DARK AND PALE CEREBELLAR PURKINJE CELLS IN THE POST RESUSCITATION PERIOD

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The study of the CNS is central to modern resuscitation science [9]. During investigation of the state of the CNS, the functional heterogeneity of neuron populations must be allowed for [5]. To understand the mechanisms of postresuscitation brain pathology it is essential to determine the state of the various neuron subpopulations and to evaluate their role in the functioning of the population as a whole.

In the investigation described below Purkinje cells (PC) of the cerebellum, whose morphological heterogeneity is manifested as the existence of pale and dark neurons, were investigated. It is considered that dimorphism of PC is connected with differences in their physiological state [13]. The ratio between the numbers of pale and dark PC is an important parameter for assessing the state of the population [1]. Systemic circulatory arrest leads to a sharp change in this ratio [2].

The aim of this investigation was to determine the level of metabolism of pale and dark PC in the cerebellum of dogs resuscitated after clinical death, in order to evaluate the role of pale and dark neurons in the dystrophic and compensatory processes taking place in the cerebellum in the postresuscitation period.

EXPERIMENTAL METHOD

PC from the cerebellar vermis of mature mongrel dogs of both sexes were investigated. Two groups of animals were studied: dogs surviving systemic circulatory arrest for 12 min due to electric shock followed by complete recovery of the neurologic status (group 1), and dogs surviving clinical death for 10 min caused by acute blood loss with disturbances of static posture (group 2). Material for investigation was taken 2 weeks after resuscitation. The right half of the cerebellar vermis was fixed in Carnoy's mixture, treated by the standard method, and cut into sagittal paraffin sections. The thickness of the sections was measured by an interferometric method [6]. The concentration of dense substances in the nucleus and cytoplasm of the PC was determined with an interference microscope [3]. The cells to be measured were tagged. Sections were stained with cresyl violet by Nissl's method to identify the type of PC (pale, dark, or morphologically changed cells). The diameters of the nucleus and cell were measured by means of an ocular micrometer, after which the area of the nucleus and cytoplasm of the PC was calculated. The dry weight of the nucleus and cytoplasm of PC was determined by the appropriate equation [3] and expressed in picograms. Two dogs of each experimental group and two intact dogs (control) were studied. From each animal 150 PC were measured. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The information obtained by determining the areas of nucleus and cytoplasm of the different types of PC is given in Table 1. Data on the dry weight of the nucleus and cytoplasm of PC are given in Table 2. In intact dogs (control) the area of the nucleus of the dark PC was 23% less than that of the pale PC ($P < 0.01$). The concentration of dense substances in the nucleus of both types of PC was the same (288.7 ± 17.3 for the pale and 298.8 ± 23.6 for the dark cells). The dry weight of the nucleus of the dark PC was lower than that of the light

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TABLE 1. Area of Nucleus and Cytoplasm (in $cm^2 \times 10^{-9}$) of Pale, Dark, and Morphologically Changed Cerebellar PC of Intact (control) Dogs and Dogs Surviving Clinical Death of Varied Etiology (M ± m)

Type of PC	Group of animals	Nucleus	Cytoplasm
Pale	Control	622,2±31,8	1394,8±49,9
	1st experimental	900,4±52,5*	1828,2±110,9*
	2nd experimental	701,3±57,1	1582,2±117,4
Dark	Control	481,7±38,5	1283,4±65,0
	1st experimental	630,6±36,9*	1546,1±67,8*
	2nd experimental	521,5±27,4	1472,1±64,3*
Morphologically changed	Control	611,6±54,2	1330,7±75,9
	1st experimental	622,5±38,1	1536,6±73,6*
	2nd experimental	493,0±24,6*	1459,5±60,6*

Legend. Here and in Table 2, *$P < 0.05$ compared with control.

TABLE 2. Dry Weight of Nucleus and Cytoplasm (in $g \times 10^{-12}$) of Pale, Dark, and Morphologically Changed Cerebellar PC of Intact (control) Dogs and Dogs Surviving Clinical Death of Varied Etiology (M ± m)

Type of PC	Group of animals	Nucleus	Cytoplasm
Pale	Control	99,8±7,8	390,9±20,2
	1st experimental	116,2±11,5	481,0±37,8*
	2nd experimental	89,0±12,5	406,0±62,5
Dark	Control	80,3±8,7	386,0±27,1
	1st experimental	103,5±8,3*	405,2±26,9*
	2nd experimental	114,3±9,8	446,8±31,0
Morphologically changed	Control	90,0±10,8	346,7±30,6
	1st experimental	89,8±7,8	424,4±27,3*
	2nd experimental	95,6±6,5	467,1±24,3*

($0.05 < P < 0.1$). The area of the cytoplasm and the concentration of dense substances in the cytoplasm of the dark (537.6 ± 25.3) and pale (504.0 ± 18.7) PC, and also the dry weight of the cytoplasm of these cells did not differ significantly. The difference in the staining properties of PC by Nissl's method was thus evidently due not to a difference in the number of ultrastructures and in the protein content, but to the fact that dark PC have a higher RNA content [2] than pale or that the protein is unequally packed in PC of different types.

In the dogs of group 1 the area of the nucleus of the pale PC was increased, but the concentration of dense substances was reduced compared with the control (288.7 ± 17.3 and 232.6 ± 18.7, respectively; $P < 0.05$). The dry weight of the nucleus of the pale PC was unchanged compared with the control. The area of the cytoplasm of the pale PC was increased, and since the concentrations of dense substances was virtually unchanged compared with the control (473.8 ± 20.8), this resulted in an increase in dry weight of the cytoplasm of these cells.

In the dark PC from dogs of group 1 the area of the nucleus was increased and the concentration of dense substances, unlike that in the pale PC, was not reduced (298.8 ± 15.2) compared with the control, so that the dry weight of the nucleus of the dark PC was increased. The area of the cytoplasm of these PC was increased and the concentration of dense substances was a little lower than in the control (471.5 ± 22.6). The dry weight of the cytoplasm of the dark PC was increased.

Hence, 2 weeks after systemic circulatory arrest due to electric shock for 12 min in dogs followed by complete recovery of the neurologic status, the dimensions of the cytoplasm of the pale and dark PC were increased, as also was its dry weight. The increase in the latter is evidence of activation of protein synthesis [4]. Consequently, it can be tentatively suggested that during this period a process of intracellular reparative regeneration is taking place in both pale and dark PC, connected with intensification of the rhythm of renewal of the ultrastructures [10], due to an increase in the rate of protein synthesis, the universal mechanism for the compensation of disturbed CNS functions [12].

After clinical death a well-marked response of the nuclei of the pale and dark PC also is found, as shown by an increase in their size. Since it is supposed [7] that one possible method of regulation of neuron function may be a temporary increase in the DNA content of the neuron, the search for hyperdiploid PC during this period becomes potentially very valuable. The solution to this problem is very interesting and important from the point of view of detection of additional compensatory mechanisms realized in neuron populations in extremal states.

It must be emphasized that the changes observed in the dogs of group 1 compared with the control were much more clearly defined in the pale PC than in the dark. For instance, the dry weight of the cytoplasm of the pale PC was increased by 23%, but that of the dark PC by only 5%. Consequently pale PC, whose metabolism changes sharply in response to the experimental procedure, exhibit considerable lability, whereas dark PC exhibit relative stability. This conclusion is in agreement with previous observations [2] showing that in these dogs the number of pale PC was significantly lowered compared with the control by 28% (evidently because of transfer of the pale PC to the morphologically changed group), whereas the number of dark PC remained constant.

In the dogs of group 2 the area of the nucleus of the pale PC was increased a little and the concentration of dense substances was lower than in the control (229.5 ± 26.2; $P < 0.05$). The dry weight of the nucleus remained at the control level. The area of the cytoplasm, its dry weight, and also the concentration of solid matter (457.2 ± 36.0) were practically the same as in the control. The area of the nucleus of the dark PC was unchanged whereas the concentration of solid matter in the nucleus was considerably increased (396.9 ± 26.5; $P < 0.01$), to correspond to the increase in dry weight of the nucleus of the dark PC. The area of the cytoplasm in these PC was increased and the concentration of solid matter unchanged (593.7 ± 30.3) compared with the control. The dry weight of the cytoplasm of the dark PC was increased.

Consequently, 2 weeks after clinical death from acute blood loss the dimensions of the nucleus of the pale PC in dogs with disturbances of static posture were very slightly increased, whereas the dimensions of the cytoplasm, and also the dry weight of both nucleus and cytoplasm agreed with the control. Consequently, pale PC, if they had changed as a result of clinical death, were by now already practically identical to the pale PC of intact dogs. However, some very significant changes affecting the dark PC were evident: a sharp increase (by 42%) in the dry weight of the nucleus, an increase in the dry weight and size of the cytoplasm. Possibly in the dogs of group 2, which had undergone more severe trauma [8], with disturbances of static posture, the intensity and velocity of repair processes in the population of cerebellar PC may have been increased. As a result of this, the response of the pale PC of these animals may have appeared sooner, and that of the dark PC may have been exhibited much more strongly than in the dogs of group 1, whose neurological status was completely restored.

It is interesting to note that 2 weeks after clinical death an increase in the dry weight of the cytoplasm was observed in the morphologically changed PC of both groups of dogs. This fact can evidently be interpreted as activation of protein synthesis aimed at repair, for the group of morphologically changed PC consists not only of cells with destructive change ("heavy sickness," vacuolation of the cytoplasm), but also cells with transient dystrophic changes (lysis of the basophilic substance to a varied degree). This group also includes swollen PC, which can be regarded as activated cells [11].

On the basis of these results a few hypotheses can be put forward regarding the role of dark and pale neurons in the response of the whole PC population to the experimental procedure. It was shown that the dimensions and dry weight of the cytoplasm of the dark and pale PC in intact dogs are identical. The only difference is that in dark PC the dry weight of the nucleus is less. On the other hand, there are distinct differences in the "strategy of behavior" of pale and dark PC in clinical death of varied severity and what is particularly important, in association with different degrees of recovery of the neurologic status. The trend of the disturbances found was the same. However, pale PC are the first to respond to the experimental procedure, whereas the dark PC make their own contribution to the overall response of the population only after the possibilities of the pale PC have become exhausted.

It can accordingly be postulated that pale and dark PC differ not only in their level of metabolism, but also in their role in the maintenance of homeostasis of the population.

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ULTRASTRUCTURAL CHARACTERISTICS OF CHANGES IN THE SENSOMOTOR CORTICAL NEUROPIL DURING LONG-TERM PROTEIN-CALORIC DEFICIENCY

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In the growing organism whose diet is deficient in protein, one of the most vulnerable organs is the brain [13, 14]. Numerous investigations on rodents (rats and mice) have been devoted mainly to the study of the effect of protein-caloric deficiency on the brain during late embryonic and early postnatal development. The reason is evidently that it is during this period that processes of neurogenesis take place actively in the brain of experimental animals. Yet from the clinical point of view it is more important to study the effect of protein-caloric deficiency on the infant at the time when he ceases to be fed on his mother's milk. It has been shown that infants between 6 months and 3 years of age most often suffer from diseases such as kwashiorkor and marasmus, which later often lead to low intelligence and mental backwardness [8]. It is at this time that processes of gliogenesis, myelinization, and growth of nerve cells and establishment of nervous connections take place in the child's brain. In the rodent brain the corresponding processes are most active during the first month of postnatal development [2, 7].

Considering the fact that most nerve cells in mice are formed during the period of embryonic development [15], i.e., significantly earlier than exposure to underfeeding began, the most significant changes might be expected in the structures of the neuropil. The study of the neuropil also seemed to be indicated in the investigation to be described below because it is in the neuropil that the main interneuronal interactions that lie at the basis of the mechanisms of integrative brain activity take place.

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