THE RELATIONSHIP OF ELECTRICAL TO METABOLIC ACTIVITY IN THE OLFACTORY BULBS OF HIBERNATING ANIMALS

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Recently the problem of the metabolic counterpart of the electrical activity of the central nervous system has acquired an importance all of its own. Changes in the specific activity of the nerve cells is associated with definite alterations in their metabolism. Adequate stimulation of receptor systems leads to reactive changes of the proteins of the cytoplasm, and to the formation of nucleoproteins [2, 3, 4, 27, 30-33]. Excitation of a neurone is accompanied by an accumulation and subsequent reduction in the cytoplasmic content of phosphomonoesterase [18, 19].

A number of isolated attempts have been made to compare the electrical and metabolic activities of the nervous system by making parallel electrophysiological and histochemical measurements [9, 13, 16-19]. The results have been contradictory, probably because of the difficulty of the task and shortcomings of the method.

We have undertaken an investigation of the relationship of electrical and metabolic activity in the olfactory capsule of hibernating animals. Winter sleep is associated with marked and maintained changes of cerebral electrical activity [10, 19, 28, 34, 36]. Apparently definite metabolic changes in the central nervous system are associated with this alteration [11, 19, 37].

The advantage of such an experiment is that the excitatory and inhibitory condition of the brain may be studied without any interference from outside; there is a profound inhibition of the main mass of central neurones, and when waking state returns they become intensely active [10, 19, 24, 28, 34, 36].

The objects of our investigation were as follows: 1) to evaluate the changes of the electrical processes in the olfactory capsule of hibernating animals under conditions of wakeful activity, number, and hibernation; 2) to compare the rhythmical processes in the olfactory capsule with the formation of histochemical substrates which would give direct or indirect information concerning the intensity of the specific activity of the central neurones.

EXPERIMENTAL METHOD

The experiments were carried out between September and April, 1960-1961, on 16 European hedgehogs (Erinaceus europaeus L.). Bipolar platinum electrodes 25-50 μ thick were introduced into the olfactory bulb under general urethane or local (novocaine) anesthesia (Fig. 1, I). The position of the electrodes was checked histologically and by x-rays. Electrical records of activity in the olfactory bulb was made by means of an ink-writing electroencephalograph. Odors were used as stimulus. The temperature of the olfactory bulb was measured by copper-constantan thermocouples inserted into the bulb and by low-temperature thermistors. At various times after the operation the animals were placed in a cold enclosure at a temperature of 4-2°.

At various stages in the experiment, at a temperature not exceeding 6°, the vault of the skull was removed and the olfactory capsule fixed for histological study later. The material was treated with Gomori for determination of acid and alkaline phosphomonoesterase, by Shabadash for examination for glycogen, by Unna-Brachet and Einarson for RNA (with control by crystalline ribonuclease).

In our choice of histochemical methods we considered results bearing on the involvement of the substrates both in the activity of the neurones [2, 3, 4, 9, 11, 13, 17-20] and in mediating the specialized function of olfaction [5, 26].

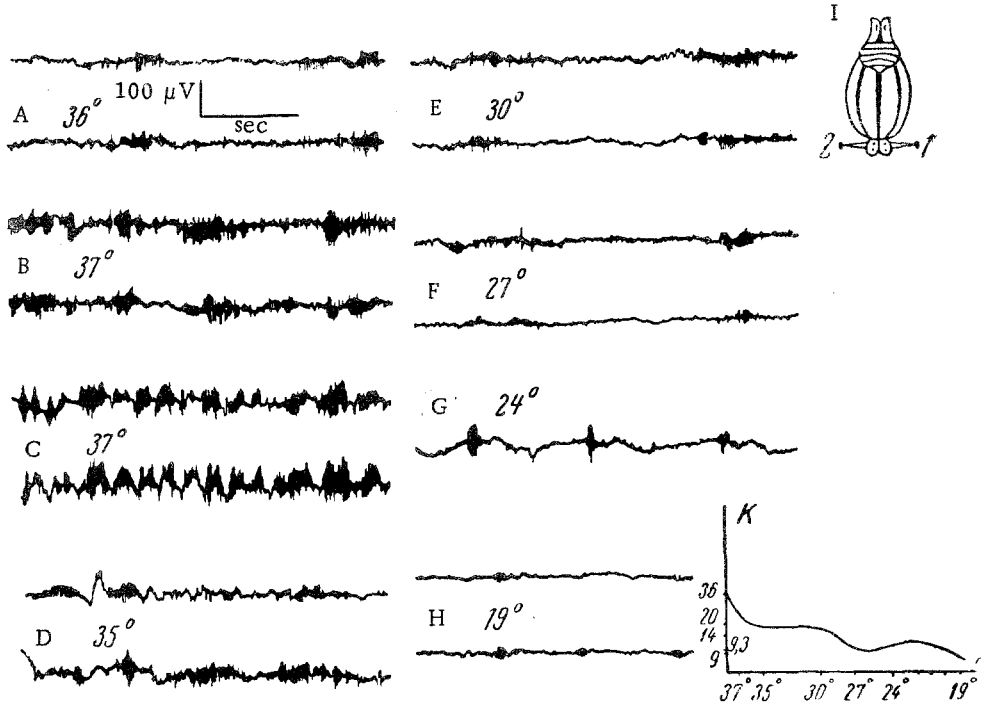

Fig. 1. Change of electrical activity of the olfactory bulb during winter sleep and torpor (A – H). I) Diagram illustrating the arrangement of the pick-up electrodes (1, 2); K) graph showing changes in the mean value of the amplitude of the potentials (MAP) during sleep and torpor. Ordinate – MAP (in μV), abscissa – temperature of the olfactory bulb (in degrees C).

EXPERIMENTAL RESULTS

The electrical activity of the olfactory bulb of hibernating animals undergoes regular changes as the temperature of the inner part of the brain falls (Fig. 1). The "spontaneous" activity of the bulb consists of bursts of regular oscillations at 40-60/sec. This form of activity is a characteristic of the mammalian olfactory bulb [1, 7, 22, 23, 29]; it dominates the electrical response for 12-16 days when the temperature falls from 37 to 24° (Fig. 1, A-F). At this time the olfactory bulb shows the following distribution of the substrates investigated histochemically: a maximum amount of RNA and phosphomonoesterase are concentrated in the olfactory glomeruli and in the glial elements of their capsule (Fig. 2, a); these substances are less abundant in the mitral cells and in the neurones with short axons (Fig. 2, d, f); the least amount is found in the layer of granular cells.

As has been explained, in the capsule of the glomeruli there are no great changes of metabolic activity associated with the change from the sleeping to the waking state.

Adequate stimulation of the olfactory receptors by camphor oil produces regular and prolonged changes of electrical activity in the olfactory bulb; they are shown by an increase in amplitude and frequency of the potentials (see Fig. 1, B, V), and also by a temperature rise of 0.5-1°. There is also a consistent increase in the amount of alkaline phosphomonoesterase and RNA in the olfactory glomeruli, and somewhat less in the mitral cells (see Fig. 2, B). It is known that the olfactory glomeruli are the structures principally concerned in receiving and "editing" the input to the olfactory analyzer.

As the brain temperature falls the amplitude of the electrical oscillation is gradually reduced without any appreciable changes in the frequency spectrum. A statistical treatment of the electrogram shows that the mean amplitude progressively falls and does not show the usual variation associated with decrease of cerebral temperature (Fig. 1, K); the frequency of the potentials begins to fall at temperature below 21° (Fig. 1, G, H). By this time the period between the separate "modulation" activities has increased, and the number of oscillations making up each "burst" has been greatly reduced.

The changes of metabolic activity are as follows. The RNA concentration in the mitral (Fig. 2, c) and short-axon neur-

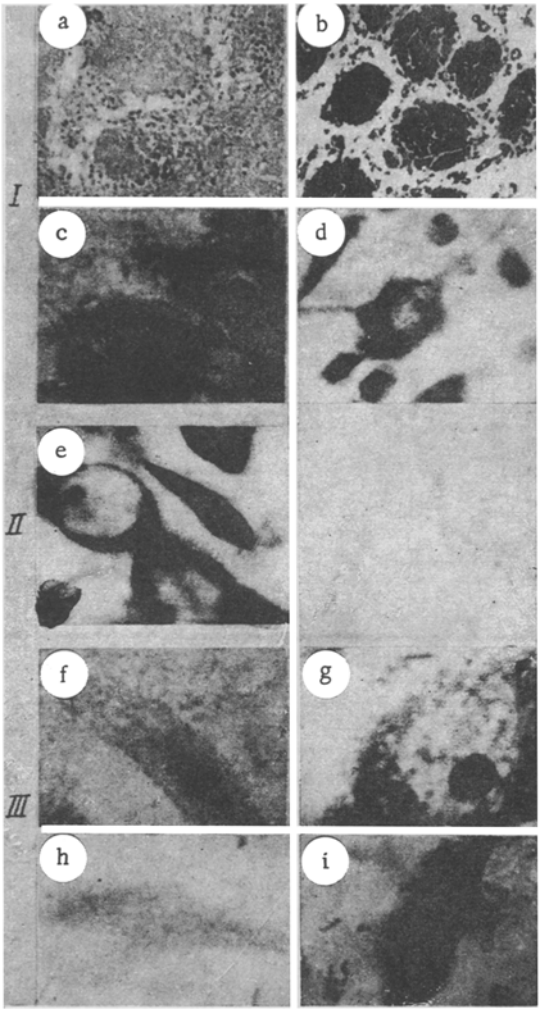

Fig. 2. Change in the amount of alkaline phosphomonesterase, RNA, and glycogen in the cells of the olfactory bulb. a) Alkaline phosphatase in the olfactory glomeruli. Ocular 15×, apochromatic objective 20×; b) ditto during prolonged olfactory stimulation. Ocular 10×, apochromatic objective 20×; c) ribonucleic acid in the mitral cells and short-axon neurones; d) during sleep (31°); e) RNA in the mitral cells during torpor (19°); f, g, h, i) glycogen in the neurones of the olfactory bulb in the waking state (f, g - 37°), during torpor (h-29°) and in deep sleep (i - 19°). For f – h: ocular 10×, immersion apochromatic objective 60×; for i: ocular 10×, immersion apochromatic objective 90×.

ones (Fig. 2, d) increased. The mitral cells were stained diffusely with pyronine (Fig. 2, e), and the cells with short axons contain RNA in the form of large granules evenly distributed in the perinuclear zone.

As sleep comes on, and particularly as it develops into torpor when the brain temperature falls to 14°, the RNA accumulates mainly in the initial segment of the axon, though at this time the normal amounts of glycogen and of alkaline phosphomonoesterase are found. During torpor the most marked increase is in the amount of glycogen in the mitral cells and in the cells with short axons, which evidently indicates a suppression of glycolysis and a profound suppression of neuronal activity.

It should be noted that the distribution of RNA and glycogen, (substances intimately related to the Nissl substance of the nerve cell) closely resembles that found in the somatochromic cells of the cerebral cortex in deep sleep induced by drugs [8].

The disappearance of electrical activity from the olfactory bulb at temperatures below 14° is associated with with stabilization of the RNA in the following gradient; mitral cells > neurones with short axons > cells of the capsule of the glomeruli; i.e., the direction is the reverse of the normal temperature conditions.

Therefore the "electrophysiological zero" for the olfactory bulb at 14° shows a large amount of RNA, glycogen, and phosphomonesterase in the cells of the first neurone of the sequence. These neurones comprise the mitral cells and the return collateral of its axon, and the short-axon neurone whose outgrowth terminates on the body of a mitral cell. This primary neurone circuit, which appears for the first time in the highly organized vertebrates [14] and which includes a feed-back connection through the return collateral of the mitral cell, shows the greatest intensity and variation of metabolic rate in winter sleep.

We must now consider how to explain the large amount of the substances investigated which collect selectively in neurones of the olfactory bulb at temperatures at which electrical activity fails.

On the one hand in all probability the condition represents an inhibitory state of all parts of the central nervous system, and on the other it may explain the constant "readiness" of the olfactory bulb and of the hippocampus (in which the content of these substances is high) to generate waking potentials. It is known that it is precisely these parts which maintain their activity at the lowest temperatures [28, 36]. These portions, in association with the diencephalon and midbrain and also with the limbic cortex appear to be responsible for the maintenance of homeostasis and for the natural awakening process.

On the basis of the results described we may put forward tentatively a scheme for the origin of the rhythmic activity of the olfactory bulb. The nature of the rhythmic oscillations is shown firstly as a modulation of the high frequency input signal from the receptor layer of the olfactory epithelium. The latter is shunted by the glial capsule of

the glomeruli which serve as an additional capacitative resistance along the path of the spread of the impulse. The form or limitation of the amplitude of the input signal which results in a true modulation is effected by the system of feedback connection of the primary neurone of the circuit and establishes the rhythm of the olfactory bulb. The modulated signal enters the next section of the analyzer.

The fact that the changes in the frequency spectrum associated with a fall of intracerebral temperature occur considerably later than the alterations in amplitude may indicate the complex nature of the structures responsible for the (primary) frequency modulation and for the amplitude modulation of the input signal. This principle is well established in cybernetics [12 et al.].

The hypothesis we have advanced must of course be confirmed by microelectrode studies and by means of an electronic model of the olfactory bulb.

SUMMARY

The work describes electrical and thermal changes in the olfactory bulb of hibernating hedgehogs (Erinaceus europaeus) during active movement, in the waking state, and in hibernation. Histochemical determinations of RNA, glycogen, and phosphomonoesterase were made for a parallel study of the metabolic activity of the cells of the bulb. It was shown that there was at first no change and later an accumulation of these substances, and at the same time electrical activity in the bulb was first reduced and then finally disappeared. The metabolic changes were most active in the first stage of the neuronal circuit of the bulb, i.e., in the mitral cell with its recurrent collateral, and in the short-axoned internuncial neurone.

We have proposed a possible function for this circuit in the establishment of the electrical rhythm of the olfactory bulb.

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