Brain Research, 374 (1986) 380–384
Elsevier

BRE 21554

Components of hypothalamic obesity: bipiperidyl-mustard lesions add hyperphagia to monosodium glutamate-induced hyperinsulinemia

ANDREW C. SCALLET and JOHN W. OLNEY

Department of Psychiatry, Washington University, School of Medicine, St. Louis, MO 63110 (U.S.A.)

(Accepted January 21st, 1986)

Key words: hypothalamus — insulin — hyperphagia — obesity — monosodium glutamate — bipiperidyl mustard — lesion — feeding

Rats with bilateral electrolytic lesions in the general region of the ventromedial hypothalamic (VMH) nucleus develop hyperinsulinemia, excessive food intake and obesity. Monosodium glutamate (MSG) destroys neurons of the arcuate hypothalamic (AH) nucleus and produces hyperinsulinemic but hypophagic obesity. Bipiperidyl mustard (BPM) primarily destroys VMH neurons, but has produced only a slight obesity even when rats were maintained on high-fat diets. In the present study, rats treated with MSG (AH lesion) were hyperinsulinemic, moderately obese and hypophagic; BPM rats (primarily VMH lesion) were not different from controls when fed standard chow diets. However, MSG/BPM rats (AH + VMH lesion) were hyperinsulinemic, massively obese and hyperphagic. Thus, two components of the electrolytic lesion syndrome previously attributed to VMH damage (hyperinsulinemia and obesity) were reproduced simply by MSG treatment alone. The third component (hyperphagia) occurred only when both AH and VMH were lesioned, suggesting that neurons in both nuclei may perform a satiety function and may be able to substitute for one another in this respect. Since MSG treatment is required for all components of both obesity syndromes described here, this underscores the importance of MSG-sensitive neurons in mechanisms of obesity. The combined treatment approach also represents the first rat model of hyperinsulinemic, hyperphagic obesity that can be entirely produced by systemic administration of neurotoxins.

It has long been known that bilateral electrolytic lesions of the rodent ventral hypothalamus can cause massive obesity and hyperphagia (excessive food intake)[9]. This animal syndrome has been widely employed as a model of human obesity[3,11,17]. Early theories attributed the obesity to hyperphagia secondary to the destruction of the ventromedial hypothalamic (VMH) nucleus[4,5], a putative satiety center. More recently, the striking hyperinsulinemia accompanying the VMH lesion has been interpreted as the primary effect of the lesion and as cause of the ensuing hyperphagia and obesity[3]. Interpretation of the hypothalamic obesity syndrome has been complicated by the fact that electrolytic VMH lesions are not anatomically specific for VMH but rather destroy fibers interconnecting several brain regions and spread locally to include the adjacent arcuate nucleus of the hypothalamus (AH)[18].

Subcutaneous administration of monosodium glutamate (MSG) to neonatal mice and rats destroys nearly all neurons in AH, except those closely bordering on VMH[13]. Intraperitoneal administration of bipiperidyl mustard (BPM) bilaterally lesions VMH and bordering portions of AH[10,12,16]. Although both toxins also produce some extrahypothalamic damage, all of the effects described here occur following electrolytic lesions restricted to the hypothalamus[2,3]. Therefore, our discussion emphasizes this region. Fig. 1 illustrates the location of hypothalamic lesions induced by MSG and BPM. Both of these approaches to chemotoxic lesioning have the advantage of producing bilateral lesions which are more symmetrical and more discretely localized to specific regions within the mediobasal hypothalamus than can be achieved by electrolytic lesioning. The MSG lesion offers the additional advantage of having axon-sparing characteristics, i.e., it removes specific neuronal cell bodies from AH without damaging axons terminating in or coursing through the region[14]. Possibly the BPM lesion is also axon-sparing but this re-

Correspondence: A.C. Scallet. Present address: Pharmacodynamics Branch, Division of Teratogenesis Research, National Center for Toxicological Research, Jefferson, AR 72079 (U.S.A.)

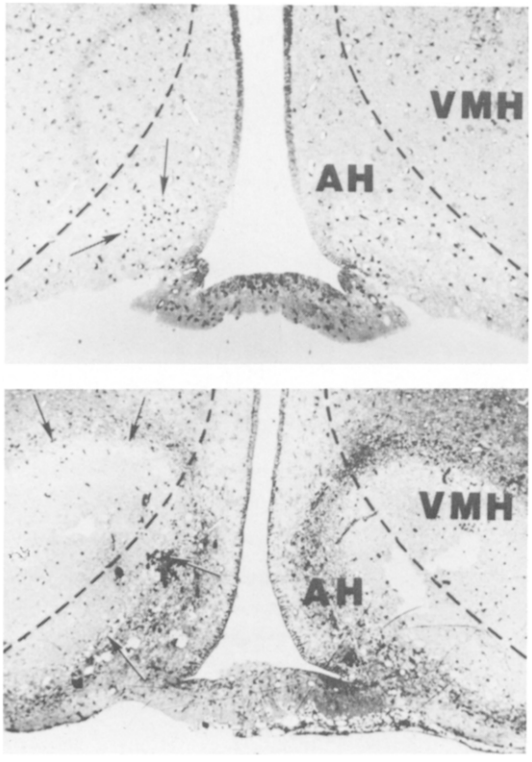

Fig. 1. Top: bilateral view of the ventral hypothalamus of a 10-day-old rat treated 6 h before sacrifice with 4 mg/g (s.c.) dose of MSG. Note the bilateral symmetry of the lesion and that the acutely necrotic neurons (arrows) are confined to the region of the AH. Bottom: similar bilateral view of the ventral hypothalamus of a 30-day-old rat treated 48 h before sacrifice with a 40 mg/kg (i.p.) dose of BPM. Note the bilaterally symmetric damage (outlined by arrows) in complementary position to the MSG lesion, straddling the dorsolateral border zone or cell-poor region between the AH and VMH (×48).

mains to be clarified. In the present experiments, we treated rats with MSG, BPM or MSG/BPM and compared the effects of each treatment on food intake, Lee Index of obesity and plasma insulin. The Lee Index (cube root of body weight divided by nose to anus length) has been widely used as a measure of obesity, since it has been shown to correlate well with the body fat content of rats bearing electrolytic lesions[1].

Newborn Sprague–Dawley derived rats from dams of the psychiatry department breeding colony received a series of 5 subcutaneous injections of either 4 mg/gm MSG or an equal volume of saline on alternate days starting one day postpartum. At 35 days of age, half of the MSG-treated and half of the saline-treated rats received an intraperitoneal injection of 40 mg/kg BPM in borate buffer vehicle[10,12,16], while the remainder of the group received merely vehicle injections. The 4 resulting groups (control, BPM, MSG, and MSG/BPM) were then housed individually with ad libitum access to pelleted Purina rat chow and water. Their weights were measured periodically until sacrificed at 31 weeks of age for histological evaluation. From 10 to 29 weeks of age, rats were observed biweekly for 24 h intakes of their standard chow in the home cage. The data were averaged for each rat to provide a single measure for statistical evaluation, since the food intake remained relatively constant during this time. At 30 weeks of age they were fasted overnight and blood was drawn by cardiac puncture for insulin determinations. Rat insulin was measured by an 18 h equilibrium radioimmunoassay employing a specific rat insulin antibody (Linco Research Inc, Eureka, MO), rat insulin standards, and a porcine insulin tracer. The assays were performed by the Diabetes Research and Training Center of Washington University School of Medicine. Routine histological evaluation was performed on cryostat sections stained with cresyl violet. In a separate study on these same brains (Scallet and Olney, in preparation), we stained alternate cryostat sections immunocytochemically for visualization and quantitation of proopiomelanocortin neurons.

The experiment was performed initially on a set of male rats and then repeated with an additional set of females. Data were grouped together and analyzed as 3-factor analyses of variance with replication/sex as one factor, MSG treatment as the second factor, and BPM treatment as the third. Significant main effects or MSG/BPM interactions were followed by individual comparisons according to the Fisher Least Significant Difference approach[7]. A total of 12 controls, 13 BPM, 15 MSG, and 15 combined MSG/BPM lesioned rats were studied for all dependent variables. Data for all-MSG/BPM-treated rats were included in the statistical analyses, but because of a clear bimodal distribution in the MSG/BPM group, the data are also shown, but not formally analyzed, for two subgroups labeled non-hyperphagic and hyperphagic[6].

At 31 weeks of age, BPM rats were not significantly different from controls on any measure. Consistent with prior reports[13,19], MSG rats compared to controls were significantly hypophagic, hyperinsulinemic and obese. The average values for MSG/BPM rats

suggest a normophagic, hyperinsulinemic and obese population (Table I). It was clear from visual inspection, however, that nearly half of the rats in the MSG/BPM group were massively obese, while the remainder were indistinguishable from MSG-alone treated rats. Analysis of food intake data for MSG/BPM rats also revealed a bimodal distribution — those rats manifesting massive obesity were markedly hyperphagic. Sorting the hyperphagic rats into one group and the remaining MSG/BPM rats into another (Table I) revealed that the latter were not different from rats treated with MSG alone (hypophagic, hyperinsulinemic, moderately obese) whereas the former were different from any other rats in the study. Indeed, they had all the characteristics of the classical 'VMH' syndrome (hyperphagia, hyperinsulinemia, massive obesity). The most obese males weighed 750–800 g (control mean = 460) at sacrifice, while the females were 380–420 g (control mean = 247). This elevation of about 60% in body weight compares favorably with published values for electrolytic VMH lesions[11].

Since BPM deletes primarily VMH neurons and VMH lesions have traditionally been linked to hyperinsulinemia[2,3], it is particularly noteworthy that BPM rats were not hyperinsulinemic. In that MSG deletes a specific population of AH neurons without damaging axons entering or passing through the mediobasal hypothalamus, it is reasonable to attribute the hyperinsulinemia associated with either MSG or MSG/BPM treatment (or indeed, to VMH electrolytic lesions) to the loss of these AH neurons and to propose that they are an important component of a neural pathway concerned with the regulation of plasma insulin. This idea is reinforced by observations that binding sites for insulin are located on AH neurons that are MSG-susceptible[20]. Whether MSG-induced obesity represents the metabolic consequences of prolonged hyperinsulinemia[3], or reflects a separate but associated disturbance attributable to loss of AH neurons, remains to be clarified. Our data do not rule out the possibility that obesity is the primary effect of AH lesioning and that hyperinsulinemia occurs secondary to the obesity. Consistent with this interpretation, although both groups of MSG/BPM rats had significant hyperinsulinemia, the massively obese group had more extreme hyperinsulinemia (Table I). It is known, however, that hyperinsulinemia occurs in the classical 'VMH' syndrome immediately after lesioning (before obesity is present) and persists even when obesity is prevented by limiting food intake[3,15,18]. Since considerable variability in the degree of fasting hyperinsulinemia was apparent in even the MSG alone group, it is also possible that those animals with the greater degree of pre-existing hyperinsulinemia became hyperphagic when BPM treated, and only then became extremely obese. Further research will be required to distinguish among these alternatives.

It is of interest that when the rat VMH is electrolytically lesioned in the weanling period, hyperinsulinemia and moderate obesity without hyperphagia may

TABLE I

Relative obesity, food intakes and plasma insulin levels exhibited by rats treated with MSG as neonates, with BPM as weanlings or with both toxic agents

Since there were no significant interactions involving sex, the male and female data were combined and are given as percent of control means*.

	Control (*n* = 12)	*BPM* (*n* = 13)	*MSG* (*n* = 15)	*MSG/BPM* (*n* = 15)	*MSG/BPM hyperphagic* (*n* = 7)	*MSG/BPM non-hyperphagic* (*n* = 8)
Lee Obesity Index	100 ± 0.7	104 ± 0.7	116 ± 1.8[a]	125 ± 3.4[b]	134 ± 1.5	117 ± 1.4
Food Intake	100 ± 7.4	91 ± 4.1	85 ± 4.3[c]	106 ± 10.0[d]	130 ± 5.1	85 ± 7.0
Insulin	100 ± 20.9	94 ± 24.3	450 ± 204.0[a]	480 ± 149.0[a]	794 ± 220.0	245 ± 53.0

* Control means, for males and females respectively, were Lee Indices, 0.290 and 0.275, insulin levels 2.0 and 0.3 ng/ml, and food intakes 26 and 14 g/day/rat.

[a] $P < 0.01$ from control and from BPM alone.

[b] $P < 0.01$ from MSG alone.

[c] $P < 0.05$ from control.

[d] $P < 0.05$ from BPM alone, and $P < 0.01$ from MSG alone.

be obtained[2,8]. Since this is identical to the effects of neonatal MSG treatment, the most likely interpretation is that this 'VMH' electrolytic lesioning approach destroys the same population of AH neurons deleted by MSG. Alternatively, the electrolytic lesion might mimic the MSG effect by severing critical afferent connections to AH neurons, thereby disrupting their insulin and/or other metabolic regulatory functions. However, any such connections must be considered insensitive to BPM treatment, since BPM lesions of the VMH failed to cause hyperinsulinemia. Since a 'VMH' electrolytic lesion results in hyperphagia if induced in adult rats, age at time of lesioning may also be a relevant factor. At the time of BPM lesioning (35 days of age), our rats were intermediate between weanling and adulthood. Whether the incidence of hyperphagia among MSG/BPM rats might be increased by postponing BPM lesioning until adulthood also warrants evaluation.

A striking aspect of our findings is the significant hyperphagia (defined as an individual food intake score greater than 2 standard deviations above the mean for the control rats) in 47% of our MSG/BPM rats and total lack of hyperphagia in a large number (n = 28) of rats treated with MSG or BPM alone (indeed both groups were relatively hypophagic). Clearly, hyperphagia is not an obligatory consequence of hyperinsulinemia, since MSG rats were hyperinsulinemic and hypo rather than hyperphagic. However, it is possible that the presence of hyperinsulinemia is necessary or permissive in order for some other factor to produce hyperphagia, since preventing hyperinsulinemia partially prevents hyperphagia in the electrolytic VMH lesion model[3,15]. Qualitative evaluation of cresyl violet sections of the hypothalami of MSG/BPM rats that did and those that did not display hyperphagic obesity revealed no obvious differences in the nature or extent of the lesions. In a quantitative study pertaining only to pro-opiomelanocortin neurons, their number was markedly, but equally, decreased in both groups. Thus, our experiments do not definitively clarify which hypothalamic neural elements, in addition to MSG-sensitive AH neurons, must be destroyed to produce hyperphagia. Since neither an AH lesion (MSG) nor VMH lesion (BPM) resulted in hyperphagia but a combined AH/VMH lesion did, it is possible that there is a functionally homologous group of neurons distributed over both AH and VMH which can substitute for one another in mediating satiety effects. If so, hyperphagia would occur only when all (or some critical mass) of such neurons had been deleted, as perhaps was achieved in 47% of our MSG/BPM rats.

Our experiments do show that by an entirely systemic chemotoxic approach in rats, we can induce a hyperinsulinemic obesity syndrome either with or without hyperphagia, the former featuring levels of insulin, food intake, and obesity comparable to the classical 'VMH' electrolytic syndrome. Although the AH obesity syndrome is less dramatic than the AH/VMH syndrome, it is no less interesting since obese humans are sometimes normo or hypophagic compared to their lean peers and hyperinsulinemia has often been found to accompany human obesity. It is noteworthy that impairment of AH neurons may play a causal role in both syndromes; this underscores the importance of AH neurons in mechanisms of obesity.

Supported by NIH Grant 20579.

1 Bernardis, L.L., Prediction of carcass fat, water and lean body mass from Lee's 'Nutritive ratio' in rats with hypothalamic obesity, *Experientia,* 26 (1970) 789–790.
2 Bernardis, L.L. and Frohman, L.A., Effect of hypothalamic lesions at different loci on development of hyperinsulinemia and obesity in the weanling rat, *J. Comp. Neurol.,* 141 (1971) 107–118.
3 Bray, G.A. and York, D.A., Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis, *Physiol. Rev.,* 59 (1979) 719–809.
4 Brobeck, J.R., Mechanisms of the development of obesity in animals with hypothalamic lesions, *Physiol. Rev.,* 26 (1946) 541–559.
5 Brooks, C.M. and Lambert, E.F., A study of the effect of limitation of food intake and the method of feeding on the rate of weight gain during hypothalamic obesity in the albino rat, *Am. J. Physiol.,* 147 (1946) 95–107.
6 Cox, C., Detection of treatment effects when only a portion of subjects respond. In S.A. Miller (Ed.), *Nutrition and Behavior,* The Franklin Institute Press, 1981, pp. 285–289.
7 Fryer, H.C., *Concepts and Methods of Experimental Statistics,* Allyn and Bacon, Boston, 1966, pp. 86–91.
8 Han, P.W., Lin, C.-H., Chu, K.-C., Mu, J.-Y. and Liu, A.-C., Hypothalamic obesity in weanling rats, *Am. J. Physiol.,* 209 (1965) 627–631.
9 Hetherington, A.W. and Ranson, S.W., Hypothalamic lesions and adiposity in the rat, *Anat. Rec.,* 78 (1940) 149–172.

10 Jagot, S.A., Webb, G.P., Rogers, P.D. and Dickerson, J.W.T., The induction of obesity in the rat with bipiperidyl mustard, *Brit. J. Nutr.*, 44 (1980) 253–255.
11 Keesey, R.E. and Powley, T.L., Hypothalamic regulation of body weight, *Amer. Sci.*, 63 (1975) 558–565.
12 Laughton, W. and Powley, T.L., Bipiperidyl mustard produces brain lesions and obesity in the rat, *Brain Research*, 221 (1981) 415–420.
13 Olney, J.W., Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate, *Science*, 164 (1969) 719–721.
14 Olney, J.W., In E. McGeer, J.W. Olney and P. McGeer, (Eds.), *Kainic Acid as a Tool in Neurobiology*, Raven Press, New York, 1978, pp. 95–121.
15 Powley, T.L. and Laughton, W., Neural pathways involved in the hypothalamic integration of autonomic responses, *Diabetologia*, 20 (1981) 378–387.
16 Rutman, R.J., Lewis, F.S. and Bloomer, W.D., Bipiperidyl mustard: a new obesifying agent in the mouse, *Science*, 153 (1966) 1000–1002.
17 Schachter, S., Obesity and eating, *Science*, 161 (1968) 751–756.
18 Sclafani, A., The role of hyperinsulinema and the vagus nerve in hypothalamic hyperphagia reexamined, *Diabetologia*, 20 (1981) 402–410.
19 Utsumi, M., Hirose, Y., Ishihara, K., Makimura, H. and Baba, S., Hyperinsulinemia and hypersomatostatinemia in hypothalamic obese rats induced by monosodium glutamate, *Biomed. Res.*, 1 Suppl. (1980) 154–158.
20 van Houten, M. and Posner, B.I., Cellular basis of direct insulin action in the central nervous system, *Diabetologia*, 20 (1981) 255–267.