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Transgenic Mice Overexpressing Urokinase-Type Plasminogen Activator in the Brain Exhibit Reduced Food Consumption, Body Weight and Size, and Increased Longevity

Ruth Miskin and Tamar Masos

Department of Biochemistry, The Weizmann Institute of Science, Rehovot, Israel.

Transgenic mice designated αMUPA overproduce in many brain sites the urokinase-type plasminogen activator (uPA), a protease implicated in fibrinolysis and extracellular proteolysis. Here we report that, compared to their parental wild-type control, αMUPA mice spontaneously consumed less food (≈20%), exhibited reduced body weight (≈20%) and length (≈6%), and also prolonged life span (≈20%). The αMUPA phenotype is thus reminiscent of experimental animals in which dietary restriction enhances longevity. Reduced eating and body weight were observed in αMUPA mice shortly after weaning, and these levels were maintained virtually throughout their lifetime. αMUPA mice also exhibited lower levels of blood sugar (≈9%), smaller litter size (≈14%), and lower birth frequency (≈10%). In the adult αMUPA brain, uPA mRNA has been localized through in situ hybridization also in neuronal cells of the hypothalamic paraventricular nucleus, a region implicated in feeding behavior. No signals of uPA mRNA could be detected in the paraventricular nucleus of control mice. It is suggested that in αMUPA mice, overproduction of uPA in brain sites controlling feeding leads to reduced food consumption that, in turn, results in retardation of growth and body weight and also in increased longevity. The αMUPA experimental model may have implications for normal mice.

PLASMINOGEN activators (PAs) are secreted serine proteases that specifically generate plasmin from the inactive zymogen plasminogen, which is abundant in body fluids. Plasmin is a trypsin-like general protease that dissolves the fibrin network of the blood clot. Plasmin is also capable of cleaving extracellular proteins either directly or through the activation of metalloprotease proenzymes. The PA-initiated proteolytic cascade has been implicated in diverse events in blood and tissues, including fibrinolysis, cell migration, ovulation, inflammation, tissue repair and remodeling, and embryonal development. Accordingly, PAs are synthesized and secreted in various cell types in response to appropriate hormones, growth factors, cytokines, and other modulators. Two distinct molecular types of PA exist in mammals, the urokinase-type (uPA) and the tissue-type (tPA), encoded by two different genes. Other important components of the plasminogen activation system are specific inhibitors of the catalytic activities and a high-affinity surface receptor for uPA found in many cell types. The multiple controls, at the level of genes and enzymes, adjust PA-initiated proteolytic cascade according to specific local and temporal needs (for reviews on the PA/plasmin system, see Reich, 1978; Danø et al., 1985; Saksela and Rifkin, 1988; Vassalli et al., 1991; Blasi, 1993; Collen and Lijnen, 1994; Carmeliet et al., 1995).

In the nervous system, PA activity has been claimed to play a role during axonal growth, development, and regeneration (for reviews, see Romanic and Madri, 1994; Smirnova et al., 1994; and an extensive list of references in Masos and Miskin, 1996). In the mature rat brain, tPA was induced during seizure-like states and has therefore been suggested to play a role in neuronal plasticity coupled to neuronal activity (Qian et al., 1993). The detection of uPA mRNA through in situ hybridization in the hippocampal formation of normal mice (Masos and Miskin, 1996) has led us to suggest that the enzyme could play a role in hippocampal functioning, including learning and memory. This notion is supported by our study on transgenic mice designated αMUPA (Miskin et al., 1990). In these mice, overexpression of uPA in sites related to learning and memory (i.e., hippocampus, amygdala and neocortex) coincides with impairment in several learning tasks (Meiri et al., 1994).

αMUPA transgenic mice were originally generated as a tool to investigate the possible effects of uPA in the eye, and have previously been described (Miskin et al., 1990, 1991). These mice carry the murine uPA cDNA linked downstream from the promoter of the murine αA-crystallin gene known to be active predominantly in the ocular lens. Unexpectedly, in addition to the lens, αMUPA mice produced transgenic uPA in an ectopic manner, specifically in nerve cells in the central nervous system (i.e., brain, spinal cord, and retina). No ectopic transgenic expression was found in the peripheral nervous system or in nonneuronal tissues. In the αMUPA brain, neuronal cells producing transgenic uPA mRNA were detected through in situ hybridization in many areas (Meiri et al., 1994; Masos, T., and Miskin, R., unpublished results). αMUPA mice thus provide a unique experimental system by which to explore consequences of excess uPA in the brain.

Here we describe several phenotypic alterations found in αMUPA mice: spontaneous reduction of food consumption, retardation of growth and body weight, and increased longevity. It is suggested that expression of transgenic uPA in

the hypothalamic paraventricular nucleus (PVN), a structure implicated in feeding control (for a review, see Morley, 1987), could lead to the above phenotypic changes.

METHODS

Transgenic mice. — Transgenic mice designated αMUPA were generated by Miskin et al. (1990) from the NIH FVB/N inbred mouse line as previously described. Histological examination of αMUPA tissues including brain did not reveal any abnormality. Throughout this study one line of αMUPA mice was used; unless stated otherwise, mice were in the homozygous state. αMUPA and parental wild-type (WT) control mice were propagated and maintained at the Weizmann Institute Transgenic Mouse Facilities according to the National Institutes of Health Guide for Care and Use of Laboratory Animals. The mice were housed as follows: either 5 mice in small cages or 10 mice in large cages, at 23°C, under a 12-h light/12-h dark cycle with water and food ad libitum, unless otherwise stated. Mice were fed with Experimental Animal Center Mice and Rat Breeding Diet, which included corn, soybeans, bran, corn oil, salts, vitamins, and amino acids (analysis: protein, 21%; carbohydrates, 48.1%; Ca, 0.8%; P, 0.6%; Mn, 95 g/ton; fat, 7%; ash, 8.5%; cellulose, 4%; humidity, 10%). Female mice were used for all experiments at the indicated ages.

In situ hybridization. — Preparation of mouse brain sections, hybridization protocol, and riboprobes at the sense and antisense orientation were as described by Meiri et al. (1994). Temperature of prehybridization, hybridization, and wash was as described by Masos and Miskin (1996).

Statistical analysis. — Methods for statistical evaluation are specified for each case. When not specifically mentioned, comparison of two groups was conducted through the unpaired *t*-test, and comparison between three or more groups was done through one-way analysis of variance (ANOVA) followed by Fisher protected least significant difference (PLSD) when needed, with a per-experiment level of significance at .05 (Winer, 1971).

Blood glucose determination. — Mouse blood samples were tested with the ONE TOUCH blood glucose monitoring system (Lifescan Inc., Mountain View, CA). For each mouse, blood sampling was conducted only once during each 24-h period.

RESULTS

αMUPA mice exhibit reduced body weight and length. — While working with αMUPA mice, we noticed that they looked smaller compared to age-matched WT control mice. We therefore determined body weight at different mouse ages for the entire available animal stock of WT and homozygous αMUPA mice which included a large number of animals derived from many litters. Results are presented in Figure 1A. Compared to WT, homozygous αMUPA mice exhibited reduced body weight throughout most of their life span. Weight differences were first noticed shortly after weaning (Figure 1A, inset) and could not be detected prior to it. The mean αMUPA body weight was 86% of control at 1–2 months of age (p-value for the t-test was .0001), 76% at 4.5–5 months ($p = .0001$), 79% at 15 months ($p = .0001$), and 87% at 18–19 months of age ($p = .0024$). At 28 months no difference between the two mouse groups could be noticed, mainly due to weight loss occurring in WT.

Body weight of heterozygous αMUPA mice (F_1) was also investigated. Body weight of F_1 mice was followed in a longitudinal manner and compared to that of age-matched WT and homozygous αMUPA mice maintained during the same 9-month period. F_1 body weight values fell roughly intermediate between those of homozygous αMUPA and WT mice (Figure 1B).

Body length of αMUPA homozygous mice was also reduced compared to WT, although to a lesser degree than

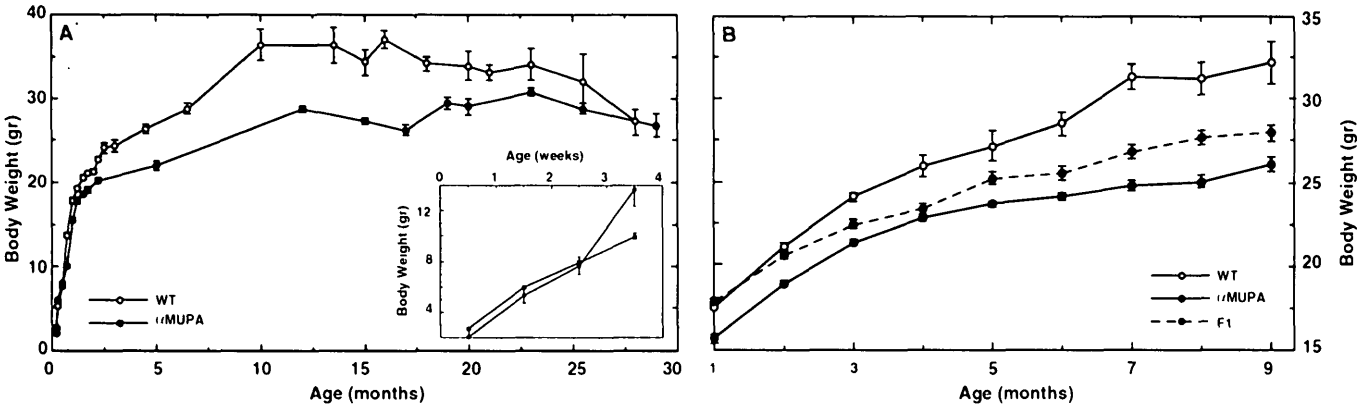

Figure 1. Measurements of body weight of homozygous and heterozygous αMUPA and WT mice at different ages. (**A**) Body weight of 114 homozygous αMUPA and 149 control WT female mice was determined at different mouse ages up to 28 months. Most mice were used for weight determination at several ages. Each point represents the mean weight ± *SE* of at least 10 mice from at least two different litters. (Inset) Mean body weight ± *SE* in the first 4 weeks. (**B**) Weight values of WT and homozygous and heterozygous (F_1) αMUPA mice. Each point represents the mean weight ± *SE* of 18–50 mice. At each age the three mouse groups were compared through the one-way ANOVA followed by Fisher protected LSD with per-experiment level of significance of $\alpha = .05$ (Winer, 1971). At 3, 5, 7, and 8 months of age all groups were significantly different ($p = .0001$ for each age). At the other ages, either no significant difference was found between all three groups, or only one group was significantly different from the two other groups.

weight (Figure 2). At 6 months of age αMUPA were about 6% shorter than WT, whereas body weight of the same group was 20% lower.

αMUPA mice exhibit reduced food consumption. — The phenotypic changes in body weight and length found in αMUPA mice prompted us to follow-up their food intake. The daily food consumption for both mouse types was determined throughout a 30- to 145-day period (Figure 3). Although consecutive measurements showed large variation, the data indicate that αMUPA consumed less food daily throughout the entire experimental period, starting immediately after weaning. Comparison of the two mouse types for their mean daily food intake in each of the age periods of 30–60, 60–90, and 90–145 days indicated that αMUPA ate, respectively, 85%, 77%, and 78% of WT consumption (p-values for the t-test were, respectively, .0001, .0005, and .0001). We also compared the mice at 16 months of age and found that αMUPA ate 69% of WT consumption (data not shown). It also appeared from the data that the daily food intake of αMUPA declined with time more than that of WT. However, this difference between the two mouse types did not quite attain significance (p = .10 for comparison of slopes in Figure 3), and further experiments are needed to critically test this issue.

To determine whether the amount of food consumed by αMUPA could account for the weight difference found between αMUPA and WT mice, a group of WT mice was fed with food availability limited to the voluntary daily consumption of aged-matched αMUPA mice. Immediately after weaning, WT mice were divided into two groups, one fed ad libitum (WT-1) and the other (WT-2) fed daily the amount consumed by aged-matched αMUPA. The amount of food was determined as described for Figure 3. Results are presented in Figure 4. During the experimental period of 145 days both αMUPA and WT mice that were fed ad libitum continued to gain weight. The WT-2 mice, which were kept on a restricted diet, exhibited body weight values intermediate between those of WT-1 and αMUPA until about 70–80 days. At that time the WT-2 mice started to lose weight, with group weight stabilizing at about 15% lighter than αMUPA. This pattern was replicated in two independent experiments.

αMUPA mice exhibit increased longevity. — Experimental dietary restriction is known to lead to increased longevity

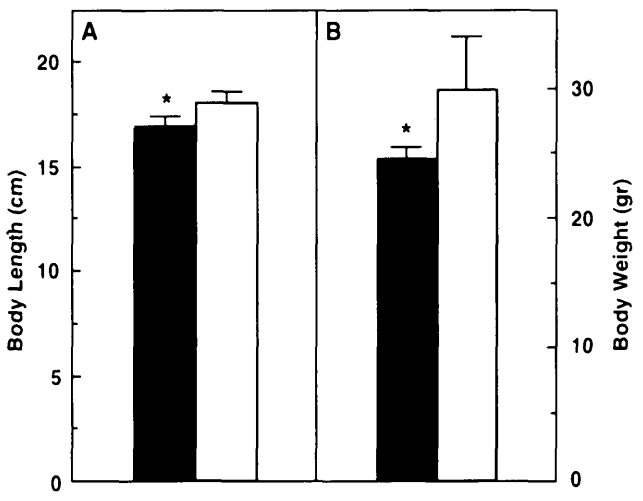

Figure 2. Measurements of body length and weight. Six-month-old female αMUPA and WT mice were examined, 17 mice in each group. Body length included tail. Columns represent mean values ± *SD*. Light columns, WT; dark columns, αMUPA. *Significantly different (p = .0001 for **A** and **B**) according to the two-group Mann-Whitney U-test (Hollander and Wolfe, 1973).

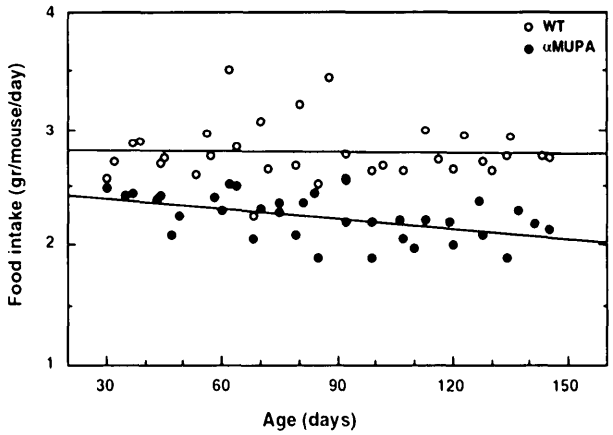

Figure 3. Determination of food consumption of αMUPA and WT mice at different mouse ages. Immediately after weaning, 20 mice of each αMUPA and WT group, including the WT-1 and αMUPA mice used in Figure 4, were fed a known excess amount of food per cage and allowed to eat ad libitum. Mice of each type were kept in two cages holding 8 and 12 mice. The points represent the average food intake per mouse per single cage. Twice a week the amount of food left in each cage was weighed, and the mean amount of food eaten per mouse per day was determined. The curves were estimated by linear regression model (using the method of least squares).

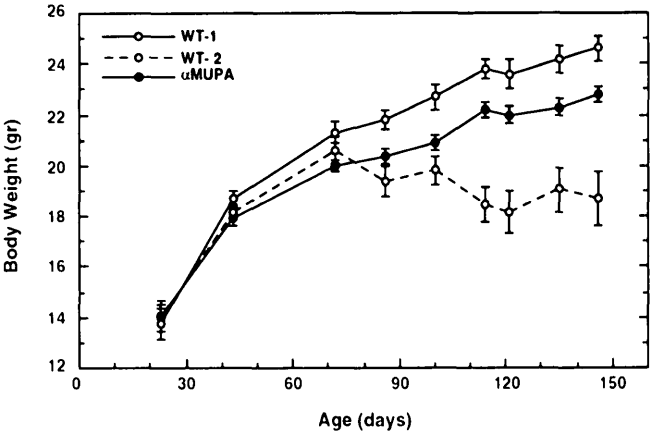

Figure 4. Effect of restricted diet on WT body weight. Immediately after weaning, 24 WT mice were divided into two equal groups (WT-1 and WT-2). WT-1 and 12 aged-matched αMUPA mice were fed ad libitum, and the amount of food eaten bv αMUPA and WT-1 was determined twice a week, as described for Figure 3. WT-2 were provided a single daily meal at 900 h that consisted of the amount of food that was last found to be consumed by αMUPA in the same experiment. Body weight measurements of all mice were conducted every 2–4 weeks. Each point represents mean body weight ± *SE*. Each one of the four last values of each group was significantly different (p = .0001) from the counterpart values of the two other groups according to ANOVA (α = .05).

in mice and rats (for reviews, see Masoro, 1992, 1993). It was therefore of interest to examine the longevity of αMUPA mice, which spontaneously eat less than non-transgenic controls. We generated survival curves for αMUPA and WT mice, 33 mice in each group, which were maintained in our stock for up to 37 months (Figure 5). At that age the surviving animals (one WT and two αMUPA) were sacrificed; therefore we did not follow the maximum length of life. The αMUPA survival curve differed significantly from that of WT (p = .0001). The ages of 75th, 50th (median), and 25th percentile survivors were significantly longer (36%, 16%, and 23%, respectively) for αMUPA compared to WT. Taken together, these and the above results indicate that in αMUPA increased longevity coincides with reduced eating.

Comparison of αMUPA and WT mice for blood glucose level and several reproduction parameters. — Reduction of plasma glucose concentration occurred in diet-restricted rats and was suggested to contribute to life-span expansion (Masoro, 1993; Masoro et al., 1995). We therefore compared αMUPA and WT for blood sugar level. Measurements were conducted for 5-month-old mice that were used in the restriction diet experiment presented in Figure 4, including the restricted diet WT-2 group. Blood samples were taken at four different times during the day, including samples of WT-2 just before the supply of their daily meal and also 2 h later. Results are presented in Table 1. WT-1 and αMUPA exhibited slight daily variations, with a highest sugar level detected at 1700 h. However, at all times αMUPA values were lower compared to WT-1, with the highest difference (12%) found at 1700 h. In WT-2, the glucose levels were at all times lower than in WT-1, except in the sample taken 2 h after eating, in accordance with published data (Masoro et al., 1995). This also was the only time when WT-2 exhibited a higher glucose level compared to αMUPA. At all other times WT-2 glucose was either similar or lower than that of αMUPA (Table 1).

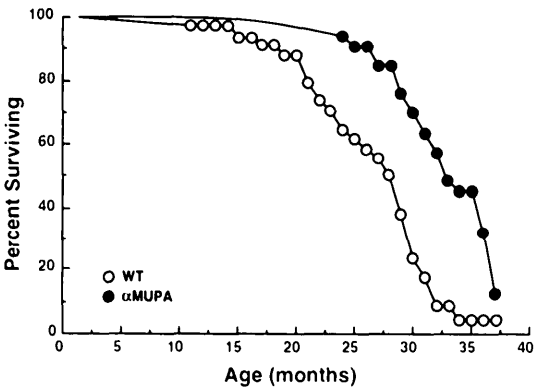

Figure 5. Survival curves of αMUPA and WT mice. Percentage of αMUPA and WT mice (33 in each group) surviving at the end of each month is indicated in the curves. At the age of 33 months one WT and seven αMUPA mice were removed for other purposes. The rest of the mice were followed up until the age of 37 months, and then the remaining animals, one WT and two αMUPA, were sacrificed. The survival curves and the 75th, 50th (median), and 25th percentile survivors were estimated using product-limit method, and the curves were compared using Wilcoxon test (Lawless, 1982) (p = .0001).

Animals kept under restricted food supply have been shown to manifest changes in female reproductive functions (Merry and Holehan, 1979). Accordingly, αMUPA and WT mice were compared for several features: litter size, frequency of births (Table 2), the age at which reproductive activities started in males and females, and duration of fertility of mates of each mouse group. αMUPA mice exhibited 12% less frequent births compared to WT. Further, the average litter size of αMUPA mice up to the age of 150 days was found to be 86% that of control. At about this age litter size started to gradually decline in both WT and αMUPA, and no significant differences between the two groups could be noticed (data not shown). The aforementioned differences between the mice were statistically significant (Table 2). By contrast, no difference between the two mouse types could be found in the start of reproductive activity and duration of fertility (data not shown).

αMUPA mice produce transgenic uPA mRNA in the hypothalamic PVN. — Whole αMUPA and WT brains were analyzed through in situ hybridization for cells producing uPA mRNA, using riboprobes that could enlight both the transgenic and the normal uPA mRNA (Masos, T., and Miskin, R., unpublished results). In the αMUPA brain, nerve cells labeled for uPA mRNA were detected in many sites. Given the results presented above, it was of interest to examine transgenic expression specifically in areas claimed

Table 1. Blood Glucose Level[a]

Mouse Group	n	Time of Blood Sampling (h) 900	1100	1700	2100	Mean, 12-h
WT-1	13	125 ± 3 A	121 ± 2 AB	139 ± 4[d]A	129 ± 2A	129 (100%)
WT-2	10	81 ± 3[b,d]B	123 ± 3[c]B	121 ± 5 B	110 ± 3B	109 (84%)
αMUPA	12	115 ± 4 A	114 ± 2 A	122 ± 2 B	118 ± 3B	117 (91%)

[a]Data are presented in mg/dL; reported as mean ± *SE*. Means within the same time followed by the same letter are not significantly different according to ANOVA, followed by Fisher's PLSD (with per-experiment level of significance .05). p-values for the ANOVA were as follows: 900 h, p = .0001; 1100 h, p = .006; 1700 h, p = .0001; 2100 h, p = .0008.

[b]Blood sample was taken before the daily meal of dietary restricted WT-2.

[c]Blood sample was taken 2 h after the daily meal of WT-2.

[d]Within the same mouse group, significantly different from means at all other times according to ANOVA followed by Fisher's PLSD (with a per-experiment level of significance of .05) (p = .0001 for WT-1 and WT-2).

to be involved in food consumption. Thus, in the hypothalamus only two nuclei were distinctly labeled for uPA mRNA: the hypothalamic PVN implicated in food consumption (Morley, 1987) and the supra-chiasmatic nucleus associated with circadian behavior (Akabayashi et al., 1994b). In other hypothalamic areas cells showing hybridization signals were randomly dispersed rather than confined to specific hypothalamic nuclei. Figure 6 shows brain sections through the PVN, where hybridization signals were detected in αMUPA (Figure 6 A–C) but not in WT mice (Figure 6E). In αMUPA, signals were seen with the antisense riboprobe but not with the sense riboprobe (Figure 6D), indicating specificity for uPA mRNA. Cells exhibiting hybridization signals could be recognized as neurons at high magnification according to morphological appearance (Figure 6B). As with other brain regions in αMUPA mice, only a fraction of the neuronal population in the PVN yielded uPA mRNA signals.

Table 2. Litter Size and Birth Frequency of αMUPA and WT Mice

	WT	αMUPA	p^a
Frequency of births[b]			
(days ± *SE*)	23.6 ± 0.7 (100%)	26.4 ± 0.9 (112%)	.0142
Litter size[c]			
(mean ± *SE*)	8.5 ± 0.2 (100%)	7.3 ± 0.2 (86%)	.0001

[a]*p*-value for the unpaired *t*-test.

[b]Thirty WT and 22 αMUPA females were mated with males of the same mouse type and followed up for frequency of births until the age of 8–10 months.

[c]Mean litter size was determined for 90 WT and 78 αMUPA litters born to the females used above.

Discussion

Our data show that the transgenic state of αMUPA mice leads to alteration of both feeding behavior and longevity. Food consumption was reduced in the transgenic mice compared to WT mice, and life span was prolonged. This inverse relation between food consumption and longevity is in accordance with the well-established findings that dietary restriction retards the aging process in laboratory rats and mice (for reviews, see Masoro, 1992, 1993). It is therefore very likely that in αMUPA the spontaneous decline in feeding is causally related not only to the retardation of growth and body weight, but also to increased longevity. It is of interest to note that in αMUPA an approximately 20% reduction in eating coincided with a 16% increase in the median length of life, while in dietary restriction experiments 40% food restriction caused an approximately 35% increase of the median life span (Masoro et al., 1995). The phenotypic manifestations of αMUPA mice are inversely related to those of the "Giant" transgenic mice carrying the gene encoding bovine growth hormone. These mice exhibited enhanced feeding and growth of body size and weight concomitantly with decreased longevity (Pendergrass et al., 1993).

αMUPA mice exhibited reduced food intake from weaning throughout at least 16 months of age. Food consumption of αMUPA mice was 90% that of WT shortly after weaning, it declined to 78% at 3–5 months, and was 69% at 16 months of age. Whether the apparent spontaneous decline with age in

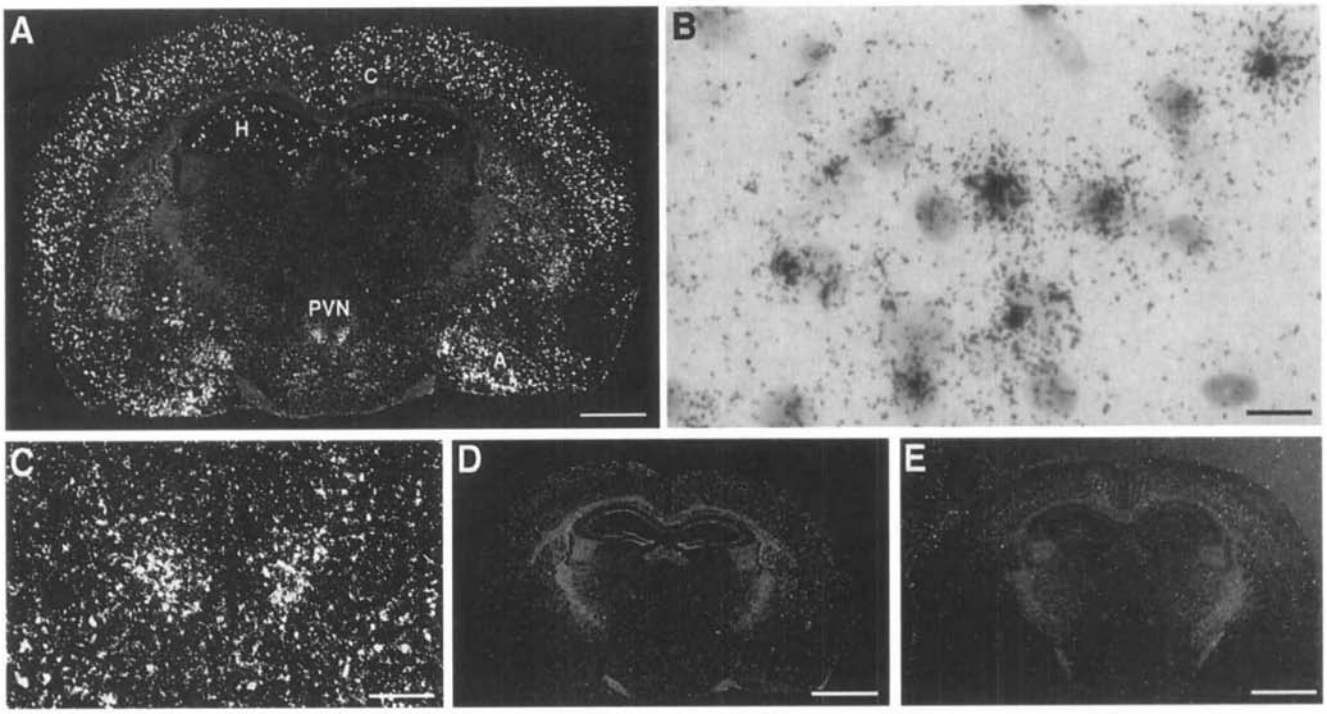

Figure 6. In situ hybridization of brain coronal sections through the hypothalamus. Coronal sections of brains of 3-month-old αMUPA and WT mice were hybridized with ^{35}S-labeled riboprobes at the sense and antisense orientations. (**A** and **D**) αMUPA sections hybridized, respectively, with the antisense and sense riboprobes. (**E**) WT section hybridized with the antisense riboprobe. (**B** and **C**) Higher magnification views of **A**. **B** was photographed in bright field illumination, and all the rest in dark field view. Abbreviations: PVN, paraventricular nucleus; C, cortex; P, hippocampus; A, amygdala. Bars = 0.9 mm (**A**); 10 μ (**B**); 0.2 mm (**C**); and 1.3 mm (**D** and **E**).

αMUPA feeding could exert any long-term beneficial effect on αMUPA aging is not known and deserves attention.

Another difference between αMUPA and WT mice was revealed when the diets of WT mice were adjusted to the reduced consumption patterns of αMUPA mice. Unlike αMUPA, the diet-restricted WT mice started to lose weight after about 70–80 days of age and became about 15% lighter than αMUPA. As both mouse groups eat the same amount of the same food, these data may indicate that αMUPA utilize the food in a more efficient manner and, furthermore, that this capability is inherent in αMUPA and was not acquired by WT mice during the experimental period of dietary restriction. Whether the survival curve of the diet-restricted WT will be similar or different from that of αMUPA is yet to be seen.

Food restriction in rodents was found to maintain many physiological processes in a youthful state and to retard age-associated diseases and senescence (Masoro, 1992, 1993; Weindruch, 1992). αMUPA mice have not yet been studied for most of these parameters, though in accordance with data from dietary restricted animals (Masoro et al., 1995), the blood glucose level of αMUPA was significantly lower than that of ad libitum-fed WT mice. Interestingly, when WT were fed the amount of food consumed by αMUPA, their mean 12-h blood sugar level was lower than that of αMUPA. This is compatible with the larger loss of body weight exhibited by the same dietary restricted WT mice. Long-term maintenance of low blood glucose levels was included among putative factors enhancing longevity upon dietary restriction (Masoro, 1993). αMUPA also exhibited two changes related to reproductive behavior: smaller litter size and less frequent births.

Food restriction in rats was reported to decrease or have no effect upon the age-related decline in several learning tasks. In no case was it found to adversely affect cognitive capacities (Means et al., 1993 and references therein). By contrast, αMUPA mice present a case where reduction in feeding was accompanied by learning deficits. We have previously shown that αMUPA were impaired in learning of spatial, olfactory, and taste aversion tasks while displaying normal sensory and motor capacities (Meiri et al., 1994). We therefore propose the hypothesis that in αMUPA the phenotypic alterations in learning and food consumption are unrelated, each resulting from transgenic expression at a different brain site. Thus, excess uPA in the hippocampus, amygdala, and neocortex could interfere with learning and memory, while transgenic expression in the hypothalamic PVN is a candidate to influence feeding behavior. This hypothesis, however, has not been critically tested.

In the αMUPA hypothalamus, transgenic uPA mRNA has now been localized in the PVN, a region implicated in the control of food intake (Kyrkouli et al., 1986; Morley, 1987; Murakami et al., 1989; Kalra et al., 1991; Akabayashi et al., 1994a). In this site no transcriptional product of the normal uPA gene could be detected. We have not looked for the level of active enzyme specifically in the PVN; however, based on analysis conducted in other αMUPA brain sites (Meiri et al., 1994), it is very likely that in the PVN transgenic uPA mRNA is translated into the active enzyme. Transgenic uPA could be envisioned to lead to reduced food intake through proteolytic intervention in the state of polypeptide neurotransmitters or surface receptors involved in the control of food consumption. Interference of uPA in the PVN-pituitary communication, whether through the above-mentioned manners or otherwise, could lead to alteration of feeding behavior. Also, it cannot be excluded that the reduced feeding in αMUPA is determined through sites implicated in feeding behavior other than the PVN, such as the amygdala and the suprachiasmatic nucleus associated with circadian rhythms (Morley, 1987; Corwin et al., 1993, Akabayashi et al., 1994b), where prominent transgenic expression was also found (Meiri et al., 1994). The ectopic transgenic expression starts relatively late in embryonic life (embryonic day 17.5; Masos, T., and Miskin, R., unpublished results), prior, however, to the accomplishment of brain development. Therefore, it is possible that transgenic interference with development, especially in the above-mentioned sites, also could affect food intake.

In the brain, extravascular plasminogen is thought to be present at a very low level (Nakajima et al., 1992; Sappino et al., 1993). Therefore, it is not clear whether uPA would exert its proteolytic effect in the αMUPA brain through plasminogen activation or via a direct cleavage of other substrate molecules, as shown in the case of chicken fibronectin (Quigley et al., 1987). We favor the latter possibility, because in the case of reduced body weight it looks as if the level of transgenic uPA is rate limiting rather than the level of the putative substrate for uPA. The dosage effect of transgenic uPA is inferred from the finding that the difference in body weight between WT and αMUPA homozygotes is approximately twice the difference between WT and αMUPA heterozygotes.

So far only one αMUPA line was generated and characterized; therefore, it could be claimed that the αMUPA phenotype is conferred by the position of transgenic insertion into the mouse genome rather than by the transgenic expression per se. Additional αMUPA lines are now in preparation to resolve this issue.

Extracellular proteases, including the blood coagulation enzyme thrombin, were claimed to be involved in normal and pathological brain functions (for reviews, see Romanic and Madri, 1994; Smirnova et al., 1994). The data presented here suggest that a protease of the fibrinolytic system could play a role in extravascular functions in the brain. Thus, the αMUPA phenotype raises the possibility that uPA in normal mice could be directly involved in biochemical brain mechanisms that determine food consumption, and secondarily to affect growth, weight, and longevity. αMUPA mice also provide a unique experimental system by which to study physiological and cellular parameters of spontaneously occurring prolonged aging, and to explore biochemical mechanisms linking food consumption and longevity.

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Address correspondence to Dr. Ruth Miskin, Department of Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel.

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