Pharmacology Biochemistry & Behavior, Vol. 15, pp. 15–19, 1981. Printed in the U.S.A.

Influence of Diet Palatability on the Noradrenergic Feeding Response in the Rat[1]

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Received 31 December 1980

SCLAFANI, A. AND J. A. TORIS. *Influence of diet palatability on the noradrenergic feeding response in the rat.* PHARMAC. BIOCHEM. BEHAV. **15**(1) 15–19, 1981.—Adult male rats which displayed a reliable feeding response to intrahypothalamic injections of norepinephrine (NE) on a chow diet were subsequently tested on one of three diets: an unpalatable quinine-adulterated meal, a palatable fat-adulterated meal, or a "neutral" unadulterated meal. The quinine diet completely blocked the NE feeding response, while the fat diet produced a small and unreliable reduction in the feeding response. When food deprived all groups increased their food intake, although the fat diet group tended to overeat, and the quinine diet group tended to undereat relative to the unadulterated diet group. The failure of the palatable fat diet to potentiate the NE feeding response does not support the hypothesis that this response mimics the ventromedial hypothalamic hyperphagia syndrome. The blocking effect of the quinine diet on NE feeding is consistent with other evidence which suggests that NE mediates the eating behavior induced by glucoprivation.

Norepinephrine feeding Paraventricular hypothalamus Diet palatability Hypothalamic hyperphagia Glucoprivation

INTRAHYPOTHALAMIC injections of norepinephrine (NE) can elicit eating in sated rats. This response has been extensively studied and is known to be mediated by alpha-adrenergic receptors located in and around the paraventricular nucleus (PVN) of the hypothalamus [15, 16, 17]. The functional significance of NE-induced feeding remains uncertain, however. In several respects the feeding behavior elicited by NE injections differs from that induced by food deprivation. For example, several studies indicate that NE induced eating, unlike deprivation induced feeding, is very dependent upon diet palatability [5], is associated with reduced rather than increased bar pressing for food on a VI schedule [6], and does not result in increased food hoarding behavior [4]. These characteristics are similar to those observed in rats with ventromedial hypothalamic (VMH) lesions, and this has led to the hypothesis that NE elicits eating by inhibiting the activity of the VMH, that is, by producing a temporary functional lesion of the VMH [6, 11, 20].

The present experiment further assessed the similarity between the noradrenergic feeding response and the VMH hyperphagia syndrome. While the influence of diet palatability on the VMH syndrome is well established [7, 9, 14, 25, 27], its influence on the NE feeding response has not been fully analyzed. Booth and Quartermain [5] reported that rats ate more of a saccharin-adulterated diet, and less of a quinine-adulterated diet after intrahypothalamic NE injections than they did after three hours of food deprivation. It is not clear from their report, however, whether quinine adulteration completely blocked or only diminished the NE feeding response because saline-baseline and unadulterated diet baseline data were not obtained. The significance of the saccharin diet results is also open to question in the absence of baseline data. Furthermore, the presumed enhanced palatability of the saccharin diet was not documented. The addition of saccharin to food does not necessarily increase its palatability, and may even reduce it at some concentrations [21,26]. In fact, the saccharin diet used by Booth and Quartermain [5] was found not to be preferred by male rats over an unadulterated diet in two-choice tests (see Method section). Also, it is not established that VMH hyperphagic rats overrespond to saccharin adulterated diets.

In the present experiment the effects of diet palatability on NE-induced and deprivation-induced eating were compared using three diets known to differ in palatability: a quinine adulterated meal, a fat adulterated meal, and an unadulterated meal. The quinine and fat diets used have been previously shown to reliably block and potentiate, respectively, VMH hyperphagia [7, 9, 25]. Furthermore, prelimi-

[1]This research was supported by grants from the PSC-BHE Research Award Program of the City University of New York. The assistance of Paul F. Aravich in this project is gratefully acknowledged. Send reprint requests to Anthony Sclafani, Department of Psychology, Brooklyn College, Brooklyn, NY 11210.

nary testing confirmed that the quinine diet was avoided, and the fat diet preferred over the unadulterated diet by male rats in two-choice tests (see Method section).

METHOD

Subjects

Twenty nine adult male rats of the CD strain (Charles River Labs, MA) with a mean body weight of 554 g were used. Data are presented for 20 of these animals. The remaining rats either died after surgery (n=3) or failed to eat in response to NE injections (n=6). The rats were individually housed in wire mesh cages kept in an air conditioned colony room under a 12:12 hr light-dark cycle. Except where noted the rats had ad lib access to Purina Lab Chow and tap water.

Surgery

The rats were anesthetized with Equi-Thesin (3 cc/kg BW) and were implanted with a unilateral 23 gauge stainless steel cannula. The tip of the cannula was positioned near the PVN using the following coordinates: 0.5 mm posterior to bregma, 0.6 mm lateral to the midline, and 8.0 mm below the skull surface. The rat was positioned in the stereotaxic instrument with the incisor bar set 3.0 mm above the ear bars. Histological confirmation of the cannula tracts is not available, but in other experiments ([1,3] and unpublished observations) in which the same surgical procedure was used, the cannula tracts were found to terminate in or near the PVN.

Drug Injections

Intrahypothalamic injections of saline or NE were administered in 0.5 μl volumes using a 30 gauge injector attached by polyethylene tubing to a Hamilton microsyringe. The NE injection contained 35 nmoles (11.8 μg) of l-norepinephrine-d-bitartrate (Sigma Chemical Co.) in 0.5 μl of saline, and was prepared immediately before each test session. When not in use the drug cannulae were occluded with a stainless steel pin.

Diets

Initially all animals were given ad lib access to Purina Lab Chow before they were tested with one of three diets: a plain, unadulterated meal, which is the powdered form of Purina Lab Chow, a fat-supplemented meal, or a quinine-adulterated meal. The fat diet was prepared by adding 35 ml of a Crisco oil-mineral oil mixture (1:1.58) to 100 g of meal according to the procedure of Corbit and Stellar [7]. The noncaloric mineral oil was included to make the fat diet isocaloric to the unadulterated Purina meal (3.6 kcal/g). The quinine diet (0.2%) was prepared by adding 0.2 g of quinine hydrochloride to 100 g of meal. The diets were presented in metal feeding cups (LC-306, Wahmann Co.), and food intake corrected for spillage was measured daily.

Preliminary tests were conducted to assess the relative palatability of these diets. The tests, which were each 4 days in length, used 10 adult male rats of the same strain as that used in the main experiment. Four rats were given a two-choice test between the 0.2% quinine diet and the plain diet. All rats strongly avoided the quinine diet in preference to the plain diet during the 24 hr/day test (2.5 vs 34.2 g/day, $p<0.01$). The remaining six rats were offered a choice between the fat diet and the plain diet during 1 hr/day and 24 hr/day tests. In both tests the rats significantly preferred the fat diet over the plain diet (10.7 vs 0.3 g/hr and 29.7 vs 2.5 g/24 hr, $p<0.01$). These six rats were also tested for their preference for the saccharin and plain diets used by Booth and Quartermain [5]. During a 1 hr/day test the rats ate significantly more of the plain diet than of the saccharin diet (14.4 vs 5.2 g/hr, $p<0.01$).

Procedure

The rats were given at least one week to recover from implant surgery. They were then given a six-day test to assess their feeding response to NE using the Purina Chow diet. Each day the rats were given fresh chow (the pellets were placed on the cage floor) and water for a one hr period prior to intrahypothalamic injections. The following procedure was used during this prefeeding period to stimulate eating and insure that the rats were satiated prior to drug testing: 15 min after food was given the rats were given a mock injection, i.e., the injector was inserted into the cannula, but no fluid injected; 30 min later the rats were briefly handled and returned to their cage. At the end of this prefeeding period the rats were injected with either saline or NE and their food intake during the next hr was recorded to the nearest 0.1 g. The rats were tested for six consecutive days using an ABABBA design. On days 1, 3 and 6 saline was injected, and on days 2, 4 and 5 NE was injected. The response to the NE injections (NE feeding score) was determined by subtracting the mean amount of food consumed during the saline tests from the mean amount consumed during the NE tests.

Based on the results of this initial feeding test 20 rats with NE feeding scores of 1.0 g or more were retained for further study. The rats were divided into three groups equated for their NE feeding scores, and were given ad lib access to one of the three test diets (Plain diet group, n=6; Fat diet group, n=7; Quinine diet group, n=7). They were given one week to adapt to their new diets. The rats were then tested for NE feeding using a procedure identical, except for the diets, to that used during the initial drug test. During the following week the feeding response of the rats to food deprivation was assessed. After fasts of 0, 6, 12, and 24 hr the rats were given access to fresh food and their intake during the subsequent 1 hr was recorded to the nearest 0.1 g.

In the final phase of the experiment all rats were returned to the Purina Chow diet. Beginning five days later they were retested for NE feeding using a procedure identical to that used in the initial drug test.

RESULTS

The results of the NE feeding tests are summarized in Fig. 1. During the initial test on the chow diet the mean NE feeding score of the 20 rats was 2.2 g. The feeding scores of the three groups were similar of course since the groups were equated on this measure. When tested on their respective diets, however, the NE feeding scores of the three groups significantly differed, $F(2,17)=12.14$, $p<0.001$. That is, the Plain diet and Fat diet groups ate more food during the NE tests than did the Quinine diet group. Also, the Plain diet group ate more than did the Fat diet group, but this difference was not statistically reliable. The negative feeding score of the Quinine diet group indicates that they actually ate less, although not significantly so, following the NE injections than they did following the saline injections. In contrast to these results, the food intake of the Plain, Fat, and Quinine diet groups during the daily 1 hr prefeeding periods did not significantly differ (4.8, 3.2, 3.7 g, respectively).

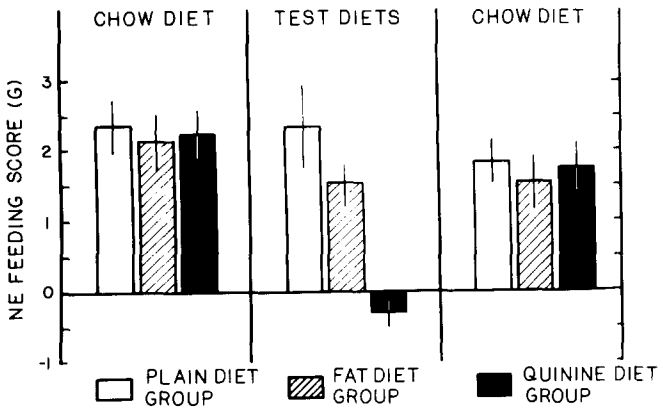

FIG. 1. Mean (±SE) NE feeding score of the Plain diet, Fat diet, and Quinine diet groups. The left and right panels present the data from the first and last NE feeding tests, respectively, when all groups had Purina Chow to eat. The middle panel presents the results of ths NE test conducted when the groups had their respective diets to eat.

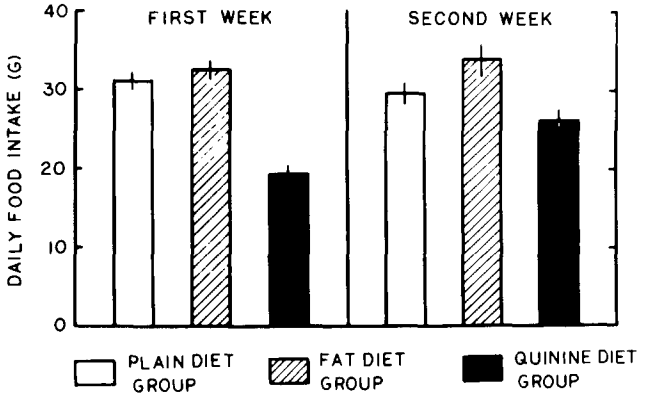

FIG. 2. Mean daily (±SE) food intake of the Plain diet, Fat diet, and Quinine diet groups during the first and second week on their respective diets. Intake data include the food consumed during the 22 hr nontest periods, and the 1 hr prefeeding periods, but not the 1 hr drug periods.

Comparison of the first two NE feeding tests indicated that the Plain diet group ate very similar amounts of the chow and meal diets, whereas the Fat diet group had a smaller feeding score during the fat diet test than during the chow diet test, although this difference (0.6 g) was not significant (Fig. 1). The feeding score of the Quinine diet group, on the other hand, was significantly less during the quinine test than during the initial chow test, $t(6)=8.48$, $p<0.01$. In the final NE feeding test the chow diet was again available, and, as indicated in Fig. 1, the feeding scores of the three groups were similar. The feeding scores obtained in this test were slightly, but not significantly less than those obtained in the initial chow test. Note in particular that the Quinine diet group fully recovered its NE feeding response when tested with the chow diet.

Figure 2 summarizes the daily food intake of the three groups during the first two weeks on their respective diets. During the first week the Quinine diet group ate significantly less than did the Plain diet and Fat diet groups, $F(2,17)=44.88$, $p<0.001$, whereas the intakes of the latter two groups were similar. The Quinine diet group increased its food intake during the second week, but significant group differences still emerged, $F(2,17)=5.81$, $p<0.05$. Individual comparisons revealed that the Quinine diet group ate less than did the Fat diet group ($p<0.05$), but not reliably less than the Plain diet group. As a result of their reduced food intake the Quinine diet group lost weight over the two week period (21.3 g), whereas the Plain diet and Fat diet groups gained weight (12.0 and 19.7 g, respectively; $F(2,17)=13.04$, $p<0.01$). However, all of the weight loss of the Quinine diet rats occurred during the first week on the diet, and their weight was stable during the NE tests conducted during the second week on the diet.

The results of the food deprivation tests, which were conducted during the third week on the diets, are presented in Fig. 3. The three groups consumed similar amounts of food under the 0 hr deprivation condition, and all groups increased their food intake with deprivation. The Fat diet group displayed the largest increase, and the Quinine diet group displayed the smallest increase in food intake as a function of deprivation. Analysis of variance confirmed that

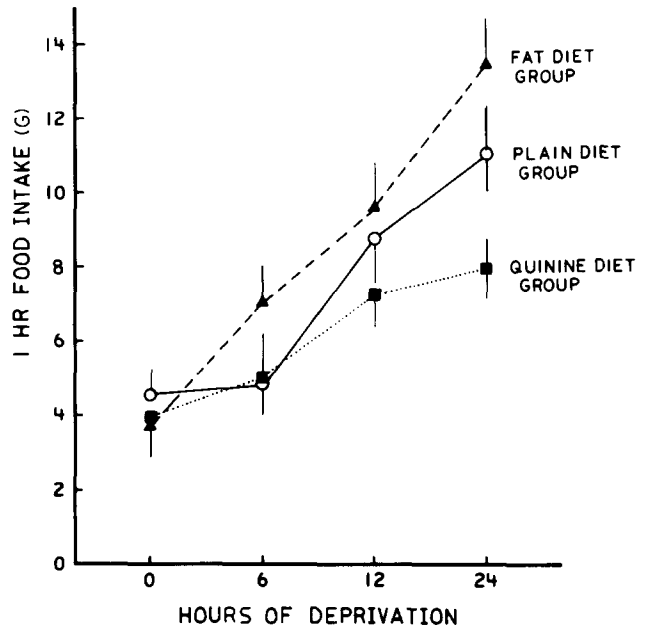

FIG. 3. Mean (±SE) one hr food intake of the Plain diet, Fat diet, and Quinine diet groups following 0 to 24 hr of food deprivation.

the deprivation effect was significant, $F(3,51)=30.46$, $p<0.001$, and further indicated that the group effect, $F(2,17)=4.25$, $p<0.05$, but not the group by deprivation interaction was also significant. Individual comparisons revealed that the Plain diet group did not differ from either of the other two groups, but that the Quinine diet and Fat diet groups differed significantly ($p<0.05$) in their postdeprivation food intakes.

DISCUSSION

The results of this experiment confirm previous findings that NE-induced feeding differs from deprivation-induced feeding [4, 5, 6]. That is, whereas food deprivation increased

the intake of the Quinine diet by 28 to 100%, NE injections produced no increase in Quinine diet intake. Furthermore, deprivation tended to increase the intake of the Fat diet more than the intake of the Plain diet, whereas NE injections tended to produce the opposite effect. It should be noted that these findings do not exclude the possibility that higher doses of NE would induce eating of the Quinine diet, or produce a greater feeding response with the high Fat diet.

Several factors may have contributed to the blocking effect of quinine on the NE feeding response. The most obvious factor is the reduction in diet palatability produced by the bitter taste of the quinine. Recent data also indicate that quinine reduces palatability by producing toxic postingestive effects [2]. The reduced ad lib food intake and body weight produced by the Quinine diet may have also influenced the rats' responsivity to NE. Recall that the groups were maintained on their test diets 24 hr/day, and the Quinine diet group underate and lost weight relative to the other groups. Finally, although unlikely, the possibility that the quinine had a direct effect on the adrenergic receptors mediating the NE feeding response cannot be excluded [19]. Whatever the mechanism, the present results indicate that quinine adulteration of food differentially affects deprivation-induced and NE-elicited feeding.

The results do not, however, clearly support the proposal that NE induced feeding is similar to the hyperphagia produced by VMH damage [5, 6, 11]. On the one hand the finding that NE induced feeding was completely blocked by quinine adulteration of the diet is consistent with this idea, since it is well known that quinine inhibits VMH hyperphagia [9, 14, 25, 27]. On the other hand, the fact that the Fat diet did not potentiate the NE feeding response, but does exaggerate VMH hyperphagia [7, 9, 25], indicates that NE induced feeding and VMH hyperphagia are not functionally similar. It is possible that other palatable diets may potentiate the NE feeding response but this remains to be demonstrated. The suggestion of Booth and Quartermain [5] that NE feeding is enhanced by the addition of saccharin to the diet is not documented by the data they report. Their results indicated that the NE feeding response is greater with a saccharin diet than with a Quinine diet, but did not demonstrate that more feeding is obtained with a saccharin diet than with an unadulterated diet.

Additional evidence that NE injections do not produce eating by inhibiting the medial hypothalamic system involved in the VMH hyperphagia syndrome is provided by a recent study of Aravich, Sclafani and Leibowitz ([3] and unpublished observations). Damage to the VMH with bilateral parasagittal knife cuts, which produced hyperphagia and obesity, was found not to alter the feeding response to intrahypothalamic NE injections. If NE induced feeding is mediated by the same fibers responsible for the VMH hyperphagia syndrome then the response should have been blocked by the knife cuts. In an earlier study Herberg and Franklin [11] reported that posterior VMH lesions inhibited NE induced feeding, and they took this as evidence that NE stimulates feeding by inhibition of the VMH. However, they also observed that damage to the anterior region of the VMH, which now appears to be the most critical focus of the hyperphagia syndrome [10,24], failed to interfere with NE feeding. Thus, the VMH mediation hypothesis of NE feeding is not well supported.

An alternative explanation of the NE induced feeding response is that it involves the glucoprivic feeding system. This hypothesis is suggested by the finding of Muller, Cocchi and Mantegazza [22] that the eating behavior produced by injections of 2-deoxy-d-glucose (2-DG) is blocked by intraventricular injections of the alpha adrenergic blocker phentolamine. Furthermore, 2-DG injections have been found to increase the turnover and release of hypothalamic NE [18,23]. Of particular relevance to the present study are the reports that the feeding responses to glucoprivic drugs (insulin, 2-DG, tolbutamide) are blocked by quinine adulteration of the diet [13,29]. Another feature of glucoprivic feeding is that it is associated with a selective increase in sugar intake [1,12]. Recent experiments indicate that intrahypothalamic injections of NE may also induce a preferential increase in sugar-containing diets, which would support the hypothesis that NE mediates the glucoprivic feeding response [8, 17, 28]. However, conflicting results have been obtained ([1], Aravich, Sclafani, and Leibowitz, in preparation), and the effects of NE injections on carbohydrate intake are being investigated further.

In summary, the results of this experiment indicate that NE-induced eating is blocked by an unpalatable quinine diet, and, unlike VMH hyperphagia, is not potentiated by a palatable high fat diet. The feeding behavior induced by glucoprivation is also blocked by quinine diets, and this and other evidence suggests that NE may mediate the glucoprivic feeding response. Inconsistent results have been obtained concerning the effects of NE on carbohydrate preference, however, and additional research is needed to establish the relationship between the noradrenergic and glucoprivation feeding systems.

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