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Inhibition of Acute Feeding Responses to Systemic 2-Deoxyglucose or Insulin in Rats Pretreated with the GABA-Transaminase Blocker Ethanolamine-O-Sulfate (EOS)

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NOBREGA, J. N. AND D. V. COSCINA. *Inhibition of acute feeding responses to systemic 2-deoxyglucose or insulin in rats pretreated with the GABA-transaminase blocker ethanolamine-O-sulfate (EOS).* PHARMAC. BIOCHEM. BEHAV. **17**(6) 1145–1148, 1982.—Acute feeding responses to 2-deoxyglucose (750 mg/kg) or insulin (12 U/kg) were examined 24 hr after intracisternal injection of the GABA-transaminase inhibitor ethanolamine-O-sulfate (EOS, 400 μg) in female rats. EOS pretreatment completely abolished acute feeding responses to both challenges. These findings complement recent research showing that central EOS can reverse chronic overeating in several experimental preparations. The present results are consistent with previous indications that EOS treatment may induce a metabolic shift away from brain glucose utilization, thus making glucoprivation irrelevant as a metabolic challenge. An alternative possibility is that EOS-induced increases of brain GABA may offset specific neural mechanisms through which these glucoprivic agents normally induce feeding.

2-Deoxyglucose Ethanolamine-O-sulfate EOS GABA Glucoprivic feeding Insulin Rat

ETHANOLAMINE-O-SULFATE (EOS) is a potent inhibitor of the enzyme GABA-transaminase, which induces lasting elevations of brain gamma-aminobutyric acid (GABA) after central or peripheral administration [5, 8, 13, 14]. Increased brain GABA levels after EOS are accompanied by dose-dependent decreases in food and water intake, which do not seem to result from inducement of taste aversion or non-specific motoric impairment [5, 6, 11, 16].

Recent work from this laboratory has shown that intracisternal EOS administration can temporarily reverse chronic overeating in rats bearing medial hypothalamic lesions, genetically hyperphagic Zucker rats, and rats given access to highly palatable diets [6]. The primary purpose of the present experiment was to investigate the feeding responses of EOS-treated rats to acute stimuli. 2-Deoxyglucose and insulin were the acute feeding stimuli used.

Quantifying the acute feeding responses of EOS-treated rats to these two classic glucoprivic stimuli was also of interest for another reason. We have recently found that intracisternal doses of EOS which significantly increase brain GABA and decrease feeding induce profound and generalized decreases in brain glucose utilization as assessed by ^{14}C-2-deoxyglucose autoradiography [16,17]. The discrepancy between the large degree of suppression of glucose metabolism in brain and the small degree of behavioral depression observed in these animals suggested that EOS may induce mobilization of metabolic fuels other than glucose [16]. If EOS-treated rats are not using glucose to maintain brain metabolic activity and behavior, they might not be expected to show acute feeding responses to either glucoprivic challenge.

METHOD

Subjects

A total of 63 female Wistar rats (Woodlyn Labs, Guelph, Ontario) were used in two separate experiments. Animals were individually housed in metal cages with wire mesh fronts and bottoms and had access to Purina Chow pellets (4% fat) and tap water at all times. Food pellets were scattered on cage floors and water was given in 100 ml graduated cylinders. Lights were on from 0800 to 2000 hr daily. Room temperature was kept at 21±1°C.

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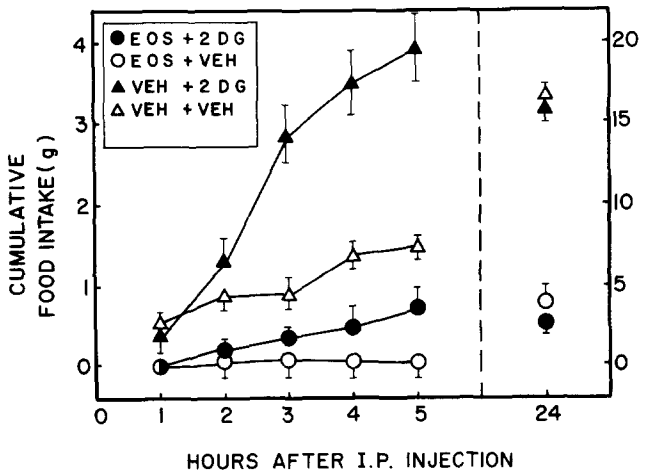

FIG. 1. Hourly food intake after 2-DG (750 mg/kg, IP) or vehicle. Injections were made 24 hr after IC EOS (400 μg) or vehicle. Each point is mean (s.e.m.) of 8 animals per group. See text for definition of abbreviations.

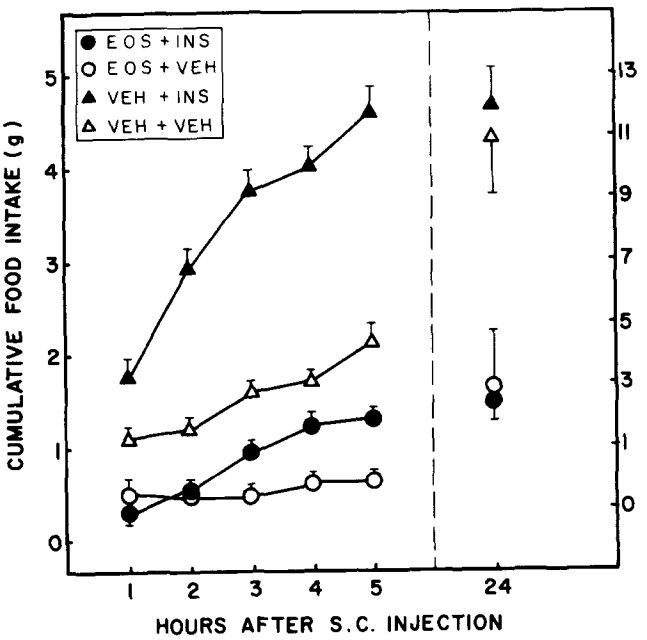

FIG. 2. Hourly food intake after insulin (12 U/kg, SC) or vehicle. Injections were made 24 hr after IC EOS (400 μg) or vehicle. Each point is mean (s.e.m.) of 8 animals per group, except for EOS-INS group, where n=7. See text for definition of abbreviations.

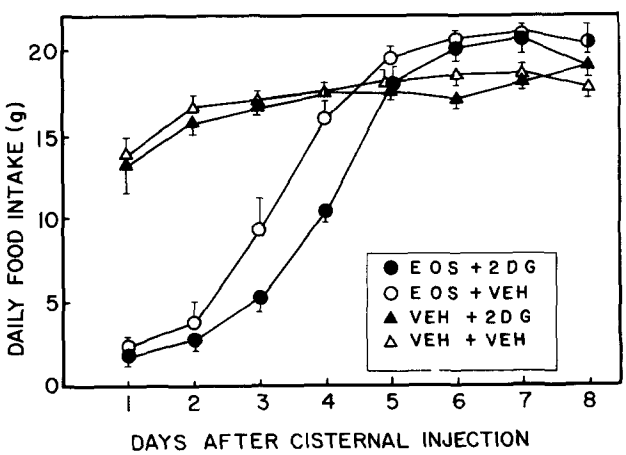

FIG. 3. Recovery of food intake after IC EOS or vehicle. Each point is mean (s.e.m.) of 8 animals per group. 2-DG testing was done after normal daily measurements on day 1. See text for definition of abbreviations.

EOS Injection

Following one week of adaptation, during which all animals were handled and weighed daily, rats were divided into two groups equated for body weight. Under light ether anesthesia animals were placed in a stereotaxic frame and received an intracisternal (IC) injection of 400 μg EOS (Calbiochem, San Diego, CA) in 20 μl deionized water or an equal volume of the deionized water vehicle. Each injection took approximately 15 seconds, after which each animal was returned to its home cage with a measured amount of food and water.

Glucoprivic Challenges

Feeding in response to glucoprivation was assessed 24 hr after the IC injections, when GABA elevation and feeding depression are at their highest points after EOS treatment (cf. [5]). Eight rats in the EOS group and 8 in the vehicle (VEH) group received a single intraperitoneal (IP) injection of 750 mg/kg 2-deoxy-D-glucose (2-DG, Sigma, St. Louis, MO) and the remaining 8 rats in each group received an equivalent amount of deionized water vehicle.

In a separate experiment, 7 EOS-treated rats and 8 VEH-treated rats received a single subcutaneous (SC) injection of 12 U/kg of zinc insulin (INS, Connaught Labs, Willowdale, Ontario) and the remaining 8 rats in each group received an equivalent amount of the deionized water vehicle.

In both 2-DG and INS experiments, food and water intakes were measured every hour for 5 hr following the systemic injections, and then once daily for 8 days. Food spillage was collected throughout and intake measures were adjusted accordingly.

RESULTS

Since in all cases water intake was highly correlated with food intake, only the latter will be reported here. As illustrated in Figs. 1 and 2, EOS pretreatment completely blocked acute feeding responses to both 2-DG and INS. All VEH-pretreated rats receiving either 2-DG or INS (VEH-2-DG and VEH-INS groups) showed significant increases in feeding over the 5-hr test periods ($ps<0.02$, t-tests vs respective VEH-VEH control groups). In contrast, animals pretreated with EOS ate no more than the VEH-VEH groups after either 2-DG or insulin. In fact, both the EOS-2-DG group and the EOS-INS group showed a tendency to eat less than the respective VEH-VEH groups, although this difference did not achieve statistical significance at the 0.05 level ($0.07>ps>0.05$). At no time were feeding responses of

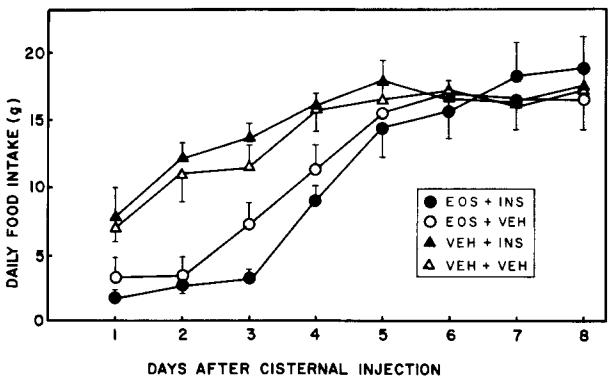

FIG. 4. Recovery of food intake after IC EOS or vehicle. Each point is mean (s.e.m.) of 8 animals per group, except for EOS-INS group, where n=7. Insulin testing was done after normal daily measurements on day 1. See text for definition of abbreviations.

EOS-2-DG or EOS-INS groups significantly different from those of the corresponding EOS-VEH groups.

Figures 3 and 4 show the recovery of food intake in the days following EOS administration. As illustrated, daily food intake returned to control levels 4–5 days after the EOS injection. Neither 2-DG nor INS produced delayed compensatory effects promoting increased food intake in EOS-treated rats in the days following the systemic injections. Instead, rats that received 2-DG or insulin on day 1 after EOS appeared to lag slightly behind the corresponding EOS-VEH groups in recovering food intake. In the case of the EOS-2-DG group vs the EOS-VEH group this difference was statistically significant on day 4 ($t=2.43$, $p<0.05$).

DISCUSSION

Previous results from this laboratory have shown that intracisternal EOS is effective in reversing excessive food intake in three animal models of chronic overeating [6], suggesting an ubiquitous action of the drug in altering central mechanisms involved in feeding. In keeping with this possibility, the present results show that EOS is equally effective in suppressing acute feeding responses induced by systemic 2-DG or insulin administration.

At least three mechanisms might be hypothesized to explain the observed EOS-induced blockade of 2-DG- or insulin-induced feeding. First, EOS might decrease reactivity to stressful stimuli, and thereby dampen responses to acute stimuli such as 2-DG and insulin. 2-Deoxy-D-glucose, in large systemic doses as used here, is known to cause sympatho-medullary and pituitary-adrenal discharge [10] and other signs usually associated with stress reactions, including analgesia [2]. Rowland [19] has suggested that feeding responses to 2-DG may also be stress-related, based on his observations that gerbils fail to eat in response to 2-DG or tail pinch. However, we have observed that the same dose of EOS which abolishes responses to 2-DG does not interfere with normal feeding responses to tail pinch [16]. This indicates that, at least in rats, these two types of feeding responses may be subserved by different mechanisms. Therefore, it seems unlikely that decreased reactivity to stress is responsible for the ability of EOS to block acute feeding responses to 2-DG and insulin.

A second possibility is suggested by our recent finding that central EOS treatment produces indices of pronounced suppression of glucose utilization in brain without a corresponding depression in behavior other than feeding [16,17]. Our recent finding of significantly elevated blood levels of the ketoacid beta-hydroxybutyrate in EOS-treated rats (unpublished observations) can be viewed as consistent with the possibility of alternate fuel mobilization in these animals. If indeed glucose is not the primary brain metabolic fuel being used by EOS-treated rats, then additional reductions in glucose availability should be of little physiological consequence to these animals and should not elicit any "compensatory" feeding responses. This notion of course rests on the assumption that feeding responses to 2-DG or insulin are solely due to the ensuing glucoprivation [3,20]. It must be noted, however, that this assumption has been seriously questioned by recent data in the case of 2-DG [7] and insulin [1] (see also [9]).

Finally, the blunted feeding effects observed might relate to specific brain areas involved in the mediation of consummatory responses to 2-DG and insulin. Evidence from several sources suggests an association between increased GABAergic activity in specific hypothalamic areas and satiety (e.g., [4, 14, 18]). Perhaps particularly relevant to our present results are the seminal observations by Kimura and Kuriyama [12] that GABA concentrations in the lateral hypothalamus of rats fall rapidly in response to insulin-induced glucoprivation. If this fall in hypothalamic GABA has any causal connection with the concomitant feeding responses normally observed after insulin administration, such feeding should not occur when whole brain GABA levels are artificially raised, as in the case of EOS-treated rats. It is thus conceivable that EOS treatment may blunt or offset a GABA-mediated hypothalamic mechanism whereby changes in glucose availability are normally translated into neural commands for the initiation or cessation of feeding (cf. [18]).

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