Conductance Changes Underlying a Late Synaptic Hyperpolarization in Hippocampal CA3 Neurons

JOHN J. HABLITZ AND ROBERT H. THALMANN

Section of Neurophysiology, Department of Neurology, Department of Cell Biology, and Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030

SUMMARY AND CONCLUSIONS

1. Single-electrode current- and voltage-clamp techniques were employed to study properties of the conductance underlying an orthodromically evoked late synaptic hyperpolarization or late inhibitory postsynaptic potential (IPSP) in CA3 pyramidal neurons in the rat hippocampal slice preparation.

2. Late IPSPs could occur without preceding excitatory postsynaptic potentials at the resting membrane potential and were graded according to the strength of the orthodromic stimulus. The membrane hyperpolarization associated with the late IPSP peaked within 140–200 ms after orthodromic stimulation of mossy fiber afferents. The late IPSP returned to base line with a half-decay time of ~200 ms.

3. As determined from constant-amplitude hyperpolarizing-current pulses, the membrane conductance increase during the late IPSP, and the time course of its decay, were similar whether measurements were made near the resting membrane potential or when the cell was hyperpolarized by ~35 mV.

4. When 1 mM cesium was added to the extracellular medium to reduce inward rectification, late IPSPs could be examined over a range of membrane potentials from -60 to -140 mV. For any given neuron, the late IPSP amplitude-membrane potential relationship was linear over the same range of membrane potentials for which the slope input resistance was constant. The late IPSP reversed symmetrically near -95 mV.

5. Intracellular injection of ethyleneglycol-bis-(β-aminoethyl ether)-N,N'-tetraacetic acid or extracellular application of forskolin, procedures known to reduce or block certain calcium-dependent potassium conductances in CA3 neurons, had no significant effect on the late IPSP.

6. Single-electrode voltage-clamp techniques were used to analyze the time course and voltage sensitivity of the current underlying the late IPSP. This current [the late inhibitory postsynaptic current (IPSC)] began as early as 25 ms after orthodromic stimulation and reached a peak 120–150 ms following stimulation.

7. The late IPSC decayed with a single exponential time course (τ = 185 ms).

8. A clear reversal of the late IPSC at approximately -99 mV was observed in a physiological concentration of extracellular potassium (3.5 mM).

9. Neither the slope conductance nor the time course of decay of the late IPSC was voltage dependent over the range of membrane potentials that were examined (-55 to -140 mV). The reversal potential of the response became less negative after perfusion of 6.5 mM potassium. This potassium elevation did not affect the time course or voltage dependence of the late IPSC.

10. These experiments illustrate that the conductance of the late IPSP is not activated by changes in membrane potential and thus support the hypothesis that the potassium conductance of the late IPSP is gated by a neurotransmitter.

INTRODUCTION

The hyperpolarization that follows orthodromic stimulation of hippocampal formation pyramidal and granule neurons in vitro has been shown to consist of at least two separate components that inhibit neuronal discharge. The first component, or early inhibitory postsynaptic potential (IPSP), corresponds to a γ-
aminobutyric acid (GABA$_A$)-mediated chloride conductance in that it is sensitive to GABA$_A$ antagonists and responds appropriately to changes in the transmembrane chloride gradient (40, 49). In contrast, the second component, or late IPSP, has a more negative reversal potential than the early IPSP, suggesting a different conductance mechanism (40, 48, 49). The late IPSP is also resistant to blockade by antagonists of GABA$_A$ action, e.g., picrotoxin or bicuculline (4, 6, 38, 48, 49, but also see Ref. 33). Similar appearing late IPSPs have been observed in the hippocampus in vivo (19) and in other telencephalic regions such as the olfactory cortex (14), piriform cortex (43), neocortex (12), and the septal area (47).

The available evidence indicates that in hippocampal formation neurons, the late IPSP is due to an increased conductance to potassium. The reversal potential of the late IPSP is negative to the presumed chloride equilibrium potential. In addition, it is sensitive to changes in the concentration of extracellular potassium (4, 49) but not of extracellular or intracellular chloride. However, experiments to characterize this potassium conductance further have yielded contradictory results. For example, evidence has been reported both for (40) and against (33, 38, 48) activation of the late IPSP by calcium, and although no channel blocking agents have been reported that specifically antagonize this response, some pharmacological effects upon the late IPSP suggest that it may have properties similar to those of various other voltage-dependent potassium currents. For example, it has been reported that the late IPSP is reduced by tetraethylammonium (4, 33), as is the delayed rectifier current (1), is altered by 4-aminopyridine (4), as is the early transient outward current or $A$-current (13, 45, 51), and can be blocked by extracellular cesium (4), as are certain anomalous rectifier currents (15, 26).

Characterization of the voltage sensitivity of the conductance of the late IPSP would eliminate some of these alternatives, but the available information is indirect and conflicting in its implications. An inability to demonstrate a clear reversal potential for the late IPSP in normal concentrations of extracellular potassium (4, 33, 40), and the decline in the slope of the relation between late IPSP amplitude and membrane potential at successively more negative membrane potentials (4, 33, 40) suggest that the underlying conductance might also decline at more negative membrane potentials. Such a decline would suggest a similarity with the conductance of the A-current (13, 22, 45, 51), the delayed rectifier current (1), the muscarine or M-current (1, 2), and potassium currents that are activated by calcium in a variety of neurons (3, 20, 21). In contrast, there are indications that the previously mentioned slope of the relation between the amplitude of the late IPSP and membrane potential does not decline as the membrane potential is made more negative but, instead, remains constant (48, 49).

Clearly, further information is needed about the membrane events that underlie the late IPSP. Thus the present experiments were designed to characterize the late IPSP in CA3 neurons. Both current- and voltage-clamp methods were used to define the amplitude, time course, and voltage sensitivity of the conductance that underlies the late IPSP. In addition, certain experiments specifically tested whether the late IPSP corresponds to certain calcium-activated potassium conductances.

CA3 neurons, orthodromically activated via stimulation of the mossy fibers, were chosen for these experiments because they are particularly suitable for the observation and control of both of the major synaptic potentials known to precede the late IPSP, namely, the classic fast excitatory postsynaptic potential (EPSP) and the early IPSP (11). Such potentials might, in principle, activate voltage-dependent conductances that could overlap or underlie the late IPSP. Although the early IPSP of many neurons is believed to originate primarily from perisomatic synapses that are readily influenced by current passed through a microelectrode lodged in the soma, this is not the case with EPSPs, which are often generated at more remote synapses. In CA3 neurons, however, the excitatory mossy fiber pathway synapses in a restricted zone of the apical dendrites that is both anatomically (9) and electrotonically (11, 28) near the soma. Thus these synapses produce EPSPs that can be accurately measured and voltage clamped, and can, in addition, provide favorable conditions under which the intrasomatic injection of the calcium buffer EGTA could affect any calcium-activated mechanism that might be regulated by the excitatory synapses.
Preliminary reports of some of these results have appeared previously (48, 50).

METHODS

Experiments were performed on hippocampal slices taken from adult rats weighing 125–200 g. Slices were prepared and maintained as described previously (25, 48). Briefly, the brain was removed after decapitation: it was cooled in ice-cold oxygenated saline, and the hippocampus was dissected free. Transverse slices, nominally 400–550 μm thick, were cut on a McIlwain tissue chopper and transferred to a chamber similar to that described by Haas et al. (23). Temperature was maintained at 33–35°C while the slices were perfused (0.5–1.5 ml/min) with an oxygenated (95% O₂–5% CO₂) artificial cerebrospinal fluid of the following composition (in mM): 124 NaCl, 1.25 NaH₂PO₄, 3.5 KCl, 2 CaCl₂, 2 MgSO₄, 26 NaHCO₃, and 10 glucose. The pH was 7.3–7.4. When alterations in potassium or divalent cation levels were made, the appropriate amount of each ion was simply added to the basic perfusate. In some experiments, picrotoxin (25–50 μM) was added to antagonize GABAₐ-mediated inhibition (25).

Intracellular recordings were obtained from neurons in the CA3 pyramidal cell layer with microelectrodes filled with 4 M potassium acetate. In experiments that employed a conventional bridge amplifier, it was found that adequate compensation for the electrode resistance could easily be achieved when passing currents < 0.5–0.7 nA. With currents exceeding this, a slow change in apparent electrode resistance was observed, which could be adequately compensated for by making all measurements after rebalancing the bridge circuit, when the resistance had stabilized. This method of compensation was not possible, however, in experiments in which measurements were taken during 2-s current pulses (Fig. 1). An electrode that was adequately balanced at the beginning of such a pulse could be several millivolts out of balance at pulse offset. This problem was evaluated and dealt with in two ways. First, whenever possible, conditions were arranged so that membrane potentials could be altered to the desired levels with currents of <0.5 nA. Second, electrode current-passing capabilities were tested after the electrode was withdrawn from the cell. Changes in potential due to current passage were measured, and when such changes exceeded 5 mV, the results from that cell were discarded. When smaller changes

---

**FIG. 1.** Appearance of the late IPSP (L) after stimulation at different membrane potentials in normal saline containing 3.5 mM K⁺. A: all records are from the same neuron. 1: at the resting membrane potential, the mossy fiber (mf) stimulus evoked a brief EPSP (E) with no action potential (see inset at faster sweep speed). The small EPSP was followed by the early IPSP and then the late IPSP. 2: after the beginning of the sweep, hyperpolarizing current was injected to set the membrane potential near the reversal potential of the early IPSP. Note that the onset of the late IPSP can be detected within ~70 ms following the stimulus (inset). 3: membrane potential set near the reversal potential of the late IPSP. 4: membrane potential set negative to the reversal potential of the late IPSP. B: different neuron. Occurrence of the late IPSP in the absence of a preceding EPSP. 1: a mossy fiber stimulus elicited no detectable EPSP at the resting membrane potential; yet, a robust late IPSP occurred. 2: same neuron as in 1. Stimuli at 4 successively greater intensities elicited 4 successively larger late IPSPs, each in the absence of a preceding EPSP.
were measured, both corrected and uncorrected results were plotted, thus defining boundaries for the contribution of this type of error. In some studies, a time-share, sample-and-hold circuit (29) was used for current and voltage clamping through a single microelectrode (3,000-Hz switching; 25% duty cycle for current injection). This system samples the membrane potential after the potential developed across the electrode during current passage has decayed, just prior to the next current pulse. Thus difficulties with bridge imbalance are avoided, and a more accurate determination of membrane potential can be made. To obtain optimal circuit performance during voltage-clamp experiments, relatively low-resistance electrodes (15–50 MΩ), carefully tested for current-passing ability and lack of rectification, were employed. During these experiments, current and voltage signals were monitored on an oscilloscope, written out on a chart recorder to provide a permanent record of membrane potential, and stored on magnetic tape for subsequent computer analysis. A second oscilloscope was used to monitor the unsampled voltage output of the headstage to ensure that the voltage transient decayed completely prior to the next voltage sample.

Orthodromic activation was accomplished via 50-μs pulses delivered to a bipolar stimulating electrode placed either in the hilus of the dentate, just superior to the CA3 pyramidal cell layer, or in the granule cell layer of the dentate gyrus. The electrode location was varied until the site yielding the largest late IPSP was determined. Stimulus strength was generally adjusted so that action potentials were not evoked at the resting membrane potential. The only exception was that during exposure to picrotoxin, an action potential was occasionally triggered in some cells at the resting membrane potential.

For analysis of voltage-clamp experiments, synaptic currents and membrane potentials were digitized (sampling at 300-μs intervals) and, in most cases, averaged (n = 4–10). To obtain an unbiased estimate of current amplitude, the amplitude of the late synaptic current was calculated as the difference between the mean of 25 points 10 ms prior to the stimulus and 25 points 100–150 ms after the stimulus. The exact time for the measurement of peak amplitude was determined by eye for each neuron and was then held constant.

RESULTS

Characteristics of orthodromically evoked hyperpolarizations in CA3 neurons

Figure 1 illustrates basic features of the late IPSP in CA3 pyramidal neurons. This figure serves to differentiate the late IPSP from the early IPSP and to document its occurrence with and without obvious preceding membrane depolarizations. As shown in Fig. 1A1, an orthodromic mossy fiber (mf) stimulus that is subthreshold for eliciting an action potential evokes two hyperpolarizing potentials. The early component (E), probably representing a chloride-dependent IPSP, peaks at 20–25 ms and is followed by a late slow hyperpolarization, or late IPSP (L). This late IPSP reaches a maximum around 140 ms (range, 120–200 ms) and returns slowly to base line within 1 s. When the membrane potential was shifted to the reversal potential for the early IPSP by passing steady hyperpolarizing current, the late IPSP could be clearly seen as a hyperpolarizing event beginning 60–80 ms after the mossy fiber stimulus (Fig. 1A2). This response peaked within 140–200 ms and had a half-decay time of ~200 ms. When the membrane potential was made more negative, it was possible to reverse the polarity of the late IPSP (Fig. 1A3 and A4). This reversal potential was always more negative than that of the early IPSP. In experiments in which the reversal potential for both responses could be determined with confidence (see METHODS), it was 73.8 ± 2.5 mV for the early IPSP and -91.2 ± 6.2 mV for the late IPSP (n = 6).

Robust late IPSPs occurred not only in the absence of action potentials, but also when no depolarizing events were detectable at the resting membrane potential (Fig. 1, B1 and B2). Successive increases in the strength of the mossy fiber stimulation produced graded increases in the amplitude of the early and late IPSPs without evoking a detectable EPSP (Fig. 1B2). It thus appears that the occurrence of the late IPSP is not dependent on a preceding membrane depolarization. The results of the voltage-clamp experiments described below also support this conclusion.

Conductance changes associated with the late IPSP

For an initial characterization of the conductance changes associated with the late IPSP, hyperpolarizing-current pulses (0.25–0.5 nA; 80–100 ms) were passed through the microelectrode to measure input resistance. By this method we estimated the conductance change near the peak of the late IPSP and monitored the time course of the decay of the conductance change. Measurements were made in each neuron at two different membrane potentials to establish whether the con-
conductance change or its decay was altered at more negative potentials. The conductance change associated with the late IPSP ($\Delta g_{\text{late IPSP}}$) was estimated according to the relation

$$\Delta g_{\text{late IPSP}} = g_{\text{late IPSP}} - g_{\text{rest}}$$

where $g_{\text{late IPSP}}$ is the input resistance at a specific time following the orthodromic stim-

ulus and $g_{\text{rest}}$ is the resting input resistance. Trains of current pulses such as those illustrated in Fig. 2, A and B were started at varying latencies following the orthodromic stimulus in an attempt to sample as many time points as possible during the late IPSP.

When elicited near the resting potential, the late IPSP was associated with a conductance change that could be measured as the deflection produced by a hyperpolarizing current pulse. The conductance increment was calculated from these deflections and curves were fitted by eye to the resulting data points. The decay half-time was calculated in terms of the conductance ($g$), input resistance ($r$), and membrane potential ($v$). The decay half-time was calculated as follows:

$$\text{Decay half-time} = \frac{\Delta g_{\text{late IPSP}}}{2g_{\text{late IPSP}}}$$

where $\Delta g_{\text{late IPSP}}$ is the conductance change during the late IPSP.

**FIG. 2.** Time course of the conductance increment during the late IPSP at 2 different membrane potentials. A: mossy fiber (mf) stimulus at the resting membrane potential (−64 mV). Two sweeps were superimposed, with and without a train of hyperpolarizing-current pulses (indicated by the lower trace). B: same as in A, but after the membrane potential had been shifted to −105 mV. C: graphic depiction of experiment in A. Current trains were started at different times following each stimulus. The conductance increment (in nS) was estimated from the deflections produced by these current pulses (see text), and curves were fitted by eye to the resulting data points. The decay half-time was calculated in terms of the conductance ($g$), input resistance ($r$), and membrane potential ($v$). D: same as in C, but with membrane potential at −105 mV. E: current-voltage relation for same neuron.
increase that averaged 15.3 nS at the peak of the response and had a half-decay time of 120 ms (Fig. 2C). When expressed in terms of resistance changes, the decay time corresponded closely with the decay of membrane potential. No significant difference in the conductance increase or decay time could be detected when neurons were hyperpolarized by an average of 34 mV) (Fig. 20). Note that at each of the two membrane potentials, the voltage deflections reflecting cellular conductance traversed a range of membrane potentials over which the slope conductance of the cell appeared constant (Fig. 2E). The results of similar experiments in six cells are summarized in Table 1.

**Effects of EGTA and forskolin**

The absence of a significant decrease in either the amplitude or time course of the conductance change associated with the late IPSP as the membrane potential was made more negative argues that the late IPSP does not result from a voltage-dependent potassium conductance that is activated by calcium (3) (also see additional current- and voltage-clamp experiments reported below that examined the voltage sensitivity of the conductance of the late IPSP). However, not all calcium-activated potassium conductances are voltage sensitive (34). Therefore, two additional experiments examined the late IPSP when the aforementioned voltage-independent potassium conductance was reduced or blocked.

The first experiment involved perfusing 25 μM forskolin, an agent that blocks the slow calcium-activated afterhyperpolarization (AHP) (36), presumably by its powerful stimulation of adenosine 3',5'-cyclic monophosphate (cAMP) synthesis under these conditions (16–18, 46). The second experiment involved intracellular injection of ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA). Although this procedure should reduce a calcium-activated conductance of any sort, the injection of EGTA in this manner has been specifically shown to block the slow calcium-activated AHP (5, 24, 44) that is generated by a voltage-independent potassium current in hippocampal neurons (34). In addition, this experiment was particularly appropriate for testing the hypothesis that an excitatory transmitter might initiate calcium entry via a voltage-independent calcium conductance (40), since the proximity of mossy fiber synapses to the soma of the CA3 neurons should be favorable for the diffusion of intrasomatically injected EGTA to the excitatory synaptic zone.

During the perfusion of forskolin, the slow AHP following a train of directly evoked action potentials was reduced or blocked (Fig. 3, A1 and A2), but a late IPSP of typical amplitude and conductance invariably remained (Fig. 3, A3 and A4). Table 2 summarizes similar experiments in six neurons, showing a lack of effect of forskolin on the late IPSP.

Impalement with EGTA-containing microelectrodes led to a characteristic sequence of events. As previously described in CA3 neurons (24), the slow AHP evoked by a train of directly elicited action potentials (Fig. 3A) rapidly declined, and, in a number of cases, was replaced by an afterdepolarization (Fig. 3B). Blockade of this AHP, usually complete by the time stable recording conditions were achieved (2–10 min), was taken as an indication that sufficient EGTA had been introduced into the cell to block a calcium-activated potassium conductance (24, 44). In contrast, neither the late IPSP nor the conductance increase associated with it decreased during this time (Fig. 3, B3 vs. B4; Table 2). In no case was the late IPSP abolished by EGTA injection; indeed, in a given neuron, the usual result

---

**TABLE 1. Conductance change during late IPSP**

<table>
<thead>
<tr>
<th>MP, mV</th>
<th>Δgs, nS</th>
<th>g</th>
<th>r</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>-66 ± 6</td>
<td>15.3 ± 6.0</td>
<td>130 ± 23</td>
<td>171 ± 37</td>
<td>195 ± 24</td>
</tr>
<tr>
<td>-100 ± 4</td>
<td>21.4 ± 7.0</td>
<td>123 ± 29</td>
<td>202 ± 40</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; summary of data from 6 neurons treated as described in legend for Fig. 2. MP, membrane potential; Δgs, conductance change; r, resistance; v, membrane potential. The late IPSP was elicited at 2 membrane potentials in each neuron: at the resting potential and near the reversal potential of the late IPSP.
FIG. 3. Failure of perfused forskolin or intracellular EGTA to block the late IPSP. A: perfused forskolin blocked an afterhyperpolarization (AHP) elicited by a depolarizing pulse (1.5 nA, 100 ms) but failed to block the late IPSP. 1
and 2: −55 mV indicates the membrane potential at which an AHP was elicited by depolarizing-current pulses. Inset
is at a faster sweep speed. Lower traces indicate current injection. Each AHP was elicited after the membrane potential
had been set 20 mV positive to the reversal potential of the early IPSP, as determined at the beginning of the experiment.
1, before perfusion of forskolin; 2, after forskolin had been perfused for 20 min. Note blockade of slow AHP. 3 and
4: −75 mV indicates the membrane potential at which the late IPSP was elicited. Each late IPSP was elicited after
the membrane potential had been set at the reversal potential of the early IPSP. Note that after forskolin had blocked
the AHP, the late IPSP was still present. B: intracellular EGTA blocked the AHP following a depolarizing pulse, but
did not block the late IPSP. 1 and 2: membrane potential was −54 mV. 1, response 1 min after impalement. 2, 13
min after EGTA was introduced into the neuron, the AHP was replaced by an afterdepolarization. 3 and 4: membrane
potential was −61 mV. 3, voltage traces with and without current pulses were superimposed. Note the reduced membrane
resistance during the late IPSP. 4, 13 min after impalement, the late IPSP (and its associated change in membrane
resistance) was still present. These records were made by a Gould chart recording of tape recorded signals played back
at one-quarter the original recording speed. The half-response time of the chart recorder, in terms of experimental
time, was ~1 ms; therefore, the action potentials were truncated.
LATE IPSP CONDUCTANCE IN CA3 NEURONS

### TABLE 2. Effects of intracellular EGTA on the late IPSP

<table>
<thead>
<tr>
<th></th>
<th>AHP Parameters</th>
<th>Late IPSP Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Input $R_s$, MΩ</td>
<td>$\Delta mV$</td>
</tr>
<tr>
<td><strong>A. Late IPSP parameters measured at reversal potential of early IPSP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21)</td>
<td>$E_{eIPSP} +20$</td>
<td>29.1 ± 9.4</td>
</tr>
<tr>
<td>Forskolin (5)</td>
<td>$E_{eIPSP} +20$</td>
<td>31.2 ± 3.6</td>
</tr>
<tr>
<td>EGTA (7)</td>
<td>$E_{eIPSP} +20$</td>
<td>37.0 ± 10.6</td>
</tr>
<tr>
<td><strong>B. All measurements at resting potential</strong></td>
<td>Same as for AHP</td>
<td>Same as for AHP</td>
</tr>
<tr>
<td>Control (13)</td>
<td>−61.3 ± 4.0</td>
<td>39.6 ± 13.0</td>
</tr>
<tr>
<td>EGTA (10)</td>
<td>60.1 ± 4.3</td>
<td>36.0 ± 11.4</td>
</tr>
<tr>
<td><strong>C. All measurements at resting potential</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGTA (15)</td>
<td>−57.0 ± 13.5</td>
<td>56.7 ± 27.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses are no. of cells. The control values given here were from different neurons that had not been injected with EGTA but had otherwise been treated similarly to the other neurons. A: late IPSP parameters were measured at the reversal potential of the early IPSP ($E_{eIPSP}$). Parameters of the afterhyperpolarization (AHP) were measured after the membrane potential had been set 20 mV positive to $E_{eIPSP}$, i.e., at approximately −55 mV. In the other 2 protocols (B and C), all measurements were made at the resting potential. From left to right: MP, membrane potential at which the AHP was measured; input $R_s$, input resistance of the neuron, measured at the same membrane potential at which the AHP was elicited; $\Delta mV$, peak amplitude of the slow afterhyperpolarization; MP, membrane potential at which late IPSP was elicited; input $R_s$, input resistance at the membrane potential at which the late IPSP was elicited; $\Delta mV$, late IPSP amplitude; $\Delta S$, conductance increment during peak of the late IPSP. Artificial CSF includes picrotoxin and cesium in C.

was that the late IPSP increased in amplitude. This increase was associated with an increase in input resistance as the impalement stabilized. The results of experiments performed with three similar protocols on 32 neurons are summarized in Table 2.

**Voltage dependence of the late IPSP**

The final series of current-clamp experiments was performed to examine the behavior of the late IPSP over a wider range of negative membrane potentials than had been possible in the previous experiments. We added 1 mM cesium to the extracellular medium to reduce certain inwardly rectifying potassium conductances (15, 26). In addition, two conductances that might overlap the late IPSP were pharmacologically blocked: 25–50 μM picrotoxin was added to block the GABA$_A$-mediated chloride conductance that probably overlaps the onset of the late IPSP (compare Fig. 4A5 with 1A1), and EGTA1A was introduced in the neuron, as previously described, to reduce potassium conductances activated by calcium (Fig. 4A6). Elevated concentrations of divalent cations (4 mM Ca$^{2+}$ and 4–6 mM Mg$^{2+}$) were perfused to suppress epileptiform burst discharges.

Under these conditions, it was possible to demonstrate a clear reversal potential for the late IPSP similar to that in normal saline [mean = −94.6 ± 3.4 (SD) mV; n = 6] (Fig. 4, A1–A5). Moreover, the relationship between late IPSP amplitude and membrane potential could be determined with confidence over the range of −60 to −140 mV, since relatively small currents were needed to polarize the cells to these values. Measurement errors associated with current passage were detectable only at the most negative membrane potentials and were easily estimated (see METHODS). In no case did the late IPSP amplitude show anomalous decreases as the membrane was made more negative (Fig. 4, B and C). The slope of the relation between late IPSP amplitude and membrane potential actually appeared to increase until the membrane potential approached a value between −60 (Fig. 4C) and −80 mV (Fig. 4B). It then remained constant over the remainder of the range of membrane potentials studied.

For any given neuron, the late IPSP amplitude-membrane potential relationship was linear over the same range of membrane potentials for which the slope input resistance was constant, a range that for different neurons
FIG. 4. Relationship of late IPSP (L) to membrane potential. Artificial CSF contained 1 mM cesium, 25 μM picrotoxin, and 4 mM each of Ca²⁺ and Mg²⁺. Recording pipette contained 240 mM EGTA in acetate. A: all records are from the same neuron. In 1–7, after the onset of each trace, the late IPSP was elicited after the membrane potential was set at the following values: 1, the resting membrane potential; 2, the reversal potential of the late IPSP; 3, negative to the reversal potential of the late IPSP; traces with and without mossy fiber stimulus were superimposed; 4, a more negative membrane potential than in 3; 5, approximately −55 mV; note absence of an early IPSP where it would normally peak, ~70 ms following the stimulus; 6, depolarizing-current pulse (1.5 nA, 0.4 s) elicited a train of action potentials but no AHP; 7, after the electrode had been withdrawn from the neuron, a current equivalent to that in trace 4 was passed. B: left, graphic depiction of experiment shown in A. Right, current-voltage relation of same neuron. The voltage deflection produced by each current pulse was measured at a time point immediately preceding the orthodromic stimulus. C: same protocol as in experiments in A and B, but different neuron. Open circles (denoted by arrows) are data points corrected for estimated error due to rectification of voltage by electrode (see text).
extended from approximately $-60$ (Fig. 4C) and $-80$ mV (Fig. 4B) to the most negative membrane potentials examined. Such a relationship is consistent with a constancy of the late IPSP conductance over the same range of membrane potentials and agrees with the invariance of the late IPSP conductance over a smaller range of membrane potentials mentioned above.

**Voltage-clamp analysis**

To ascertain the voltage dependence of the conductance of the late IPSP more directly, we proceeded with a voltage-clamp analysis of the associated current. This analysis also allowed better determination of the onset and time course of the membrane currents that underlie the late IPSP and provided additional

---

**FIG. 5.** Evoked synaptic currents recorded from a CA3 neuron bathed in normal saline containing 3.5 mM K⁺. **A**: examples of the membrane potential during the synaptic response. Note that the voltage is well controlled during the late IPSC (L). **B**: clamp currents recorded at the indicated membrane potential. Each voltage and current trace is an average of 5–7 records. **C**: plot of late synaptic current ($I_{nA}$) as a function of holding potential ($V_h$). In this figure and in Figs. 6 and 7, the dotted line represents the result of a least-squares linear regression analysis (see text for further results of this analysis).
support for the potassium dependence of the response in CA3 neurons.

Typical synaptic responses evoked by mossy fiber stimulation are shown in Fig. 5. The voltage traces are shown in Fig. 5A and the evoked currents in Fig. 5B. At a holding potential of $-55$ mV, there was an initial outward current, representing the GABA-mediated chloride-dependent IPSC or early IPSC (E), which decayed exponentially with a time constant, in this cell, of 14.6 ms. This decay was interrupted by the onset of a second outward current, or the late IPSC (L) that peaked $\sim 130$ ms after the stimulus. This current corresponds to the late IPSP seen in voltage recordings. When the holding potential was set at $-105$ mV, the initial current was inward, representing a reversed early IPSC, and the late IPSC was near its reversal point. Increasing the holding potential to $-127$ mV resulted in a reversal of the late IPSC and an increase in the amplitude of the early IPSC.

Note that although the late IPSP was well clamped, a portion of the early IPSP was unclamped at the most depolarized and hyperpolarized holding potentials. This error would lead to an overestimate of the duration (37) and an underestimate of the peak current of the early inhibitory postsynaptic current (IPSC) at these holding potentials. We therefore did not quantitate the behavior of the early IPSC with respect to membrane potential.

The current-voltage relation for the late IPSC, shown in Fig. 5C, reveals a constant slope conductance over the range examined. These measurements were well fitted by the dotted line, which represents the results of a least-squares linear regression analysis. The indicated reversal potential in this case was $-103$ mV, and the conductance increase calculated from the slope of the regression line was 12 nS ($r = 0.98$). Data from similar experiments are summarized in Table 3.

Synaptic currents were also examined when picrotoxin (50 $\mu$M) was perfused to reduce the possible overlap of the early and late IPSCs (Fig. 6). This blockade of the large conductance of the early IPSC also made possible a more adequate clamp of the synaptic potentials that preceded the late IPSC (Fig. 6A). Under these conditions, at $-60$ mV, mossy fiber stimulation resulted in an early inward current followed by a slow outward current, corresponding to the late IPSC (Fig. 6B). As the holding potential was made more negative, the early inward current, most likely representing an excitatory postsynaptic current (EPSC), increased in amplitude. The slow outward current declined in amplitude and reversed symmetrically near $-100$ mV (Fig. 6B). A plot of late current amplitude as a function of membrane potential (Fig. 6C) was well fitted by a straight line, indicating a slope conductance of 22.5 nS ($r = 0.99$).

Late IPSP in increased extracellular potassium

A series of experiments was performed in which extracellular potassium was raised to 6.5 mM. This allowed us to examine more closely the behavior of the late IPSC at membrane potentials negative to its reversal potential, as illustrated in Fig. 7. The pattern of synaptic currents observed in 6.5 mM potassium was similar to that recorded in 3.5 mM potassium, except that the late IPSC now reversed at $-81$ mV rather than at approximately $-99$ mV (see Table 3 for averaged reversal potentials in several such experiments). This de-

<table>
<thead>
<tr>
<th>Bath Medium</th>
<th>Reversal Potential, mV</th>
<th>Peak Conductance, nS</th>
<th>Decay Time, $\tau$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 K⁺</td>
<td>$-99.3 \pm 10.6$ (16)</td>
<td>$13.3 \pm 3.9$ (16)</td>
<td>$165.9 \pm 39$ (11)</td>
</tr>
<tr>
<td>6.5 K⁺</td>
<td>$-77.3 \pm 7.3$ (10)</td>
<td>$22.46 \pm 3.0$ (10)</td>
<td>$197.8 \pm 55$ (17)</td>
</tr>
<tr>
<td>3.5 K⁺ + PTX</td>
<td>$-100.5 \pm 6.0$ (3)</td>
<td>$17.8 \pm 3.8$ (3)</td>
<td>$189.7 \pm 48$ (16)</td>
</tr>
<tr>
<td>6.5 K⁺ + PTX</td>
<td>$-79.3 \pm 2.6$ (3)</td>
<td>$15.86 \pm 4.2$ (3)</td>
<td>$187.4 \pm 38$ (5)</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; nos. in parentheses are no. of cells. Concentrations of potassium (K⁺) given in millimoles; + PTX $= 50$ $\mu$M picrotoxin also present. Each of the 4 bath media were applied to different groups of neurons in which the orthodromic stimulus was adjusted so as not to elicit action potentials.
FIG. 6. Late IPSCs recorded in the presence of the antagonist of GABA_A action, picrotoxin. The concentration of Ca^{2+} and Mg^{2+} was raised to 4 mM, and low stimulus strengths were used to avoid triggering epileptiform burst discharges. A: membrane potentials during synaptic activation. B: synaptic currents recorded at the indicated membrane potentials. No early outward current was observed at -60 mV due to picrotoxin's antagonism of GABA_A-mediated inhibition. The fast inward current probably represents the mossy fiber (mf) excitatory postsynaptic current (EPSC). A prominent late IPSC is observed arising from the decay of the EPSC. Averages of 8 traces are shown. C: plot of late IPSC amplitude ($I_{\text{nA}}$) as a function of holding potential ($V_h$).

polarization of the reversal potential in elevated extracellular potassium is consistent with the evidence from other cell types that the late IPSP is dependent on a potassium flux.

The late IPSC was sometimes reduced after the holding potential had been maintained positive to -55 mV for prolonged periods. This could have been a result of ionic shifts and possibly other changes during such depolarization. To minimize these effects, we used relatively brief epochs of depolarization and discarded results that may have been affected
by such hysteresis (as determined by inter-
mittent measurements at -60 mV). In addi-
tion, we employed a protocol in which neurons
were held near their normal resting potential
for 7 s and stepped to a new holding potential
for only 1 s, as illustrated in Fig. 7A. Ortho-
dromic stimulation was then given 70–100 ms
after the onset of the step. To correct for ac-
tivation of voltage-dependent currents during
the step command, records during such a step
in the absence of synaptic stimulation were
digitally subtracted from those in its presence
(Fig. 7B). Figure 7C shows a plot of late IPSC
amplitude obtained in such a manner as a

![Graph showing synaptic currents in 6.5 mM K⁺.](image)

**Fig. 7.** Synaptic currents in 6.5 mM K⁺. **A:** membrane voltage responses. This neuron was held at -75 mV and
then stepped to the indicated potentials for 800 ms; orthodromic stimuli were applied 100 ms after onset of the step.
**B:** membrane currents recorded in response to the protocol described above. Each record in A and B is an average
of 5–10 traces. **C:** plot of late IPSC amplitude (I(nA)) vs. holding potential (V_H(mV)).
FIG. 8. Time course of the late IPSC. 

A: estimation of the time of onset of the late IPSC. Upper trace: example of a late IPSC recorded when the membrane potential ($V_H$) was held at the reversal potential for the early IPSC. The arrow beneath the trace indicates the onset of the late IPSC, which, in this neuron, occurred within 35 ms after the stimulus. Lower trace: averaged ($n = 5$) record obtained from a neuron bathed in 6.5 mM K+ and 50 µM picrotoxin. The membrane potential was held at $-120$ mV to increase the amplitude of the late IPSC. The onset of the late IPSC was within 28 ms of the mossy fiber stimulus. 

B: different neuron. Analysis of the time course of decay of the late IPSC. Averaged records were obtained at 2 membrane potentials. A single exponential function was fitted to the data for the period between the vertical lines. For clearer visibility, the same function is also displayed parallel to the curve that was fitted through the late IPSC. The decay time constants at $-55$ and $-95$ mV were 247 and 226 ms, respectively. 

C: same neuron as in B. Semilog plot of decay time constant as a function of membrane potential.
function of membrane potential. The late IPSC in this neuron had a reversal potential of \(-76\) mV and a slope conductance of 23 nS \((r = 0.99)\) (also see Table 3).

The two protocols for setting the holding potential gave essentially identical results. More importantly, the behavior of the late IPSC as a function of membrane potential was the same under the two procedures; i.e., the slope conductance of the late IPSC showed no tendency to decline with increasingly negative membrane potentials and was usually constant between approximately \(-60\) and \(-130\) mV. When apparent deviations from constancy occurred, they were in the direction of increases, not decreases, in slope conductance with more negative holding potentials.

Thus both voltage- and current-clamp methods estimated the average peak conductance increase during the late IPSP or late IPSC at between 13 and 23 nS (compare Tables 3 and 1), and, more importantly, neither method gave indication of a decline in this conductance as the membrane potential was made more negative.

**Onset and time course of the late current**

Although our current-clamp experiments suggested that the late IPSP began within 70–100 ms of the synaptic stimulation, the results of the voltage-clamp measurements showed that the underlying current began considerably earlier.

When the membrane potential was held at the reversal potential of the early IPSC, an outward current could be detected as early as 35 ms following synaptic stimulation (Fig. 84, upper trace). In 20 neurons, the average time of onset of the late IPSC was within 45 \pm 6 ms after stimulation (range, 35–60 ms). If the early IPSC was blocked by picrotoxin, the late IPSC was detectable even earlier. For example, the lower trace in Fig. 84 illustrates a recording made in 6.5 mM potassium and 50 \(\mu\)M picrotoxin at a holding potential of \(-120\) mV. The initial inward current represents an early EPSC that decays with a time constant of 3.9 ms. Within 28 ms following the stimulus, before the EPSC has decayed completely, the late IPSC is detectable. In three such experiments, the time of onset of the late IPSP occurred within 28 \pm 5 ms (range, 22–40 ms). Thus the membrane events that give rise to the late IPSP are evident soon after the time of the peak of the early IPSP and overlap with that potential.

To examine the time course of decay of the late IPSP, a single exponential curve was fitted to the decay phase of the response using the method of least squares. As shown in Fig. 8B, the decay phase was well described by a single exponential function. This was true whether the response was an outward current (Fig. 8B, upper trace) or was reversed to an inward current by holding at negative membrane potentials (Fig. 8B, lower trace). When the decay time constant was plotted as a function of membrane potential (Fig. 8C), no appreciable voltage dependence was observed. For this reason, all measurements of decay time in a given bathing medium were pooled and are summarized in Table 3. To compare the decay of the late IPSC with that of the conductance of the late IPSP that had been measured under current clamp (Table 1), half-decay times of the late IPSC were extrapolated from the mean time constants of decay given in Table 3. These values ranged from 114 (3.5 mM potassium) to 136 ms (6.5 mM potassium), and appeared to be in reasonable agreement with the half decay of the conductance as estimated from current-clamp data (see Table 1). Thus these voltage-clamp measurements support our current-clamp estimates of both the duration and voltage insensitivity of the conductance that underlie the late IPSP.

**DISCUSSION**

These experiments employed quantitative current- and voltage-clamp techniques to provide new information concerning the time course, conductance change, and voltage dependence of a late hyperpolarizing postsynaptic potential in hippocampal CA3 pyramidal neurons. The late IPSP in these neurons is similar to the late IPSP in CA1 pyramidal neurons (4, 38) and in dentate granule neurons (49) in that it is insensitive to GABA\(_A\) antagonists and is associated with an increased conductance to potassium (31–33). Our results further indicate that 1) the potential has an earlier onset than was previously thought; 2) the amplitude of the response is linearly related to membrane potential, reversing near the presumed potassium equilibrium potential; 3) the conductance increase associated with the
hyperpolarization is voltage independent over the range of potentials examined; and 4) the decay time of the conductance and current associated with the hyperpolarization is also voltage independent over the range of membrane potentials examined. Our data further indicate that the late IPSP is not due to a voltage- or calcium-dependent potassium conductance triggered by a preceding voltage transient. Instead, the late IPSP must represent a synaptic potential that results from the activation of transmitter-gated potassium channels.

Time course
Consistent with findings of earlier studies of synaptically evoked late hyperpolarizing potentials (4, 33, 39, 48, 49), we found that such responses in CA3 neurons began within 50–100 ms following orthodromic stimulation, considerably later than the onset of the early IPSP under similar conditions [see also the results of Andersen et al. (7) and Fujita (19)]. Using voltage-clamp techniques and experimental manipulations that minimize distortion from other synaptic conductances, we further showed that, although the late IPSC has its peak well after that of the early IPSC (11), it begins earlier than was previously evident, typically within 35 ms after the stimulus. Indeed, it can sometimes be observed within 25 ms. These measurements establish only conservative estimates for late IPSP onset, since low stimulus strengths were employed to avoid evoked action potentials and associated conductances and since even the reduced EPSPs that were elicited by these stimuli may have obscured the earliest portions of the late IPSC. Thus, as proposed by Alger (4), and by Newberry and Nicoll (38), the late IPSP occurs early enough to play a role in the curtailment or actual termination of the epileptiform discharges that occur when GABA_A inhibition is attenuated. More importantly, the late IPSP could play a significant role in normal neuronal integration. Previous studies have shown that action potentials are inhibited during the late IPSP, even when certain inhibitory potentials that could overlap the response are blocked or reversed in polarity (31, 32, 48). [See Satou et al. (43) for such a result in pyriform cortex in vivo.] The implication that the late IPSP accounts for the inhibition is further supported by our observation that the current underlying the response decays according to a single exponential function, even in the absence of pharmacological treatment. Thus, under the conditions of our experiments, no additional inhibitory currents need be postulated to account for the decay of the late IPSP or the observed decrease in neuronal excitability.

Voltage sensitivity
In previous studies of the late IPSP in hippocampal neurons, a clear reversal potential was not demonstrable when physiological levels of extracellular potassium were employed; furthermore, the slope of the relation between late IPSP amplitude and membrane potential declined with both depolarization and hyperpolarization relative to the resting membrane potential (4, 33, 40). Although these results are consistent with rectification of the conductance of the late IPSP, we believe that such nonlinearities are much more likely to be due to the shunting of the late IPSP by the activation, at different holding potentials, of steady-state conductances that are in parallel with the conductance of the late IPSP. The current-voltage relation of hippocampal neurons is not linear, and most cells show an increase in input conductance with either depolarization or hyperpolarization relative to the resting membrane potential (27). In our study of CA3 neurons, nonlinearities in the relation between late IPSP amplitude and membrane potential occurred only when there were accompanying changes in the steady-state slope conductance. When external cesium was applied to these cells to reduce the increases in input conductance with either depolarization or hyperpolarization relative to the resting membrane potential (15, 26), the cells could be polarized over a wide range of negative membrane potentials with little change in input conductance. In such cells it was possible to demonstrate a linear relation between late IPSP amplitude and membrane potential over the range of −60 to −140 mV, and it was also possible to show a clear reversal potential for the late IPSP.

Our findings are consistent with a constant conductance of the late IPSP over the range of membrane potentials studied. Measurements with both current- and voltage-clamp methods repeatedly showed that the conduc-
tance of the late IPSP was relatively constant over the same range and did not decline at more negative membrane potentials. In addition, the time course of the response showed no tendency to change as a function of membrane potential. This voltage insensitivity of the time course and conductance of the late IPSP, together with other findings in this study, has a direct bearing on a number of hypotheses concerning the identity of the underlying conductance.

Possible correspondence with other potassium conductances

One such hypothesis is that the late IPSP results from a voltage-dependent conductance activated by a preceding voltage transient, such as an EPSP or an early IPSP. At least on a qualitative basis, it might appear that several potassium conductances could be activated following synaptic stimulation. For example, the depolarization induced by the preceding EPSP might activate a delayed rectifier current (1), an A-current (22, 51), or a calcium-activated potassium current (3, 10). Such depolarizing triggers have been particularly difficult to rule out in previous studies of CA1 (4) or granule (50) neurons because the electrotonic distance between the recording site and the generation site of the EPSP has not been well defined. An alternative hypothesis is that the hyperpolarization caused by the early IPSP could activate a Q-current (26) or a fast inwardly rectifying potassium current (15), or might, by removing inactivation of an A-current, lead to its activation (12, 22, 45, 51).

In the light of our data, both of these hypotheses are unlikely. First, the relative voltage insensitivity of the time course and peak amplitude of the conductance underlying the late IPSP does not match the characteristics of most of the above-mentioned currents, particularly those whose conductance, time course, or both, show a sharp decline as the membrane potential is made more negative. Second, typical late IPSPs or IPSCs can occur in the absence of a preceding depolarization, as first reported in granule neurons (48), or when preceding depolarizations are reduced or prevented by voltage clamp. These observations are particularly significant in CA3 neurons because of the electrotonic proximity of the excitatory mossy fiber synapses to the intrasomatic recording electrode (11, 28). Characteristic late IPSPs also occurred when the possibility of a preceding hyperpolarization was minimal, such as when the early IPSP was blocked by picrotoxin, null ed by appropriate adjustment of the membrane potential, or reversed in polarity (at membrane potentials as negative as −140 mV). Perhaps more conclusively, typical late IPSPs also occurred when preceding hyperpolarizations were minimized under voltage-clamp conditions in the presence of picrotoxin.

A different hypothesis is that the conductance of the late IPSP is regulated by a neurotransmitter but, like the M-current, is also influenced by membrane potential (1). Thus the conductance could correspond to one of the major voltage-dependent conductances but could also require a neurotransmitter for its activation. Our finding that the conductance of the late IPSP does not decline at negative membrane potentials argues against the participation of the delayed rectifier current, the A-current, the M-current, several currents that are activated by calcium, or, indeed, any conductance that declines at more hyperpolarized levels. However, we cannot rule out an inwardly rectifying conductance with the same degree of confidence, because an apparent slight increase in the late IPSP conductance was sometimes observed at more negative membrane potentials. It has been reported (19a) that baclofen, a ligand for the GABAB receptor that may be responsible for the late IPSP, induces responses in cultured hippocampal neurons that show anomalous increases in slope conductance upon hyperpolarization. However, the inwardly rectifying conductances that have been described in temporal lobe neurons are blocked by external cesium (15, 26), and one of these, the Q-current (26), has a reversal potential at least 40 mV positive to that of the late IPSP. In our experiments, typical late IPSPs were elicited in the presence of 1 mM external cesium. Thus the conductance of the late IPSP does not closely correspond to either of these inwardly rectifying currents.

Is the late IPSP activated by calcium?

Another hypothesis is that the late IPSP is caused by a calcium influx that is initiated either by a depolarizing potential (the EPSP) or
by direct transmitter gating of a calcium channel (40, 49). However, the voltage sensitivity of the late IPSP clearly distinguishes it from the voltage sensitivity of calcium-activated potassium currents whose conductance or time course declines with more hyperpolarized membrane potentials (1, 10). This sensitivity, together with the occurrence of the late IPSP in the absence of a depolarizing event, would appear to eliminate the possibility that the late IPSP could depend on a voltage-activated calcium conductance that is simply triggered by a preceding EPSP, and would also make less likely the possibility that the late IPSP might arise from the transmitter regulation of such a voltage-sensitive channel.

Alternatively, transmitter binding might regulate a calcium-activated potassium conductance that lacks this voltage sensitivity (40); indeed, such a relatively voltage-insensitive potassium conductance underlies the slow AHP following a train of directly evoked action potentials (34). However, we have shown that both EGTA injections and forskolin applications block such slow AHPs in CA3 neurons without affecting the appearance of the late IPSP. Similar results have recently been reported in CA1 neurons (4, 38). We therefore believe that the previously mentioned calcium-activated potassium conductances are probably not responsible for the late IPSP.

It is possible that the conductance of the late IPSP is a calcium-regulated potassium conductance of a type other than previously described. If a recently reported reduction of the late IPSP by diacylglycerol agonists (8) depends on protein kinase c (41), then, by analogy with other systems, calcium, possibly at quite low concentrations (30, 42), may play a role in the regulation of the late IPSP.

Activation by a neurotransmitter

In conclusion, these experiments have shown that the conductance of the late IPSP is unlikely to be activated by a preceding membrane potential and therefore support the hypothesis that the late IPSP must instead be activated by a neurotransmitter (e.g., 38, 48), at least over the range of membrane potentials that we could examine. This view easily accommodates the major characteristics of the response that we have observed, such as its increased conductance, well-defined reversal potential, decay according to a single exponential function, and, most importantly, its relative lack of voltage sensitivity over the range of membrane potentials examined. Although our experiments did not address the identity of the transmitter involved, they may nevertheless aid in its discovery, since the transmitter should produce a response that mimics the late IPSP with respect to these properties. In this regard, Newberry and Nicoll (39) found that iontophoretic application of the GABA$_B$ ligand baclofen mimics the late IPSP in producing an increased conductance to potassium. It would be of interest to determine whether the response of these neurons to baclofen mimics the additional properties of the late IPSP that have been described here since, in cultured hippocampal neurons, this response was recently reported to display some inward rectification over the same range of membrane potentials that were examined in the present experiments (19a).

ACKNOWLEDGMENTS

The authors thank Drs. John A. Connor, Philip E. Hockberger, and Frank J. Lebeda for their comments on this manuscript. We also thank Dr. Daniel Johnston for providing the single-electrode voltage clamp used in several of the experiments.

This work was supported by National Institute of Neurological and Communicative Disorders and Stroke Grants NS-21713 (R. H. Thalmann), NS-18145, and NS-22373.

Requests for reprints should be addressed to: Robert H. Thalmann, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Received 29 September 1986; accepted in final form 25 February 1987.

REFERENCES


37. MAGLEY, K. L. AND STEVENS, C. F. The effect of...


