DIRECT ACTION OF ANGIOTENSIN II ON CENTRAL NEURONS

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Response of nerve cells of the somatosensory and visual cortex and anterior hypothalamus of rabbits and also of the isolated circumesophageal nerve ring of *Helix pomatia* to microiontophoretic administration of angiotensin II (A-II) were studied. Responses of the rabbit brain neurons to A-II were characterized by a marked increase in the frequency of spike discharges which depended on the dose of the drug injected. Neurons of the anterior hypothalamus had a lower threshold of response than cortical neurons. Application of A-II to the soma of the recorded snail cells caused a marked but reversible decrease in the membrane potential level. The resistance of the membrane under these circumstances was reduced by two to four times. These results are evidence of the direct action of A-II on central neurons.

KEY WORDS: *Angiotensin II; neurons of the cerebral cortex; neurons of the anterior hypothalamus.*

Angiotensin II (A-II), a natural octapeptide with high biological activity, is one of the leading hypertensive factors and regulators of the water and salt balance of the organism. It is ascribed an essential role in the genesis of various forms of arterial hypertension [1]. According to the widely held view A-II has an influence on many systems of the body and chiefly on the smooth-muscle apparatus of the blood vessels [2, 6]. At the same time the possibility of local synthesis and inactivation of A-II in the CNS has been demonstrated [4, 5]. Nevertheless, the mechanisms of the central action of A-II still remain virtually unstudied. In particular, the question of the possibility of a direct action of A-II on central neurons still remains unanswered.

The object of the present investigation was accordingly to study the action of microiontophoretically applied A-II on neurons of the mammalian and molluscan CNS.

EXPERIMENTAL METHOD

In acute experiments on unimmobilized, unanesthetized rabbits, fixed in a stereotaxic apparatus, extracellular recordings were made by means of multichannel glass microelectrodes of spike potentials (through the central channel of the microelectrode, filled with 2-5 M NaCl solution) of neurons of the somatosensory and visual areas of the cortex, and also of the anterior hypothalamus. The side channels of the multichannel microelectrode were filled with aqueous solutions of NaCl (a 2.5 M solution for control polarization) and with A-II (0.005 M, pH 7.6; Ciba). Injection of A-II into the zone of the recorded neurons was carried out by means of anionic currents with the strength of 5-60 nA and the duration of injection varied from 1 to 60 sec. The bioelectrical activity was amplified and recorded by means of the UPT-2 amplifier, S1-18 oscilloscope, and the Amplior IITR four-channel tape recorder, with subsequent recording on an ink-writing electroencephalograph with reduction of the playback speed of the magnetic tape by eight times.

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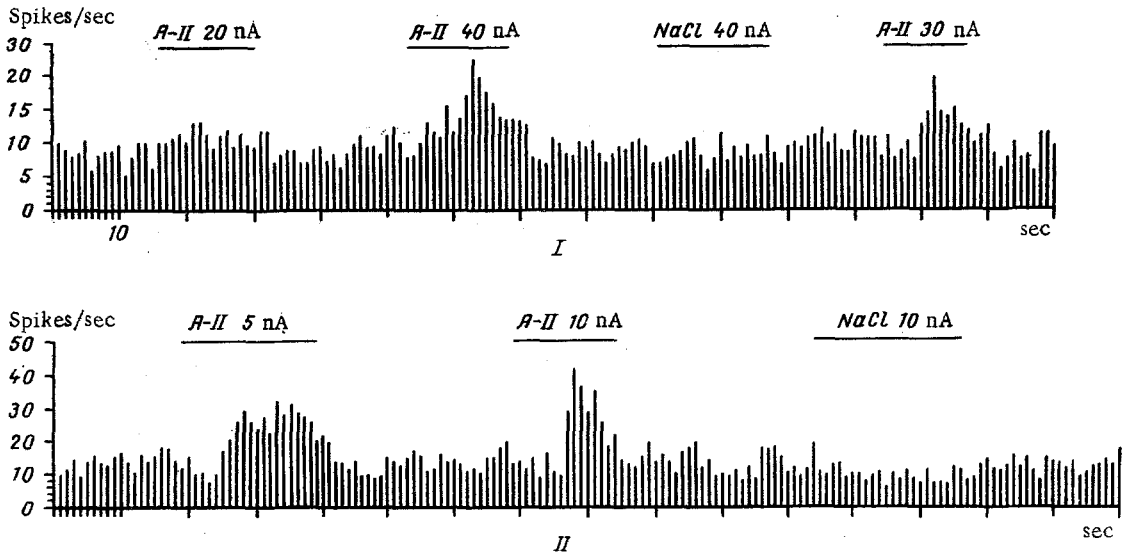

Fig. 1. Responses of neurons in different parts of rabbit brain to microiontophoretic injection of A-II: 1) frequency histogram of spontaneous spike activity and responses during injection of A-II by currents of different strength, and also extracellular polarization of visual cortical neuron; 2) frequency histogram of spontaneous spike activity and of responses to injection of A-II by currents of different strength and also of intracellular polarization of anterior hypothalamic neuron. Horizontal lines mark injection of substances.

In the experiments on *Helix pomatia* the large superficial neurons of the subesophageal complex of the isolated circumesophageal nerve ring, placed in hemolymph, were investigated. Bioelectrical activity was recorded by means of glass capillary microelectrodes filled with 2.5 M potassium citrate solution, with a tip about 1 μ in diameter and a resistance of 15-25 MΩ. The IMA-01 instrument (Tesla) was used as amplifier and a dual-beam universal indicator (Disa Electronic) as the display unit. A camera made by the same firm was used to photograph the potentials. Microiontophoretic application of the drugs to the soma of the nerve cells was carried out be means of multichannel microelectrodes fixed to a separate micromanipulator. The conditions of administration were similar to those described above for rabbits. In some cases A-II, in concentrations of $5 \cdot 10^{-9}$-$5 \cdot 10^{-10}$ g/ml was injected into the hemolymph bathing the isolated circumesophageal nerve ring of the snail.

EXPERIMENTAL RESULTS

In six rabbits, 47 neurons of the sensomotor and visual areas of the cortex and of the anterior hypothalamus were investigated. The experiments showed that about one third of recorded neurons possessed marked sensitivity to microiontophoretically injected A-II. Responses of the nerve cells to A-II were characterized by a marked increase in the frequency of spike discharges with a latent period of 0.5-7 sec, and they were observed for 0.5-10 sec after the end of administration of the peptide. The intensity of the responses to A-II depended on the dose of the substance injected (Fig. 1). With some neurons, a phenomenom of densensitization was observed: It took the form of a decrease in spike frequency during continued administration of the drug (Fig. 1) or a decrease in the intensity of responses to repeated injections. In some case phasic responses to A-II were observed. After a period of increased frequency of spikes, a period of a fairly prolonged (for several seconds) reduction in the frequency of spike activity was observed.

It was noted that the threshold of sensitivity of the neurons tested to A-II differed greatly. However, neurons of the anterior hypothalamus had a lower threshold of response than the cortical neurons (Fig. 1).

Neurons sensitive to microiontophoretically injected A-II were thus found in the rabbit CNS. Nevertheless, it cannot be categorically stated on the basis of the results of this series of experiments that A-II acted directly on the nerve cells. Its effect could have

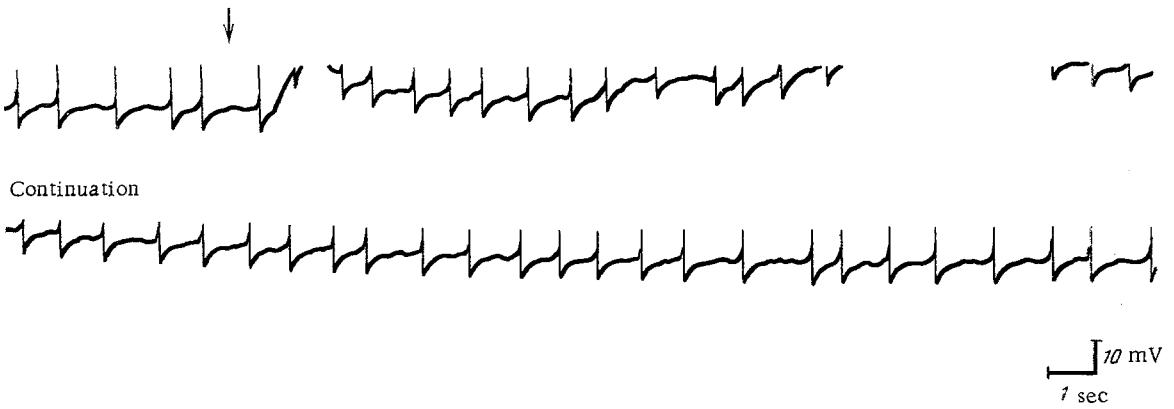

Fig. 2. Responses of *Helix pomatia* neuron to injection of A-II into hemolymph. Arrow marks time of injection of A-II. High-voltage part of spikes and reduction of membrane potential by more than 14 mV not recorded because of high degree of amplification.

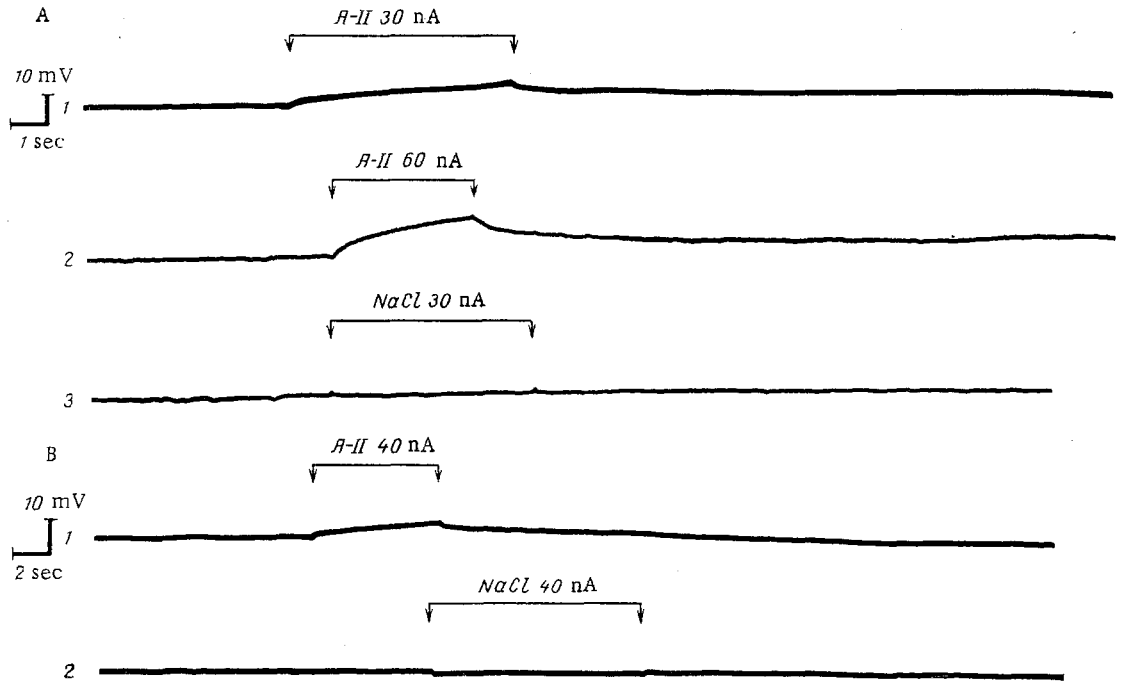

Fig. 3. Responses of *Helix pomatia* neurons to microiontophoretic injection of A-II. Horizontal lines with arrows above records of unit activity mark time of injection of substance. A) Unit responses during a change in strength of injecting current. 1) Decrease in membrane potential by 4 V in response to injection of A-II by a current of 30 nA; 2) decrease in membrane potential by 10 mV following increase in strength of current to 60 nA; 3) control polarization. B) Change in level of membrane potential of another neuron in response to injection of A-II with prolonged afterhyperpolarization after injection of peptide ceased. 1) Response to injection of A-II; 2) polarization.

been transmitted through adjacent microvessels. However, taking data in the literature [3, 7] into account, it can be postulated that the observed responses of the cortical and hypothalamic neurons of the rabbits were due to the direct action of angiotensin.

To rule out the possibility of an indirect action of A-II on the neurons through their primary effect on microvessels experiments were carried out on neurons of mollusks, for during microiontophoretic injection of the peptide toward the soma of neurons of the isolated circumesophageal nerve ring the possibility of a vascular action of the drug was completely

eliminated. Altogether 23 neurons of the isolated subesophageal complex of *Helix pomatia* were tested. The experiments showed that injection of A-II into the hemolymph in a concentration of $5 \cdot 10^{-10}$ g/ml caused a sharp but reversible decrease in membrane potential and an increase in frequency or inhibition of spike activity in all the neurons tested. The resistance of the membrane under these circumstances was reduced by 2-4 times (Fig. 2). The use of a higher concentration of A-II led to changes in bioelectrical activity of the nerve cells similar to those described above, but this time irreversible.

Microiontophoretic injection of the peptide toward the soma of the recorded snail cells caused a marked but reversible decrease of membrane potential, corresponding to the strength of the injecting current, in some of them (Fig. 3A). Repeated injections of the drug could lead to a less marked response. In three neurons a phenomenon of prolonged afterhyperpolarization was observed after the end of A-II injection. A record of the level of the membrane potential of one neuron of the subesophageal complex of *Helix pomatia* is shown in Fig. 3 B. After the end of injection of A-II, accompanied by a fall of membrane potential, prolonged afterhyperpolarization up to 5 mV was recorded.

The writers consider that the results of these experiments are evidence of the direct action of A-II on central neurons.

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