GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of the Dipeptides Gly-Pro and Pro-Gly, Glycine, and Proline on Cardiotropic Effects of Acetylcholine

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The dipeptides Gly-Pro and Pro-Gly, glycine, and proline do not affect contractile activity of the Straub cardiac preparation of the frog. Gly-Pro and glycine augment, while Pro-Gly moderates the effects of acetylcholine. The effect of proline was insignificant. Equimolar amino acid mixtures and mixtures of the dipeptides decrease the effects of acetylcholine. Direction and the degree of Gly-Pro effect attests to specific action of this peptide on cardiac activity.

Key Words: *Gly-Pro; Pro-Gly; acetylcholine; heart*

Recent years yielded ample evidence on participation of many peptide regulators in various processes in the normal and pathological conditions [3,6,7]. However, the study of functional role of peptide regulation and its interpretation are complicated by a number of specific features of endogenous peptides, most prominent of them being the cascade character of their action [5]. In the process of proteolytic catabolism the final stage of cascade effect of regulator peptides can be mediated by the most simple oligopeptides. Oligopeptides that are the most resistant to proteolysis contain proline: Gly-Pro, Pro-Gly, Pro-Gly-Pro, and Gly-Pro-Gly-Gly.

These amino acid sequences are present in various regulator peptides: enterostatin, β-casomorphine, lulliberin, β-lipotropin, gastrin, tachykinins, etc. [7,13]. The probable precursors of these oligopeptides could also be such connective tissue peptides as collagen and elastin [11,14]. The simplest oligopeptides are shown to enter the enterocytes during digestion, so it is probable that some oligopeptides enter the blood [8,10,12]. Unfortunately, there are no data on tissue and plasma contents of the simplest proline-containing peptides. However, an endogenous cyclic dipeptide, cPro-Gly, was identified recently in the rat brain, and it was shown to have an antiamnestic activity [9]. The simplest oligopeptides composed of proline and glycine modulate nociception, blood coagulation, and the work of mucous membrane of the alimentary tract [1,2,4]. The physiological basis of their action is poorly understood.

Our aim was to study the influence of the dipeptides Gly-Pro and Pro-Gly, and their constituent amino acids on the effects of norepinephrine (NE) and acetylcholine (Ach) using the classical models of the rat deferent duct and the isolated frog heart preparation.

MATERIALS AND METHODS

The study was carried out on rat deferent duct [6] and on the Straub cardiac preparation of the frog. The oligopeptides Gly-Pro, Pro-Gly, Pro-Gly-Pro, and Gly-Pro-Gly-Gly (Sigma) as well as amino

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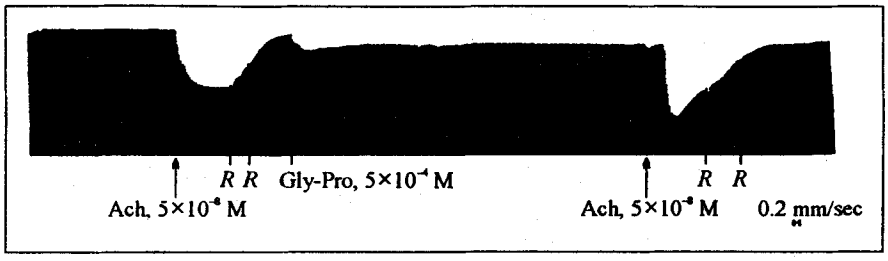

Fig. 1. Influence of Gly-Pro on the effects of acetylcholine (Ach). Rinsing of cardiac preparation is marked by *R*.

acids glycine and proline were used in the concentration range 10^{-3}-5×10^{-9} M, while NE and Ach were tested in concentrations of 10^{-6} g/ml and 5×10^{-8}-5×10^{-9} M, respectively. The degree of inhibitory effect of Ach on isolated heart was evaluated by comparing the amplitude of the response measured in control conditions and against the background of cardiac perfusion with the test substances. The results were statistically analyzed using Student's *t* test.

RESULTS

Analysis of the effects of dipeptides and amino acids on deferent duct revealed no significant changes in its muscular tone. We did not find any changes in the responses evoked by electrical stimulation. There were no significant changes in the amplitude of contraction induced by EN. These data indicate that the studied agents do not affect the β-adrenergic structures in this experimental model.

In the next series of experiments, the oligopeptides and their constituent amino acids were studied on isolated heart of the frog. In concentrations of 10^{-3}-10^{-8} M these agents virtually did not affect the amplitude and heart rate, although they slightly modulated the effects of Ach (Fig. 1). The effect depended on concentration (Fig. 2, *a*) and duration of perfusion (Fig. 2, *b*). The most demonstrative was the test of the effects of Ach after a 10-min preliminary perfusion of the heart by the test substance. The dipeptides had opposite modulating influences on the effects of Ach (Fig. 3). Thus, Gly-Pro (5×10^{-7} or 5×10^{-6} M) increased the inhibitory effect of Ach by 14±2% and 28±4%, respectively. By contrast, Pro-Gly (5×10^{-6} M) decreased the effect of Ach by 7±2%. The same but smaller opposite modulating effects were revealed when the heart was perfused by glycine (8±3%); proline demonstrated a similar tendency.

Other proline-containing peptides, Pro-Gly-Pro and Gly-Pro-Gly-Gly, did not produce significant changes in the amplitude of the heart beat against the background of Ach.

An unexpected result was obtained when studying the effect of the solutions composed the amino acid mixtures. Perfusion of isolated heart by solution containing equimolar concentrations of glycine and proline decreased the inhibitory effect of Ach, the decrease being more pronounced than that caused by proline or Pro-Gly alone (Fig. 3). A similar decrease of the inhibitory effect of Ach was observed

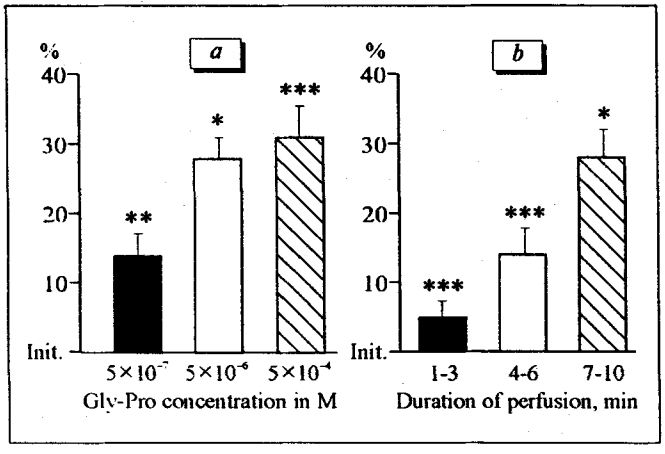

Fig. 2. Potentiation of the acetylcholine effects depending on (*a*) Gly-Pro concentration and (*b*) duration of perfusion of the heart with 5×10^{-6} M Gly-Pro. *$p<0.001$, **$p<0.01$, and ***$p<0.02$ in comparison with the initial values. Here and in Fig. 3: *Init.* corresponds to the ratio of the amplitudes of acetylcholine responses in test and control conditions.

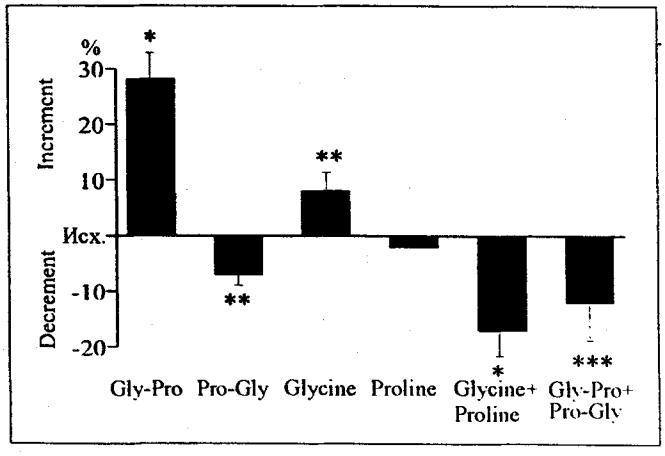

Fig. 3. Influence of Gly-Pro, Pro-Gly, glycine, proline, and equimolar mixtures of these amino acids and peptides on the effects of acetylcholine. Final concentration of all substances, the mixtures included, was 5×10^{-6} M. *$p<0.001$, **$p<0.02$, and ***$p<0.05$ in comparison with the initial values.

when the heart was perfused with solution containing both Gly-Pro and Pro-Gly.

While interpreting the results obtained, we should note that the peptides modify the effect of Ach to a greater extent than their constituting amino acids. Gly-Pro and glycine induced an unidirectional enhancement of the effects of Ach, while Pro-Gly or proline decreased them. Similar complex and opposite effects of the tested dipeptides and their constituent amino acid may be explained by participation of various receptor systems in these effects. The necessity of relatively prolong exposition of Gly-Pro to attain the strongest effects makes the interpretation of the data even more difficult. Presumably, it is related to the specificity of the processes induced by the receptors for Gly-Pro and Ach in the cells. One cannot exclude the possibility of transmembrane transport of Gly-Pro into the cells with subsequent triggering of some intracellular mechanisms.

Comparison of the direction and degree of the effects of Gly-Pro and other substances attests to a certain specificity of Gly-Pro influence on cardiac activity under the chosen experimental conditions. Together with other data on the effects of Gly-Pro and Pro-Gly [1,2,4], our findings will help to outline the search for the corresponding receptors.

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