ACTION OF PARASYMPATHETIC AGENTS ON RENIN SECRETION *IN VITRO*

V. I. Fedorov

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Numerous histochemical and immunofluorescence studies have shown [3] that besides adrenergic nerve fibers, the juxtaglomerular apparatus, blood vessels, and tubules in the renal cortex are innervated also by sympathetic nerves with acetylcholinesterase activity. Attempts to induce changes in renin secretion by the action of acetylcholine under intact conditions [8, 11] proved unsuccessful. However, an increase in renin secretion was found in some investigations in response to depression of parasympathetic activity: bilateral vagotomy [5, 15], application of cold to the vagus nerve [5, 10], and injection of nicotinic (N) cholinolytics and ganglion blockers [6]. At the same time, activation of renin secretion was observed in response to injection of a muscarinic (M) cholinomimetic [13] and renin secretion was depressed after bilateral vagotomy [7, 12]. All these results were interpreted by the authors cited either as hypotensive or sympathico-adrenal activation of renin secretion or as abolition of tonic parasympathetic inhibition of adrenergic renal nerves.

To study the possibility of a direct effect of parasympathetic agents on renin secretion, in the investigation described below experiments were carried out *in vitro*.

EXPERIMENTAL METHOD

Renin secretion by slices of rat renal cortex was studied by the method [9] in the modification [4]. The kidneys were taken under pentobarbital anesthesia. The weight of each slice was 25-100 mg and the volume of incubation medium 500 µl. Incubation was carried out for 60 min in the presence of α- and β-adrenoblockers dihydroergotamine and propranolol, in final concentrations of 10^{-4} M and 10^{-6} M, respectively. The dose was chosen on the basis of previous observations [14], showing that the adrenoblockers, in these concentrations, suppress renin secretion stimulated by adrenomimetics, without affecting it themselves. These findings are in agreement with my own preliminary investigations. A 20-µl sample of the incubated material was taken 30 min after the beginning of incubation for subsequent determination of the initial rate of renin secretion, and one of the parasympathetic agents for testing was added to the incubation medium. Nicotinic cholinomimetics nicotine and cytosine, the N cholinolytic adiphenine, ganglion blockers benzohexonium and kvateron, which under these experimental conditions can be regarded as N cholinolytics, the M cholinomimetics aceclidine and pilocarpine, M cholinolytics atropine and platyphylline, and cholinesterase inhibitors galanthamine and neostigmine were studied. Each substance was added in a quantity creating one of the chosen concentrations of the drug (10^{-8} M, 10^{-6} M, or 10^{-4} M). The volume injected was 20 µl. At the end of incubation 20 µl of the sample was taken for analysis and frozen, and the tissue was weighed. On the day of analysis 500 µl plasma from nephrectomized rats, diluted 1:4 with 0.2 M maleate buffer (pH 6.0), 5 µl of 0.3 M solution of phenylmethylsulfonyl fluoride, and 25 µl of a 2 mM solution of EDTA-Na_2 to inhibit activity of converting enzyme and angiotensinases, were added to the corresponding sample as substrate for renin. The samples were incubated for 60 min at 37°C. The quantity of angiotensin I formed during incubation was estimated by radioimmunoassay using standard kits from "Clinical Assays," USA. Preliminary tests showed that samples of incubation medium, untreated with renin substrate, like plasma which had not been in contact with incubation medium, did not contain angiotensin I, and in the reaction of renin with its substrate angiotensin I production was a linear function of time. The total quantity of renin secreted during every 30 min of incubation of the slice

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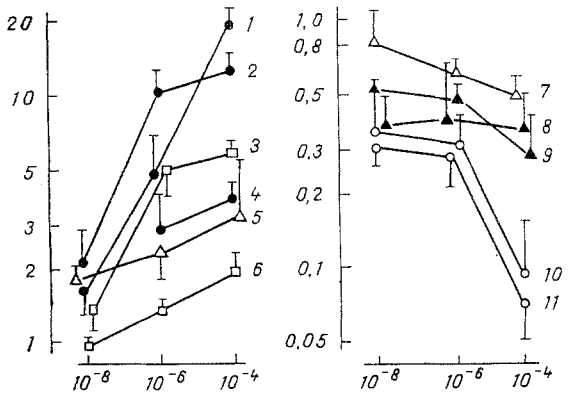

Fig. 1. Intensity of renin secretion by slices of rat renal cortex under the influence of parasympathetic agents. Abscissa, concentration of drugs added (in M); ordinate, intensity of renin secretion (in calculated units, plotted on logarithmic scale). Empty triangles — M cholinomimetics; filled triangles — M cholinolytics; empty circles — N cholinomimetics; filled circles — N cholinolytics; squares — anticholinesterase drugs. 1) Adiphenine; 2) benzohexonium; 3) neostigmine; 4) kvateron; 5) aceclidine; 6) galanthamine; 7) pilocarpine; 8) platyphylline; 9) atropine; 10) cytosine; 11) nicotine.

was calculated with a correction for the weight of tissue and volume of incubation medium tested and of medium in which renin reacted with the substrate, and was expressed in nanograms of angiotensin I formed per hour of incubation of the renin substrate with an aliquot of the test incubation medium, per milligram tissue. The intensity of renin secretion by the slice under the influence of a parasympathetic agent was calculated by the equation:

$$I = \frac{A - A_0}{A_0},$$

where I is the intensity of renin secretion, A the total quantity of renin secreted throughout the incubation period, and A_0 the total quantity of renin secreted in the first 30 min of incubation.

EXPERIMENTAL RESULTS

Renin secretion when no drugs were added was similar in every 30 min of incubation, in agreement with data in the literature [4]. The results of experiments with addition of various parasympathetic agents to the incubation medium are illustrated in Fig. 1. It will be clear that N cholinomimetics and their antagonists had a marked effect on renin secretion (kvateron less so). Their effects were opposite, and mainly dose-dependent. In the case of M cholinomimetics and M cholinolytics and the anticholinesterase drugs, differences (not significant) were observed between the action of different concentrations (neostigmine was an exception in some respects). Their effects were significantly weaker than those of the previous group of substances. The opposite direction of the effects of M and N cholinolytics or of M and N cholinomimetics, with the exception of pilocarpine, will be noted. However, we know [1] that pilocarpine can increase the sensitivity of cholinergic structures to N cholinomimetics. As regards cholinesterase inhibitors, neostigmine affects renin secretion in the same direction as aceclidine and, in this case, it can be regarded as a M cholinomimetic; galanthamine, on the other hand, evokes neither muscarine- nor nicotine-like effects, and for that reason its quite weak effect on renin secretion is evidently the vector sum of opposite effects.

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CHANGES IN NEURONAL TRACE RESPONSES OF THE SENSOMOTOR CORTEX AND PUTAMEN INDUCED BY HALOPERIDOL

L. A. Zhavoronkova and E. V. Rusinova

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Mechanisms of action of haloperidol are mainly studied in behavioral experiments on animals during conditioning [3, 5] and also on models such as amphetamine stereotypy, rotatory responses to electrical stimulation of the caudate nucleus, etc. [7]. However, there is no information on the effect of haloperidol on the ability of neurons of different structures to form and preserve trace responses.

The aim of this investigation was to compare the action of haloperidol on formation and reproduction of conditioned trace responses by neurons of the putamen and sensomotor cortex. The reasons why these brain structures were chosen were, first, that the basal ganglia and, in particular, putamen are the point of application of the action of haloperidol [2], and second, that the putamen and neocortex have many direct connections [1, 6].

EXPERIMENTAL METHOD

Experiments were carried out on 25 waking, unimmobilized rabbits. Trace activity of 122 neurons in the putamen and 121 neurons in the sensomotor cortex was analyzed during formation and reproduction of a conditioned reflex to time. Motor conditioned reflexes to time were formed by combinations of an acoustic stimulus (conditioned stimulus – clicks: 10/sec) with electrodermal stimulation – EDS (unconditioned stimulus – square pulses: 40/sec, 4-6 V) applied at a constant 30-sec interval, and reproduction of these responses was tested with omissions of combinations at assigned intervals. Clicks were applied for 2 sec, whereas EDS was applied after isolated action of the acoustic stimuli for 1.5 sec, and it continued for 0.5 sec [4]. Unit activity was recorded during 10 combinations, a series of 15 omissions of stimuli, and a repeated series of combinations and omissions in intact animals and in animals receiving haloperidol. Haloperidol was injected intravenously in a dose of 0.2 mg/kg into animals to which about 40-50 combinations had been presented. The significance of differences in responses of the cells was determined by the Wilcoxon–Mann–Whitney U test.

EXPERIMENTAL RESULTS

Changes in trace activity of neurons in the putamen and sensomotor cortex under the influence of haloperidol were diametrically opposite in character. In the putamen the number of neurons exhibiting trace responses after injection of haloperidol increased by 21% whereas

Laboratory of Neuronal Mechanisms of Plastic Processes, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Rusinov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 307-308, March, 1984. Original article submitted March 22, 1983.

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