Physiology & Behavior, Vol. 60, No. 3, pp. 1039–1042, 1996

0031-9384/96 $15.00 + .00

ELSEVIER

PII S0031-9384(96)00135-7

BRIEF COMMUNICATION

Short-Term Consumption of a Diet Rich in Fat Decreases Anxiety Response in Adult Male Rats

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Received 20 September 1995

PRASAD, A. AND C. PRASAD. *Short-term consumption of a diet rich in fat decreases anxiety response in adult male rats.* PHYSIOL BEHAV **60**(3) 1039–1042, 1996.—Short- and long-term changes in the composition of dietary macronutrients [protein (P), carbohydrate (C), and fat (F)] alter neurochemistry and behavior in animals. We examined whether short-term intake of a diet rich in P, C, or F affected their anxiety response (AR). AR of Sprague–Dawley rats was measured in an elevated plus maze. Rats were placed in the black compartment facing the wall opposite the aperture, and the time (max. 360 s) it took to enter the white compartment with all four paws was noted. Rats were fed Purina chow and tap water unless otherwise indicated. On repeated testing (three times on the same day) AR increased and, consequently, most rats spent the entire 360 s in the dark. Whereas most rats exhibited low anxiety response in trial 1, which increased during successive trials (low–high group), some exhibited high initial anxiety that remained unchanged (high–high group). To determine whether macronutrients may alter AR, groups of low–high and high–high rats were tested three times on the same day and then put on a P, C, or F diet for 7 days. On day 8, they were again tested for AR in a single trial and the results compared with those of the third trial of the previous test (pre-C: 302 ± 39, post-C: 294 ± 42, $p > 0.05$; pre-P: 305 ± 35, post-P: 297 ± 43, $p > 0.05$; pre-F: 321 ± 17, post-F: 241 ± 24sec, $p = 0.009$; $n = 30$; mean ± SEM). The results show that a diet rich in F, but not P or C, decreases AR in rats.

Dietary fat Dietary carbohydrate Dietary protein Anxiety Hormones

DIETARY macronutrients are important determinants of brain function (24,28). However, studies into the role of diet in adult brain function are few and are limited to long-term dietary manipulations. For example, long-term changes in the composition of macronutrients in diet have been shown to have significant effects on brain chemistry, behavior, and pharmacological responses in adult animals. For example, rats consuming a diet high in protein (50% calories from casein) for 20 weeks exhibit hyperactivity, decreased anxiety, hyperalgesia, and hyperresponsiveness to noxious stimuli and dopaminergic agonists (19,20), amplification of motor cortical potentials (5), and increased spine density in the cortex and striatum (6). Long-term changes in the dietary protein/carbohydrate ratio have been shown to alter the distribution of a variety of neurotransmitters and their receptors in rats (11,15). In addition, long-term consumption of a diet low in fat alters brain serotoninergic responsiveness in Cynomolgus monkeys (18). Therefore, it will be of interest to determine whether short-term dietary manipulations alter behavior. Such information could be helpful in modulating therapeutic efficacies of a variety of CNS-active drugs. To this end, we have chosen to examine the effect of high fat diet on the behavior of rats in the elevated plus maze test, a putative measure of anxiety. Whereas the elevated plus maze test for the measurement of anxiety is a fairly reliable, inexpensive, and simple, it has many limitations that must be considered when analyzing the results (7). Some of these include: i) confounding of anxiolytic and anxiogenic effects of drugs that may also affect motor activity, and ii) marked day to day variation in the baseline measurements.

METHOD

Animals and Diet

Outbred adult male Sprague–Dawley rats (250–300 g) were purchased from Hilltop Laboratories (New York, NY). Animals were housed in a temperature- and light-controlled (20–21°C, 12-h light) room with free access to Purina Chow and water unless specified otherwise. For dietary manipulations, rat chow was withdrawn and rats were presented with feeding jars containing one of the three diets that derived 90% of its total calories from protein, carbohydrate, or fat and the remainder from the

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TABLE 1
COMPOSITION OF DIETS

Ration	Diets (g/kg)		
	Carbohydrate	Fat	Protein
Casein*	48.4	86.7	834.4
Corn starch*	417.9	34.7	19.1
Sucrose	417.9	34.7	19.1
Corn oil†	21.4	38.5	21.2
Crisco†	—	635.9	—
Cellulose‡	48.3	86.7	48.3
Salt mixture§	33.8	60.7	33.8
Vitamin mixture¶	9.7	17.3	9.7
DL-Methionine	0.7	1.3	12.5
Choline bitartrate	1.9	3.5	1.9
Total (g)	1,000	1,000	1,000
Macronutrient (kcal %)			
Carbohydrate	90	5	5
Fat	5	90	5
Protein	5	5	90
Caloric density (kcal/kg)	3,768	6,762	3,724

*ICN Pharmaceuticals, Cleveland, OH.
† Proctor and Gamble, Cincinnati, OH.
‡ Alphacel nonnutritive bulk (ICN Pharmaceuticals).
§ Briggs salt mixture (ICN Pharmaceuticals).
¶ Vitamin fortification mixture (ICN Pharmaceuticals).

other two macronutrients in equal proportion (Table 1). All experiments were initiated at least 7 days after arrival of the rats in the animal care facility. The rats were brought to the behavioral laboratory 24 h before testing.

The Elevated Plus Maze and Measurement of Anxiety

The elevated plus maze is made of wood and has two opposite open and closed arms set in a plus configuration. The open and closed arms are painted in cream and black, respectively. The dimensions of the open arms are 50 × 10 cm (length × width) and the black arms are 50 × 10 × 40 cm (length × width × height). The whole maze is elevated 50 cm off the ground. The open arms are exposed to a 25.2 lx light, and the black compartments are covered at the top.

To measure the anxiety response, rats were placed in the black compartment facing the wall opposite the aperture and the time it took the animal to exit the black compartment was noted. The experiment was terminated at 360 s and the maximum time of 360 s was noted. The rat was then taken out, and the maze was wiped with alcohol and allowed to dry. Then the next rat was put into the maze. The rats underwent three successive trials on the same day at intervals of 60–90 min. Food and water were available ad lib between testings.

Statistics

The data are presented as mean ± SEM and are analyzed statistically by analysis of variance followed by a *t*-test.

RESULTS

Naive outbred male Sprague–Dawley rats were subjected to three consecutive tests on a single day in the elevated plus maze. The results show a wide rat-to-rat variation in anxiety response in the first trial, in which the mean time spent in the dark compartment was 89 ± 19 (n = 45) seconds with a range from 5–360 s and a median of 22 s. Most of subjects (68%) exited the dark compartment in less than 30 s. However, some rats (16%) took 330–360 s to exit the dark compartment. The time other subjects spent in the dark varied between the two groups. While on the open arm, rats spent most of the time sniffing or exploring the platform. The time spent in the dark compartment in trials 1, 2, and 3 increased on repeated testing at an interval of 60–90 min. Whereas the mean time spent in the dark compartment increased on repeated testing, individual rats exhibited significant differences in the patterns of response on repeated testing. For example, although most rats exhibited low anxiety responses in trial 1 that increased during successive trials (low–high group), some exhibited high (high–high group) or low (low–low group) initial anxiety that remained unchanged; however, a small population (12%) did not show any reproducible pattern and was eliminated from further study.

Rats exhibiting low–high or high–high patterns of anxiety were divided into three groups (n = 30 per group) and placed on a high-fat, high-carbohydrate, or high-protein diet (see Table 1) for 7 days. On day 8, rats were tested in the elevated plus maze once to determine whether dietary manipulation had decreased their anxiety response. The data presented in Fig. 1 show a significant decrease in the anxiety response in rats on a high-fat diet (pre-F: 321 ± 17, post-F: 241 ± 24 s, p = 0.009; n = 30; mean ± SEM). In contrast, rats on high-carbohydrate or high-protein diets did not show a significant change in the anxiety response (pre-C: 302 ± 39, post-C: 294 ± 42, $p > 0.05$; pre-P: 305 ± 35, post-P: 297 ± 43, $p > 0.05$).

DISCUSSION

The biologic mechanisms underlying the expression of fear, anxiety, and panic behavior are poorly understood; therefore, it is not easy to provide a clear explanation for the anxiolytic action of high-fat diet. Most of our understanding of a possible neurochemical basis for anxiety has emerged from a long history of experience with the use of various pharmacological agents (alcohol, bromides, belladonna alkaloids, opiates, barbiturates, ben-

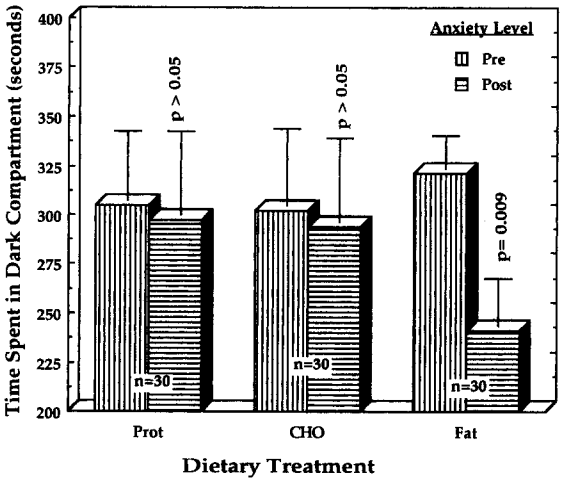

FIG. 1. Short-term consumption of a diet rich in fat but not carbohydrate or protein decreases anxiety in rats selected for high anxiety. Prot, CHO, and Fat refer to protein, carbohydrate, and fat diets, respectively, whose composition is given in Table 1. The data are presented as mean ± SEM.

zodiazepines, and many others) in the treatment of anxiety disorders (12). Based on the therapeutic efficacy of γ-aminobutyric acid (GABA) agonists in the treatment of anxiety disorders, the cortical benzodiazepine–GABA receptor–chloride channel complex has been considered one of the major sites for anxiety regulation (12). In pharmacological studies in animals and/or humans, however, a variety of agents endogenous to the brain have been shown to attenuate or augment anxiety behavior (1,2,8,14,22,25,27,29). These include corticosteroids (2,14), cholecystokinin (8,22,29), pentagastrin (1), and corticotropin-releasing hormone (CRH) (25) as anxiogenic agents, and neuropeptide Y (27) as anxiolytic agent. Other human studies in the search for peripheral chemical markers of anxiety have also yielded interesting data. These include: i) a significant positive correlation between anxiety level and plasma concentrations of gastrin and insulin in healthy women (26); ii) an increase in plasma concentrations of kynurenine with increasing severity of anxiety (21); and iii) a differential noradrenergic and hormonal (growth hormone, prolactin, luteinizing hormone, and melatonin) response to physical exercise in adolescents with high or low senses of anxiety, guilt, self-esteem, and tolerance to frustration (13). Although the above data suggest there is a psychoneuroendocrinological antithesis to anxiety behavior, a direct causal relationship between hormone levels and anxiety has not yet been established.

Considering the multiplicity of endogenous mediators that may alter the anxiety response, it is possible that consumption of a diet rich in fat may alter one or more of these mediators, thus decreasing anxiety. To this end, we surveyed the literature for data on the neurochemical and neuroendocrine effects of high dietary fat intake. Our literature review uncovered information on changes in plasma levels of a variety of hormones, including gastric inhibitory polypeptide (10), glucagon (3), corticosterone/cortisol (4,9,16,17), and T_3 (23) following acute/chronic ingestion of a high-fat diet by humans, dogs, and rodents. Of all the hormonal changes linked to a high-fat diet, hypercorticosteronemia/hypercortisolimia during chronic consumption of a fat-rich diet appears to be the most consistent (4,9,16,17).

In conclusion, it is tempting to speculate that hypercorticosteronemia secondary to a diet rich in fat may decrease neuronal CRH levels, possibly via a feedback control of "central (hippocampo)–hypothalamo–pituitary–adrenal axis," resulting in decreased anxiety. In addition, it is possible that the changes in corticosterone and/or other hormone levels may directly alter neuronal levels of anxiety-modulating agents.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Air Force Office of Scientific Research (DEPSCoR-94-NL-102). The authors thank Anne Compliment for her superb editorial assistance.

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