Angiotensin II in the hippocampus. A histochemical and electrophysiological study[1]

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Summary. The presence of the renin angiotensin system in the hippocampus is shown by immunohistochemistry. Intra- and extra-cellular recordings revealed that angiotensin II and III excite CA 1 pyramidal cells by disinhibition. The effect is antagonized by [Sar[1], Thr[8]]-A II.

The octapeptide angiotensin II (A II) is known as the circulating effector peptide of the renin-angiotensin system. It is classically linked to the systemic control of blood pressure[5,6]. On the other hand, A II was shown to have central effects[7,8] and to be present in brain tissue[9,10]. Centrally administered A II induces drinking[11] and stimulates the secretion of vasopressin and ACTH[12,13]. Furthermore, central A II was reported to disrupt learning and retention performance in rats[14,15]. The multiple physiological responses elicited through centrally administered A II were reported to be mediated by hypothalamic structures and circumventricular organs (for review see Schelling et al.[16]). Wide-spread distribution of renin[17,18] and angiotensins[19,20] in the brain, however, suggested the possibility that the renin-angiotensin system may play some hitherto unrecognized functions in regions outside the hypothalamus and circumventricular organs. Recently, Celio et al.[21] using immunohistochemical methods have demonstrated the localization of renin in granular and pyramidal cells of mouse hippocampus. We report here histochemical evidence for the presence of A II in hippocampal pyramidal cells of the rat and our conclusions from extra- and intracellular recordings from pyramidal neurones in the CA 1 area that angiotensins enhance excitatory transmission by a disinhibitory mechanism.

The sensitivity of the peroxidase-antiperoxidase method, and the use of colchicine to block peptide transport in the nerve cell, enable us to localize A II in pyramidal cells of the hippocampal formation. 60 µg of colchicine were applied intracerebrally 48 h previous to perfusion of the animal with Bouin's fluid. The brain was then embedded in paraffin. Longitudinal sections 5 µm thick were incubated with a 1:5000 dilution of A II antiserum and then processed by the unlabeled antibody technique[22]. Immunoreactive cell bodies in the hippocampal formation are detectable in CA 1 and CA 3 regions of the pyramidal layer (figure 1) and in the stratum granulosum of the area dentata. The immunostaining is specific for angiotensin as it was selectively abolished by preadsorption with synthetic A II.

Electrophysiological experiments were carried out on the hippocampal slice, a preparation which 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[23,24]. 11 rats were decapitated, the hippocampus dissected free and transverse slices 450 µm thick were cut with a tissue chopper and transferred to a perfusion chamber[25]. The perfusion fluid was at 34 °C, pH 7.4 and contained (mM): Na 150, K 6.25, Cl 133, Ca 2.0, Mg 2.0, HCO_3 26, SO_4 2, HPO_4 1.25, glucose 10 and was equilibrated with 95% O_2/ 5% CO_2.

Extracellular epsp's which reflect excitatory postsynaptic potentials (epsp's) occurring in the apical dendrites of CA 1 pyramidal cells after stimulation of the strata radiatum and lacunosum (RAD and LAC, figure 2, A) were recorded from 16 different slices before, during and after perfusion with A II and A III. These peptides caused a dose-dependent increase of the potentials but had no effect on the input volleys. At an A II concentration of 10^{-6} M the average increase was 16 ± 4 (SD) %, at 5×10^{-6} M 38 ± 17% and at 10^{-5} M 50 ± 20%. In some slices the effect was repeated several times without desensitization. The effect was antagonized by [Sar[1], Thr[8]]-A II, given in the same concentration (3 experiments; figure 2, B). A similar effect was also seen in 3 experiments from recording in the dentate area after stimulation of the perforant path fibres.

In the CA 1 soma layer (PYR, figure 2, A) synaptically and antidromically elicited population spikes which represent synchronous action potentials of many cells were recorded after stimulation of the stratum lacunosum and the alveus (ALV) respectively. A III enhanced synaptically evoked potentials and caused the appearance of multiple spikes. Antidromically elicited spikes were unaffected. A 2nd antidromic shock was given after a latency of 20 msec. At this time recurrent inhibition usually reduces the 2nd spike. During perfusion with A III (2×10^{-6} M) this reduction was abolished or even replaced by a facilitation in 4 out of 6 experiments.

Intracellular records were obtained with potassium acetate filled microelectrodes in 9 CA 1 pyramidal neurones (resting potentials 65–77 mV, action potentials 80–112 mV and input resistances 22–48 MΩ). A II and A III depolarized 4 cells by up to 13 mV. The resting potential of 5 cells was unaffected, in 4 of those, however, as in the depolarized neurones, the rate of spontaneous firing increased. Analysis of sequences of excitatory and inhibitory postsynaptic potentials (ipsp's), after stimulation of the afferent fibres revealed that the epsp's were prolonged but not increased by the angiotensins. Ipsp's were reduced (figure 2, C). This intracellularly measured effect was also reversed by an angiotensin antagonist, saralasin, [Sar[1], Ala[8]]-A II. Little change was observed in the membrane conductance, which was measured by constant current injection via a bridge circuit (figure 2, C, right). This finding and the double shock experiment described above suggest that disinhibition is the basis of the observed excitation.

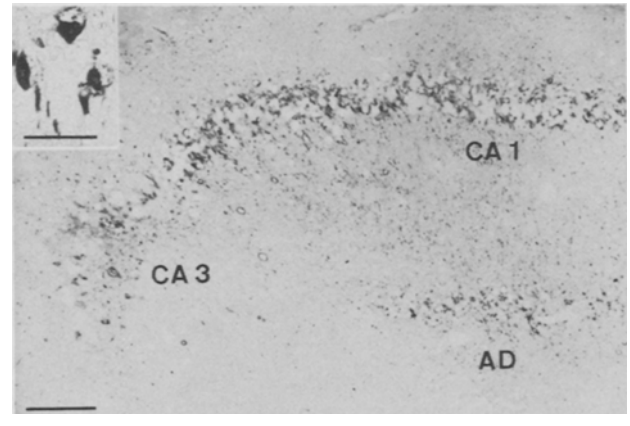

Fig. 1. Immunohistochemical localization of A II-like immunoreactivity in the rat hippocampus. Pyramidal cells in the CA 1 and CA 3 area and granular cells in the dentate area (AD) are stained. The inset shows a higher magnification of A II immunoreactive cells in CA 1. Bars indicate 200 µm and 35 µm respectively.

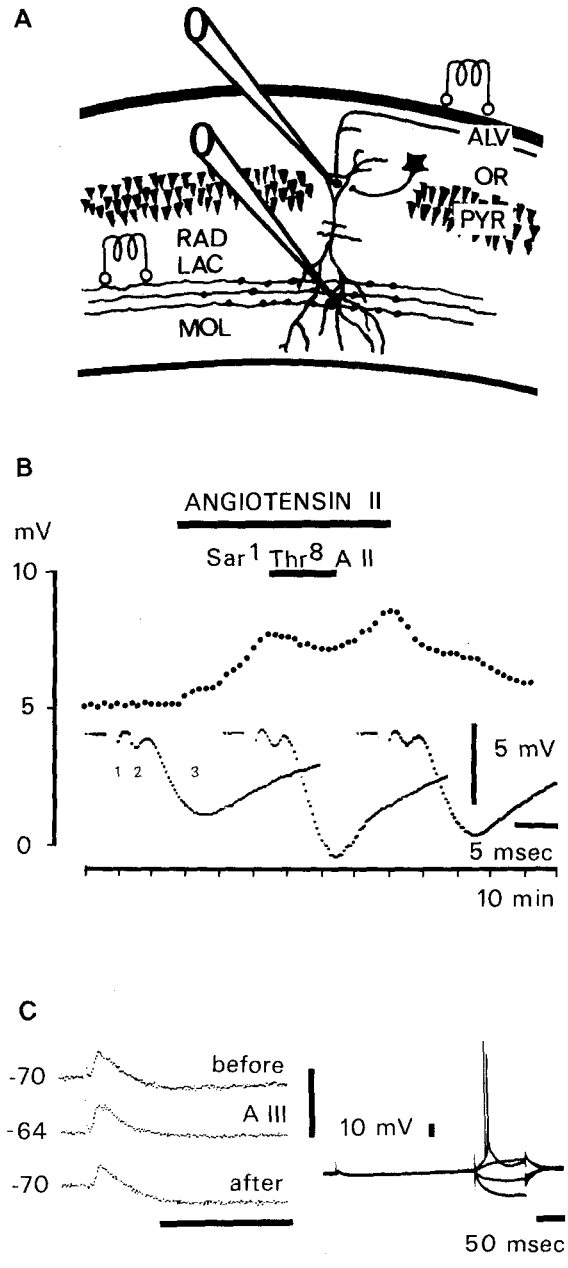

Fig. 2. Electrophysiological actions of angiotensins in the hippocampus. *A* Schematic drawing of the CA 1 area. Microelectrodes are inserted in the apical dendritic and the somatic region for extra- und intracellular recording. Local stimulation is indicated by the coil symbols. A pyramidal cell and an interneurone (black) with dendrites and axons are shown. The strata are alveolare (ALV), oriens (OR), pyramidale (PYR), radiatum (RAD), lacunosum (LAC) and moleculare (MOL). *B* Points in the upper trace indicate the averaged amplitudes of extracellularly recorded epsp's at the CA 1 apical dendrites after stimulation of the Schaeffer collaterals and commissural fibres (45 µA, 0.2 msec). The inserted traces give examples before, during and after the action of A II. 8 extracellular epsp's are averaged, 1: stimulus artefact, 2: input volley, 3: extracellular epsp. Angiotensin II and the antagonist [Sar^1, Thr^8]-A II were added to the perfusion fluid during the times indicated by black bars (10^{-5} M). *C* Intracellular recording from a CA 1 pyramidal neurone which was depolarized by 6 mV during the perfusion with A III. The ipsp is reduced and the pure epsp shape appears. The rising phase is unchanged. Left: averaged epsp-ipsp sequences before, during and 25 min after A III. (5×10^{-6} M for 10 min). Right: original record showing epsp-ipsp sequence at the beginning and voltage deflections after intracellular injection of ± 0.5 and ± 1.0 nA, 100-msec pulses at the end of the trace.

We have thus provided evidence for the presence of the renin-angiotensin system in the hippocampus and a specific action of A II and A III. A large proportion of the fibres stimulated in our experiments originates from ipsilateral CA 3 and contralateral CA 1 pyramidal cells. A subpopulation of both these cell types displayed a strong immunoreactivity to renin and angiotensin II antibodies. The transmitter released from the en passant boutons which is responsible for the recorded epsp's is most probably an excitatory amino acid[23]. It may well be that angiotensin is released together with this transmitter from the same or separate varicosities in order to modulate its efficacy. The angiotensin effect could be through inhibition of inhibitory interneurones or an interference with inhibitory transmission. A similar excitatory action of opioid peptides in the hippocampus has been ascribed to disinhibition[26] and to presynaptic facilitation[27]. Disinhibitions like the one we describe here cause 'epileptic' behaviour, e.g. multiple population spikes. As angiotensin has been shown to disrupt learning[14,15], it is of interest in this context that convulsions cause amnesia and that long term potentiation which is related to memory processes in the hippocampus is lost following seizures[28].

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