PHYSIOLOGY

Cascade Afteraction of Delta Sleep-Inducing Peptide in Rats

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Delta sleep-inducing peptide is found to alter markedly the levels of substance P, β-endorphin, and corticosterone in the hypothalamus and blood plasma of rats, suggesting that the long-lasting stress-mitigating effects of this peptide are due to the considerable changes it causes in the content of other oligopeptides and hormones, involving them in various processes. Thus, DSIP itself appears to act only as a trigger, initiating a cascade of interdependent molecular reactions that correlate with the degree of resistance to stress.

Key Words: *delta sleep-inducing peptide; aftereffects; substance P; β-endorphin; corticosterone; resistance to emotional stress*

Our previous experiments showed that administering delta sleep-inducing peptide (DSIP) to animals makes them more resistant to emotional stress [5,10]. For Wistar and August rats, a positive correlation was found, using enzyme-linked immunosorbent and radioimmunosorbent assays, between the levels of DSIP, substance P, and β-endorphin, on the one hand, and resistance to emotional stress, on the other [3,8]. DSIP was shown to raise substance P levels in the hypothalamus of August rats predisposed to such stress [4,7]. The half-life of the DSIP molecule has been estimated to be 7-8 min in human plasma [9] and 3-4 min in rat plasma. This suggests that the stress-mitigating effects observed for animals injected with DSIP are determined not only by the activity of this peptide, but also by the activity of those endogenous biologically active substances which are produced in the body under its influence. Some authors have called attention to a cascade type of action exhibited by oligopeptides.

In view of the foregoing, the present study was undertaken to see how substance P, β-endorphin, and corticosterone levels would vary following injection of DSIP into animals differing in genetically determined resistance to emotional stress.

MATERIALS AND METHODS

For the tests, which ran from April to June, 1993, a total of 75 male Wistar rats (body weight 300-310 g) and 75 August rats (180-220 g) were used. The animals were kept in the vivarium under natural lighting conditions and had free access to water and food. As previous work has shown, Wistar rats are more resistant than August rats to emotional stress [5]. The control rats of both strains (15 animals of each) were injected with 0.5 ml of physiological saline intraperitoneally and decapi-

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TABLE 1. Substance P Levels in the Hypothalamus (pmol/g) and Blood Plasma (pg/ml) of Wistar and August Rats at Different Times after DSIP Injection

Wistar rats (n=75)			August rats (n=75)		
Group	Hypothalamus	Plasma	Group	Hypothalamus	Plasma
Control (n=15)	231.6±17.8°	32.5±5.7°	Control (n=15)	164.0±3.2	24.3±5.0
			After DSIP injection		
1 h (n=18)	208.5±45.3	41.2±3.0	1 h (n=20)	258.5±16.8**	36.8±2.2**
6 h (n=20)	305.0±40.7*	51.6±5.5**	6 h (n=19)	262.0±14.6**	37.0±6.6**
24 h (n=22)	212.8±20.8	47.0±4.6**	24 h (n=21)	228.6±11.6	31.0±5.0*

Note. Here and in Tables 2 and 3: *p<0.05 and **p<0.001 relative to control rats; °p<0.05 relative to August rats.

tated 60 min later, after which blood samples were taken from the hypothalamus for measurement of blood levels of substance P, β-endorphin, and corticosterone. The 120 test rats of both strains (60 animals of each) received an intraperitoneal injection of DSIP (Serva) in a dose of 60 nmol per kg body weight [6]. At 1, 6, or 24 h postinjection, the rats were decapitated, and their hypothalamuses were dissected out and immediately frozen in liquid nitrogen. The peptides to be tested were extracted with acetic acid [3]. Concurrently, blood samples were collected from the rats and plasma was obtained by centrifugation in the presence of EDTA. Substance P and the immunoreactivity of β-endorphin in the hypothalamus and plasma samples were measured radioimmunologically using standard kits (Amersham). Corticosterone was measured using a highly specific antiserum produced in the Laboratory of Endocrinology at the Institute of Experimental Pathology and Therapy, Moscow. Concentrations of the test substances were calculated by a method of transformation. The results were analyzed statistically using the nonparametric Mann-Whitney U test.

RESULTS

As shown in Table 1, the hypothalamic concentration of substance P recorded for Wistar rats was 10% lower 1 h after DSIP injection, 31.7% higher after 6 h, and 8.2% lower after 24 h as compared to the control value, while its plasma concentration was 26.8%, 58.3%, and 44.6% higher, respectively.

In the control group of August rats, hypothalamic and plasma levels of substance P were lower by 29.2% and 25.2%, respectively, than in their Wistar counterparts.

At 1, 6, and 24 h after DSIP administration, hypothalamic levels of substance P in the test August rats were 57.8%, 59.8%, and 39.4% higher, respectively, than in the controls, while its plasma levels were 51%, 52%, and 27.8% higher.

The hypothalamic level of β-endorphin in the test Wistar rats was 42.5% below the control value 1 h after DSIP injection and 54.7% below it at 6 h, but after 24 h it had risen sharply to exceed the control value by 74.3%. Similarly, plasma levels of β-endorphin in these rats were 27.9% and 35.3% below the control value at 1 and 6 h postinjection and 121.4% above it at 24 h. The control August rats had a lower hypothalamic level (by 8.2%) and a higher plasma level (by 105.6%) of β-endorphin than did the Wistar controls (Table 2).

In the test August rats, the hypothalamic level of β-endorphin virtually did not differ from the control value at 1 h after DSIP injection but fell far below it later (by 68.8% at 6 h and 50.8% at 24 h), while the plasma levels of this substance were 46% below the control value at 1 h, 21% above it at 6 h, and 76% below at 24 h.

As can be seen in Table 3, the control Wistar rats had a lower plasma concentration of corticosterone than the August controls. After DSIP in-

TABLE 2. β-Endorphin Levels in the Hypothalamus (pmol/g) and Blood Plasma (pg/ml) of Wistar and August Rats at Different Times after DSIP Injection

Wistar rats (n=75)			August rats (n=75)		
Group	Hypothalamus	Plasma	Group	Hypothalamus	Plasma
Control (n=15)	622.0±64.8°	33.7±7.4°	Control (n=15)	571.3±33.2	69.3±6.7
			After DSIP injection		
1 h (n=18)	357.8±58.7**	24.3±2.8	1 h (n=20)	576.0±16.8	37.4±14.7
6 h (n=20)	281.6±34.8**	21.8±4.5*	6 h (n=19)	178.0±28.7**	83.8±29.9*
24 h (n=22)	1084.0±253.7**	74.6±26.4**	24 h (n=21)	280.8±54.5**	15.1±6.3**

TABLE 3. Corticosterone Levels (μg%) in Blood Plasma of Wistar and August Rats at Different Times after DSIP Injection

Wistar rats (n=75)		August rats (n=75)	
Group	Plasma	Group	Plasma
Control (n=15)	8.0±2.5°	Control (n=15)	36.1±7.37
	After DSIP injection		
1 h (n=18)	5.5±3.8*	1 h (n=20)	15.6±1.3**
6 h (n=20)	16.4±6.1**	6 h (n=19)	20.8±6.9*
24 h (n=22)	12.3±5.1**	24 h (n=21)	22.2±6.3*

jection, the plasma corticosterone level was 68.7% below the control value at 1 h and 105% and 57.8% above it at 6 and 24 h, respectively, in the test Wistar rats and 43.2%, 57.6%, and 61.5% above it in the test August rats.

Thus, as these experiments have shown, Wistar rats, which are more resistant to emotional stress than August rats, also contain higher concentrations of substance P in the hypothalamus and blood plasma as well as a higher β-endorphin concentration in the hypothalamus. August rats, on the other hand, have higher plasma levels of β-endorphin and corticosterone. These findings agree with our previous studies [3,7], in which enzyme-linked immunosorbent assays and radioimmunoassays demonstrated higher levels of β-endorphin, substance P, and DSIP in the hypothalamus of Wistar rats. The higher β-endorphin and corticosterone concentrations found in the plasma of August rats suggest that the hypophyseoadrenal mechanism is activated in this strain. As shown previously [11], adenocytes of the anterior pituitary produce β-endorphin when expression of the proopiomelanocortin gene is activated, so that the plasma level of β-endorphin rises. Our present experiments indicate that the administration of DSIP brings about marked changes in the hypothalamic and plasma levels of substance P and β-endorphin and the plasma level of corticosterone. This, in turn, can be taken as evidence that the long-lasting antistress effects observed after DSIP administration are determined by substantial alterations in the concentrations of other oligopeptides and hormones that become involved in the various reactions under the influence of DSIP. Apparently, DSIP only acts as a trigger to initiate a cascade of interdependent molecular reactions, which, as our experiments suggest, differ in the two strains used. One indication of this difference is the observed variation in the hypothalamic concentration of substance P after DSIP injection: Wistar rats had the highest concentrations of this substance at 6 h postinjection and much lower concentrations at 1 and 24 h, whereas August rats showed progressive increases in its concentration from 1 to 24 h. Moreover, the plasma level of substance P after DSIP injection continued to rise throughout the 24-h observation period only in Wistar rats (in August rats it had fallen by 24 h after the earlier rises at 1 and 6 h).

It follows, then, that Wistar rats, which are more resistant to emotional stress than August rats, exhibit smaller variations in the hypothalamic content of substance P over time and longer-lasting changes in its blood content after DSIP administration.

The hypothalamic concentration of β-endorphin was markedly increased at 24 h after DSIP injection in both strains, but the largest increase occurred at 24 h postinjection in Wistar rats and at 6 h in August rats. The changes in plasma β-endorphin in Wistar rats at 6 and 24 h were the opposite of those recorded for August rats: its concentration had fallen by 6 h followed by a rise at 24 h in the former rats and had risen sharply by 6 h followed by a decline in the latter.

However, the greatest differences between the two strains after DSIP injection were detected in plasma levels of corticosterone. These were above the control value at 6 and 24 h postinjection in Wistar rats and below it at all three times in their August counterparts. As the blood level of corticosterone characterizes stress resistance [2], this observation indicates that in August rats the mechanisms via which resistance to stress arises are stimulated by DSIP to a lesser extent than in Wistar rats.

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Use of Peripheral Electrography to Evaluate Synchronism of Electrical Activity in Different Parts of the Gastrointestinal Tract

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Use of the technique of peripheral electrography to study electrical activity in various parts of the gastrointestinal tract in dogs after fasting and during digestion is described, and it is shown that a computerized spectral analysis of peripheral electrograms makes it possible to monitor the cycle of fasting periodic gastrointestinal activity. In the process of digestion, the cycle of fasting periodic activity is disrupted and nonadjacent areas of the gastrointestinal tract exhibit synchronous electrical activity.

Key Words: *gastrointestinal tract; migrating myoelectric complex; rhythms; peripheral electrography; spectral analysis*

The recording of electrical activity from the body surface remains problematic. In particular, it is not clear whether the method of peripheral electrography (PEG) can be applied to evaluate peak activity of smooth muscle in the gastrointestinal tract (GIT).

It has been shown that the signal from the GIT recorded with PEG in the low-frequency region carries mainly information about the intensity of peak electrical activity in parts of the GIT [5,6]. Indeed, as indicated by data obtained from intracellular electrodes, the repolarization of a membrane potential in smooth muscle is a fairly long process - taking an amount of time of the order of that of a slow wave (i.e., the total cellular current is not equal to zero). In addition, the action potentials arising at the crests of slow waves are grouped in clusters so that the intensity of their generation in smooth-muscle tissues of the GIT is traceable on the body surface at frequencies equal to basal rhythms of the GIT (0.04-0.35 Hz).

Until now, the periodic activity of the GIT has been studied chiefly by electromyographic methods using implanted electrodes [1,4,8]. The purpose of this study was to evaluate by the noninvasive technique of PEG to what extent the electrical activity in various parts of the GIT is synchronous during fasting periodic activity and in the process of digestion.

MATERIALS AND METHODS

Mongrel dogs of both sexes weighing 15-30 kg were used. In chronic experiments, peripheral electrograms were recorded for 3 h after 18 h of food deprivation and during digestion following the intake of a standard breakfast.

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