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QUININE SUPPRESSES EXTRACELLULAR POTASSIUM TRANSIENTS AND ICTAL EPILEPTIFORM ACTIVITY WITHOUT DECREASING NEURONAL EXCITABILITY *IN VITRO*

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Abstract—The effect of quinine on pyramidal cell intrinsic properties, extracellular potassium transients, and epileptiform activity was studied *in vitro* using the rat hippocampal slice preparation. Quinine enhanced excitatory post-synaptic potentials and decreased fast- and slow-inhibitory post-synaptic potentials. Quinine reduced the peak potassium rise following tetanic stimulation but did not affect the potassium clearance rate. Epileptiform activity induced by either low-Ca^{2+} or high-K^+ artificial cerebrospinal fluid (ACSF) was suppressed by quinine. The frequency of spontaneous inter-ictal bursting induced by picrotoxin, high-K^+, or 4-aminopyridine was significantly increased. In normal ACSF, quinine did not affect CA1 pyramidal cell resting membrane potential, input resistance, threshold for action potentials triggered by intracellular or extracellular stimulation, or the orthodromic and antidromic evoked population spike amplitude. The main effects of quinine on intrinsic cell properties were to increase action potential duration and to reduce firing frequency during sustained membrane depolarizations, but not at normal resting membrane potentials. This attenuation was enhanced at increasingly depolarized membrane potentials.

These results suggest that quinine suppresses extracellular potassium transients and ictal activity and modulates inter-ictal activity by limiting the firing rate of cells in a voltage-dependent manner. Because quinine does not affect 'normal' neuronal function, it may merit consideration as an anticonvulsant.

Key words: epilepsy, anticonvulsant, quinidine, non-synaptic, rat.

Extracellular potassium transients are a ubiquitous feature of epileptic seizure activity (Fertziger and Ranck, 1970). Intense neuronal activity in an epileptic focus induces a rise in extracellular potassium which, in turn, further depolarizes neurons, triggering more potassium release. This regenerative process has been suggested to facilitate the initiation of seizures and account for the all-or-none nature and prolonged duration (several seconds) of ictal bursts (Fertziger and Ranck, 1970; Jensen and Yaari, 1988; Bikson et al., 1999). Between seizures, neuronal populations in an epileptic focus may remain active, generating brief (lasting hundreds of ms) inter-ictal bursts.

Quinine has been shown to attenuate extracellular potassium transients *in vitro* and *in vivo* (Zetterstrom et al., 1995; Smirnov et al., 1999) by a yet unknown mechanism. We, therefore, investigated the effect of quinine on spontaneous ictal and inter-ictal epileptiform activity *in vitro* to determine the role of extracellular potassium build-up in epileptogenesis. In addition, the effect of quinine on pyramidal cell properties was characterized to elucidate the mechanism of quinine-induced potassium transient suppression. A portion of these results has previously been presented in abstract form (Bikson et al., 2001a).

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Abbreviations: 4-AP, 4-aminopyridine; ACSF, artificial cerebrospinal fluid; AHP, after-hyperpolarizing potential; EPSP, excitatory post-synaptic potential; IPSP, inhibitory post-synaptic potential.

EXPERIMENTAL PROCEDURES

Transverse hippocampal slices (400 μm) were prepared from male Sprague–Dawley rats (Harlan OLAC, Bicester, UK; 180–225 g; anesthetized with ketamine and medetomidine). All efforts were made to minimize both the suffering and the number of animals tested (in accordance with UK Animal Scientific Procedures Act 1986). The slices were stored submerged in a holding chamber filled with 'normal' artificial cerebrospinal fluid (nACSF) consisting of (in mM): 125 NaCl, 26 $NaHCO_3$, 3 KCl, 2 $CaCl_2$, 1.0 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose. After >60 min slices were transferred to an interface recording chamber (35°C).

Micropipettes (2–10 MΩ), 'field electrodes', were filled with ACSF and placed in the CA1 or CA3 pyramidal cell layers. Recordings of extracellular concentration of potassium ions ($[K^+]_o$) were performed using double-barrelled ion-sensitive microelectrodes, using either the valinomycin ion exchanger FLUKA 60398 or the equivalent of CORNING 477317 (for description see Alvarez-Leefmans et al., 1992), and a custom

made high-impedance differential amplifier. Preparation of the electrodes is described in detail elsewhere (Köhling et al., 1993). In brief, one channel of thick-septum theta-glass electrode (Kugelstaedter, Germany; tip diameter 2–4 μm) was backfilled with 152.25 mM NaCl solution as the reference channel. The ion-sensing channel was backfilled with 100 mM KCl solution, and its tip was silanized and loaded with ion exchanger. The ion selective electrode resistance was 10–20 GΩ, the field electrode resistance was 10–15 MΩ. The electrodes were calibrated using solutions with $[K^+]$ varying between 1 and 100 mM against a fixed background of 152.25 mM NaCl, and had a slope of 52–61 mV per decade change in $[K^+]$.

Intra- and conventional extracellular signals were amplified and low-pass-filtered (1–10 kHz) with an Axoclamp-2B or -2A (Axon Instruments, Union City, USA) and Neurolog NL-106 and NL-125 amplifiers (Digitimer, Hertfordshire, UK) and digitized using a Power 1401 and Signal software (Cambridge Electronic Design, Cambridge, UK). Single (0.5–10 V, 200 μs) or tetanic (20 pulses at 100 Hz) stimulation was applied via a bipolar Nichrome wire electrode (50 μm) using a DS2 isolated stimulator (Digitimer). Unless otherwise stated, results are reported as mean ± standard deviation; n = number of slices. CA1 pyramidal cells were accepted in this study if they had an input resistance >25 MΩ (average 31 ± 2.6 MΩ) and overshooting action potentials. Unless otherwise stated, all cells were held at −60 mV during stimulation protocols. Post-synaptic potentials in response to orthodromic stimulation were measured at the maximum subthreshold stimulus intensity. The early inhibitory post-synaptic potential (IPSP) amplitude was the maximum negativity. The late IPSP amplitude was measured 100 ms after stimulation. Action potential duration was measured at 50% peak amplitude. Action potential maximum frequency was determined from peak-to-peak times. Null hypothesis rejected at $P < 0.05$.

'Low-Ca^{2+}' ACSF consisted of (in mM): 125 NaCl, 26 $NaHCO_3$, 5 KCl, 0.2 $CaCl_2$, 1.0 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose. 'High-K^+' ACSF consisted of (in mM): 125 NaCl, 26 $NaHCO_3$, 8 KCl, 1.0–1.2 $CaCl_2$, 1.0 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose. 'Picrotoxin' ACSF was made by adding 100–200 μM picrotoxin (Sigma) to nACSF (with 5 mM K^+). '4-AP' ACSF was made by adding 70 μM 4-aminopyridine (4-AP) to nACSF. Quinine (Sigma) was dissolved directly into the perfusate. Unless otherwise stated 200 μM quinine was applied for 60 min.

RESULTS

Effect of quinine on evoked non-synaptic after-discharges

Incubation of hippocampal slices in low-Ca^{2+} ACSF blocks synaptic transmission but enhances neuronal excitability and non-synaptic interactions such that a prolonged epileptiform after-discharge can be elicited by a single antidromic stimulus (Taylor and Dudek, 1982). After-discharges consisted of trains of high-frequency population spikes in the CA1 pyramidal cell layer (Fig. 1), reflecting synchronized fast firing of pyramidal cells (Taylor and Dudek, 1982). After >20 min, quinine (200 μM) increased the duration of individual population spikes and markedly reduced the number of spikes in the after-discharge (Fig. 1). However, even after 60 min, when all but the first two population spikes were completely suppressed, the amplitude of the first population spike was $84 \pm 8\%$ control ($n = 3$).

This result suggests that a majority of cells still fired a single action potential in response to stimulation but could not continue firing at the high rate required to sustain an after-discharge. The failure of after-discharges to develop could be a result of population spike widening (see below), a suppression of the associated extracellular potassium increase (see below), or a combination of the two effects. The suppression of after-discharges by quinine was only partially reversible even after >120 min wash.

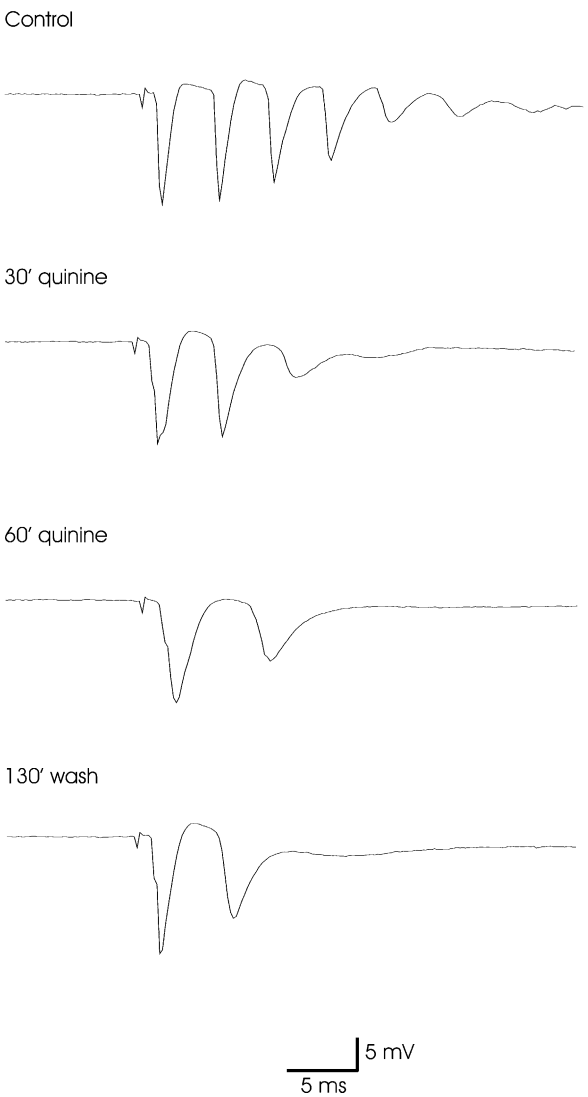

Fig. 1. Effect of quinine (200 μM) on after-discharges evoked in low-Ca^{2+} solution. Activity was evoked by alvear stimulation and recorded in the CA1 pyramidal cell layer with a field electrode before, 30 and 60 min after addition of quinine, and after 130 min wash of quinine.

Effect of quinine on spontaneous non-synaptic ictal epileptiform activity

In slices exposed to low-Ca^{2+} ACSF, spontaneous non-synaptic epileptiform activity was observed (Jefferys and Haas, 1982; Yaari et al., 1986a). Low-Ca^{2+} ictal epileptiform activity can last as long as electro-graphic seizures *in vivo* and has been used for screening non-synaptic anticonvulsant properties of drugs (Heinemann et al., 1985). After ~25 min in quinine (200 μM), low-Ca^{2+} bursts occurred more frequently but were dramat-

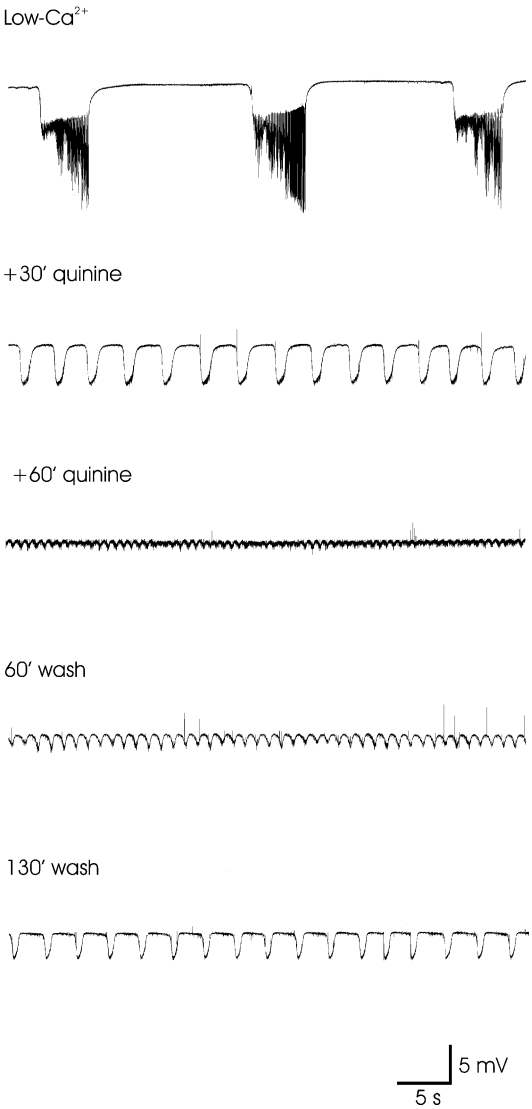

Fig. 2. Effect of quinine (200 μM) on spontaneous non-synaptic epileptiform activity. Low-Ca^{2+} bursting was monitored in the CA1 region with a field electrode before, 30 and 60 min after addition of quinine, and 60 and 130 min after wash of quinine.

ically shortened (amplitude, duration, and frequency were 98 ± 17%, 54 ± 13%, and 268 ± 105% control respectively). After 60 min, quinine either completely suppressed bursting or reduced the amplitude and duration of spontaneous activity such that the residual bursting was too small and high-frequency to be classified as ictal ($n = 5$, Fig. 2). The suppression of low-Ca^{2+} activity by quinine was partially reversible. Lower concentrations of quinine (< 100 μM) can suppress low-Ca^{2+} bursts in a completely reversible manner, given sufficient application and wash time.

Effect of quinine on spontaneous picrotoxin, 4-AP, and high-K^+ induced bursting

The effect of quinine (200 μM) on spontaneous inter-ictal epileptiform activity was tested using the picrotoxin and 4-AP models (Hablitz, 1984; Rutecki et al., 1987). Incubation of slices in picrotoxin ACSF led to the development of spontaneous inter-ictal activity in CA3 and CA1 (Fig. 3). Addition of quinine had no significant effect on peak spontaneous burst amplitude in CA3 (96 ± 37% control, $n = 4$) or CA1 (88 ± 51% control, $n = 4$). In 2/4 slices spontaneous burst frequency increased and burst duration decreased (Fig. 3), while in the remaining slices, spontaneous event frequency and duration were not affected.

Before addition of quinine, stimulation of the Schaffer collaterals in picrotoxin ACSF elicited an after-discharge in CA1 and CA3 that resembled a spontaneous inter-ictal burst and consisted of a train of four to five population spikes. After addition of quinine for 60 min, there was no significant effect on the amplitude of the first evoked population spike in CA1 (93 ± 12% control, $n = 4$) or CA3 (116 ± 80% control, $n = 4$). However, in slices where spontaneous burst duration was significantly shortened, subsequent population spikes were also attenuated (Fig. 3, right). Quinine (60 min) similarly increased the frequency of 4-AP-induced inter-ictal bursting but also decreased burst amplitude in CA1 and CA3 to 54 ± 5% and 62 ± 18% control, respectively (data not shown, $n = 2$).

Consistent with previous reports (Jensen and Yaari, 1988), perfusion of slices in high-K^+ medium resulted in the development of spontaneous ictal activity in CA1 and inter-ictal activity in CA1 and CA3 ($n = 4$). After 20 min in quinine (200 μM), spontaneous inter-ictal burst amplitude and duration were attenuated (Fig. 4) and the frequency of ictal activity in CA1 increased. After > 40 min in quinine inter-ictal activity in CA3 was abolished; however, very high-frequency 'mini-population spikes' persisted (Fig. 4, 30 min inset), suggesting that cells continued to fire but did not syn-

Table 1. Effect of quinine on intrinsic properties of CA1 pyramidal cells

Current	First AP amplitude	First AP duration	Second AP amplitude	Second AP duration	Max. freq.	Average freq.
0.6 nA	82 ± 13	170 ± 48	64 ± 23	342 ± 212	48 ± 26	47 ± 20
0.8 nA	84 ± 10	171 ± 41	61 ± 20	408 ± 370	56 ± 16	62 ± 49
1 nA	84 ± 8.0	149 ± 29	57 ± 22	1523 ± 1527	79 ± 44	49 ± 27
1.5 nA	83 ± 10	181 ± 64	54 ± 22	2559 ± 1611	50 ± 14	36 ± 23
2 nA	86 ± 10	169 ± 40	55 ± 19	2447 ± 1526	46 ± 23	30 ± 18

Quinine (200 μM) was applied for 60 min. The effects on first action potential amplitude (first AP amplitude), first action potential duration (first AP duration), second action potential amplitude (second AP amplitude), second action potential duration (second AP duration), maximum action potential frequency (Max. freq.), and average action potential frequency (Average freq.) in response to 200 ms depolarizing pulses of varied amplitude are reported as percent control (average for six neurons).

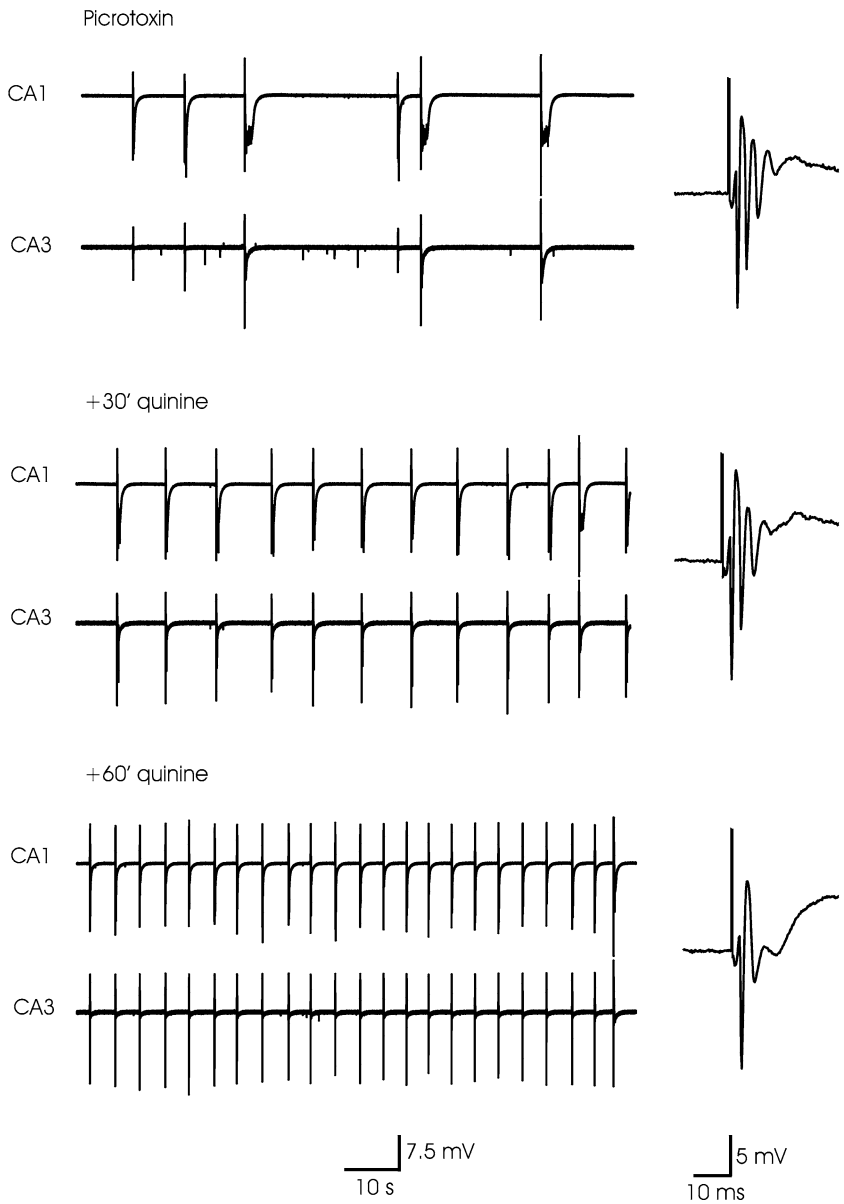

Fig. 3. Effect of quinine (200 μM) on spontaneous picrotoxin-induced epileptiform activity (left) and after-discharges (right) in the same slice. Activity was monitored with field electrodes in the CA1 (top) and CA3 (bottom) regions before and 30 and 60 min after addition of quinine.

chronize into bursts (Draguhn et al., 1998). These mini-events in CA3 were blocked in 3/4 slices after 60 min in quinine. After 60 min, ictal activity was suppressed; however in 2/4 slices mini-population spikes persisted in CA1 (Fig. 4, 60 min inset). In high-K^+ medium, after 60 min in quinine, the peak-evoked orthodromic response in CA1 and antidromic response in CA3 were $94 \pm 2\%$ and $83 \pm 25\%$ control, respectively ($n = 2$).

Effect of quinine on extracellular potassium transients

The effect of quinine on extracellular potassium transients induced by high-frequency stimulation in the CA1 region was determined in nACSF. Consistent with previous studies (Smirnov et al., 1999), addition of quinine (200 μM) reduced the peak extracellular potassium concentration rise in response to high-frequency stimulation (Fig. 5) without a consistent effect on the rate of rise or decay. Significant suppression of the peak potassium rise was observed after 20 min ($57 \pm 12\%$ control) and maximal suppression ($28 \pm 10\%$ control) was reached after ~40 min in quinine.

Effect of quinine on synaptic function and intrinsic membrane properties of CA1 pyramidal cells

The effect of quinine on CA1 pyramidal cell intrinsic properties was studied in nACSF. Application of quinine (200 μM) for 60 min had no significant effect on cell input resistance (31 ± 2.7 MΩ control, 32 ± 4.4 MΩ quinine; $n = 4$) or resting membrane potential (56 ± 3.5 mV control, 48 ± 4.3 MΩ quinine; $n = 4$). After 60 min expo-

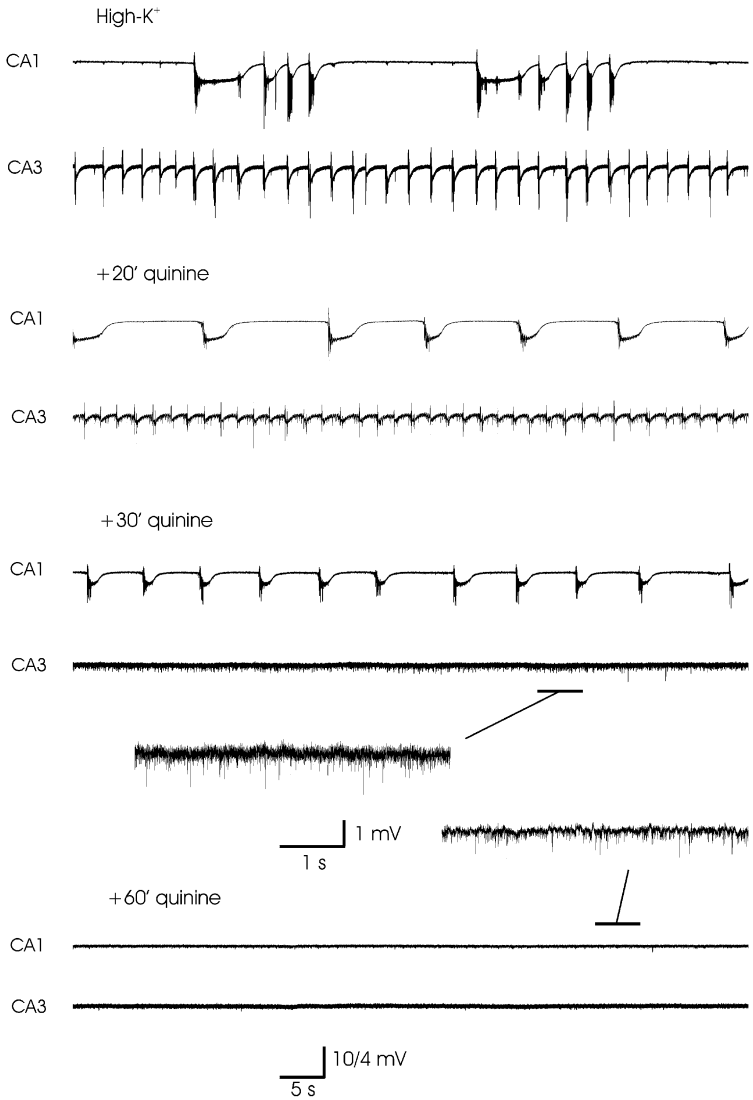

Fig. 4. Effect of quinine (200 μM) on spontaneous high-K^+ epileptiform activity. Activity was monitored with field electrodes in the CA1 (top) and CA3 (bottom) regions before and 20, 30, and 60 min after addition of quinine.

sure to quinine, the amplitudes of the evoked orthodromic and antidromic population spikes were not significantly changed (average change 113 ± 35% of control, $n = 7$, and 107 ± 19% of control, $n = 3$, respectively). Quinine had no effect on action potential threshold as determined by intracellular current injection (0.55 ± 0.2 nA control, 0.45 ± 0.1 nA quinine) or by an orthodromic stimulation.

Quinine decreased both the maximum and average firing rate of neurons in response to a 200-ms depolarizing pulse (Fig. 6A). The relative reduction in average firing frequency increased significantly with larger depolarizing steps (Table 1). Quinine slightly attenuated the size of the first action potential and increased its width (Fig. 6). The second action potential amplitude was substantially depressed and dramatically widened. The depression of the second action potential, but not of the first one, increased with increased current injections (Table 1). Thus, in response to a depolarizing step, the main effect of quinine was an increase in action potential duration and inter-spike interval, and a consequent reduction in firing rate. The degree of 'widening', and hence reduction in firing frequency relative to control, was a function of membrane potential and the time from the previous action potential (Fig. 6B).

In four of six pyramidal cells, quinine (60 min) increased the duration of action potentials, evoked by a current step, to the extent (> 30 ms) that they resembled prolonged Ca^{2+}-like spikes (Fig. 7A). This effect was most pronounced with large current injection (see above), was never observed in the first action potential (see above), and generally lead to a disruption of (normal) action potential generation for the remainder of the current step.

We investigated the hypothesis that quinine reduces firing rate by interfering with voltage- and time-dependent action potential repolarization, which has been shown to limit the firing rate of pyramidal cells (Schwartzkroin and Prince, 1980). We, therefore, determined the effect of quinine on the ability of neurons to

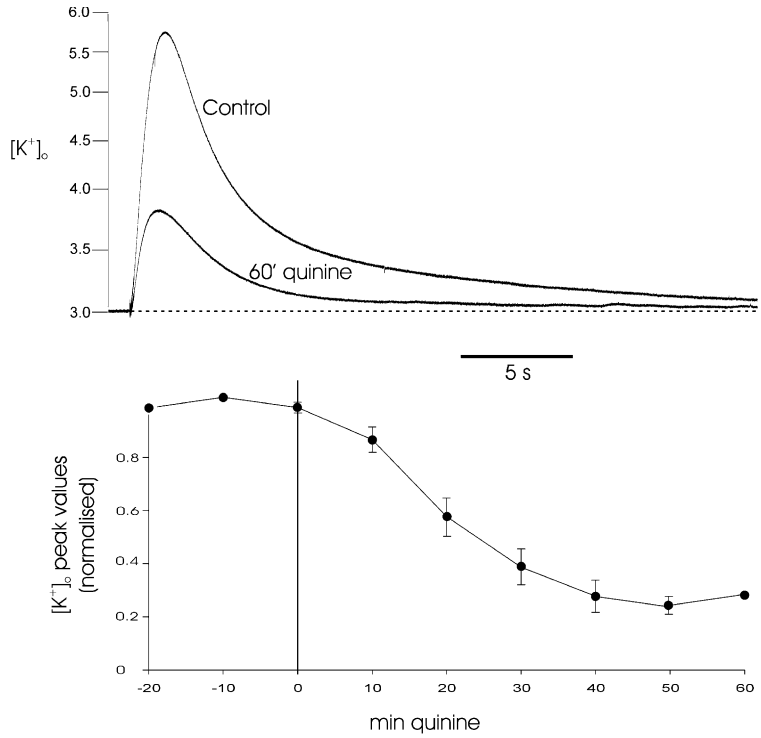

Fig. 5. Effect of quinine (200 μM) on extracellular potassium transients induced. Example of potassium transients induced by tetanic (20 pulses at 100 Hz) stimulation (top) before and 60 min after addition of quinine. Summary of effect of quinine on extracellular potassium transient time course for eight slices (bottom).

follow a train of high-frequency pulses. In contrast to a constant depolarizing step (Fig. 7A), stimulation using pulses allows the membrane potential to approach baseline during the inter-pulse period. Even after >60 min in quinine all neurons tested were able to fire action potentials at >50 Hz (Fig. 7B). Hyperpolarizing a neuron enhanced its ability to follow a high-frequency (>70 Hz) pulse train while depolarization in quinine, but not control solution, resulted in significant attenuation and eventual failure of repetitive firing. Thus quinine suppresses the ability of cells to fire high-frequency action potentials during sustained depolarization, but not necessarily at hyperpolarized levels (compare Fig. 7A with Fig. 7B).

The analysis of quinine's effect on the Ca^{2+}-mediated slow after-hyperpolarizing potential (AHP) was complicated by the dramatic effect of quinine on action potential duration which would, in turn, enhance Ca^{2+} influx (Lin et al., 1998) and thus indirectly enhance AHP size. While 200 μM quinine had no consistent effect on AHP amplitude, larger concentrations of quinine (≥ 1 mM) can suppress its underlying current (data not shown, Yoshida et al., 1986).

The effect of quinine (200 μM) on synaptic function was studied by evoking subthreshold responses via orthodromic stimulation (Fig. 8). After 60 min, quinine increased the peak excitatory post-synaptic potential (EPSP) amplitude ($125 \pm 15\%$ control) but not peak EPSP slope ($106 \pm 7\%$ control, $n = 3$). Fast and slow IPSP amplitude decreased substantially in the presence of quinine to $42 \pm 43\%$ and $25 \pm 34\%$ control respectively ($n = 3$). The increase in EPSP size and duration could reflect enhanced excitatory transmission or a decrease in inhibitory transmission function (Fig. 8).

Addition of quinine (200 μM, 60 min) to nACSF under normal (<10 Hz) stimulus paradigms did not induce spontaneous inter-ictal or ictal bursting ($n = 18$) or spontaneous or evoked intracellular bursts ($n = 6$).

DISCUSSION

The main finding of this study is that quinine increased ictal epileptiform burst frequency and decreased ictal burst amplitude and duration until spontaneous activity was blocked. In contrast, only burst frequency and duration were affected during picrotoxin-induced inter-ictal epileptiform activity bursting. 4-AP-induced inter-ictal burst amplitude was slightly attenuated while high-K^+ inter-ictal activity was almost completely suppressed. Quinine suppressed repetitive neuronal firing evoked by current injection at depolarized but not at normal resting membrane potentials. The effect of quinine on evoked low-Ca^{2+} and picrotoxin-induced after-discharges closely resembled its effect on intrinsic cell properties (compare Fig. 1 and Fig. 3 right with Fig. 7A).

The goal of anticonvulsant drug development is to eliminate seizures with limited or no side effects (e.g. to dissociate epileptic mechanisms from normal neuronal function, Hochman et al., 1995). The following discussion addresses the relationship between quinine's effect on intrinsic cell properties and its effect on each epilepsy model tested and whether quinine's mechanism of action suggests a specific targeting of epileptic mechanisms.

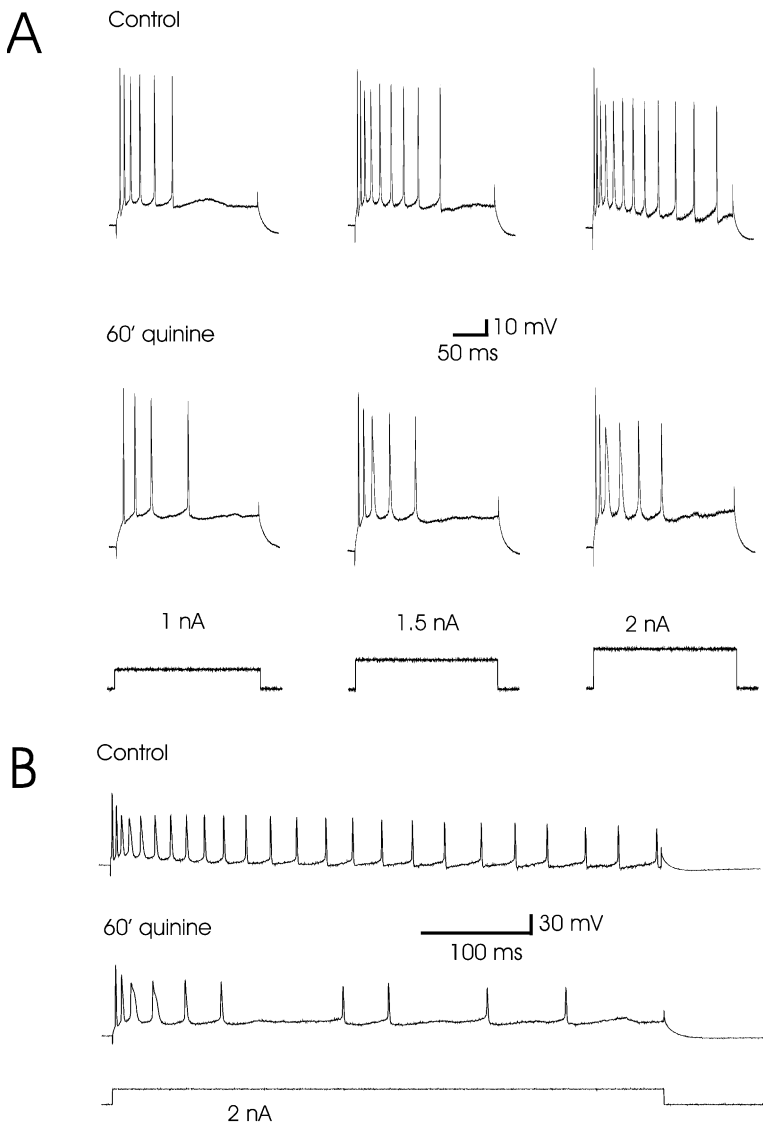

Fig. 6. Effect of quinine (200 μM) on CA1 pyramidal cell firing properties. Response of single CA1 pyramidal cell to (A) short and (B) long depolarizing steps of various amplitudes (same cell as A).

Effect of quinine on intrinsic cell properties and potassium transients

In dissociated bullfrog sympathetic neurons, quinine can block the A-type, M-type, and delayed rectifier-type potassium (I_K) currents (Imai et al., 1999). Its effect on the delayed rectifier-type current was to cause a hyperpolarizing shift in the inactivation curve without significantly affecting the activation curve (Kotani et al., 2001). Similarly, Lin et al. (1998) reported that in cultured spiral ganglion neurons quinine blocked I_K, in a voltage-dependent manner, which resulted in a broadening of action potentials. In dissociated hippocampal pyramidal neurons, quinidine (an analog of quinine), attenuated the A-type and the delayed rectifier-type potassium current (Oyama et al., 1992). The effect of quinine on CA1 pyramidal cell intrinsic properties (Fig. 6) in many respects resembles that of other delayed rectifier-type potassium channel blockers such as TEA, which similarly broadens spikes and slows firing rate in a voltage- and time-dependent manner (Schwartzkroin and Prince, 1980). High concentrations of quinine can also attenuate sodium conductances (Yoshida et al., 1986; Lin et al., 1998). The small decrease in action potential amplitude observed in the present study is consistent with an antagonism of sodium channels and may contribute to the suppression of epileptiform activity (Yaari et al., 1986b). However, the dramatic effects of quinine on action potential duration and firing frequency, and on epileptiform activity waveform are not consistent with an action on sodium channel function (Schneiderman and Schwartzkroin, 1982; Yaari et al., 1986b).

In sharp contrast to quinine, addition of other potassium channel antagonists (such as Ba^{2+}, TEA, 4-AP) to

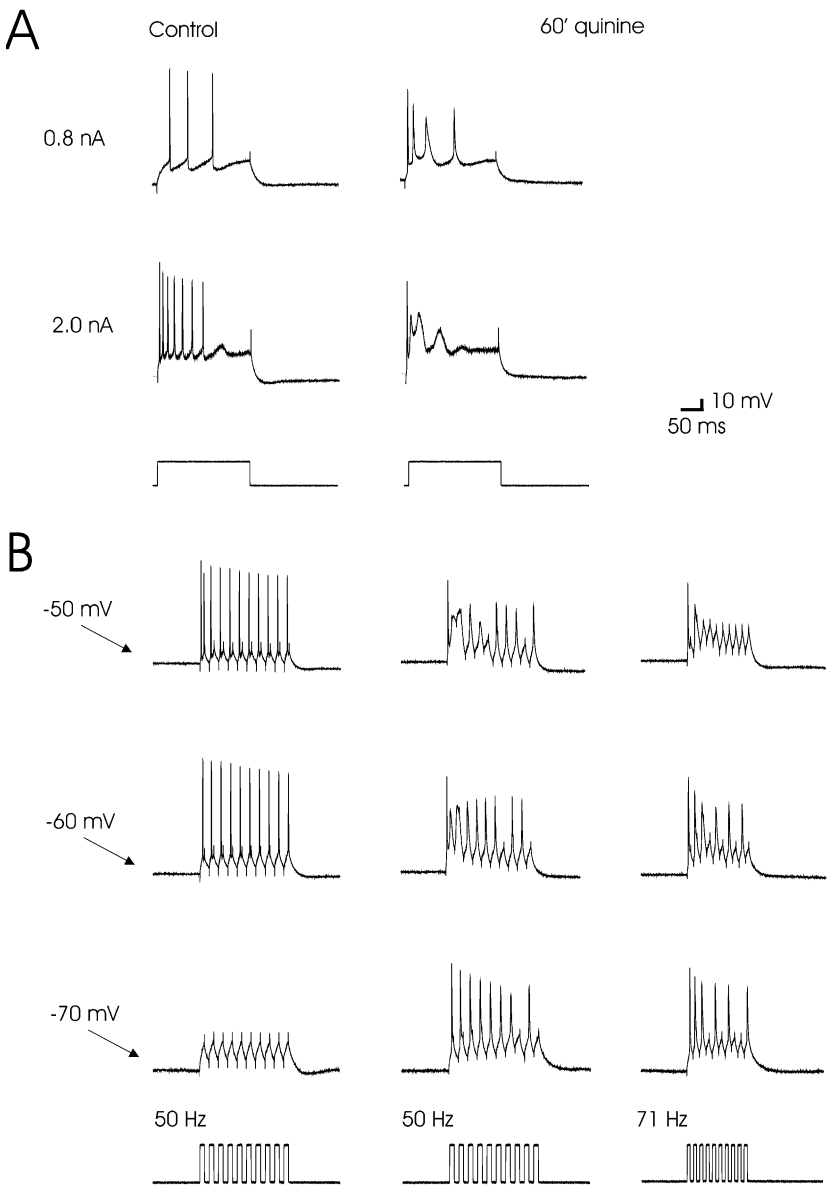

Fig. 7. Effect of quinine (200 μM) on intrinsic CA1 pyramidal cell firing properties. Response of CA1 pyramidal cell to (A) constant depolarizing steps of various amplitude and (B) a train of depolarizing pulses applied at different membrane holding potentials (same cell as A). Note that the pulses used were subthreshold in control at −70 mV.

nACSF can induce spontaneous intracellular bursts and epileptiform activity (Schwartzkroin and Prince, 1980; Hotson and Prince, 1981; Rutecki et al., 1987). While quinine shortened the duration of 4-AP- and picrotoxin-induced bursting, TEA increased the duration of 4-AP- and bicuculline-induced bursting (Domann et al., 1994). Similarly, while quinine suppresses extracellular potassium transients and ictal epileptiform activity (which is facilitated by these transients), other potassium channel antagonists (including Ba^{2+}, TEA, and 4-AP) either do not affect or enhance extracellular potassium transients and facilitate icto-genesis (Jones and Heinemann, 1987; Gabriel et al., 1998; Bikson et al., 1999). The unique effects of quinine may reflect observations that quinine shifts the voltage-dependent kinetics of certain potassium channels rather than reducing the peak K^+ conductances (Lin et al., 1998; Kotani et al., 2001).

It has been suggested that the large extracellular transients, associated with seizures, result from a positive feed-back between potassium accumulation and neuronal depolarization (Fertziger and Ranck, 1970; Jefferys and Haas, 1982; Yaari et al., 1986a; Jensen and Yaari, 1988). Though recent studies have shown that neuronal firing is not required for the maintenance of large potassium transients (Bikson et al., 2001b), high-frequency action potentials are required to initiate a potassium rise. The frequency of this firing could be critical in establishing whether a potassium rise will be sufficient to become regenerative. Quinine, by reducing the peak firing rate of neurons, could thus suppress the initiation of regenerative K^+ transients and ictal bursts.

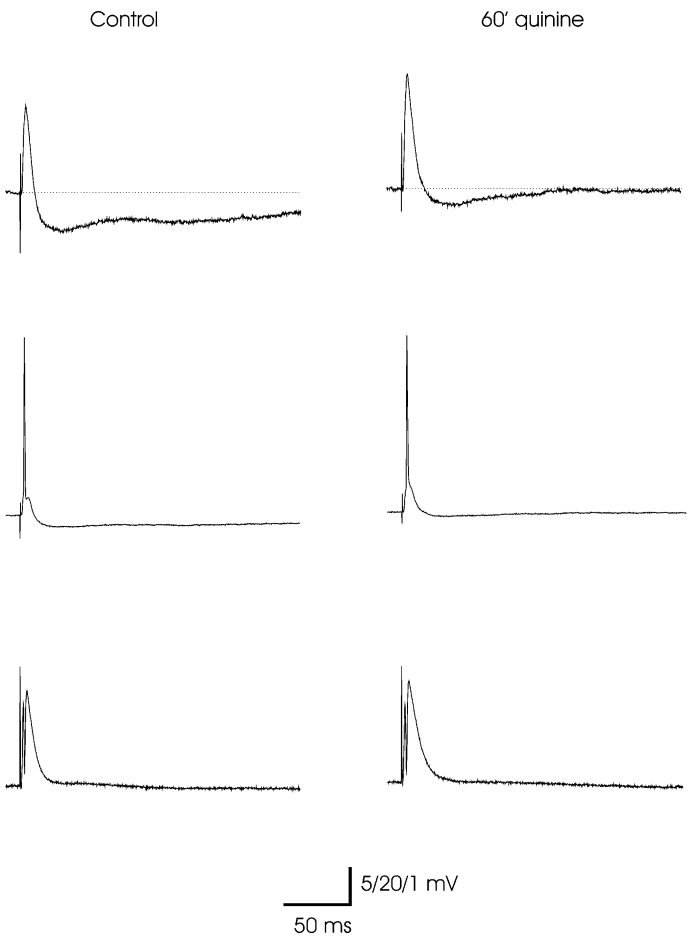

Fig. 8. Effect of quinine (200 μM) on evoked synaptic function. Response of CA1 pyramidal cell to just sub- (top) and supra (middle) threshold orthodromic stimulation. Population spike (bottom) in response to same suprathreshold stimulation.

Modulation of ictal and inter-ictal epileptiform activity

The modulation of inhibitory and excitatory synaptic function by quinine (Fig. 8) would be expected to increase network excitability; rather than to depress it. The suppression of low-Ca^{2+} activity, under conditions where synaptic transmission is blocked, illustrates that quinine can suppress bursting via a non-synaptic mechanism. The modulation of picrotoxin-induced bursting, where $GABA_A$ receptors are blocked, similarly shows that the increase in inter-ictal burst frequency cannot be explained by an effect on inhibitory transmission.

Several lines of evidence suggest that quinine does not modulate epileptiform activity by changing neuronal excitability. In nACSF, 200 μM quinine had no effect on cell resting membrane potential, action potential threshold, or evoked population spike size. In every model of epileptiform activity tested, quinine caused bursts to increase in frequency and decrease in duration. Treatments that decrease neuronal excitability decrease burst frequency, while drugs that increase neuronal excitability increase burst frequency without necessarily modulating burst amplitude or duration (Rutecki et al., 1987; Bikson et al., 1999). Thus, the effect of quinine on burst duration cannot be explained by a simple decrease in excitability, while the effect of quinine on event frequency argues against a reduction of neuronal excitability by quinine. However, even if neuronal excitability is not changed, an increase in event frequency could result from a decreased refractory period due to shortened (and hence less robust) bursts.

Intracellularly, inter-ictal bursts are characterized by a paroxysmal depolarization shift – a large membrane depolarization surmounted by high-frequency action potentials. Quinine dramatically affected cell firing during depolarization (Fig. 1) and could thus prematurely terminate a paroxysmal depolarization shift, thereby decreasing epileptiform event duration. Quinine can modulate repetitive firing not only during a transient depolarization but also if the cell is tonically depolarized (Fig. 2). Pyramidal cell resting membrane potential is not dramatically changed during perfusion with picrotoxin or 4-AP ACSF (Hablitz, 1984; Rutecki et al., 1987). In contrast increasing extracellular potassium (from 3 to 8 mM) can depolarize cells ~15 mV; the reduction in extracellular Ca^{2+} (from 2 to 1 mM) shifts the transmembrane potential sensed by voltage-gated channels another ~10 mV (Hille, 1992). Thus, the suppression of high-K^+ inter-ictal bursting by quinine is consistent with the interruption of repetitive firing at a depolarized resting membrane potential.

Ictal events differ from inter-ictal activity in that they

require potassium accumulation. Since the clearance of potassium produces the slow DC shift characteristic of ictal bursts, the size and waveform of the DC shift directly reflect the extracellular potassium transient (Yaari et al., 1986a; Bikson et al., 1999). Thus, the gradual decrease in ictal burst amplitude by quinine reflects an incremental reduction in the extracellular potassium transient size. It is important to emphasize that quinine does not directly suppress potassium transients, but does so indirectly by modulating neuronal firing (Figs. 6 and 7).

Clinical relevance

Quinine, an alkaloid commonly found in tonic beverages (Weinred, 2001), is used clinically in the treatment of malaria (Warrell, 1999) and as an antispasmodic drug (Ebell, 1995). Quinine has been shown to possess anticonvulsant properties *in vivo* during certain types of seizures (Wambebe et al., 1990). *In vitro*, in the presence of convulsants, quinine does not suppress low-frequency neuronal firing but rather interrupts the regenerative process required for ictal burst development. Epilepsy is characterized by recurrent seizures, which are the key targets for anticonvulsants. In fact, *in vitro* studies have shown that inter-ictal bursting can suppress icto-genesis (Jensen and Yaari, 1988; Barbarosie and Avoli, 1997). We show here that quinine (by virtue of its mechanism of action) can selectively suppress the generation of ictal activity.

Most anticonvulsants are believed to suppress seizures by reducing neuronal excitability or limiting repetitive neuronal firing through actions on neurotransmitter, Ca^{2+} channel, or Na^{+} channel function (Yaari et al., 1986b; Brodie and Dichter, 1997). Despite the critical and multifaceted role of K^{+} channels in the generation and regulation of abnormal neuronal activity (Fertziger and Ranck, 1970; Gabriel et al., 1998; Bikson et al., 1999), no drugs antagonizing potassium channel function are commonly used in the treatment of seizures because previous *in vitro* studies have indicated that potassium channel blockers are pro-convulsants. Interestingly, retigabine, a potassium channel agonist (Rundfeldt and Netzer, 2000), is active against several animal models of seizures (Rostock et al., 1986), and is undergoing clinical testing. The results of this study suggest a novel approach for the treatment of seizures using potassium channel antagonists based on quinine analogs.

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