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ACTION OF RENIN ON EFFECTS OF ELECTRICAL STIMULATION OF THE VENTROMEDIAL HYPOTHALAMUS

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KEY WORDS: renin; lateral cerebral ventricles; ventromedial hypothalamus; ventricular extrasystoles.

The renin—angiotensin system plays an important role in the regulation of vascular tone [1, 7], for renin is formed in the kidneys when the circulation in them is reduced. Renin, as an enzyme, has been shown to activate angiotensinogen — a protein found in the blood — and to convert it into angiotensin I, from which is subsequently formed angiotensin II, which has high pressor activity [3, 8].

Recent investigations have shown that the brain has its own renin—angiotensin system, which contains the same components as the renin—angiotensin system of plasma [4-6, 10, 11] and which evidently participates in the central regulation of arterial pressure (BP), water and electrolyte homeostasis, and other functions of the body. Consequently, many workers have directed their efforts toward the study of different effects of the biologically active substances in the renin—angiotensin system of the plasma, when applied centrally [12-14].

The object of this investigation was to study the action of renin, injected into the lateral cerebral ventricles, on some autonomic parameters and on the effects of electrical stimulation of negative emotiogenic centers of the hypothalamus.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male Chinchilla rabbits weighing from 1.5 to 2.5 kg. The animals were immobilized in a frame and bipolar nichrome electrodes were implanted in the ventromedial nuclei of the hypothalamus, with an interpolar distance of 0.3-0.5 mm. Electrical stimulation of the hypothalamus was carried out with square pulses 1 msec in duration, frequency 50 Hz, and strength 100-300 μA. Renin was injected through a cannula into the right lateral cerebral ventricle in doses of 10, 20, and 30 μg/kg in a volume of 20 μl physiological saline. Partially purified renin from the dog's kidney (batch No. 246, provided by Dr. E. Hass, Cleveland, Ohio), was used in the experiments.

Respiration and BP in the femoral artery were recorded in all the experimental animals by means of piezoelectric and strain-gauge transducers connected to a Mingograf-34 (Siemens-Elema, Sweden). The ECG also was recorded in standard lead II. In some experiments the EEG was recorded in several cortical regions: sensomotor, parietal, and visual, on an eight-channel electroencephalograph of the EEG-80 type (Medicor, Hungary).

At the beginning of each experiment background values of BP, ECG, respiration, and EEG were recorded. Changes in these parameters were then studied in response to stimulation of the ventromedial nuclei of the hypothalamus. Renin was then injected into the lateral ventricle and the time course of changes in the above parameters was analyzed for 2 h. Against

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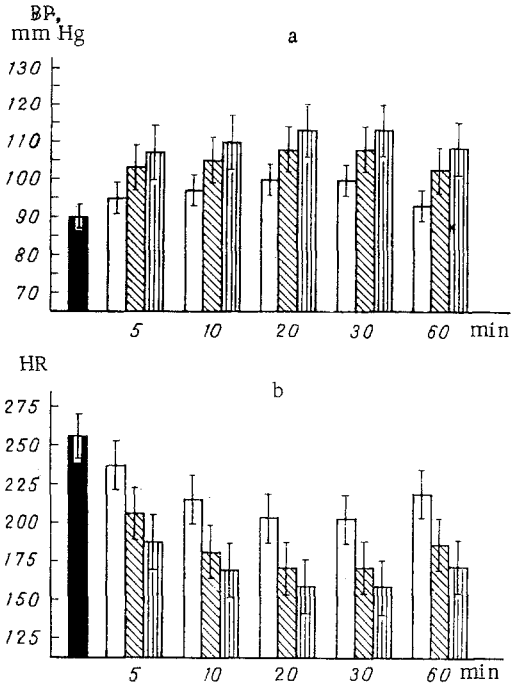

Fig. 1. Dose-dependent effect of intraventricular injection of renin on BP (a) and HR (b). Abscissa, time (in min). Black columns denote initial value, unshaded columns — renin, 10 μg/kg; obliquely shaded columns — renin, 20 μg/kg; vertically shaded columns — renin, 30 μg/kg.

the background of the central action of renin, cardiovascular responses to stimulation of negative emotiogenic centers of the hypothalamus were recorded every 10 min. During the experiment, repeated injections of renin were given into the lateral ventricle of some animals.

The location of the electrodes was verified by the projection method in frozen brain sections at intervals of 180 μ.

EXPERIMENTAL RESULTS

Injection of renin into the lateral ventricle in doses of 10 to 30 μg/kg caused various changes in the general BP level and heart rate (HR) with a latent period of 4 ± 0.3 min (Fig. 1). The maximal effect of renin on cardiovascular functions was observed after 24 ± 1.3 min, and their background values were restored 104 ± 5 min after the intraventricular injection. When renin was injected in a dose of 10 μg/kg, BP rose by 10 ± 0.8 mm Hg and HR fell by 53 ± 2 beats/min; when renin was injected in a dose of 20 μg/kg BP increased by 18 ± 1.8 mm Hg and HR fell by 84 ± 3 beats/min; a dose of 30 μg/kg caused BP to rise by 23 ± 2.6 mm Hg and HR to fall by 100 ± 3.6 beats/min. BP and HR reached 97% of their maximal and minimal values, respectively, 10 min after injection of renin. In the course of 10 ± 2.2 min a steady increase in BP and marked bradycardia were observed. In six animals (38%) characteristic waves of rise and fall of BP with a frequency of 18 ± 1.4/min and with an amplitude of 120-140 mm Hg appeared during this period. During the next 50 ± 2.3 min BP and HR gradually returned to their initial values. A second injection of renin into the lateral ventricle gave rise to similar changes in cardiovascular functions.

When renin was injected in doses of 20 and 30 μg/kg not only a rise in BP and fall in HR were observed, but also considerable changes in the ECG. For instance, in 12 animals (75%) during the period of stable elevation of BP and marked bradycardia, enlargement of the T wave (giant T wave) was observed. The period of all of BP and normalization of HR was characterized by the appearance of a negative T wave (Fig. 2). In nine animals (56%) of this group cardiac arrhythmias also were observed to appear during the period of stable elevation of BP and marked bradycardia, whereas in three animals (19%) spontaneous attacks of extrasystoles occurred. In four animals (25%) no significant changes were found on the ECG at the time of maximal elevation of BP and marked bradycardia, but a fine muscle tremor was observed.

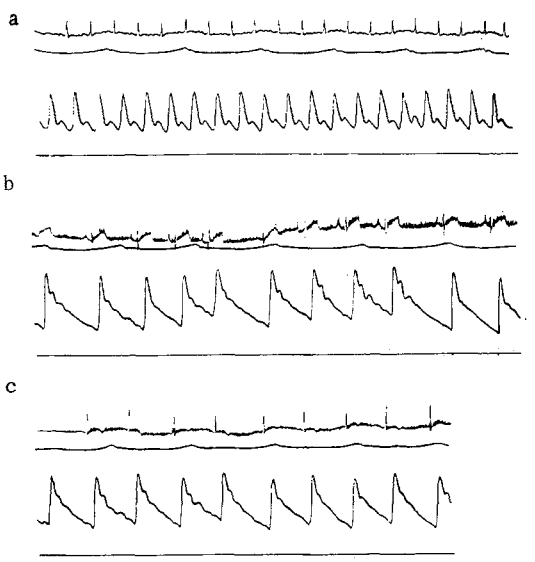

Fig. 2. Time course of changes in autonomic parameters and ECG after intraventricular injection of renin. From top to bottom: ECG in standard lead II, respiration, BP; a) background trace, b) trace recorded 25 min, c) 60 min after intraventricular injection of renin.

Intraventricular injection of renin in doses of 10 and 30 μg/kg caused changes in respiration rate in all the animals. For instance, in six animals (38%), in response to central application of renin, slowing of the respiration rate by 36 ± 2.3 was observed, whereas in 10 animals (62%), conversely, the rate was increased by 30 ± 2.1.

In eight animals, besides recording autonomic parameters, cortical electrical activity also was recorded. Intraventricular injection of renin in doses of 20 and 30 μg/kg was shown to cause desynchronization of the cortical EEG against a background of slow-wave, high-amplitude electrical activity; the desynchronization was formed 0.8 ± 0.2 min before the rise of BP and fall of HR.

Electrical stimulation of the ventromedial hypothalamus for 3 sec caused BP of the intact animals to rise by 23 ± 1.2 mm Hg and HR to fall by 19 ± 1.5/min. After intraventricular injection of renin in doses of 20 and 30 μg/kg in the period of rise of BP and fall of HR, similar stimulation of the ventromedial hypothalamus caused an additional rise of BP by 15 ± 1.4 mm Hg and a fall in HR by 14 ± 1.3/min. Stimulation of the ventromedial hypothalamus during the period of stable elevation of BP and of marked bradycardia, it will be noted, did not cause any additional increase in BP or bradycardia, despite the changes in respiration.

The latent period of the pressor response to stimulation of the ventromedial hypothalamus, both in intact animals and after intraventricular injection of renin, remained unchanged throughout the experiment at 1.1 ± 0.05 sec. No statistically significant difference likewise could be found between the time when the pressor response reached a maximum and the duration of the response in intact animals and in animals after injection of renin. Against the background of the central action of renin, the threshold of stimulation of the ventromedial hypothalamus was lowered. For instance, whereas the threshold of stimulation of the ventromedial hypothalamus producing the maximal pressor response in intact animals was 180 ± 8 μA, after intraventricular injection of renin in doses of 20 and 30 μg/kg the strength of stimulation of the ventromedial hypothalamus required to produce the maximal pressor response was 120 ± 6 μA. Against the background of the central action of renin in five animals (31%) with an initial BP level of 76 ± 0.8 mm Hg, in response to stimulation of the ventromedial hypothalamus multiple ventricular extrasystoles appeared (Fig. 3). In another 11 animals (69%) with an initial BP of 95 ± 1.2 mm Hg, stimulation of the ventromedial hypothalamus against the background of the central action of renin caused no disturbances of cardiac activity.

To compare the effects of the central and peripheral action of renin, renin was injected intravenously into two animals in a dose of 100 μg/kg. Under these circumstances a transient

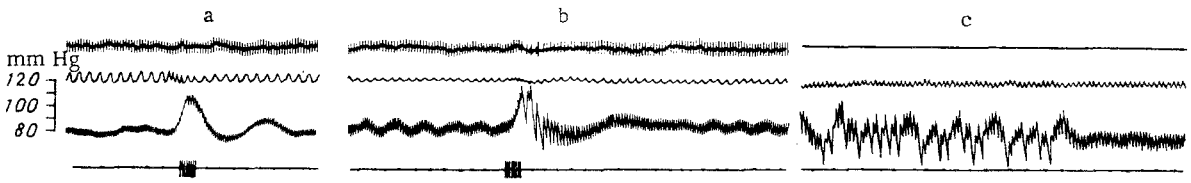

Fig. 3. Effect of stimulation of ventromedial hypothalamus on cardiovascular responses during the central action of renin. From top to bottom: ECG, respiration, BP, marker of stimulation of ventromedial hypothalamus. a) Background trace; b) trace recorded 15 min after intraventricular injection of renin; c) spontaneous appearance of grouped ventricular extrasystoles.

rise of BP by 12 mm Hg was observed after a latent period of 3 min (the duration of the response was 6 min), and HR fell by 24 beats/min. The threshold, magnitude, and duration of the pressor response to stimulation of the ventromedial hypothalamus remained unchanged in these experiments compared with the corresponding values in intact animals.

These experiments thus showed that renin obtained from the dog kidney, injected into the lateral cerebral ventricles, exerts high biological activity, manifested as a considerable and prolonged rise of BP, a fall of HR, disturbance of cardiac activity with the appearance of arrhythmias and extrasystoles, lowering of the threshold of stimulation of the ventromedial hypothalamus, and changes in respiration and the EEG. Injection of renin into the **bloodstream in doses** much greater than those used for central administration caused no significant changes in autonomic parameters. It can accordingly be concluded that the renin–angiotensin system of the brain is a regulatory factor relative to the renin–angiotensin system of the blood plasma and plays a leading role in the central regulation of BP. The experiments also showed that central application of renin increases the excitability of structures in the central nervous system. Under these conditions even brief stimulation of the negative emotiogenic centers of the hypothalamus acts as a factor provoking the onset of ventricular extrasystoles. There is evidence in the literature that the threshold of onset of ventricular fibrillation is lowered in dogs by stimulation of the posterior hypothalamus [9, 15], and that the threshold of onset of ventricular extrasystoles, paroxysmal ventricular tachysystole, and fibrillation is lowered during stimulation of negative emotiogenic centers of the hypothalamus [2]. The experimental results indicate that a similar lowering of the thresholds of onset of ventricular extrasystoles can be achieved by the intraventricular injection of renin, one of the components of the renin–angiotensin system.

In the present investigation renin was administered centrally in doses evidently much greater than the physiological level of natural renin, entering the renin–angiotensin system of the brain. As a result, different changes and disturbances of cardiovascular functions could be detected. It can accordingly be postulated that in different natural and also experimental situations the renin–angiotensin system of the brain undergoes activation, with the release of biologically active substances including renin, which lead to stable elevation of BP and to a disturbance of cardiovascular functions.

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ROLE OF PERIPHERAL ADRENERGIC STRUCTURES IN DISORDERS OF MOTOR COMPONENTS OF OPERANT BEHAVIOR IN RATS WITH EMOTIONAL STRESS

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KEY WORDS: adrenoreceptors; motor skills; emotional stress.

Disturbances of the fine motor skills in man during emotional stress are realized to some extent through activation of adrenergic structures of skeletal muscles [4]. It has been shown that substances activating peripheral β-adrenoreceptors (in doses without any central action) or stimulating release of endogenous catecholamines into the blood stream, can disturb reproduction of motor programs formed previously [3].

The aim of this investigation was to continue the study of the role of development of disturbances of precise movements during emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 220-350 g. The effect of emotional stress and of the test substances on ability to reproduce precise motor skills was studied by a modified Sidman's operant activity method [12]. To avoid electric shocks applied through the electrode floor of the chamber, the animal had to press a pedal with a force of not less than 3 g and not more than 5 g. In that case the painful electrical stimulation (ac pulses, 50 Hz, stabilized amplitude 0.5 mA, duration 1 sec, following frequency once every 5 sec) was interrupted for 20 sec. If the pedal was not released during the 20-sec interval and pressed again with the required force, electrical stimulation was resumed. If the force of pressure on the pedal exceeded 5 g, at the beginning of the period of interruption of electric shocks the animal received extra punishment in the form of a series of dc pulses (1.5 mA, 5 msec, 50 Hz) for a duration of 2 sec. Experiments were carried out on 36 rats trained beforehand in this type of operant activity. Training was given in two stages: First the animals were taught operant activity in accordance with Sidman's usual program. During training the strength of pulses applied to the electrode floor was 1 mA. After the skill of avoiding electric shocks (allowing not more than 5-7% of shocks applied to the electrode floor) had been achieved the animals were taught to work according to a modified program. Experiments began on animals which, in the course of 60 min of activity by the modified program, did not press too strongly on the pedal more than 10 times. As the criterion of accuracy of reproduction of the preformed motor skill, the number of times the animal pressed too strongly during a 60-min experimental session was used.

To evaluate the central effects of the compounds, the method of recording electrical activity in the sensomotor cortex, lateral area of the hypothalamus, and the dorsal hippocampus through chronically implanted electrodes was used. The electrodes were inserted stereotaxically, taking coordinates from the atlas [8]. Experiments began 3-4 days after the operation. The EEG was analyzed by integration (epoch of analysis 10 sec) within frequency bands of 2-4, 4-8, 8-13, 13-20, and 20-30 Hz. The method of constructing histograms of distribution by number of intervals of different duration between pressings on the pedal during

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