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Physiology of GABA Inhibition in the Hippocampus

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Application of GABA to hippocampal pyramidal cells causes three types of responses. Low doses applied at the soma cause a chloride-and bicuculline-sensitive hyperpolarization mediated by $GABA_A$ receptors. Low doses applied in the dendrites cause primarily a bicuculline-sensitive depolarization which may also be mediated by chloride-dependent $GABA_A$ receptors. Higher doses of GABA elicit a bicuculline-resistant hyperpolarization which is due to the opening of K+ channels by $GABA_B$ receptors. The coupling of $GABA_B$ receptors to K+ channels requires a GTP binding protein which may directly activate the K+ channel. Stimulation of afferent fibers in the hippocampus elicits a fast and slow IPSP. The fast IPSP is due to the activation of $GABA_A$ receptors and it is proposed that the slow IPSP is due to the activation of $GABA_B$ receptors. Under conditions of enhanced release of GABA or enhanced responsiveness of $GABA_A$ receptors with barbiturates a very slow (100's of msecs to secs) bicuculline-sensitive depolarizing IPSP can be recorded. The different properties of these putative GABAergic IPSPs make it likely that they subserve distinct functions in the hippocampus.

The role of GABA in synaptic transmission in the hippocampus is well established and initial analysis revealed that the basic properties are remarkably similar to those found in other brain regions. The most prominent action of GABA in the CNS involves an increase in membrane conductance to chloride ions. This action is blocked by a number of quite selective antagonists, such as bicuculline. However, it has become clear that GABA may have actions which do not involve chloride ions and which are resistant to the action of known GABA antagonists. The first clear evidence that GABA might act on more than one receptor was provided by Bowery (1982) who described a presynaptic inhibitory action of GABA which was resistant to GABA antagonists. The bicuculline-sensitive receptors were referred to as $GABA_A$ receptors, whereas the bicuculline-insensitive receptors were referred to as $GABA_B$ receptors. In the present paper the multiple actions of GABA on hippocampal pyramidal cells will be reviewed and these actions will then be related to the inhibitory synaptic potentials that are recorded from these cells.

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Finally, the fact that other transmitters besides GABA can cause inhibition in the hippocampus, in some cases by actually using the same ionic channels, will be discussed.

Actions of GABA

The type of response that one obtains by local application of GABA onto pyramidal cells depends critically on the location of the pipette, as well as on the amount of GABA ejected (Alger and Nicoll, 1982b; Andersen, Dingledine, Gjerstad, Langmoen, and Mosfeldt Laursen, 1980; Blaxter and Cottrell, 1985; Inoue, Matsuo, and Ogata, 1985b; Newberry and Nicoll, 1985). When the pipette is positioned very close to the somata of a pyramidal cell and a very small amount of GABA is iontophoretically ejected, a pure hyperpolarization is recorded associated with a large increase in membrane conductance (see Figure 1A2, S). The reversal potential for this action is approximately –70mV. This hyperpolarizing action is entirely blocked by GABA antagonists, such as

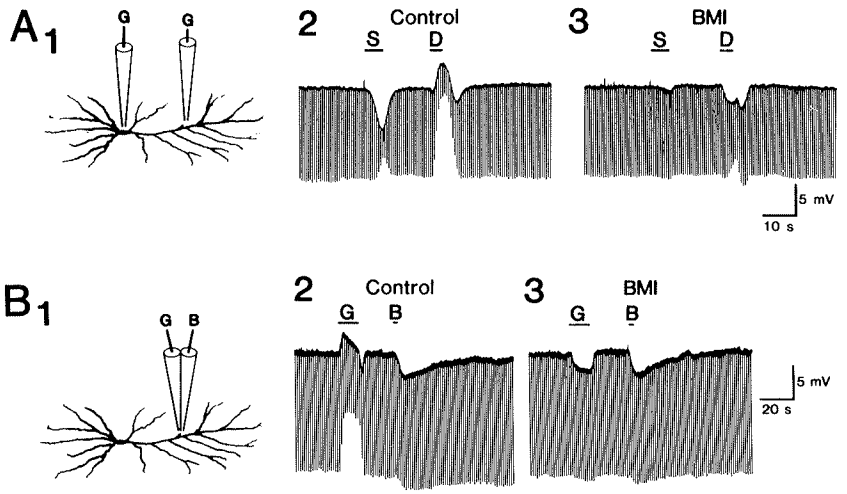

Figure 1: *Actions of GABA on hippocampal pyramidal cells.* A1, diagram of placement of ionophoretic electrodes. A2 and 3, somatic (S) and dendritic (D) GABA applications were repeated after superfusion with bicuculline methiodide (BMI) (100 μM) for 20 min. B1, diagram of placement of double-barreled ionophoretic electrode. B2 and B3, responses to GABA (G) and baclofen (B) before (B2) and 25 min. after superfusing BMI (100 μM) (B3). Resting potentials were –55(A) and –62 mV(B). From Newberry and Nicoll, 1985. Reprinted with permission of The Physiological Society, © 1985, Oxford, England.

bicuculline (Figure 1A3), and picrotoxin and results from an increase in chloride conductance, since shifting the chloride gradient across the membrane shifts the reversal potential for this response. The response can be

mimicked by such $GABA_A$ agonists as muscimol and THIP. If one increases the amount of GABA ejected at the soma—or positions the pipette into the dendritic field—a depolarizing component to the GABA response appears. When applied in the dendritic region the depolarizing response predominates and is associated with a large increase in membrane conductance (see Figure 1A2, D). The evidence that this response may also involve an increase in chloride conductance is based on the following observations. First, the response is blocked by $GABA_A$ antagonists and $GABA_A$ receptors are usually coupled to a chloride conductance mechanism. However, $GABA_A$ agonists such as muscimol and THIP appear to be weaker at eliciting this response than GABA, raising the possibility that some differences in $GABA_A$ receptors might exist (Alger and Nicoll, 1982b; Newberry and Nicoll, 1985). Second, the reversal potential for the response shifts when the concentration of chloride in the extracellular medium is reduced (Blaxter and Cottrell, 1985; Inoue et al., 1985b; Newberry and Nicoll, 1985). Third, furosemide which blocks chloride transport in other systems, blocks both the dendritic depolarizing responses as well as the somatic hyperpolarizing responses, but does not alter the underlying increase in membrance conductance (Misgeld, Deisz, Dodt, and Lux, 1986).

When higher amounts of GABA are ejected in the dendritic region, the depolarization is often followed by a hyperpolarization (see Figure 1). This hyperpolarization is resistant to GABA antagonists and can be studied in relative isolation after blockade of the depolarizing component (Figure 1A3 and 1B3) [Blaxter and Cottrell, 1985; Inoue et al., 1985b; Newberry and Nicoll, 1985]. It is associated with an increase in membrane conductance, and has a reversal potential of about –90mV in a superfusing medium containing 5.4 mM K+ (Figure 2A). Increasing the extracellular concentration of K+ causes a depolarizing shift in the reversal potential (Figure 2B), while shifting the chloride gradient has little effect on the hyperpolarization. Thus, it can be concluded that GABA can open potassium channels by a bicuculline-resistant action. This action can be closely mimicked by the selective $GABA_B$ agonist, baclofen (Figure 1B2 and B3) [Gahwiler and Brown, 1985; Newberry and Nicoll, 1984b, 1985]. As would be expected, baclofen responses are unaffected by $GABA_A$ receptor antagonists (Figure 1B3). It has been reported that the response to baclofen is antagonized by 4-aminopyridine while the response to GABA is unaffected (Inoue et al., 1985a; Ogata, Inoue, and Matsuo, 1987). We, however, have not observed this sensitivity of the baclofen response to low doses of 4-aminopyridine (Nicoll, unpublished observation).

Inhibitory Postsynaptic Potentials

Antidromic stimulation of the alveus, at least at moderate stimulus intensities, results in a fast IPSP that peaks at approximately 50 ms and lasts for 200 to 300 ms (Figure 3A, Anti). This IPSP is blocked by GABA antagonists and

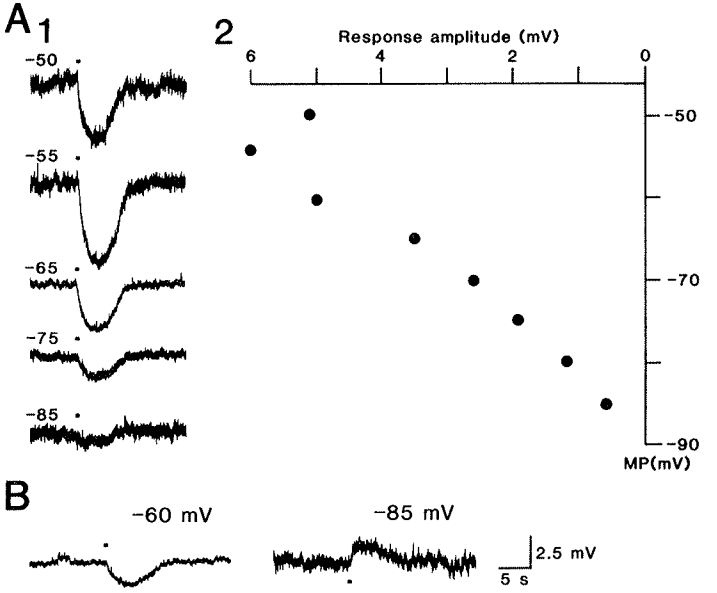

Figure 2: *Effect of altering membrane potential on the bicuculline-resistant GABA response.* A shows responses to the pressure ejection (20 lbf/in^2 for 200 ms) of GABA onto the dendrites in the presence of bicuculline methiodide (100 μM). A2 is a graph of the response partially illustrated in A. In B the extracellular potassium was doubled to 10.8 mM. All responses are from the same cell. The calibration in B also applies to A. Resting potential =−65mV. From Newberry and Nicoll, 1985. Reprinted with permission of The Physiological Society, © 1985, Oxford, England.

has a reversal potential of approximately −70mV (Alger and Nicoll, 1982a; Dingledine and Gjerstad, 1980), and is proposed to be generated by a recurrent pathway (Alger and Nicoll, 1982a). Stimulation of afferent fibers in stratum radiatum at low intensities results in an IPSP similar in every respect to that evoked by antidromic stimulation. However, stronger stimulation evokes a slower and later component which peaks at about 200 ms and lasts about 1 second (Alger, 1984; Newberry and Nicoll, 1984a, 1985; Nicoll and Alger, 1981; Thalmann, 1984) (Figure 3A ortho and B). This response is referred to variously as the slow or late IPSP or the late hyperpolarizing potential (LHP). This potential is resistant to GABA antagonists and has a reversal potential of approximately −90 mV. These features, which are identical to the $GABA_B$ receptor mediated action of GABA, suggest that GABA may be the transmitter responsible for the slow IPSP. A selective $GABA_B$ antagonist, which currently does not exist, would be of great value in testing such an hypothesis. However, an alternative approach is to examine the effects of selective blockers of GABA uptake on the duration of the slow IPSP. In this

regard, it has been found (Dingledine and Korn, 1985) that the GABA uptake blocker cis-4-hydroxynipicotic acid does, indeed, prolong the slow IPSP.

Under normal conditions synaptic activation does not evoke any depolarizing GABA mediated events. There are two conditions however which have been found that reveal depolarizing GABA mediated synaptic potentials. If one greatly enhances and prolongs $GABA_A$ responses with pentobarbitol a long lasting (many seconds) depolarizing potential can be recorded following stimulation of excitatory afferents (Alger and Nicoll, 1982a). This potential is localized to the dendrites and can be blocked by GABA antagonists. Since the potential is evoked by orthodromic stimulation of afferent fibers, but not by antidromic stimulation of pyramidal cell axons, it appears to be generated by a feedforward class of interneurons. It has recently been found that if one enhances the release of GABA with 4-aminopyridine (4-AP) a similar GABA mediated depolarizing IPSP can be recorded (Avoli and Perreault, 1987). Why are depolarizing IPSPs not observed under normal conditions? Two possible explanations are as follows. First, synaptically released GABA might not have access to the GABA receptors responsible for the depolarization. This explanation could explain the 4-AP experiments. However, the results with pentobarbital, which acts by enhancing $GABA_A$ responses, suggests that the receptors responsible for the depolarization are normally activated by synaptically released GABA. The second, and more plausible explanation, is that the receptors responsible for the depolarizing

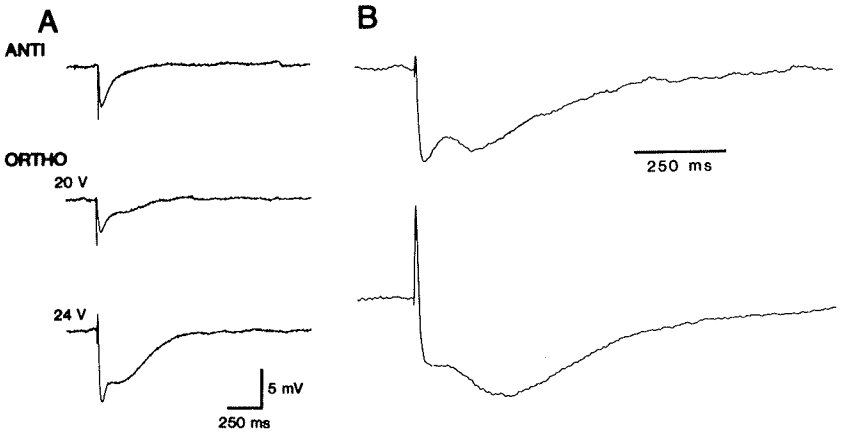

Figure 3: *The synaptically evoked potentials in the CA1 pyramidal cell.* A, comparison of the potential evoked by antidromic (anti) and orthodromic (ortho) stimulation in S. radiatum. The slow IPSP was prominent only after orthodromic stimulation. Resting membrane potential =−56 mV. From Newberry and Nicoll, 1984a. Reprinted with permission of The Physiological Society, © 1984, Oxford, England. B, other examples of the two component IPSP recorded from two pyramidal cells.

responses are normally activated by synaptic release of GABA on the dendrites, but that the very powerful overlapping somatic hyperpolarization masks the dendritic response. Only when the action and/or release of GABA is greatly prolonged do the dendritic events become manifest.

Coupling of GABA Receptors to Ion Channel

It is clear that the $GABA_A$ receptor and the chloride channel are very tightly coupled and are likely to be part of the same macromolecular complex. Electrophysiological studies show that GABA opens chloride channels in a membrane patch only when it is present in the patch pipette. In addition, channel opening occurs extremely rapidly in less than a millisecond. Biochemical studies show that the GABA binding site and a binding site likely to be associated with the chloride channel copurify. On the other hand, the coupling of $GABA_B$ receptors to K+ channels appears to be indirect. First, the delay and slow time course for the $GABA_B$ response suggests that there is a delay between the binding of GABA to the receptor and the opening of the channel. Second, the K+ channels opened by $GABA_B$ receptors are also opened by other receptors such as 5-HT (Andrade, Malenka, and Nicoll, 1986) and adenosine receptors (Andrade and Nicoll, unpublished observations). This sharing of K+ channels can be demonstrated by the nonadditivity of the maximal outward currents elicited by these agonists. What might indirectly couple $GABA_B$ (as well as other receptors) to these K+ channels? We have considered and ruled out established diffusable second messenger candidates such as cAMP, and phosphatidyl inositol breakdown (Andrade et al., 1986). Although increases in intracellular calcium have been proposed to play a role in mediating baclofen responses (Blaxter and Carlen, 1985), our evidence, with blockers of calcium activated K+ conductances and intracellular injection of EGTA, strongly suggests that the baclofen response is not due to a calcium activated potassium conductance (Andrade et al., 1986). However, a variety of experimental evidence indicates that the response does require a GTP binding protein. Thus, the response is blocked in hippocampi that have been treated previously with pertussis toxin (see Figure 4). In addition, the hydrolysis resistant GDP analog, GDPβS antagonizes the action of baclofen, while the GTP analog, GTPγS mimics the action. These findings, therefore, suggest that a mechanism similar to that reported for muscarinic inhibition of the heart (Breitwieser and Szabo, 1985; Pfaffinger, Martin, Hunter, Nathanson, and Hille, 1985), also exists for $GABA_B$ responses in the hippocampus. Specifically, it is suggested that the $GABA_B$ receptor is coupled to the K+ channel via a pertussis toxin-sensitive GTP binding protein that is able to directly open the K+ channel. Such a mechanism would also provide a means by which different neurotransmitter receptors could converge onto the same ion channel.

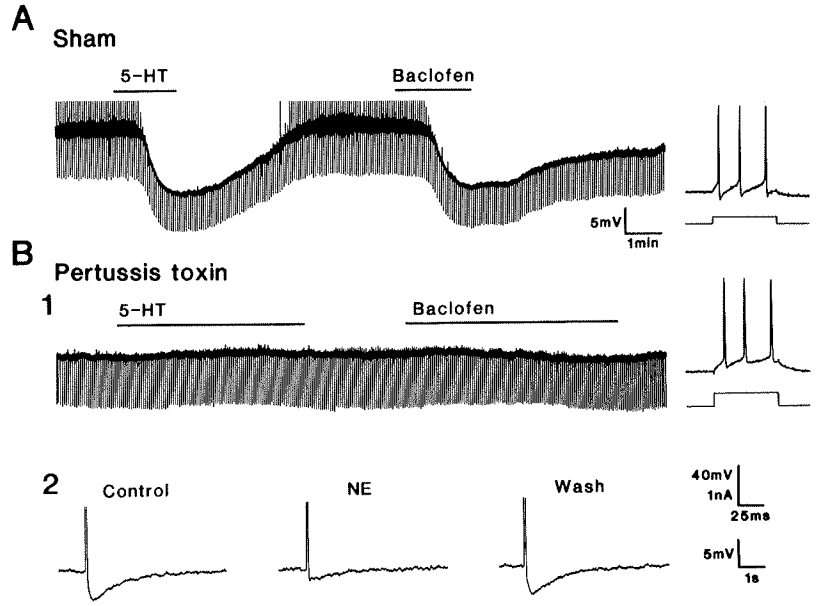

Figure 4: *Pertussis toxin blocks the action of 5-HT and baclofen.* A, Pyramidal cell responses to bath applied 5-HT (30 μM) and baclofen (30 μM) are shown in a cell from a hippocampal slice taken from an animal sham injected with bovine serum albumin (1.5 μg) (Sham). Downward deflections are hyperpolarizing responses to constant current pulses. The resting membrane potential was –60 mV. B_1, 5-HT and baclofen responses from a slice taken from an animal injected with pertussis toxin (1.5 μg). The action potentials recorded from the two cells are shown to the right. B_2, shows that the same cell, which did not respond to 5-HT or baclofen, responds normally to 5 μM norepinephrine (NE) with a decrease in the size of the AHP. The resting membrane potential was –60mV. Calibrations below A apply to the 5-HT and baclofen responses in A and B_1. Calibrations for the AHP's are to the right of B_2. From Andrade, Malenka, and Nicoll, 1986. Reprinted with the permission of *Science*, *234*, 1261–1265, 1986, ©AAAS.

Conclusion

In conclusion, we propose that there are two types of GABA mediated inhibition in the hippocampus. Occupation of $GABA_A$ receptors opens Cl– channels which are part of a single macromolecular complex and results in the fast IPSP. This fast IPSP is hyperpolarizing and generated largely at the soma by a class of interneurons activated by recurrent collaterals of pyramidal cells. $GABA_A$ receptors are also proposed to be activated by a feedforward pathway ending on the dendrites, which, in the presence of barbiturates, appear as a slow depolarization. Under normal conditions this depolarizing input is masked by the more powerful somatic input. Pyramidal cell membranes also possess

$GABA_B$ receptors which are indirectly coupled to K+ channels via a GTP binding protein. This receptor may mediate the slow IPSP. It is unclear whether the $GABA_A$ and $GABA_B$ receptors are present together in the same subsynaptic membrane or whether they are present at different synapses. However, the fact that $GABA_B$ responses are more readily evoked in the dendrites whereas $GABA_A$ responses are more readily evoked at the soma, raises the possibility that there may be some segregation of receptor types. The coupling of two IPSPs to two different ion channels using different mechanisms suggests that they may serve distinct functions in both the normal and abnormal operation of the hippocampus.

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