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Adrenal Involvement in Scrapie-Induced Obesity[1] (42774)

YONG S. KIM, RICHARD I. CARP, SHARON M. CALLAHAN, AND HENRYK M. WISNIEWSKI

New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York 10314

Abstract. In previous studies we found an increase in body weight during the preclinical phase of disease in certain scrapie strain–mouse strain combinations. The effect was augmented by injection into the hypothalamus. In the present study, we found an increase in food consumption (compared to the normal mouse brain injection group) for both the 139A and the ME7 scrapie groups, although only the ME7 group showed an increase in body weight. In a scrapie strain–mouse strain combination that showed an increase in body weight, the adrenal gland was the only organ that showed a significant increase in weight. The titer of scrapie in the adrenals was comparatively low. Adrenalectomy prevented the increase in body weight in two strains of mice injected with the ME7 scrapie strain. The results suggest that scrapie-induced obesity depends on an effect of scrapie on the hypothalamic–pituitary–adrenal axis.

Scrapie is the archetype of the unconventional slow infection diseases (1, 2). The causative agent has been passaged experimentally in various small animals, including mice (3, 4). In previous studies, we showed a significant increase in body weight during the preclinical phase of scrapie disease in certain combinations of scrapie strain and mouse strain (5, 6). This finding, together with the appearance of an altered glucose tolerance in many of these obese mice (7, 8), suggests that this system has value as a model of obesity/diabetes syndromes in humans.

In previous studies, we showed that C57BL mice injected with ME7 by either the routine intracerebral (ic) route or a stereotaxic method into the cortex failed to show an increase in body weight, whereas mice injected into the hypothalamus did show a weight increase (9). In groups of SJL mice injected with ME7, there was an increase in weight in both the cortex and the hypothalamus injection groups (compared to mice injected with normal mouse brain); however, the increase started earlier and reached significantly greater levels in the group injected in the hypothalamus. If we assume that the initial effect of scrapie agent vis-à-vis the weight increase is on the hypothalamus, then there are a variety of mechanisms that could bring about the end result, obesity. One possible mechanism is through the satiety or food consumption centers and another is through an effector gland such as the adrenals. In the present study we analyzed food and water consumption and organ weights of mice from scrapie strain–mouse strain combinations that showed an increase in body weight and from combinations that did not show an increase. The effect of adrenalectomy on the weight increase was analyzed and the scrapie agent titer of the adrenals was determined.

Materials and Methods. *Mice.* Female, weanling SJL/J and C57BL/6J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Mice were given food and either water or NaCl (9 mg/ml) *ad libitum.* All mice were housed in rooms in which the temperature, humidity, and light cycle (12 hr on, 12 hr off) were automatically controlled.

Scrapie strains. The ME7 scrapie strain was obtained from Dr. Alan Dickinson of the AFRC & MRC Neuropathogenesis Unit, Edinburgh, Scotland, and the 139A strain was obtained from Dr. Richard Kimberlin of the same laboratory. Scrapie material used for

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injections was prepared by passage of strains by ic injection of 0.03 ml of 1% (w/vol) brain homogenates from mice clinically sick with scrapie disease. C57BL mice were used for passaging of both ME7 and 139A.

Preparation of homogenates. Homogenates (10% w/vol) were prepared in phosphate-buffered saline (PBS) by 20 strokes of a hand-operated Ten Broeck homogenizer and stored at −70°C. Just prior to injection, the homogenates were thawed and diluted 1:10 in PBS. Normal mouse brain homogenates for injection of control mice were prepared from uninfected C57BL mice.

Assay of infectivity. Serial 10-fold dilutions of 10% homogenates were prepared in PBS. Appropriate dilutions were injected ic into female, weanling C57BL mice. Endpoint titers were determined by the Reed–Muench calculation (10).

Stereotaxic microinjections. Stereotaxic microinjections were carried out under general anesthesia (sodium pentobarbital, 70 mg/kg ip). After mounting the mice in a stereotaxic instrument (Stoelting Co.), the choice of the right side for injection was made arbitrarily, since no cerebral dominance has been established in small rodents. The coordinates used for the ventromedial hypothalamic nucleus were A—1.8, L0.5, and H+5.2 (11). Using a 30-gauge stainless steel needle, 5 μl of a 1% brain homogenate was injected.

Monitoring weight and consumption of food and water. Mice were ear-punched for identification and weighed just prior to injection. Mice were then weighed early in the afternoon at approximately 2 week intervals. Food consumption was determind by weighing the food for each cage on the same day each week. Water consumption was determined by weighing the contents of water bottles each week.

Monitoring for clinical disease. Mice were monitored for clinical disease starting at 10 weeks postinjection (pi). This was done by observing their activity levels and competence on an apparatus consisting of a series of parallel bars (3 mm in diameter) placed 7 mm apart (5). With the scrapie strain–mouse strain combinations used, the initial clinical findings were a reduction in activity and/or the ability of mice to traverse the parallel bars. The scrapie incubation period was designated as the day pi when the mouse had shown clinical symptoms of the disease for the third consecutive week.

Adrenalectomy procedure. For adrenalectomy, mice were anesthetized with sodium pentobarbital (70 mg/kg) and then adrenalectomized by the lumbar approach (12). Sham-operated mice were exposed to the same operative procedure, except for removal of the glands. Mice were adrenalectomized 2–3 weeks prior to injection. After adrenalectomy, mice were given NaCl solution (9 mg/ml) in place of tap water for drinking.

Results. Previous results with hypothalamic injection led us to an investigation of the effect of scrapie agent on functions controlled by this brain region, such as food and water consumption. Body weight and the consumption of food and water were analyzed in SJL mice injected stereotaxically in the hypothalamus with either normal mouse brain, 139A or ME7 (Fig. 1). The results show that 139A failed to cause an increase in body weight compared to mice injected with normal mouse brain, whereas mice injected with ME7 began to show a significant increase in weight ($P < 0.05$ by Student's t test) starting at 67 days pi and continuing to the end of the experiment (Fig. 1a). The increase in weight was significant approximately 41 days prior to the first appearance of typical signs of clinical disease (9).

The data on water consumption showed no significant differences among the groups (data not shown). ME7 caused an increase in food consumption (Fig. 1b) starting at approximately 67 days pi and continuing throughout the incubation period. The surprising result was that increased food consumption was also seen in 139A injected mice; the increase also started at 67 days pi and continued until mice were sacrificed at the end of the experiment.

In an earlier study we established that the weight of a number of organs (brain, spleen, liver, and kidney) in mice that showed an increase in total body weight (5) was the same or decreased compared to organ weights of mice injected with normal mouse brain. We expanded this study to include a number of additional organs, including certain endocrine glands. These data (Table I)

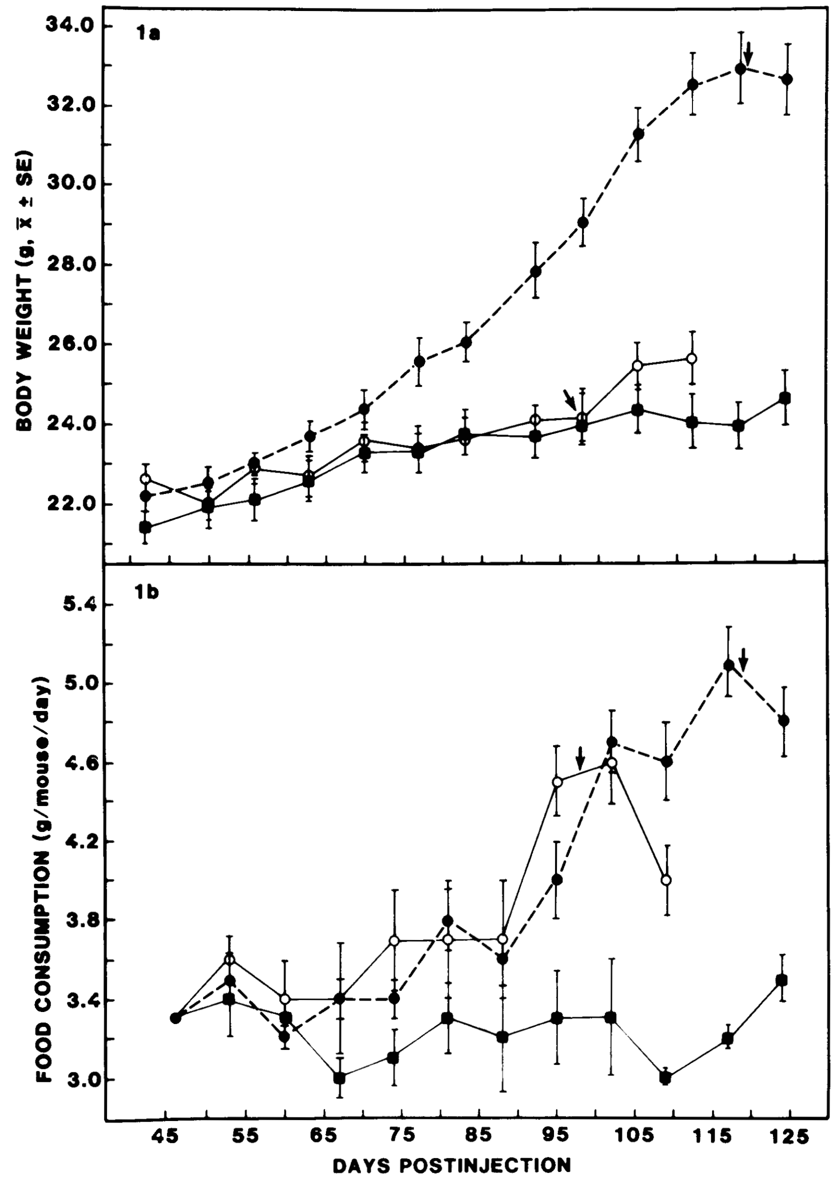

FIG. 1. Body weight in grams (a) and food consumption (b) at various times after injection with normal mouse brain (■); scrapie strain 139A (○), or scrapie strain ME7 (●). Each point represents the average value of 13–15 mice. Arrows indicate the average incubation period for each scrapie strain.

confirm previous findings with the organs noted above and show that the weights for three additional organs (pancreas, uterus, and ovary) remained the same or decreased compared to organ weights of controls. The single exception to this pattern was the statistically significant weight increase of the adrenal glands in the ME7-SJL group. Histopathological examination of the adrenals revealed that the increased weight was due to marked enlargement of the zona fasciculata and slight enlargement of the zona glomerulosa of the cortex. The cells did not reveal any cytopathic changes. Adrenal weight decreased in the 139A-SJL group. A study was then initiated to analyze further the role of

TABLE I. ORGAN WEIGHTS OF SJL MICE INJECTED WITH ME7, 139A AND NORMAL MOUSE BRAIN

	Inoculum		
Organ[a]	Normal mouse brain	ME7	139A
Brain	455 ± 5[b]	418 ± 5[c]	400 ± 6[c]
Liver	1510 ± 80	1290 ± 50[c]	1020 ± 30[c]
Kidney	395 ± 15	375 ± 11	300 ± 6[b]
Spleen	217 ± 3	98 ± 4[c]	90 ± 7[c]
Pancreas	198 ± 8	201 ± 14	208 ± 13
Uterus	145 ± 10	157 ± 16	150 ± 8
Ovary	9.0 ± 0.3	6.5 ± 0.2[c]	6.4 ± 0.4[c]
Adrenal gland	6.9 ± 0.4	8.6 ± 0.6[d]	6.3 ± 0.4

[a] Organs from mice injected intracerebrally with ME7 or 139A were obtained within 1 week of the end of the incubation period. The mice injected with normal mouse brain were sacrificed at a time pi equal to that of the scrapie injected mice.
[b] Average weight in mg, mean ± SE; the number of animals in each injection group was 10.
[c] Significant difference from controls: $P < 0.01$.
[d] Significant difference from controls: $P < 0.05$.

the adrenal gland in the increase in body weight.

Adrenalectomized or sham-operated SJL mice were injected ic with ME7. The results (Fig. 2a) show that adrenalectomy prevented the body weight increase in the ME7-SJL combination. The increase in weight seen in sham-operated animals was similar to that seen in unoperated mice (data for unoperated mice not shown). In a second experiment, C57BL mice were injected with ME7 stereotaxically in the hyopthalamus. The results (Fig. 2b) showed a weight increase in hypothalamically injected, sham-operated C57BL mice, but not in mice that had been adrenalectomized prior to injection.

The titer of scrapie agent in adrenal glands was low in both the ME7-SJL group ($10^{4.8}$ LD_{50}/g of tissue) and the 139A-SJL group (10^6 LD_{50}/g of tissue). As a point of reference these titers represent between 0.06 and 1% of the titers in brain tissues.

Discussion. The increase in food consumption (compared to mice injected with normal mouse brain) seen in the ME7-SJL group of mice was expected in view of the increase in body weight. Previous studies have documented a correlation between increases in body weight and increases in food and water consumption after scrapie infection (13). In these studies (13), A_2G mice injected ic with ME7 showed an increase in body weight starting at 9 weeks and continuing until the 17th week, with clinical disease beginning at that time. Increased food consumption began at approximately 10 weeks pi and reached a maximum at the 17th week. In the same study, a group of mice (22A-IM) that failed to show a preclinical increase in body weight did not eat or drink more than controls. Therefore, the fact that the 139A-SJL group of mice showed an increase in food consumption (Fig. 1b), but not in their body weight was surprising. The failure to gain weight in this group might indicate that food consumption and weight gain are mediated by two different pathways: A food consumption pathway might be affected in the same way in both groups leading to an increase in food intake, whereas another pathway, for example involving metabolic rate, might be affected differently in the two groups of mice.

The neuroendocrine system plays a role in infections with conventional viruses (14). For example, lymphocytic choriomeningitis infection of C3H/st mice at birth produces profound growth retardation, low blood glucose, and death because of a deficit of growth hormone production and activity (15). Virus replicates in the cells that make growth hormone. The virus effect is produced without cell necrosis or inflammation. Although there is no loss of vital functions, there is a loss of "differentiation" or "luxury" functions (15). In another study, reovirus-in-

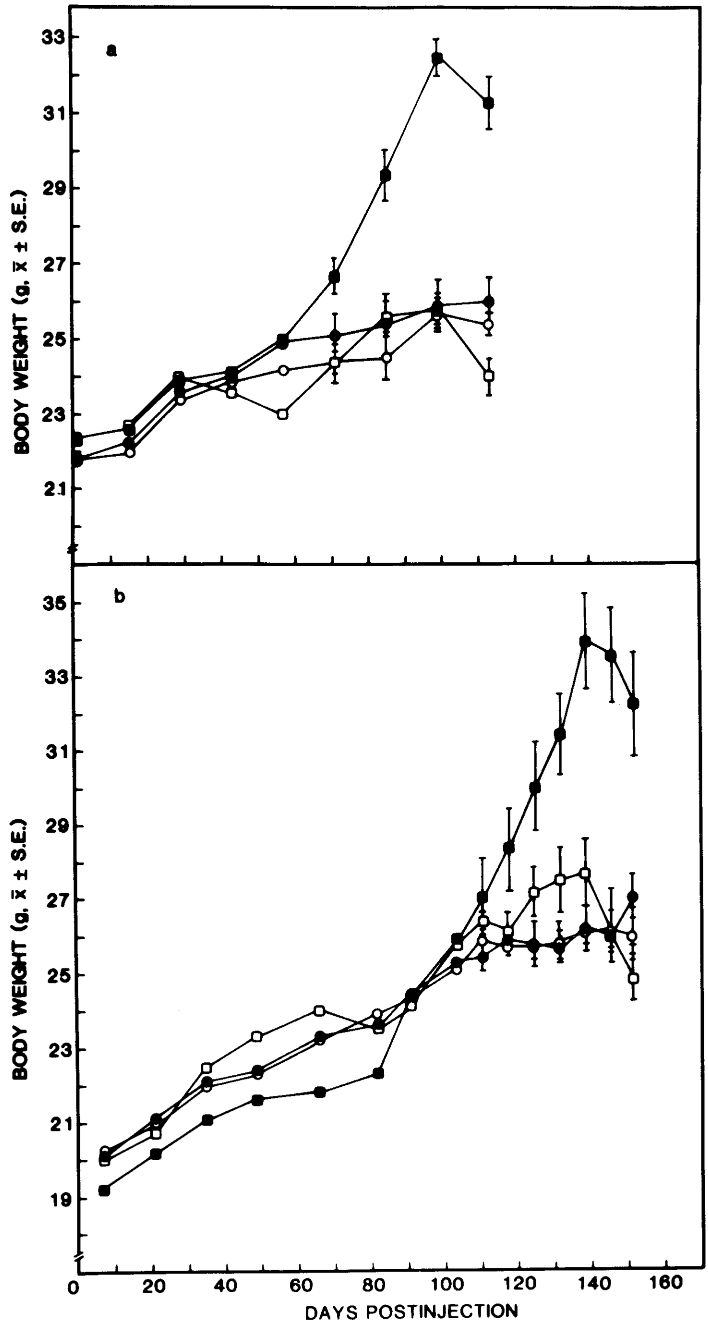

FIG. 2. Body weight of (a) SJL mice injected ic and (b) C57BL mice injected stereotaxically in the hypothalamus. Key: (■) ME7 injected, sham-operated mice; (□) ME7 injected, adrenalectomized mice; (●) normal mouse brain injected, sham-operated mice; (○) normal mouse brain injected, adrenalectomized mice. Each point represents the average value of 12–15 mice.

fected mice developed an autoimmune polyendocrine disease (16). The animals developed mild diabetes mellitus and retarded growth (17). Lyons *et al.* (18) suggested that the obesity seen in some (approximately 30%) mice infected with canine distemper virus was related to disruption of catecholamine pathways.

Effects on a neuroendocrine system in scrapie disease were suggested in a report in which histopathological changes in the ovaries were described (19). The author concluded that this was related to a neuroendocrine system effect rather than a direct effect of scrapie agent on the ovaries because scrapie infectivity in the ovaries was negligible. A weight increase was not reported.

A number of our findings indicate the importance of neuroendocrine damage in the development of scrapie-induced weight increase. First, stereotaxic work has demonstrated that the hypothalamus plays a role as it does in a number of obesity syndromes (9). Second, there are alterations in glucose metabolism that occur during the preclinical phase of the disease (7, 8). Aberrant glucose tolerance test (GTT) results were shown to parallel, essentially, the increase in weight. Altered GTT did not occur in a scrapie strain–mouse strain combination that did not show the weight increase. Third, the weight of the adrenals was significantly greater (because of cortical enlargement) than that of controls in mice that showed a body weight increase but not in a scrapie strain–mouse strain combination that failed to show an increase in body weight. This was true even though there was a reduction in the weight of virtually all visceral organs. Fourth, adrenalectomy prevented the increase in body weight. These findings indicate that scrapie-induced obesity is based on an effect on the hypothalamic–pituitary–adrenal axis.

Three points indicate that the effect is probably not due to direct action of scrapie agent on the adrenals: (i) the effect of hypothalamic injection; (ii) comparatively low scrapie infectivity titers in the adrenals, particularly in the scrapie strain–mouse strain combination that showed an increase in body weight (ME7-SJL); and (iii) the only changes seen in the adrenals were a marked enlargement of the zona fasciculata and a slight enlargement of the zona glomerulosa of the cortex. Adrenal effects are probably mediated through the levels of ACTH produced by the pituitary which, in turn, are a function of hypothalamic changes. Future work will establish if replacement therapy using mineralocorticoid or glucocorticoid can counter the effect of adrenalectomy. We will also compare the levels of ACTH, insulin, and adrenal hormones in scrapie strain–mouse strain combinations that show the weight increase and in those combinations that do not.

The importance of adrenal function in other obesity syndromes has been documented (20, 21).

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