DELTA SLEEP-INDUCING PEPTIDE IN BLOOD AND HYPOTHALAMUS OF RATS DIFFERING IN TOLERANCE TO EMOTIONAL STRESS

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UDC 612.826.4.018.06:612.821.7].06:613.863

KEY WORDS: acute emotional stress, blood, hypothalamus, delta sleep-inducing peptide.

Delta sleep-inducing peptide (DSIP) has been shown to increase the tolerance of animals to emotional stress substantially [1, 3, 7, 8]. Intravenous injection of DSIP [8] lowers the blood pressure of spontaneously hypertensive rats sensitive to stress. Intraventricular injection of DSIP blocks hypertensive reactions arising in rabbits to stimulation of negative emotiogenic zones of the ventromedial hypothalamic nucleus [6] and improves the microcirculation in the cerebral cortex of cats [5, 6]. DSIP has been shown to strengthen parasympathetic and weaken sympathetic influences on the heart [2, 4]. In patients with secondary hypertension injections of DSIP after discontinuation of other forms of treatment maintained the blood pressure at its normal level [8].

With discovery of the antistressor properties of DSIP, it is interesting to examine its role in the mechanisms of tolerance of animals to emotional stress. For this purpose it was decided to determine the DSIP concentrations in animals tolerant and predisposed to emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on 39 adult male Wistar rats. A metal plate was fixed with "Noracryl-100" plastic under ether anesthesia to the previously widely scalped skull.

Tolerance of the rat to emotional stress was determined beforehand by studying the animal's behavior in the open field test in the tail flick test in a "Varimex" instrument, and by determining the character of changes in the electrocardiogram, rhevasogram, blood pressure, and respiration rate in response to stressor stimuli. Of all the animals studied in the experiments 13 were identified as tolerant and 11 as predisposed to emotional stress. Blood and hypothalamic levels of DSIP were determined in these animals. In addition, a group of 15 rats tolerant and predisposed to emotional stress were subjected to stress for periods of 1.5 and 3 h. For this purpose the rats were placed in Plexiglas constraining cages, and their heads were fixed by the metal plate to a stereotaxic apparatus. Electrical stimulation was applied to the ventromedial hypothalamus of the animals, alternating with electrodermal stimulation. A specially developed program of stimulation was devised for 3 h with alternating current of threshold strength, voltage 1.4 V, frequency 50 Hz, and pulse duration 1 msec. Each stimulation lasted 30 sec or 1 min. The threshold voltages of the electric current for stimulation of the ventromedial hypothalamus were chosen on the basis of a change of not less than 20% in the initial value of the blood pressure. After the experiments the animals of all the above-mentioned groups were decapitated on a guillotine and blood and hypothalamic tissue were taken simultaneously for biochemical tests.

The DSIP concentration in acetic acid extract of blood and hypothalamus was determined by solid-phase enzyme immunoassay.

Brain preparations were homogenized in 400 µl of 2N acetic acid. The samples were subjected to acetic acid extraction for 10 min on a boiling water bath, followed by cooling and centrifugation for 10 min at 10,000g. The supernatant was lyophilized. For DSIP determination the dry residue was weighed and dissolved in 1 ml of EIA buffer, pH 7.5.

Laboratory of Physiology of Emotions, P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR K. V. Sudakov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 264-266, September, 1988. Original article submitted January 4, 1988.

Antiserum to DSIP was obtained by immunizing rabbits with conjugates of DSIP and hemocyanin, synthesized with the aid of carbodiimide. The DSIP conjugate was emulsified in an equal volume of Freund's complete adjuvant and injected subcutaneously and intradermally into up to 100 points over the whole body. The animals were reimmunized with the DSIP-conjugate, mixed with Freund's incomplete adjuvant, 2 weeks later. Blood was taken 1 week after reimmunization.

The quantity of DSIP in the experimental samples was determined by test EIA using 96-well polystyrene panels ("Titertek"). To obtain quantitative information, preliminary calibration was carried out against standard solutions of DSIP. The intensity of the reaction was estimated by means of an M-580 reader ("Dynatech," Switzerland).

EXPERIMENTAL RESULTS

The investigations revealed different concentrations of DSIP in the blood and hypothalamus of animals tolerant and susceptible to emotional stress. High blood and hypothalamic levels of DSIP were observed in animals tolerant to emotional stress.

Exposure to emotional stress for 1.5 h caused a parallel increase in blood and hypothalamic DSIP levels in animals tolerant and predisposed to stress. The DSIP concentration in the blood increased more rapidly in the tolerant animals, whereas in the hypothalamus the DSIP level was almost equally increased compared with the background in animals both tolerant and predisposed to stress. The blood DSIP level in the tolerant rats was increased by 171.5% compared with the background, but by only 21.79% in the hypothalamus. In the predisposed animals the DSIP level in the blood was increased by 81.26% compared with the background, but by 101.4% in the hypothalamus. Thus the DSIP concentration in tolerant animals showed a greater increase in the blood, whereas in predisposed rats the increase was greater in the hypothalamus.

After exposure to stress for 3 h the DSIP concentration in the blood was lower by 50.85% compared with its value after 1.5 h of stress, whereas in predisposed animals it was 16.24% lower. The DSIP concentration in the hypothalamus continued to rise in both groups until the 3rd hour of exposure to stress. In the tolerant animals the DSIP level was 70.18% higher than after 1.5 h of stress, whereas in the predisposed animals it was 81.25% higher.

It can be supposed that, together with other oligopeptides, DSIP is a factor determining the tolerance of animals to emotional stress.

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