification of resting conductance (Connors *et al.* 1982), responses during depolarizing and hyperpolarizing current steps were treated separately in the linear portion (up to 0·4 nA). Division of conductances in depolarizing and hyperpolarizing directions yields the rectification ratio. This method certainly represents an oversimplification of the possible interactions between synaptic conductances and the complex time- and voltage-dependent components which might be activated. Although only a first approximation of the underlying synaptic conductance is gained, the data to be presented below indicate that this method provides sufficient resolution for the issues addressed here.

RESULTS

General properties

A total of thirty-five neurones recorded from layers II and III was selected from a larger sample using the criteria of neuronal input resistance (R_{in}) of at least 20 MΩ, stability of membrane potential for at least 20 min, and spike height of at least 90 mV (estimated from the oscilloscope). Membrane potential (E_m) of accepted neurones varied from −67 to −84 mV (−75·8 ± 5·2 mV, mean ± S.D., $n = 35$) and R_{in} from 22 to 180 MΩ (46 ± 27 MΩ, $n = 35$). Spike amplitudes were measured in some of the neurones and ranged from 101 to 115 mV (107 ± 7·2 mV, $n = 11$). Moreover, only neurones with sizeable IPSPs and negligible drift of E_m (below 3 mV) were used. Recordings from neurones accepted on the basis of these fairly stringent criteria typically lasted 1–2 h. In most neurones an apparently pure EPSP was evoked with low intensity of stimulation, but an inhibitory component became apparent with increased stimulus intensity. In some neurones the IPSP was only discernible as an accelerated decay phase of the EPSP when the neurone was at resting E_m (see Fig. 1*A* and 3*B*); in others a distinct hyperpolarization predominated (Fig. 3*A*). This variation depended upon the difference between resting E_m and the equilibrium potential of the IPSP (E_{IPSP}) and the contribution of the EPSP. A late inhibitory component with a peak hyperpolarization of about 3 mV, 100 ms after the stimulus, was usually present at higher stimulus intensities (see Fig. 3). The general properties of the two hyperpolarizing components in neocortical neurones have been described (Connors & Malenka, 1985; Connors, Malenka & Silva, 1988) and further elaboration of the ionic mechanisms involved is presented elsewhere (Thompson, Deisz & Prince, 1986, 1988*a*, *b*). By analogy to hippocampal pyramidal cells (Newberry & Nicoll, 1985) and to previous evidence in neocortex (Connors & Malenka, 1985), the early IPSP fulfils the criteria for a $GABA_A$ receptor-mediated event, while the late hyperpolarizing component is presumably mediated by a different receptor ($GABA_B$) and ionic mechanism. Therefore we shall refer to the early and late components as $IPSP_A$ and $IPSP_B$, respectively. Here we will focus on those points pertinent to the mechanisms of IPSP depression during low frequency of stimulation (between 0·1 and 2 Hz).

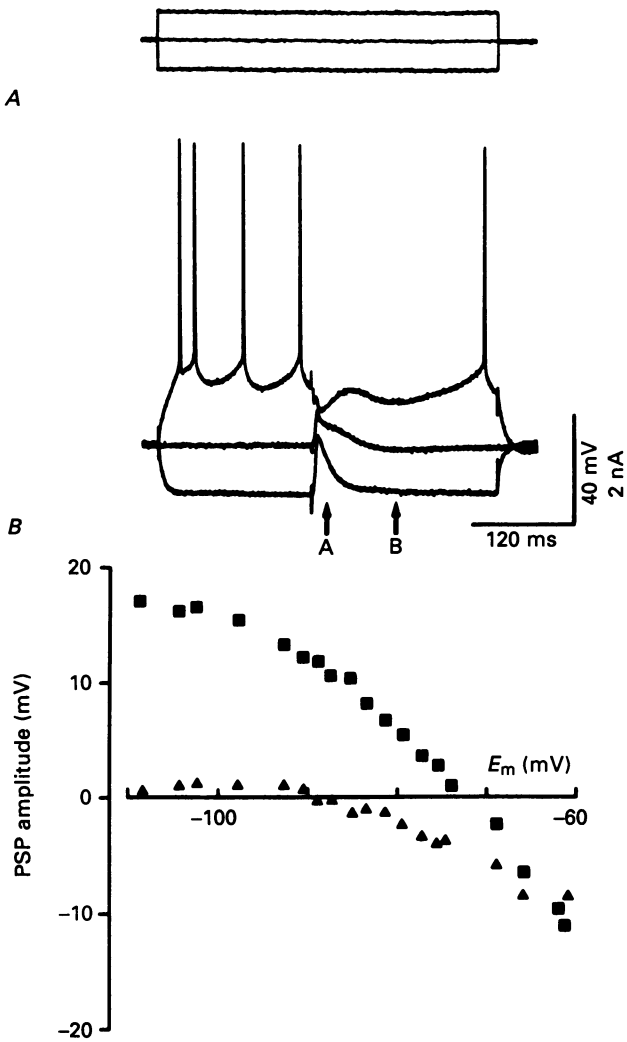

Fig. 1. Voltage dependence of synaptic components. *A*, changes in membrane potential and evoked synaptic responses (bottom traces) induced by current injection (top traces). Membrane potential (E_m, −82 mV) was measured about 5 ms before the stimulation (obvious from the truncated artifacts) and at the peak of the early (arrow A) and late component (arrow B). *B*, the amplitudes of the synaptic components determined at A and B have been plotted *versus* membrane potential to determine the reversal potential for the early (E_{IPSP_A}, −73 mV) and late component (E_{IPSP_B}, −88 mV). A, ■; B, ▲.

Owing to the small and variable difference between resting E_m and E_{IPSP_A} the IPSP amplitude as such is of little, if any, use in quantifying the parameters of inhibition. Therefore we always determined the IPSP amplitudes at various E_m values to obtain the conductances activated (see Methods). However, the temporal overlap of EPSP, $IPSP_A$ and $IPSP_B$ not only distorts the amplitude of each synaptic component but also precludes the exact determination of the reversal potential. Therefore, the terms 'reversal potential' or 'synaptic conductance' used here refer to the 'apparent'

value. Depolarizing the membrane by current injection increased the amplitudes of both IPSPs. When the membrane was progressively hyperpolarized the EPSP and inverted IPSP usually merged into a depolarizing transient (see Fig. 1*A*). The voltage dependence of the synaptic responses exhibited considerable variation. In some neurones the relationship between the amplitude of both IPSPs and E_m was fairly linear, whereas in the majority there was a marked increase in IPSP amplitude with depolarization resulting in a curvature of the voltage–amplitude relationship (Fig. 1*B*). This difference of voltage dependence of IPSPs between neurones suggests that $GABA_A$ receptors are not a homogeneous population. The E_{IPSP_A} varied between -66 and -82 mV ($-73{\cdot}0 \pm 4{\cdot}4$ mV, $n = 27$), being slightly less negative than E_m, partly due to the EPSP contribution. The synaptic conductance (G_{IPSP_A}) determined in the depolarizing direction at low frequencies (0·1–0·25 Hz) was between 12 and 970 nS (193 ± 250 nS, $n = 27$). Although this represented a large variability in G_{IPSP} between neurones, the changes in G_{IPSP} during changes in stimulus frequency in a given cell were consistent and reproducible. At more negative E_ms, G_{IPSP_A} was always considerably smaller with values between 6 and 136 nS ($33{\cdot}5 \pm 30$ nS, $n = 22$). This approximately sixfold higher conductance at depolarized E_m values cannot be attributed to the temporal overlap with the EPSP. Within the current range examined, plots of EPSP amplitudes *versus* E_m were linear so that EPSP voltage dependence cannot account for relationships as shown in Fig. 1*B*. Furthermore, if voltage-dependent components of the EPSP were present (Thomson, 1986) but undetected, an opposite deviation of the IPSP–E_m relationship at depolarized E_ms would be expected.

The reversal potential of $IPSP_B$ was between -80 and -98 mV ($-88{\cdot}6 \pm 6{\cdot}1$ mV, $n = 18$), always more negative than that for $IPSP_A$. The conductance of $IPSP_B$ (G_{IPSP_B}) was also not constant at different membrane potentials and higher values were obtained in the depolarizing direction (24 ± 19 nS, $n = 18$) compared with hyperpolarized levels (10 ± 11 nS). Changes in apparent R_{in} in the depolarizing direction (Connors *et al.* 1982) were insufficient to account for the pronounced rectification of the synaptic events. For example the average rectification ratio (see Methods) in our cells was $1{\cdot}16 \pm 0{\cdot}27$ ($n = 30$), while the mean rectification of conductance was 5·8 and 2·4 for $IPSP_A$ and $IPSP_B$, respectively. Furthermore, the rectification of the IPSPs was independent of the rectification of the membrane, suggesting that it was instead due to inherent properties of synaptically activated channels, resulting in a relatively higher conductance at more depolarized levels.

In order to test this possibility more directly and to avoid the complicating issue of an underlying EPSP, the current–voltage characteristics of GABA responses were compared with those of the $IPSP_A$. As described previously in hippocampal (Andersen, Dingledine, Gjerstad, Langmoen & Mosfeldt Laursen, 1980; Thalmann, Peck & Ayala, 1981) and neocortical neurones (Connors & Malenka, 1985), GABA induces either hyperpolarizing or depolarizing potential changes dependent upon the location of the GABA-containing electrode. Application of short GABA pulses (20 ms) usually evoked only small potential changes at resting E_m, but when E_m was depolarized by current injection, a progressively increasing hyperpolarizing response was evoked. In Fig. 2*B* the disproportionate increase in the GABA response at more depolarized E_ms is illustrated (cf. first and second traces). A comparable family of

responses to current injections from this neurone during orthodromic stimulation is shown in Fig. 2*A*. The current–voltage relationship of GABA responses, plotted in Fig. 2*C*, reveals that the conductance of the GABA response (◆) is indeed non-linearly related to E_m, yielding a much larger conductance at depolarized potentials. A similar relationship is obtained for the $IPSP_A$ (■). Since GABA was applied within

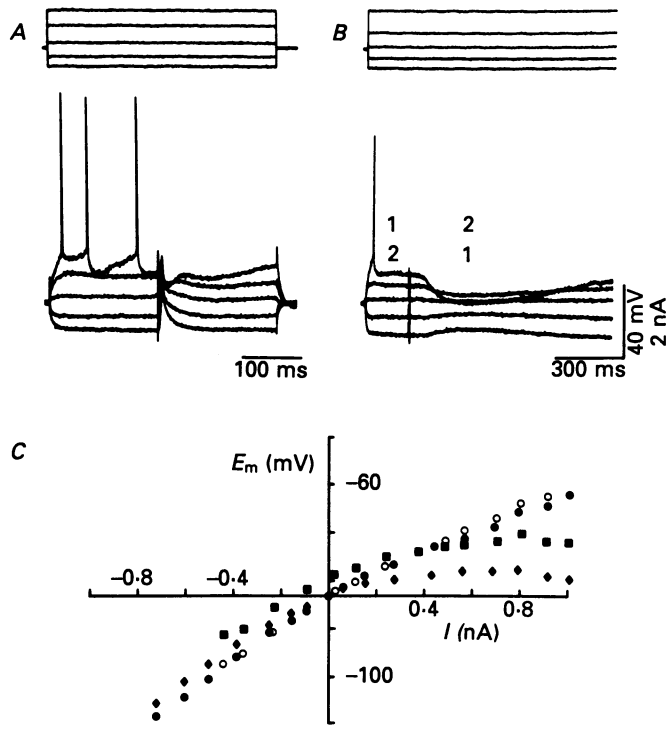

Fig. 2. Comparison of GABA-induced potential and conductance changes with synaptic events. *A*, depolarizing current pulses of various amplitudes (top traces) applied to a neurone while stimulating the white matter. The time of stimulation is obvious from the stimulus artifact. Note the IPSP amplitude in the top trace undershoots that evoked at the lower intensity of intracellularly injected current. *B*, records from the same neurone shown in *A* during application of 20 ms GABA pulses from a 2 mM-GABA-containing ejection electrode. The amplitude of the action potential is truncated by the digitization. The first and second trace (from top to bottom) show cross-over. *C*, current–voltage relationship of the records shown in *A* and *B*. The current–voltage relationships of the passive membrane before synaptic stimulation (○) and before GABA application (●) coincide. The peak voltage attained during the IPSP (■) parallels that of the peak GABA responses (◆). Note the decreased slope in the depolarizing direction indicating an approximately fivefold higher conductance.

50 μm from the somatic recording site (see Methods), a localized action is assumed. Therefore, electrotonic decrement appears unlikely to account for the rectification. The average rectification of GABA responses of 4·5 was similar to that of the $IPSP_A$ (5·8). In those neurones where a small EPSP component and a fairly small depolarizing late component of the GABA response allowed a reliable estimate of IPSP and GABA reversal potentials, the reversal potentials of $IPSP_A$ and the early hyperpolarizing GABA response were in fairly good agreement.

Frequency dependence of IPSPs

At low frequencies of stimulation (below 0·2 Hz) the amplitudes of both IPSPs were usually stable. However, the amplitudes of both components were attenuated in some neurones when a second stimulus was applied within 5–10 s. With repetitive stimulation the decline in response from the first to the second stimulus was largest; subsequent stimulations had smaller effects and finally, after about five to ten stimuli, the depression reached a steady state. Examples of such effects in two neurones are illustrated in Fig. 3. In the neurone of Fig. 3*B* (E_m, −78 mV), the amplitude of $IPSP_A$ is slightly affected at a stimulus frequency of 0·1 Hz, with little effect on the $IPSP_B$. In the neurone of Fig. 3*A* (E_m, −68 mV), $IPSP_A$ was not affected while $IPSP_B$ was attenuated after the third stimulus at 0·1 Hz. At a frequency of 1 Hz, $IPSP_A$ and $IPSP_B$ were reduced in amplitude (Fig. 3*A* and *B*). The functional consequences of frequency-dependent IPSP depression were particularly obvious when synaptic events were evoked during constant depolarizing current pulses. At low frequencies both early and late components of IPSP blocked the action potentials, elicited by suprathreshold depolarizing current pulses. Occasionally a single action potential was generated in the late part of the current pulse (about 230 ms after the stimulus), coincident with the decay of the $IPSP_B$. At stimulus frequencies of, for example, 1 Hz, the same intensity of orthodromic stimulation no longer prevented action potential generation and repetitive action potentials could be evoked.

The attenuation of $IPSP_A$ at 1 Hz as shown in Fig. 3*A* could be either due to a net flux of Cl^- during the inhibitory event leading to an elevation of intracellular Cl^- or to a decreased conductance. The latter seems more likely since a comparable alteration of $IPSP_A$ occurs when the IPSP peak is close to resting E_m (Fig. 3*B*), suggesting a smaller net flux of Cl^-. In order to distinguish between these alternatives, we determined the E_{IPSP} during a series of orthodromic stimulations at a given frequency. The plot of IPSP amplitudes *versus* E_m shown in Fig. 3*C* for stimulations at 0·1 and 1 Hz indicates that the $IPSP_A$ amplitude is reduced at 1 Hz at all potentials with a decline of the apparent E_{IPSP_A} of about 5 mV. On average the E_{IPSP_A} decreased from $-72{\cdot}9 \pm 4{\cdot}8$ to $-67{\cdot}5 \pm 6{\cdot}6$ mV ($n = 12$) for the tenfold change in the stimulation frequency. The amplitudes of $IPSP_B$ were also reduced at every potential and the reversal potential decreased by 3 mV at stimulus frequencies between 0·1 and 1 Hz. On average, however, E_{IPSP_B} was only slightly reduced from $87{\cdot}1 \pm 6{\cdot}5$ to $85{\cdot}5 \pm 5{\cdot}7$ mV ($n = 10$). The decreased slope of the E_m–IPSP amplitude plots in Fig. 3*C* indicates that a decreased synaptic conductance contributes significantly to the decline in IPSP amplitudes. A change in E_{IPSP} in contrast would have been expected to cause an approximately parallel shift in the E_m–IPSP amplitude plots.

The changes in both synaptic components at two different stimulus frequencies are illustrated in Fig. 4 for a neurone with a membrane potential of −78 mV at 0·1 Hz and −79·8 mV at 1 Hz. Comparing the records reveals that the EPSP–IPSP sequence is little affected by increased frequency at more negative membrane potentials (lower traces in *A* and *B*). At depolarized levels, however, both IPSP components are reduced in amplitude at 1 Hz with a concomitant decrease in duration. The current–voltage relationship of the neurone shown in Fig. 4*A* and *B* is plotted in Fig.

4*C* and *D*. At 1 Hz stimulation the IPSP amplitudes are much smaller at each potential in the depolarized direction, but coincide at more negative membrane potentials (compare the difference between open and filled squares and triangles in Fig. 4*C* and *D*, respectively). Although the slopes of the IPSPs *versus* current at first

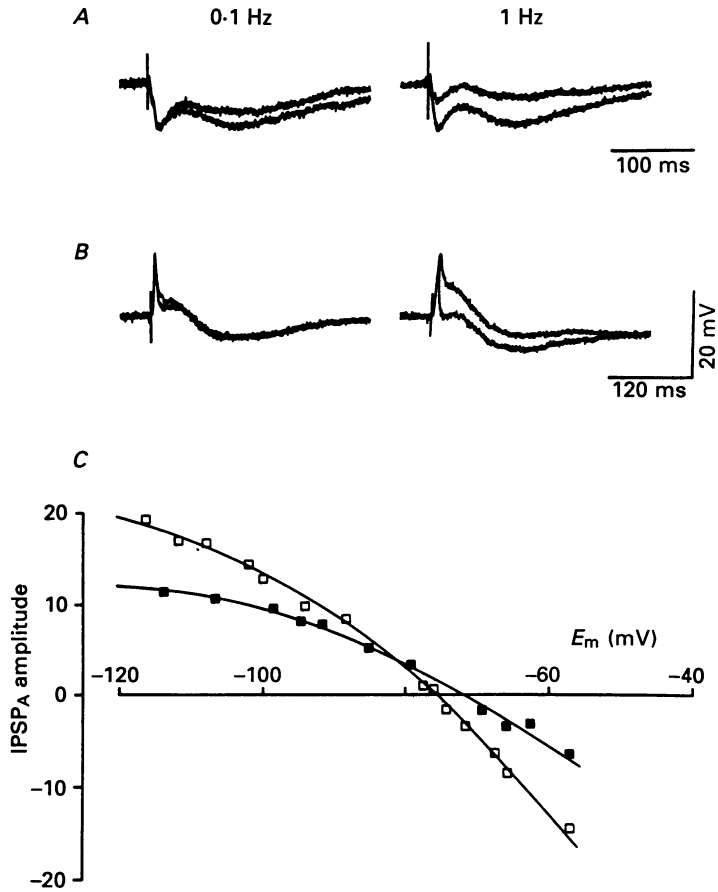

Fig. 3. Frequency dependence of synaptic components at resting E_m. The first and third PSPs of two neurones during a series with two frequencies of stimulation have been superimposed. *A*, neurone at a resting E_m of −68 mV. *B*, another neurone at a resting E_m of −78 mV. Stimulus frequency as indicated. *C*, the amplitudes of IPSP$_A$ of the neurone shown in *A* have been plotted *versus* membrane potential for stimulation frequencies of 0·1 Hz (□) and 1 Hz (■). The predominant component of the decreased IPSP amplitude appears to be the decrease in slope of the IPSP *versus* E_m relationship rather than a decline in reversal potential. A shift in reversal potential, in contrast, would produce parallel curves.

sight do not appear much different, the conductance is in fact reduced by about 60% from 89 to 34 nS and from 32 to 10·5 nS for IPSP$_A$ and IPSP$_B$, respectively, when the stimulus frequency is increased from 0·1 to 1 Hz. On average the conductance decreased from 145±134 to 30±19 nS and from 25±19 to 9·8±7·6 nS, i.e. 79 and 61% for the early and late component, respectively. Neither E_m nor R_{in} was consistently affected by changes of stimulus frequency between 0·1 Hz (E_m

$-75{\cdot}9 \pm 4{\cdot}6$ mV, R_{in} $55{\cdot}5 \pm 41{\cdot}9$ MΩ) and 1 Hz (E_m $-75{\cdot}4 \pm 4{\cdot}5$ mV, R_{in} $50{\cdot}2 \pm 41{\cdot}2$ MΩ). The synaptic conductance of $IPSP_A$ has been plotted in Fig. 5 *versus* the frequency of stimulation for measurements from three neurones. In all neurones the synaptic conductance falls monotonically as the frequency of stimulation is increased.

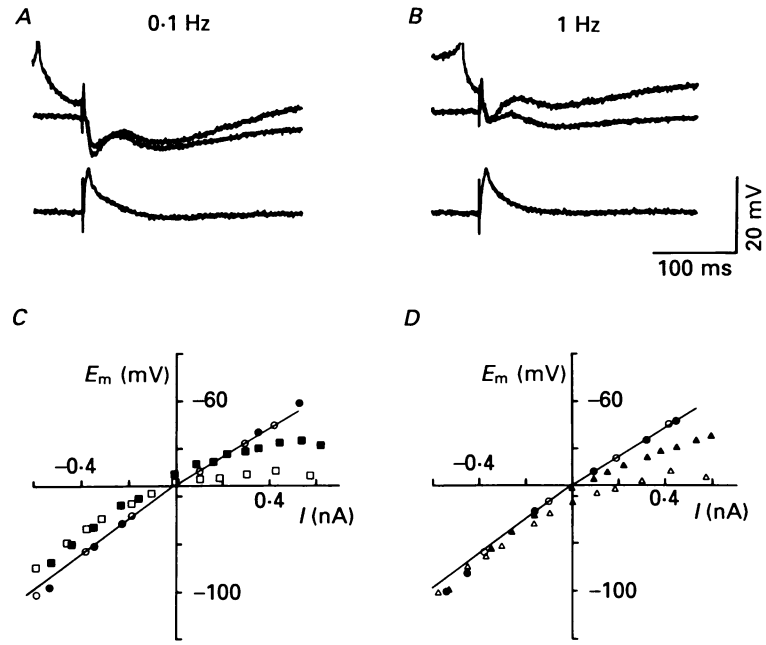

Fig. 4. Frequency dependence of synaptic components at different E_m values. *A*, records of synaptic components at different membrane potentials obtained by current injection of +0·73, +0·30 and −0·46 nA in a neurone with a mean E_m of −78·1 mV at a stimulation frequency of 0·1 Hz. *B*, records of the same neurone as shown in *A* at a stimulation frequency of 1 Hz, about 30 min later (E_m, 79·8 mV) with identical current injections. *C*, current–voltage relationship of the neurone illustrated in *A* and *B*. The membrane potential attained before the stimulus (circles) has been plotted *versus* the injected current at 0·1 Hz (○) and at 1 Hz (●). The potential attained at the early component has been plotted *versus* injected current at a stimulus frequency of 0·1 Hz (□) and at 1 Hz (■). *D*, the open and filled circles refer to E_m as in *C* and the open and filled triangles to the potential of the late component at 0·1 and 1 Hz, respectively. The linear regression of the data in the right quadrants indicates an R_{in} of 28 and 27 MΩ, an apparent synaptic conductance of the early component of 89 and 34 nS, and 32 and 10 nS for the late component at 0·1 and 1 Hz, respectively.

As mentioned above, in addition to the decline in amplitude the duration of the $IPSP_A$ also appeared to be reduced. A direct estimate of duration was not feasible, owing to the late component and the intervening depolarization, together with the small amplitude of the $IPSP_A$ at resting E_m. Therefore we determined the time constant at more hyperpolarized levels, when the contribution of at least the $IPSP_B$ voltage transient was negligible. The time constant of the membrane, measured from semilogarithmic plots of membrane hyperpolarization to near −90 mV, was 9 ms (see Fig. 6). Increasing the stimulus frequency yielded an

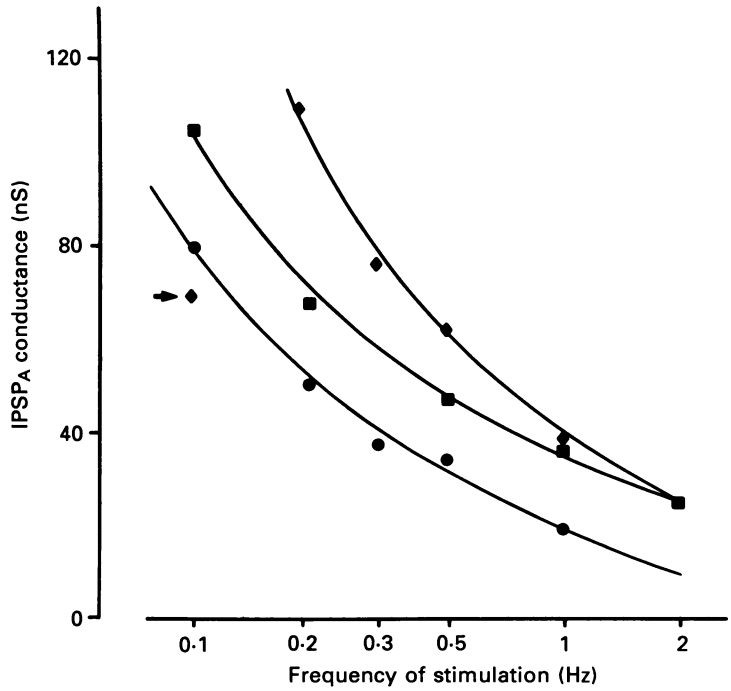

Fig. 5. Frequency dependence of IPSP$_A$ conductance. The conductance of the IPSP$_A$ has been plotted *versus* the logarithm of the frequency of stimulation for three neurones represented by different symbols. The conductance determination at 0·1 Hz of the neurone represented by ◆ (arrow) was preceded by a series of 20 ms GABA pulses at 0·1 Hz.

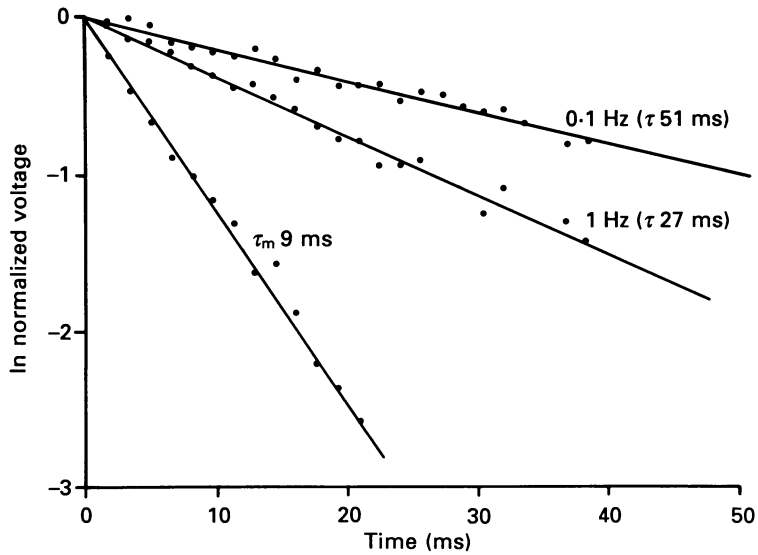

Fig. 6. Frequency dependence of IPSP time course. Semilogarithmic plot of membrane potential changes following hyperpolarizing current pulses and of synaptic potentials. Time constant for hyperpolarization of the membrane from resting E_m to around -90 mV is 9 ms at 0·1 and 1 Hz. Increasing frequency of stimulation decreases the time constant of the synaptic potential from 51 to 27 ms.

indistinguishable regression line for the membrane time constant, as expected from the unaltered R_{in}. The time constant of the IPSP decay was much longer, 51 ms (see Fig. 6). At 1 Hz the $IPSP_A$ decay time constant was reduced to 27 ms, representative of the mean values, which decreased from $55{\cdot}4 \pm 5{\cdot}2$ ms at 0·1 Hz to $29{\cdot}8 \pm 16{\cdot}6$ ms at 1 Hz ($n = 5$, $P < 0{\cdot}005$, Student's paired t test).

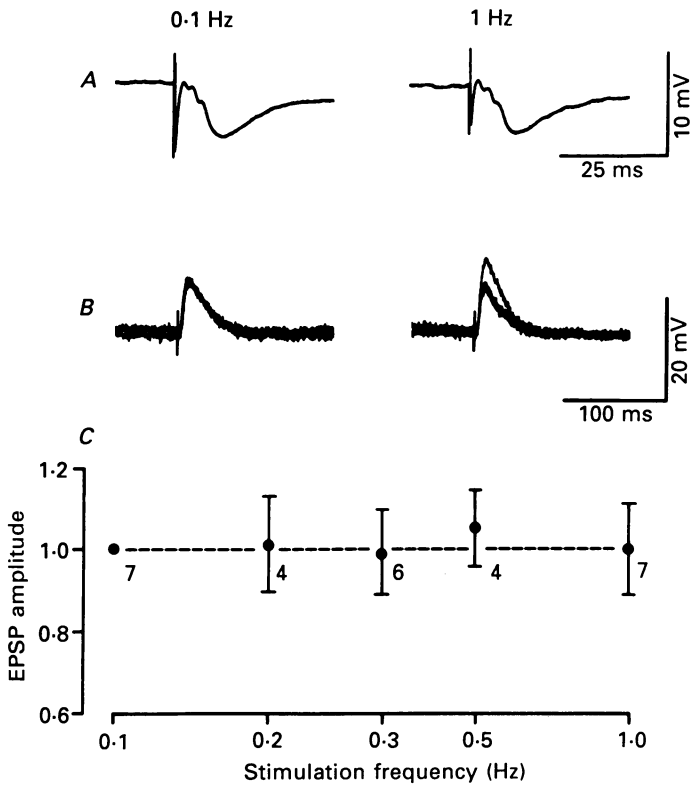

Fig. 7. Frequency dependence of field potentials and EPSPs of pyramidal cells. *A*, field potential average of about ten records at 0·1 and 1 Hz stimulation. *B*, single EPSP records at 0·1 and 1 Hz stimulation. The apparent increase in amplitude of some EPSPs shown for the records at 1 Hz stimulation illustrates the increased variability of EPSP amplitudes observed in some neurones at higher stimulus frequencies. *C*, frequency dependence of normalized EPSP amplitudes. Numbers refer to number of cells, with typically about twenty records measured for a given frequency in each neurone. The averages for a given frequency have been normalized to the value obtained at 0·1 Hz for the individual neurones. The standard deviation (vertical bars) represents the standard deviation of the averaged mean values.

Frequency dependence of local circuits

The decline of the IPSP during repetitive stimulation, in the face of an essentially unaltered ionic gradient (see above), might be brought about by several possible factors. Synaptic transmission at the inhibitory synapse *per se*, or at the excitatory synapses converging onto inhibitory interneurones may exhibit a frequency-dependent depression. The latter may be inferred from the parallel decline of EPSPs and IPSPs in spinal cord (Curtis & Eccles, 1960). In neocortex the frequency

dependence of EPSPs has not been determined, but available data from other central neurones suggest that excitatory transmission begins to decrease at 1 Hz (Miles, 1986). As a first estimate we measured the frequency dependence of the field potential changes. The peak amplitudes of the initial negativity of the field potential evoked by white matter stimulation and recorded at 400–700 μm below the pial surface were measured in seven slices. The amplitudes ranged from 0·9 to 4·5 mV. The

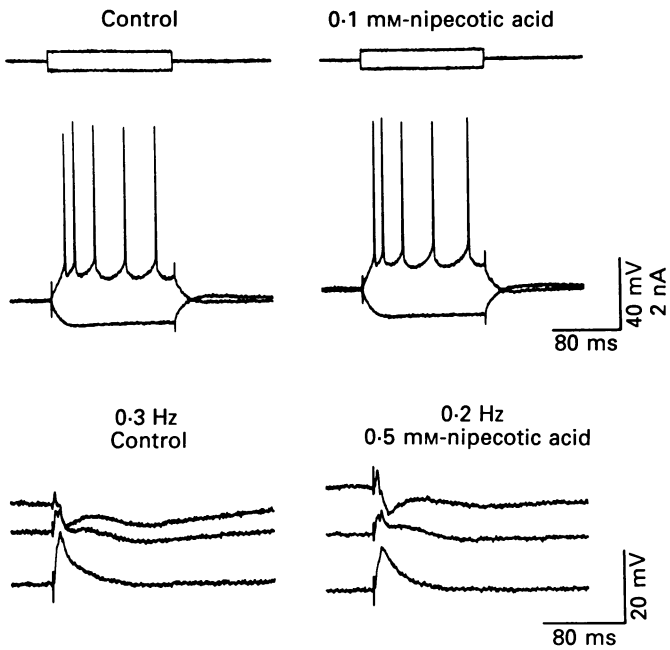

Fig. 8. Effects of nipecotic acid on membrane properties and synaptic potentials. Top, responses of a neurone to depolarizing and hyperpolarizing current pulses (top traces) in the absence and presence of 100 μM-nipecotic acid. Bottom, synaptic potentials in control and after wash-in of nipecotic acid. The currents injected are 0, +0·3 and −0·5 nA in the control and in the presence of 500 μM-nipecotic acid. E_m was −78 mV in control and in nipecotic acid during these sequences. Despite the lower rate of stimulation, the attenuation of IPSPs is particularly evident at resting and depolarized E_ms.

average amplitude at 0·1 Hz of 3·28 ± 1·03 mV decreased to 3·14 ± 1·02 mV at 1 Hz ($n = 7$), i.e. by about 4%, indicating that there was no large alteration in the effectiveness of the subcortical stimulus in evoking synaptic events at the frequencies employed. The amplitude of field potentials represents a summation of various evoked synaptic events on pyramidal cells and interneurones and may not reflect subtle changes in synaptic events. Thus the frequency dependence of EPSPs of pyramidal cells was determined. In each neurone three to five frequencies of stimulation were applied and the amplitudes of ten to twenty EPSPs measured and normalized to the value obtained at 0·1 Hz. At 1 Hz the mean normalized amplitude was 100 ± 12% ($n = 7$), suggesting no significant effects in this frequency range (see Fig. 7). Moreover, the action potentials of pyramidal cells are also not significantly affected in the same frequency range (Deisz & Prince, 1987) and interneurones are

capable of firing at much higher rates (McCormick *et al.* 1985). Although these experiments provide some evidence for robust spike generation and synaptic transmission up to 1 Hz, additional effects such as progressive blockade at axonal branch points at higher frequencies in interneurones cannot be ruled out. At any rate, these results do not support a frequency dependence of EPSPs in the network

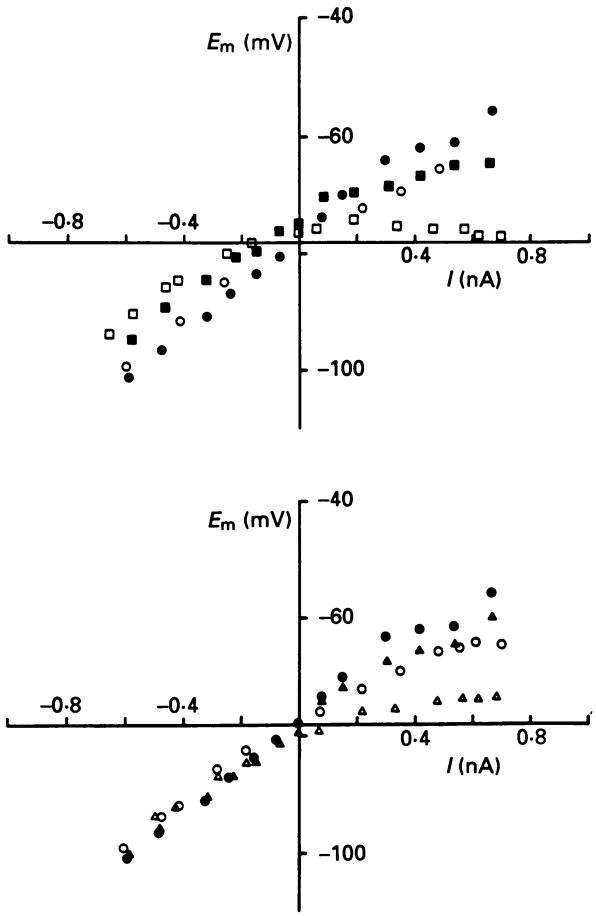

Fig. 9. Current–voltage relationships in the presence and absence of nipecotic acid. The current–voltage relationship of the membrane (circles). $IPSP_A$ (squares, upper plot) and $IPSP_B$ (triangles, lower plot) before (open symbols) and after the wash-in of 500 μM-nipecotic acid (filled symbols) from the neurone shown in the bottom part of Fig. 8.

between the stimulation site and the input to inhibitory interneurones. More substantial changes in the EPSPs on pyramidal cells and interneurones would be expected considering that up to tenfold decreases in inhibitory synaptic conductance were observed.

Effects of nipecotic acid

The decline of IPSP conductance at higher frequencies, in spite of a marginal frequency dependence of local circuits involved, suggests that it may be localized at

the inhibitory synapse as such. If the depression of the IPSPs at higher frequencies were related to residual GABA in the synaptic cleft, which might reduce presynaptic release, a reduction of GABA uptake should enhance the effects. In order to test this possibility we added nipecotic acid (50–500 μM) to the bathing solution. This agent produced no detectable effect on R_{in}, membrane rectification or action potential generation in seven neurones (Fig. 8). The amplitudes of both $IPSP_A$ and $IPSP_B$

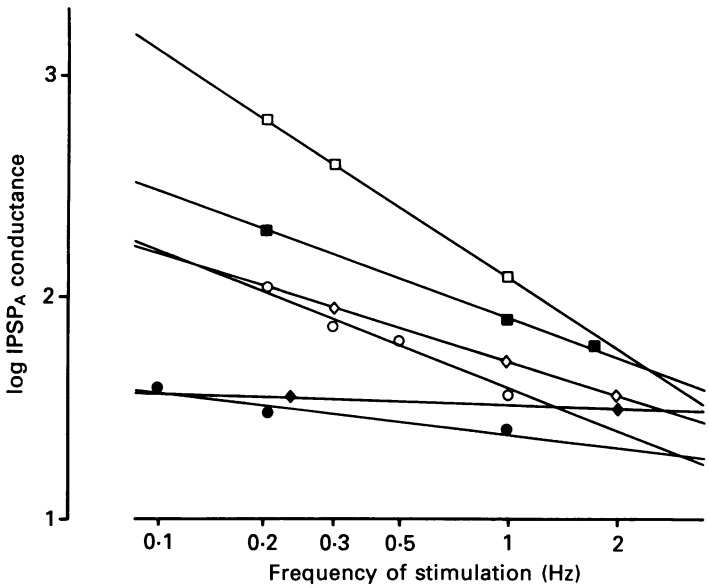

Fig. 10. Effects of nipecotic acid on frequency dependence of $IPSP_A$. The logarithm of $IPSP_A$ conductance has been plotted *versus* the logarithm of the frequency of stimulation for three neurones before (open symbols) and after (filled symbols) bath application of nipecotic acid. The resting membrane resistance was 31·8, 49·8 and 31·2 MΩ for the neurones represented by squares, circles and diamonds, respectively. Note the intersection near about 2 Hz, indicating that the effects of nipecotic acid are smaller at higher frequencies of stimulation.

were reduced and the durations of the IPSPs were shortened (see Fig. 8). The effect was mostly reversible. The alteration of the current–voltage relationship of a neurone before and during application of 500 μM-nipecotic acid is illustrated in Fig. 9. The increased slopes of the IPSP–current relationships in the depolarizing quadrant (cf. open (control) and filled (500 μM-nipecotic acid) symbols of Fig. 9) indicate a reduced conductance for both components. Remarkably, there was no significant difference in IPSP components in the presence of nipecotic acid when IPSPs were measured at hyperpolarized E_ms. The E_{IPSP_A} at the intersection of open circles and open squares decreased slightly with nipecotic acid.

Comparable effects were observed with 50 μM-nipecotic acid. In one neurone, for instance, a synaptic conductance of 89 nS was determined at 0·2 Hz before bath application of nipecotic acid; however, after about 20 min in nipecotic acid, a conductance of 39 nS was obtained, corresponding to a 56% decrease. At higher concentrations up to 500 μM, IPSPs were further reduced (80%, $n = 3$). In one

of the two neurones tested with 100 μM-nipecotic acid, the IPSP was reduced by 89% at 0·2 Hz; in the other one, IPSPs were reversibly abolished with nipecotic acid so that a quantitative evaluation of the effect was not performed. The large scatter of the data prevented a clear delineation of a dose–response curve and therefore we pooled the data. In six neurones with sizeable IPSPs before and during nipecotic acid application, the mean synaptic conductance decreased from 453 ± 443 to 130 ± 170 nS, i.e. by 60–90% (71 ± 14%). The decrease in synaptic conductance in the presence of nipecotic acid at low frequencies was significant ($P < 0{\cdot}05$ level, Student's paired t test).

The attenuation and even loss of IPSPs in the presence of nipecotic acid is similar to previous reports from crayfish stretch receptor (Aickin & Deisz, 1981) and hippocampal neurones (Dingledine & Korn, 1985). In order to test whether the depression of both inhibitory synaptic potentials was an unspecific effect as proposed for hippocampal neurones (Dingledine & Korn, 1985) or related to a decrease in GABA uptake, we determined the frequency dependence of the IPSP in the presence of nipecotic acid. If the effects of nipecotic acid were unspecific, a constant depression at all frequencies would be anticipated, while an effect of nipecotic acid due to a prolongation of the GABA transient in the cleft would be expected to produce an altered frequency dependence. In the presence of nipecotic acid, the $IPSP_A$ amplitude is severely attenuated at low frequencies. At 1 Hz stimulation, however, nipecotic acid causes only a slight further decrease. The neurones had a mean synaptic conductance of 50 ± 42 nS in control solution and 60 ± 37 nS in nipecotic acid, an insignificant difference. The effects of different frequencies of stimulation on the $IPSP_A$ of three neurones in the presence of nipecotic acid are summarized in Fig. 10.

Effects of nipecotic acid on local GABA responses

The alteration of frequency dependence by nipecotic acid would be compatible with a prolonged GABA transient at the inhibitory synapse, rather than a non-specific depression of GABA responses, as a possible cause of the IPSP attenuation. To further eliminate postsynaptic sensitivity changes we measured the alteration of GABA-induced conductance by nipecotic acid. GABA responses from such an experiment are illustrated in Fig. 11 before and during application of nipecotic acid. Close inspection reveals that the hyperpolarizing GABA response is slightly larger in the presence of nipecotic acid. The current–voltage relationship indicates that the conductance is indeed increased by 10–30% (19 ± 8%, $n = 4$) in the presence of nipecotic acid. When GABA was applied at different frequencies, the conductance increase remained almost constant, i.e. the GABA conductance determined at 0·1 Hz was 95 nS and at 1 Hz was 91 nS, suggesting that with low concentrations of GABA and frequencies up to 1 Hz no significant desensitization occurs.

Both the reversal potential and current–voltage relationship of the late depolarizing component of the GABA response were unaltered by nipecotic acid. The similarity in current–voltage relationships of both GABA responses (see Fig. 11) suggests a similar receptor-conductance mechanism with different ionic gradients (Misgeld, Deisz, Dodt & Lux, 1986), in line with previous evidence for a pharmacological similarity of hyperpolarizing and depolarizing GABA responses in

hippocampal neurones (Thalmann, Peck & Ayala, 1981). However, a passive depolarization of pyramidal neurones by the potassium efflux from surrounding glial cells depolarized by GABA (Kettenmann & Schachner, 1985) has to be considered too. Either of these mechanisms may also account for the transient depolarization (see Figs 1*A* and 2*A*) between the two IPSP components.

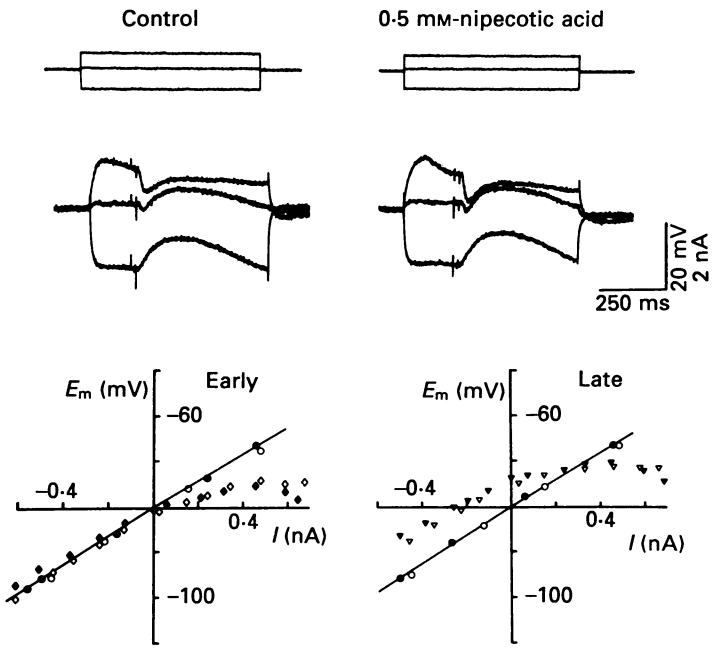

Fig. 11. Effects of 500 μM-nipecotic acid on GABA responses. Top, superimposed records of changes in membrane potential (bottom traces) evoked by 20 ms GABA pulses at different membrane potentials obtained by current injection (top traces). Bottom, current–voltage relationship of the membrane (circles) and of the early (diamonds) and late (triangles) GABA responses in the presence (filled symbols) and absence (open symbols) of nipecotic acid.

The results so far suggest that at higher frequencies of stimulation, particularly after application of nipecotic acid, GABA may accumulate in the synaptic region and reduce release. We have tested the possibility that GABA may reduce GABA release directly by determining the current–voltage relationship before and after application of GABA by short pressure pulses. GABA in fact reduced the efficacy of subsequent IPSPs at a time when the postsynaptic conductance and membrane potential effects of the individual GABA responses had recovered (see Fig. 5).

Effects of baclofen

The reduction of IPSPs may be brought about by various presynaptic effects of GABA, a shunting of the membrane by an increase in Cl^- conductance, or a more specific effect by alterations of Ca^{2+} spikes (Dunlap, 1981). We used baclofen, an agonist for $GABA_B$ receptor sites which reduces transmitter release (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980) and Ca^{2+}-mediated events

(Deisz & Lux, 1985; Holz, Rane & Dunlap, 1986; Bowery & Williams, 1986) to test these alternatives. Pressure pulse application of a racemic mixture of 100 μM-baclofen caused a slow transient hyperpolarization of the postsynaptic membrane of up to 4 mV in all seven neurones tested. The effect was dose dependent; the hyperpolarization increased in amplitude and duration when baclofen was applied

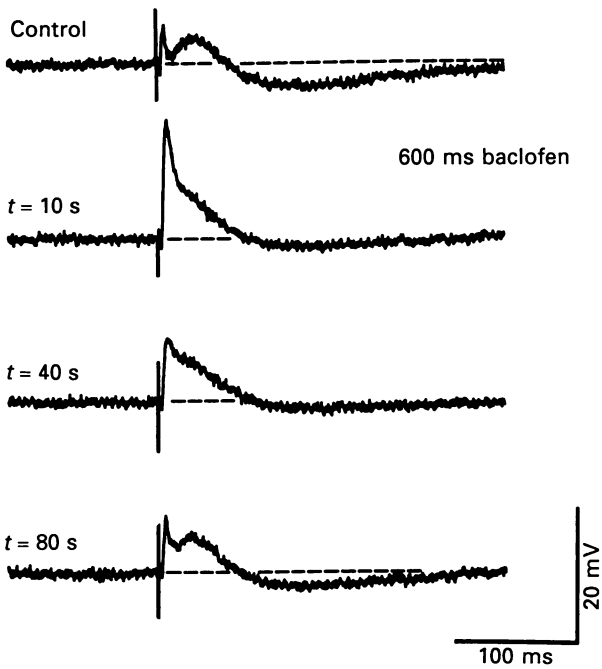

Fig. 12. Changes in the compound EPSP–IPSP after a brief pulse of baclofen to the soma of a pyramidal cell. The application was located less than 40 μm from the somatic recording site. The records shown illustrate the selective depression of the IPSPs following a pressure ejection of 600 ms from an electrode containing 100 μM-baclofen, which corresponds to an ejected volume of about 6 pl (see Methods). The change in E_m induced by baclofen was about 3 mV at the peak of the response, far too small to account for the observed effects on the synaptic components. Note that even after 80 s the $IPSP_A$ and $IPSP_B$ have not fully recovered. t is the time after baclofen application.

for longer times. The baclofen-induced conductance change was in the order of 5–10 nS, much smaller than the GABA-induced conductance. Repetitive application of baclofen caused a constant effect. These findings are similar to those reported previously in neocortical neurones (Connors & Malenka, 1985) and are probably due to a $GABA_B$-mediated increase in K^+ conductance (Newberry & Nicoll, 1985; Howe, Sutor & Zieglgänsberger, 1987). Despite these comparatively small postsynaptic effects, IPSPs were markedly attenuated by baclofen. The time course of changes of the IPSP at resting E_m is illustrated in Fig. 12. The two components of inhibition are suppressed while the EPSP is enhanced. Eighty seconds after baclofen application both $IPSP_A$ and $IPSP_B$ had not fully recovered. When constant depolarizing current pulses slightly above threshold were applied to monitor excitability changes, the membrane depolarization failed to evoke an action potential during the application

of baclofen. Constant depolarizations with slightly increased current pulses indicated essentially unaltered excitability. The firing property of a neurone before and after application of a 200 ms pulse of 100 μM-baclofen is illustrated in Fig. 13*B*. Despite the maintained generation of action potentials during stimuli of sufficient intensity, the IPSP is virtually abolished within a few seconds of application (cf. response in

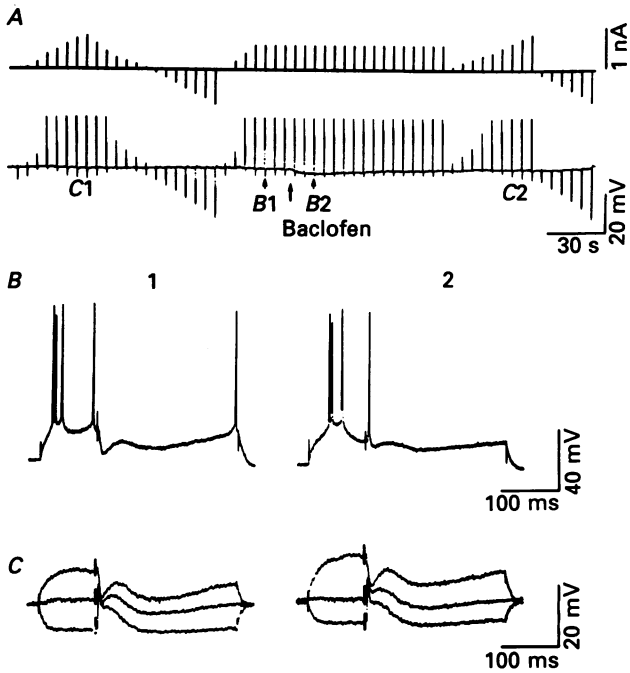

Fig. 13. Pen recording showing part of an experiment. During the white matter stimulation at low frequency (0·2 Hz) a series of depolarizing and hyperpolarizing current pulses have been applied to determine the current–voltage characteristic. In order to monitor membrane excitability directly during the pressure application of baclofen (600 ms pulse from a 100 μM-containing electrode), constant suprathreshold depolarizing current pulses of 350 ms duration (about 0·6 nA) were applied at the same frequency, timed so that the orthodromic stimulus fell in the midst of each depolarizing current pulse. Traces shown in *B* are from the same experiment as in *A* taken at the times indicated. Within 10 s after baclofen application the IPSP is abolished but the excitability is maintained (cf. *B*1 and *B*2). The set of recordings shown in *C* was obtained before and after the application of baclofen. Note that even after about 2 min, when membrane potential has recovered, IPSP amplitudes are still depressed.

Fig. 13 *B*1 and *B*2). An identical set of current pulses and orthodromic stimuli 2 min after application of baclofen reveals that the depression of the IPSP continues for minutes (see Fig. 13*C*). Comparison of the membrane and IPSP slope conductances before and after the application of baclofen indicated that both IPSPs were still attenuated after 2 min when postsynaptically detectable effects had dissipated (see Fig. 14).

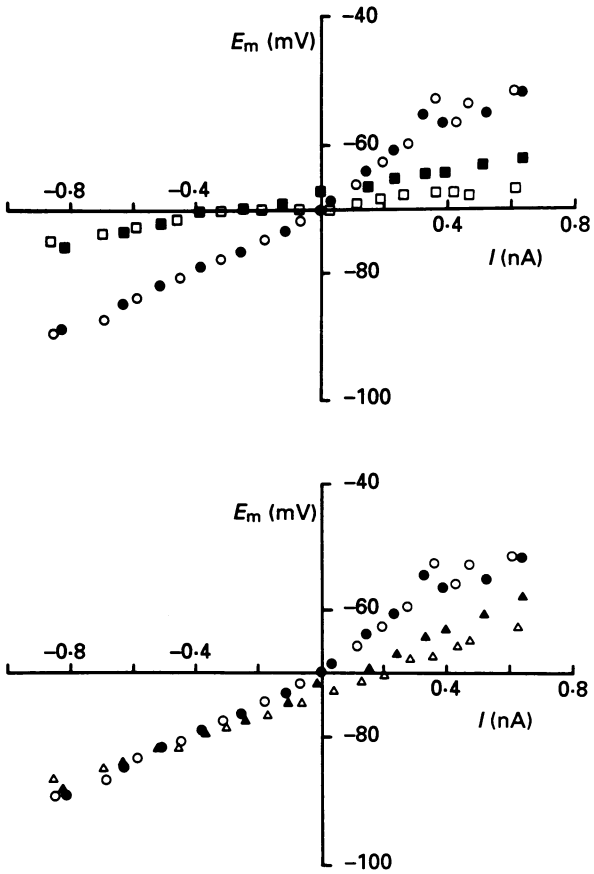

Fig. 14. Current–voltage relationship before (open symbols) and about 2 min after (filled symbols) application of a minute quantity of baclofen. The membrane potential attained has been plotted *versus* the injected current amplitude both before orthodromic stimulation (circles) and at the peak of the $IPSP_A$ (rectangles, upper plot) and $IPSP_B$ (triangles, lower plot).

DISCUSSION

General properties of the IPSPs

In accord with previous studies of hippocampal (Newberry & Nicoll, 1985) and neocortical neurones (Connors & Malenka, 1985; Howe *et al.* 1987; Connors *et al.* 1988), two distinct components of inhibition can be evoked by orthodromic stimulation of sufficient intensity. The differences in reversal potentials and conductances found here corroborate previous conclusions that these events are mediated by entirely different conductance mechanisms, an early Cl^--dependent and a late K^+-dependent component (see Thompson, Deisz & Prince, 1988*b*). Before discussing the mechanisms contributing to the pronounced frequency dependence, we would like to emphasize some features of IPSPs pertinent to the issues addressed here. The sixfold higher apparent conductance of IPSPs at depolarized compared to hyperpolarized levels of E_m might be due to the superposition of the EPSP and the

two IPSP components or to membrane rectification. But the similarity of the IPSP current–voltage relationship with that of perisomatic GABA responses suggests that the marked sensitivity of IPSP conductance to E_m is rather due to rectifying properties of the GABA–receptor channel complex (Segal & Barker, 1984, see also Ashwood, Collingridge, Herron & Wheal, 1987). Since the changes in IPSP conductance in our experiments were much greater than the single GABA channel conductance increase in hippocampal neurones over an even larger voltage range (Gray & Johnston, 1985), we speculate that a voltage dependence of gating of GABA-activated channels (Dudel, 1977; Dudel, Finger & Stettmeier, 1977; Deisz, 1983) may significantly contribute to our results.

It is interesting to note that the time constant of the inhibitory synaptic event significantly exceeds the membrane time constant, hence it is probably determined by the time course of the synaptic current. In crayfish muscle the duration of inhibitory current can be accounted for by the open time of GABA-activated channels (Dudel, 1977). However, the time course of the inhibitory current inferred here would be considerably longer than the channel open time determined in vertebrate central neurones (Segal & Barker, 1984). Therefore we suggest that the transmitter transient contributes significantly to the time course of the IPSP. It is noteworthy that separate mechanisms for the rate-limiting process of inhibitory current decay and the voltage dependence have been suggested previously (Onodera & Takeuchi, 1979). This is analogous to our proposal that the transmitter transient governs the IPSP time course and channel gating governs the voltage dependence of the inhibitory conductance. Given the conclusion that the decay of the IPSP reflects the GABA concentration in the synaptic cleft, the decrease of the IPSP time constant at higher frequencies would support our hypothesis that GABA release is reduced (see below).

Contribution of local circuits to frequency dependence of IPSPs

The marked depression of both inhibitory components at comparatively low rates of stimulation corroborates and extends previous investigations on frequency-dependent depression of IPSPs (Curtis & Eccles, 1960; Ben-Ari *et al.* 1979; McCarren & Alger, 1985). This attenuation occurs in a frequency range where no significant effects on EPSPs are detectable, rendering a causal link to EPSP depression unlikely (Curtis & Eccles, 1960; Miles, 1986). It is possible that EPSPs of inhibitory interneurones exhibit a more pronounced frequency dependence than those of pyramidal cells. Certainly both interneurones (McCormick *et al.* 1985) and pyramidal cells (Deisz & Prince, 1987) can generate Na^+ and Ca^{2+} action potentials at high frequencies, although discharge properties of axonal terminals are unknown. Transduction of a given excitatory input into release of GABA by inhibitory interneurones may be a limiting factor at higher stimulation rates. However, presumed GABAergic interneurones in cortex have relatively linear membrane properties and apparently stable and linear relationships between depolarization and spike output. The stability of Ca^{2+} spikes of pyramidal cells (Deisz & Prince, 1987) in the frequency range investigated here, if accepted as an index for presynaptic processes, excludes the Ca^{2+} transient as a limiting factor. It could be argued that the Ca^{2+} currents of interneurones may be more susceptible to depression at higher

frequencies, since Ca^{2+} currents of different neurones have been shown to display marked differences in their frequency dependence (Jia & Nelson, 1986). However, a frequency-dependent attenuation of Ca^{2+} action potentials may not apply directly to the much briefer periods required for release.

Frequency-dependent decline of ionic gradients

The marginal frequency effects on the reversal potential observed here appear at variance with previous evidence (McCarren & Alger, 1985) suggesting a use-dependent deterioration of the Cl^- gradient as an essential factor for the IPSP decline. But this effect is difficult to distinguish from the concomitant elevation of extracellular K^+. Elevated extracellular K^+ affects the IPSP reversal potential in hippocampal pyramidal cells (Alger & Nicoll, 1983) and it increases intracellular Cl^- activity in the crayfish stretch receptor (Deisz & Lux, 1982) by interfering with a K^+–Cl^- co-transport system (Aickin *et al.* 1982). Moreover, Cl^- extrusion in neocortical neurones recently was shown to be reduced by extracellular K^+ elevation (Thompson, Deisz & Prince, 1986, 1988*a*). The rather low experimental temperature used in earlier experiments (McCarren & Alger, 1985) might have impaired temperature-sensitive transport mechanisms such as Cl^- extrusion (Thompson *et al.* 1986, 1988*b*) and the Na^+–K^+ pump (Thompson & Prince, 1986), thus aggravating effects of extracellular K^+ accumulation. The relevant ionic gradients in the neocortex appear little affected at 37 °C, as indicated by the unaltered E_m, R_{in} and small effects on the reversal potentials. The slight decline in the E_{IPSP_A} illustrates a general problem of reversal potential determination, but does not necessarily imply a change in the driving force. An accurate determination of the reversal potentials depends mainly upon two factors: a pure inhibitory response or at least a constant proportion of 'contamination' and an ohmic behaviour of the transmitter-evoked conductance; however, neither of these prerequisites is satisfied in our case. The current–voltage relationship (Figs 2 and 9) indicates that the IPSP is deviating from an ohmic behaviour such that a higher conductance is found at more depolarized levels. This voltage dependence itself would result in apparent changes of the reversal potential when the conductance is changing. Moreover, the proportion of EPSP and $IPSP_B$ contamination is altered when the IPSP conductance decreases, resulting in an apparent decline of E_{IPSP_A}. Therefore the apparent shift of the reversal potential at higher frequencies of stimulation can be explained by the temporal overlap of EPSP and IPSP together with a decrease of a voltage-dependent conductance, rather than by a change in ionic gradient.

Contribution of postsynaptic sensitivity changes to IPSP decline

A crucial experiment for excluding postsynaptic sensitivity changes as a cause for the decline in IPSP conductance would be its comparison with GABA-induced conductance before and after a series of IPSPs. Such a comparison is not possible within the required accuracy, since GABA application reduced IPSP. Secondly, such a comparison would depend on unrestricted and constant access of exogenously applied GABA to receptor sites. The enhancement of GABA responses after reduction of GABA uptake described here and in other types of neurones (Curtis *et al.* 1976; Brown & Galvan, 1977; Deisz & Dose, 1983; Gallagher *et al.* 1983;

Dingledine & Korn, 1985; Hablitz & Lebeda, 1985) suggests that the saturable GABA uptake system limits diffusion. Therefore, access of exogenous GABA to postsynaptic sites is probably neither unrestricted nor constant. Thirdly, the same population of receptors must be activated by synaptically released and exogenously applied GABA. But comparison would be distorted, even if exogenous GABA were to reach the same population of receptors and activate the same increase in conductance. Exogenous GABA would require locally higher concentrations than the widespread synaptic input (Houser, Vaughn, Hendry, Jones & Peters, 1984), hence the local changes in gradient and desensitization may be entirely different.

Due to the inherent limitations outlined above, the extent of desensitization contributing to frequency-dependent depression of the $IPSP_A$ conductance could not be satisfactorily elucidated, but accounts probably for less than 20%. The constant effect of baclofen implies that the $GABA_B$ receptors show little, if any, desensitization, hence the reduction of the $IPSP_B$ conductance by 60% is presynaptically mediated and the higher reduction of $IPSP_A$ (by 80%) could reflect an additional component of desensitization. However, the monomolecular $GABA_B$ response (Hill & Bowery, 1981) will be less affected by decreased release than the bimolecular $GABA_A$ reaction. From these considerations we propose that desensitization of $GABA_A$ receptors accounts for less than 10% of the frequency-dependent depression.

Nipecotic acid effects on frequency dependence of IPSPs

The decline of inhibitory transmission in the presence of nipecotic acid is in accord with evidence from hippocampus (Hablitz & Lebeda, 1985; Dingledine & Korn, 1985) and crayfish stretch receptor (Aickin & Deisz, 1981). The mechanism for this decline is probably related to an increase in extracellular GABA due to decreased reuptake. However, other alternatives have been proposed, which might affect the interpretation of our data.

First, blockade of GABA uptake may enhance depletion of presynaptic stores, which under normal conditions might occur only at higher frequencies. Although depletion might decrease the quantal content with unaltered probability for release, it has been shown that, at higher stimulation rates, the quantal content remains constant and the probability of release decreases (Korn, Mallet, Triller & Faber, 1982; Korn, Faber, Burnod & Triller, 1984). If nipecotic acid produced a depletion of presynaptic GABA in our experiments, a use-dependent depression of evoked responses at higher frequencies would be expected. This is contrary to the more pronounced effects at lower frequencies that we observed (Fig. 10). In addition, the similarity of IPSP attenuation by nipecotic acid and GABA supports a release-related effect.

Secondly, it has been proposed that nipecotic acid depresses IPSPs through non-specific effects, unrelated to actions on GABA uptake (Dingledine & Korn, 1985). This conclusion was based upon the observation that a derivative of nipecotic acid, 4-OH-isonipecotic acid, decreased inhibition without an effect on GABA uptake (Schousboe, Thorbeck, Hertz & Krogsgaard-Larsen, 1979). However, considering the possible differences in action among such structural analogues, this observation does not rule out a nipecotic acid-induced depression of inhibition by blockade of uptake.

Thirdly, inhibitory synaptic transmission may be decreased by the uptake of nipecotic acid, which subsequently hinders release of GABA as proposed by Lerma, Herreras, Herranz, Munoz & del Rio (1984). These authors used concentrations of nipecotic acid (10 mM) that were much higher than those employed in the present experiments (< 1 mM). Such high concentrations might cause intracellular accumulation of nipecotic acid and exert effects unrelated to blockade of uptake. However, such an internal blockade of release by nipecotic acid should produce a constant effect at different frequencies and does not readily account for the pronounced effects at low frequencies (see Fig. 10).

The pronounced depression of the IPSP at low frequencies with little effect at higher frequencies (Fig. 10) could be due to the altered time course of GABA concentration in the synaptic cleft. If slow components of the GABA concentration decay under normal conditions lasted a few seconds, reduction of GABA uptake should further slow the decline. In effect the interval during which a preceding release would have an effect on subsequent release would be prolonged. In contrast to hippocampal neurones (Hablitz & Lebeda, 1985; Dingledine & Korn, 1985), the duration of the IPSP was not prolonged in our study. It could be argued that the lack of prolongation may be due to the more complex PSPs in neocortical neurones, such that a slight prolongation of IPSPs is buried in the depolarizing and hyperpolarizing transients. But to the contrary, a rather remarkable shortening of the IPSPs was observed. This would be compatible with a decreased release, if the time course of the transmitter determines the duration (see above). The negative feed-back mechanism of GABA on subsequent release proposed previously (Aickin & Deisz, 1981) would therefore not only account for the frequency-dependent decrease of IPSP conductance but also for the effects of nipecotic acid.

Possible actions of GABA and baclofen

The decrease in inhibitory conductance at higher frequencies, after GABA or baclofen application, or in the presence of nipecotic acid, with little if any effect on R_{in}, suggests that the GABA concentration required to exert the proposed presynaptic effect is substantially smaller than that required to induce a postsynaptic conductance. Therefore, neither the $GABA_A$- nor $GABA_B$-induced conductances can account fully for the frequency-dependent depression. Rather, another type of $GABA_B$ receptor, modulating Ca^{2+} currents, may be involved (Deisz & Lux, 1985; Holz *et al.* 1986). Comparing GABA dose–response curves reveals that the half-maximal concentration for $GABA_A$ conductance increase is about 50 μM (Deisz & Dose, 1983; Ozawa & Yuzaki, 1984), like the half-maximal concentration required for the decrease in Ca^{2+} current in dorsal root ganglion cells of about 50 μM (Deisz & Lux, 1985). Despite this similarity, the presynaptic sensitivity appears to be much higher. The effect on release may be potentiated by additional factors. For instance, the pronounced slowing of the Ca^{2+} current onset in the presence of GABA (Deisz & Lux, 1985) might effectively contribute to a reduction of the relevant current time integral. During the short interval required for release, the total effect may thereby be larger than expected from the attenuation of peak currents. Alternatively, the GABA effects may be mediated by second messengers which also could account for the long-lasting effects.

Implications for abnormal neuronal discharges

The lack of development of abnormal discharges in the presence of a decreased inhibition at higher frequencies of stimulation or in the presence of nipecotic acid appears to be at variance with the ease of eliciting epileptogenic discharges in the presence of convulsant drugs (Gutnick, Connors & Prince, 1982). Postsynaptic GABA responses reduced by antagonists not only would shorten the effective length constant of dendrites but also could unmask bursting of individual neurones (Wong & Prince, 1979). When the spatial and temporal pattern of inhibition is impaired without a decreased postsynaptic efficacy, however, several mechanisms may compensate for it. Reduced re-uptake would elevate the cleft concentration and tend to increase the effective length constant. In addition, GABA 'spill over' from the synaptic cleft may reach non-synaptic areas allowing a more widespread and more effective control of both low- and high-threshold Ca^{2+} currents (Deisz & Lux, 1985) and other inward rectifying processes.

In conclusion the present experiments provide an unifying account of several previous observations: (1) the reduction by GABA of IPSPs in tissue culture (Nelson, Ransom, Henkart & Bullock, 1977) and hippocampal slices (Andersen *et al.* 1980); (2) the frequency dependence of the IPSP (Ben-Ari *et al.* 1979; McCarren & Alger, 1985); (3) the decrease of the IPSP by baclofen (Misgeld, Klee & Zeise, 1984; Peet & McLennan, 1986); and (4) reduction of IPSPs by nipecotic acid (Dingledine & Korn, 1985). All of these effects may be explained by a negative feed-back of GABA on its own release (Aickin & Deisz, 1981).

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