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INTRACELLULAR OBSERVATIONS ON THE DISINHIBITORY ACTION OF ACETYLCHOLINE IN THE HIPPOCAMPUS

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Abstract—Intracellular recording (with KCl microelectrodes) from CA1 and CA3 hippocampal neurons in rats under urethane anaesthesia has revealed two kinds of facilitatory actions of acetylcholine (applied microiontophoretically). One was a mild depolarization (mean +12 mV) accompanied by a rise in input resistance (mean + 14%). The reversal potential for this effect was much more negative than the resting potential, and it differed from the reversal potential of the inhibitory synaptic potential by a mean of 65 mV. It was therefore concluded that one action of acetylcholine tends to reduce K conductance, as in neocortical neurons. The second effect is a reduction in potency of inhibitory synaptic potentials—evoked by fimbrial or entorhinal stimulation—made evident by a 62% average reduction in the conductance increase recorded near the peak of inhibitory potentials.

Since acetylcholine did not depress the inhibitory potency of iontophoretic applications of γ-aminobutyrate, it was concluded that acetylcholine must reduce the release of γ-aminobutyrate either by a direct action on inhibitory terminals or by inhibition of inhibitory interneurons. The former appears more likely.

EXTRACELLULAR studies in the hippocampus (KRNJEVIĆ, REIFFENSTEIN & ROPERT, 1980; 1981) have shown that acetylcholine (ACh) strongly facilitates the initiation of population spikes by fimbrial stimulation, apparently because ACh reduces the powerful inhibitory input that normally prevents or limits pyramidal cell discharges. Most of this extracellular evidence, however, was of an indirect nature, so it became important to know what happens to IPSPs and the accompanying conductance changes when ACh is applied to hippocampal neurons. Since GABA is very probably the main inhibitory agent released by inhibitory pathways in the hippocampus (STORM-MATHISEN, 1977; ANDERSEN, DINGLEDINE, GJERSTAD, LANGMOEN & LAURSEN, 1980; BEN-ARI, KRNJEVIĆ, REIFFENSTEIN & REINHARDT, 1981*b*), clearly it was also of interest to find out whether the action of GABA on hippocampal neurons is significantly altered by ACh. The present article describes observations that bear on these points.

EXPERIMENTAL PROCEDURES

All the experiments were performed on Sprague-Dawley rats weighing 250–300 g, anaesthetized with urethane (2.5 g/kg). Fine glass microelectrodes (containing 3 M KCl, 1 M K citrate, or a mixture of 1 M K citrate and 1 M KCL (4:1) with a 3–4 barrelled iontophoretic electrode attached, were inserted into areas CA1 and CA3 of the dorsal hippocampus. Responses of hippocampal neurons were evoked by stimulating either the fimbria or the entorhinal cortex with stereotaxically-placed metal electrodes. Intracellular records were obtained most often from the superficial or deep pyramidal layer, readily identified by the characteristic field potentials evoked especially by fimbrial stimulation (as well as by marking record sites with local release of Pontamine Sky Blue in some experiments). We therefore presume that our intraneuronal records were obtained mainly (if not exclusively) from pyramidal cells. The techniques used in these experiments for the preparation of microelectrodes, and the recording of membrane potential and resistance have been recently described in other papers (KRNJEVIĆ, LAMOUR, MACDONALD & NISTRI, 1979; BEN-ARI, KRNJEVIĆ, REIFFENSTEIN & REINHARDT, 1981).

RESULTS

Intracellular recordings from 20 cells were considered to yield particularly meaningful data because clear IPSPs (and large conductance changes) could be evoked reproducibly by fimbrial or entorhinal stimulation, iontophoresis of GABA produced unambiguous conductance increases (proving that the tip of the extracellular iontophoretic micropipette was sufficiently close), and ACh could be released for 30–60 s by iontophoretic currents of at least 100 nA. In the majority of cases, resting potentials were well below the likely 'normal' value; but, as indicated in Tables 1 and 2, the pooled results from all 20 neurons (22 applications of ACh) do not differ in any major respect from the changes observed in three neurons that had

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Abbreviations: ACh, acetylcholine; E_{ACh}, reversal potential for effect of ACh; E_{ipsp}, reversal potential for IPSP; E_{Cl}, Cl^- equilibrium potential; E_K, K^+ equilibrium potential; GABA, γ-aminobutyric acid; G_{Cl}, Cl^- conductance; G_K, K^+ conductance; IPSP, inhibitory post-synaptic potential; R_{in}, input resistance; V_m, membrane potential.

TABLE 1. EFFECTS OF ACETYLCHOLINE ON RESTING MEMBRANE POTENTIAL (V_m) AND INPUT RESISTANCE (R_{in}) OF HIPPOCAMPAL PYRAMIDAL NEURONS. ACh WAS APPLIED FOR ABOUT 1 MIN BY IONTOPHORETIC CURRENTS OF 80–160 nA

Cell	Control		Maximum change during ACh applications			E_{ACh}
	V_m	R_{in}	ΔV_m	ΔR_{in}	ΔR_{in}	(mV)
	(mV)	(mΩ)	(mV)	(MΩ)	(%)	
1.	−78	21.6	+5.7	+4.3	+20	−100
	−83	25.3	+6.7	+1.4	+6	−117
2.	−65	7.0	+32	+0.7	+10	− 95
3.	−74	6.5	+9	+0.3	+5	—
Means of 22 observations (±S.E.)	−37.7 (±4.5)	6.1 (±1.3)	+12.2 (±2.2)	+0.87 (±0.25)	+13.6 (±3.4)	−89.9 (±6.19) $n = 10$

resting potentials of at least −65 mV (they are listed separately in the Tables).

Effects produced by acetylcholine

If one examines the oscilloscope traces of Fig. 1, two kinds of action of ACh become apparent.

The upper traces (A–C) were obtained from a cell having a poor resting potential (initially only −32 mV) and an input resistance of 4.4 MΩ, which are typical of many cells encountered in such experiments (Table 1, cf. also BEN-ARI *et al.*, 1981 and ECCLES, NICOLL, OSHIMA & RUBIA, 1977). Nevertheless, as reported elsewhere (BEN-ARI *et al.*, 1981), large IPSPs and conductance changes are often recorded under these conditions. In the present case, the resistance fell to 1.6 MΩ when measured 12 ms after the positive peak of the IPSP. During the application of ACh (160 nA), there was a gradual depolarization, by a maximum of 12 mV, and the resting resistance rose by 14%, to 5.0 MΩ. At the same time the IPSP diminished somewhat in amplitude and the resistance minimum during the IPSP increased by 63%, to 2.6 MΩ. About two min after the end of the release of ACh, there was partial recovery of the resting potential (to −25 mV), but the resistance, both at rest and during the IPSP rebounded, to 3.7 and 1.0 MΩ respectively.

The lower traces in Fig. 1 (D–F) illustrate recordings from a cell with an initial resting potential of −78 mV (D), and an unusually high resistance (at least 22 MΩ). Near the peak of the large IPSP, the resistance was greatly reduced (to a minimum of only 3.8 MΩ). In E, recorded after 40 s of ACh release (100 nA) there was a depolarization by 6 mV and a rise in resting resistance to at least 25 MΩ; the cell now discharged repetitively in response to the entorhinal stimulus. The minimum of resistance near the peak of the IPSP rose to 6.3 MΩ and the two other sets of current pulses also show a reduced conductance increase during the later phase of the IPSP. All these changes were reversible, as indicated by the traces in F, recorded 95 s later.

Comparable data from two other cells are shown graphically in Fig. 2. The first cell (A) received two applications of ACh, which caused a sufficient depolarization to reverse the IPSP (initially made positive by Cl^- leakage from the microelectrode). Both cells show, in addition to depolarization, some increase in input resistance, both at rest and during the IPSP (open and filled triangles respectively).

The changes in resting potential and resistance, on the one hand, and the changes in IPSPs probably have a different significance and therefore will be described in more detail separately.

Acetylcholine effect on 'resting' neuronal membrane

In 21 tests out of 22, ACh caused some depolarization of the presumed pyramidal cells. The relatively slow onset and long duration of this effect—typical of the muscarinic excitatory actions of ACh on central

TABLE 2. EFFECTS OF ACETYLCHOLINE ON PEAK INHIBITORY SYNAPTIC POTENTIAL AMPLITUDE (ΔV_{IPSP}), RESISTANCE CHANGE (ΔR_{IPSP}), OR CONDUCTANCE CHANGE (ΔG_{IPSP}), AS WELL AS ON PEAK RESISTANCE CHANGE ELICITED BY IONTOPHORETIC APPLICATIONS OF γ-AMINOBUTYRIC ACID. IPSPs WERE EVOKED IN HIPPOCAMPAL PYRAMIDAL CELLS BY FIMBRIAL OR ENTORHINAL STIMULATION

Cell	Control values				Maximum changes during ACh release			
	ΔV_{IPSP}	ΔR_{IPSP}	E_{IPSP}	ΔR GABA	$\Delta(\Delta V_{IPSP})$	$\Delta(\Delta R_{IPSP})$	$\Delta(\Delta G_{IPSP})$	$\Delta(\Delta R_{GABA})$
	(mV)	(MΩ)	(mV)	(MΩ)	(mV)	(%)	(%)	(%)
1.	+28	−17.5	−38	—	−5	+10	−44	—
	+32	−21.8	−47	−14.2	−7	−16	−66	+9.8
2.	+30	−5.5	−11	−3.5	−12	−27	−34	+45
3.	+26	−1.25	+30	−0.75	−11	−80	−90	+15.0
Means of 22 observations (±S.E.)	+12.0 (±2.22)	−4.03 (±1.12)	−20.8 (±4.2)	−4.28 (±1.82)	−4.8 (±1.08)	−41.2 (±7.2)	−62.6 (±4.3)	+64.6 (±20.9) $n = 7$

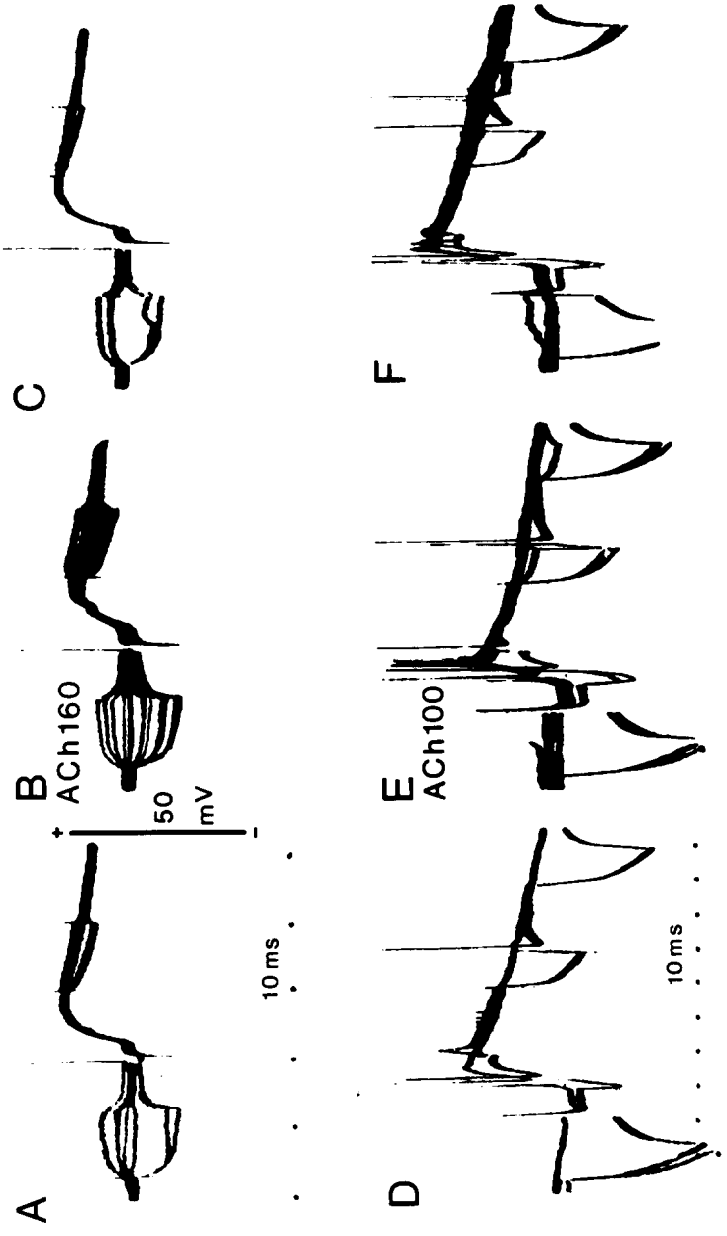

FIG. 1. Acetylcholine reduces the conductance increase observed normally during inhibitory synaptic potential. Intracellular recording with KCl electrodes from two hippocampal neurons. Regular series of 10 current pulses were injected into cell at rest (at left, before stimulus artifact), and also during IPSP, evoked by entorhinal stimulation. In A–C, currents were incremented in equal steps in the range −3.0 to +2.0 nA. Initial resting potential was −32 mV and it rose to −28 mV during application of ACh (160 nA) in (B); trace C shows recovery from effects of ACh. Traces D–F were obtained from a neuron with a much better resting potential initially (−79 mV); it rose to −73 mV after 40 s of ACh application (E), and showed a rebound to −83 mV subsequently (F). Four sets of current pulses were injected into this cell, one during 'rest' and three at various points during IPSP. Currents injected were incremented regularly in range −2.0 to 0 nA. In all records, superimposed traces include responses evoked by maximum inward and outward injected currents (but not all 10 current pulses).

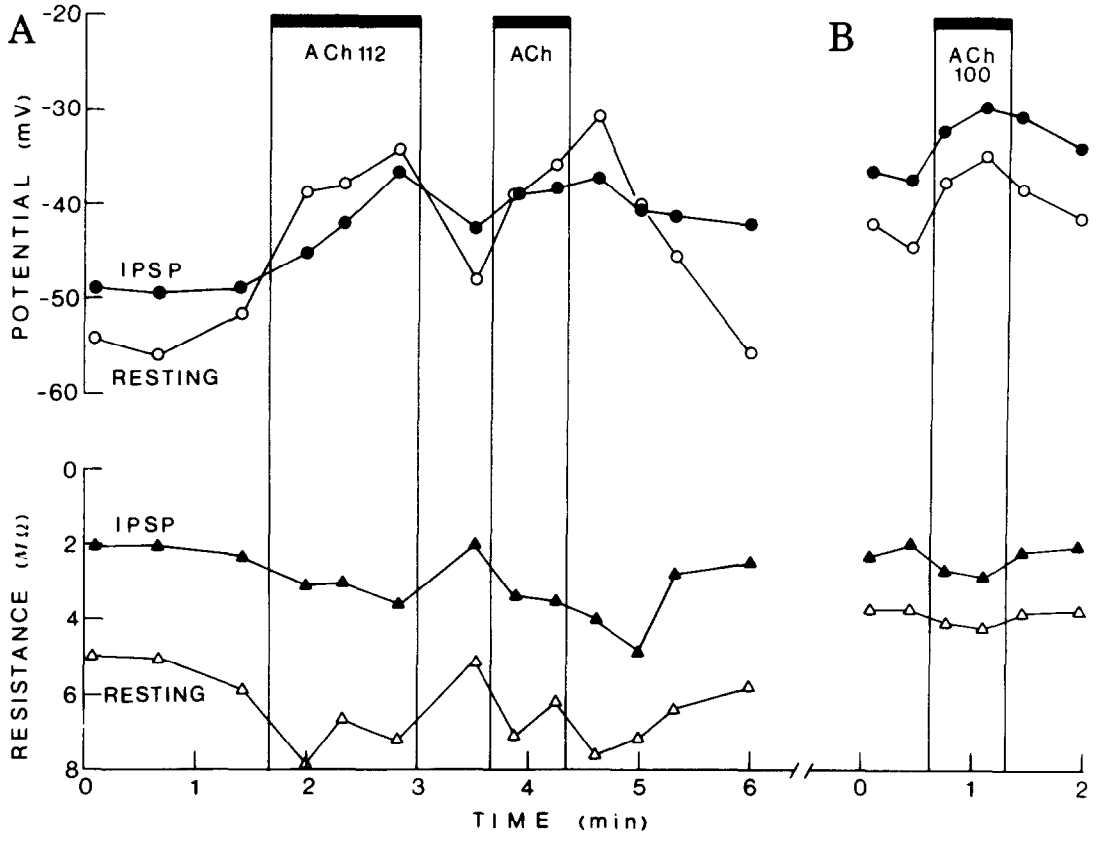

FIG. 2. Further examples of depolarizing action of acetylcholine on two separate neurons (A and B). In A, note reversal of positive IPSP, and in both A and B, increase in resistance at rest and at peak of IPSP.

neurons (KRNJEVIĆ, PUMAIN & RENAUD, 1971; KRNJEVIĆ, 1975)—are evident in the traces of Fig. 4A and B. As set out in Table 1, the mean of all 22 observations indicated a change of +12.2 mV (S.E. 2.2), accompanied by an equally significant 13.6% (S.E. 3.4) increase in input resistance (Figs 1–3). These overall means differ little from the results obtained from the three best cells in the same series, also given in Table 1 (cells 1–3). It is curious that the mean depolarization is practically identical with that observed in neocortical cells (KRNJEVIĆ *et al.*, 1971) as well as in hippocampal slices (DODD, DINGLEDINE & KELLY, 1981); but the mean increase in resistance is less than half what was reported in the previous studies.

From the points of intersection of the current-voltage lines that were used to calculate input resistance, one can obtain some idea of the reversal potential for the action of ACh (Fig. 3). When resistance changes are relatively small, and intersections can be found only by extrapolation, the accuracy of this estimate is liable to be quite low, except when the recording is exceptionally free of noise. For this reason, meaningful estimates of E_{ACh} could not be obtained in all experiments. Nevertheless, as would be expected from a depolarizing action associated with a rise in resistance, points of intersection were consistently more negative than the resting potential, the mean of 10 estimates being −89.9 mV. Figure 3 illustrates the method of obtaining E_{ACh}, as well as the consistent finding with KCl-containing microelectrodes that for the same neurons, the IPSP reversal potential (E_{ipsp}) was much more positive (Table 2). Indeed, on the average E_{ACh} and E_{ipsp} differed by 64.5 mV (S.E. 5.5, $n = 10$).

These results therefore indicate that the depolarizing action of ACh on hippocampal neurons already reported by KELLY, DODD & DINGLEDINE, 1979; DODD *et al.*, 1981 and very recently by BENARDO & PRINCE, 1981) is most probably mediated by a reduction in K outward current, as in neocortical (KRNJEVIĆ, PUMAIN & RENAUD, 1970, 1971) and sympathetic neurons (WEIGHT & VOTAVA, 1970; BROWN & ADAMS, 1980) and not by a reduction in Cl^- conductance. The evidence that Cl^- ions are probably not involved in this effect is particularly significant because, as already mentioned at the beginning of the Results, ACh also seems to interfere with the Cl^- mediated IPSPs.

Acetylcholine effects on synaptic inhibition

The best quantitative index of the strength of synaptic inhibition is the amount of increase in input conductance at the peak of the IPSP. Unfortunately, the conductance changes can only be calculated, from the observed resistance changes; unlike the latter, they are critically dependent on the accuracy of

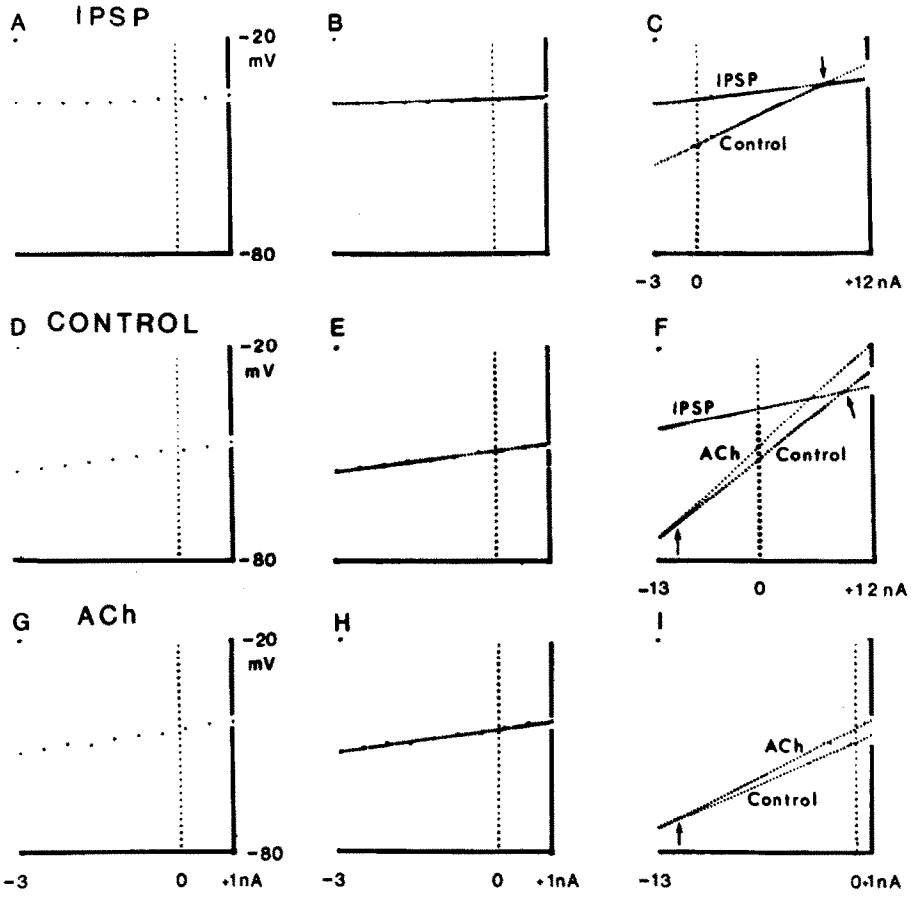

FIG. 3. Current voltage points and lines of best-fit obtained by technique illustrated in Figure 1. D, E control points and corresponding line indicate resting potential of −50 mV and resistance of 1.6 MΩ; G, H points and line of best fit recorded from same cell during ACh application (80 nA); the depolarization (by 5.3 mV, upward shift) and increased resistance (by 0.5 MΩ, steeper slope) are more evident when control and 'ACh' lines are superimposed, as in I, note relatively negative point of intersection of extrapolated lines, giving a reversal potential of −69 mV (arrow). By contrast points A, B (and corresponding line) obtained during IPSP show very low resistance (0.4 MΩ) and a very positive reversal potential (−33 mV, cf. arrow in C). Large difference between reversal potentials for IPSP and ACh (arrows) is particularly obvious in F, where the control IPSP and 'ACh' lines are superimposed. Note current (horizontal) scale is same for A, B, D, E, G and H.

bridge balance when the resistance is estimated in the ordinary way (the possible variations in conductance become enormous as the resistance approaches zero). In view of this unavoidable problem, in all cases variations in IPSP efficiency are expressed both in terms of observed resistance changes and the calculated conductance changes.

By these criteria, ACh consistently lowered the efficiency of IPSPs. In 20 cases out of 22, the fall in resistance associated with the IPSP was clearly reduced during applications of ACh. As shown in Table 2, the mean overall change was a reduction by −41.2% (S.E. ± 7.2). When the results were calculated in terms of conductance, in every instance the IPSP conductance increase was diminished by ACh, the mean reduction being by −62.6% (S.E. ± 4.3). A notable feature of this effect was its quick onset and a rapid recovery of the IPSP after the end of the application of ACh (Fig. 5).

Site of disinhibitory action of acetylcholine: interaction with γ-aminobutyrate

An obvious possible explanation for the reduced effectiveness of inhibition is that ACh lowers the inhibitory potency of GABA, the probable physiological transmitter of inhibition in the hippocampus (STRAUGHAN, 1975; STORM-MATHISEN, 1977; ANDERSEN *et al.*, 1980; BEN-ARI *et al.*, 1981). This could happen if ACh either reduced the affinity of GABA receptors or interfered with Cl^- fluxes.

In most of the experiments described here, GABA was applied to the same neurons, in order to test the utility of the iontophoretic electrodes as well as the responsiveness of the observed neurons. It was clear from such tests that applications of ACh did not produce any gross diminution in the inhibitory action of GABA. More systematic tests were performed on six neurons by applying the same iontophoretic dose of

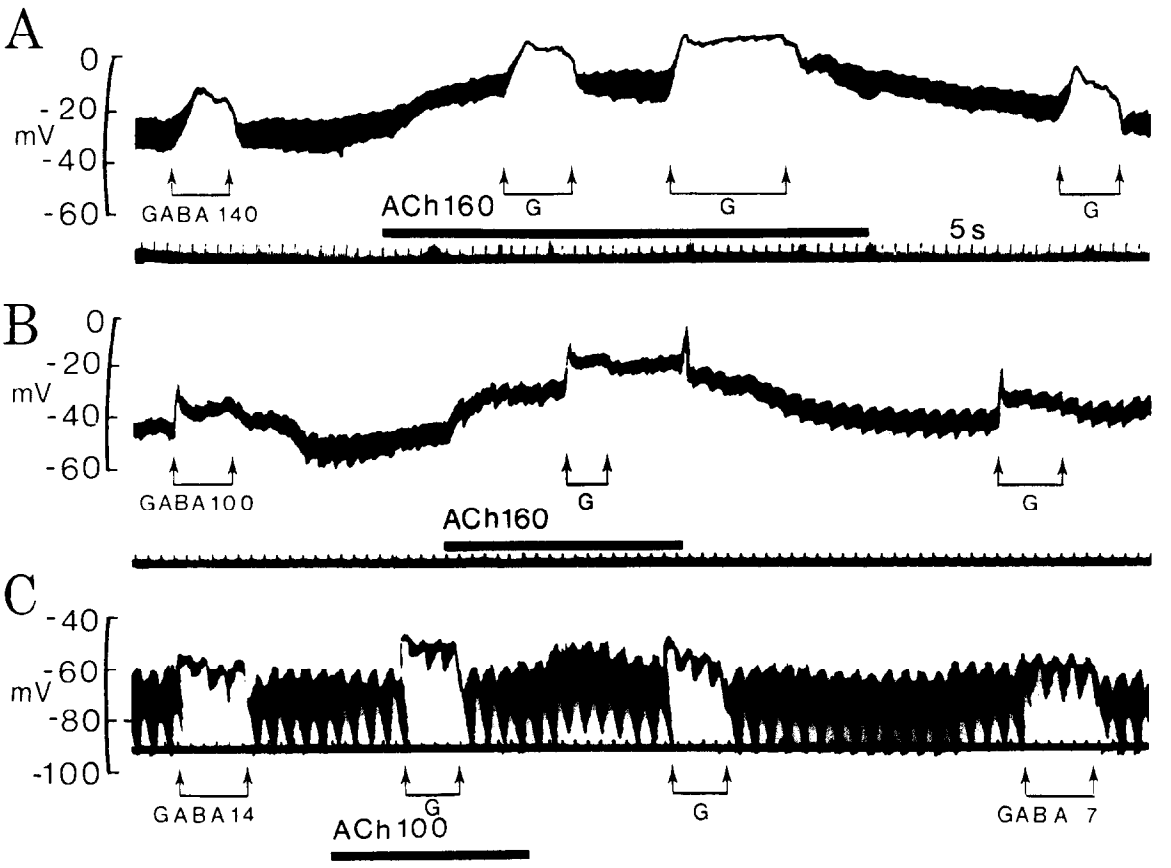

FIG. 4. Polygraph traces show that acetylcholine does not depress action of γ-aminobutyrate (G). A, B, C are from separate cells. Thick trace in A and B is caused by superimposed IPSPs, as well as resistance testing pulses. Only latter were applied in C.

GABA before, as well as during and after a prolonged application of ACh.

Examples of the responses evoked by GABA from three different cells are given in Fig. 4. These polygraph traces record the potential changes evoked by applications of GABA and ACh: in addition, superimposed on the base-line are positive IPSPs, and, especially in the lowest trace (C), incrementing series of hyperpolarizing pulses whose amplitude reflects in input resistance. It is evident from these traces that the depolarizing and membrane short-circuiting actions of GABA were not diminished in the presence of ACh. Indeed, as in C, GABA seemed to evoke an even greater depolarization in the presence of ACh.

A more quantitative description of such an experiment is provided by Fig. 5. The main graph (larger symbols) illustrates the typical, prolonged depolarization evoked by ACh, associated with a small reduction in resting conductance (down by only about 10%, cf. open triangles) and a much greater proportional reduction in IPSP conductance (full triangles). The superimposed small symbols indicate the effects produced by three equal iontophoretic currents of GABA: initially, there was a marked rise in conductance, followed by the characteristic 'fading' (BEN-ARI, KRNJEVIĆ & REINHARDT, 1979; BEN-ARI *et al.*, 1981), as well as a striking parallel depression of the IPSP conductance increase. During the release of ACh, there was no diminution of the conductance increase evoked by GABA; if anything, this tended to be somewhat greater than before. None of the six neurons tested in this way showed any reduction in GABA effectiveness; on the contrary, in 5 cases out of 6, GABA became more effective. Judging by the peak change in resistance evoked by GABA, its potency rose by a mean of 64.6% (S.D. 55.4, $n = 6$).

DISCUSSION

Our observations provide evidence for two kinds of modulatory actions of ACh, both of which would tend to enhance the firing of pyramidal cells in response to various synaptic inputs.

Postsynaptic modulation by reduction in K currents

This kind of action was originally observed in neocortical neurons (KRNJEVIĆ *et al.*, 1970; 1971), as well as in sympathetic ganglion cells (WEIGHT & VOTAVA, 1970). The similar characteristics of the excitatory effects of ACh in the neocortex and hippocampus (KRNJEVIĆ & PHILLIS, 1963; BISCOE & STRAUGHAN, 1966; STRAUGHAN, 1975) pointed to a similar mechanism of operation; and this was strongly supported by the finding that the depolarizing effect of ACh on

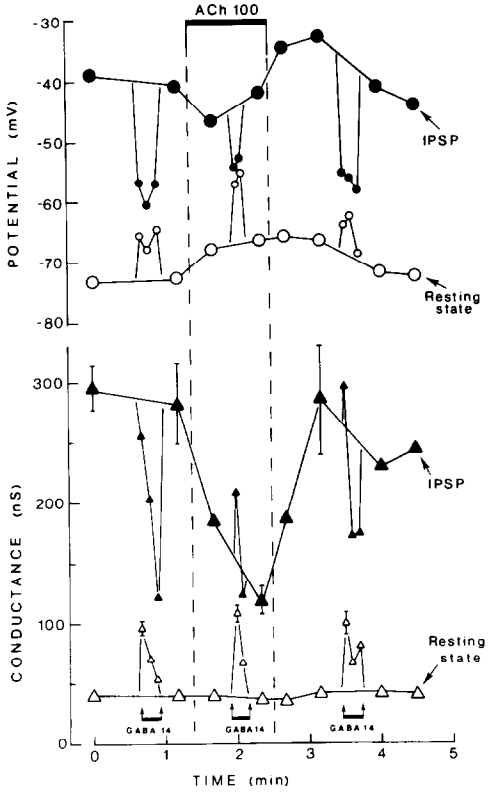

FIG. 5. Graph showing that acetylcholine has a depolarizing action (open circles), associated with a small fall in input conductance (open triangles) and a marked depression of inhibitory synaptic potential; but acetylcholine does not reduce the conductance and potential changes evoked by applications of γ-aminobutyrate. All iontophoretic currents are in nA.

pyramidal cells in hippocampal slices (*in vitro*) is very clearly associated (and in fact strongly correlated) with an increase in input resistance (KELLY *et al.*, 1979; DODD *et al.*, 1981; BENARDO & PRINCE, 1981). These authors, however, made no attempt to distinguish between two possible mechanisms of action: a reduction in G_K and a reduction in G_{Cl}. The present results show that even when E_{Cl} is much more positive than the resting potential—owing to Cl^- leakage– E_{ACh} remains relatively negative, being in the region of the probable value of E_K; on the average E_{ipsp} differed from E_{ACh} by over 60 mV. This strongly supports the idea that in the hippocampus, as in the neocortex, ACh acts by reducing G_K.

In various experiments, there appears to be at least a rough correlation between the initial resting potential and the depolarizing efficiency of ACh. If the latter is defined by the ratio of the ACh-evoked depolarization (ΔV) to the associated ΔR, one finds that the *in vitro* experiments of DODD *et al.* (1981), where the mean resting potential was −74 mV and the cells had a particularly high resistance (33 MΩ), $\Delta V/\Delta R$ was only about 1 mV/MΩ. Whereas in the present series, with a mean resting potential of −38 mV, $\Delta V/\Delta R$ was 14 mV/MΩ; in the experiments on the neocortex, the values of Vm and $\Delta V/\Delta R$ were intermediate (KRNJEVIĆ *et al.*, 1971). Presumably, these variations in $\Delta V/\Delta R$ reflect variations in the inward currents that were unmasked by ACh-mediated inactivation of outward K current. If hippocampal neurons have a voltage-dependent K current with the characteristics of the muscarine sensitive M-current described in frog sympathetic neurons by BROWN & ADAMS (1980)—the K-conductance reaching a peak at a membrane voltage near −30 mV—then one could expect a particularly large effect of ACh when the 'resting' potential is relatively poor. What is more surprising is that the mean voltage change observed under these different conditions remains the same, being very close to +12 mV in all three sets of experiments, in the neocortex (KRNJEVIĆ *et al.*, 1971), the hippocampal slice (DODD *et al.*, 1981), and the hippocampal neurons *in situ*. This may be quite fortuitous; or it may indicate that the depolarizing effect is in some way self-limiting, perhaps because with increasing depolarization, Na inactivation diminishes the underlying depolarizing drive.

A very general role for this kind of modulatory mechanism is suggested by evidence that other endogenous agents may also have a comparable action: substance P on spinal neurons (KRNJEVIĆ, 1977; NOWAK & MACDONALD, 1980) and in the myenteric plexus (KATAYAMA, NORTH & WILLIAMS, 1979); luteinizing hormone-releasing hormone and angiotensin in amphibian sympathetic ganglia (ADAMS & BROWN, 1980; JAN, JAN & KUFFLER, 1980); as well as serotonin in the myenteric plexus (WOOD & MAYER, 1978; JOHNSON, KATAYAMA & NORTH, 1980), and serotonin and noradrenaline in the facial motor nucleus (VANDERMAELEN & AGHAJANIAN, 1980). In all cases, a facilitation of cell firing seems to be brought about by a reduction in K currents.

Modulation by disinhibition

The other (and newer) aspect of the facilitation of hippocampal firing by ACh is evidently an indirect mechanism of disinhibition. By confirming a depression of IPSP conductance increase, the intracellular experiments support the conclusion already drawn from the extracellular observations (KRNJEVIĆ *et al.*, 1980, 1981) that ACh somehow reduces the effectiveness of IPSPs.

This effect cannot be explained by the postsynaptic facilitatory action already discussed: a depression of K conductance should leave unchanged the IPSP conductance increase (mainly due to Cl conductance, KELLY, KRNJEVIĆ, MORRIS & YIM, 1969; ECCLES *et al.*, 1977) but it should enhance the IPSP resistance fall. For example, the mean observed increase in resting resistance produced by ACh (from 6.1 to 7.0 MΩ, Table 1) should augment the IPSP resistance change from the mean observed value of −4.0 MΩ (Table 2) to −4.9 MΩ (a 22.5% *increase*). The actual mean ACh effect was a 41% *reduction* in IPSP resistance change.

There was no evidence that ACh depresses the in-

hibitory action of GABA on hippocampal neurons; therefore it is most likely that ACh reduces the liberation of GABA by a presynaptic action. It is of interest that methacholine diminishes GABA release in the cat's neocortex (REIFFENSTEIN, 1979). One possible side-effect is a reduction in on-going desensitization of GABA receptors which could explain why GABA appears to be potentiated by ACh.

Site of presynaptic acetylcholine action

One obvious possibility is that ACh inhibits the firing of inhibitory neurons, as in the reticular nucleus of the thalamus (BEN-ARI, DINGLEDINE, KANAZAWA & KELLY, 1976). There is some evidence that a comparable cholinergic mechanism of disinhibition also operates in the neocortex (KRNJEVIĆ, 1974; STERIADE, 1979). Alternatively, ACh may interfere with GABA release from inhibitory terminals. Many authors have reported a depressant effect of ACh on presynaptic terminals. The most extensively studied is the autoregulation of ACh release in the cortex, which also appears to be mediated by presynaptic muscarinic receptors (MITCHELL, 1963; POLAK, 1970; DUDAR, 1977; SZERB, 1978). Some non-cholinergic terminals may also be affected be ACh (YAMAMOTO & KAWAI, 1967; SHEPHERD, LORENZ, TYCE & VANHOUTTE, 1978; TAKAGI & YAMAMOTO, 1978). Particularly relevant are the observations of HOUNSGAARD (1978), who found that ACh depresses excitatory transmission at axo-dendritic synapses in the hippocampal slice, probably by a presynaptic inhibition-like depolarizing effect on the excitatory terminals.

Either an inhibition of inhibitory interneurons or a depression of transmitter release from inhibitory terminals would explain the disinhibitory effects observed here. The sharp localization of this disinhibitory action near the pyramidal layer (KRNJEVIĆ *et al.*, 1980; 1981), where the inhibitory terminals are particularly concentrated (ANDERSEN, ECCLES & LØYNING, 1964; RIBAK, VAUGHN & SAITO, 1978), as well as our failure to find interneurons that are inhibited by ACh (Y. BEN-ARI, K. KRNJEVIĆ & N. ROPERT, unpublished observations), seem to point towards the perisomatic inhibitory terminals as the main site of ACh action.

The axons of the septo-hippocampal pathway are the most likely physiological source of ACh release (LEWIS, SHUTE & SILVER, 1967; DUDAR, 1977; STORM-MATHISEN, 1977; LYNCH, ROSE & GALL, 1978). According to the histochemical evidence, their terminals are concentrated on either side of the pyramidal cell layer (STORM-MATHISEN, 1977; LYNCH *et al.*, 1978; VIJAYAN, 1979). Hence they would be especially well placed to produce disinhibition rather than disfacilitation (the effect to be expected at axo-dendritic excitatory synapses, HOUNSGAARD, 1978). This is in agreement with the facilitatory effect of medial septal stimulation observed by ALVAREZ-LEEFMANS & GARDNER-MEDWIN (1975), as well as in our own experiments (ROPERT, BEN-ARI, KRNJEVIĆ & POLC, 1980; KRNJEVIĆ & ROPERT, 1981).

There has been much speculation about the functional role of the septohippocampal cholinergic input and its likely involvement in the phenomenon of the theta rhythm (STUMPF, 1965; TEITELBAUM, LEE & JOHANNESSEN, 1975; ANDERSEN, BLAND, MYHRER & SCHWARTZKROIN, 1979). Our observations add no further light on this aspect of the problem, but they help one to understand what could be an important mechanism of modulation at central synapses.

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