BIOPHYSICS AND BIOCHEMISTRY

Effect of the Hypothalamic Cardioactive Protein-Hormonal Complex on Choline Sensitivity of the Small Intestine and Contraction of the Vas Deferens in Rats

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A cardioactive protein-hormonal complex capable of increasing the sensitivity of the small intestine to acetylcholine about 2.5 times was isolated from magnocellular nuclei of the hypothalamo-neurohypophyseal system. Moreover, this complex enhanced contraction of the vas deferens caused by transmural stimulation, exogenous noradrenalin, and phenylephrine. The findings indicate release of transmitters from cholinergic and adrenergic neurons under the influence of the complex.

Key Words: *noradrenergic transmitter; cardioactive protein-hormonal complex*

Progress attained in investigations of the neurochemical aspects of interactions between the neural and hormonal regulation factors, notably studies of the molecular mechanisms of hormonal interactions with transmitters and modulators of synaptic transmission in the hypothalamus, has contributed greatly to recent advances in neuroendocrinology. Three cardioactive protein-hormonal complexes formed from noncovalently bound cardioactive neurohormones and their protein carriers have been isolated from the hypothalamic magnocellular nuclei [7,8]. The protein-neurohormone C (PNC) complex, which has proved to be an active regulator of various metabolic processes in the brain and some internal organs, specifically, in the heart [9], has been studied in detail. Studies of the *in vivo* and *in vitro* effects of this complex on the heart have revealed a manifest coronarodilating action developing with a long latent period and persisting for a long time.

In this research we studied the effect of PNC on intestinal smooth muscles and on contraction of the vas deferens of rats, as well as on acetylcholine-receptor protein interactions.

MATERIALS AND METHODS

Preparations of cardioactive protein-hormonal complex isolated from bovine hypothalamus and puri-

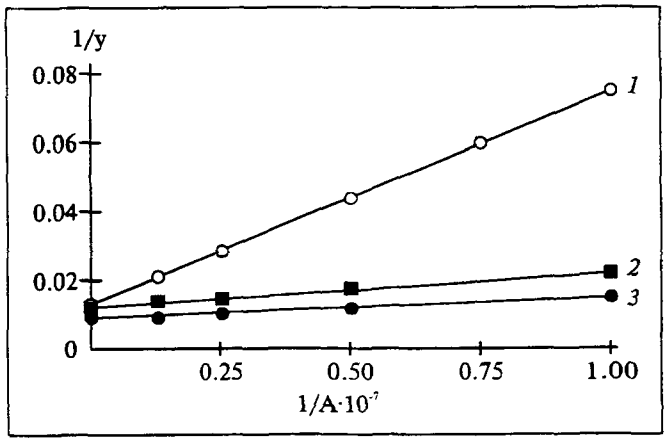

Fig. 1. Relationship between effect of AC and its concentration in health (*1*), under the action of PNC (*2*), and after washing free of PNC (*3*). Here and in Figs. 2 and 3: abscissa: inverse concentrations, ordinate: inverse values of the effect.

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TABLE 1. Effect of PNC on Choline Sensitivity of Rat Small Intestine Fragment

Experimental conditions	K_{AC}, $\times 10^{-7}$ M	Maximal reaction, mm
In health	2.47±0.83	45.8±12.03
During exposure to PNC	0.97±0.12*	57.6±13.31
After washing free of PNC	0.7±0.04*	66.1±17.97

Note. Here and in Table 2: an asterisk shows $p<0.1$ *vs.* the norm. Data of 5 experiments are presented.

fied after a scheme we developed previously [7] to an electrophoretically homogenous state were used.

Experiments were carried out with outbred male white rats weighing 250 g. The animals were stunned with electric current and decapitated. Fragments of the small intestine and vas deferens were prepared as described previously [4,6].

The sensitivity of the small intestine fragments to acetylcholine (AC) was assessed by the kinetic method [5]. Effects of PNC added to incubation media in a concentration of 0.005 to 0.01 μg were estimated after 40 min of incubation with the small intestine preparation.

The postganglionic sympathetic nerve endings were irritated by transmural stimulation of the vas deferent [1]. Ring electrodes were used [10] with 15-Hz pulses of 0.08 to 0.8 msec duration generated for 5 sec by an ISE-0.1 electrostimulator at a submaximal voltage. The principal parameters of the adrenergic reaction, the constant (K) and maximal reaction, were calculated as described previously [5]. Synaptosomes from the cerebral cortex were isolated as reported elsewhere [11].

Protein was measured after Lowry [12]. The protein concentration varied from 0.3 to 0.4 mg/ml per sample during assessment of the absorption spectrum. Absorption spectra were measured using a Unicam SP-800 recording spectrophotometer at wavelength 240 to 300 nm. Before measuring, the cholinesterase inhibitor eserine sulfate was added in a concentration of 10^{-5} M to samples diluted 1:6 with phosphate buffer at pH 6.6. After measurement of the reference spectrum, acetylcholine chloride in a concentration of 10^{-6} M was added to both cuvettes.

The results were statistically processed using Student's *t* test.

RESULTS

A 40-min incubation of a small intestine fragment with PNC increased its sensitivity to AC. The evident increase of the "step" of the accumulation curve, particularly when low AC concentrations were used, proves this. The curve plotted in the double inverse coordinate system (Fig. 1) shows this increase of choline reactivity as a reduced angle of the curve slope, this indicating a relative increase in the number of active receptors. The constant of cholinoreceptors (K_{AC}) reliably decreases in the presence of PNC to 40% of the reference value, on average. It may be deduced that the sensitivity of the fragment to AC increases approximately 2.5 times, with the maximal reaction value being virtually the same (Table 1).

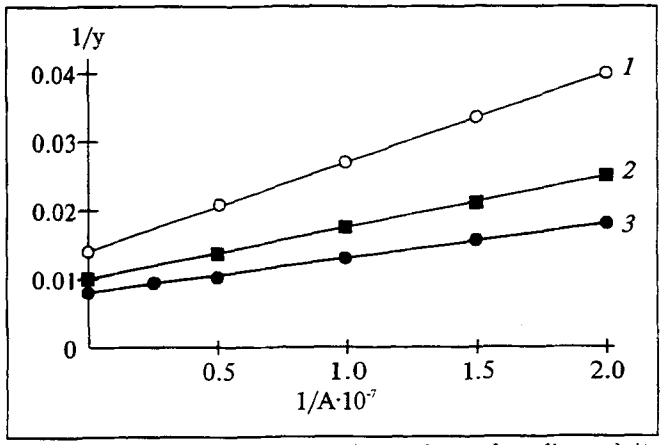

Fig. 2. Relationship between effect of noradrenalin and its concentration in health (*1*), under the action of PNC (*2*), and after washing free of PNC (*3*).

It is noteworthy that the sensitivity of the small intestine fragment to AC did not normalize after a 30-min washing from PNC in Tyrode's solution. Since the effect of PNC is not dose-dependent, one may assume that this complex causes specific biochemical shifts in tissues. Washing free of the compound causing these shifts did not lead to rapid normalization.

AC is known to reduce the absorption spectrum of cholinoreceptor protein in the ultraviolet 240 to

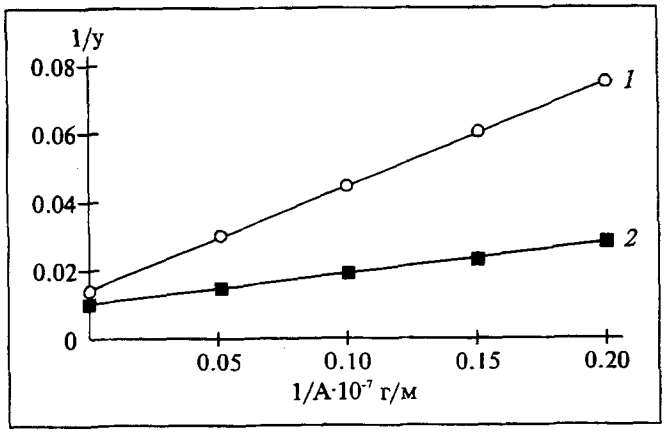

Fig. 3. Relationship between effect of phenylephrine and its concentration in health (*1*) and under the action of PNC (*2*).

TABLE 2. Effect of PNC on Contraction of Rat Vas Deferens Caused by Noradrenalin and Transmural Stimulation

Experimental conditions	Transmural stimulation, mm	Noradrenalin, $K \times 10^{-7}$ M	Maximal reaction, mm
In health	48±1.9	10.94±3.12	78.5±8.29
During exposure to PNC	56±2.9*	4.27±0.99*	94±10.6
After washing free of PNC	55±6.2	3.8±1.19	102.5±10.6

300 nm band during interaction with its active center, with the maximal changes observed in the 260 nm band. Our studies showed that low-molecular-weight cardioactive compounds released from PNC, which we denoted as nC_{7a}, nC_{7b}, nC_{10a}, and nC_{10b} (multiple forms of neurohormone C) [3], simulate the effect of AC. Moreover, administration of these compounds in physiological doses in parallel with AC potentiated the hypochromatic effect of AC. These changes are particularly evident in the synaptosomes of the cerebral cortex and cardiac tissue extract, where the hypochromatic effect is 2-3 times increased. The mechanism of the hypochromatic effect of these compounds is still unclear. Certain conformational changes in the active center of cholinoreceptor protein may contribute to it.

A study of the effect of PNC on the contraction of the rat vas deferens caused by transmural stimulation and noradrenalin and phenylephrine showed a varying degree of increase of receptor sensitivity to these agents (Figs. 2 and 3). The constant of the adrenergic reaction of the vas deferens was 2.5 times increased after 40-min incubation with PNC (Table 2), whereas the maximal reaction value increased only negligibly, similarly as with transmural stimulation. The reaction to phenylephrine changed in the same direction and was more (approximately 3 times) pronounced.

It is noteworthy that changes in all the parameters were more expressed after washing (Table 2).

These findings and the results we obtained previously for potassium ion stimulation of noradrenalin released from the synaptic endings of the hypothalamus [2] permit us to assume that in this case, as before, this transmitter is released from adrenergic neurons under the influence of PNC. It is also possible that PNC directly acts upon the cell membrane, thus increasing the sensitivity of receptors incorporated in the membrane to specific effects of transmitters. This increase of transmitter sensitivity, in turn, may be an alterative explanation for the coronarodilating action of PNC.

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