Pharmacology Biochemistry & Behavior, Vol. 19, pp. 883–886, 1983. © Ankho International Inc. Printed in the U.S.A.

Attack Stress and IgE Antibody Production in Rats

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Received 8 March 1983

ITO, Y., K. MINE, Y. AGO, T. NAKAGAWA, M. FUJIWARA AND S. UEKI. *Attack stress and IgE antibody production in rats.* PHARMACOL BIOCHEM BEHAV **19**(5) 883–886, 1983.—The effect of stress on production of immunoglobulin E (IgE) in rats was investigated, the IgE being titrated by passive cutaneous anaphylaxis (PCA) reactions. In rats, exposed to attack by other rats made aggressive by intraventricular injections of 6-OHDA for one hour per day for three consecutive days before the first immunization, there was no difference in the titer of IgE as compared to the control rats, but, in the rats exposed to stress procedure before the second immunization, the production of IgE was significantly suppressed.

Rat   Aggressive behavior   Stress   Immunoglobulin E (IgE)

EMOTIONAL stress may contribute to the onset and progression of allergic diseases such as bronchial asthma, rhinitis and urticaria [1, 14, 32], but the mode of such effects remains unknown. In animal experiments, both humoral and cell mediated immune responses in various animal species were suppressed [7, 9, 19, 25, 26, 28, 31] and immune responses were either not affected [18] or were increased [20,24] by various types of stress.

With regard to humoral response, there have been reports on the effect of stress on antibody formation of IgG and IgM, but the production of IgE, which is considered to play an important role in atopic diseases, has been given little attention. Kamoshita *et al.* seem to have pioneered this work [10]. Russmussen *et al.* reported the effect of emotional stress on anaphylaxis [21], however, they investigated changes in the susceptibility of animals to the chemical mediators released in anaphylaxis and not the change in production of IgE. We investigated the effect of emotional stress on the production of IgE using a new stress model, in which animals are exposed to the attack by other animals that are artificially made aggressive by intraventricular injection of 6-OHDA [16].

METHOD

Subjects

All rats were obtained from Kyushu University Institute of Experimental Animals. Thirty male Wistar rats were used for 6-OHDA treatment, and the body weight of the rats at the time of intraventricular injection ranged from 200 to 250 g.

After this treatment, the animals were housed in isolation for one month. The animals used for passive cutaneous anaphylaxis (PCA) reactions were male Wistar rats weighing 160~180 g. As the stressed rats and control group, forty male Wistar rats weighing 200~250 g were used and were housed in groups of five per cage, except for the period of stress exposure. Food and water were supplied ad lib. Throughout the experiment the animal room was maintained at a temperature of 23±1°C.

Illumination was provided on a 12-hour light-dark cycle with lights on from 7:00 a.m. to 7:00 p.m.

Antigens

Dinitrophenyl coupled-Ascaris extracts (DNP-Asc) and killed Bordetella pertussis were used. Ascaris protein was extracted from Ascaris suum [27]. DNP conjugate was prepared according to the method of Eisen *et al.* [4].

Procedure

Stress overload was then carried out. Twenty-four rats were made aggressive, according to the method already reported [16]. Briefly, male Wistar rats given 500 μg of 6-OHDA intraventricularly were housed in isolation for one month and their tails were continuously pinched by metal clips during the paired housing period. Nontreated Wistar rats were placed in the same cage with the aggressive rats. Fighting behavior was induced throughout one hour of paired housing and the fighting posture of the two rats was recorded at 5 min intervals commencing 5 min after the start of paired housing a total of twelve times for one hour, according to the definitions of Grant and Mackintosh [8], Miczek [15] and Carlini [3]. For each rat, the numbers of aggressive posture were recorded as the dominant score and the total number of

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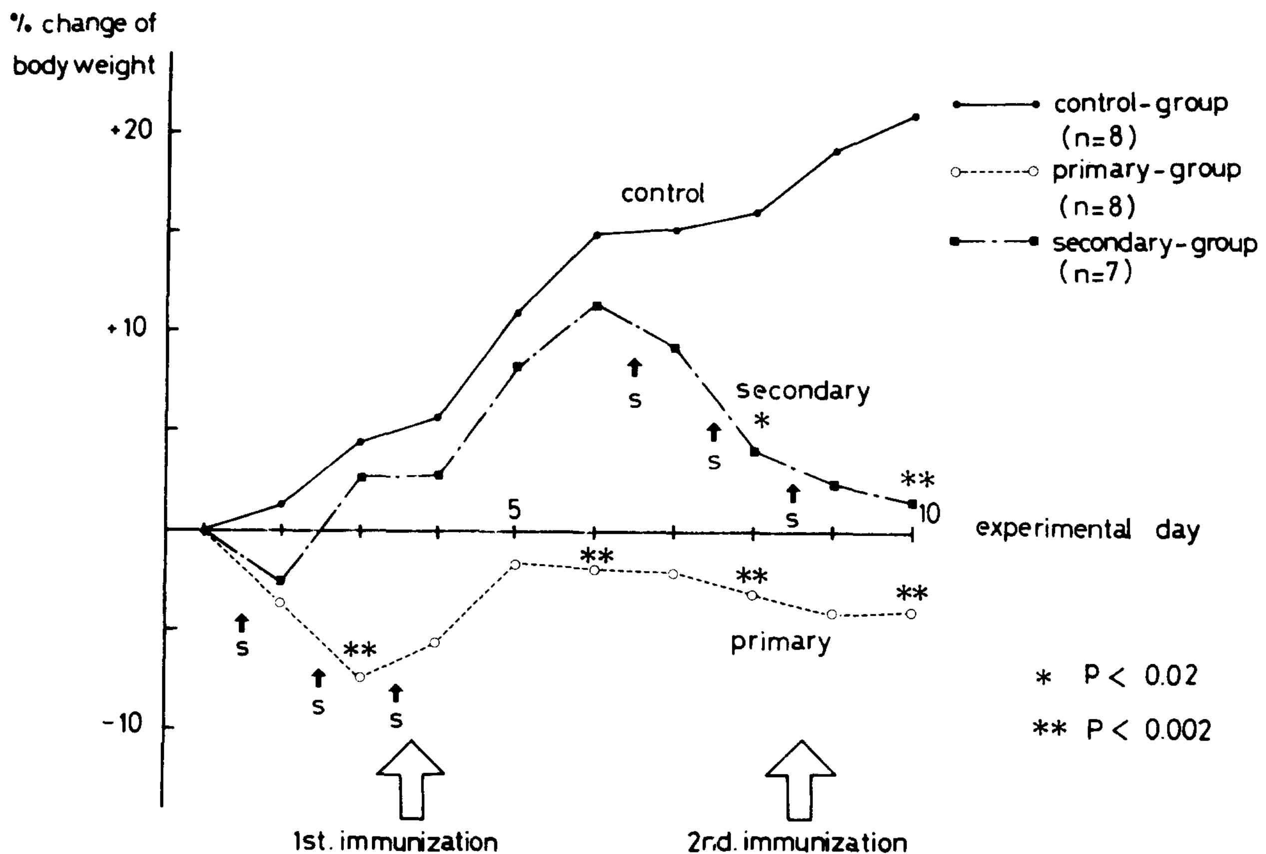

FIG. 1. Experimental procedure and change in body weight in each experimental group. S represents the stress exposure for one hour per day.

defensive upright, immobile crouch and submissive supine posture was recorded as the subordinate score. Any rat attacked by a 6-OHDA-treated rat with a subordinate score of ≦8 in fighting for one hour was excluded from the experiment. Paired housing was consistently conducted between the hours of 9:00 a.m. to 10:00 a.m. Twenty-four normal Wistar rats were grouped into three. Eight rats were designated as the controls, primary and secondary groups, respectively. The primary group was exposed to the attack of 6-OHDA-treated rats for one hour per day for three consecutive days until the first immunization and the secondary group for three consecutive days until the second immunization. All rats of each group were weighed daily.

All the rats of three groups were immunized simultaneously, according to the method of Tada and Okumura [29]. DNP-Asc 1.0 mg mixed with 10^{10} killed *B. pertussis* was injected into the four footpads.

Five days later a booster dose of 0.5 mg of DNP-Asc alone was given into the dorsum. According to Tada and Okumura the IgE antibody was first detected seven days after the primary immunization and reached the maximum on the 8–10th days [29]. In both the primary and secondary group, immunization was performed immediately after one hour of paired housing on the third day of stress. Eight days after the first immunization, blood samples were obtained from aorta after laparotomy under ether anesthesia and immunoglobulin E (IgE), immunoglobulin G (IgG), and immunoglobulin M (IgM) were titrated.

The IgE was titrated by PCA test, according to the method of Tada and Okumura [29]. IgG and IgM were titrated using laser immunoassay [11]. Immediately after blood samples had been obtained, bilateral adrenal glands were removed and weighed. To investigate changes in levels of plasma corticosterone, 16 normal male Wistar rats (8—control; 8—stress exposed) were used without immunization.

Eight rats were decapitated and blood specimens were obtained immediately after the same stress procedure used for the primary and secondary groups. The levels of plasma corticosterone were determined using radioimmunoassay after corticosteroids had been separated from blood specimens using Sephadex LH-20 chromatography [22]. The percent changes of mean body weight in control, primary and secondary groups on the third, sixth, eighth and tenth experimental days were compared using the Mann-Whitney U-test. Other statistical analyses were performed using Student's *t*-test.

RESULTS

6-OHDA-treated rats violently attacked the intact rats during the one hour of paired housing, and fighting episodes

TABLE 1

EFFECT OF STRESS ON WEIGHT OF ADRENALS IN EACH EXPERIMENTAL GROUP

Group	N	Weight of adrenals (mg ± S.E.)	Weight of adrenals (mg/100 g body weight ± S.E.)
Control	8	49.6 ± 1.0	19.2 ± 1.2
Primary	8	72.2 ± 4.8*	38.0 ± 4.0*
Secondary	7	71.0 ± 4.2*	34.2 ± 3.0*

*Significantly different from control ($p<0.01$).

TABLE 2

EFFECT OF STRESS ON THE LEVEL OF PLASMA CORTICOSTERONE

Group	Plasma corticosterone level (μg/dl ± S.E.)
Control	2.4 ± 0.4
Stressed	44.9 ± 3.0*

*Significantly different from control ($p<0.001$).

were equally observed, as in our previous report [16]. The 6-OHDA-treated rats consistently played a dominant role, and no rat attacked by 6-OHDA-treated rats showed a subordinate score of ≦8.

One rat in the secondary group died two days after the second immunization. In both the primary and secondary groups, the rats lost weight during the stress procedure, and weight gain was retarded throughout the experiment (Fig. 1).

Both the primary and secondary groups showed marked increases in adrenal weights compared to the controls ($p<0.01$) (Table 1).

The levels of plasma corticosterone of the stressed groups were extremely higher than in the controls ($p<0.001$) (Table 2).

The PCA titer in the controls was 134.0 (32–256), 94.0 (16–256) in the primary group, and 31.4 (8–64) in the secondary group.

The secondary group had a significantly lower titer, as compared to the control group ($p<0.05$) (Fig. 2). In the secondary group, PCA titer was lowered regardless of the degree of adrenal hypertrophy or loss of body weight. Thus in the group stressed before the second immunization, the pro-

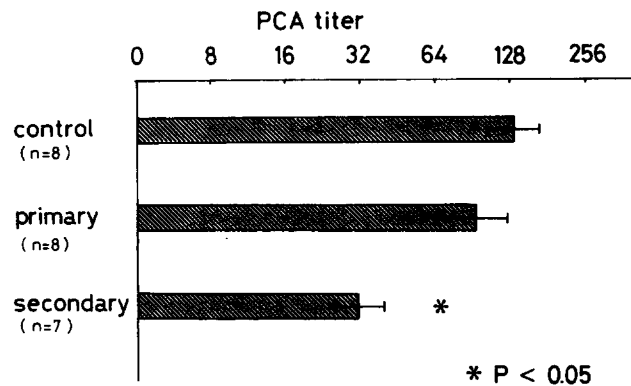

FIG. 2. Titer of IgE in each experimental group. Horizontal lines indicate the standard error.

duction of IgE was suppressed, but in both the primary and secondary groups, there was no difference in the levels of IgG and IgM, as compared to the controls (Table 3).

DISCUSSION

There are several reports suggesting that stress may reduce antibody response other than IgE [7, 9, 25, 26, 28, 31].

Under the conditions in the present experiment, psychological stress suppressed IgE formation against DNP-Asc without influencing the levels of IgG and IgM.

Our data confirm the notion that emotional stress modifies the atopic disease responses in which IgE plays a role. Adrenal weights and plasma corticosterone levels were highly increased in the group exposed to the same stress.

Considering that stress caused adrenal hypertrophy to the same extent in both the primary and the secondary groups, plasma corticosterone would also be as high as that at the time of immunization after stress exposure in both groups. But in the primary group, plasma corticosterone levels might be normal or rather low as the result of the rebounds.

Glucocorticosteroids suppress immune responses [5], and several workers noted that stress-induced immunosuppression is due to increased levels of glucocorticosteroids [7, 9, 17].

However, it has been shown that IgE antibody formation is enhanced by glucocorticosteroids in rats [30].

Amkraut and Solomon [2] reported that the serum corticosterone level was increased double or triple and IgE but not IgG was increased in rats stressed by food limitation. Therefore, suppression of IgE in the secondary group may

TABLE 3

EFFECT OF STRESS ON PLASMA IgG AND IgM LEVELS IN EACH EXPERIMENTAL GROUP

Group	N	IgG (mg/dl ± S.E.)	IgM (mg/dl ± S.E.)
Control	8	127.0 ± 18.6	14.2 ± 1.9
Primary	8	118.1 ± 17.3	17.0 ± 2.4
Secondary	7	126.3 ± 11.2	14.9 ± 1.7

not be related to hyperactivity of the pituitary-adrenal system. Malnutrition accompanied by weight loss may lead to a suppression of antibody formation.

However, such can probably be ruled out as the levels of IgG and IgM were not influenced by stress exposure.

It has been reported that minor tranquilizer diazepam is effective in alleviating the immunosuppression by restraint stress [7,10]. Stress may influence the function of the immunologic system via the central nervous system and the endocrine system [23].

Macris *et al.*[13] reported that PCA was markedly suppressed by anterior hypothalamic lesioning in rats.

Stimulation or destruction of hypothalamic region alters antibody responses [12]. In the present experiment, central nervous system may be important in the suppression of IgE production by attack stress, in which more psychological and less physical factors may play a role than other stresses (restraint, cold, overcrowding, food limitation) do.

Kamoshita *et al.* reported that the production of IgE in mice was explicitly inhibited by restraint stress prior to the first immunization, but not prior to the second immunization [10].

It is known that IgE antibody production is performed in co-operation with various immunocompetent cells such as T cells (helper, suppressor), B cells and macrophage, which show different responses to stress among species of animals, antigens, timing of stress, etc. [7,23].

These might cause controversial results between ours and those of Kamoshita *et al.* or of Amkraut and Solomon.

The present result was that stress exposure before the secondary immunization but not the primary suppressed IgE formation. We speculate that these results suggest that sensitized cells may be more responsive to stress than intact ones

ACKNOWLEDGEMENT

This study was supported by Japanese Ministry of Education, Science and Culture, Grant-in-Aid for Scientific Research. We are grateful to M. Ohara for critical review of the manuscript.

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