PLASMA β-ENDORPHIN-LIKE IMMUNOREACTIVITY AND ITS VARIATIONS IN BABOONS

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Endogenous opioid peptides are implicated in many physiological processes, including formation of the response of the organism to stress [1]. One of the most important components of the system of endogenous opioid peptides in β-endorphin. An important physiological role has been ascribed to β-endrophin circulating in the blood stream [4]. Formed as a result of hydrolysis of β-lipotropin, it enters the blood stream from the anterior and intermediate lobes of the pituitary [6]. The plasma β-endorphin concentration in animals of different species varies within considerable limits. In cats, for instance, it is measured in hundreds of picograms per milliliter [2], in rats in tens of nanograms per milliliter, and in man from units to tens of picograms per milliliter [5, 9].

The aim of this investigation was to determine the level of β-endorphin-like immunoreactivity (β-ELIR) in the blood plasma of baboons and to study its changes in certain situations.

EXPERIMENTAL METHOD

Experiments were carried out on six baboons (*Papio hamadryas*) weighing 5.5-8.5 kg. Between 5 and 7 days before the beginning of the experiments electrodes were implanted into various brain structures of the baboons under pentobarbital anesthesia (40 mg/kg, intravenously). Stainless steel electrodes 0.8 mm in diameter were insulated over their whole length except the tip (0.5 mm). Immediately after the operation the animals were placed in primatologic chairs, in which they remained throughout the experiment (3-4 weeks). Blood (3-4 ml) was taken from the cubital vein of the animals by means of a polyethylene syringe and quickly transferred into a polyethylene test tube (after preliminary addition of EDTA), kept on ice. After centrifugation the blood plasma was frozen and stored at -20°C until required for analysis. Blood was taken between noon and 4 p.m. For radioimmunoassay of β-ELIR in the blood plasma, a standard kit from INC (USA) and the appropriate technique were used. In some cases radioimmunoassay of total β-ELIR/β-lipotropin-like immunoreactivity (β-LLIR) also was carried out with the aid of a standard kit from "Seragen" (USA). In this case, by substracting the β-ELIR from the total β-ELIR + β-LLIR, the level of β-LLIR could be determined. Electrical stimulation (ES) of the brain structures was carried out by monopolar square pulses with a duration of 10 msec, frequently 50 Hz, and current strength 4-5 μA. The animals were killed by injection of large doses of pentobarbital. Hemorrhagic shock was produced by bleeding from the brachial vein of anesthetized animals (chloralose, 80 mg/kg, intravenously). The position of the electrodes was verified histologically in serial brain sections fixed beforehand in formalin solution, and cut on a freezing microtome. The sections were photographed without preliminary staining.

EXPERIMENTAL RESULTS

The background plasma β-ELIR level of the baboons, in a state of quiet wakefulness, was 8.0 = 1.0 fmoles/ml, and the minimal and maximal values were 5 and 11 fmoles/ml respectively (28.0 ± 5.0 pg/ml, n= 10). The total level of β-ELIR and β-LLIR was 134 ± 24 pg/ml (minimum 84 pg/ml, maximum 162 pg/ml, n = 11). By subtraction, the β-LLIR level was 90 ± 10 fmoles/ml (from 5 to 11 fmoles/ml, 104.0 ± 19.0 pg/ml, n = 7). The ratio of the molar concentration of β-ELIR to that of β-LLIR averaged 0.9 ± 0.1 (0.8-1.2).

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Blood samples were taken 3 times from two animals, on alternate days, over a period of 7 days. The background β-ELIR level and the calculated β-LLIR level remained stable at 9.0 ± 1.0 fmoles/ml (the average concentration in all the samples was 9 fmoles/ml).

After establishment of the background β-ELIR level in two animals blood samples were taken 30 sec after the beginning of ES of the paraventricular-perifornical region of the medial hypothalamus, which is considered to participate in the mechanism of the autonomic component of emotional reactions [8]. ES of this region was accompanied by marked restlessness of the animals, elevation of their mean arterial pressure (BP) from 100 to 190 mm Hg, tachycardia, and tachypnea. The plasma β-ELIR level rose by 189% (from 9.0 ± 1.0 to 26.0 ± 9.0 fmoles/ml, $n = 4$; $P < 0.05$), and at the same time a small increase was observed (by 12.6%, from 143.0 ± 6.0 to 161 ± 11.0 pg/ml) in the toal β-ELIR + β-LLIR concentration. The calculated level of β-LLIR fell from 9.0 ± 1.0 to 6.0 ± 2.0 fmoles/ml, and the β-ELIR/β-LLIR ratio rose from 1 to 4.9.

Blood was taken from two animals after ES of the mammillary region of the hypothalamus. Changes in the autonomic parameters also took place in these animals, although by a lesser degree: hypertension (from 98 to 150 mm Hg), tachypnea, and tachycardia. In these cases there was a small (not significant) increase (by 66%, from 6.0 ± 1.0 to 10.0 ± 2.0 fmoles/ml) in the β-ELIR concentration, a small increase in the total β-ELIR + β-LLIR concentration (from 102.0 ± 17.0 to 114.0 ± 12.0 pg/ml), and the calculated value of the β-LLIR concentration fell (not significantly) from 7.0 ± 1.0 to 6.0 ± 1.0 fmoles/ml; the β-ELIR/β-LLIR ratio rose from 1.6 to 0.9.

In another two animals changes in the β-ELIR level and the total β-ELIR + β-LLIR level were studied during the development of hemorrhagic shock. Blood samples were taken 3 times from these animals: before the beginning of bleeding, 20 min after induction of sleep by chloralose, in the course of which the mean BP was maintained by repeated bleedings at the level of 50 mm Hg, and 60 min after the end of bleeding (both these animals died with signs of increasing shock).

Before the beginning of bleeding the β-ELIR level was 9.0 ± 1.0 fmoles/ml (31.0 ± 1.0 pg/ml), the total β-ELIR + β-LLIR concentration was 152.0 ± 1.3 pg/ml, and the β-LLIR level was 10.0 ± 1.0 fmoles/ml (121.0 ± 13.0 pg/ml). The β-ELIR/β-LLIR ratio under these circumstances was 0.85.

After 20 min the β-ELIR level had risen by 300% (to 36.0 ± 3.0 fmoles/ml, 126.0 ± 11.0 pg/ml; $P < 0.001$). The total β-ELIR + β-LLIR concentration was virtually unchanged (155.0 ± 47.0 pg/ml). The β-LLIR level had fallen to 3.0 ± 2.0 fmoles/ml (39.0 ± 25.0 pg/ml). The β-LLIR level in one animal during this period was virtually zero. The β-ELIR/β-LLIR ratio was over 10. The β-ELIR concentration 60 min after the end of bleeding had risen by a further 47% compared with its previous level (to 53.0 ± 2.0 fmoles/ml, 184 pg/ml; $P < 0.05$).

The blood plasma of baboons (*P. hamadryas*) thus contains measurable amounts of both β-ELIR and β-LLIR (8.0 ± 1.0 and 9.0 ± 1.0 fmoles/ml respectively). The circulating blood levels of β-ELIR and β-LLIR under normal conditions are sufficiently stable and resemble those in man [5, 9]. However, whereas in man the molar concentration of β-lipotropin is twice or three times higher than the β-endorphin level [9], in baboons these parameters are virtually equal.

The paraventricular-perifornical region of the medial hypothalamus, where the bodies of opiatergic neurons are located [3], is known on the one hand to be closely connected with the secretory function of the pituitary gland [7], and on the other hand, to participate in the autonomic mechanism of emotional reactions [8]. It has also been established that a rise of the plasma β-endorphin level accompanies the formation of a stressor state [1, 2]. The experiments described above showed that stimulation of this region, giving rise to autonomic shifts in animals characteristic of stress, also leads to a considerable increase in the β-endorphin concentration in the blood plasma, and under these circumstances the β-ELIR/β-LLIR ratio moves in favor of β-ELIR. At the same time, stimulation of the mammillary region of the hypothalamus does not cause such marked changes in the β-ELIR and β-LLIR levels. This can be interpreted as evidence that the paraventricular and perifornical zones of the medial hypothalamus are also involved in β-endorphin release during stress.

An even greater increase in the plasma β-ELIR concentration is observed during the development of hemorrhagic shock; in this case the β-LLIR level falls by an even greater degree.

All these data show that elevation of the plasma β-ELIR level accompanies stress formation, including the development of a state of shock in baboons. A definite role in the regulation of the plasma β-endorphin level may be played by the paraventricular-perifornical region of the hypothalamus. Elevation of the β-endorphic level probably takes place on account of increased hydrolysis of β-lipotropin and also on account of its increased synthesis in the pituitary.

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MECHANISM AND TIMES OF DEVELOPMENT OF HYALINE MEMBRANES IN ACUTE RESPIRATORY FAILURE DUE TO TRAUMA

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The question whether hyaline membranes (HM)of the lungs in adults with an acute respiratory failure syndrome are a feature of the disease itself or a complication developing during treatment remains unsolved. Some workers have observed the formation of HM in the shock lung syndrome only after artificial ventilation and transfusion of an inappropriate volume of fluid [8, 9, 12]. The problem of the times of development of HM, which is important for an understanding of the pathogenesis of respiratory failure, also is being discussed. Electron-microscopic investigations of the lungs of persons dying after chest injury, with the clinical picture of acute respiratory failure, revealed HM 1-7 days after death or later [5-7, 11, 12, 14].

In the investigation described below the possibility of HM formation in the absence of treatment was studied at the ultrastructural level on an experimental model of traumatic shock lung.

EXPERIMENTAL METHOD

A model of chest trauma accompanied by respiratory failure, and excluding any lesion of the vertebral column and spinal cord, was created in 78 guinea pigs weighing 300 g. The control consisted of 50 intact guinea pigs. The morphological investigations at the macroscopic, microscopic, and ultrastructual levels, were undertaken 1, 6, 12, 24, 48, and 72 h after trauma (13 animals at each time). Besides ordinary transmission microscopy, in order to study the lung surfactant, in 30 cases (five animals at each time) the animal was perfused with 3.6% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) through the pulmonary artery [15]. To detect glycosamino glycans of the surfactant and hypophase, ruthenium red was injected as a component of the fixing mixtures [2, 10].

EXPERIMENTAL RESULTS

In 100% of cases the clinical picture of respiratory failure was obtained. At the macroscopic and microscopic levels the typical picture of shock lung was discovered: a combination

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