ANOXIC CHANGES IN cAMP LEVEL IN THE CAT CEREBRAL CORTEX

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Cyclic nucleotides are ascribed an important role in intracellular adaptive reactions to extremal factors [1, 3, 5]. Marked changes have been found in the cAMP concentration in different brain formations during ischemia [2, 9]. We have virtually no information on changes in the cortical cAMP level in mammals in response to hypoxia. The problem of a possible connection between cAMP concentrations and bioelectrical activity of brain neurons at different stages of disturbance and restoration of the oxygen supply still remains unsolved.

The aim of the investigation described below was to study the dynamics of the cAMP concentration, with parallel recording of neuronal spike activity in the cat cerebral cortex during a 5-min period of anoxia and the early postanoxic period.

EXPERIMENTAL METHOD

Experiments were carried out on 65 male cats anesthetized with pentobarbital (30 mg/kg), immobilized with tubocurarine, and artificially ventilated. Anoxia was induced by disconnecting the artificial respiration apparatus for 2.5 or 5 min. The skull was trephined and the brain surface permanently moistened with McIlwain's solution at a temperature of 37.5 ± 0.5°C. A biopsy specimen (10-20 mg) was taken from the visual or sensomotor areas of the animals' cerebral cortex before interruption of the oxygen supply and also at different periods of anoxia and reoxygenation. To determine the cAMP level, brain tissue was homogenized in ten volumes of 0.6 M perchloric acid. The extract was separated by centrifugation at 12,000 rpm for 15 min on a K-24 refrigeration-centrifuge. The cAMP level in the extract was determined by a radioisotopic method of competitive binding with protein, using kits from "Amersham" (England). Protein was determined by Lowry's method. The individual dynamics of the cAMP concentration was studied and changes in its level evoked by anoxia were compared with the original (basal) values for the given animal. As a parallel measure, in each experiment the time course of unit activity of the brain neurons was estimated by two methods: by continuous recording of activity of one or more neurons, or the number of spontaneously active neurons was determined in the tracks during insertion of the microelectrodes from the surface to a depth of 2 mm, at different stages of anoxia and recovery. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

After interruption of the oxygen supply, changes took place in phases of the neuronal spike discharge (NSD) of the cortex, expressed as a change of pattern during the first tens of seconds, and the development of hyperactivation after 60-90 sec, followed by inhibition and disappearance (after 90-150 sec) of action potentials. At different stages of asphyxia characteristic changes were found in the cAMP concentration. After 1, 1.5, and 2.5 min of anoxia, the time course of the cAMP concentration was similar in type in all the animals studied. The initial fall of the cAMP level toward the first minute (by 44%) was replaced by a rise in the hyperactivation phase (by 98% toward

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1.5 min), followed by a fall after 2.5 min. At the 5th minute of anoxia two approximately equal groups of animals with opposite changes in their cAMP concentration could be distinguished. In group 1 a marked increase (by 123%) in the cAMP concentration was observed, whereas in group 2 there was some decrease (by 25%) compared with the preanoxic level. The fact will be noted that in these groups of animals, which also were characterized by different degrees of functional recovery, basal cAMP concentrations in the cerebral cortex differed significantly. Whereas the average control level in the whole sample of animals studied (65) was 48.4 pmoles/mg protein, in the cats of group 1 it was almost 40% less (29.9 pmoles/mg protein), but in group 2 it was close to the mean value (53.5 pmoles/mg protein). Meanwhile in four cats which did not survive 5 min of asphyxia, a high basal level of cAMP was found (81 pmoles/mg protein), which was 68% higher than the mean control value for the whole sample of animals.

In the early period of reoxygenation (up to 30 min) after anoxia for 2.5 and 5 min differences were found in the rate and degree of respiration of the spontaneous NSD, and also in the changes in the cortical cAMP concentration. After 2.5 min of anoxia NSD appeared comparatively quickly (during the first 5 min of reoxygenation). Toward the 15th-20th minutes of restoration of the oxygen consumption hyperactivation of the neurons developed. The number of spontaneously active neurons in the tracks at the 20th-30th minutes was twice the number observed initially. After 5 min of anoxia, recovery took place more slowly. NSD as a rule did not appear before 8-10 min. Depending on the dynamics of restoration of NSD the animals could be divided into two groups. In the first group, toward the 30th minute of reoxygenation the number of spontaneously active neurons was about half of the preanoxic value. Meanwhile in the second group a tendency was noted for more complete restoration of NSD.

Toward the 30th minute of reoxygenation after 2.5 min of anoxia the cAMP level fell to 63% of the control value. In animals surviving anoxia for 5 min, and classed in the first group because of the character of restoration of their NSD, the cAMP concentration toward the 30th minute of reoxygenation remained high, 90% above the preanoxic level. Meanwhile, in the second group of animals the cAMP concentration fell to 56% of the initial values by this time.

The results are evidence that involvement of one of the secondary key messengers (cAMP) has an influence in the response of the cerebral cortex to disturbance and restoration of the oxygen supply, and also that correlation exists between changes in its concentration and the time course of neuronal spike activity under these conditions. Considering data on the important role of cAMP in the modulation of synaptic transmission [4, 7] and of excitability of neurons in the mammalian brain [6, 8, 10], it must be considered that the changes revealed in the cAMP concentration reflect changes in the functional state of the neurons evoked by anoxia, and they may evidently be concerned in their initiation. This conclusion is confirmed by the appearance of phasic changes in the cAMP level during anoxia and subsequent reoxygenation, corresponding to a definite time course of NSD. Analysis of individual differences in the changes in cAMP concentration evoked by 5 min of anoxia, and the character of postanoxic restoration, revealed their dependence on the basal level of the cAMP concentration in the cerebral cortex of different animals. Thus, cAMP-dependent intracellular processes play an important role not only in the mechanisms of response of brain neurons to disturbance of the oxygen supply, but also in the individual differences in their manifestation.

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