CORRELATION BETWEEN DOSE AND PHARMACOLOGICAL EFFECTS OF CARDIAC PEPTIDES

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Peptides isolated from bovine heart (CP – cardiac peptides), with mol. wt. of under 15 kD, possess biological activity on the heart of laboratory animals. This action is exhibited under normal conditions by large doses, but in hypoxia and ischemia, by very small doses. The writers previously observed a protective action of CP in isoprenaline-induced myocardial necrosis [3] and in experimental myocardial infarction caused by ligation of the left coronary artery [2, 4], and the effects of the preparation on the course of myocardial infarction have been shown to depend on its concentration and mode of administration [4].

The aim of this investigation was to study correlation between the dose and pharmacological effect of CP on different models, with a view to establishing schedules of dosage for clinical use.

EXPERIMENTAL METHOD

A model of myocardial infarction was created on 120 noninbred albino rats weighing 160-180 g by ligation of the left coronary artery. Some rats received CP intraperitoneally after the operation. The size of the myocardial infarct (MI) was measured gravimetrically [1]. The hearts of guinea pigs weighing 230-250 g were obtained under thiopental anesthesia (75 mg/kg) for retrograde perfusion by Langendorff's method. The guinea pigs first were given an injection 500 U heparin. Perfusion with Tyrode solution was given without electrical stimulation and at a constant rate of flow of the solution of 10 ml/min; its temperature was +30°C and it was saturated with carbogen. The pH of the solution was adjusted with sodium bicarbonate to 7.4. CP were added intermittently in an initial concentration of $2 \cdot 10^{-8}$-$2 \cdot 10^{-4}$ g/ml to the top reservoir (air trap), and this was followed by continuous dilution and rinsing to remove the preparation. Contractions were recorded by means of a latex balloon placed in the left ventricle, which was connected to a pulse recorder, from which the signal was transmitted to a "Malysh" electrocardiograph. Cardiomyocytes were obtained by Vahouny's method [8]. The oxygen consumption of the isolated cardiomyocytes was recorded polarographically, using a Clark's electrode at +30°C. The capacity of the polarographic cells 1 ml. The rate of oxygen consumption by the cardiomyocytes was expressed in nanogram-atoms oxygen per minute per milligram protein. The protein concentration was determined by Lowry's method. The cardiomyocyte suspension was oxygenated by saturation with carbogen at room temperature.

EXPERIMENTAL RESULTS

The isolated guinea pig heart responded in a dose-dependent manner to intermittent addition of different concentrations of CP, followed by their dilution and rinsing; the effect began to appear with a concentration of

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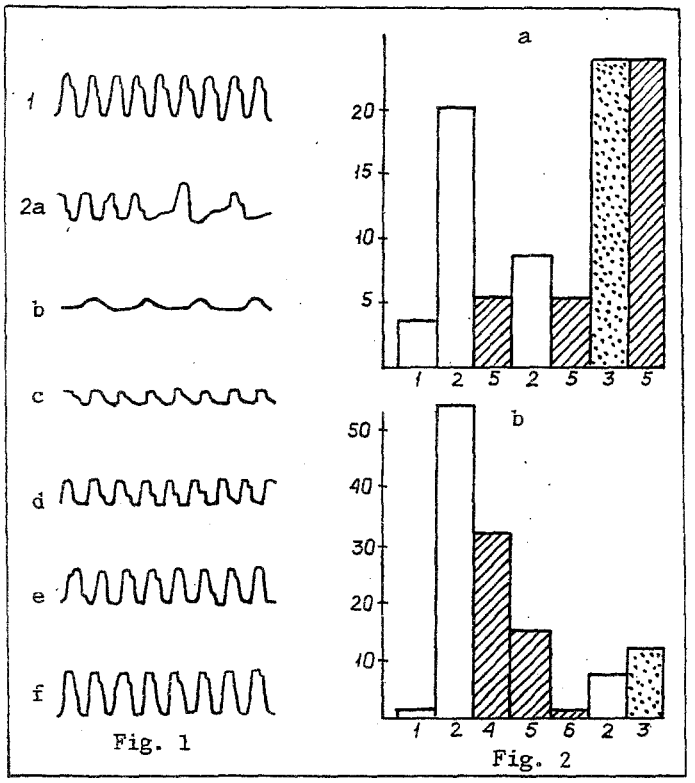

Fig. 1 Fig. 2

Fig. 1. Response of isolated guinea pig to heart to intermittent administration of $2 \cdot 10^{-4}$ g/ml CP followed by dilution and rinsing. 1) before addition of CP; 2: a-e) after addition of CP: a) a few seconds (amplitude already being reduced, transition to a slower rhythm can be seen), b) 2 min, c) 3 min (restoration of frequency, amplitude still remains low), d) 5 min, e) 10 min, f) 15 min after addition of CP (restoration of original values of parameters of contraction).

Fig. 2. Respiration of isolated rat cardiomyocytes in presence of additives: a) high, b) low CP concentration; 1) endogenous level (without additives), 2) addition of 100 μmoles sodium succinate, 3) addition of 500 μmoles NADH, 4) addition of 10^{-10} g/ml CP, 5) addition of 10^{-8} g/ml CP, 6) addition of 10^{-6} g/ml CP. Ordinate, rate of oxygen uptake (in ng-atoms/min/mg protein).

$2 \cdot 10^{-8}$ g/ml and more, and took the form of a decrease in the amplitude of contractions. The heart rate then decreased, but as the preparation was rinsed out, the previous amplitudes were restored. After rinsing for 15 min the parameters of contractile function were restored (Fig. 1). If the heart was exposed to total ischemia for 25 min and reperfusion began in the presence of CP, contractile function also was found to depend on the concentration of CP. For instance, in a concentration of 10^{-6} or, in particular of $5 \cdot 10^{-6}$ g/ml, a dose-dependent negative inotropic action was observed, whereas with CP in a concentration of $5 \cdot 10^{-7}$ g/ml in the perfusion fluid or lower, reversal of the effect was observed, namely improvement of parameters of contractility, an increase in the amplitude of contractions, and reduction of postischemic contracture [2]. In other words, the use of small doses of CP led to improvement of the parameters of contractile function, and this was seen particularly clearly in the presence of ischemia. The use of large doses of CP, however, leads to inhibition of myocardial contractility, i.e., it can be concluded that increasing the dose of the preparation leads to overdosage. It will also be evident that the ischemic myocardium is more sensitive to an increase in the CP concentration.

The most probable explanation of the observed effects of CP on the isolated heart is provided by data on their effects on energy metabolism in the cardiomyocytes. For instance, rat cardiomyocytes, when under conditions of oxygen deficiency, become more sensitive to the addition of exogenous succinate. In other words, the rate of oxygen

TABLE 1. Dimensions of MI as a Percentage of Mass of Left Ventricle 24 h after Coronary Occlusion in Rats Treated with Various Doses of CP

MI + physiological saline, control	MI + CP				
	10^{-4} g/kg	$5 \cdot 10^{-4}$ g/kg	10^{-3} g/kg	$5 \cdot 10^{-3}$ g/kg	$5 \cdot 10^{-4}$ g/kg
55,8±1,6	55,3±1,7	51,8±1,6	52,1±2,8	55,2±5,2	50,6±1,0*

Legend. *$p < 0.05$ compared with control.

consumption of a suspension of hypoxic cardiomyocytes rises in response to addition of succinate, and the more severe their hypoxic damage, the greater the rise. This hyperactive oxidation of succinic acid may run the risk not only of exhaustion of substrates of mitochondria with a low level of energization, but also of further uncoupling of oxidative phosphorylation [5, 7]. Inhibition of oxidation of succinic acid therefore becomes essential should an energy deficit develop during deep hypoxia. Stimulation of oxygen consumption induced by the addition of succinate to the cardiomyocyte suspension is considered to be an indicator of cell damage [6]. CP begin to exhibit an inhibitory action of respiration of cells existing under hypoxic conditions, in the presence of succinate added in a concentration of 10^{-13} g/ml, and with a further increase in concentration their effects are progressively enhanced (Fig. 2). It is important to note that oxygenation of the cell suspension with carbogen abolishes the effect of stimulation of respiration by succinate, so that the action of CP is comparable with that of oxygen, and can be regarded as a positive fact. However, with an increase in CP concentration in the medium to 10^{-6} g/ml or more, their inhibitory action begins to be manifested, not only on succinate-dependent, but also on NADH-dependent oxidation, so that with high concentrations of the preparation this may lead to almost total inhibition of respiration (Fig. 2a). In the presence of lower concentrations NADH-stimulated respiration is not inhibited by CP, unlike succinate-dependent respiration (Fig. 2b).

Thus a parallel is observed between the dose-dependent effects of CP on the two different models. CP in a concentration of $5 \cdot 10^{-7}$ g/ml or lower has a positive action on the contractile function of the heart and promotes optimization of respiration under conditions of oxygen deficiency. CP in a concentration of 10^{-6} g/ml or higher caused definite and dose-dependent inhibition of the contractile function and respiration of the cardiomyocytes, or even their total inhibition.

The results are in agreement with those of investigations of the effect of CP on the course of myocardial infarction in rats (Table 1). When CP was used in doses of $5 \cdot 10^{-4}$ and 10^{-3} g/kg a tendency was noted for the ischemic focus in the myocardium to decrease in size, whereas in a dose of $5 \cdot 10^{-3}$ g/kg this effect was canceled out. The effects of higher concentrations of CP were not studied on this particular model. However, doses causing death of mice have been found: $LD_{50} = 255 \pm 12$ mg/kg by intraperitoneal injection, which is more than 50 times higher than the therapeutic doses which may be used. Nevertheless, the results obtained in experiments with MI are evidence that overdosage of the preparation can occur, i.e., with an increase in the dose of CP the positive effects are initially enhanced, later depressed, and eventually negative aftereffects may also arise. It has been shown that repeated injections of CP in a dose of $5 \cdot 10^{-4}$ g/kg gave the strongest positive effect and led to a significant reduction in size of the myocardial lesion (Table 1).

Peptide bioregulators contained in the heart, in certain concentrations have a favorable effect of oxidative phosphorylation in cells in the ischemic zone, normalized contractile function, and delay cell death in the ischemic myocardium. However, increasing the dose leads to disappearance of the positive effect, and to subsequent enhancement of the negative effect of this therapy.

The fact that the effects of CP observed on different models are repeatable and unidirectional is evidence that the preparation can be used in clinical practice. However, the clinical use of CP to correct ischemic states requires establishment of optimal doses and modes of administration.

Since CP exhibit their action of myocardial function as a result of each injection and since repeated injections do not potentiate the negative effect (negative effects are exhibited if large doses are given in a single stage, or if the concentration of the preparation is constantly increased), in our view the use of small doses of the preparation at definite time intervals is indicated. Treatment is best given during a period of time when local ischemic lesions

arise in the myocardium as a result of acute (myocardial infarction) or chronic (ischemic heart disease) course of the disease.

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EFFECT OF NALOXONE ON ETHANOL-INDUCED MEMBRANE-BOUND ENKEPHALIN CONVERTASE ACTIVATION IN THE RAT MESENCEPHALON AND HYPOTHALAMUS

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The brain enkephalin system may be involved in the pathogenesis of alcoholism through its role both in the regulation of pathological addiction to ethanol and also, evidently, in the formation of tolerance to it [6, 9]. Recent investigations have revealed several general principles which suggest that the formation of these symptom-complexes is associated with lowering of the sensitivity of the system for enkephalin formation and release to the stimulating action of ethanol [1, 5, 10]. However, the fine mechanisms of the action of ethanol on enkephalin metabolism and, consequently, on the processes lying at the basis of adaptation of enkephalin neurotransmission to ethanol, require a closer study. One interesting object from this point of view is enkephalin convertase (carboxypeptidase E, H; E.C. 3.4.17.10). This carboxypeptidase B-like peptide hydrolyse catalyzes the final stage of processing of enkephalins, removing the hexapeptide precursor arginine or lysine from the C-end of the molecule, and activated by cobalt ions [7, 8, 12]. It was shown previously that as a result of chronic alochol intake by rats the activity of this enzyme in the brain changes significantly, and that the most marked changes are observed in the mesencephalon and hypothalamus [2]. The absence of any such effect in vitro [2] suggests that changes in enkephalin convertase activity in alcoholic intoxication may be quite complex in nature and may be connected with adaptation of the enkephalin system to long term exposure to ethanol.

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