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BRIEF COMMUNICATION

Effects of Dietary Fat on Pain Threshold, Thermoregulation and Motor Activity in Rats

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YEHUDA, S., C. E. LEPROHON-GREENWOOD, L. M. DIXON AND D. V. COSCINA. *Effects of dietary fat on pain threshold, thermoregulation and motor activity in rats.* PHARMACOL BIOCHEM BEHAV 24(6) 1775–1777, 1986.—Groups of young male Sprague-Dawley (albino) or Long-Evans (hooded) rats were fed the same semi-purified diets containing 20% (w/w) fat in the form of soybean oil vs. lard, or a reference diet of standard Purina Chow (4.5% mixed fats) for 21 days. Behavioral testing after this time revealed that albino rats fed the diet containing soybean oil had increased paw-lick latencies on a 58°C hot plate compared to chow-fed rats. In addition, both strains fed the diet containing soybean oil were protected from hypothermia induced by placing animals in a 4°C cold room for 60 min following systemic injection of 10–15 mg/kg d-amphetamine. Rats of both strains fed the lard diet displayed paw-lick latencies similar to those shown by rats fed chow and hypothermic changes intermediary to those shown by rats fed soybean oil vs. chow diets. Horizontal crossings as well as rearings in a 15 min test of open field activity were the same for all diet groups within strains. No substantial differences were observed in the number of calories consumed, amount of body weight gained or basal colonic temperatures across diet conditions. The results suggest that a soybean oil-based diet can alter physiological mechanisms which mediate these indices of pain perception and thermoregulation. More generally, they indicate that qualitative changes in dietary fat content may be capable of altering certain behavioral states.

Dietary fat   Soybean oil   Lard   Hot plate   Pain   Rat   Thermoregulation   Amphetamine   Open field activity

SINCE the initial publication of evidence that variations in tryptophan can alter levels of brain serotonin [2], there have been many subsequent studies which have demonstrated the capacity of dietary manipulations to modify brain biochemistry (e.g., [8]) and behavior (e.g., [9]). Most of this research has focused on the effects of changing brain neurotransmitter metabolism by varying the amount or availability of precursors for neurotransmitter synthesis or co-factors (vitamins and minerals) necessary for optimal enzyme activity. However, much less is known about the effects of other types of diets or nutrients on brain biochemistry and behavior. To the best of our knowledge, the potential capacity of dietary lipids to influence brain biochemistry and behavior has been largely ignored. This relationship may be important to study since fatty acids derived from diets are able to penetrate into the brain and modify the lipid composition of neuronal membranes [3]. As a secondary effect, such changes may alter the degree of membrane fluidity [7] and/or functional states of specific membrane-bound proteins [4,6] which ultimately contribute to brain neuronal processes.

The experiments reported here represent a first attempt to determine if modifying the quality of dietary lipids can alter aspects of behavior. To achieve that purpose, relatively rudimentary (i.e., unlearned) behavioral processes were chosen for investigation in rats. The specific behavioral indices quantified were: (1) paw-lick latencies after a single placement on a thermoelectric hot plate, (2) colonic hypothermia induced by exposure to environmental cold [10], and (3) horizontal

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crossings and rearings in a single test of open field exploration. In order to assess the potential generality of any such behavioral changes, two separate animal strains were investigated. The results obtained show that changing the source of dietary lipid can significantly alter paw-lick latencies and amphetamine-induced hypothermia.

METHOD

Subjects were male Sprague-Dawley (albino) and Long-Evans (hooded) rats weighing 100–120 g (approximately 30–40 days old) at the beginning of these investigations. Rats were housed individually in hanging stainless steel, wire-mesh cages contained in a well ventilated room maintained at 22°C±1°C. Light was provided from 0800 to 2000 hr daily. Rats of each strain were divided into one of three diet conditions based on equivalent starting body weights. Two experimental semi-purified diets of equal caloric density (4.486 kcal/g) were used, each containing the same amounts of protein (casein: 270 g/kg), carbohydrate (corn starch: 402 g/kg), fiber (Alphacel: 50 g/kg) and vitamin/mineral mix (76 g/kg). Each diet contained 200 g/kg fat from one of two sources: (1) soybean oil (polyunsaturated, vegetable fat), or (2) lard (saturated, animal fat). The third diet used was Purina Lab Chow, which is totally different in its caloric density (3.6 kcal/g) and nutrient composition (228 g/kg protein, 525 g/kg carbohydrate and 38 g/kg fiber, all from grain sources; approximately 82 g/kg vitamins and minerals; plus 45 g/kg mixed fats, largely from vegetable sources). However, it was included to serve as a general reference diet since it is so widely used to feed experimental animals. Throughout these studies rats had free access to weighed amounts of their assigned diets and to tap water. Body weights were recorded across these feeding conditions.

Following three weeks of feeding these diets to different groups of rats, three categories of behavior were tested, one on each of three different days. On the first day, motor activity was measured during a 15 min session in an automated open field apparatus (see [5] for description). The number of horizontal crossings (detected by infrared photobeams) and the number of rearings (monitored by video camera and recorded on video tape) were scored for each subject. On the second day, pain threshold was measured by placing each rat on a thermoelectric hot plate (Omnitech Analgesiometer, model AMTI; Columbus, OH) set at 58°C±0.5°C and recording the latency (to the nearest $^1/_{10}$ sec) to lick a paw. On the third day, the basal colonic temperature of each rat was measured (YSI tele-thermometer, model 43TA; Yellow Springs, OH), then it was injected with d-amphetamine sulfate (Smith, Kline & French, Montreal, Canada) IP and placed immediately into a 4°C cold room for 1 hr. Colonic temperatures were re-measured at 30 and 60 min after injection. In the first series of experiments with albino rats, a dose of 15 mg/kg amphetamine was used. However, significant stereotypies developed during and after testing. In order to minimize these undesirable side effects, only 10 mg/kg of drug was used in later tests with hooded rats. Previous work has shown that either dose of amphetamine is effective in producing reliable hypothermia in rats exposed to cold environmental temperatures [10].

All behavioral tests were conducted between 1000 and 1400 hr. The number of rats tested on any particular measure varied both within and across experimental replications. The total numbers tested per strain and diet treatment are indicated in the data table (see below). All testing was performed by an experimenter who was unaware of the diets fed to individual subjects. When more than one group of animals was tested on a particular measure, data were pooled within strains for that treatment condition. Comparisons across diet treatments were analyzed by one-way Analyses of Variance (ANOVAs). When significant F ratios were obtained, post-hoc tests were performed (studentized Newman-Keuls corrected for harmonic means). Levels of p reported represent two-tailed distributions.

RESULTS

All results are summarized in Table 1. The amounts of energy consumed over the three week feeding period preceding behavioral testing did not vary significantly across diet groups for albinos. However, hooded rats fed the lard diet consumed slightly more than those fed the soybean oil diet, with chow-fed controls consuming intermediary amounts. Despite these slight differences in intake, all groups from both strains gained the same amount of weight over this period. Furthermore, no differences were observed in either strain for the number of horizontal crossings or rearings in the open field test. However, significant variations in paw-lick latencies were observed for albino rats depending on the diet fed. Post-hoc tests revealed that rats consuming the soybean oil diet displayed longer latencies than chow-fed controls. However, lard-fed rats did not vary significantly from either group. Unlike albino rats, no significant variatons in paw-lick latencies were observed for hooded rats as a function of diet.

Basal colonic temperatures before tests of amphetamine-induced hypothermia in the cold did not vary significantly for albino rats. However, hooded rats fed either semi-purified diet displayed the same slight but significant elevations compared to chow-fed controls. Both 30 min and 60 min after receiving amphetamine plus cold exposure, there were highly significant changes in colonic temperature across diet treatments for both strains. Aside from the 30 min time period for albinos, post-hoc tests revealed that each diet group per strain was significantly different from the other. For both strains, the magnitude of hypothermia was greater for rats fed chow compared to those fed lard. Rats fed soybean oil showed no signs of hypothermia; rather, their colonic temperatures increased compared to basal measures.

DISCUSSION

There were two main findings in the present study: (1) albino rats fed the soybean oil diet displayed elevated paw-lick latencies, and (2) rats of both strains fed the soybean oil diet were protected against amphetamine-induced hypothermia in the cold. The latter results provided clear delineations among the three diet groups regardless of rat strain tested. However, the former results were less convincing given that differences were only seen for albinos, and then only between soybean oil- vs. chow-fed controls.

In contrast to these findings, indices of horizontal and vertical locomotor activity in a novel environment appeared unaltered by these dietary treatments. The latter findings argue against the likelihood that non-specific changes in behavioral arousal or general responsivity were responsible for these effects. Similarly, the significant effects of the soybean oil diet seem unrelated to simple changes in caloric intake, body weight gain or basal colonic temperature. These variables either did not differ across groups or were not consistently associated with a particular behavioral change between strains. The fact that lard-fed rats displayed some re-

TABLE 1

MEAN ± S.E.M. (n) OF ALL VARIABLES TESTED FOR BOTH RAT STRAINS

Diet	3 Week Diet Feedings		15 Min Open Field Activity		58° Hot Plate	Colonic Temp (°C) After Amphetamine		
	Food Intake (kcal)	Weight Gain (g)	Line Crossings	Rearings	Paw-Lick Latencies (sec)	Basal	Change After 30′	Change After 60′
Albino Strain								
Soybean	1789 ± 36	170 ± 2.5	783 ± 20	63.5 ± 4.2	14.8 ± 1.5*	37.6 ± 0.3	+0.8 ± 0.2*	+1.0 ± 0.4*
oil	(28)	(28)	(13)	(6)	(23)	(6)	(6)	(6)
Lard	1761 ± 93	166 ± 6.3	756 ± 48	51.0 ± 4.9	9.9 ± 1.0*†	37.8 ± 0.3	−0.9 ± 0.4†	−1.1 ± 0.3†
	(12)	(12)	(11)	(6)	(13)	(6)	(6)	(6)
Chow	1767 ± 64	165 ± 2.9	859 ± 27	62.2 ± 8.0	7.7 ± 0.4†	37.4 ± 0.5	−1.8 ± 0.5†	−2.3 ± 0.4‡
	(11)	(11)	(11)	(5)	(6)	(5)	(5)	(5)
p	NS	NS	NS	NS	<0.01	NS	<0.001	<0.001
Hooded Strain								
Soybean	1862 ± 25*	176 ± 5.6	802 ± 21	93.0 ± 4.6	9.9 ± 0.7	37.5 ± 0.1*	+1.1 ± 0.1*	+1.5 ± 0.1*
oil	(22)	(22)	(16)	(16)	(12)	(6)	(6)	(6)
Lard	2026 ± 59†	177 ± 6.4	747 ± 35	97.0 ± 6.3	10.1 ± 0.9	37.5 ± 0.1*	−1.1 ± 0.1†	−1.5 ± 0.1†
	(22)	(22)	(16)	(16)	(12)	(6)	(6)	(6)
Chow	1915 ± 27*†	163 ± 3.3	745 ± 29	102 ± 6.0	11.8 ± 1.5	37.0 ± 0.1†	−2.2 ± 0.2‡	−2.9 ± 0.3‡
	(20)	(20)	(16)	(16)	(10) (5)	(5)	(5)	(5)
p	<0.02	NS	NS	NS	NS	<0.01	<0.0001	<0.0001

p Values for one-way ANOVAs across diet conditions. NS=not significant. All *p* values represent two-tailed distributions. Group means with different superscripts vary significantly ($p<0.05$) on Newman-Keuls post-hoc tests.

duction of amphetamine-induced hypothermia suggests that increasing the quantity of dietary fat may confer some protective effect against this treatment. However, the fact that rats fed the same amount of fat in the form of soybean oil showed no hypothermia indicates that the quality of dietary fat can be a powerful factor in modulating thermoregulation in response to such pharmacological perturbation.

We are presently unable to offer a definitive explanation for these diet-dependent effects. It is conceivable that variations in fatty acid quality produced by feeding these different lipids might have altered the composition of neural membranes in the central nervous system [3]. This, in turn, may have modified important functional processes which normally mediate these responses at a synaptic level. Further work is in progress to elucidate this possibility. Regardless of the exact mechanism responsible, these results indicate a novel, potentially important role for dietary fat quality in the control of certain behavioral and/or physiological states.

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