ELECTROENCEPHALOGRAPHIC ANALYSIS OF HYPOTHALAMO-RETICULO-LIMBIC INTERRELATIONS UNDER THE INFLUENCE OF CORTICOSTEROIDS AND NEUROPEPTIDES

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UDC 612.822.3.014.46:/615.357:577.175.53 + 615.357.175.82

KEY WORDS: stress, delta sleep-inducing peptides, aggressive-defensive behavior.

Delta sleep-inducing peptide (DSIP), isolated from the brain of sleeping rabbits [6] and identified [8] chemically as a nonapeptide with hypnogenic, somnogenic, and antistressor properties [2, 3], is also a regulator of circadian rhythms and of the functional state of the CNS and, in addition, it can increase resistance to emotional stress [2]. It has been suggested [4] that the brain contains a specific system, consisting of the septum (lateral nuclei), the dorsal hippocampus, the ventromedial hypothalamus, the frontal regions of the

TABLE 1. Correlation Coefficient Matrix between EEG of Dorsal Hippocampus (Hip), Basal Amygdala (AB), Lateral Septal Nucleus (NSL), Ventromedial Hypothalamus (HVM), and Mesencephalic Reticular Formation (NRT) of Rabbits (n = 10) before and after Intravenous Injection of DSIP in a Dose of 30 nmoles/kg

Experimental conditions	Time, min	Brain structure	1 Hip	2 NSL	3 AB	4 HVM	5 NRT
Background		I Hip	1.00				
		II NSL	0,4 (100%)	1.00			
		III AB	0,5 (80%)	0,6 (30%)	1.00		
		IV HVM	0,5 (80%)	0,5 (70%)	0,5 (70%)	1.00	
		V NRT	0,5 (60%)	0,7 (20%)	0,4 (90%)	0,6 (80%)	1.00
DSIP		I Hip	1.00				
		II NSL	0,6 (60%)	1.00			
		III AB	0,7 (100%)	0,7 (100%)	1.00		
		IV HVM	0,2 (100%)	0,2 (100%)	0,4 (100%)	1.00	
		V NRT	0,6 (100%)	0,6 (30%)	0,4 (100%)	0,4 (100%)	1.00
	15	I Hip	1.00				
		II NSL	0,7 (60%)	1.00			
		III AB	0,7 (100%)	0,7 (100%)	1.00		
		IV HVM	0,2 (100%)	0,2 (100%)	0,4 (100%)	1.00	
		V NRT	0,6 (100%)	0,6 (100%)	0,4 (100%)	0,45 (100%)	1.00
	30	I Hip	1.00				
		II NSL	0,6 (60%)	1.00			
		III AB	0,6 (60%)	0,8 (100%)	1.00		
		IV HVM	0,2 (100%)	0,2 (100%)	0,4 (100%)	1.00	
		V NRT	0,7 (60%)	0,7 (60%)	0,4 (100%)	0,4 (100%)	1.00
	45	I Hip	1.00				
		II NSL	0,7 (30%)	1.00			
		III AB	0,6 (100%)	0,8 (80%)	1.00		
		IV HVM	0,2 (100%)	0,2 (100%)	0,2 (100%)	1.00	
		V NRT	0,7 (100%)	0,7 (60%)	0,2 (100%)	0,2 (100%)	1.00
	60	I Hip	1.00				
		II NSL	0,7 (100%)	1.00			
		III AB	0,7 (100%)	0,8 (60%)	1.00		
		IV HVM	0,4 (100%)	0,4 (100%)	0,4 (100%)	1.00	
		V NRT	0.7 (100%)	0.7 (100%)	0,4 (100%)	0,4 (100%)	1.00

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 259-262, September, 1986. Original article submitted April 4, 1985.

0007-4888/86/0009-1161$12.50

TABLE 2. Spearman's Coefficients of Correlation (ρ) of Changes in Individual EEG Rhythms and Blood Corticosterone (DSIP) Level in Rats in a State of Functional Rest and during ADB

Brain Structure	Spearman's coefficient of correlation for EEG rhythms										
	σ		ν		α		β		EEG		
	DSIP		corticosterone								
Hip Background	—0,6	0,2	—0,6	—0,8	—0,6	—0,3	—0,1	—0,2	—0,1	0,1	0,2
ADB	—0,7	—0,6	—0,7	—0,4	0,6	0,6	0,4	0,8	0,1	0,2	0,9
NSL Background	—0,5	0,1	0,2	—0,1	0,5	0,2	0,7	0,1	0,5	0,1	0,2
ADB	—0,1	—0,9	—1	0,5	—0,7	—0,9	0,1	0,2	0,9	—0,3	0,9
AB Background	—0,2	0,1	—0,1	0,8	—0,1	0,6	—0,3	—0,6	0,5	0,7	0,2
ADB	—0,6	—0,6	0,8	0,6	0,8	0,6	0,9	0,9	0,6	0,6	0,9
HVM Background	—0,3	—0,9	—0,5	—0,8	—0,1	—0,8	—0,1	—1,00	—0,4	—1,0	0,2
ADB	—0,3	—0,3	—0,4	0,2	—0,1	0,6	—0,6	—0,2	—0,6	—0,1	0,9
NRT Background	—0,3	0,7	—0,8	0,4	—0,1	0,2	0,3	0,6	—0,1	—0,1	0,2
ADB	0,1	0,2	—0,1	—0,1	—0,9	1,00	—0,4	—0,9	0,7	—0,1	0,9

cortex, and the pituitary, as well as certain other formations all of which share a common DSIP-dependent neurotransmission system.

The aim of this investigation was to study intercentral relations between the hypothalamo-reticulo-limbic structures (HRLS) of the brain under the influence (systemic and local) of DSIP, in animals in a state of functional rest and exposed to stress (the formation of aggressive-defensive behavior (ADB), accompanied by elevation of the ACTH and corticosteroid levels), and to compare this effect with the character of changes in intercentral relations between these same brain structures under the influence of stress-inducing ACTH. of corticosteroids and ACTH.

EXPERIMENTAL METHODS

Chronic experiments were carried out on 10 male Chinchilla rabbits weighing 3-3.5 kg and on 60 adult male albino rats weighing 150-200 g. As a first step bipolar nichrome wire electrodes, covered with standard insulation (diameter of tip 0.18 μ, resistance 20 kΩ) were inserted into the animals in order to record brain potentials in symmetrical structures on the right and left sides: dorsal hippocampus, basal amygdala, lateral septal nucleus, ventromedial hypothalamus, mesencephalic reticular formation, and ventromedial thalamus, and also into the frontal cortex, using coordinates from a stereotaxic atlas [5, 7].

Potentials were recorded in unrestrained animals and behavioral reactions were noted before and after injection of DSIP in doses of 30 nmoles/kg (intravenously) and 60 nmoles/kg (intraperitoneally), medrol (methylprednisolone) in doses of 5 and 8 mg/kg (intravenously) and 10 mg/kg (intraperitoneally), ACTH (5 U), and hydrocortisone (12.5 mg/kg, systemically). Cannulas for microinjections of the test substances, in a volume of not more than 0.02 ml, also were inserted into the lateral septal nucleus, dorsal hippocampus, and lateral ventricles. At the end of the experiments the location of the electrodes and cannulas in the brain structures chosen for investigation was verified histologically. DSIP, synthesized at the M. M. Shemyakin Institute of Bioorganic Chemistry by a team of workers headed by V. T. Ivanov, was dissolved in sterile distilled water immediately before injection. A Medicor-8 eight-channel ink-writing electroencephalograph was used as the recording instrument and a Medicor-8 wide-band analyzer-integrator as the analyzing instrument. The time constant of the high frequency filters was 0.3 sec, corresponding to a boundary frequency of 0.5 Hz. The boundary frequency of the low frequency filters was set at 70 Hz. The duration of analysis was 120 sec. The EEG was recorded on magnetic tape on a TEAK seven-channel tape recorder and then led into a Plurimat C computer for spectral, cross-correlation, and coherence analysis of the EEG by a standard program. Momentary values of the EEG were calculated in 5-sec cuts, at "quantization step" intervals of 0.025 sec. Graphs of correlation functions were plotted by means of a graph plotter connected to the computer. Functions whose coefficient of correlation was 0.4 or higher were regarded as correlating processes. Altogether 3221 cross-correlograms, 2221 coherence analysis graphs, and 863 spectrograms of the rabbit and rat EEG were analyzed. The corticosterone concentration also was determined by a radioimmunologic method of competitive binding, and the plasma DSIP of the rabbits and rats was determined by the method in [1]. Corticosterone and DSIP concentrations after a single injection of the peptide, and also during formation of ADB in the rats, by unavoidable nociceptive stimulation,

TABLE 3. Changes in Plasma Corticosterone and DSIP Levels in Rats after ADB Formation Preceded by Cold Stress (CS) and Injection of DSIP (M ± m)

Experimental conditions	Corticosterone, μg %	P<0,05	DSIP, fmoles/ml	P<0,05
Background	4,73±0,5		388,1±41,06	
ADB	48,5±1,5	0,05	242,3±31,88	0,05
CS	99,5±9,1	0,05	241,4±37,9	0,05
DSIP	3,64±0,7	0,25	422,1±41,2	0,05
DSIP + ADB	31,1±4,4	0,05	241,4±35,42	0,05
DSIP + CS	50,8±3,9	0,05	321,4±72,9	0,01

Legend. Results of 10 investigations shown.

were determined in the general population of the animals 15-30 min after injection or after the onset of ADB. An amino-acid cocktail was injected in the control, and DSIP, ACT, or corticosteroids in the experiment. The results were subjected to statistical analysis by Student's test and by Spearman's rank correlation method.

EXPERIMENTAL RESULTS

After injection of DSIP under conditions of functional rest correlation was strengthened in the spectrum of the delta-rhythm between bioelectrical processes in the limbic and reticular brain structures, whereas correlation was weakened between these structures and the ventromedial hypothalamus (Table 1). It will be clear from Table 1 that 15 min after injection of DSIP the coefficient of cross correlation between the EEG of the dorsal hippocampus and lateal septal nucleus increased from 0.4 (100%) to 0.7 (60%), whereas between the EEG of these structues and the ventromedial hypothalamus it decreased from 0.5 (80%) to 0.2 (100%). The level of statistical significance was taken to be 0.5.

Meanwhile investigation of intercentral relations in HRLS of the brain after systemic injection of stress-inducing doses of ACTH and glucocorticoids (hydrocortisone, methylprednisolone) revealed the consolidating character of the action of these hormones on reticulolimbic relations with the ventromedial hypothalamus. Because of the tendency for the action of DSIP and the hormones of adaptation (ACTH, glucocorticoids) on the intercentral relations between brain structures to be opposite in character when injected systemically, it was necessary to study correlation between the spectral density of the EEG rhythms and DSIP and corticosterone levels both in a state of functional rest and in response to endogenous elevation of the adaptation hormone levels during stress (ADB). These investigations, conducted on rats, showed that in a state of functional rest the highest rank correlation coefficient was observed between delta-activity in the hippocampus and septum and the endogenous plasma DSIP level (Table 2). This may be taken as evidence of the selective effect of DSIP on the delta-activity of these structures in a state of functional rest. Meanwhile, for corticosterone, the highest rank correlation was observed with the hippocampal theta-rhythm, increasing during stress (ADB), in good agreement with the known fact that corticosterone binds selectively with hippocampal receptors.

The stress response to ADB led to an increase in the coefficient of correlation between the hippocampal theta-rhythm and the blood corticosterone level, whereas highest correlation between the delta-rhythm and DSIP was observed in the septum. This fact is probable evidence of the selective sensitivity of the septum to DSIP, and of its increase under stress conditions.

It is particularly important to note the increase in the coefficient of correlation between the DSIP and corticosterone levels during stress (ADB). The rank correlation coefficient increased from 0.2 to 0.9 and became statistically significant. This indicates close correlation between the endogenous DSIP level and the corticosterone level. Characteristically, during stress (ADB) the increase in the plasma endogenous corticosterone concentration (from 4.73 ± 0.5 to 48.5 ± 1.5 μg%) was accompanied by lowering of the endogenous DSIP level (from 388.1 ± 41.06 to 242.3 ± 31.88 fmoles/ml). The results are statistically significant and are particularly interesting because they indicate the possible use of DSIP as a factor reducing aggressiveness.

Meanwhile injection of DSIP before ADB formation led to a fall of the corticosterone level from 4.73 ± 0.5 to 3.64 ± 0.7 μg%. ADB formation after preliminary administration of DSIP was accompanied by a smaller rise in the plasma corticosterone level than during ADB without administration of DSIP (Table 3).

The time course of brain electrical activity of the rats receiving DSIP before ADB compared with that during ADB alone indicates reorganization of brain function in these situations in the diametrically opposite direction. The spectrum of theta-activity in the hippocampus in rats in a state of ADB (in boxing posture) was increased like it was after administration of ACTH and glucocorticoids in stress-inducing doses. The value of the coefficient of coherence for the delta-rhythm between hippocampus and septum was increased 15 min after injection of DSIP, and coefficients of correlation of limbico-reticular structures were increased 30 min after injection.

Investigation of intercentral relations between brain structures on the basis of the results of correlation analysis of the EEG thus revealed the consolidating character of action of the endogenous and exogenous stress-inducing level of ACTH and glucocorticoids and the uncoupling character of the action of DSIP on brain structures. Comparative analysis of the action of glucocorticoids and ACTH (stress factors) and of DSIP as a factor increasing resistance to stress, showed that DSIP is a regulator of brain function which differs in principle in the character of its action from stress factors.

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MECHANISM OF EXERCISE HYPERVENTILATION: A HITHERTO DISREGARDED FACTOR

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UDC 612.766.1-08:612.216.2 + 616.24-008.61-02:612.766.1

KEY WORDS: diaphragm, inspiration, pyramidal tract, hyperventilation.

Mechanisms of hyperventilation during exercise remain unexplained [1-3, 9]. Exercise is not accompanied by any significant change in the partial pressures of CO_2 or O_2 in arterial blood [1-2, 9]. The influence of the cerebral cortex on the respiratory center explains only the initial, stepwise intensification of respiration [7]. The results of studies of afferent influences from working muscles are highly contradictory so far as the explanation of exercise hyperventilation is concerned [5, 6]. The present investigation was motivated by the following observation, made in clinical pratice. A bilateral lesion of the respiratory fiber tract of the spinal cord below C1, along which impulsation spreads from the res-

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(Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 262-264, September, 1986. Original article submitted December 10, 1985.

0007-4888/86/0009-1164$12.50