COMBINED MORPHOLOGICAL AND BIOCHEMICAL STUDY OF CATECHOLAMINE METABOLISM IN THE MYOCARDIUM DURING FIBRILLATION AND AUTOLYSIS

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Recent investigations of the mechanisms of sympathetic regulation of the human heart have been carried out with the aid of modern methods of analysis of levels of catecholamines (CA) and some of their derivatives in the blood [6, 7, 9] and in biopsy material from the myocardium obtained from patients during operations on the heart [7, 8]. However, interpretation of the results is beset by definite difficulties. By analysis of subendocardial biopsy material no final conclusions can be drawn regarding the state of CA metabolism in all parts of the heart, nor can the results be compared with those of analysis of control material; as a rule patients investigated in this way have severe forms of cardiac pathology, and effects of previous medication cannot be ignored. The shortcomings of clinical investigation mentioned above can be avoided if the results of analysis of early autopsy material are taken into account. A combined and morphological and biochemical study of autopsy material has led, in particular, to determination of the pattern of distribution of CA in the heart and their metabolism in the myocardium in persons dying as a result of trauma or suddenly from ischemic heart disease [2]. For instance, it has been shown by neuromorphometric methods that in cases of sudden death the catecholamine-storing function of the nerve plexuses is depressed in all parts of the heart but to different degrees, and this is accompanied by redistribution of the neurotransmitter in sympathetic axons; this process, moreover, is unconnected with the mechanism of death and with postmortem changes [11]. Nevertheless, at this stage of the investigation it was impossible to judge the total CA content in the myocardium or the particular features of its metabolism and, in particular, the relations between their intraneuronal and extraneuronal metabolic pathways. In order to solve these problems it was necessary to develop and introduce an original method of analysis of autopsy material, using high-pressure high-performance liquid chromatography and determining 20 components of CA metabolism in the same specimen [3]. It was shown that characteristic features of CA metabolism in persons dying suddenly from ischemic heart disease, unlike those dying from trauma, are not only the unequal fall of the CA concentration, but also the presence of large quantities of products of extraneuronal CA metabolism in the myocardium [2]. Nevertheless, these studied also require experimental confirmation. In particular, it is not yet clear how the mechanism of death itself and the postmortem changes affect the concentration of CA and their precursors and metabolites in the heart, i.e., to what degree the results of analysis of the autopsy material reflect the state of CA metabolism during life or immediately before death.

The above-mentioned problems can be solved by experiments involving reproduction of fibrillation of the ventricular myocardium, the commonest mechanism of death, and in the presence of autolysis, with fixation of autopsy material during its course. The investigation described below was undertaken for this purpose.

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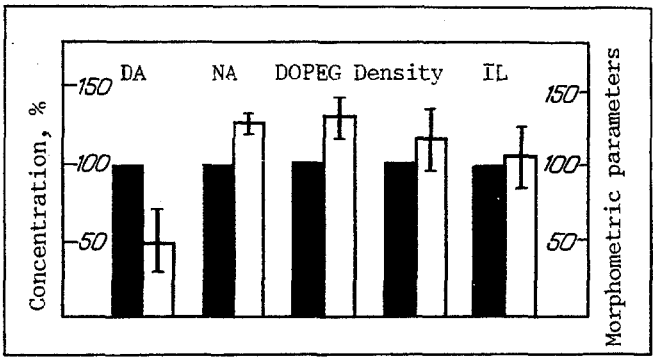

Fig. 1

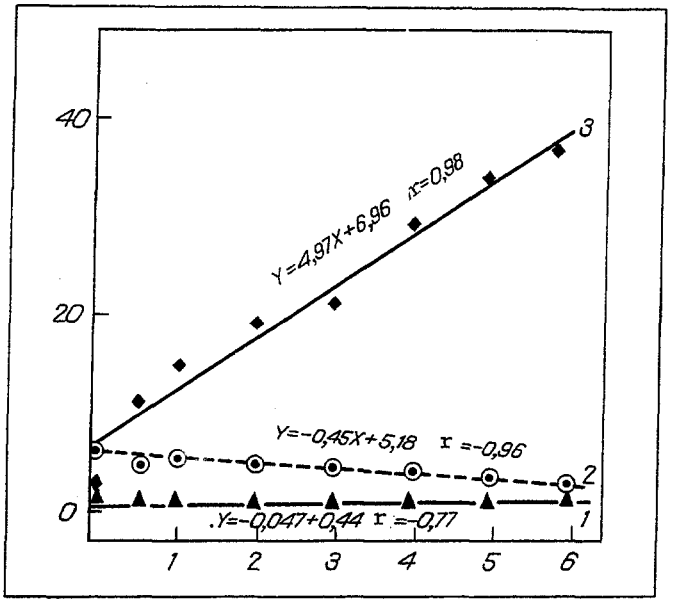

Fig. 2

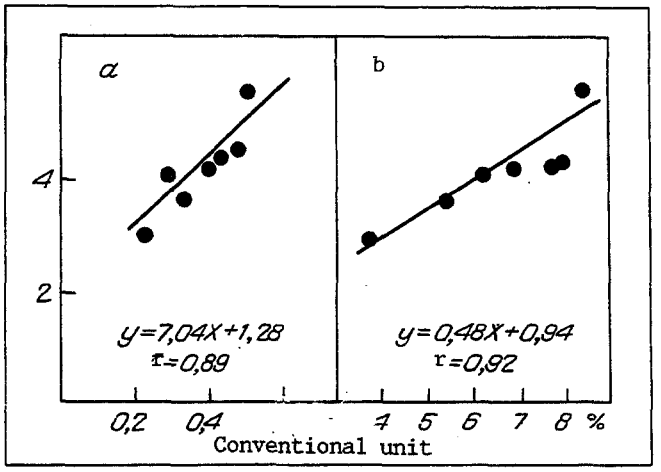

Fig. 3

Fig. 1. Changes in concentrations of dopamine (DA), noradrenalin (NA), dihydroxy-phenylethylene-glycol (DOPEG), and parameters of density and intensity of luminescence (IL) of adenergic nerve plexuses in left ventricular myocardium of dogs (n = 6) during ventricular fibrillation (in %). Black columns — 1st minute of fibrillation; unshaded columns — 5th minute of fibrillation.

Fig. 2. Concentrations of dopamine (1), noradrenalin (2), and dihydroxyphenylethylene-glycol (3), in left ventricular myocardium of dogs 1, 2, 3, 4, 5, and 6 h after death (n = 6). Abscissa) time after death (in h); ordinate) concentration of substances (in mmoles/g tissue).

Fig. 3. Correlation between noradrenalin concentration in left ventricular myocardium of dogs and intensity of luminescence (a) and density of adrenergic nerve plexuses (b) during autolysis. Abscissa: a) intensity of luminescence (in conventional units); b) density of nerve plexuses (in %); ordinate) NA concentration (in mmoles/g tissue).

METHODS

Experiments were carried out on mongrel dogs weighing 15-17 kg. Ventricular fibrillation was induced in six animals under pentobarbital anesthesia (35 mg/kg, intramuscularly) by electrical stimulation of the right ventricular myocardium at threshold strength during one cardiac cycle. Pieces of the cardiac layer of the left ventricular myocardium

were taken at the beginning of the 1st and end of the 5th minutes of fibrillation and fixed in liquid nitrogen. The state of metabolism of DA and their content in the nerve plexuses 0, 0.5, 2, 3, 4, 5, and 6 h after death were studied in five dogs which, under general anesthesia, were given an intracardiac injection of 10.0 ml of a 1% solution of potassium chloride to prevent fibrillation. Concentrations of CA, their precursors and metabolites were determined by HPLC with electrochemical detection [3]. The CA concentration in adrenergic nerve plexuses in adjacent areas of the myocardium was estimated by a quantitative neurohistochemical method with incubation of slices in glyoxylic acid solution [5]. The density of the nerve plexuses was determined by a dot planimetric method with incubation of slices in glyoxylic acid solution [5]. The density of the nerve plexuses was determined by a dot planimetric method [4] and their intensity of luminescence (IL), reflecting the average CA concentration in individual nerve terminals, was calculated by means of a spectrophotometric attachment to the LYUMAM FMÉL-1A microscope, by a technique of photometry elaborated previously [1]. The results are given in conventional units. Methods of analysis of variance and correlation analysis were used.

RESULTS

Toward the end of the 5th minute of fibrillation an increase in the noradrenalin (NA) and 3,4-dihydroxyphenyl-ethylene-glucol (DOPEG) concentrations relative to the 1st minute, on average by 20 and 25%, respectively, was found in all the animals, whereas the dopamine (DA) concentration in the specimens was reduced on average by 50% (Fig. 1). Concentrations of the remaining CA in the myocardium, including products of their extraneuronal metabolism, did not exceed the threshold of sensitivity of the method in all the animals. Neurohistochemical analysis of neighboring areas of the myocardium revealed no significant differences in mean values of density and IL of the sympathetic nerve plexuses in the early and late stages of fibrillation (Fig. 1).

The results of chromatographic analysis of the concentrations and spectrum of CA, their precursors and metabolites in myocardium during autolysis, are given in Fig. 2. In the course of the experiment, the qualitative composition of CA and their derivatives was unchanged in all the animals, and no new components appeared. Just as during fibrillation, changes in concentrations of the substances analyzed during autolysis were found only in the case of DA, Na, and DOPEG. The NA concentration fell successively by about 50% in the course of 6 h, whereas the DOPEG concentration rose during this period by 4-5 times as a result of oxidation of NA. Attention is drawn to the linear character of changes in concentrations of the substances depending on the duration of autolysis, which is confirmed by high values of the coefficients of correlation of the straight line on the graph for DOPEG ($r = 0.98$) and the negative values for NA ($r = -0.96$) and for DA ($r = -0.77$). A parallel neuromorphometric analysis of the catecholamine-containing structures of these specimens also revealed a successive decrease in IL and in the density of the nerve plexuses, which became significant 3 h after death — from 0.533 ± 0.035 to 0.304 ± 0.025 ($p < 0.01$) for IL and from 8.3 ± 0.08 to 6.1 ± 0.5 ($p < 0.05$) for the density parameters. The positive correlation between these values and the NA concentration is evidence that the latter discovered in the myocardium during 6 h of autolysis is mainly in nerve endings (Fig. 3).

Comparison of the control and experimental data thus leads to the conclusion that the qualitative composition of the myocardial CA and of their metabolites is unchanged in fibrillation and autolysis. Changes in concentrations of NA, DA, and DOPEG were found during these processes, whereas concentrations of the remaining CA derivatives were unchanged. These observations confirm the view that in sudden cardiac death there is an intravital decrease in the NA concentration in the heart and a strengthening of the role of its extraneuronal inactivation pathway, which is unconnected with the mechanism of death or with postmortem changes and it is therefore a characteristic feature of CA metabolism in the myocardium of persons dying suddenly from ischemic heart disease.

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