Antibodies to Delta Sleep-Inducing Peptide in Ultralow Doses: Effect on the Behavior of Male Mice with Anxiety and Depressive Syndrome

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We studied the effect of antibodies to delta sleep-inducing peptide in ultralow doses on the behavior of male mice with anxiety and depressive syndrome resulting from competitive interactions. The behavior of animals was studied in the elevated plus-maze, partition, and forced swimming tests. The preparation produced a strong anxiolytic effect, which was especially pronounced in animals with anxiety and depressive syndrome.

Key Words: *anxiety and depressive syndrome; antibodies; ultralow doses; delta sleep-inducing peptide; anxiety*

Our previous studies showed that potentiated antibodies (PAB) in ultralow doses *in vitro* modulate functions of S100 protein providing integrative activity of the central nervous system [5,6]. Experimental and clinical observations indicate that PAB to S100 protein (PAB-S100) relieve the withdrawal syndrome in patients with alcohol dependence [4]. The search for new preparations that belong to endogenous peptidergic stress-limiting factors and possess anxiolytic and antidepressant properties attracts much attention [7,10,13].

Delta sleep-inducing peptide (DSIP) is one of the stress-limiting compounds [1,14]. DSIP is probably involved in the regulation of functional activity in the hypothalamic-pituitary-adrenal system. Glucocorticoids affects the level of DSIP [17,18]. The content of DSIP changes in patients with mental disorders [8, 9,16]. For example, plasma DSIP concentration in depressive patients with suicidal acts surpasses that in healthy donors [18].

Here we studied the effects of PAB to DSIP (PAB-DSIP) on the behavior of animals with anxiety and depressive syndrome resulting from chronic emotional stress (social defeats in daily competition).

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6J mice weighing 26-28 g and aging 2.5-3 months. The animals were bred in a vivarium of the Institute of Cytology and Genetics and had free access to food and water.

The model of sensory contact was used to produce anxiety and depressive syndrome in male mice [2,11]. The animals were maintained under conditions of daily social competition for 20 days. By the symptoms, sensitivity to antidepressants and anxiolytic preparations, and neurochemical changes in the brain, the state of repeatedly defeated males was similar to anxious and depressive disorders in people [11,12]. The experimental group included 20 defeated animals. Male mice (n=20) kept in individual cages over 5 days before the start of experiments served as the control

To study therapeutic activity of PAB-DSIP, 10 animals with anxiety and depressive syndrome from each group intraperitoneally received the aqueous solution of polyclonal rabbit antibodies in a single dose of 10 ml/kg (mixture of homeopathic dilutions C12+ C30+C200). Other animals from each group received an equivalent volume of distilled water (intragroup control). Treatment was performed immediately after 20-day modeling of anxiety and depressive syndrome.

Animal communication in the partition test was studied 1 h after single administration of PAB-DSIP or distilled water. The behavior of mice in the elevated plus-maze (EPM) and Porsolt's forced swimming test was assayed 5 days after treatment. Agonistic (competitive) interactions were terminated in the postinjection period.

The partition test is analogous to the test of social interactions [11]. The degree of animal's communicability was determined by the reaction to a partner in the adjacent department of a chamber separated by a

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0007-4888/03/supp-0140$25.00

transparent partition with holes. The test proceeded in 3 stages: activation (5 min), testing of the reaction to a familiar partner (5 min), and testing of the reaction to an unfamiliar partner (5 min). We recorded the number of approaches to and total time spent near a partition, which reflected the reaction to a partner in the adjacent department.

Anxiolytic activity of the preparation was determined in EPM. The maze consisted of 2 open arms (25×5×30 cm) and 2 closed arms (25×5×30 cm) set at right angle to each other and elevated above floor level (50 cm). The following 12 parameters were recorded over 5 min: number of entries into the open arms, center, and closed arms (% of the total number of entries), time spent in the open arms, center, and closed arms (% of the total testing time), and parameters for evaluation of risk (number of looks below the maze, peeping out from the closed arms, and transitions from one closed arm to another).

The Porsolt's test reflects the sensitivity to antidepressants. The total time of active and passive behavior in a glass with water (height 20 cm, inner diameter 9 cm, 25±1°C) was recorded for 5 min (sec).

The results were analyzed by Mann-Whitney test.

RESULTS

The partition test showed that after repeated social defeats the degree of communicability in defeated animals was markedly reduced (Table 1). The number of approaches to a partition decreased, which reflected suppression of locomotor activity. The reduction of communicability was considered to be related to an increase in the degree of anxiety. The EPM test showed that in experimental animals receiving distilled water the time spent in the open arms and center of the maze decreased, while the time spent in the closed arms increased compared to the control (Table 2). These changes were observed even 5 days after termination of competitive interactions. These animals displayed low number of entries into various compartments. Our results show that the psychopathogenic social factor produced general anxiety and decreased locomotor activity of animals (behavioral deficiency), which reflects the development of anxiety and depressive syndrome [2,11,12].

PAB-DSIP produced a strong anxiolytic effect on control and experimental animals in EPM (Table 2). In control animals the number of entries into the open arms increased, while the number of entries into the closed arms decreased. The preparation enhanced locomotor activity of mice and increased the number of looks below the maze. Animals of the main group were more sensitive to PAB-DSIP. In mice receiving PAB-DSIP we observed an increase in the time spent in the open arms, incidence of transitions between arms, and total number of entries. Besides this, after administration of PAB-DSIP the time spent in the closed arms decreased compared to animals treated with distilled water. However, these changes were insignificant due to the wide scatter of the data. Some animals receiving PAB-DSIP reached the terminal part of the closed arms. These changes reflect a decrease in the fear of open spaces (anxiolytic effect) and stimulation of exploratory activity.

The time of active swimming in the Porsolt's test decreased in mice receiving PAB-DSIP (Table 3). On the face of it, the preparation seemed to produce the pro-depressant effect under aversive conditions. However, the time of passive swimming reflecting depressiveness of animals remained unchanged. It may be suggested that a decrease in the time of active swimming over the first minutes resulted from the anxiolytic effect of PAB-DSIP that relieved the panic reaction during unavoidable stress.

Published data show that DSIP content changes in patients with psychoemotional and psychopathological disorders (Alzheimer's disease and Parkinson's disease) [8], Cushing's syndrome [9], and schizophrenia [16]. It is hypothesized that DSIP triggering cascade molecular processes produce various effects on animals with different resistance to emotional stress [15].

TABLE 1. Effect of Single Treatment with PAB-DSIP on Communicative Behavior of C57Bl/6J Mice in the Partition Test (n=20, $M \pm m$)

Parameter	Control	Experiment
Number of approaches to and looks at partition		
familiar partner	11.82±1.54	1.00±0.45^{+}
unfamiliar partner	11.73±2.04	1.20±0.73^{+}
Time spent near partition, sec		
familiar partner	93.64±9.71	1.20±0.73^{+}
unfamiliar partner	135.45±19.45*	1.80±0.97^{+}

Note. *p<0.05 compared to the reaction to an unfamiliar partner; ^{+}p<0.001 compared to the control.

TABLE 2. Behavior of Male Mice in the EPM Test 5 Days after Treatment with PAB-DSIP (n=20, $M\pm m$)

Parameter	Control		Experiment	
	distilled water	PAB-DSIP	distilled water	PAB-DSIP
Number of entries into the open arms, %	8.67±2.16	15.06±1.07*	10.06±4.04	15.49±3.36
Time spent in the open arms, %	6.19±1.90	8.35±1.48	1.41±0.62*	6.83±2.13^{+}
Number of entries into the center, %	48.77±0.42	50.00±0.40	45.17±2.53	45.55±1.14
Time spent in the center, %	19.69±0.85	19.07±2.08	6.43±2.02**	12.99±1.99^{+}
Number of entries into the closed arms, %	42.56±2.13	34.94±1.28*	44.78±6.06	38.96±3.80
Time spent in the closed arms, %	74.12±1.85	72.57±2.85	92.16±2.33**	80.17±2.71^{++}
Number of transitions	6.67±2.51	4.17±0.95	1.33±0.61	3.13±0.61^{+}
Number of looks	1.67±0.61	5.67±1.28*	1.67±0.56	3.13±0.67
Number of peeping out	3.50±0.62	5.83±1.19	4.33±0.61	8.25±1.67
Number of reaching the terminal part	0.33±0.21	0.79±0.29	0.00±0.00	0.75±0.25^{++}
Defecation rate	0.67±0.49	0.71±0.47	0.67±0.33	0.88±0.44
Total number of entries	31.67±4.08	27.43±1.41	10.00±1.93**	19.50±3.42

Note. *p<0.05 and **p<0.01 compared to control animals receiving distilled water. ^{+}p<0.05 and ^{++}p<0.01 compared to experimental animals receiving distilled water.

These peculiarities probably attest to various effects of PAB-DSIP on experimental and control animals in the Porsolt's tests and its strong anxiolytic action in EPM. These animals differ in stress reactivity and involvement of oligopeptides and hormones that regulate the influence of DSIP.

TABLE 3. Behavior of Male Mice in the Porsolt's Test 5 Days after Single Treatment with PAB-DSIP ($M\pm m$, sec)

Group	Swimming time	
	active	passive
Control		
distilled water	117.10±8.09	177.0±8.7
PAB-DSIP	106.78±5.94	185.56±7.45
Experiment		
distilled water	140.25±13.62	159.00±12.93
PAB-DSIP	104.88±11.80*	192.25±12.30

Note. *p<0.05 compared to experimental animals receiving distilled water.

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