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Angiotensin Excites Hippocampal Pyramidal Cells by Two Mechanisms

H. L. Haas,[1,2] D. Felix,[3] and M. D. Davis[4]

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SUMMARY

1. The mechanism of action of angiotensins was studied on CA 1 pyramidal cells in hippocampal slices of the rat. Extracellular field potentials, single-cell action potentials, and intracellular excitatory and inhibitory postsynaptic potentials (epsps and ipsps) were recorded.

2. Angiotensin II and Des-Asp[1]–angiotensin II added to the perfusion fluid caused a dose-dependent increase in extracellularly recorded epsps and synaptically evoked population spikes. Either the neurons were depolarized by angiotensins and their firing frequency of action potentials increased or the membrane potential was unaffected.

3. Local application of angiotensins caused a depolarization associated with a conductance increase which was resistant to synaptic isolation.

4. Evoked and spontaneous inhibitory postsynaptic potentials were reduced by angiotensins, but the effects of γ-aminobutyric acid (GABA) on soma and dendrites were unchanged.

5. All angiotensin effects were blocked by the specific antagonist Sar[1], Ala[8]–angiotensin II (saralasin).

6. It is concluded that angiotensins excite CA 1 pyramidal cells by a direct and a disinhibitory mechanism.

INTRODUCTION

Angiotensin II (A II), the circulating effector peptide of the renin–angiotensin system, is classically linked to the systemic control of blood pressure and plays a role in

[1] Neurophysiology Laboratory, Neurochirurgische Universitätsklinik CH-8091 Zürich, Switzerland.
[2] To whom correspondence should be addressed.
[3] Institute for Brain Research, University of Zürich, CH-8029 Zürich, Switzerland.
[4] Department of Zoology, University of Washington, Seattle, Washington 98195.

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pituitary function (see Peach, 1977). On the other hand, A II was shown to have central effects (Felix and Schlegel, 1978; Phillips *et al.*, 1979; Severs and Daniels-Severs, 1973), and there is increasing evidence of an endogenous isorenin–angiotensin system in the brain. The existence of such a system, however, has been questioned by other authors (Reid, 1977; Ramsay, 1979). Of broad interest is the fact that angiotensin produces profound effects on physiological and behavioral mechanisms. Centrally administered A II induces drinking (Epstein *et al.*, 1970) and Fitzsimons (1972) has proposed that angiotensin is a thirst hormone. Furthermore, A II was reported to disrupt retention performance and learning in rats (Köller *et al.*, 1979; Morgan and Routtenberg, 1977). The multiple physiological effects were reported to be mediated by hypothalamic and circumventricular structures (Phillips *et al.*, 1977; Schelling *et al.*, 1980). The evidence so far implies that angiotensin is formed intracellularly (Ganten *et al.*, 1971) and is concentrated in nerve terminals (Fuxe *et al.*, 1976). The widespread distribution of renin (Hirose *et al.*, 1980; Inagami *et al.*, 1980) and angiotensins (Changaris *et al.*, 1978; Fuxe *et al.*, 1976) in the brain, however, indicates that the brain renin–angiotensin system may also play some hitherto unknown functions in regions outside the hypothalamus and circumventricular organs. Celio *et al.* (1980) demonstrated renin-like immunoreactivity in granular and pyramidal cells of mouse hippocampus, and in a recent report we have shown histochemical evidence for the presence of A II in pyramidal cells of the CA 1 and CA 3 regions of the rat hippocampus (Haas *et al.*, 1979).

Earlier data demonstrate that the biologically active A II as well as Des-Asp^1–A II (A III) excite neurons in different areas of the brain such as the supraoptic region (Nicoll and Barker, 1971), subfornical organ (Felix, 1976; Felix and Schlegel, 1978), and septal area (Huwyler and Felix, 1980). The actions of both the octapeptide and the heptapeptide are blocked by saralasin (Phillips and Felix, 1976). Furthermore, a slowing of cellular activity was noted when saralasin was applied alone to those neurons. These results demonstrate that angiotensin has a specific action on certain brain cells.

From the microionophoretic experiments it was not possible to decide whether A II shows transmitter-like or modulating activity. In the present study we have investigated the mechanism of action of angiotensin at the membrane level in the hippocampal slice using extra- and intracellular recording techniques. This preparation offers unique advantages for such a study because drugs can be applied in known concentrations by perfusion, the sites for stimulation and recording are under visual control, and the anatomy and functional connections are well established (Andersen, 1975). Renin- and angiotensin-like immunoreactivity has been reported to be present in the hippocampus (Celio *et al.*, 1980; Haas *et al.*, 1980). Preliminary results of this study have been reported (Haas *et al.*, 1980).

METHODS

Thirty-five male Wistar rats of about 100–150 g were decapitated, the brain was removed, and transverse slices 450 μm thick were cut from the hippocampus with a tissue chopper. Slices were transferred to two chambers, representing a double version of our previously described recording chamber (Haas *et al.*, 1979) and perfused with a

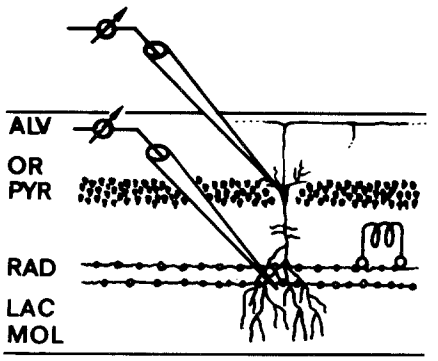

Fig. 1. Schematic diagram of the experimental situation in the CA 1 area of the rat hippocampal slice. Extracellular recordings were made with both pipettes; intracellular recordings, only from pyramidal cell bodies. Local stimulation through a bipolar electrode is indicated by a coil symbol. Strata are alveus, oriens, pyramidale, radiatum, and lacunosum-moleculare.

fluid containing (m*M*) 150 Na, 6.25 K, 134 Cl, 2.5 Ca, 2.0 Mg, 26 HCO_3, 2 SO_4, 1.25 HPO_4, and 10 glucose at pH 7.4 and 32°C. The fluid level was adjusted to the upper surface of the slices and a warmed, moistened gas mixture (95% O_2 and 5% CO_2) was deflected over the surface of the slices. The perfusion fluid was completely exchanged in less than 2 min. Drugs were applied for at least 10 min to allow equilibration in the whole slices which were, although covered by a thin fluid film, underfused. The normal perfusion fluid and the fluids containing drugs were chosen from pressurized reservoirs by means of a three-way tap. Three-barreled pipettes were used for the ionophoretic administration of γ-aminobutyric acid (GABA; 0.2 *M*, pH 4) and DL-homocysteic acid (DLH; 0.2 *M*, pH 7.7), and appropriate controls for current or pH artifacts were made with 0.2 *M* NaCl. A II and A III (10^{-5}–10^{-4} *M*) were also locally applied from micropipettes held in a separate manipulator by microdrops (nl–μl), diffusion, or pressure ejection from the pipette orifice into the slice. All peptides were obtained from Senn Chemicals. The experimental situation is explained in Fig. 1. Extracellular recording was carried out with 2 *M* NaCl-filled micropipettes having resistances of 1 to 10 mΩ in the apical dendritic or somatic areas of CA 1 pyramidal cells. Intracellular recording was by means of micropipettes filled with 3 *M* potassium acetate or potassium chloride; resistances were 40–100 mΩ. Local stimulation was bipolar with a constant current (30–150 μA, 0.2 msec, 0.2 Hz). Recording and current injection were performed with a high-input impedance amplifier (WPI). Signals were amplified, displayed on an oscilloscope, and stored on magnetic tape for later analysis. Action potentials were counted with the aid of a window discriminator and a ratemeter. Synaptic potentials and extracellularly recorded field potentials were routinely averaged and their amplitudes measured from zero to peak, except for the population spikes which were measured as shown in Fig. 3, left inset. The neighboring positive peaks were connected by a straight line and the amplitude was determined on a vertical line passing through the peak.

RESULTS

Field Potentials

Stimulation of the strata radiatum and lacunosum-moleculare evoked negative potentials of up to 26 mV. These potentials are thought to reflect largely excitatory

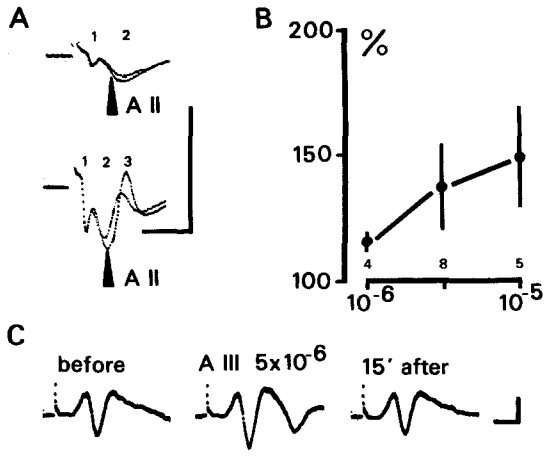

Fig. 2. Effects of angiotensin on field potentials. (A) Averages of extracellular epsps (eight sweeps) before and during perfusion with angiotensin II (A II; 10^{-6} *M*). (1) Input volleys; (2) extracellular epsps; (3) population spikes. Stimulation was 60 μA in upper traces and 80 μA in lower traces. (B) Mean increase in epsp amplitudes during perfusion with different concentrations of A II (10^{-6}, 5×10^{-6}, 10^{-5} *M*). The number of experiments is indicated (4, 8, 5); the vertical bars represent the standard deviation. (C) Synaptically evoked population spikes (eight sweeps averaged) recorded from the pyramidal layer before, during, and after perfusion with A III (5×10^{-6} *M*). Calibration in A and C, 10 mV and 10 msec.

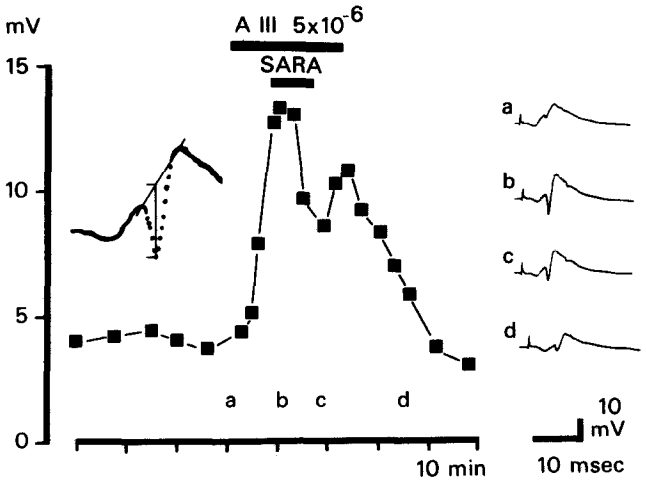

Fig. 3. Interaction of angiotensin III (A III) and saralasin. The drugs were applied during the time indicated by black bars at a concentration of 5×10^{-6} *M*. Filled squares represent the amplitudes of population spikes recorded in the pyramidal layer. Representative original potentials are shown on the right. The inset on the left explains the method of measurement of averaged (eight sweeps) population spikes.

postsynaptic potentials (epsps) in the apical dendrites of pyramidal cells (Andersen *et al.*, 1977). Such extracellular epsps were recorded in 19 slices from eight rats. Seventeen of those were enhanced by up to 70% in a dose-dependent manner by 10^{-6}–10^{-5} *M* A II. This is illustrated in Fig. 2. On the left (A) averaged extracellular epsps before and during perfusion with A II are superimposed; on the right (B) the mean increase at different concentrations of A II is shown. Although a fading of the angiotensin effect was sometimes observed with prolonged exposure, the perfusion could be repeated with the same result on several slices. The input volleys which represent synchronous action potentials in the afferent fibers (Fig. 2A, 1) were unaffected. The enhancement of the epsps led to the appearance of or an increase in the size and occasionally the number of synaptically evoked population spikes, as illustrated in Fig. 2A, 3. This effect was also observed in six experiments with recording in the pyramidal layer (Fig. 2C). The increase in the epsps and population spikes was reduced by up to 50% in all of five experiments when the angiotensin blockers, [Sar^1, Thr^8]-A II and [Sar^1, Ala^8]-A II (saralasin), were added at a concentration equal to that of the perfusion fluid (Fig. 3). Similar results were obtained when field potentials were recorded in the dentate area after stimulation of perforant path fibers (five experiments). Saralasin alone at 10^{-5} *M* had no effect on extracellular epsps. Antidromically evoked population spikes in the pyramidal layer after stimulation of the alveus were unaffected by perfusion with angiotensin.

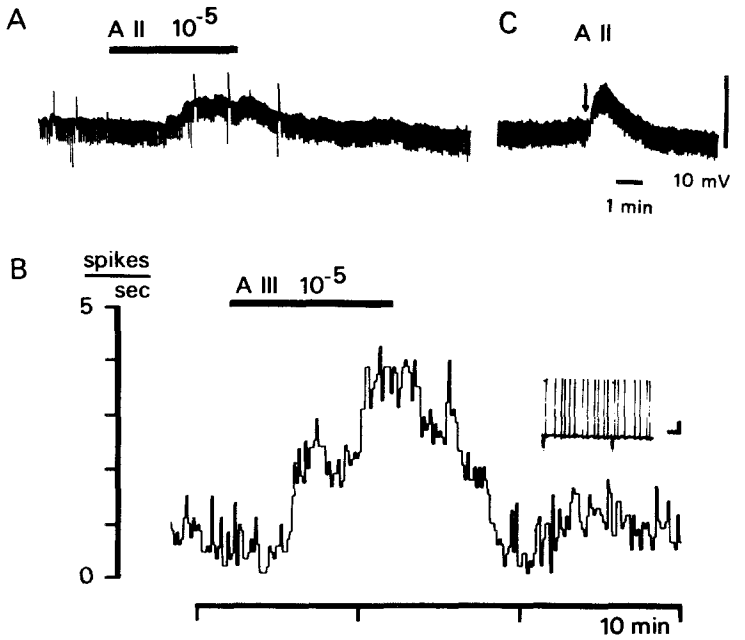

Fig. 4. Effect of angiotensins on membrane potential and firing rate of CA 1 pyramidal neurons. (A, B) Perfusion with angiotensins (10^{-5} *M*) during the time indicated by bars above traces. The inset in B shows 7 sec of the original record (calibration, 10 mV and 1 sec). Deflections in traces A and C are from constant-current injection and reduced by the pen-recorder time constant. C illustrates the response to a local microdrop application at the arrow.

Intracellular Recording

Intracellular records were obtained from 43 CA 1 pyramidal neurons having resting potentials of 70.8 ± 5.4 (SD) mV, action potentials of 92.7 ± 16.1 mV, and input resistances of 37.7 ± 9.3 MΩ. When angiotensins were added to the bath about half of the cells tested (11 of 23) were depolarized by 7.2 ± 6.3 mV, and their firing rate and the number of action potentials elicited by depolarizing current injection were increased up to several fold (Figs. 4A, B). In the remaining cells the resting potential was not measurably affected, but in three of them an increased firing was also observed. The membrane conductance was routinely measured by the injection of constant-current pulses of 0.2- to 2.0nA amplitude and 100- to 200-msec duration. No consistent changes were observed in this parameter during perfusion with angiotensin. In three cells a sudden massive depolarization lasting more than 30 sec occurred during perfusion with A II. The depolarization started with a burst of action potentials followed by a massive drop of the membrane resistance and spike inactivation. Such long-lasting depolarization shifts were not observed spontaneously. In two further cells shorter depolarization shifts lasting a few seconds were observed after A II (see Fig. 7). When angiotensins were applied locally from a micropipette by a drop (Fig. 4C) or by pressure injection into the slice (Fig. 5), 9 of 10 cells were rapidly depolarized and the membrane conductance was increased by 23 ± 15%. The onset of the depolarization was often within less than 1 sec after the application of a microdrop. This excitation was often followed by a hyperpolarization and depression of spontaneous activity lasting for up to 10 min (Figs. 5 and 7). In three cells tested during the presence of tetrodotoxin (10^{-6} g/ml), weaker and purely depolarizing effects were observed (Fig. 5). This indicates that part of the excitatory angiotensin effect occurs directly on the pyramidal cell membrane, while the later hyperpolarization may be indirectly mediated.

Stable intracellular recordings could not be obtained in a medium containing 0.2 m*M* Ca and 4 m*M* Mg, but the effect of angiotensin perfusion (two cells) or local application (13 cells) was investigated on the spontaneous firing of extracellularly recorded cells in the pyramidal layer: the frequency of action potentials was enhanced up to severalfold in nine cells, and in three of these the excitation was followed by a depression when large drops were administered. Four cells were unaffected and two were depressed.

Saralasin was added to the perfusion fluid (10^{-6}–10^{-5} *M*) during the recording from seven cells. In all cases the angiotensin effects (perfusion, one cell; local application, six cells) were completely and reversibly blocked in the absence of direct effects of saralasin (Fig. 6). The depolarization evoked by pressure ejection of

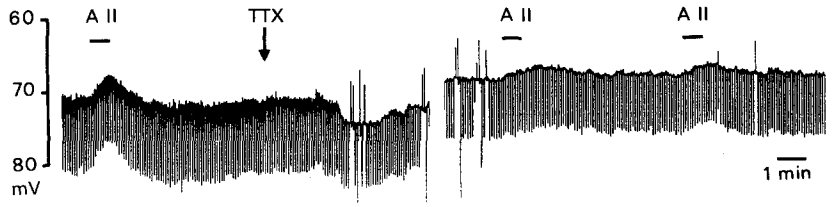

Fig. 5. Angiotensin action on membrane potential before and during the presence of tetrodotoxin (TTX). TXX (10^{-6} g/ml) was added at the arrow; the gap represents 10 min. (Pen recorder; action potentials are not shown; fast voltage deflections are reduced.)

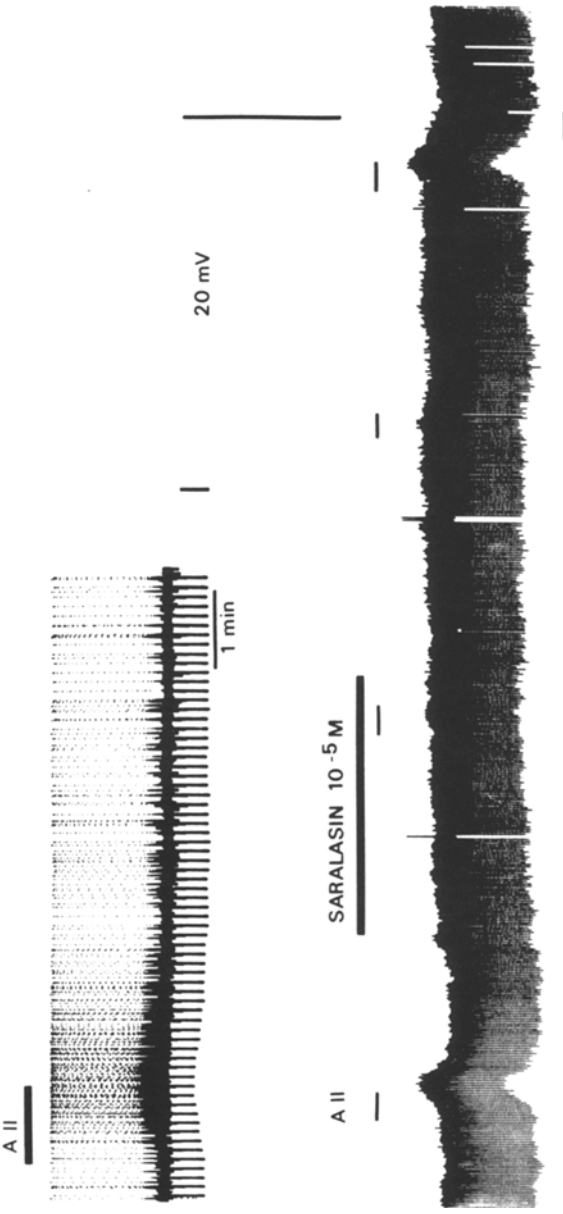

Fig. 6. Block of the A II action by saralasin. A II (10^{-4} *M*) was applied locally by pressure ejection; saralasin was added to the perfusion fluid. Upper trace: A II action filmed with a fiber optic recorder. Upward deflections are action potentials; downward deflections are from −0.5-nA current injection. Lower trace is from a pen recorder.

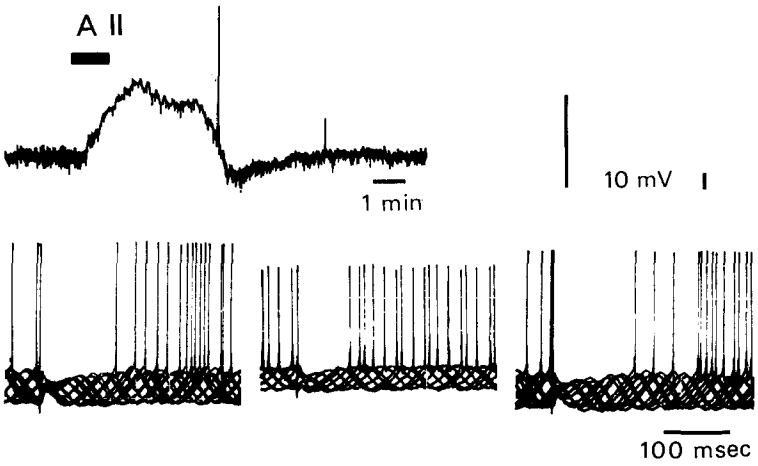

Fig. 7. Action of A II on inhibition after orthodromic stimulation. Upper trace: membrane potential; A II was ejected by pressure during the time indicated by a bar. Note the two depolarization shifts (fast upward deflections). Lower traces are 10 superimposed pictures each, before (left), during (middle), and 12 min after (right) the A II action. An alternating current of 10 Hz was continuously injected, forming an envelope which illustrates the ipsp at two voltage levels. The width of the trace is proportional to the membrane resistance at any point. During A II an increase in the conductance but a decrease in the inhibitory postsynaptic conductance change and the pause in firing can be seen.

carbachol (Haas, 1982) was unaffected even by prolonged (up to 60-min) perfusion with saralasin. With such prolonged administrations of saralasin, three cells were hyperpolarized and their firing was reduced. The latter was also seen in four further cells recorded extracellularly. In these cases the firing evoked by ionophoretically applied DL-homocysteic acid was also reduced.

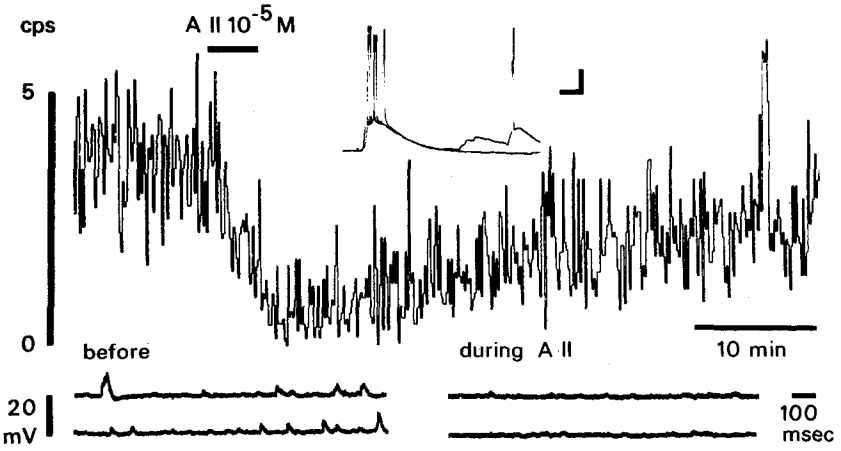

Fig. 8. Action of A II on spontaneous depolarizing ipsps. A KCl-filled recording electrode was used; the inset shows epsp–ipsp sequence after stratum radiatum stimulation (calibration, 20 mV and 20 msec). Spontaneous ipsps were counted and displayed as a frequency vs time diagram. cps; counts per second. Sample records are shown below.

The effects of angiotensins on epsp–inhibitory postsynaptic potential (ipsp) sequences after stimulation of the stratum radiatum were observed in eight neurons. When compared at identical voltage levels the rising phase of the epsps was unchanged but they were slightly increased and their decay was slowed; the ipsp was always reduced. This was particularly clearly demonstrated by continuously monitoring the conductance caused by the ipsp (Fig. 7). Recurrent ipsps after alveus stimulation were also reduced (two cells).

Ten cells were recorded with KCl-filled electrodes in order to investigate spontaneous depolarizing ipsps (Alger and Nicoll, 1980; Buckle and Haas, 1982). Only one of these was depolarized (6 mV); four were hyperpolarized (4.8 ± 3.4 SD mV) while the membrane potential of five cells remained unaffected by perfusion with A II. In six of these cells spontaneous depolarizing potentials were counted, and in five cells the number of these presumable ipsps was reduced to 35.4 ± 22.4% of the control frequency (Fig. 8). As the stimulus-evoked and spontaneous ipsps are probably mediated to a large extent by GABA (Andersen *et al.*, 1980; Curtis and Johnston, 1974), their reduction might be due to an angiotensin–GABA antagonism. This possibility was tested by applying GABA ionophoretically at regularly spaced intervals to the apical dendrites or the soma, where depolarizing and hyperpolarizing effects were seen as previously described by Andersen *et al.* (1980). These effects were not modified by angiotensin (10^{-5} *M*).

DISCUSSION

In a previous paper histochemical evidence for the presence of the renin–angiotensin system in CA 1 and CA 3 pyramidal cells of the rat hippocampus has been provided (Haas *et al.*, 1980). A subpopulation of both these cell types displayed a strong immunoreactivity to renin (Inagami *et al.*, 1980) and angiotensin II (Haas *et al.*, 1980) antibodies. A large proportion of the fibers stimulated in our experiments is axons from ipsilateral CA 3 and contralateral CA 1 pyramidal neurones. The transmitter released from these fibers which is responsible for the recorded epsps is most probably an excitatory amino acid (Andersen, 1975). Angiotensin may be released together with this transmitter from varicosities of the same or separate axons.

The direct excitatory action of angiotensin faded sometimes when the cells were still exposed to the peptides and was often, especially after large drop applications, followed by a longer-lasting hyperpolarization. These observations could be attributed to a sequential activation of different elements in the slices but may also be explained in part by a desensitization. This relatively rapid excitation looks like a classical transmitter action and could account for quick information transfer. The onset of the disinhibitory action was also fast as judged by the effect on spontaneous ipsps but was much longer lasting.

A reduction of hippocampal ipsps is also produced by opioid peptides (Gähwiler, 1980; Nicoll *et al.*, 1980; our own unpublished observations). As we have excluded direct antagonism between GABA and the peptides, or another change in postsynaptic sensitivity to GABA, the action probably does take place on interneurones. This could be through somatic inhibition of inhibitory interneurons or through a presynaptic

interference with inhibitory transmission. We have not been able so far to record satisfactorily from identified interneurons in order to test the former possibility, but the spontaneous synaptic potentials probably mirror the firing of such interneurons. The increase in extracellularly and intracellularly recorded epsps, which is also observed with opioid peptides (Haas and Ryall, 1980), penicillin (Dingledine and Gjerstadt, 1980), and bicuculline (Haas, unpublished), is best explained by a reduction of a feed-forward ipsp unmasking the epsp (Dingledine and Gjerstadt, 1980).

As at least some of the presumed angiotensinergic cells are entirely present in the slice and probably spontaneously active, one might expect to see an effect of angiotensin antagonists on actions of naturally released angiotensin. Although we have not detected such an action on extracellular epsps—it may have been below the detection threshold—we found a depressant effect of perfused saralasin on spontaneous and amino acid-evoked firing of CA 1 pyramidal cells. Further experiments are necessary to investigate the specificity of this effect (Gähwiler and Dreifuss, 1980) and its relevance for hippocampal synaptic transmission.

A reduction of inhibition like the one we have described here can cause epileptiform behavior also in the slice (Schwartzkroin and Prince, 1980). In our experiments the multiple firing of population spikes and the depolarization shifts may be considered epileptic signs. Angiotensin has been shown to disrupt learning, and it is well known that convulsions cause amnesia and that long-term potentiation, which is related to memory processes in the hippocampus, is lost following seizures (Hesse and Teyler, 1976).

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