a similar damaging effect on Ca-ATPase but also have similar mechanisms of action: in both cases polypeptide is damaged, fulfilling the catalytic function (Fig. 2) [3].

Earlier studies on the influence of HChE and LPO, induced in vitro, on the structure and function of the SR membrane [1, 4] also point out the principle role of LPO in the process of molecular pathogenesis during HChE in SR membranes. In both cases the efficiency of operation of the Ca-pump is reduced, viscosity of the membranes increased, the fatty acid composition of the phospholipids modified, membrane proteins undergo oligomerization, and the number of free SH-groups is reduced. Finally, it was shown that addition of the natural antioxidant TP to the diet simultaneously with ChS sharply lowers the level of LPO products and prevents disturbance of functional activity of the Ca-pump of SR.

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PHARMACOLOGICAL EFFECT OF EXOGENOUS HISTONES ON MUSCLES

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Histones are interesting as a test object because not only are they components of the eukaryotic chromosomal apparatus, but they also give rise to various pharmacological effects [1]. They attract even closer attention because histones have certain of the features which distinguish the primary structure of many regulatory peptides: a high prevalence of arginine, lysine, and proline and a correspondingly more frequent proximity of these amino acids in the primary structure.

Comparison of the amino-acid sequences of individual fractions of histones and of known regulatory peptides, polypeptide growth factor and hormones, undertaken by the writers with the aid of a computer, showed that histones contain tetrapeptide sequences identical to regulatory peptides and other protein regulators. Histones also contain longer fragments which are homologous with various peptides. The COOH-end of histone H4, for example, consists of

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the opiate-like sequence Tyr-Gly-Phe-Gly-Gly. The fragment A-B-Pro/Val-Pro/Val (where A denotes a dicarboxylic and B a basic amino acid), which is common to a number of peptide hormones and kinins [2], is contained in histones H2a, H3, and H4. The discovery of these determinants in the composition of histones led the authors to decide to investigate whether the corresponding purified fractions of exogenous histones have any effect on contractile function of muscles, and whether the opiate-like fragment of histone H4 affects opiate receptors. The results of an analysis of these effects of histones are given in this report.

EXPERIMENTAL METHOD

As a first step roughly purified fractions of calf thymus histones were isolated [4]. The histones were additionally purified by chromatography on CM-cellulose [5], and this was followed by analysis of their homogeneity by electrophoresis in 15% polyacrylamide gel with 6 M urea and 0.9 N CH_3COOH [6]. Histone H4 supplied by the firm "Boehringer" (West Germany) also was used in the experiments.

The pharmacological effect of histones on heart muscle was evaluated by studying their effect on the contraction rate of isolated atria of Wistar rats weighing 150-200 g. Ligatures were applied to the auricle of each atrium. One of them was fixed in a constant-temperature bath and perfused with Ringer's solution aerated with carbogen, the other was connected to a transducer based on a KTD-2B strain-gauge resistor. Atrial contractions with an initial strength of 1-1.5 g were recorded under isometric conditions at 31.5°C. Mechanical tension was transformed into electrical signals, which were recorded on an N-339 automatic recording voltmeter. Changes in frequency and strength of contractions were estimated as percentages of their initial levels.

The peptide Tyr-Gly-Phe-Gly-Gly was synthesized by a solid-phase method, using Merrifield resin. Anhydrous HF was used for clearing. The end products were isolated by high-efficiency liquid chromatography.

To determine the opiate activity of this peptide and of histone H4 itself, its effect on contractions of the guinea pig ileum (GPI), induced by electrical stimulation, was studied. Experiments were carried out on inbred animals of both sexes weighing 200-350 g. The animals were killed by cervical dislocation, after which the ileum was quickly removed. Segments of ileum measuring 2-3 cm long were placed in a constant-temperature cuvette (37.2°C) with Ringer's solution, aerated with a mixture of 95% O_2 and 5% CO_2. The segment of ileum was stimulated electrically by the indirect field method through parallel platinum plates, with square pulses 1 msec in duration and with a frequency of 0.1-0.2 Hz. After rinsing of the preparation for 20 min submaximal contractions were obtained by choice of potential difference (10-30 V). All stimulated contractions were completely abolished by perfusion with a solution containing 1 μM atropine. The preparations for testing were placed in the cuvette after the amplitude of contractions of the ileum had stabilized.

EXPERIMENTAL RESULTS

In a concentration of 10^{-12}-10^{-10} M the histones had virtually no effect on the frequency or strength of atrial contractions. Data on the effects of histones on the contraction frequency in a concentration of 10^{-9}-10^{-6} M are given in Table 1. All the tested histone fractions increased the spontaneous atrial contraction rate and the effect was dose-dependent. Fractions H1, H2a, and H3 had maximal chronotropic action in a concentration of 10^{-6} M, and H4 in a concentration of 10^{-7} M.

The histone fractions increased the strength of spontaneous atrial contractions in the following descending order: H4 > H1 > H2a > H3. It can therefore be postulated that the action of the histones is not determined by features such as the density of their positive charge or the type of their dominant basic amino acid. In fact, histone H4 has the least, and histone H1 the greatest density of basic amino acids in its molecule. Meanwhile histones H4 and H3 are similar in their arginine-lysine ratio, but with respect to order of activity they occupy extreme positions. This suggests that the intensity of the chronotropic effect of the histones is determined by specificity of their primary structure. It is possible that the strongest effect of histone H4 is due to the presence of a fragment common for vasoactive regulatory peptides in its sequence.

In a concentration of 10^{-8}-10^{-5} M the synthetic pentapeptide Tyr-Gly-Phe-Gly-Gly from histone H4 caused a dose-dependent reduction of amplitude of electrically stimulated contractions of GPI similar to that of Leu- and Met-enkephalins, and its action was virtually in-

TABLE 1. Effect of Histone Preparation on Spontaneous Contraction Rate of Isolated Rat Atria (M ± m)

Histone concentration, M	Increase in frequency of atrial contractions, % of initial values			
	H1	H2a	H3	H4
10^{-9}	4±2 (n=6)	0 (n=4)	3±2 (n=6)	4±2 (n=3)
10^{-8}	8±3 (n=6)	8±3 (n=5)	2±4 (n=5)	22±8 (n=7)
10^{-7}	13±3 (n=7)	14±6 (n=5)	11±3 (n=5)	31±11 (n=4)
10^{-6}	27±8 (n=6)	18±4 (n=6)	12±5 (n=5)	13±5 (n=5)

Legend. Number of experiments shown in parentheses.

stantaneous. The effect reached a maximum after 30 sec, and 2-3 min after administration of the preparation the amplitude of contractions of GPI returned to its initial level. EC_{50} in all experiments was about $2.5 \cdot 10^{-6}$ M. Besides inhibiting contractions of GPI, the pentapeptide in a concentration of 10^{-8}-10^{-5} M caused a dose-dependent reduction of its tone. In all experiments the action of the pentapeptide on the amplitude of contractions of GPI was abolished by naloxone in a concentration of 10^{-7}-10^{-6} M; in the same concentrations naloxone prevented the inhibitory action of the pentapeptide on the amplitude of contraction of GPI. Consequently, pentapeptide Tyr-Gly-Phe-Gly-Gly possesses opiate activity, and we suggest that it be called historphin, a name which reflects its origin from histone and its possession of opiate activity.

Histone H4 (in a concentration of 10^{-6}-$2 \cdot 10^{-6}$ M) increased only the tone of GPI, which was accompanied by a sharp decrease in the amplitude of the contractions. Addition of naloxone (10^{-7}-$2 \cdot 10^{-6}$ M) reduced the amplitude of contractions of the GPI muscle preparation even more.

The results are thus evidence that exogenous histones have a distinct chronotropic action on atrial muscle and that the strongest effect is associated with histone H4. Histone H4 also increased muscle tone of the ileum, while reducing the amplitude of contractions, and its COOH-terminal pentapeptide possesses opiate activity.

In the attempt to explain the chronotropic effect of histones on heart muscle and also hypertonia of the muscles of the small intestine under the influence of histone H4, the recently discovered modulation by polylysine of a special phosphatase which dephosphorylates the light chain of myosin and phosphorylase α in muscles must be recalled [3]. It has been shown that histone H1 is the regulatory factor of this phosphatase [7]. It is possible that exogenous histones change the relationship between phosphorylation and dephosphorylation in the muscle and, accordingly, change its functional activity.

The discovery of an opiate peptide in histone H4 is interesting also for the reason that histone, both exogenous and endogenous, can serve as the source of regulatory peptides and, through their intermediary, can affect various functions of the body.

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