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RESEARCH ARTICLE

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Extracellular K^+ accumulations and synchronous GABA-mediated potentials evoked by 4-aminopyridine in the adult rat hippocampus

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Abstract Transient changes in extracellular potassium concentration ($[K^+]_o$) and field potentials were evoked by 4-aminopyridine (4-AP; 50–100 μM) and recorded with ion-selective microelectrodes in CA1b, CA3b and dentate sectors of adult rat hippocampal slices. Long-lasting field potentials recurred at a frequency of ≈1/60 s (0.016±0.003 Hz) in association with increases in $[K^+]_o$ which were largest and most sustained in the dendritic regions where afferent fibers terminate (dentate>CA1>CA3) and in the hilus. In stratum radiatum of CA1 or stratum moleculare of the dentate these fields had a peak amplitude of 1.4±0.29 mV, duration 8.3±1.6 s, and were accompanied by increases in $[K^+]_o$ of 1.8±0.22 mM that lasted 32±5.5 s (n=17 slices). Interictal epileptiform potentials, which were brief (<0.2 s) and more frequent at ≈1/3 s (0.30±0.02 Hz) were also present in CA1, CA3 and the hilus and associated with small increases in $[K^+]_o$ (≤0.5 mM, duration ≤2 s). Interictal activity was blocked by 6-cyano-7-nitroquinoxalone-2,3-dione (CNQX; 5–20 μM); the slow, less frequent potentials were resistant to both CNQX and DL-2-amino-5-phosphonovaleric acid (APV; 50 μM) and reversibly blocked (or attenuated by ≈80%) by bicuculline methiodide (BMI) (25–100 μM). The BMI-sensitive potentials were also abolished by baclofen (100 μM), an effect which was reversed by 2-OH-saclofen (100 μM). Focal application of KCl or GABA in the absence of 4-AP evoked long-lasting field and $[K^+]_o$ potentials which were similar to those evoked by 4-AP but more sustained. The proportional relationship between the amplitudes of field and K^+ potentials with GABA closely resembled that observed for 4-AP; in contrast the slope of KCl-evoked responses was lower. Our results demonstrate that in the adult rat hippocampus 4-AP induces in many different regions accumulations of $[K^+]_o$ in synchrony with the long-lasting field potentials, which are known to correspond to an intracellular long-lasting depolarization of the pyramidal cells. These changes are smaller than those which occur in the immature rat hippocampus – which may be related to differences in Na-K-ATPase and susceptibility to seizures. These events involve the activation of $GABA_A$ receptors, are under the modulatory control of $GABA_B$ receptors, and likely arise from the activity of GABAergic interneurons and/or afferent terminals. The long-lasting field potentials appear to reflect mainly the direct depolarizing actions of GABA and to a much more limited extent the associated accumulation of $[K^+]_o$.

Key words Ion-selective microelectrodes · Long-lasting field potentials · 4-Aminopyridine · GABA (γ-aminobutyric acid) · Extracellular $[K^+]$

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Introduction

A well-recognized mechanism in the production of synchronous epileptiform discharges is the decrease or loss of GABA (γ-aminobutyric acid)-mediated inhibition (Prince 1978; Ribak et al. 1979; McCarren and Alger 1985; Avoli 1988). Several factors might be implicated in the fading of the hyperpolarizing response of neurons to GABA and depolarizing shifts of the reversal potentials for GABA (E_{GABA}) and inhibitory postsynaptic potentials (E_{IPSP}), including anion redistribution and a decline in driving force for Cl^- (McCarren and Alger 1985; Thompson and Gähwiler 1989a,b,c); a decrease in $GABA_A$ receptor affinity, secondary to $[Ca^{2+}]_i$ increase (Inoue et al. 1986); modulation of transmitter release at specific presynaptic sites by $GABA_B$ and μ opioid receptors (Thompson et al. 1993); as well as activity-evoked accumulation of extracellular K^+ (McCarren and Alger 1985). Indeed extracellular K^+ accumulation has long been considered to play a role in epileptogenesis (Green

1964; Lux 1974; Moody et al. 1974; Sypert and Ward 1974; Fisher et al. 1976; Heinemann et al. 1977; Prince 1978; Somjen 1979; Krnjevic and Morris 1981; Jensen and Yaari 1988; Taylor 1988).

GABA's depolarizing effects – which if sufficiently strong can be excitatory (Michelson and Wong 1991) – are more often observed in dendritic regions and when concentrations of GABA are high (Alger and Nicoll 1982; Avoli 1992), are associated with increases in $[K^+]_o$ (Segal and Gutnick 1980; Barolet and Morris 1991), and may significantly contribute to the initiation of ictal epileptiform activity (Avoli et al. 1993). Since 4-aminopyridine (4-AP) increases neuronal excitability and produces convulsant/epileptiform activity, while at the same time preserving synaptic inhibition, it offers a useful model of focal epilepsy for examining the role of GABAergic mechanisms (McCarren and Alger 1985; Perreault and Avoli 1989). Two types of synchronous recurrent potentials are induced by 4-AP throughout the hippocampus. The first consists of brief interictal epileptiform discharges which occur at approximately 0.6 Hz and are sensitive to excitatory amino acid receptor antagonists (Perreault and Avoli 1989, 1991, 1992). The second is a long-lasting field (which corresponds to an intracellular depolarization of principal cells) which is generated at a much lower frequency, in the presence or absence of excitatory synaptic transmission, and has been attributed to the activity of GABAergic interneurons (Perreault and Avoli 1989, 1991, 1992). Previous studies have demonstrated that 4-AP can evoke $GABA_A$-mediated excitatory synaptic recruitment of GABAergic interneurons (Michelson and Wong 1991), and that in the dendritic regions of the cerebellum it produces large increases in $[K^+]_o$ (Nicholson et al. 1976). Perreault and Avoli (1992) proposed that the long-lasting field potentials induced by 4-AP occur in close association with $[K^+]_o$ accumulations and that these could importantly contribute a positive feedback role to the recruitment of activity and propagation of discharges. In recent studies by Louvel et al. (1994) such changes in $[K^+]_o$ have been demonstrated in dendritic regions of CA3 in the juvenile rat hippocampus.

To further assess the K^+ accumulation hypothesis, we have carried out experiments in different regions of the adult rat hippocampal slice to quantify and correlate changes in $[K^+]_o$ and the synchronous field potentials during 4-AP evoked activity, and to determine the contribution of receptors for GABA. Some of the results have been previously reported in abstract form (Morris et al. 1993a,b).

Materials and methods

Preparation of slices

Experiments were performed using mature Sprague-Dawley rats (150–350 g) which were housed under controlled environmental conditions and killed by guillotine decapitation, in accordance with regulations of the Canadian Council of Animal Care and the National Institutes of Health. The brain was rapidly removed from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF). After separation of the hemispheres, the hippocampus was dissected free and transverse slices (400–450 μm) were cut, as previously described in detail by Perreault and Avoli (1991, 1992). The slices were placed in chilled ACSF and then in ACSF equilibrated with 95% O_2/5% CO_2 in a holding chamber at room temperature for periods of ≥1 h. They were subsequently transferred to a Haas-type interface recording chamber (Medical Systems Corporation) and perfused at rates of ≤3 ml/min with oxygenated ACSF (at 31–32°C and pH 7.4) which contained (in mM): 120 NaCl, 3.3 KCl, 1.23 NaH_2PO_4, 1.3 $MgSO_4$, 1.8 $CaCl_2$, 26 $NaHCO_3$ and 11 glucose.

Drug applications

4-Aminopyridine was added to the ACSF bath perfusate in a concentration of either 50 or 100 μM. In some experiments additions of one or more of the following compounds were also made: 6-cyano-7-nitroquinoxalone-2,3-dione (CNQX; 5–20 μM), DL-2-amino-5-phosphonovaleric acid (APV; 50 μM), bicuculline methiodide (BMI; 25–100 μM), DL-baclofen (100 μM) and 2-OH-saclofen (100 μM). In experiments in which KCl (0.15 M) or GABA (10/20 mM) was applied by pressure ejection (Picospritzer, General Valve Corporation) from a single-barrel electrode [Dagan Prism FLG12 capillary (Dagan), tip diameter ≈5 μm, resistance 20 MΩ, inserted into the slice to positions estimated to be <100 μm from the tip of the recording electrode] no 4-AP was included in the bath ACSF. All drugs were obtained from Sigma, with the exception of CNQX and 2-OH-saclofen, which were purchased from Tocris Neuramin.

Recording and stimulation

Ion-selective microelectrodes were prepared from double-barrel theta borosilicate capillary tubing (R & D Scientific), as previously described (Barolet and Morris 1991; Morris et al. 1985, 1991) using the Potassium Ionophore I-Cocktail B valinomycin membrane (Selectophore 60398, Fluka), which has high selectivity for K^+ relative to acetylcholine (Oehme and Simon 1976; Krnjevic et al. 1982b; Barolet and Morris 1991). Electrodes were broken back to give a tip diameter ≈5–8 μm and resistance ≈8–15 MΩ for the 0.15 M NaCl-filled reference channel. They were calibrated before and after recording at 31.5°C in solutions of ACSF in which concentrations of KCl were varied and the amount of NaCl was adjusted to maintain a constant total activity for NaCl and KCl. The voltage signal from each barrel was led to a Keithley Model 604 differential amplifier (Instron); both the differential output between K^+ and reference signals and the single-ended reference (field) voltage were recorded on a Grass model P7 chart recorder. The electrodes were highly selective for K^+relative to 4-AP, APV, baclofen, BMI, CNQX and 2-OH-saclofen. In tests with the fixed interference method (Guilbault et al. 1976), calibration curves showed no differences in the absence or presence of a constant background of these compounds in the concentrations used in experiments.

Recordings were made in different regions in CA1b, CA3b and the dentate gyrus of the hippocampus, following the insertion of the ion-selective electrodes to depths in the slice of 25–100 μm. Identification of recording sites was assessed by visual inspection of trans-illuminated slices and from the characteristics of field potentials evoked at the beginning of each experiment by stimulation in stratum radiatum using a bipolar concentric electrode (Model MCE-100, Rhodes Medical Instruments; distributor David Kopf Instruments). The evoked field potentials were recorded on an oscilloscope and/or with an Axotape 1.1 program (AXON Instruments). Data were expressed as mean±SE and analyzed using either Student's *t*-test or Mann-Whitney *U*-test, Pearson correlation coefficient (*r*), and linear regression and covariance (*F*), and interpreted as significant at a level of $P \leq 0.05$.

Results

Changes in extracellular field and K^+ potentials

The application of 4-AP evoked within 15–45 min two recurring types of field potentials: (i) long-lasting transients of duration 8.32±1.61 s at a frequency of 0.009–0.044 Hz (0.016±0.003, n=17 slices), i.e., ≈1/60 s, and (ii) brief (<0.2 s) interictal-like epileptiform discharges at 0.19–0.33 Hz (0.297±0.019, n=14 slices), i.e., ≈1/3 s. In recordings made in either stratum radiatum of CA1 or the dentate molecular layer, the first, more sustained type of potential, which has been correlated with an intracellularly recorded long-lasting depolarization (Perreault and Avoli 1989, 1991, 1992) was accompanied by increases in $[K^+]_o$ of 1.8±0.22 mM (above the resting level of 3.6±0.05 mM, which was unaltered by the presence of 4-AP) and of 32±5.5 s duration (n=7–20 observations in each of 17 slices). These changes in $[K^+]_o$ corresponded to increases in K^+ potential (V_K) of 8.6±1.0 mV. Although in some experiments maximal peak levels of $[K^+]_o$ and ΔV_K of 9–11 mM and 20–24 mV respectively were observed (cf. Fig. 5), it should be emphasized that the average values reported here represent measurements of sequential responses, which varied considerably in amplitude, over periods ≥10–15 min. Figure 1A shows an example of the long-lasting type of field potential (V_F, in upper trace) and potassium signal (V_K), which were evoked by 4-AP and recorded in the region of termination of perforant path fibers in the dentate gyrus. The onset of the initial fast negative potential of the field potential (indicated by arrow 1) consistently preceded that of the large $[K^+]_o$ change but frequently appeared at the same time as a small early increase in $[K^+]_o$. The slow positive component of the field reached a maximum approximately 1 s earlier than the peak $[K^+]_o$ increase, and had a briefer total duration (ratio≈1:4). The slow component of the field appeared to consist of two phases (marked by arrows 2 and 3 in Fig. 1A): an early one of approximately 1–2 s duration, followed by a second, smaller and slower phase.

Earlier studies have addressed the proposed role of extracellular ion accumulations in the generation of slow potentials in synaptic regions of the central nervous system (Barron and Matthews 1938) by demonstrating the proportional relationship of stimulus-evoked changes in focal (field) and K^+ potentials in the dorsal horn and cuneate nucleus (Kriz et al. 1974; Krnjevic and Morris 1975, 1981). If K^+ plays a primary role in depolarizing neuronal membrane the field potential changes would be expected to be much smaller than the trans-membrane response and the potassium potentials. If the ionic

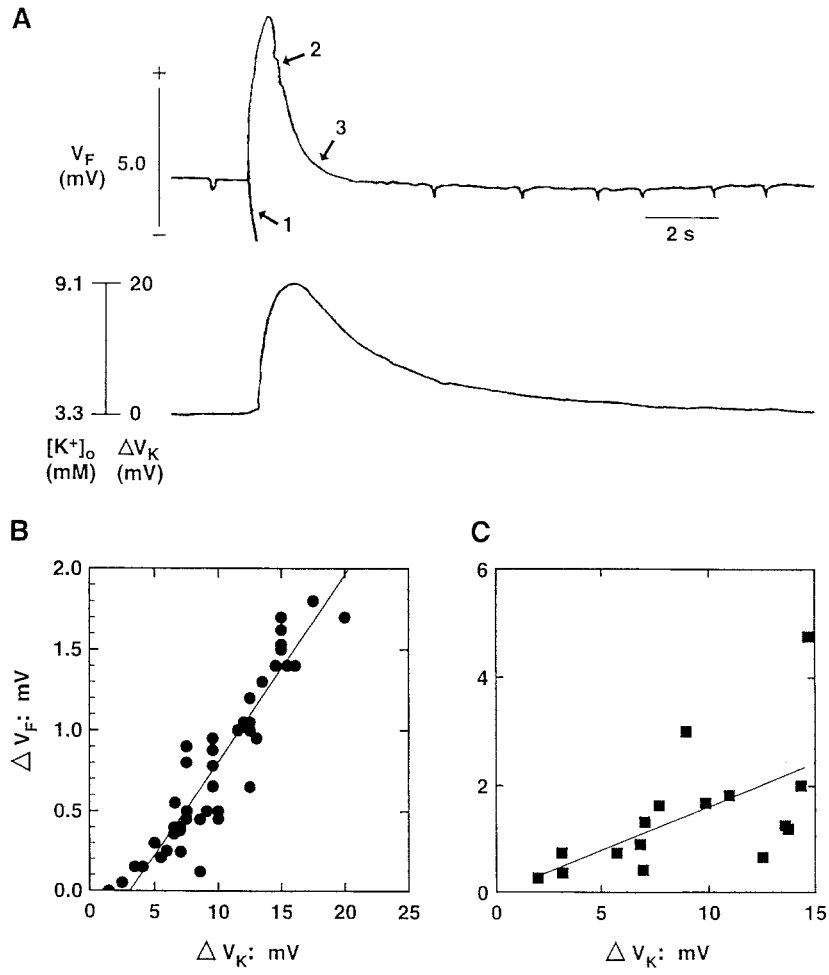

Fig. 1 **A** Correlation of synchronous, recurring long-lasting potentials, evoked by 4-AP. DC voltages recorded extracellularly with an ion-selective microelectrode in the stratum moleculare of the dentate gyrus: the *upper trace* shows a long-lasting field potential (V_F) recorded from the reference channel; below is the potassium voltage signal (V_K), differentially recorded at the same time from the ion-sensing and reference channels. (*Arrow 1* marks an initial fast component; *arrows 2* and *3* indicate early and late phases of the slow component.) **B** Correlation of maximal changes in slow potentials (ΔV_F and ΔV_K) evoked by 4-AP (50–100 μM) and recorded in dentate stratum moleculare before, during and after changes induced pharmacologically with 100 μM BMI in the presence of 5 μM CNQX in one slice (r=0.930, P≤0.0001, n=46; slope of regression =0.114±0.007). **C** Correlation of changes in long-lasting field and K^+ potentials (ΔV_F and ΔV_K) recorded in CA1 stratum radiatum or stratum moleculare of dentate in 16 different experiments (r=0.597, P<0.01; slope=0.146±0.058)

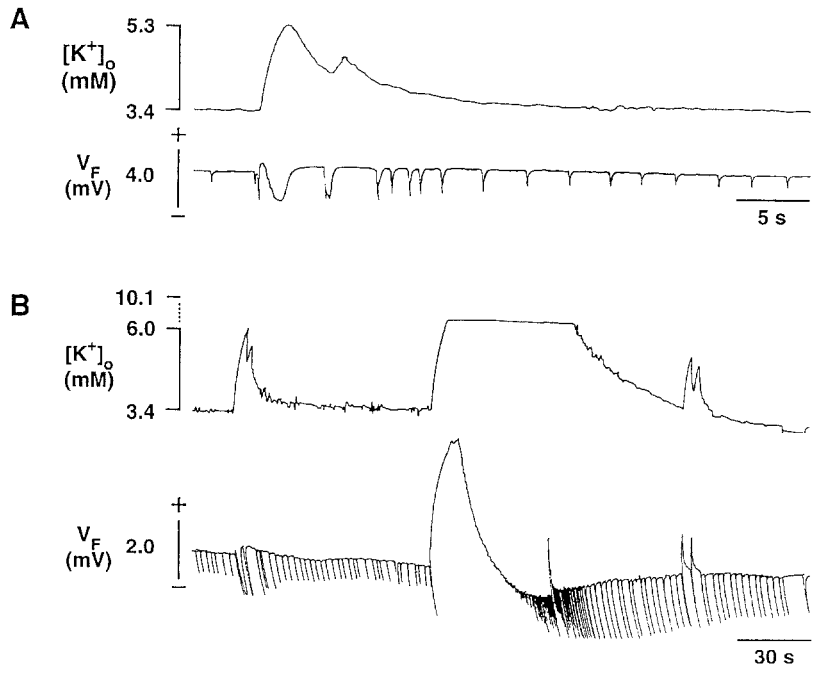

Fig. 2A,B Examples of complex slow potentials evoked by 4-AP (100 μM) and recorded in stratum moleculare of the dentate in one experiment. **A** Superimposed smaller, secondary increase on decline of $[K^+]_o$ increase; the prolonged change in $[K^+]_o$ is accompanied by a series of slow field potentials (V_F) which progressively decrease in duration and amplitude. **B** High-frequency brief interictal and low-frequency slow field potentials (V_F), accompanied by a random prolonged ictal event. *Upper trace* shows slow $[K^+]_o$ changes; maximal $[K^+]_o$ was not recorded, but estimated to reach approximately 10 mM

changes arise secondary to the depolarizing effects of a transmitter, such as GABA, the difference might be expected to be relatively smaller (Krnjevic and Morris 1981). Accordingly, a similar analysis was carried out in the present experiments and showed that the peak amplitude of the slow component of the field potential was 1.4±0.29 mV (n=16 slices) – approximately one-sixth that of the K^+ peak voltage shift of 8.6±1.0 mV (n=17 slices). The graphs in Fig. 1 shows the interdependence of the maximal long-lasting field ΔV_F) and K^+ (ΔV_F) potential changes (with slopes of 0.114 and 0.146, respectively in B and C). Figure 1B shows the correlation of 46 sets of observations recorded in the molecular layer of the dentate in a single slice (r=0.930, P<0.0001) during experimentally-induced changes; C presents similar data from recordings in CA1 stratum radiatum or the stratum moleculare of the dentate from 16 different slices (r=0.597, $P\leq$0.01).

Figure 2 illustrates doublets of long-lasting increase in $[K^+]_o$ which were recorded in stratum moleculare of the dentate and observed in this and other locations in many experiments. In Fig. 2A the superimposition of a second increase on the decline in the K^+ potential shift was associated with a sequence of seven or eight progressively briefer and decrementing slow field potentials – a feature commonly observed. The lower trace of Fig. 2B shows an example of the sporadic occurrence of an apparent ictal event (slow potential shift=5 mV and 30 s in duration, followed by after-discharge) in association with both the interictal and slow type of recurring potentials. This was accompanied by a large increase in $[K^+]_o$, which was not fully recorded but estimated to reach approximately 10 mM.

The interictal field potentials could be recorded in many locations throughout most slices and in CA1, CA3 and the hilus were often associated with small, discrete and relatively constant increases in the K^+ signal of ≤2 mV (representing $\Delta[K^+]_o \leq 0.5$ mM) and ≤2 s duration. Interictal field activity similar to that illustrated in the lower traces in Fig. 2A and B can also be seen in Figs. 3 and 5, where it is accompanied by changes in $[K^+]_o$. As previously described (Perreault and Avoli 1989, 1991, 1992), interictal potentials frequently closely precede or merge with, and therefore appear to trigger, the slower long-lasting field potentials (see examples in Figs. 2 and 3). When interictal activity was associated with distinct $[K^+]_o$ changes, the onset of the larger and slow $[K^+]_o$ increases could often be seen to arise from or be superimposed on a small elevation (Figs. 1A, 3C, 3D). Both the interictal field potentials and the associated small $[K^+]_o$ accumulations could be abolished by the glutamate receptor antagonist CNQX (see Fig. 5B); however, they persisted in the presence of the $GABA_A$ receptor antagonist BMI when CNQX was absent.

Distribution of slow $[K^+]_o$ potentials

In seven hippocampal slices recordings were made in different locations within the CA1b, CA3b and dentate sectors, after the magnitude and frequency of the synchronous activity evoked by 4-AP were stabilized. Figure 3 shows examples of the recurring increases in $[K^+]_o$ and field potentials recorded in different layers of CA1b in one experiment. Progressively greater and longer-lasting increases in the longer duration, slower frequency type of $[K^+]_o$ transient were observed as the recording electrode was moved further away from the alveus (ALV) to stratum oriens (SO), stratum pyramidale (SP), and the distal dendritic region of stratum radiatum (SR).

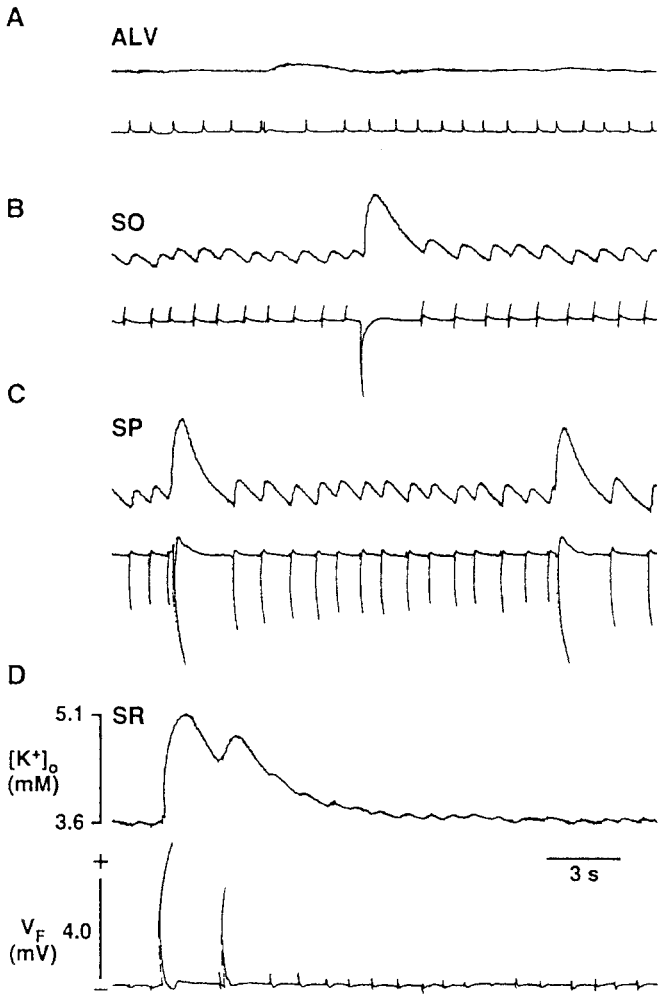

Fig. 3 Slow potentials evoked by 4-AP (100 μM) and recorded in different regions of the hippocampus in one experiment. **A,B,C,D** were recorded respectively from alveus (*ALV*), stratum oriens (*SO*), stratum pyramidale (*SP*) and dendritic region of stratum radiatum (*SR*) of CA1b

The largest $[K^+]_o$ accumulations in association with the long-lasting field potential were recorded in most experiments in the stratum moleculare of the dentate gyrus (SM), followed by those in the dendritic regions of stratum radiatum of CA1 and CA3 and stratum lacunosum moleculare (SLM) of CA1, and the hilus (H). In addition, changes evoked in CA3 generally appeared to be smaller, with amplitudes of approximately 30–70% as compared with those recorded in CA1 at corresponding locations, but with the exception of stratum oriens these were not significantly different. In contrast, the small, brief and higher-frequency $[K^+]_o$ increases, which accompanied the interictal fields, exhibited no specific localization.

The differential distribution of the slow $[K^+]_o$ changes, summarized in the histogram plot in Fig. 4 for 14 different recording locations (average of 6–14 consecutive values for each of 4–7 slices) in the hippocampus, shows the significantly greater $[K^+]_o$ accumulation in the dentate molecular layer as compared with the hilus and granule cell layer within the dentate and for all other locations in CA3 and CA1, with the exception of the SLM.

The time-course of the slow $[K^+]_o$ accumulations associated with the 4-AP-evoked long-lasting field potentials in different subregions of the hippocampus was more fully characterized and compared in some experiments in which measurements were recorded at fast paper speed. Table 1 summarizes data from three locations (stratum pyramidale and distal dendritic region of stratum radiatum in CA1 and the stratum moleculare in the dentate) in one slice. The $[K^+]_o$ changes were greater and rise and fall times were faster in the region of perforant path termination in the molecular layer of the dentate than in either the stratum radiatum or stratum pyramidale of CA1. However, latencies to the peak of K^+ increase for different locations in this and other experiments showed small and variable differences (≈0.3–0.5 s). A notable difference was the longer duration of ΔV_K in CA1 stratum radiatum or stratum moleculare when compared with that in CA1 stratum pyramidale (25.8±3.8 s vs 15.4±2.36 s; $P<0.005$, $n=8$ slices).

Pharmacological features

In 13 experiments the competitive $GABA_A$ receptor antagonist BMI (25–100 μM) was applied for periods of 37±8 min ($n=13$ slices) following and during the application of 4-AP-containing ACSF and evoked no change in resting $[K^+]_o$. In several experiments the slice was also exposed to excitatory amino acid receptor antagonists, either CNQX (5 μM, $n=1$ or 20 μM, $n=3$) alone or in combination with APV (50 μM, $n=3$), before and during the presence of BMI.

BMI (25–100 μM) rapidly produced a complete block of the 4-AP-evoked long-lasting field and K^+ potentials in six experiments (in four of which glutamate antagonists were present). This occurred within 2–6 min, endured a further 10–15 min, and within 3–30 min of washout showed onset of reversal. Figure 5 illustrates recordings from stratum radiatum of CA1 in one experiment which show the blocking effect of BMI in the presence of CNQX (5 μM) and APV (50 μM). The application of the glutamate receptor antagonists in this and other experiments blocked the fast interictal and small $[K^+]_o$ changes and did not alter the frequency of the slow potentials but sometimes decreased their amplitude.

In the remaining experiments, the slow $[K^+]_o$ potentials were not fully abolished by BMI (25–100 μM) but were attenuated to 22±5.0% ($n=7$). In this group the effect of BMI was observed in the absence of CNQX and/or APV in four slices. In three experiments exposure to BMI was for only 11–14 min; in four others BMI applications of between 45 min and 2½ h produced no further change in the steady attenuated state after the first 15 min, although the frequency of the slow potentials was decreased by approximately 70%.

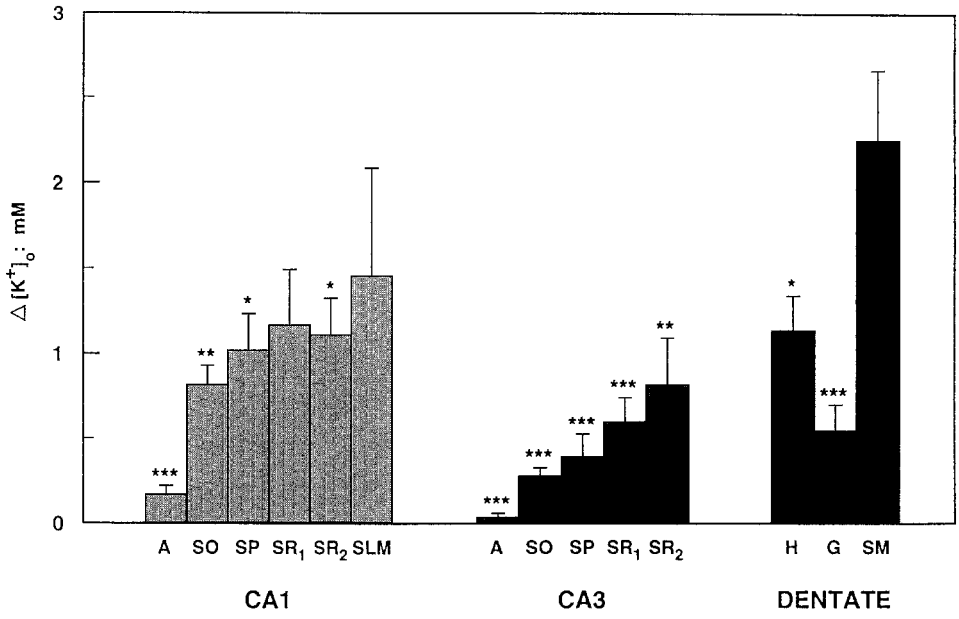

Fig. 4 Differential distribution of magnitude of slow $\Delta[K^+]_o$ associated with long-lasting field potentials evoked by 4-AP (100 μM) in different regions of CA1b, CA3b and the dentate sectors of the hippocampus. *Shaded columns* are means of the means (±SE) of 6–14 observations at a single location (n=4–7 slices). *A* alveus, *SO* stratum oriens, *SP* stratum pyramidale, SR_1 mid stratum radiatum, SR_2 dendritic region of stratum radiatum, *SLM* stratum lacunosum moleculare, *H* hilus, *G* stratum granulosum, *SM* stratum moleculare. * P<0.05, ** P<0.01, *** P<0.001 indicate significant difference for individual sites as compared with SM (t-test)

Table 1 Comparison of $[K^+]_o$ changes associated with synchronous long-lasting field potentials evoked by 4-aminopyridine (4-AP) in stratum pyramidale and stratum radiatum of CA1 and in the stratum moleculare of the dentate in one experiment (data from five or six sequential synchronous potentials recorded in one location)

	ΔV_F (mV)	ΔV_K (mV)	$\Delta[K^+]_0$ (mM)	ΔV_K Rate of Rise (mV/s)	ΔV_K Rate of Fall (mV/s)	Latency to Peak ΔV_K (s)
CA1 stratum pyramidale (n=6)	0.23±0.15*	2.62±0.36***	0.41±0.07***	1.63±0.13***	0.59±0.08***	1.58±0.11
CA1 stratum radiatum (n=5)	0.15±0.03	2.61±0.28***	0.41±0.05***	1.56±0.18***	0.57±0.09***	1.67±0.05
Dentate stratum moleculare (n=5)	0.54±0.04	11.0±0.47	2.20±0.12	10.59±0.18	5.74±0.34	1.04±0.46

*$P\leq0.05$, ***$P\leq0.0001$ indicate significant difference as compared with stratum moleculare of dentate

We also examined the influence of the $GABA_B$ receptor agonist baclofen (100 μM) on 4-AP-induced potentials that were recorded in the region of perforant path fiber termination in the dentate or in stratum radiatum of CA1 in three additional experiments. There were no detectable changes in the resting level of $[K^+]_o$ in the presence of baclofen. Within 5 min baclofen blocked the interictal and long-lasting field potentials (as previously reported by Siniscalchi and Avoli 1992) and also the accompanying $[K^+]_o$ increases. This effect was readily reversed by the addition of the competitive $GABA_B$ receptor antagonist, 2-OH-saclofen (100 μM) (Fig. 6).

Focal applications of K^+ and GABA

In three experiments responses to the focal application of KCl by pressure ejection (50 psi, 10–100 ms duration, 0.15 M) were recorded in stratum radiatum of CA1 and the stratum moleculare of the dentate in the absence of 4-AP. These ejections produced ΔV_K of 13.1±1.5 mV (n=32 observations) (equivalent to a $[K^+]_o$ increase of 4.5 mM) and field potential shifts ≤3 mV in amplitude. During these artificial increases in $[K^+]_o$ the onset of the $[K^+]_o$ change relative to that of the accompanying field (Fig. 7A) was similar to that for the 4-AP-evoked slow potentials (Figs. 1A, 8A), and the early fast component of the field potential evoked with KCl often appeared to resemble that of the long-lasting field induced by 4-AP – which suggests the early activation of interneurons and a role in the initiation of the slow potential (cf. Figs. 7 and 8A). Although the durations of responses to KCl (32.1±6.52 s and 88.2±0.005 s, n=32 for fields and $[K^+]_o$ respectively) were longer than those of the slow synchronous 4-AP-evoked potentials, their ratios (1:3–4) and the intervals (1 s) between peaks were similar. The peak voltage shifts of the field and potassium potentials evoked by KCl were significantly correlated (r=0.932, n=30, $P\leq0.001$) (Fig. 7C); however, the slope of the ΔV_F:ΔV_K relationship (0.068±0.005) was not as steep as that for 4-AP (0.146±0.058, n=16 slices) (see Fig. 1C), with a significant difference (F=45.55, f=1,46, $P\leq0.001$).

In four other experiments focal applications of GABA (50 psi, 100 ms, 10/20 mM) in stratum radiatum of CA1

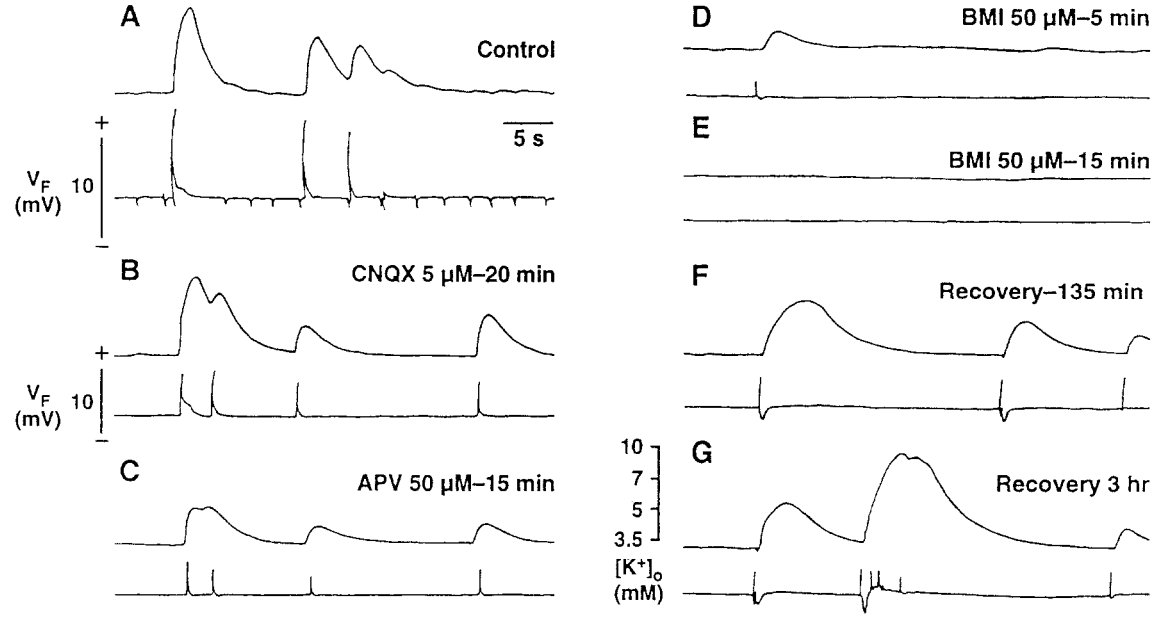

Fig. 5A–F Antagonism by bicuculline methiodide (BMI) of long-lasting field and $[K^+]_o$ potentials evoked by 4-AP (100 µM) and recorded in stratum radiatum of CA1 in the presence of glutamate receptor antagonists (CNQX, 5 µM; APV, 50 µM). The slice was continuously perfused with ACSF containing 4-AP; after control recording (**A**) the perfusant sequentially included CNQX (5 µM) for 20 min (**B**), CNQX and APV (50 µM) for 15 min (**C**), and CNQX with APV and BMI (50 µM) for 15 min (**D**). Recovery was observed during washout of all antagonists. Note the block of interictal fields and associated $[K^+]_o$ changes in the presence of CNQX; field potential (V_F) in **A** is recorded at different gain from those in **B–F**

and in the stratum moleculare of the dentate gyrus in the absence of 4-AP evoked voltage increases for potassium of 18±2.4 mV (n=26) (average $[K^+]_o$ increase of 6.6 mM) and for the associated fields of ≤8 mV. These potentials also resembled the 4-AP-induced slow synchronous K^+ and field potentials with respect to their onset, inter-peak interval of 1.43±0.41 s (n=23), and ratio of duration of 1:3 (22.4±3.30 s and 68.7±17.5 s, n=26 respectively). The correlation between maximal amplitudes of field and potassium changes was significant (r=0.505, n=26, P≤0.01) and the slope of ΔV_F:ΔV_K was 0.132±0.046 (Fig. 7D). This slope was significantly greater than that for focal KCl responses (F=21.88, f=1,56, P≤0.001), but showed no significant difference when compared to that for 4-AP (F=1.00, f=1,40, P>0.05).

Discussion

K^+ accumulations and interictal activity

The epileptiform discharges evoked by 4-AP were similar to those previously described in vivo (Szente and Pongracz 1981) and in vitro (Galvan et al. 1982; Voskuyl and Albus 1985; Rutecki et al. 1987; Perreault and Avoli 1989, 1991, 1992), but of somewhat longer duration. They were frequently associated with distinct but small (≤0.5 mM) accumulations of extracellular K^+, which were not consistently present, localized to or larger in any specific hippocampal region. These appear to be similar to those in the immature animal, since Louvel et al. (1994) reported an increase of 0.5 mM $[K^+]_o$ in association with interictal discharges recorded in stratum radiatum of CA3, although this was described for only one experiment. Our demonstration of the concomitant block of the $[K^+]_o$ and interictal field potentials by the glutamate receptor antagonist CNQX, and persistence in the presence of the $GABA_A$ receptor antagonist BMI, indicates that they are closely related, not generated by activity of GABAergic interneurons and do not contribute in a

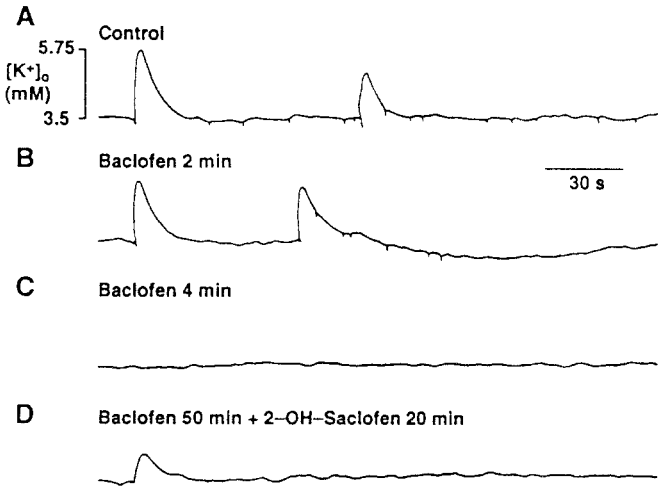

Fig. 6A–D Effect of baclofen (100 µM) on $[K^+]_o$ potentials evoked by 4-AP (100 µM) in stratum moleculare of dentate in one experiment. **A** Control. **B** Last responses recorded during application of baclofen. **C** Recording at 4 min of baclofen exposure. **D** 2-OH-saclofen (100 µM) antagonized interference by baclofen; reversal began within a few minutes, although amplitude and frequency were not fully recovered at 20 min

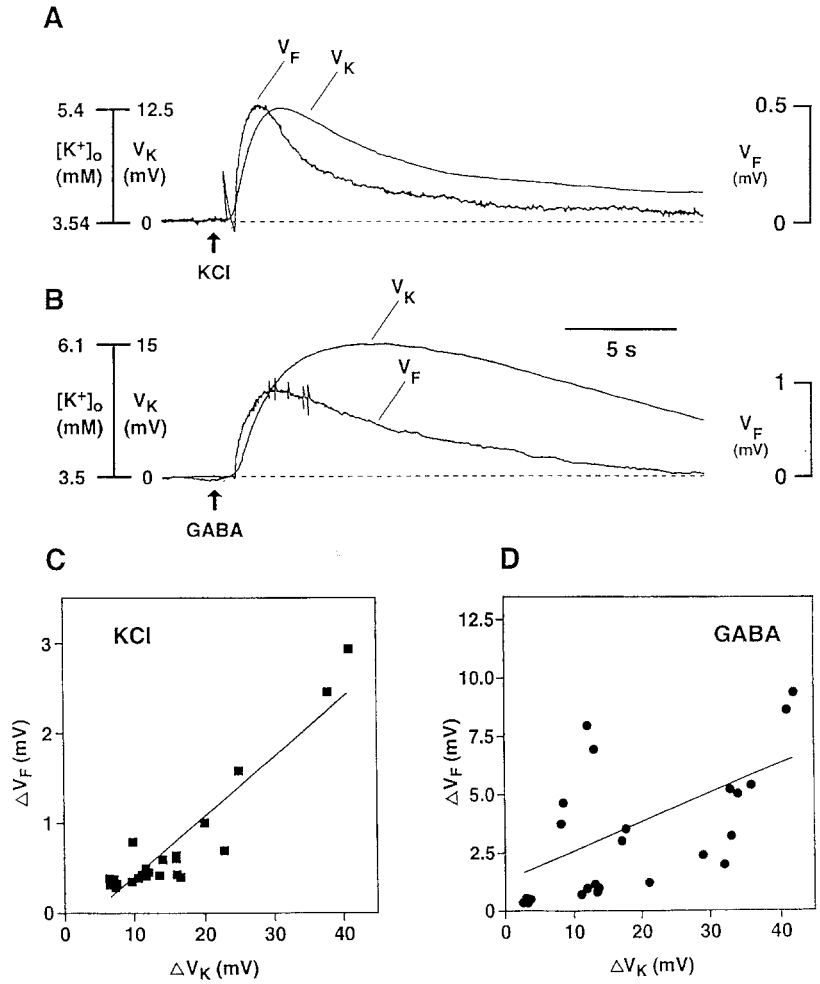

Fig. 7A–D Slow potential (V_F and V_K) changes induced in stratum moleculare of the dentate by focal application of K^+ or GABA. At times marked by *arrows* 0.15 M KCl (**A**) or 10 mM GABA (**B**) was applied by pressure ejection (50 psi, 100 ms from a pipette with tip diameter 5–7 µm, inserted into the slice to a position close to the tip of the recording electrode). (V_F and V_K scales have positive polarity upward.) **C,D** Relation of V_F and V_K changes evoked by 0.15 M KCl (r=0.932, n=32, $P \leq 0.001$); slope of regression=0.068±0.005) and 10 mM GABA (r=0.505, n=26, P<0.01; slope=0.132±0.046), respectively

major way to the other, long-lasting type of field potential that is revealed by 4-AP.

K^+ accumulations and long-lasting potentials

The presence of 4-AP evoked significant accumulations of $[K^+]_o$ in association with the recurrent long-lasting field potentials, which have been correlated with a long-lasting intracellular depolarization (LLD) of the principal cells in the hippocampus (Perreault and Avoli 1989, 1991, 1992). These slow K^+ potentials were much larger and longer in duration and interval (by a factor of approximately 20), than those which occurred in synchrony with the faster, higher-frequency epileptiform fields. The average $[K^+]_o$ increases which we report for all regions in the adult are much smaller than those observed in the CA3 dendritic regions in the juvenile hippocampus (Louvel et al. 1994) – 1.8 mM compared with 7.9 mM, respectively. This may reflect a greater variability of sequential potentials in the adult, and be due to differences in maturation of Na-K-ATPase and related to the greater susceptibility to seizures during development.

In the present experiments we show that the amplitude of the long-lasting fields was approximately one-sixth that of the K^+ potentials, and under conditions in which they varied the relationship between the voltage shifts showed close correlation. This relationship is quite similar to that previously demonstrated in studies in synaptic regions of termination of primary afferent fibres in the dorsal column nuclei and dorsal horn of the spinal cord, in which it was concluded that activity-induced accumulations of K^+ could contribute to the field potentials and presynaptic depolarization, without excluding the influence of an early GABA-mediated depolarization (Kriz et al. 1974; Krnjevic and Morris 1975, 1981). The current results suggest, as previously hypothesized (Prince 1978), that an extracellular K^+ accumulation – which is insensitive to glutamate receptor antagonists but sensitive to BMI, and can be attributed to the activation of GABAergic interneurons and $GABA_A$ receptors – may indeed contribute to further depolarization of principal cells and glia.

Comparison of the time-course of the slow potentials evoked by 4-AP may provide some insight into their mechanisms. The superimposition of extracellular recordings from one experiment (Fig. 8A) and of the intracellular LLD and extracellular field recorded in the same region in an experiment from our earlier study (Fig. 8B) allows comparison of changes in V_K, V_F and membrane potential (V_M). Our recordings have consistently demonstrated an earlier onset for both the fast and slow compo-

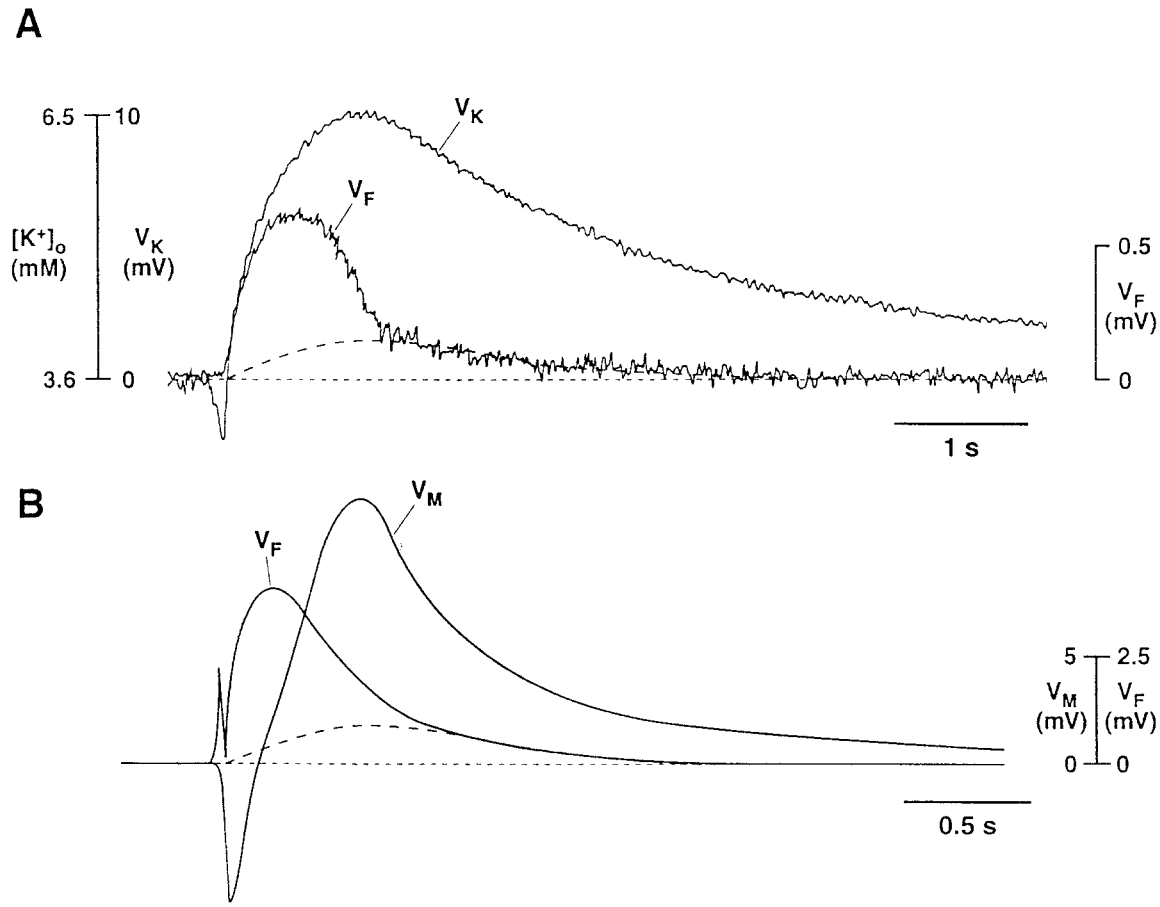

Fig. 8 **A** Comparison of time-course of extracellular long-lasting potentials evoked by 4-AP and recorded in stratum moleculare of the dentate. V_K (potassium signal) is superimposed on V_F (field), with correction for an estimated lag in response time of the K^+-sensitive electrode (V_K trace shifted to left by 100 ms). **B** Comparison of time-course of intracellularly recorded spontaneous long-lasting depolarization (LLD) of V_M and concomitant extracellular field (V_F) evoked by 4-AP in stratum granulosum of dentate. (Reproduced with permission from Perreault and Avroli (1992), with V_F and V_M redrawn and superimposed; note that the LLD is preceded by a hyperpolarizing inhibitory postsynaptic potential.) *Dashed curves* in **A** and **B** indicate the potential contribution of the late phase of the slow component of V_F; V_F and V_K scales have positive polarity upward

nents of the long-lasting field component as compared with the $[K^+]_o$ potential. The relatively slow response time of K^+-sensitive microelectrodes (≤0.2 s) (Dufau et al. 1982; Krnjevic and Morris 1981) may underestimate the onset and time-course of $\Delta[K^+]_o$. However, even taking this artifact into consideration, the difference observed suggests that the fast field potential may reflect a very early event – activity in interneurons or afferent terminals (cf. the acceleration of neuronal activity described by Ives and Jeffreys 1990) – which would generate a localized $[K^+]_o$ increase that could contribute to the initiation of the intracellular LLD and long-lasting field. The intracellularly recorded LLD (V_M) and $[K^+]_o$ profiles appear similar – with peaks later than that of the early phase of the slow field and longer durations (cf. Fig. 8A and B). The peak of the later low-amplitude component of the field (indicated by dashed curves) occurs at approximately the same time as that for potassium or V_M.

Role of GABA receptors

The increases in $[K^+]_o$ which are associated with the long-lasting fields evoked by 4-AP were readily blocked or markedly attenuated by the $GABA_A$ receptor antagonist BMI – an effect observed with or without CNQX and APV and similar to that reported for the juvenile hippocampus (Louvel et al. 1994). The antagonism of AMPA/kainate and NMDA receptors had no effect on the frequency of the slow potentials and either no or little influence on their amplitude. The persistence of the slow potentials in the absence of excitatory synaptic transmission and their sensitivity to BMI support the hypothesis that the $[K^+]_o$ change, like the intracellular long-lasting depolarization, is largely generated by post-synaptic activation of $GABA_A$ receptors – although there may be some contribution from the repetitive firing of the interneurons and the action potentials which are generated ectopically in the axons of pyramidal cells (Segal 1987; Michelson and Wong 1991; Perreault and Avoli 1992). In experiments where slow field and K^+ potentials were attenuated to approximately 20% amplitude by BMI, additional factors might contribute to their formation. Although other transmitters may be involved, the residual response may still be GABA-mediated since both

$GABA_B$ and $GABA_C$ receptors are insensitive to BMI (Barolet et al. 1985; Taylor 1988), and electrogenic uptake of GABA will also persist.

GABA release and accumulation in or near dendritic regions can, especially at high concentrations, activate extrasynaptic and synaptic $GABA_A$ receptors to evoke depolarizing responses of neurons – which are more sensitive to bicuculline than are the hyperpolarizing responses (Alger and Nicoll 1982; Perreault and Avoli 1989) – and glia (MacVicar et al. 1989), as well as a secondary $[K^+]_o$ increase due to transmitter uptake and/or outward co-transport of K^+ with Cl^- (Barolet and Morris 1991; Morris et al. 1983). GABA accumulation may therefore contribute both directly and indirectly to the LLD and slow field potentials.

In the present experiments GABA evoked slow field and K^+ potentials quite similar to those observed with 4-AP but also showing a resemblance to those evoked by KCl. In each case the K^+ change lagged behind that of the field potential and the ratios of durations and interpeak intervals were similar. However, the proportional relationship between field and K^+ for responses to GABA was similar to that for the synchronous 4-AP potentials; while their slopes were both significantly greater than that for KCl. These observations lead us to suggest that 4-AP-evoked long-lasting field and intracellular potentials may reflect the combined effects of GABA and K^+, with (i) an involvement of $[K^+]_o$ increase due to activity of GABAergic interneurons and presynaptic terminals in the initiation of very early changes, (ii) GABA making the major contribution to the production of the early large phase and peak of the slow component of the field and intracellular LLD, as a result of its direct depolarizing actions, and (iii) a potential influence of $[K^+]_o$ accumulation, secondary to $GABA_A$ receptor activation and/or uptake and delayed restorative mechanisms, in sustaining and prolonging the slow potentials.

Our results with applications of baclofen confirm the modulatory effect of $GABA_B$ receptor activation on the interictal and long-lasting field potentials (Siniscalchi and Avoli 1992) and further show that the associated $[K^+]_o$ increases are similarly affected. Differences between these observations and the selective block of interictal activity described by Watts and Jefferys (1993) may be due to differences in recording sites and the concentration of baclofen (100 μM compared with 2 μM). The fact that baclofen did not induce increases in resting $[K^+]_o$ in the presence of 4-AP but still interfered with the production of the slow potentials suggests that the major effect is not mediated by an increase in K^+ conductance and may be presynaptic – with activation of $GABA_B$ receptors and inhibition of GABA release from interneurons, as suggested by Siniscalchi and Avoli (1992).

Localization of K^+ accumulations and long-lasting potentials

$[K^+]_o$ accumulations were recorded in association with 4-AP-induced slow field potentials in all locations of the hippocampus, although with low or negligible amplitude in the alveus. These findings are in agreement with the previous localization of 4-AP-evoked synchronous long-lasting potentials (Perreault and Avoli 1989, 1991, 1992). The largest K^+ transients were mapped in regions where fibers of the perforant pathway terminate on granule cell dendrites in the stratum moleculare of the dentate. Large increases were also recorded in dendritic regions of the hilus and stratum radiatum. In CA1 and CA3 the changes in stratum pyramidale were lower in amplitude than those in the apical dendrites, while those in the granule cell layer of the dentate were smaller than those in the hilus or dendrites of the molecular layer. In contrast, in CA1 and CA3 the maximal changes during stimulus-evoked and ictal activity are in stratum pyramidale or at the border between stratum oriens and stratum pyramidale (Fisher et al. 1976; Benninger et al. 1980; Krnjevic et al. 1982a,b). Although GABA-evoked accumulations of $[K^+]_o$ have been reported as largest in stratum pyramidale (Segal and Gutnick 1980; Barolet et al. 1985), more recent and detailed analyses show significantly greater changes in stratum radiatum than in stratum pyramidale (Müller et al. 1989; Obrocea and Morris 1993) – an observation consistent with the differential distribution of 4-AP-induced $[K^+]_o$ changes.

The localization of larger accumulations of $[K^+]_o$ in dendritic regions than in the cell body layers might be due to a differential presence of GABAergic interneurons (e.g., in stratum radiatum, stratum lacunosum moleculare, and the hilus) (Amaral 1978; Buhl et al. 1994; Lacaille et al. 1987; Lux 1974; Miles and Wong 1987; Misgeld and Frotscher 1986) and glia cells. Factors which would promote larger $[K^+]_o$ change in such regions include the large surface-to-volume area of fine fibers, high firing frequency of inhibitory interneurons (Michelson and Wong 1991), and the presence/activation of depolarizing extrasynaptic $GABA_A$ receptors on dendrites (Alger and Nicoll 1982) and glia (MacVicar et al. 1989).

In summary, 4-AP evokes increases in $[K^+]_o$ in the adult rat hippocampus which are significantly correlated with synchronous long-lasting field potentials. They are maximally localized in multiple dendritic regions, present in the absence of excitatory synaptic transmission, sensitive to $GABA_A$ receptor antagonism, and under the modulatory influence of $GABA_B$ receptors. It is concluded that the long-lasting field potentials, which are associated with an intracellular depolarization and arise from the activity of GABAergic interneurons and activation of $GABA_A$ receptors, reflect mainly the direct depolarizing actions of GABA and to a much lesser extent the influence of the associated and secondary $[K^+]_o$ accumulation.

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References

Alger BE, Nicoll RA (1982) Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied in vitro. J Physiol (Lond) 328:125–141

Amaral DG (1978) A golgi study of cell types in the hilar region of the hippocampus in the rat. J Comp Neurol 182:851–914

Avoli M (1988) GABAergic mechanisms and epileptic discharges. In: Avoli M, Reader TA, Dykes RW, Gloor P (eds) Neurotransmitters and cortical function. Plenum Press, New York, pp 187–205

Avoli M (1992) Synaptic activation of $GABA_A$ receptors causes a depolarizing potential under physiological conditions in rat hippocampal pyramidal cells. Eur J Neurosci 4:16–26

Avoli M, Psarrapoulou C, Tancredi V, Fueta Y (1993) On the synchronous activity induced by 4-aminopyridine in the CA3 subfield of juvenile rat hippocampus. J Neurophysiol 70:1018–1029

Barolet AW, Morris ME (1991) Changes in extracellular K^+ evoked by GABA, THIP and baclofen in the guinea-pig hippocampal slice. Exp Brain Res 84:591–598

Barolet AW, Kish SJ, Morris ME (1985) Identification of extrasynaptic binding sites for [^{3}H]GABA in peripheral nerve. Brain Res 358:104–109

Barron DH, Matthews BHC (1938) The interpretation of potential changes in the spinal cord. J Physiol (Lond) 92:276–321

Benninger C, Kadis J, Prince DA (1980) Extracellular calcium and potassium changes in hippocampal slices. Brain Res 187: 165–182

Buhl EH, Halasy K, Somogyi P (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. Nature 368:823–828

Dufau E, Acker H, Sylvester D (1982) Triple-barrelled ion-sensitive microelectrode for simultaneous measurements of two extracellular ion activities. Med Progr Technol 9:33–40

Fisher RS, Pedley TA, Moody WJ, Prince DA (1976) The role of extracellular potassium in hippocampal epilepsy. Arch Neurol 33:76–83

Galvan M, Grafe P, ten Bruggencate G (1982) Convulsant actions of 4-aminopyridine on the guinea-pig olfactory slice. Brain Res 241:75–86

Green JD (1964) The hippocampus. Physiol Rev 44:561–608

Guilbault GG, Durst RA, Frant MS, Freiser H, Hansen EH, Light TS, Pungor E, Rechnitz G, Rice NM, Rohm TJ, Simon W, Thomas JDR (1976) Recommendations for nomenclature of ion-selective electrodes. Pure Appl Chem 48:127–145

Heinemann U, Lux HD, Gutnick MJ (1977) Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. Exp Brain Res 27:237–243

Inoue M, Oomura Y, Yakushiji T, Akaike N (1986) Intracellular calcium ions decrease the affinity of the GABA receptor. Nature 324:156–158

Ives AE, Jefferys JGR (1990) Synchronization of epileptiform bursts induced by 4-aminopyridine in the in vitro hippocampal slice preparation. Neurosci Lett: 112:239–245

Jensen MS, Yaari Y (1988) The relationship between interictal and ictal paroxysms in an in vitro model of focal hippocampal epilepsy. Ann Neurol 24:591–598

Kriz N, Sykova E, Ujec E, Vyklicky L (1974) Changes of extracellular potassium concentration induced by neuronal activity in the spinal cord of the cat. J Physiol (Lond) 238:1–15

Krnjevic K, Morris ME (1975) Correlation between extracellular focal potentials and K^+ potentials evoked by primary afferent activity. Can J Physiol Pharmacol 53:912–922

Krnjevic K, Morris ME (1981) Electrical and functional correlates of changes in transmembrane ionic gradients produced by neural activity in the central nervous system. In: Zeuthen T (ed) Application of ion-selective microelectrodes. Elsevier, Amsterdam, pp 195–216

Krnjevic K, Morris ME, Reiffenstein RJ (1982a) Stimulation-evoked changes in extracellular K^+ and Ca^{2+} in pyramidal layers of the rat's hippocampus. Can J Physiol Pharmacol 60: 1643–1657

Krnjevic K, Morris ME, Reiffenstein RJ, Ropert N (1982b) Depth distribution and mechanism of changes in extracellular K^+ and Ca^{2+} concentrations in the hippocampus. Can J Physiol Pharmacol 60:1658–1671

Lacaille J-C, Schwartzkroin PA (1988) Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. I. Intracellular response characteristics, synaptic responses, and morphology. J Neurosci 8:1400–1410

Lacaille J-C, Mueller AL, Kunkel DD, Schwartzkroin PA (1987) Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. J Neurosci 7:1979–1993

Louvel J, Avoli M, Kurcewicz I, Pumain R (1994) Extracellular free potassium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. Neurosci Lett 167: 97–100

Lux HD (1974) The kinetics of extracellular potassium: relation to epileptogenesis. Epilepsia 15:375–393

MacVicar BA, Tse FW, Crichton SA, Kettenman H (1989) GABA-activated Cl^- channels in astrocytes of hippocampal slices. J Neurosci 9:3577–3583

McCarren M, Alger BE (1985) Use-dependent depression of IPSPs in rat hippocampal pyramidal cells in vitro. J Neurophysiol 53:557–571

Michelson HB, Wong RKS (1991) Excitatory synaptic responses mediated by $GABA_A$ receptors in the hippocampus. Science 253:1420–1423

Miles R, Wong RKS (1987) Latent synaptic pathways revealed after tetanic stimulation in the hippocampus. Nature 329:724–726

Misgeld U, Frotscher M (1986) Postsynaptic-GABAergic inhibition of non-pyramidal neurons in the guinea-pig hippocampus. Neuroscience 19:193–206

Moody WJ, Futamachi KJ, Prince DA (1974) Extracellular potassium activity during epileptogenesis. Exp Neurol 42:248–263

Morris ME, DiCostanzo GA, Barolet A, Sheridan PJ (1983) Role of K^+ in GABA (gamma-aminobutyric acid)-evoked depolarization of peripheral nerve. Brain Res 278:127–135

Morris ME, Krnjevic K, MacDonald JF (1985) Changes in intracellular free Ca ion concentration evoked by electrical activity in cat spinal neurons in situ. Neuroscience 14:563–580

Morris ME, Leblond J, Agopyan N, Krnjevic K (1991) Temperature dependence of extracellular ionic changes evoked by anoxia in hippocampal slices. J Neurophysiol 65:157–167

Morris ME, Obrocea GV, Avoli M (1993a) Changes in [K^+] and synchronous GABA-mediated potentials revealed by 4-aminopyridine in rat hippocampus. Can J Physiol Pharmacol 71: Axiv–Axv

Morris ME, Obrocea GV, Avoli M (1993b) Role of K^+ in GABA (γ-aminobutyric acid)-mediated synchronous potentials evoked in rat hippocampus by 4-AP (4-aminopyridine). Soc Neurosci Abstr 19:1668

Müller W, Misgeld U, Lux HD (1989) γ-Aminobutyric acid-induced ion movements in the guinea pig hippocampal slice. Brain Res 484:184–191

Nicholson C, ten Bruggencate G, Senekowitsch R (1976) Large potassium signals and slow potentials evoked during aminopyridine or barium superfusion in cat cerebellum. Brain Res 113: 606–610

Obrocea GV, Morris ME (1993) Comparison of changes evoked by anoxia and GABA (γ-aminobutyric acid) in $[K^+]_o$ and $[Cl^-]_o$ in the guinea pig hippocampus. Can J Physiol Pharmacol 71: Axviii

Oehme M, Simon W (1976) Microelectrode for potassium ions based on a neutral carrier and comparison of its characteristics with a cation exchanger sensor. Anal Chim Acta 86:21–25

Perreault P, Avoli M (1989) Epileptiform bursts induced by 4-aminopyridine (4AP) in the rat hippocampus: possible mechanisms. Soc Neurosci Abstr 15:1213

Perreault P, Avoli M (1991) Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. J Neurophysiol 65:771–775

Perreault P, Avoli M (1992) 4-Aminopyridine induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. J Neurosci 12:104–115

Prince DA (1978) Neurophysiology of epilepsy. Annu Rev Neurosci 1:395–415

Ribak CE, Harris AB, Vaughn JE, Roberts E (1979) Inhibitory GABAergic nerve terminals decrease at sites of focal epilepsy. Science 205:211–214

Rutecki PA, Lebeda FJ, Johnston D (1987) 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. J Neurophysiol 57:1911–1924

Segal M (1987) Repetitive inhibitory postsynaptic potentials evoked by 4-aminopyridine in hippocampal neurons in vitro. Brain Res 414:117–130

Segal M, Gutnick MJ (1980) Effects of serotonin on extracellular potassium concentration in the rat hippocampal slice. Brain Res 195:389–401

Siniscalchi A, Avoli M (1992) Modulation by $GABA_B$ receptors of spontaneous synchronous activities induced by 4-aminopyridine in the rat hippocampus. Neurosci Lett 148:159–163

Somjen GG (1979) Extracellular potassium in the mammalian central nervous system. Annu Rev Physiol 41:159–177

Sypert GW, Ward AA (1974) Changes in extracellular potassium activity during neocortical propagated seizures. Exp Neurol 45:19–41

Szente M, Pongracz F (1981) Comparative study of aminopyridine-induced seizure activities in primary and mirror foci of cat's cortex. Electroencephalogr Clin Neurophysiol 52:353–367

Taylor CP (1988) How do seizures begin? Clues from hippocampal slices. Trends Neurosci 11:375–378

Thompson SM, Gähwiler B (1989a) Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in vitro. J Neurophysiol 61:501–511

Thompson SM, Gähwiler B (1989b) Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide and membrane potential on E_{Cl} in hippocampal CA3 neurons. J Neurophysiol 61:512–523

Thompson SM, Gähwiler B (1989c) Activity-dependent disinhibition. III. Desensitization and $GABA_B$ receptor-mediated presynaptic inhibition in the hippocampus. J Neurophysiol 61: 524–552

Thompson SM, Capogna M, Scanziani M (1993) Presynaptic inhibition in the hippocampus. Trends Neurosci 16:222–226

Voskuyl RA, Albus H (1985) Spontaneous epileptiform discharges in hippocampal slices induced by 4-aminopyridine. Brain Res 342:54–66

Watts AE, Jefferys JGR (1993) Effects of carbamezipine and baclofen on 4-aminopyridine-induced epileptiform activity in rat hippocampal slices. Br J Pharmacol 108:819–823