# Intraventricular Injection of Clonidine, Norepinephrine, and 5-Hydroxytryptamine in Genetically Obese (ob/ob) and Lean Mice: Differential Effects on Food Intake and Macronutrient Selection

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BY

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## Abstract

$\alpha_2$-Noradrenergic stimulation of the hypothalamic paraventricular nucleus [PVN] is known to enhance feeding, particularly of carbohydrate, in the satiated rat. PVN microinjection of 5-hydroxytryptamine (5-HT), however, decreases carbohydrate intake. More recent research with the $\alpha_2$ agonist clonidine [CLON] indicate that peripheral injection of this compound produces a reliable and dose-dependent eating response, associated with a preferential increase in carbohydrate ingestion, in 6-h meal-feeding genetically obese (ob/ob) mice. Further, the stimulatory effect of CLON is blocked by yohimbine pretreatment. The present study measured the effects of central administration of CLON and norepinephrine [NE] on macronutrient self-selection in genetically obese and lean mice maintained under free-feeding conditions. Following adaptation to single-energy source diets (simultaneous access) of carbohydrate, protein, and fat, pre-satiated obese (n=7) and lean (n=7) mice were injected with CLON [10-20 nmol ICV] or sterile physiological saline, in counterbalanced order, approximately 1 h (16h00) before the start of the dark cycle, in a 12 h L-D cycled colony room. Macronutrient intakes were assessed at 1 h and 2 h postinjection. Mice received several injections at each dose, and all intake scores represented an average of these test scores. Similar tests were conducted with arterenol bitartrate [NE, 40-80 nmol ICV] in a separate group of mice. In an additional study, 5-HT [35-140 nmol ICV] was injected in obese and lean mice that were mildly food-deprived for 2 h at the start of the active feeding cycle. Food intake, after injection of CLON, NE, or 5-HT, was also examined in mice maintained on a diet of standard rodent chow. Results indicated that both CLON and NE increased feeding, associated with a dramatic and selective increase in carbohydrate intake in both phenotypes, although obese mice showed an enhanced behavioural

response to noradrenergic stimulation. Intake of chow was also potentiated, an effect that was again most evident in the ob/ob. Moreover, the CLON and NE-induced potentiation of carbohydrate intake resulted in a significant decrease in the percent concentration of fat and protein ingested in both phenotypes. In contrast, the effects of centrally administered 5-HT on food intake and diet selection were generally opposite to those of CLON and NE. Injection of 5-HT decreased feeding, particularly of carbohydrate and chow, in a dose-dependent manner; however, obese mice showed a reduced sensitivity to the anorectic effects of 5-HT. Further, the reduction in carbohydrate intake in both phenotypes after 5-HT treatment resulted in an increase in the percent energy from fat and protein. Although these findings provide increasing support for the role of NE and 5-HT in the control of feeding and macronutrient-specific appetite in mice, the altered sensitivity of the ob/ob to both noradrenergic and serotonergic stimulation may reflect abnormal monoamine function in this mutant.

# Intraventricular Injection of Clonidine, Norepinephrine, and 5-Hydroxytrytptamine in Genetically Obese (ob/ob) and Lean Mice: Differential Effects on Food Intake and Macronutrient Selection

The genetically obese (ob/ob) mouse exhibits multiple physiological and behavioural abnormalities including excessive food intake, increased adiposity, hyperinsulinemia, and hyperglycemia (Bray & York, 1979; Mrosovsky & Melynk, 1982; Sclafani, 1984; Storlien, 1984). Although the mechanisms underlying these effects remain unknown, an abnormality in central nervous system (CNS) function may contribute to the hyperphagia of the ob/ob mouse (Bray & York, 1971; Callahan, Beales, & Oltmans, 1984; Kuprys & Oltmans, 1982). The ob/ob has elevated brain 5-hydroxytryptamine (5-HT) (Garthwaite, Martinson, Tseng, Hagan, & Menahan, 1980), as well as decreased cholecystokinin (CCK) and increased CCK receptors in the cerebral cortex (Hays & Paul, 1981; Straus & Yalow, 1979). Evidence of hypothalamic abnormalities in the ob/ob include increased $\alpha_1$-noradrenergic receptor density (Oltmans, Lorden, Callahan, Beales, & Fields, 1981), decreased neuronal size in several hypothalamic nuclei (Bereiter & Jeanrenaud, 1979), altered dendritic orientation in the lateral and ventromedial nucleus (Bereiter & Jeanrenaud, 1980), increased paraventricular norepinephrine (NE) (Oltmans, 1983), and reduced metabolism of hypothalamic NE and 5-HT (Lorden, Fillingim, & Hayley, 1986). Therefore, multiple abnormalities in neural systems putatively involved in the control of feeding exist in this mutant.

Hypothalamic noradrenergic mechanisms believed to contribute to altered caloric intake in the genetically obese mouse have also been implicated in energy intake regulation of nonpathological models. Specifically, noradrenergic receptor mechanisms in the hypothalamic paraventricular

nucleus (PVN) are implicated in the physiological control of food intake (Hoebel, 1984; Leibowitz, 1980; Morley & Levine, 1985; Stanley, Schwartz, Hernandez, Hoebel, & Leibowitz, 1989). Norepinephrine injected into this nucleus elicits feeding in satiated rats and enhances eating in hungry rats (Leibowitz, Brown, Tretter, & Kirschgessner, 1985; Lichtenstein, Marinescu, & Leibowitz, 1984; Towell, Muscat, & Willner, 1989), an effect which is blocked by selective $\alpha_2$-noradrenergic antagonists and also by discrete electrolytic lesions of the PVN (Goldman, Marino, & Leibowitz, 1985; Leibowitz, Hammer, & Chang, 1983). The $\alpha_2$-noradrenergic agonist clonidine (CLON) has similarly been shown to stimulate feeding when injected intraperitoneally in different species, including the ob/ob (Currie & Wilson, 1991a; Currie & Wilson, 1990), or directly into the PVN of the rat (Leibowitz et al., 1985; McCabe, De Bellis, & Leibowitz, 1984; Sanger, 1983; Schlemmer, Elder, Casper, & Davis, 1981; Shor-Posner, Azar, Volpe, Grinker, & Leibowitz, 1988; Weiss & Leibowitz, 1985). Moreover, long-term feeding patterns are altered by chronic PVN infusion of NE or CLON, which produces a potentiation of daily food intake, associated with an increase in meal size but not the number of eating bouts, and an enhanced body weight gain (Leibowitz, 1988; Leibowitz, Roosin, & Rosenn, 1984; Lichtenstein et al., 1984; Shor-Posner, Grinker, Marinescu, & Leibowitz, 1985). Further, administration of CLON or NE is associated with an alteration in macronutrient selection, or an increase in carbohydrate ingestion (Cheung, Dietz, Alexander, Ade, Brennan, & Leibowitz, 1990; Currie & Wilson, 1989; Leibowitz et al., 1985; Shor-Posner et al., 1988; Yee, MacLow, Chan, & Leibowitz, 1987). Given that hypothalamic catecholamines are implicated in the control of macronutrient-specific appetite, the present study investigated the central neurochemical regulation of feeding and diet selection in the genetically obese mouse,

a mutant which exhibits hyperphagia and abnormal macronutrient preference.

The Genetically Obese Mouse

The genetic aberration in the ob/ob is an autosomal recessive trait, a single point mutation in the genetic code leading to the production of a defective peptide (Connolly & Carnie; 1984; Storlien, 1984). The homozygous recessive ob/ob displays marked obesity, involving increases in both size and number of adipocytes (Faust, Johnson, Stern, & Hirsch, 1978; Johnson & Hirsch, 1972). The rate of lipogenesis is increased in the liver and fat (Assimacopoulos-Jeannet, Singh, Le Marchand, Loten, & Jeanrenaud, 1974). In contrast, the homozygous dominant (+/+) wild type and the heterozygous +/ob are phenotypically lean.

The ob/ob is hyperphagic which is manifested in an increased caloric intake (Bray & York, 1979; Sclafani, 1984). Obese mice typically maintain an elevated energy intake regardless of their pattern of nutrient intake (Romsos & Ferguson, 1982), although hyperphagia is usually not found when obese mice are placed on restricted feeding (Currie & Wilson, 1991; Jagot, Dickerson, & Webb, 1982). Adult obese mice prefer to self-select a higher proportion of energy from fat than from protein or carbohydrate (Castonguay, Rowland, & Stern, 1985; Currie & Wilson, 1991; Romsos, Chee, & Bergen, 1982). Obese mice allowed to self-select from diets varying in protein and carbohydrate consume as much protein as lean mice (Chee, Romsos, & Bergen, 1981; Chee, Romsos, Bergen, & Leveille, 1981; Romsos, Chee, & Bergen, 1982). When given access to diets varying in protein and fat, both obese and lean mice reduce their intake of protein (Romsos, Chee, & Bergen, 1982). The reduction in protein intake appears to be secondary to a greater preference for a high-fat diet rather than a high-carbohydrate diet, and suggests that a nonprotein energy source can affect self-selected protein intake.

Energy intake derived from carbohydrate, fat, and protein averages 12-18%, 66-68%, and 15-20%, respectively, for obese mice and 25-28%, 53-60%, and 12-22% for lean mice (Currie & Wilson, 1991a; Romsos & Ferguson, 1982), suggesting that both obese and lean mice self-select more energy from fat, although the ob/ob appears to maximize fat intake resulting in a greater increase in body weight gain. However, increased adiposity has not been attributed to overeating alone since the ob/ob remains obese even when pair-fed with lean littermates (Stock & Rothwell, 1982).

The ob/ob is also hyperinsulinemic and exhibits glucose intolerance (Dubuc, 1977; Storlien, 1984). Elevated plasma insulin concentrations are associated with an increase in the number and size of insulin-secreting beta cells of the pancreas (Storlein, 1984). Marked insulin resistance is associated with progressive loss of insulin receptors (Herberg & Coleman, 1977). As a result, the hypoglycemic effect, normally associated with elevated levels of circulating insulin, is not present in the ob/ob mutant. Also, enzymes involved in gluconeogenesis, found to be decreased in the hyperinsulinemic state, remain elevated in the ob/ob, an abnormality which occurs early in development and may be associated with the loss of insulin receptor sites in the liver (Coleman, 1978).

Another significant component of the ob/ob syndrome is the thermogenic defect (Seydoux, Rochner-Jeanrenaud, Assimacopoulos-Jeannet, & Jeanrenaud-Girardier, 1981). A chronically lower colonic temperature and resting metabolic rate at ambient temperatures below thermoneutrality suggest that the ob/ob is hypothermic and hypometabolic, as indicated by a lowered rate of oxygen consumption (Boisonneault, Hornshuh, Simons, Romsos, Leveille, 1978; Carlisle & Dubuc, 1984; Thurlby & Trayhurn, 1978). Preweaning obese mice exhibit defective

thermogenesis as well as differences in energy metabolism, which are evident as early as 5 days postpartum (Boisonneault et al., 1978), suggesting that abnormalities in both thermoregulation and metabolism are not secondary to obesity. That is, defective physiological thermoregulation precedes the divergence in body mass of lean and obese mice. Hypothermia, therefore, may be a primary contributor to the development of obesity, as a greater percentage of the diet is diverted to fat rather than energy for thermogenesis. By expending less energy on thermoregulatory thermogenesis, obese mice store energy intake as white fat, resulting in gross adiposity (Thurlby & Trayhurn, 1979; Trayhurn & James, 1980). The lowered expenditure of energy on thermoregulatory heat production leads to an increased metabolic efficiency which appears to underlie the development and maintenance of obesity.

Hogan & Himms-Hagen (1980) have identified a mitochondrial defect in brown adipose tissue of reduced binding of purine nucleotides and a failure of the ob/ob to respond to cold stress with an increase in purine binding. Reduced NE turnover in brown adipose tissue is also characteristic of the ob/ob (Knehans & Romsos, 1982). Therefore, an important mechanism in heat production, nonshivering thermogenesis, involving extra-muscular sources or areas of brown adipose tissue, is defective in the ob/ob mouse (Trayhurn, Goodbody, & James, 1982). As a result, the ob/ob displays an impairment in increasing heat production. The reduction in nonshivering thermogenesis, in turn, is manifested in a decreased metabolic rate and lowered colonic temperature (Batt & Hambi, 1982; Trayhurn & James, 1980).

In addition to these symptoms, biochemical and neuroanatomical abnormalities have been cited as evidence of a CNS defect in the ob/ob mouse. Altered neuroanatomical organization in the CNS includes reduced brain mass and cortical brain volume and significantly reduced soma

cross-sectional areas in the ventromedial hypothalamus (VMH) of the ob/ob in comparison to lean mice (Bereiter & Jeanrenaud, 1980; Bereiter & Jeanrenaud, 1979). Elevated levels of hypothalamic NE (Feldman, Blalock, & Zern, 1979; Lorden, Oltmans, & Margules, 1975; Oltmans, 1983), pituitary dopamine (DA) (Lorden & Oltmans, 1977), brain 5-HT (Garthwaite, Martinson, Tseng, Hagan, Menahan, 1980), and ß-endorphin (Govoni & Yang, 1981) have also been detected in the ob/ob mouse. Hypothalamic NE levels are increased in the PVN and VMH (Oltmans, 1983), nuclei which have been implicated in the regulation of feeding (Hoebel, 1984; Leibowitz, 1986; Leibowitz et al., 1985). However, peripheral injection of reserpine, which acts to deplete catecholamines by affecting the ability of the adrenergic vesicle to store transmitter, does not reduce endogenous levels of hypothalamic NE in the ob/ob to the same extent as in lean mice (Oltmans, Olsauskas, & Comaty, 1980). Therefore, increased NE levels and altered sensitivity to pharmacological treatment in the ob/ob could involve various neurochemical mechanisms, including abnormal release mechanisms of the presynaptic neuron, modified storage properties, or altered reuptake mechanisms.

An increase in $\alpha_1$-noradrenergic receptor density is found in the ob/ob, although no differences in $\alpha_2$ receptor density and affinity are observed in obese mice and lean controls (Callahan et al., 1984; Oltmans et al., 1981). No significant differences in either receptor number or affinity for $\alpha$-adrenergic receptors in the cortex or for dopaminergic receptors in the cortex or corpus striatum are evident between the two phenotypes (Oltmans et al., 1981). However, a decrease in cholecystokinin (Straus & Yalow, 1979) and an increase in cholecystokinin receptors (Hays & Paul, 1981) have been found in the cerebral cortex of obese mice, suggesting that multiple abnormalities in neural systems involved in the control of feeding exist in the ob/ob.

Neural Mechanisms of Feeding and Satiety

The medial and lateral hypothalamus in conjunction with forebrain and hindbrain structures, and with peripheral autonomic-endocrine systems, are believed to play an important role in the control of energy balance via hunger and satiety mechanisms (Hoebel, 1984; Leibowitz, 1985; Oomura, 1988; Towell et al., 1989; Weiss & Leibowitz, 1985). More specifically, the hypothalamus monitors and integrates the complex sensory and metabolic input concerning the nutritional status of the organism and transduces this information into appropriate quantitative and qualitative adjustments in food intake (Bray, 1989; Hernandez & Hoebel, 1989; Leibowitz, 1988; Schwartz, McClane, Hernandez, & Hoebel, 1989).

Research from brain-cannula mapping studies has identified central brain sites which are sensitive to injected neurotransmitters and may therefore be physiologically active in food intake regulation. The hypothalamus, in contrast to extrahypothalamic forebrain structures, has repeatedly been shown to be most responsive, and in addition, selective sensitivity within this brain region has been identified (Leibowitz, 1978; Leibowitz 1987; Leibowitz et al., 1985; Matthews, Booth, & Stolerman, 1978). Although the hypothalamus appears to be a primary brain structure in the mediation of monoamine and neuropeptide effects on food intake, however, it does not operate autonomously in this process, but functions as part of a whole-brain circuitry, organized at various integrative and interacting levels, and in close association with peripheral autonomic and endocrine systems, which are essential in providing neural, metabolic, and hormonal information required to maintain energy and nutritional homeostasis (Leibowitz, 1987; Luiten, ter Horst, & Steffens, 1987; Steffens & Strubbe, 1987; Siviy, Kritikos, Atrens, & Shepherd, 1989; Tempel, Bhakthavatsalam, Shor-Posner, Dwyer, & Leibowitz, 1985).

Hetherington & Ranson (1940) and Anand & Brobeck (1951) originally identified syndromes of feeding dysfunction and altered body weight, and localized these syndromes to the ventromedial and lateral hypothalamic nuclei. Bilateral destruction of the lateral hypothalamus (LH) was shown to result in aphagia, or the absence of feeding (Anand & Brobeck, 1951); whereas, hyperphagia and increased adiposity resulted from bilateral hypothalamic lesions to the dorsomedial and ventromedial (VMH) nuclei (Hetherington & Ranson, 1940). From this, Stellar (1954) introduced the concept of dual-centre neural control of feeding, a model which specified the basic pattern of excitatory mechanisms in the LH that subserve hunger (and referred to as a feeding centre), and inhibitory mechanisms in the VMH that subserve satiety (satiety centre). Feeding motivation was controlled by the integration of internal signals such as stomach fullness and external signals such as taste. Stimulation of the LH feeding system initiated feeding; whereas, lesioning resulted in the LH starvation syndrome and lowered body weight. Stimulation of the medial region or satiety system interrupted feeding, and lesioning resulted in the hypothalamic hyperphagia syndrome.

Subsequent research, however, determined that lesions to the ventral noradrenergic bundle ascending from brainstem nuclei and projecting through the VMH were necessary to produce hyperphagia (Gold, 1973), and NE was identified as the putative neurotransmitter in this hypothalamic satiety mechanism (Ahlskog & Hoebel, 1973). Loss of noradrenergic fibres in the ventral bundle termination areas, and not simply diffuse VMH damage, therefore, contributed to the development of hypothalamic obesity. Further, given that active feeding was maintained by an intact LH, destruction of this brain region should result in a permanent deficit in feeding. However, the aphagia appears to be only transitory as recovery of feeding generally occurs,

possibly due to the fact that not all cells within the LH, or fibres passing through the LH, are destroyed (Grossman, 1984; Teittlebaum & Epstein, 1962). As a result, importance has been placed on the role of cells within the hypothalamus as well as fibres of passage projecting through the VMH and LH which may be implicated in hypothalamic regulation of energy intake. For example, the LH fibre system includes a complex feedback mechanism involving the extrapyramidal motor system, suggesting that sensorimotor dysfunction may be implicated in aphagia via dopaminergic nigrostriatal damage (Grossman, 1975).

Hoebel (1984) has outlined brain mechanisms in the control of feeding which include monoamines, opioids, and gut-brain peptides. The neurophysiological profile of the LH feeding system, or more specifically the perifornical LH region, is distinguished by dopaminergic and ß-adrenergic inhibitory influences. Epinephrine, norepinephrine, and dopamine have an effect on feeding which is inhibitory. The medial hypothalamus, and in particular the PVN, is characterized by $\alpha$-adrenergic inhibition and serotonergic excitation of the satiety system. (The PVN rather than the VMH is identified as the medial hypothalamic structure involved in satiety control). Norepinephrine reduces satiety through disinhibition; whereas 5-HT acts to facilitate satiety. It is further hypothesized that various neuropeptides exert an influence on feeding by acting on lateral and medial hypothalamic nuclei (Hoebel, 1984; Leibowitz, 1988; Leibowitz, 1987; Leibowitz, 1986; Oomura, 1988).

Hypothalamic Control of Food Intake

Noradrenergic receptor mechanisms in the hypothalamic PVN, therefore, have a physiological role in the control of feeding. Norepinephrine injected directly into this nucleus at near physiological doses stimulates food intake in satiated rats, and enhances feeding in hungry rats

(Leibowitz et al., 1985; Lichtenstein, Marinescu, & Leibowitz, 1984). This effect is blocked by selective $\alpha_2$-noradrenergic receptor antagonists and by discrete electrolytic lesions of the PVN (Goldman, Marino, & Leibowitz, 1985; Leibowitz, Hammer, & Chang, 1981). The specific noradrenergic innervation of the PVN that controls feeding appears to derive, in part, from NE-containing neurons in the locus coeruleus and subcoeruleus. The efferent projection of this neurocircuit follows a periventricular course as it projects to hindbrain structures, including the dorsal vagal complex, to the peripheral autonomic nervous system (Weiss & Leibowitz, 1985).

Acute injection of NE is most effective in initiating a feeding response in rats when injected directly into the PVN region, producing the strongest feeding response at the lowest threshold dose (Leibowitz, 1978). The ingestive response is similar to natural feeding in terms of magnitude and duration. Chronic NE injection into the PVN results in hyperphagia and increased body weight gain, although feeding induced by central NE administration is attenuated following discrete lesions to the PVN, suggesting the integrity of the PVN is essential in order to demonstrate a normal feeding response (Leibowitz, Roosin, & Rosenn, 1984). In addition, destruction of PVN noradrenergic innervation by the neurotoxin 6-hydroxydopamine (6-OHDA) results in hypophagia (Shor-Posner, Azar, Jhanwar-Uniyal, Filart, & Leibowitz, 1986), implying that hyperphagia following PVN electrolytic lesioning cannot be attributed to damaged noradrenergic afferents, but reflects damage to PVN efferent projections through which NE may act to control satiety.

The $\alpha_2$-noradrenergic agonist CLON has similarly been shown to stimulate feeding via an $\alpha_2$ postsynaptic receptor mechanism in the PVN (Leibowitz et al., 1985; Shor-Posner, Azar, Volpe, Grinker, & Leibowitz, 1988). Long-term feeding patterns are altered by chronic infusion of NE

or CLON directly into the PVN, which produces a potentiation of daily food intake, associated with an increase in meal size but not the number of eating bouts, and an enhanced weight gain (Leibowitz, et al., 1984; Lichtenstein et al., 1984). Specifically, analysis of meal patterns has consistently shown that NE increases meal size and rate of ingestion and not meal frequency, suggesting that the effect of this neurotransmitter is primarily to maintain feeding and delay satiety (Shor-Posner, Grinker, Marinescu, & Leibowitz, 1985; Stanley, Schwartz, Hernandez, Hoebel, & Leibowitz, 1989).

Recent studies have provided increasing evidence as to the precise function of PVN NE in the control of natural feeding behaviour. Both central and peripheral pharmacological treatment have been shown to alter macronutrient selection, suggesting that specific brain neurotransmitters function to balance the proportion of carbohydrate, fat, and protein consumed (Kanarek, Marks-Kaufman, Ruthazer, & Gualtieri, 1983; Leibowitz et al., 1984). Leibowitz et al. (1985) have shown that endogenous NE acting on $\alpha_2$-noradrenergic receptors in the PVN regulates carbohydrate ingestion in the rat. Destruction of PVN noradrenergic innervation following 6-OHDA treatment results in specific disturbances in feeding patterns and nutrient selection (i.e., deficits in food intake and carbohydrate selection) in association with lowered levels of NE in the PVN (Shor-Posner et al., 1988). Again, the effect of NE apparently occurs through inhibition of PVN satiety neurons that have direct control of carbohydrate intake as well as altering total daily caloric intake and body weight gain. These findings, therefore, suggest an important role for the medial hypothalamus or PVN $\alpha_2$-noradrenergic system in controlling normal feeding behaviour as well as macronutrient selection.

The link between hypothalamic NE and food intake, particularly carbohydrate ingestion, is

believed to be related to the finding that NE, to express its physiological function, must act in close association with two circulating hormones, the adrenal glucocorticoid, corticosterone, and the pancreatic hormone, insulin, both of which appear to function synergistically with central neurotransmitters (Leibowitz, 1987). PVN-NE elicited feeding is abolished by adrenalectomy and attenuated by dissection of vagal afferents to the pancreas (Leibowitz, Roland, Hor, & Squillari, 1984; Roland, Bhakthavatsalam, & Leibowitz, 1986). In addition, the PVN plays an important role in the control of corticosterone release, and it is now believed that corticosterone may have a positive feedback to this nucleus to control its noradrenergic innervation and $\alpha_2$ receptor population. Circulating corticosterone in the range of 2-15 $\mu$g/100 ml significantly up-regulates these receptors and in a precise, dose-dependent manner with short latency, permits NE to function normally in its short-term regulation of meal size in relation to energy (carbohydrate) reserves (Leibowitz, 1986). Further, the concentration of $\alpha_2$ receptors in the PVN, as opposed to other hypothalamic sites, is strongly positively correlated with circulating glucose concentrations (Jhanwar-Uniyal, Papamichael, & Lebowitz, 1988).

From this, it is hypothesized that the noradrenergic system of the paraventricular hypothalamus, as part of its overall effort to rapidly replenish body energy stores, becomes physiologically active under conditions involving energy expenditure (i.e., food deprivation, stress, or onset of the active period of the diurnal cycle where hepatic glycogen stores may be low and blood glucose levels may decline) (Leibowitz, 1987). Several studies suggest that PVN NE acts to initiate feeding and consequently restore carbohydrate reserves. Specifically, in the nocturnal rat, the initial eating bout that normally occurs at the beginning of dark onset is associated with a sharp unimodal peak of circulating corticosterone, of $\alpha_2$-receptor density

exclusively in the PVN, and of responsiveness to PVN NE and CLON infusion; a release in medial hypothalamic NE in association with feeding; and a natural increase in meal size, rate of feeding, and preference for carbohydrate (Bhakthavatsalam & Leibowitz, 1986; Jhanwar-Uniyal, Roland, & Leibowitz, 1986; Leibowtiz, 1988; Tempel et al., 1985; Towell et al., 1985).

Food deprivation appears to activate the PVN noradrenergic system, as evidenced by enhanced release of medial hypothalamic or PVN NE in deprived rats, in addition to a rapid, dramatic, and highly site specific down-regulation of $\alpha_2$ receptors in the PVN (Jhanwar-Uniyal, Fleischer, Levin, & Leibowitz, 1982; Jhanwar-Uniyal & Leibowitz, 1986). A close relationship exists between blood glucose concentration and hypothalamic NE turnover, and the changes that occur in $\alpha_2$-receptors, NE turnover, and glucose levels after acute food deprivation are reversed by a brief period of food ingestion (Jhanwar-Uniyal et al., 1988; Smythe, Grunstein, Bradshaw, Nicholson, & Compton, 1984). The importance of the PVN adrenal-dependent $\alpha_2$-noradrenergic system in monitoring and replenishing carbohydrate stores after acute food deprivation becomes apparent in PVN-lesioned or adrenalectomized rats, which exhibit disturbances in carbohydrate ingestion and in the ability to produce adequate compensatory feeding in response to food deprivation (Bhakthavatsalam & Leibowitz, 1986; Shor-Posner, Azar, Insingna, & Leibowitz, 1985).

There is also substantial evidence for the involvement of 5-HT in the regulation of feeding and in the control of macronutrient-specific appetite (Bendotti & Samanin, 1986; Blundell, 1986; Leibowitz, Weiss, & Shor-Posner, 1988; Leibowitz, Weiss, Shor-Posner, Walsh, & Tempel, 1987; Luo, Ransom, & Li, 1990; Nicolaidis, 1986). Injection of 5-HT or drugs which act via the release and uptake blockade of endogenous 5-HT are effective in suppressing feeding, in

contrast to reports of increased consumption following injection of 5-HT antagonists (Blundell, 1984; Garattini, Mennini, & Scmanin, 1987; Leibowitz et al., 1988; Pollock & Rolland, 1981). Additional evidence suggests that depletion of 5-HT by neurotoxin injection or micro-knife cuts may produce hyperphagia and weight gain (Bendotti & Samanin, 1986; Rowland, 1986; Waldbillig, Bartness, & Stanley, 1981). Therefore, lesions and depletion of the brain serotonergic system may lead to an increase in food intake.

The medial hypothalamic PVN is believed to have an important role in the control of satiety in which PVN 5-HT may function in opposition to PVN NE to control satiety signals (Fletcher & Patterson, 1989; Leibowitz, 1985; Leibowitz, Weiss, Walsh, & Viswanath, 1989; Weiss, Papadakos, Knudson, & Leibowitz, 1986). Direct competition between these neurotransmitters has been observed biochemically in the hypothalamus, with the release of 5-HT inhibited by NE via $\alpha_2$-receptors (Chesselet, 1984). Administration of 5-HT or the 5-HT releasing compound dl-norfenfluramine directly into the PVN is effective in causing a potent suppression of feeding in hungry rats (Fletcher & Paterson, 1989; Garattini et al., 1987; Leibowitz, 1986; Shor-Posner et al., 1986). Injection of 5-HT into this nucleus is particularly effective in suppressing food intake under conditions of deprivation, drug-elicited and spontaneous feeding (Leibowitz, Weiss, Shor-Posner, Walsh, & Tempel, 1987; Massi & Marini, 1987; Weiss & Leibowitz, 1988). Serotonergic stimulation of the PVN can inhibit the feeding response elicited by PVN administration of NE (Weiss, Papadakos, Knudsen, & Leibowitz, 1986). Also, 5-HT injected into the PVN produces a suppression of feeding that is characterized by a change in meal size rather than frequency of meals consumed, and by a change in preference for dietary nutrients, in particular carbohydrate intake is reduced but protein intake is spared or even enhanced

(Leibowitz, Weiss, Walsh, & Viswanath, 1989; Shor-Posner, Grinker, Marinescu, Brown, & Leibowitz, 1986). The feeding patterns exhibited by rats after PVN serotonergic administration, therefore, are opposite to those obtained after $\alpha_2$-noradrenergic stimulation of the PVN. In addition, peripheral injection of serotonergic agonists have also been shown to selectively suppress appetite for carbohydrate (Blundell, 1984; Luo & Li, 1990; Luo & Li 1991; Nathan & Rolland, 1987).

The apparent antagonistic interaction between 5-HT and NE, possibly in conjunction with catecholaminergic innervation to the LH, may provide potential neurochemical substrates for regulating the flow of body energy and nutrients and, in particular, for balancing the ingestion of specific macronutrients. In monitoring the need for carbohydrate and protein, hypothalamic 5-HT may function as a ratio sensor, by translating amino acid concentration into neurotransmitter function, and hypothalamic NE may be involved in monitoring and responding to information concerning energy or carbohydrate levels (Leibowitz, 1985; Symthe et al., 1984). It has been proposed that at the start of the active period, when energy stores are low, an increase occurs in medial hypothalamic PVN $\alpha_2$-noradrenergic activity and consequently, a potentiation of carbohydrate intake results (Leibowitz, 1986; Leibowitz, 1988; Stanley et al., 1989). Since brain 5-HT synthesis, relative to catecholamine synthesis, is increased following carbohydrate ingestion (Wurtman & Fernstrom, 1976), a subsequent change in preference for protein then occurs as a result of increased satiety for carbohydrate (Shor-Posner, Grinker, Marinescu, Brown, & Leibowitz, 1986). With protein ingestion, the reverse neurochemical pattern develops, resulting in an increase in hypothalamic catecholamine synthesis, and a return to preference for carbohydrate.

Central 5-HT has also been implicated in the coordination of circadian timing systems in addition to its involvement in medial hypothalamic feeding and food-anticipatory rhythms (Kordon, Hery, Szafarczyk, Ixart, & Assenmacher, 1981; Rosenwasser & Adler, 1986). These findings sugggest that 5-HT in the medial hypothalamus may act in a phasic, circadian-related manner, exerting its influence primarily on carbohydrate ingestion at the start of the active feeding cycle (Leibowitz et al., 1989; Stanley et al., 1989). Anatomical studies have shown that the PVN is one of three hypothalamic nuclei, including the VMH and the suprachiasmatic nucleus, innervated by serotonergic fibres from the midbrain raphe nuclei (Sawchenko, Swanson, Steinbusch, & Verhofstad, 1983), and having a relatively dense concentration of 5-$HT_{1B}$ receptors (Pazos & Palacios, 1985), the receptor subtype believed to mediate 5-HT-induced hypophagia in the rat (Bendotti & Samanin, 1987; Hutson, Donohoe, & Curzon, 1988). Again, it is believed that these nuclei interact closely in coordinating temporal patterns of macronutrient ingestion where 5-HT acts at the beginning of the active feeding cycle to exert an inhibitory effect on the carbohydrate-rich meal that occurs naturally at dark onset, and while inducing satiety for carbohydrate, promotes the ingestion of protein in the next meal of the active cycle.

Statement of the Problem

Biochemical abnormalities in the hypothalamus of the genetically obese mouse have been cited as possible evidence of a central nervous system defect contributing to obesity and alterations in feeding behaviour (Callahan et al., 1884; Oltmans, 1983; Lorden et al., 1986). Further, reports of brain neurochemical and neurophysiological abnormalities in several other rodent models of genetically transmitted obesity, including the db/db mouse and the fa/fa rat, are consistent with such a hypothesis (Finkelstein, Kim, Awad, Leibowitz, & Jhanwar-Uniyal, 1988;

Levin & Sullivan, 1979; Lorden, 1979; Lorden et al., 1975). With respect to noradrenergic function, Callahan et al. (1984) have reported that peripherally administered yohimbine and rauwolscine ($\alpha_2$ antagonists) significantly reduced intake of standard rodent chow in both ob/ob and lean mice. In contrast, CLON, the $\alpha_2$ agonist, increased food intake in obese mice at doses which did not affect intake in lean mice, suggesting that obese mice are more sensitive to the hyperphagic effect of clonidine. However, higher doses of CLON resulted in the apparent suppression of intake in both phenotypes.

More recent studies have shown that CLON reduces total energy intake in meal-feeding obese and lean mice primarily through the suppression of carbohydrate and fat consumption (Currie & Wilson, 1991a; Currie & Wilson, 1988), while lower doses of this $\alpha_2$ agonist selectively increase the ingestion of carbohydrate and augment total caloric intake (Currie & Wilson, 1991b; Currie & Wilson, 1990; Currie & Wilson, 1989). These studies have also shown that obese mice appear to be more sensitive to CLON's anorectic and orexigenic effects on diet selection, possibly suggesting an enhanced pharmacological sensitivity of an $\alpha_2$-noradrenergic mechanism in genetic obesity. The specificity of the CLON-induced potentiation of carbohydrate intake in the ob/ob is consistent with previous reports of alterations in long-term feeding patterns by chronic PVN infusion of NE or CLON, resulting in increased food intake and enhanced body weight gain (Leibowitz, Roosin, & Rosenn, 1984; Lichtenstein et al., 1984). In addition, CLON may also potentiate caloric intake of fat in ob/ob and lean mice (Currie & Wilson, 1990), although the effect appears to be less dramatic than the CLON-induced carbohydrate preference. In fact, the proportion of fat and protein ingested is actually reduced following CLON treatment, emphasizing the differential effects of this $\alpha_2$ agonist on macronutrient intake. In contrast,

treatment with the $\alpha_2$-receptor antagonist yohimbine decreases feeding, particularly of carbohydrate and fat, in both phenotypes (Currie & Wilson, 1989). Yohimbine pretreatment, resulting in competitive $\alpha_2$ receptor antagonism, eliminates CLON's robust and selective increase in carbohydrate intake (Currie & Wilson, 1991b; Currie & Wilson, 1990).

However, previous research examining drug effects on energy intake in the ob/ob have typically employed a restricted feeding regimen and a peripheral route of drug administration, both of which contribute to the difficulty in making precise inferences about the hypothalamic defect in the ob/ob. Given the elevated levels of hypothalamic NE in the ob/ob, it is possible that a genetically determined abnormality in the PVN-noradrenergic system may result in an impaired satiety control mechanism and subsequent hyperphagia. Altered neurochemical mechanisms in this brain region could permit an increase in the amount of neurotransmitter reaching the postsynaptic membrane. Elevated PVN NE could result in an increase in postsynaptic $\alpha_2$ receptor stimulation which could, in turn, promote hyperphagia. However, it is also possible that an aberrant serotonergic mechanism may also contribute to a defect in satiety in the ob/ob. Both NE and 5-HT are implicated in the physiological control of food intake, and both neurotransmitters are increased in the central nervous system of the ob/ob and demonstrate impaired hypothalamic metabolism (Garthwaite et al., 1980; Leibowitz et al., 1985; Leibowitz et al., 1989; Lorden et al., 1986; Oltmans, 1983). The present study, therefore, examined the effects of centrally administered CLON, NE, and 5-HT on spontaneously motivated feeding and diet selection in ob/ob and lean mice. It was anticipated that centrally administered NE and CLON would potentiate caloric intake, reflected in an increased preference for carbohydrate, particularly in the ob/ob. Norepinephrine and CLON would, therefore, selectively increase

carbohydrate intake resulting in an overall increase in total caloric intake. Although both $\alpha_2$ agonists could also alter fat and protein consumption, a decrease in the actual proportion of fat and protein consumed was expected to result from the substantial increase in the amount of carbohydrate ingested by mice. Further, central injection of 5-HT was also expected to be associated with distinct changes in food consumption and appetite for a specific macronutrient, suppressing carbohydrate intake in hungry obese and lean mice, by increasing endogenous serotonergic activity.

## Method

### Subjects

Genetically obese (C57Bl/6J, ob/ob, N=34) and lean (C57Bl/6J, +/?, N=34) adult male mice (Jackson Laboratory, Bar Harbor, ME, USA), aged 10 weeks at the start of the experiment, were individually housed and tested in hanging wire cages (24 x 18 x 18 cm). Mice were maintained under controlled light (lights on 05h00-17h00) and temperature (23°C). Body weights averaged 56.96 g for obese mice and 30.03 g for lean mice.

### Diets

Three single-energy source diets were presented in circular aluminum containers with a stainless steel cover with four 1-cm holes, to allow access to the macronutrient but minimize food spillage. Previous work with these diets has shown them to be generally palatable, to permit normal body weight gain, and to yield consistent day-to-day intake scores following an adaptation of one to two weeks (Currie & Wilson, 1991; Shor-Posner, Grinker, Marinescu, Brown, & Leibowitz, 1986; Tempel, Shor-Posner, Dwyer, & Leibowitz, 1989). The carbohydrate ration was composed of 43.9% dextrin (ICN Pharmaceuticals), 43.9% starch (St.

Lawrence Starch Ltd.), 5% fibre (ICN), 4% minerals (ICN), 3% vitamins (ICN), and 0.2% choline (ICN). The fat diet consisted of 70.5% lard (Tenderflake), 10% corn oil (Mazola), 8% minerals, 6% vitamins, 5% fibre, and 0.5% choline. The protein component was composed of 86.3% vitamin-free casein (ICN), 5% fibre, 4% minerals, 3% vitamins, 1.5% methionine (ICN), and 0.2% choline. Calculation of caloric density was based on caloric coefficients of 3.7 kcal/g for carbohydrate and protein, and 7.7 kcal/g for fat. Standard laboratory stock diet, Agway Prolab R-M-H 3000, consisted of 51% carbohydrate, 22% protein, 11% moisture, 6% ash, 5% fat, and 5% fibre, yielding a calculated metabolizable energy of 3.5 kcal/g.

Surgeries

Mice were stereotaxically implanted under pentobarbital anesthesia (7 mg/100 g body weight IP Nembutal, Allen & Hanburys), with unilateral 22-ga. cannulae (Plastics One Inc., Roanoke, VA, USA) aimed at the left ventricle. A Kopf stereotaxic instrument fitted with a non-traumatic adaptor for mice (Slotnick, 1972) was used. The interaural scalp hair was clipped and the shaved scalp was then swabbed with 70% EtOH and sterile saline. An incision was then made off-midline (since the lateral suture is typically not closed in mice) to expose the skull from the nasal bone to the occiput. The scalp was retracted and the underlying fascia removed to clearly expose the bregma and lambda cranial points. Leveling the skull so that bregma and lambda are even is of critical importance. The tip of the cannula was positioned at the appropriate anterior/posterior coordinate as well as the lateral coordinate, the entry hole was marked, and then drilled through the skull using a medium dental burr to expose the dura. Three additional holes were drilled in each of the remaining quadrants of the skull into which a stainless steel screw, measuring 2.0 mm in length, was placed. Curved forceps and a small screw driver were

used to position each screw into the skull at a depth of approximately 0.4 mm. The screws were secured to the 0.2 mm thick skull to minimize cerebral damage and avoid surgical trauma. The dura was pierced with a sharp needle and the cannula was then placed at the appropriate coordinates: 2.9 mm anterior from the interaural line, 0.8 mm lateral to midline, and 2.2 mm ventral to the surface of the brain (Lehman, 1974). By lowering the outer or guide cannula together with the inner or injector cannula in the inserted position, it was possible to ascertain during the actual surgical procedure, whether or not the injector was in the ventricle, since sterile physiological saline would flow in a small volume down the attached tubing (connected to the injector cannula) and into the ventricle, when the cannula was properly implanted. Each implant was secured with acrylic cement applied initially as a thin layer over the screws and to a clean and dry skull. Successive layers of acrylic cement were applied to completely secure the cannula implant. The incision was closed using OO silk surgical suture. A stainless steel stylette was used to keep each cannula patent. All mice received a postoperative intramuscular injection of ethacilin (Rogar STB) at a dose of 0.0066 ml/100 g body weight. A one-week postoperative recovery period, with frequent handling, was permitted before drug tests were initiated, during which time food and water intakes were monitored to ensure adequate recovery from surgery. All ICV injections were administered through a 28-ga. injector cannula inserted into, and protruding 1 mm beyond, the guide cannula. These procedures enabled the cannula assembly to remain patent for periods exceeding one month.

Design and Procedure

Experiment 1. The effects of clonidine hydrochloride (CLON, Sigma), an $\alpha_2$ agonist, and arterenol bitartrate (NE, Sigma), on total energy intake and diet selection, were assessed in

ob/ob and +/? mice. After an initial adaptation of two weeks to the colony room with ad-lib access to stock diet, mice were subsequently adapted to macronutrient diets for three weeks. Following surgery, and prior to the start of actual drug administration, all mice were allowed a one-week recovery period, during which time 24-h macronutrient intakes were measured to ensure that each animal was consuming adequate amounts of each diet (relative to presurgical intakes). Vehicle injections (0.9% physiological saline) were given during postoperative diet adaptation in order to acclimate mice to the test procedure. Mice were maintained and tested under free-feeding conditions with water also available ad lib.

Clonidine (10,20 nmol) dissolved in sterile physiological saline was injected through the implanted ICV cannula in a volume of 2 $\mu$l and infused over 60 s. An additional 60 s was allowed for the solution to diffuse from the cannula and into the cerebrospinal fluid. Drug or vehicle (VEH) injections were administered in counterbalanced order. Because regular and repeated testing on the same set of animals has been found to be important if not essential for obtaining stable baseline food intake scores and reliable drug effects (Leibowitz et al., 1985), mice ($n=7$) received a total of 9 tests (3 drug tests at each dose and 3 saline tests), and all food intake scores represent an average of these test scores.

Mice were initially given fresh diets 1 h before ICV injections to ensure maximal satiation. Injection of CLON or saline was then administered approximately 1 h before the start of the dark period (16h00). Immediately following drug treatment, mice were replaced in the home cage together with preweighed amounts of fresh macronutrient--carbohydrate, fat, and protein. Macronutrient intakes were determined at 1 h and 2 h postinjection. A similar series of testing was conducted with ICV NE (40,80 nmol) using a separate group of cannulated obese and lean

mice (n=7). In addition, CLON and NE were administered to mice (n=5) maintained on rodent chow to assess drug effects on this high-carbohydrate diet. Independent variables included phenotype (obese, lean), drug treatment (dose in nmols), and test interval (1, 2 h postinjection), with dependent measures of total energy intake, carbohydrate, fat, and protein intake measured to the nearest .001 g and converted to kilocalories. Cannula placement was verified by post mortem injection of black ink. Histological examination revealed particles of ink in the cerebral ventricles.

Experiment 2. Obese and lean mice (n=5) were deprived of food for 2 h at the beginning of the dark period (17h00-19h00), and then immediately injected with 5-hydroxytryptamine creatinine sulphate complex (5-HT, Sigma) (35,70,105,140 nmol), dissolved in sterile physiological saline vehicle, to determine the effect of ICV serotonergic stimulation on energy intake and macronutrient preference. Central injections were given through implanted cannulae in a volume of 2 $\mu$l. With the exception of the 2-h food deprivation period, procedures similar to the first experiment were followed. Briefly, mice received 2 drug tests at each dose, administered on separate days, and in counterbalanced order. Macronutrient intakes were assessed at 1 h and 2 h postinjection. The effects of 5-HT were also examined in a separate group of mice (n=5) maintained on rodent chow. Independent variables for this study included phenotype (obese, lean), drug treatment, and test interval (1 h and 2 h postinjection), with dependent measures of total energy intake, carbohydrate, fat, and protein intake, expressed in kilocalories.

Statistical Analysis

Statistical analyses were determined using one- and three-way analyses of variance (ANOVA)

with repeated measures followed by post hoc Tukey tests for individual mean comparisons (Hays, 1981). ANOVA were performed on cumulative caloric intake measures, as well as percent change from saline scores, calculated by subtracting the drug score from the saline score and dividing this difference by the saline score (% saline intake). Because of differential baselines in energy intake, the percent saline intake measure allowed for interphenotypic comparisons. In addition, percent concentration scores which represent the percent of each diet ingested relative to total energy intake (proportion of total intake) were examined.

## Results

### Baseline Energy Intake and Macronutrient Diet Selection

Macronutrient diet selection and food intake scores for 24-h free-feeding ob/ob and lean mice, prior to drug treatment, are shown in Table 1. Obese mice were hyperphagic on both macronutrient, $F(1,36)=5.35$, $p<.03$, and lab chow diets, $F(1,28)=5.73$, $p<.028$, ingesting significantly more kilocalories in comparison to lean mice. Although both obese mice, $F(2,36)=37.58$, $p<.0001$, and lean mice, $F(2,36)=38.61$, $p<.0001$, self-selected a higher proportion (percent concentration) of energy from fat than from protein or carbohydrate sources, ob/ob mice appeared to maximize fat intake, consuming more kilocalories in the form of fat, $F(1,36)=13.26$, $p<.002$, but fewer kilocalories of carbohydrate, $F(1,36)=6.46$, $p<.02$, compared to lean mice. Protein intakes did not differ significantly between phenotypes. Free-feeding obese mice exhibited a marked preference for fat but tended to consume proportionally similar amounts of carbohydrate and protein. Lean mice, although showing a similar but reduced fat preference, consumed more of the carbohydrate diet than the protein ration ($p<.05$). Analysis of macronutrient intake patterns just prior to and immediately following dark onset

Table 1

Mean (±SE) Caloric Intake in 24-h Free-Feeding Obese and Lean Mice

| | | Macronutrient | | | |
|---|---|---|---|---|---|
| | | Carbohydrate | Fat | Protein | Total |
| Obese | kcal | 3.25(0.36)* | 9.64 (0.56)* | 3.54(0.58) | 16.43(0.74)* |
| | % Concentration | 19.8(2.1)* | 59.4(3.6)* | 20.8(3.0) | |
| Lean | kcal | 4.37(0.26) | 7.21(0.36) | 2.90(0.28) | 14.48(0.39) |
| | % Concentration | 30.2(1.7) | 49.9(2.3) | 19.9(1.8) | |
| | | R-M-H 3000 Prolab Diet (kcal) | | | |
| Obese | | | | | 14.84(0.89)* |
| Lean | | | | | 12.52(0.39) |

*$p < .05$ vs. intake in lean mice

(under baseline saline conditions) indicated that lean mice altered their diet selection toward an increase or preference for carbohydrate, and a reduction in the percent concentration of fat and protein ingested, $F(2,26)=19.84$, $p<.0001$. Obese mice showed a similar, but less dramatic, shift in diet preference associated with a modest reduction in fat and protein intake and an increase in the percent concentration of carbohydrate, $F(2,26)=16.16$, $p<.0001$. However, the ob/ob still exhibited a significant preference for fat, while carbohydrate was preferred more than protein. With respect to overall caloric intake, obese mice were hyperphagic at dark onset, eating more calories than lean mice on the lab chow diet, $F(1,18)=5.19$, $p<.03$. Obese mice feeding on macronutrient tended to ingest, overall, more kilocalories than lean mice, although the effect was not statistically significant.

Effects of $\alpha_2$-Noradrenergic Agonists on Food Intake

Table 2 shows the effects of CLON on the intake of rodent chow diet. Again, obese mice were hyperphagic, ingesting significantly more kilocalories of energy than lean mice, irrespective of treatment condition, $F(1,8)=49.11$, $p<.0001$. Clonidine increased feeding and caloric intake in ob/ob mice at both the 1 h and 2 h measure, $F(2,16)=14.92$, $p<.0002$, with the 10 nmol dose eliciting the most dramatic orexigenic response. Although food intake was also increased in lean mice, there was no reliable difference between the two clonidine doses. Analysis of percent change scores, representing the change in magnitude of feeding in comparison to saline values within a phenotype, revealed a significant three-way interaction, $F(2,16)=8.31$, $p<.003$. Food intake was dramatically enhanced 1 h postinjection by over 436% of saline values for ob/ob mice treated with 10 nmol CLON, and by 380% following injection of 20 nmol CLON. Caloric intake was also increased in lean mice but represented an increase of only 229% from

Table 2

Food Intake (kcal) in Obese and Lean Mice Following ICV Injection of Clonidine

| Phenotype | CLON Dose | | 1 h | 2 h |
|---|---|---|---|---|
| Obese | Saline | kcal | 0.17(0.02)‡ | 0.61(0.09)‡ |
| | 10 nmol | kcal | 0.91(0.05)†‡ | 1.32(0.14)†‡ |
| | | % Saline | 436.5(29.7)‡ | 151.6(63.9) |
| | 20 nmol | kcal | 0.81(0.02)†‡ | 1.19(0.11)†‡ |
| | | % Saline | 379.9(34.5)‡ | 128.9(60.8) |
| Lean | Saline | kcal | 0.13(0.01) | 0.40(0.10) |
| | 10 nmol | kcal | 0.39(0.05)† | 0.70(0.05)† |
| | | % Saline | 229.2(70.6) | 124.1(52.6) |
| | 20 nmol | kcal | 0.34(0.06)† | 0.59(0.04)† |
| | | % Saline | 182.3(66.8) | 89.9(44.9) |

†$p < .05$ vs. saline intake of same phenotype
‡$p < .05$ vs. intake in lean mice

saline for mice treated with 10 nmol CLON and 182% following injection of 20 nmol CLON. Although intake analyzed as a percent change from saline remained elevated 2 h postinjection for mice treated with CLON, there were no differences between phenotypes.

Intraventricular injection of NE also increased food intake (Table 3), particularly in obese mice 1 h following drug treatment, $F(2,16)=4.44$, $p<.03$, representing an increase of 439% of saline values for ob/ob mice injected with 40 nmol NE and 384% following 80 nmol NE, $F(2,16)=11.19$, $p<.0009$. Again, ob/ob mice consumed significantly more kilocalories than lean mice regardless of treatment, $F(1,8)=27.20$, $p<.0008$. Caloric intake was also increased in lean mice by approximately 200% of saline injection values. Intake remained elevated for both phenotypes across the 2-h test, but did not differ between phenotype on the 2-h postinjection measure.

Effects of ICV Clonidine on Energy Intake and Diet Selection

As illustrated in Figure 1, intraventricular administration of CLON potentiated feeding and differentially altered macronutrient diet selection. Total energy intake increased in both obese and lean mice, $F(2,24)=4.45$, $p<.02$, representing increases of over 219-267% for obese mice treated with CLON (20 and 10 nmol doses, respectively) and 133-143% for similarly treated lean mice 1 h postinjection, $F(2,24)=5.84$, $p<.008$ (Figure 2). Caloric intake remained elevated on the 2 h measure for both phenotypes in comparison to saline values. Associated with the increase in overall energy intake, which was most evident in obese mice, $F(2,24)=3.57$, $p<.04$, was a significant increase in carbohydrate intake, $F(2,24)=55.45$, $p<.0001$. Clonidine 10 nmol ICV elicited a more robust response in obese mice, in comparison to the 20 nmol dose. Although there was no differential dose-effect in lean mice, both the 10

Table 3

Food Intake (kcal) in Obese and Lean Mice Following ICV Injection of Norepinephrine

| Phenotype | NE Dose | | 1 h | 2 h |
|---|---|---|---|---|
| Obese | Saline | kcal | 0.24(0.09)‡ | 0.69(0.15)‡ |
| | 40 nmol | kcal | 0.90(0.10)†‡ | 1.10(0.16)†‡ |
| | | % Saline | 439.2(129.3)‡ | 139.5(100.4) |
| | 80 nmol | kcal | 0.81(0.09)†‡ | 1.08(0.13)†‡ |
| | | % Saline | 384.6(119.1)‡ | 140.0(105.9) |
| Lean | Saline | kcal | 0.08(0.02) | 0.34(0.04) |
| | 40 nmol | kcal | 0.21(0.04)† | 0.66(0.08)† |
| | | % Saline | 219.6(98.7) | 99.5(28.3) |
| | 80 nmol | kcal | 0.18(0.02)† | 0.55(0.06)† |
| | | % Saline | 171.4(65.4) | 68.7(26.3) |

†$p < .05$ vs. saline intake of same phenotype
‡$p < .05$ vs. intake in lean mice

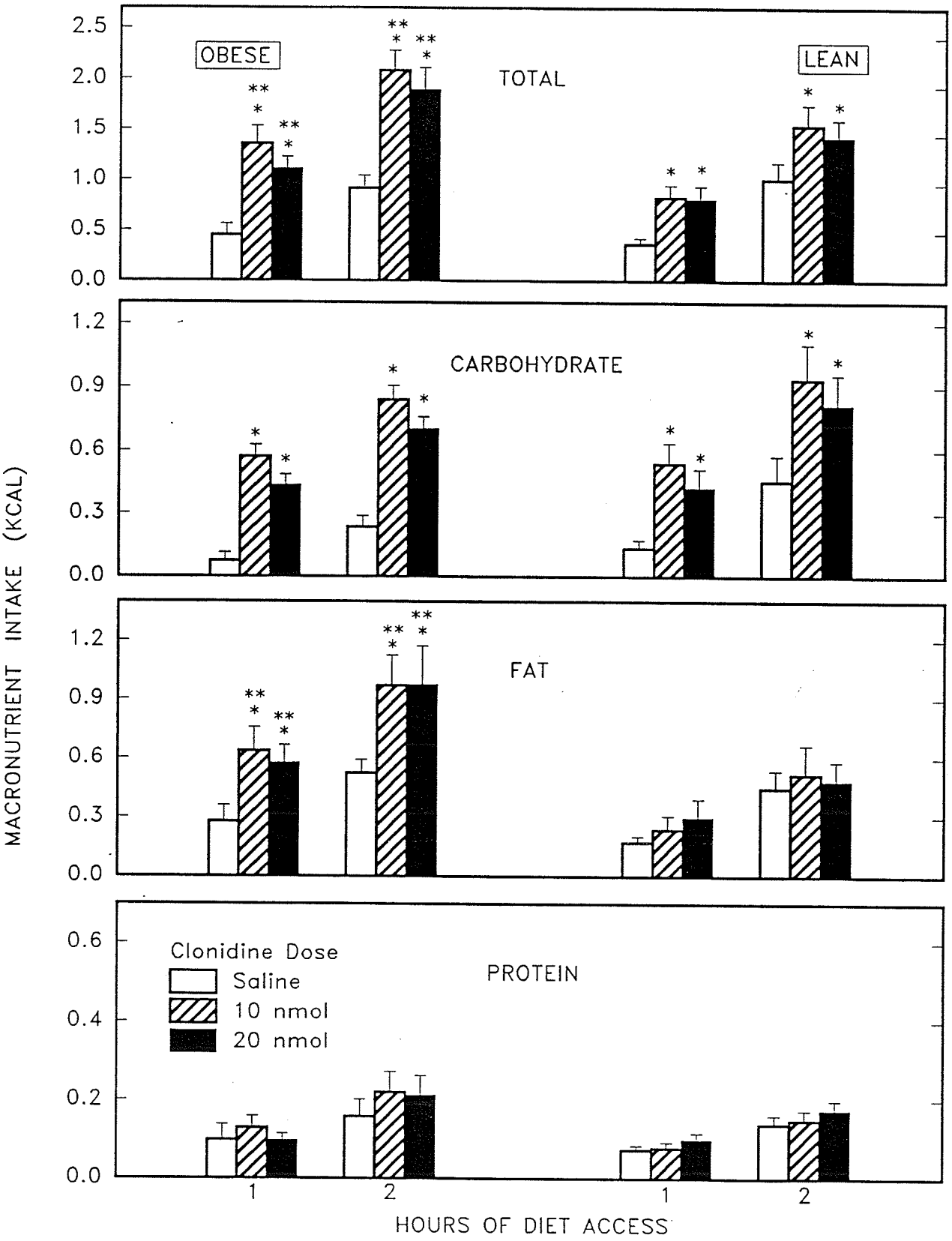


FIG. 1. Effects of central injection of clonidine on energy intake and diet selection in genetically obese and lean mice. Clonidine increased total caloric intake and intake of carbohydrate in both phenotypes. Fat intake was potentiated in obese mice. (*p<.05 for within phenotype comparisons, **p<.05 for between phenotype comparisons).

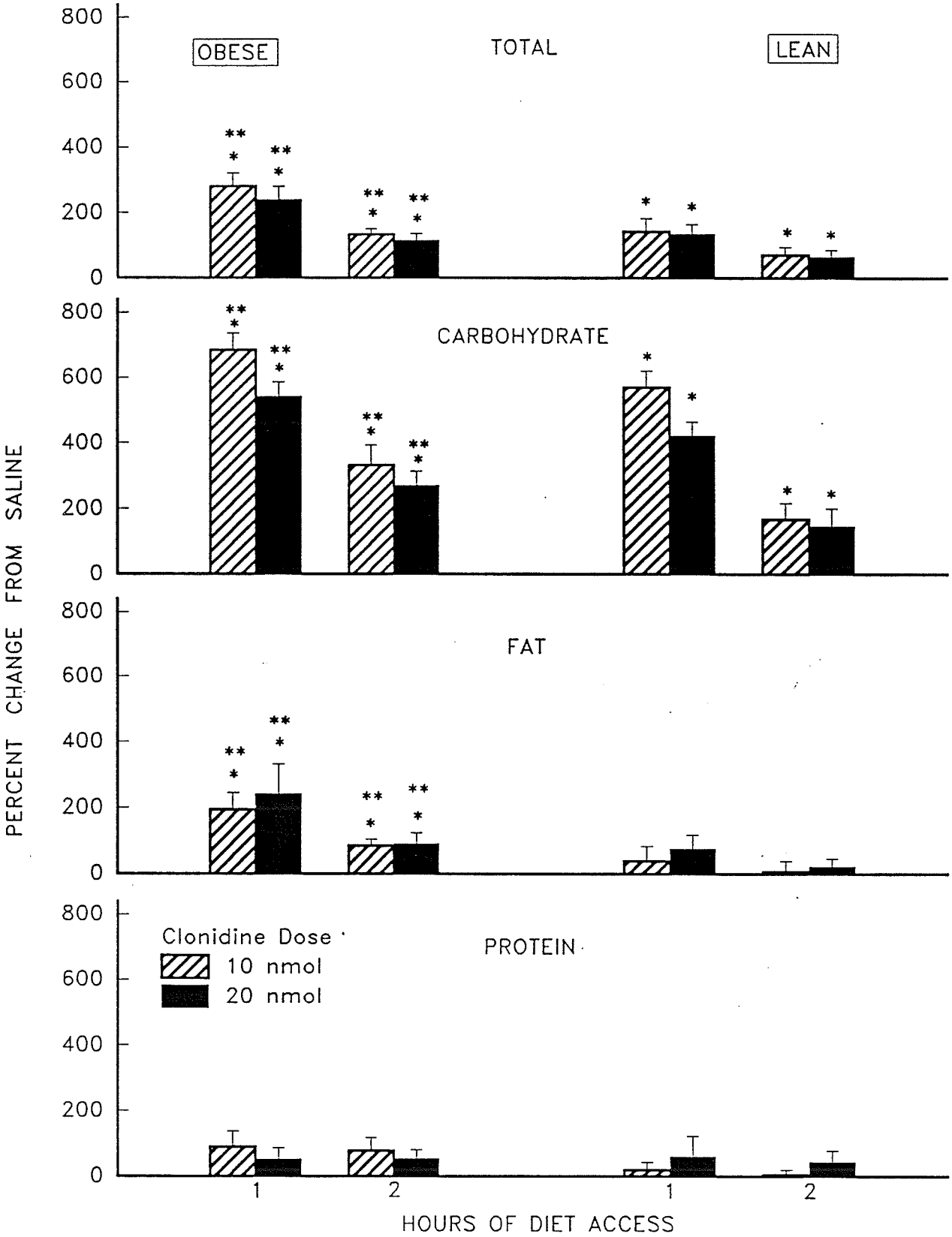


FIG. 2. Change in macronutrient intake after ICV clonidine injection. Clonidine increased total energy intake and ingestion of carbohydrate, particularly in obese mice, although intake was also potentiated in lean mice. Fat intake was enhanced in obese mice only. (*$p<.05$ for within phenotype comparisons, **$p<.05$ for between phenotype comparisons).

nmol and 20 nmol doses significantly increased carbohydrate ingestion. Percent change scores indicated that CLON increased 1-h carbohydrate intake by 572% of saline values in lean mice at the 10 nmol dose, and by over 683% in the ob/ob, $F(2,24)=11.48$, $p<.0003$. In fact, obese mice showed an enhanced response to CLON treatment at all doses and time intervals tested, in comparison to lean mice. Injection of CLON also increased intake of fat in the ob/ob, $F(2,24)=4.06$, $p<.02$, resulting in significant increases when analysed as a percentage of saline intake, $F(2,24)=6.98$, $p<.004$, but did not alter fat intake in lean mice, or protein intake in either phenotype.

The shift in the pattern of nutrient selection toward a substantial increase in preference for the carbohydrate diet after CLON injection resulted in a significant increase in the percent concentration or proportion of carbohydrate ingested relative to the other macronutrients. This carbohydrate preference was found in both obese and lean mice across the entire 2-h test, $F(2,24)=4.88$, $p<.01$ (Table 4). However, ob/ob mice tended to consume, proportionally, less carbohydrate than lean mice, irrespective of treatment condition, $F(1,12)=4.67$, $p<.03$. Although caloric intake of fat was also enhanced by CLON in the ob/ob, the effect was considerably smaller than the potentiation of carbohydrate intake, and consequently, a decline in the proportion of calories ingested from fat was obtained for both phenotypes, $F(2,24)=13.10$, $p<.0001$. Further, because obese mice tended to consume more fat independent of treatment condition, $F(1,12)=4.87$, $p<.03$, the percent concentration of energy derived from fat after CLON treatment was lower in lean mice. Again, CLON had little effect on the caloric intake of protein, resulting in a decrease in the proportion of protein consumed for both phenotypes, $F(2,24)=5.19$, $p<.01$.

Table 4

Percent Concentration of Carbohydrate, Fat, and Protein Ingested Following ICV Injection of Clonidine in Obese and Lean Mice

| Phenotype | CLON Dose | Hours of Diet Access | |
|---|---|---|---|
| | | 1 h | 2 h |
| Obese | | | |
| Carbohydrate | Saline | 21.9(3.8)‡ | 26.1(3.8)‡ |
| | 10 nmol | 44.8(4.1)†‡ | 44.7(4.0)†‡ |
| | 20 nmol | 40.8(4.1)†‡ | 40.0(4.6)†‡ |
| Fat | Saline | 56.4(3.2)‡ | 57.4(3.0)‡ |
| | 10 nmol | 43.7(4.2)†‡ | 44.8(3.4)†‡ |
| | 20 nmol | 49.2(3.5)†‡ | 49.2(3.2)†‡ |
| Protein | Saline | 21.7(4.4) | 16.5(3.4) |
| | 10 nmol | 11.5(1.6)† | 10.5(1.7)† |
| | 20 nmol | 10.0(1.4)† | 10.8(1.5)† |
| Lean | | | |
| Carbohydrate | Saline | 31.1(7.2) | 41.2(6.6) |
| | 10 nmol | 62.9(6.0)† | 60.6(7.4)† |
| | 20 nmol | 52.0(6.1)† | 54.5(5.5)† |
| Fat | Saline | 48.4(4.0) | 44.2(5.2) |
| | 10 nmol | 27.3(6.3)† | 30.5(7.7)† |
| | 20 nmol | 34.5(6.8)† | 32.4(4.7)† |
| Protein | Saline | 20.5(3.9) | 14.6(2.9) |
| | 10 nmol | 9.8(2.3)† | 8.9(3.0)† |
| | 20 nmol | 13.5(2.1)† | 13.1(2.5) |

†$p < .05$ vs. saline control of same phenotype
‡$p < .05$ vs. intake in lean mice

Effects of ICV Norepinephrine on Feeding and Macronutrient Selection

Central injection of norepinephrine also increased feeding and altered nutrient intake patterns in ob/ob and lean mice (Figure 3). Total caloric intake was potentiated in both phenotypes across the 2 h test, $F(2,24)=5.36$, $p<.01$, representing an increase of over 74% and 67% for obese mice treated with 40 and 80 nmol NE respectively and 51-63% for similarly treated lean mice, 1 h postinjection, $F(2,24)=20.42$, $p<.0001$, with slightly smaller increases 2 h postinjection (Figure 4). This increase in total energy intake was again associated with a substantial increase in carbohydrate intake for both phenotypes at 1 h postinjection, $F(2,24)=28.54$, $p<.0001$, resulting in an increase of over 381% for obese mice treated with 40 nmol NE, and 340% for ob/ob mice following 80 nmol NE, relative to saline intakes, $F(2,24)=4.21$, $p<.04$. Intake in lean mice increased by over 175-210%. Carbohydrate intake remained elevated 2 h postinjection; however, there was no significant difference between phenotypes. Injection of NE did not alter caloric intake of fat or protein. (However, obese mice did ingest more calories from protein than lean mice, $F(1,12)=14.11$, $p<.002$).

Norepinephrine also produced a shift in diet selection resulting in an increase in the percent concentration of carbohydrate ingested, $F(2,24)=10.07$, $p<.0007$ (Table 5). Obese mice, however, ingested proportionally less carbohydrate irrespective of treatment condition, $F(1,12)=5.78$, $p<.02$. Although obese mice consumed proportionally more fat than lean mice prior to drug treatment, NE reduced the percent concentration of fat ingested by obese and lean mice, $F(2,24)=9.89$, $p<.0007$, to similar values. The percent concentration of protein was also reduced in both phenotypes, $F(2,24)=6.41$, $p<.005$, an effect that was largely found at 1 h postinjection. Lean mice treated with 40 nmol NE also showed a reduced preference for protein

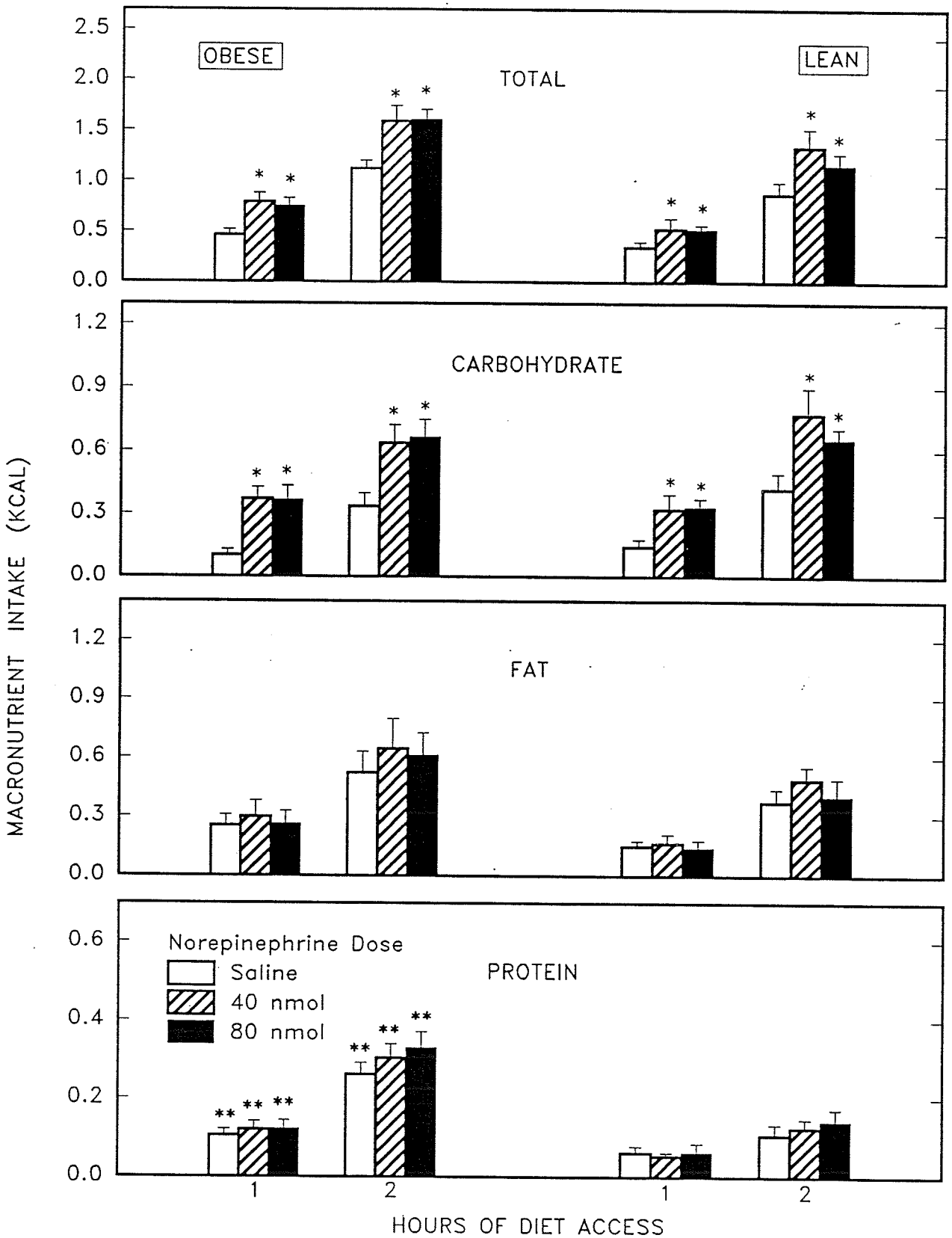


FIG. 3. Effects of intraventricular injection of norepinephrine on energy intake and diet selection in obese and lean mice. Norepinephrine elicited a significant increase in total intake associated with a specific enhancement of carbohydrate ingestion in both phenotypes. (*$p<.05$ within phenotype comparisons, **$p<.05$ between phenotype comparisons).

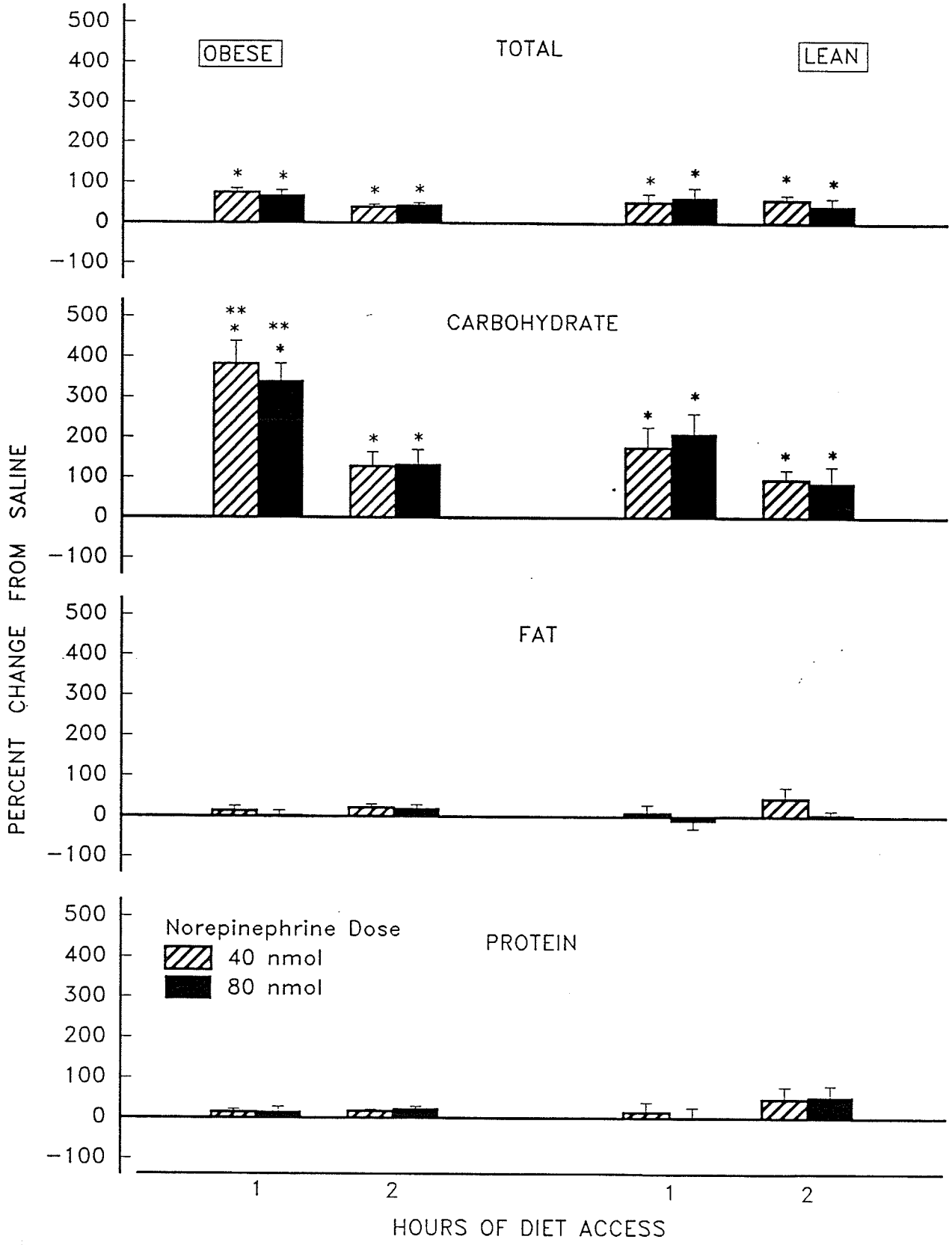


FIG. 4. Change in macronutrient intake after ICV norepinephrine injection. NE increased total energy intake and selectively enhanced carbohydrate ingestion, particularly in obese mice. (*p<.05 for within phenotype comparisons, **p<.05 for between phenotype comparisons).

Table 5

Percent Concentration of Carbohydrate, Fat, and Protein Ingested Following ICV Injection of Norepinephrine in Obese and Lean Mice

| Phenotype | NE Dose | Hours of Diet Access | |
|---|---|---|---|
| | | 1 h | 2 h |
| Obese | | | |
| Carbohydrate | Saline | 24.7(6.9)‡ | 29.8(5.3)‡ |
| | 40 nmol | 49.5(5.0)†‡ | 41.6(4.9)†‡ |
| | 80 nmol | 49.6(6.1)†‡ | 41.8(4.9)†‡ |
| Fat | Saline | 52.2(5.3)‡ | 47.7(4.0)‡ |
| | 40 nmol | 35.1(6.3)† | 38.9(3.9)† |
| | 80 nmol | 34.2(6.8)† | 37.9(4.3)† |
| Protein | Saline | 23.1(2.9) | 22.5(1.2)‡ |
| | 40 nmol | 15.4(2.2)† | 19.5(1.8)‡ |
| | 80 nmol | 16.2(2.4)† | 20.3(1.7)‡ |
| Lean | | | |
| Carbohydrate | Saline | 37.5(5.2) | 45.5(2.9) |
| | 40 nmol | 59.4(4.0)† | 54.9(3.5)† |
| | 80 nmol | 61.9(5.4)† | 55.5(3.5)† |
| Fat | Saline | 243.4(2.9) | 41.6(2.0) |
| | 40 nmol | 29.1(3.1)† | 35.6(2.9)† |
| | 80 nmol | 25.6(5.9)† | 32.0(4.1)† |
| Protein | Saline | 19.1(5.9) | 12.9(3.2) |
| | 40 nmol | 11.5(2.1)† | 9.5(1.5)† |
| | 80 nmol | 12.5(4.5)† | 12.5(2.7) |

†$p < .05$ vs. saline control of same phenotype
‡$p < .05$ vs. intake in lean mice

2 h postinjection. Furthermore, obese mice ingested a greater proportion of energy from protein (2 h postinjection) than lean mice, even after NE treatment.

Effects of Central Injections of 5-Hydroxytryptamine on Food Intake

Table 6 shows the effects of 5-HT on the intake of rodent chow diet following a 2-h fast. Baseline measures under saline conditions indicated that lean mice ingested more kilocalories than obese mice, $F(1,8)=29.29$, $p<.0006$. Central injection of 5-HT, 70-140 nmol, however, reduced food intake in a dose-dependent manner in obese and lean mice, $F(4,32)=7.77$, $p<.0002$. Intake in lean mice was significantly lower than caloric intake in ob/ob mice, suggesting that obese mice were less affected by 5-HT's anorectic action. In fact, analysis of percent change scores indicated that food intake in lean mice, following injection of 5-HT, was significantly lower compared to ob/ob mice, at each dose and time interval tested, $F(4,32)=12.65$, $p<.0001$, with the exception of the lowest 5-HT dose, 35 nmol ICV, which did not significantly alter food intake in either phenotype.

Effects of ICV 5-Hydroxytryptamine on Feeding and Diet Selection

Intraventricular injection of 5-HT (70-140 nmol) also suppressed feeding and altered macronutrient intake in obese and lean mice (Figure 5). Total energy intake, which did not differ significantly between phenotypes prior to drug treatment, decreased in a dose-dependent manner, $F(4,32)=11.58$, $p<.0001$, although treatment with 35 nmol 5-HT failed to significantly alter energy intake. The most effective anorectic dose, 140 nmol 5-HT, suppressed intakes by 43% in obese mice and by over 50% in lean mice across the 2-h test, $F(4,32)=163.40$, $p.<0001$ (Figure 6). 5-HT also reduced carbohydrate intake in both phenotypes over the 2-h postinjection period. The reduction in intake was again dose-dependent, $F(4,32)=3.66$, $p<.01$.

Table 6

Food Intake (kcal) in Obese and Lean Mice Following ICV Injection of 5-Hydroxytryptamine

| Phenotype | 5-HT Dose | | 1 h | 2 h |
|---|---|---|---|---|
| Obese | Saline | kcal | 2.36(0.09)‡ | 3.76(0.07)‡ |
| | 35 nmol | kcal | 2.32(0.11)‡ | 3.80(0.05)‡ |
| | | % Saline | -1.6(0.6) | 1.0(1.7) |
| | 70 nmol | kcal | 1.91(0.05)†‡ | 3.48(0.01)†‡ |
| | | % Saline | -18.4(4.2)‡ | -7.4(1.5)‡ |
| | 105 nmol | kcal | 1.81(0.02)†‡ | 2.56(0.04)†‡ |
| | | % Saline | -22.6(3.7)‡ | -31.9(1.5)‡ |
| | 140 nmol | kcal | 1.40(0.03)†‡ | 1.80((0.13)†‡ |
| | | % Saline | -40.3(2.4)‡ | -51.7(4.3)‡ |
| Lean | Saline | kcal | 2.54(0.06) | 4.36(0.08) |
| | 35 nmol | kcal | 2.59(0.14) | 4.24(0.14) |
| | | % Saline | 1.8(5.7) | -2.8(2.1) |
| | 70 nmol | kcal | 1.71(0.10)† | 3.04(0.13)† |
| | | % Saline | -32.7(6.0) | -30.0(3.5) |
| | 105 nmol | kcal | 1.40(0.06)† | 2.42(0.05)† |
| | | % Saline | -44.8(2.2) | -44.5(2.6) |
| | 140 nmol | kcal | 0.67(0.02)† | 1.57(0.06)† |
| | | % Saline | -73.5(0.6) | -63.2(2.8) |

†$p < .05$ vs. saline intake of same phenotype
‡$p < .05$ vs. intake in lean mice

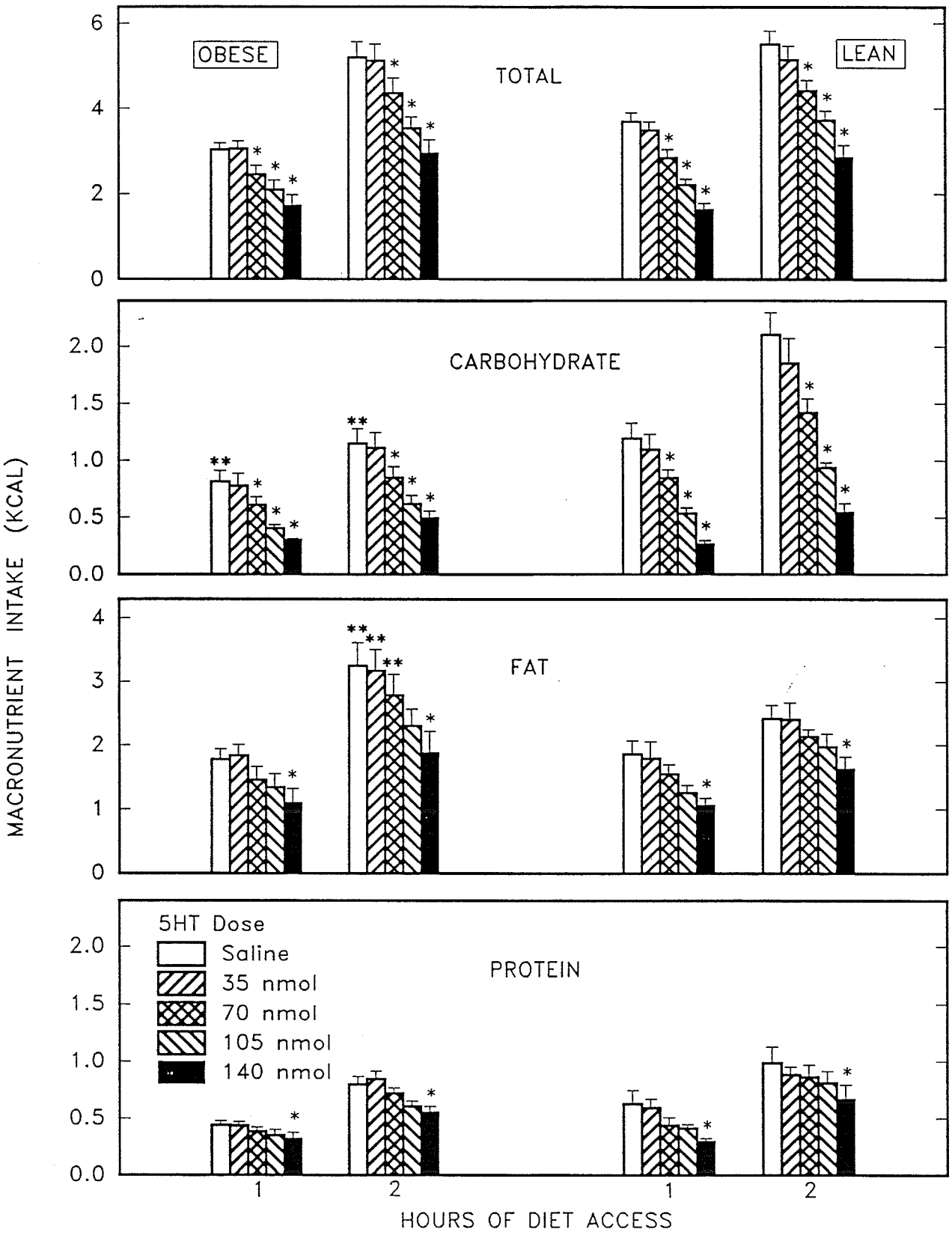


FIG. 5. Effects of ICV 5-HT on macronutrient intake in obese and lean mice. 5-HT (70-105 nmol) reduced total energy intake associated with a preferential decrease in carbohydrate intake. Injection of 140 nmol 5-HT reduced total intake and intake of carbohydrate, protein, and fat. (*p<.05 within phenotype comparisons. **p<.05 between phenotype comparisons).

Although saline intake scores for carbohydrate were lower for obese than lean mice, indicating that ob/ob mice ingested fewer kilocalories of carbohydrate following a brief deprivation period of 2 h, 140 nmol of 5-HT decreased carbohydrate intake in lean mice to the extent that intakes no longer differed significantly between phenotypes. When intake of this macronutrient was analysed as a percentage of saline intake, suppression of carbohydrate intake appeared to be more pronounced in lean mice, particularly at the higher 5-HT doses tested, $F(4,32)=2.53$, $p<.04$. Measures of caloric intake under saline conditions indicated that obese mice ingested more fat than lean mice, $F(1,8)=12.05$, $p<.008$. However, fat intake was only suppressed after injection of 140 nmol 5-HT, $F(4,32)=5.80$, $p<.001$. Percent change scores also showed a reduction in fat following injection of 140 nmol 5-HT compared to saline values, with no interphenotypic differences, $F(4,32)=37.49$, $p<.0001$. Similarly, 140 nmol 5-HT also decreased protein intake in obese and lean mice, $F(4,32)=3.34$, $p<.02$, representing a significant decrease when analysed as a percent change from saline, with no effect for phenotype, $F(4,32)=32.40$, $p<.0001$.

Central injection of 5-HT also resulted in a change in the percent concentration of carbohydrate, fat, and protein ingested relative to total energy intake (Table 7). The percent concentration of carbohydrate (although higher in lean mice prior to drug treatment), decreased in both phenotypes following 5-HT injection, $F(4,32)=4.60$, $p<.004$. Proportional intake of carbohydrate decreased in lean mice after injection of 70-140 nmol 5-HT. In obese mice, however, percent concentration of carbohydrate was reduced only after injection of 105-140 nmol 5-HT. The percent energy derived from fat increased in lean mice after 5-HT (105-140 nmol) injection, but remained relatively unaltered in the ob/ob (although ob/ob mice did

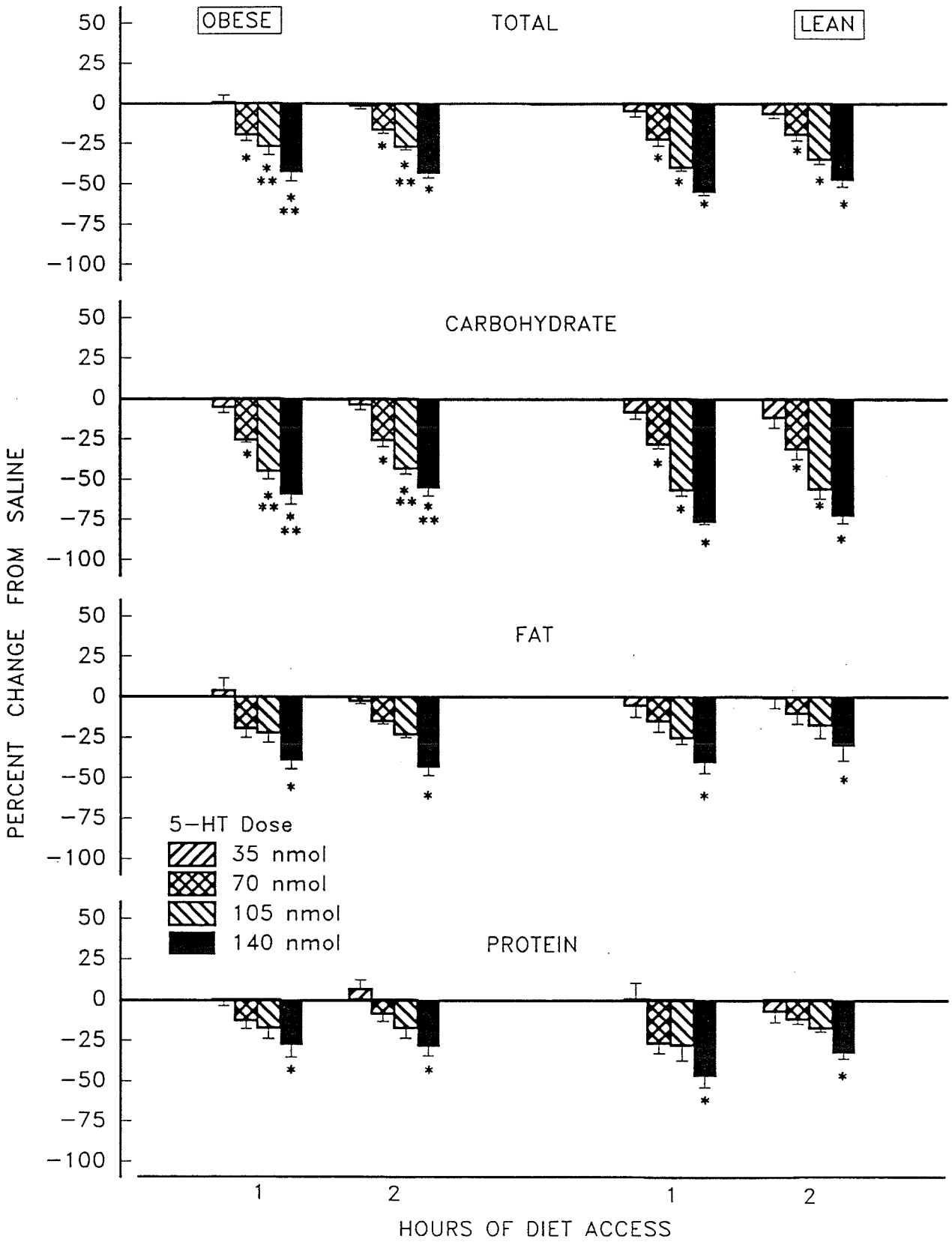


FIG. 6. Change in macronutrient intake after ICV 5−hydroxytryptamine injection. 5−HT (70−140 nmol) reduced total energy and carbohydrate intake in both phenotypes, although lean mice were more sensitive to 5−HT's anorectic effect. Injection of 140 nmol 5−HT also suppressed fat and protein intake. (*$p<.05$ within phenotype, **$p<.05$ between phenotype).

Table 7

Percent Concentration of Carbohydrate, Fat, and Protein Ingested Following ICV Injection of 5-Hydroxytryptamine in Obese and Lean Mice

| Phenotype | Macronutrient | | | | | |
|---|---|---|---|---|---|---|
| 5-HT Dose | Carbohydrate | | Fat | | Protein | |
| | 1 h | 2 h | 1 h | 2 h | 1 h | 2 h |
| Obese | | | | | | |
| Saline | 27.0‡ (2.2) | 22.2‡ (1.9) | 58.2‡ (2.8) | 61.7‡ (3.2) | 14.8 (1.8) | 16.1 (2.5) |
| 35 nmol | 25.4 (3.3) | 21.6‡ (2.0) | 59.7‡ (2.8) | 61.2‡ (2.9) | 14.9 (2.0) | 17.2 (2.1) |
| 70 nmol | 25.6 (3.8) | 19.6‡ (1.8) | 58.4‡ (3.4) | 63.0‡ (2.8) | 16.0 (1.7) | 17.4 (2.1) |
| 105 nmol | 20.2† (2.4) | 17.7†‡ (2.1) | 62.7‡ (3.1) | 64.5‡ (3.0) | 17.1 (2.2) | 17.8 (1.9) |
| 140 nmol | 18.8† (2.3) | 18.1† (3.0) | 62.3‡ (3.8) | 61.8‡ (5.2) | 18.9† (2.6) | 20.1† (2.7) |
| Lean | | | | | | |
| Saline | 33.9 (2.3) | 38.0 (2.1) | 50.7 (5.0) | 44.3 (3.7) | 15.4 (1.7) | 17.7 (2.0) |
| 35 nmol | 31.9 (4.4) | 35.9 (3.2) | 50.9 (46.7) | 46.8 (3.8) | 17.2 (2.4) | 17.3 (1.2) |
| 70 nmol | 28.8† (1.3) | 30.9† (1.3) | 55.7 (3.5) | 49.7 (2.5) | 15.5 (2.3) | 19.4 (1.7) |
| 105 nmol | 24.4† (2.1) | 25.4† (1.9) | 56.8† (2.7) | 52.7† (2.4) | 18.8† (1.0) | 21.9† (1.8) |
| 140 nmol | 16.7† (1.2) | 19.5† (2.6) | 64.9† (2.1) | 56.9† (1.5) | 18.4† (1.0) | 23.6† (1.4) |

†$p < .05$ vs. saline control of same phenotype
‡$p < .05$ vs. intake in lean mice

consume proportionally more fat prior to drug treatment), $F(4,32)=4.36$, $p<.006$. Higher doses of 5-HT also increased the percent concentration of protein ingested in lean (105-140 nmol) and obese (140 nmol only) mice, $F(4,32)=17.00$, $p<.0001$. The increases in percent energy from fat and protein, therefore, appeared to result from the reduction in carbohydrate intake in both phenotypes.

## Discussion

These results provide support for the role of NE and 5-HT in the control of feeding and macronutrient-specific appetite in genetically obese and lean mice. Intraventricular injection of CLON and NE increased feeding associated with a dramatic and selective enhancement of carbohydrate ingestion, and a decrease in the proportional intake of fat and protein. The opposite effect was found after 5-HT injection. 5-HT suppressed carbohydrate intake while sparing or even enhancing the percent energy selected as fat and protein. CLON and NE also potentiated intake of rodent chow, a high carbohydrate diet; whereas, injection of 5-HT suppressed chow intake in both phenotypes. Obese mice, however, exhibited an altered sensitivity to both noradrenergic and serotonergic stimulation in comparison to lean mice. The change in carbohydrate intake was significantly greater in the ob/ob after ICV injection of CLON and NE but reduced after injection of 5-HT. While these findings are consistent with previous reports which have shown that systemic CLON injection potentiates feeding, particularly of carbohydrate, in meal-feeding ob/ob and lean mice (Currie & Wilson, 1991a; Currie & Wilson, 1989), the present study is the first to demonstrate that centrally administered neurotransmitter or receptor-selective agonists can evoke changes in energy intake and diet selection in free-feeding ob/ob and lean mice. Previous research has also shown that

intraperitoneal injection of $\alpha_2$ receptor antagonists attenuates food intake in meal-feeding mice (Callahan et al., 1984; Currie & Wilson, 1989). Again this anorectic effect is associated with a reduction in carbohydrate intake. In addition, pretreatment with the $\alpha_2$ antagonist yohimbine can eliminate CLON's robust and selective increase in carbohydrate intake (Currie & Wilson, 1991b; Currie & Wilson, 1990). Taken together, these observations suggest that CLON and NE potentiate feeding through selective interaction with the $\alpha_2$ receptor. Further, the increase in feeding only occurs after injection of relatively low doses of CLON, a condition also favouring selective interaction with the $\alpha_2$ receptor subtype.

The increased sensitivity of the ob/ob to systemic CLON treatment could result from various factors associated with a peripheral route of drug administration including a greater absolute amount of drug administered on a mg/kg basis, altered storage and release of CLON from enlarged white fat depots, or impaired drug clearance in comparison to lean mice. However, the present study shows that ob/ob mice are still more sensitive to the orexigenic effects of CLON when this $\alpha_2$ agonist is administered centrally. Several factors may in fact contribute to the heightened sensitivity of the ob/ob to $\alpha_2$-noradrenergic stimulation. Although hypothalamic $\alpha_2$ receptors, particularly in the PVN, are up-regulated by corticosterone and down-regulated following adrenalectomy in the rat (Jhanwar-Uniyal & Leibowitz, 1986), the efficacy of NE to increase total caloric intake and, more specifically, carbohydrate intake, through an $\alpha_2$-noradrenergic mechanism, is dependent on intact adrenocortical function (Leibowitz, Roland, Hor, Squillari, 1984). The ob/ob mouse has elevated corticosterone levels across the 24-h day (Saito, Shimomura, & Bray, 1983), and displays enhanced sensitivity to glucocorticoid-induced food intake (McGinnis, Walker, & Margules, 1987). Chronically elevated plasma corticosterone

levels, which appear as early as 17 days postpartum in the ob/ob (Dubuc, 1977), could, therefore, permit enhanced $\alpha$ receptor number, affinity, or both, to determine the ob/ob's response to low doses of CLON (Currie & Wilson, 1991a). Therefore, interphenotypic differences in corticosterone may contribute to the increased sensitivity of the ob/ob to the stimulating effects of CLON on food intake and diet selection. Further, the ob/ob has 58% more hypothalamic $\alpha_1$ receptors than does its lean control (Oltmans et al., 1981) determined by the binding of [$^3$H]-WB-4101, a highly selective $\alpha_1$ antagonist. Although comparable binding assays for $\alpha_2$ receptors have failed to identify any differences in $\alpha_2$ receptor density or affinity between obese and lean mice (Callahan et al., 1984), both $\alpha_1$ and $\alpha_2$ densities were determined in pooled samples, following unspecified feeding regimens or age groups, which may have masked significant intrahypothalamic differences between groups. In another model of genetic obesity, the fa/fa rat, both $\alpha_1$ and $\alpha_2$ receptor density is elevated, principally in the PVN, compared to lean controls (Finkelstein et al., 1988), which suggests that differences in $\alpha_2$ receptor density may, in fact, exist between obese and lean mice. Further, while hypothalamic $\alpha_1$ receptor density is increased in the ob/ob, injection of the $\alpha_1$ agonist phenlyephrine has little effect on feeding (Currie & Wilson, unpublished data), again suggesting a specific behavioural effect associated with $\alpha_2$-noradrenergic receptor function.

There is also evidence to suggest that circulating glucose, in addition to corticosterone, may be involved in modulating receptor activity in the medial hypothalamus, and may in turn influence feeding behaviour in the ob/ob. The concentration of $\alpha_2$ receptors in the PVN is highly positively correlated with circulating glucose (Jhanwar-Uniyal et al., 1988), and the link between glucose and $\alpha_2$-noradrenergic receptor mechanisms is consistent with the findings that

several types of physiologically-induced increases in blood glucose are blocked by $\alpha$-receptor antagonists (Leibowitz et al., 1988). Moreover, reduced levels of glucose in the brain have been shown to potentiate hypothalamic NE activity (Smythe et al., 1984), which in turn increases levels of circulating blood glucose (Chafetz, Parko, Diaz, & Leibowitz, 1986), and medial hypothalamic administration of glucose, similar to gastric carbohydrate loads, suppresses the release of endogenous NE, specifically in the PVN (Myers & McCaleb, 1980). It has also been demonstrated that blood glucose declines naturally just prior to meal initiation in spontaneously feeding rats (Leibowitz, 1986). These findings, therefore, suggest a close association between $\alpha_2$-noradrenergic activity in the PVN, circulating glucose levels, and feeding behaviour. Although fewer studies have focused on the pancreatic hormone, insulin, in relation to the hypothalamic $\alpha_2$-noradrenergic feeding system, there is some evidence to suggest that insulin may act synergistically with NE in stimulating feeding in the rat. Insulin is known to preferentially enhance carbohydrate intake (Kanarek, Marks-Kaufman, & Lipeles, 1980), as well as alter the release of medial hypothalamic NE (McCaleb, Myers, Singer, Willis, 1979). Loss of neuronal innervation to the pancreas through vagotomy significantly alters the effect of NE in the PVN on feeding (Sawchenko, Gold, & Leibowitz, 1981). In the ob/ob, alterations in energy intake may be directly or indirectly related to this mutant's hyperglycemic and hyperinsulinemic state. Although the nature of the relationship between the increased levels of ciculating glucose and insulin to the hypothalamic $\alpha_2$-noradrenergic feeding system of the ob/ob remains to be determined, it is possible that increases in circulating glucose and insulin have an exaggerated impact on PVN $\alpha_2$ receptors and/or NE neurotransmission producing an alteration in feeding behaviour.

In addition to the increased sensitivity of the ob/ob to noradrenergic stimulation, in the current study, this mutant also demonstrated a reduced sensitivity to the anorectic effects of 5-HT. While moderate doses of 5-HT selectively decreased carbohydrate consumption in ob/ob and lean mice, intake of this macronutrient was decreased significantly more in lean mice. Other studies have shown that 5-HT is effective in reducing carbohydrate ingestion in the rat, independent of whether baseline carbohydrate intake scores are high or low, and of whether the other macronutrients, protein or fat, are more or less preferred relative to carbohydrate (Leibowitz et al., 1989; Leibowitz et al., 1987; Shor-Posner et al., 1986). This argues for behavioural selectivity in the action of PVN 5-HT, particularly in light of the finding that preference for protein is potentiated by 5-HT injection (Leibowitz et al., 1989). In the ob/ob, the reduced sensitivity to central 5-HT's anorectic action on carbohydrate may be associated with the increased levels of CNS 5-HT (Garthwaite et al., 1980), and/or reduced hypothalamic metabolism of this indoleamine (Lorden et al., 1986), or possibly result from 5-HT's interaction with endogenous peptides, including amino acids, glucose, and hormones (e.g., corticosterone). Given the apparent interactive role of NE and 5-HT on energy intake, and more specifically on carbohydrate ingestion in the rat, the ob/ob's heightened sensitivity to $\alpha_2$-noradrenergic effects on feeding may be directly related to this mutant's reduced sensitivity to 5-HT's anorectic action. This could involve a number of neurochemical mechanisms, including abnormal release properties of the presynaptic neuron, modified storage, altered reuptake, or enhanced postsynaptic receptor activity, all of which could be influenced by endogenous glucocorticoids, opioid peptides, glucose, and insulin, which display abnormal functioning in the ob/ob (Bray & York, 1979; Govoni & Yang, 1981; Margules, Moisset, Lewis, Shibuya, & Pert, 1978;

Sclafani, 1984; Storlien, 1984).

The possibility that PVN 5-HT acts, in part, by opposing the action of endogenous NE at $\alpha_2$-noradrenergic receptors has received support from various neurochemical, pharmacological, and behavioural studies (Leibowitz et al., 1988; Leibowitz et al., 1987). In particular, there is now extensive evidence revealing a specific role for NE in stimulating the first carbohydrate-rich meal of the dark cycle in the rat. The pattern of effects revealed by PVN NE injection are generally opposite to those produced by 5-HT. In vitro studies have also shown direct antagonism between 5-HT and $\alpha_2$-noradrenergic receptor function in the rat hypothalamus (Galzin, Moret, & Langer, 1984), and lesion studies have demonstrated that the medial hypothalamus has an important function in balancing the proportion of carbohydrate and protein in the diet, and in temporal feeding patterns across the light-dark cycle (Sclafani & Aravich, 1983; Shor-Posner et al., 1985). Again, these studies also demonstrate that 5-HT in conjunction with NE provides a neurochemcial substrate for this control. The present study suggests that endogenous NE and 5-HT may also function to control carbohydrate intake in mice. Stimulation of $\alpha_2$-noradrenergic receptors promotes carbohydrate ingestion; whereas, serotonergic neurotransmission inhibits intake of this nutrient. Systematic investigations of brain noradrenergic and serotonergic neurotransmission in ob/ob and lean mice are therefore warranted in order to define the precise nature of these effects including anatomical localization and physiological function. For example, the administration of an $\alpha_2$-receptor antagonist, prior to central injection of CLON or NE, should attenuate the CLON-induced potentiation of carbohydrate intake shown in the current study, if this effect is $\alpha_2$-receptor mediated. Similarly, injection of 5-HT antagonists should block the anorectic effects of 5-HT. In addition, if CLON,

NE, and 5-HT are acting within the hypothalamus to elicit changes in food intake, then electrolytic or neurotoxin lesion of the medial hypothalamus should eliminate these effects.

Several studies have attempted to examine the relationship of the elevated hypothalamic NE content of the ob/ob to alterations in food intake (i.e., hyperphagia) and obesity. Although central catecholamine depletion resulting from neurotoxin lesion produces only a transient weight loss followed by recovery and normal weight gain in the ob/ob (Lorden, 1979), other studies have shown alterations in feeding behaviour associated with a change in NE synthesis and in hypothalamic NE content in this mutant (Batt, Wilson, & Topping, 1978; Kuprys & Oltmans, 1982; Oltmans et al., 1980). Reserpine treatment, for example, reduces food intake in the ob/ob associated with a reduction in the hypothalamic NE content (Oltmans et al., 1980), although obese mice exhibit an incomplete or delayed catecholamine depletion compared to lean mice. The differential sensitvity of the ob/ob, may, therefore reflect modified characteristics of the storage vesicle and could have important implications for a number of neuronal functions including transmitter storage and release. Amphetamine treatment also produces a dose-dependent decrease in food intake in the ob/ob, and is associated with a reduction in hypothalamic NE levels (Kuprys & Oltmans, 1982). Further, the elevated hypothalamic NE levels of the ob/ob do not appear to be a secondary consequence of obesity (Oltmans, Lorden, & Margules, 1976). These findings are largely based on earlier reports, which have typically employed a peripheral route of drug administration, or monitored food intake in restricted feeding paradigms, or focused exclusively on body weight gain/loss without measuring caloric intake. Although they should be interpreted cautiously, they do provide some insight concerning the nature of the neurophysiological defect in the ob/ob in relation to feeding behaviour.

Bilateral electrolytic lesion of the ventromedial hypothalamus has been shown to increase body weight in lean mice associated with an increase in the percentage of body fat (Chlouverakis, Bernardis, & Hojnicki, 1973). Although obese mice fail to show an increase in body weight in response to medial hypothalamic lesion, small increases in body fat are evident. While the cause of this deficient response to VMH damage is uncertain, it is possible that the maximum storage capacity of adipocytes is a limiting factor (i.e., the capacity of the adipocytes to store fat is near its limit and expansion beyond this limit cannot occur). Obese mice are capable of expanding both the number and size of their adipocytes as obesity develops, however, the obesity induced by VMH lesion is due entirely to adipose cell enlargement (Johnson & Hirsch, 1972). Because damage to the VMH can result in a modest increase in body fat in the ob/ob, it is argued that the medial hypothalamus, prior to lesioning, is functional, and that homeostatic mechanisms involved in the regulation of body fat in the ob/ob are operative, but that this regulation is performed at a higher level of body fat (Chlouverakis, 1972). The results of the present study also suggest that the medial hypothalamus remains functionally intact in the control of macronutrient-specific appetite, although the ob/ob does exhibit differential sensitivity to neuropharmacological treatment.

Obese mice, compared to lean mice, typically consume a greater proportion of daily caloric intake from fat when given simultaneous access to carbohydrate, fat, and protein sources (Currie & Wilson, 1991; Romsos & Ferguson, 1982), a finding also demonstrated in the present study. Free-feeding lean mice, although showing a similar but reduced fat preference, consume more carbohydrate than protein. However, recent studies in other rodent species have shown that macronutrient preference varies as a function of time across the nocturnal cycle. For example,

at the onset of the dark period, the rat exhibits a burst of eating associated with a strong preference for carbohydrate. This carbohydrate preference is subsequently replaced later in the nocturnal cycle by an increased preference for protein and fat, and a reduction in carbohydrate (Leibowitz at al., 1989; Tempel et al., 1989). As indicated in the present study, analysis of macronutrient intake patterns in genetically lean mice, at dark onset, show a similar shift in nutrient selection toward an enhanced preference for carbohydrate and a reduction in fat and protein. Obese mice also exhibit an increased preference for carbohydrate, and a reduction in fat and protein, although fat remains the diet of choice. While the enhanced fat preference appears to account for the increase in overall energy intake in the ob/ob, the proportional increase in carbohydrate intake and the subsequent reduction in protein intake at dark onset are characteristic of the feeding pattern of the nocturnal rodent. Because of a strong preference for fat, however, the percent energy derived from carbohydrate is lower in the ob/ob compared to lean mice. Recent studies have shown that opiate receptors acting on multiple hypothalamic sites, including the PVN, potentiate feeding associated with a selective enhancement of fat (Marks-Kaufman, 1982; Shor-Posner, Azar, Filart, Tempel, & Leibowitz, 1986). In the ob/ob, opiate receptor antagonism reduces feeding (Margules et al., 1978), particularly of fat (Gilson & Wilson, 1989). Also, opioid peptides may act in association with PVN NE and 5-HT to control appetite for specific macronutrient (Leibowitz, 1986; Leibowitz, 1985). However, future research must attempt to identify the potential interaction among medial hypothalamic NE, 5-HT, and opioid peptides in appetite regulation, which may be particularly complex in the ob/ob, given this mutant's profoundly altered neurochemical profile.

Although the results of the current study are consistent with a role for $\alpha_2$-noradrenergic and

serotonergic receptors in the control of feeding and macronutrient-specific appetite in obese and lean mice, obese mice exhibited a heightened sensitivity to the orexigenic effects of CLON and NE but diminished behavioural response to 5-HT's anorectic action. This is consistent with previous reports of impaired satiety in the ob/ob (Coleman, 1973; Strohmayer & Smith, 1979). For example, previous research has shown that obese mice exhibit abnormal feeding and postprandial behavioural responses to food deprivation, particularly in the dynamic phase of development (Strohmayer & Smith, 1979). In the present study, although free-feeding obese mice were hyperphagic on the chow diet, mildly food-deprived obese mice subsequently ingested fewer kilocalories of this high carbohydrate stock diet than did food-deprived lean mice. Similarly, obese mice ingested fewer kilocalories from the single-energy source carbohydrate diet than lean mice following a brief deprivation period, although intake of this macronutrient did not differ significantly between phenotypes under free-feeding conditions. In addition, more recent research provides evidence of a behavioural deficit after carbohydrate ingestion in the obese Zucker rat. Unlike normal rats, obese rats fail to adjust short-term energy and carbohydrate intake in response to a carbohydrate load given intragastrically (van Zeggeren & Li, 1990). While the response of the ob/ob to a similar carbohydrate preload is not known, the results of this study suggest that altered food intake and selection behaviour of the ob/ob may also be due, in part, to a delay in response to the physiologic and metabolic changes arising from macronutrient ingestion.

In summary, the genetically obese mouse exhibits alterations in feeding behaviour and abnormal macronutrient preference. However, both obese and lean mice exhibit a shift in nutrient selection, at the onset of the dark period, toward an increased preference for

carbohydrate and a reduction in fat and protein. At this time, central injection of CLON and NE potentiate an already enhanced preference for carbohydrate; whereas 5-HT suppresses ingestion of this macronutrient. While these results suggest that NE and 5-HT may function in the regulation of carbohydrate intake in mice, obese mice, however, showed a differential sensitivity to 5-HT and NE-induced changes in diet selection. Although the mechanisms underlying the ob/ob's altered response to neuropharmacological treatment remain to be determined, the altered neurochemcial profile of this mutant, including increased PVN-NE, elevated corticosterone, circulating glucose, and insulin may be contributing factors. Future research, therefore, should be directed at examining the variations in the diurnal pattern of macronutrient ingestion in obese and lean mice, in relation to neurotransmitter function. In light of the results obtained in the present study, additional systematic investigation of brain NE and 5-HT neurotransmission would appear warranted in order to identify the anatomical localization and physiological function of these effects, and to identify the neural mechanisms implicated in the control of diet selection in mice under normal and pathological conditions.

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