References and Notes

1. O. Stutman, *Science* **183**, 534 (1974); L. Schjerven, K. Elgjo, O. H. Iversen, V. Palbo, in *Proceedings of the First International Workshop on Nude Mice*, J. Rygaard and C. O. Poulsen, Eds. (Fischer, Stuttgart, West Germany, 1974), p. 265; H. C. Outzen, R. Custer, G. Eaton, R. Prehn, *J. Reticuloendothel. Soc.* **17**, 1 (1975); L. Anderson, J. Budinger, R. Maronpot, R. Good, *Cancer Res.* **38**, 137 (1978); O. Stutman, *J. Natl. Cancer Inst.* **62**, 353 (1979); M. Gershwin and R. Ikeda, *Dev. Comp. Immunol.* **2**, 529 (1978); S. Yuspa, C. Viguera, R. Nims, *Cancer Lett.* **6**, 301 (1979); S. Yuspa, E. Spangler, R. Donahoe, S. Geusz, E. Ferguson, M. Wenk, H. Hennings, *Cancer Res.* **42**, 437 (1982).
2. O. Stutman, in *The Nude Mouse in Experimental and Clinical Research*, J. Fogh and B. Giovanella, Eds. (Academic Press, New York, 1978), p. 411.
3. The mice were maintained as an isolated colony in a room with vertical laminar airflow (25°C; humidity, 40 to 60 percent) with a 12-hour light-dark cycle. They were housed in filter-topped plastic cages (one to five mice per cage) with hardwood shavings as bedding and given Purina Autoclavable Laboratory Chow and acidified water. Once a week a ball of whole wheat bread mixed with condensed milk was placed in each cage as a dietary supplement. All materials were sterilized by autoclaving, and sterile procedures were used for all manipulations.
4. F. Arndt, in *Organic Synthesis*, A. Blatt, Ed. (Wiley, New York, 1943), p. 461.
5. Stutman (*2*) exposed nude mice transplacentally to ENU and did not report skin tumors. The mouse strain was CBA/H and the treatment was given somewhat later in gestation (day 16 or 17) than in our experiment.
6. G. Krueger, D. Chambers, J. Shelby, *J. Exp. Med.* **152**, 1329 (1980).
7. Supported by NIH grants CA 22498 and CA 08748.

* Present address: Laboratory of Comparative Carcinogenesis, National Cancer Institute, Fort Detrick, Frederick, Md. 21701.

29 April 1982; revised 16 July 1982

Damage to Hypothalamic Dopaminergic Neurons Is Associated with Development of Prolactin-Secreting Pituitary Tumors

Abstract. *Old female rats with spontaneous prolactin-secreting pituitary tumors (prolactinomas) and young females with prolactinomas produced by prolonged estrogen treatment had damaged tuberoinfundibular dopaminergic neurons. Since these neurons inhibit the function of pituitary prolactin-secreting cells, their destruction may lead to development of prolactinomas.*

Prolactin-secreting pituitary tumors are frequently observed in aging female and male rats, with an incidence of up to 80 percent in old Sprague-Dawley females. Prolactinomas can be induced in young rats by prolonged administration of estrogen. The mechanism responsible for the development of these tumors has been elusive (*1*), but we now report evidence that such tumors are associated with a loss of hypothalamic dopaminergic neurons, which normally inhibit the function of prolactin-secreting cells (lactotrophs) in the anterior pituitary. Hypothalamic dopamine is produced mainly in tuberoinfundibular dopaminergic (TD) neurons of the arcuate nucleus in the medial basal hypothalamus. Most of these neurons terminate in the external layer of the median eminence (ME), from which dopamine is released into the portal vessels that go to the anterior pituitary (*2*).

Sprague-Dawley and Wistar Furth female rats were obtained from Harlan Research Industries and maintained under controlled conditions of light, temperature, and feeding (*3*). Of the 44 Sprague-Dawley females, 31 developed spontaneous prolactinomas at 26 to 28 months of age, as indicated by a significant increase in the mean weight of the anterior pituitary and in the concentration of serum prolactin (Table 1). Histological examination revealed that these pituitaries were hemorrhagic, with disruption of parenchymal cells and capillary integrity. The mean weight of the prolactinomas was more than three times greater than that of the pituitaries of tumor-free anestrous females 24 to 27 months of age (old controls) or diestrous females 3 to 4 months of age (young controls). Serum prolactin concentrations in old rats with prolactinomas were approximately six times higher than in old controls and 13 times higher than in young rats. In old control rats pituitary weights and serum prolactin concentrations were also significantly higher than in young rats. The mean concentration of dopamine in the ME of old rats with prolactinomas was significantly lower than in old controls, and the dopamine concentration in the ME in both groups of old rats was lower than that in young rats (Table 1).

Dopamine fluorescence (*4*) in the external layer of the ME was not as intense in old rats as in young rats, and was even less intense in old rats with prolactinomas (Fig. 1). In addition, both groups of old rats showed degeneration of dopaminergic neurons in the arcuate nucleus, as indicated by the presence of distorted fluorescent fibers and deposits of punctate autofluorescent material—usually considered indicative of phagocytic cells and lipofuscin (*4, 5*). Increased cellular lipofuscin is often seen in tissues of aged individuals but does not necessarily signify decreased cellular function. The presence of distorted fibers and phagocytic cells indicates neuronal degeneration (*4*). There is a significant reduction in pituitary portal blood dopamine in old rats, and the reduction is greater in old rats with prolactinomas (*6*). These observations suggest that functional dopaminergic neurons are lost during aging and that this loss increases as animals develop spontaneous prolactinomas.

Damage to TD neurons also was found in young rats with prolactinomas produced by long-term estrogen treatment (*7*) (Table 1 and Fig. 1). Compared to effects in young control rats, this treatment resulted in significantly greater anterior pituitary weight and serum prolac-

Table 1. Median eminence (ME) dopamine, serum prolactin, and anterior pituitary weight in Sprague-Dawley and Wistar Furth rats with and without prolactin-secreting tumors. Dopamine and prolactin concentrations were measured by radioenzymatic assay (*19*) and by radioimmunoassay (*3*), respectively. Numbers of rats are given in parentheses; values are means ± standard errors. Overall significance ($P < .001$) was determined by analysis of variance: for ME data, $F(4, 27) = 9.69$; for prolactin data, $F(4, 68) = 9.08$; and for AP data, $F(4, 62) = 28.48$. Subsequent t-tests were used to compare individual groups (all differences significant at $P < .05$, except ME data of old and young Sprague-Dawley anterior pituitary).

Group	Tumor type	Dopamine in ME (ng/mg protein)	Serum prolactin (μg/ml)	Anterior pituitary weight (mg)
Young Sprague-Dawley and Wistar Furth	None	127.1 ± 21.9 (8)	0.054 ± 0.006 (17)	9.3 ± 0.3 (22)
Old Sprague-Dawley	None	54.5 ± 3.6 (6)	0.129 ± 0.012 (12)	12.1 ± 0.6 (10)
Old Sprague-Dawley	Anterior pituitary	37.2 ± 1.7 (6)	0.702 ± 0.078 (19)	40.9 ± 10.2 (11)
Young Sprague-Dawley	Anterior pituitary (estrogen-induced)	32.4 ± 4.3 (6)	1.375 ± 0.230 (14)	59.2 ± 4.0 (8)
Young Wistar Furth	MtT · W_{15}	67.8 ± 7.2 (6)	7.780 ± 2.904 (11)	6.5 ± 0.4 (16)

0036-8075/82/1112-0684$01.00/0

tin concentration, a significantly lower concentration of dopamine in the ME, and a markedly lower intensity of dopamine fluorescence in the external layer of the ME and in the arcuate nucleus. The low concentration of dopamine in the ME may be the result of damage to TD neuron terminals originating in the arcuate nucleus. Long-term estrogen treatment was also reported to result in specific cytopathological changes in the arcuate nucleus (*8*). The tumorigenic action of estrogen on the anterior pituitary may not be due solely to its effect on the hypothalamus, since estrogen can produce prolactinomas in pituitaries grafted underneath the kidney capsule (*9*). Estrogen can also directly block the inhibitory action of dopamine on the pituitary (*10*). Elimination of dopamine inhibition by cutting the medial basal hypothalamus can lead to hyperplasia of lactotrophs in rats, even in the absence of ovarian steroids (*11*). Therefore, a blockade of the inhibitory action of dopamine on the pituitary may be an initial stage of estrogen action, which is then followed by unhindered growth of lactotrophs. It also is possible that estrogen stimulates the release of a pituitary growth-stimulating factor from the hypothalamus (*9*).

In rats with spontaneous or estrogen-induced prolactinomas there was a progressive increase in circulating prolactin and a loss of TD neurons (Table 1 and Fig. 1). We tested whether chronically high levels of circulating prolactin can contribute to degeneration of TD neurons by subcutaneously implanting a small amount of a prolactin-secreting pituitary tumor (MtT · W_{15}) in young Wistar Furth females (*12*). Within 8 weeks the average weight of the transplanted tumor was approximately 40 g and serum prolactin was approximately 7.8 μg/ml (Table 1). These rats showed a significantly lower concentration of dopamine in the ME than young control rats (Table 1) (*13*). Degeneration of TD neurons was also evident in the tumor-bearing rats (Fig. 1), as indicated by the presence of distorted fibers and autofluorescent phagocytic cells (*4*). However, after short-term administration of prolactin to rats, dopamine turnover in the ME and dopamine levels in the pituitary portal vessels are increased (*14*). Therefore, prolactin appears to be neurotoxic only when its concentration in the blood is chronically elevated. We believe that the neurotoxic action of continuously high levels of circulating prolactin may have been partially responsible for the reduction of dopamine activity in the old rats, and in the young rats after prolonged estrogen administration.

In old rats a progressive loss of TD neurons was associated with the development of prolactinomas (Table 1 and Fig. 1). The neurotoxic action on TD neurons in old rats may be due initially to the recurrent action of prolactin and estrogen during the numerous estrous cycles and subsequently to the chronic elevation in blood prolactin after cycling has ceased. During each estrous cycle of the rat, both estrogen and prolactin show a preovulatory surge (*1*). Therefore, the marked loss of TD neurons due to chronically elevated levels of circulating prolactin, estrogen, or both may represent an initial step that leads to sustained alteration in the mechanism controlling prolactin secretion. Since a close correlation may exist between the secretory and mitotic processes in lactotrophs (*15*),

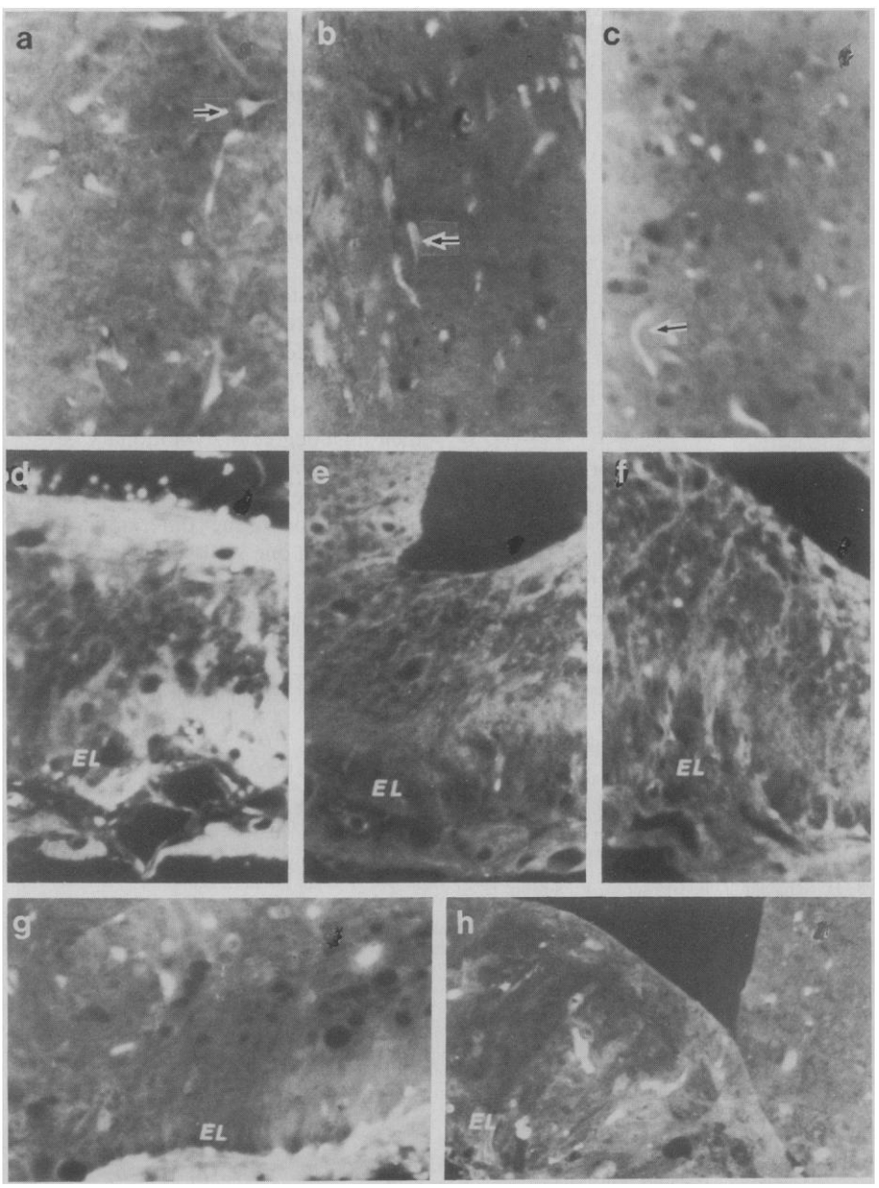

Fig. 1. Fluorescence photomicrographs of coronal sections (*20*) through the hypothalamus (middle level) of representative female rats bearing or not bearing prolactinomas. (a) Bright catecholamine fluorescence in cell bodies (arrow) and fibers in the arcuate nucleus of a 4-month-old, diestrous Wistar Furth rat. (b and c) Distorted arcuate nucleus neurons (arrows) in young Wistar Furth rats bearing an MtT · W_{15} tumor for 4 (b) or 8 (c) weeks. (d) Normal catecholamine fluorescence in nerve terminals of the external layer (*EL*) of the ME in a 3-month-old, diestrous Sprague-Dawley rat (*21*). (f and g) Reduced catecholamine fluorescence in the external layer of the ME in an acyclic Wistar Furth rat bearing an MtT · W_{15} tumor for 8 weeks (f) and in an old, anestrous Sprague-Dawley rat (g). (e and h) Marked reduction of dopamine fluorescence in the external layer of the ME in Sprague-Dawley rats bearing a spontaneous prolactinoma (e) or an estrogen-induced prolactinoma (h). Magnifications: (a) to (c) ×480; (d) to (h) ×240.

the unhindered stimulus to prolactin secretion may cause the development of a tumorous pituitary in rats. There is no evidence that prolonged exposure to estrogen is responsible for development of prolactinomas in humans (*16*). However, there is some evidence that dopaminergic activity may be reduced in prolactinemic patients (*17*) and that catecholamines are decreased in the brains of elderly individuals (*18*). Hence, damage to TD neurons may be associated with the development of human prolactinomas (*1*).

DIPAK K. SARKAR
PAUL E. GOTTSCHALL
JOSEPH MEITES
Neuroendocrine Research Laboratory, Department of Physiology, Michigan State University, East Lansing 48824

References and Notes

1. J. Meites, in *The Prostatic Cell: Structure and Function*, G. P. Murphy, A. A. Sandburt, J. P. Karr, Eds. (Liss, New York, 1981), part B, p. 1; *Neuroendocrinology* **34**, 151 (1982).
2. A. Björklund, B. Flack, A. Nobin, U. Stenevi, in *Neurosecretion: The Final Neuroendocrine Pathway*, E. Knowles and L. Vollrath, Eds. (Springer-Verlag, New York, 1974), p. 209.
3. D. A. Van Vugt, P. W. Sylvester, C. F. Aylsworth, J. Meites, *Endocrinology* **108**, 2017 (1981).
4. The procedure used was that of D. K. Sarkar, G. C. Smith, and G. Fink [*Brain Res.* **213**, 335 (1981)], except that fixed tissues were embedded in glycol methacrylate and thinner sections (3 μm) were used for better resolution of cellular detail. The fluorescence intensity was graded on a semiquantitative scale by D.K.S., who had no knowledge of which treatment the animals had received. Four to five brains were examined for each treatment group and fluorescence was rated as zero, markedly reduced, reduced, or normal. The results represent means of these observations.
5. G. E. Hoffman and J. R. Sladek, Jr., *Neurobiol. Aging* **1**, 27 (1980).
6. D. K. Sarkar, P. E. Gottschall, Q. W. Xie, J. Meites, in preparation.
7. Young cyclic females were ovariectomized and, 4 to 6 weeks later, subcutaneously implanted with an estradiol 17β (Sigma)–containing Silastic capsule (Dow Corning) 10 mm in length [D. K. Sarkar and G. Fink, *J. Endocrinol.* **86**, 511 (1980)].
8. H. Schipper, J. R. Brawer, J. F. Nelson, L. S. Felicio, C. E. Finch, *Biol. Reprod.* **25**, 413 (1981); J. R. Brawer, F. Naftolin, J. Martin, C. Sonnenschein, *Endocrinology* **103**, 501 (1978).
9. C. W. Welsch, T. Jenkins, Y. Amenomori, J. Meites, *Experientia* **27**, 1350 (1971).
10. K. H. Lu, Y. Koch, J. Meites, *Endocrinology* **89**, 229 (1971); V. Raymond, M. Beaulieu, F. Labrie, J. Boissier, *Science* **200**, 1173 (1978); G. A. Gudelsky, D. D. Nansel, J. C. Porter, *Endocrinology* **108**, 440 (1981).
11. M. J. Cronin, C. Y. Chueng, R. I. Weiner, P. C. Goldsmith, *Neuroendocrinology* **34**, 140 (1982).
12. Inbred Wistar Furth cycling rats (2 to 3 months old) were given the transplants in the dorsal neck region. The rats became acyclic 2 to 3 weeks after receiving the transplant.
13. During the preparation of this report, a brief report appeared by J. W. Simpkins, C. A. Hodson, P. S. Kalra, and S. P. Kalra [*Life Sci.* **30**, 1349 (1982)], showing that transplantation of a MtT · W_{15} tumor reduced the concentration of dopamine in male rat hypothalamus.
14. G. A. Gudelsky, J. W. Simpkins, G. P. Mueller, J. Meites, *Neuroendocrinology* **22**, 206 (1976); O. M. Cramer, C. R. Parker, J. C. Porter, *Endocrinology* **105**, 636 (1979).
15. H. M. Lloyd, J. D. Meares, J. Jacobi, *Nature (London)* **255**, 497 (1975); M. Pawlikowski, *Life Sci.* **30**, 315 (1982).
16. M. F. El Etreby, in *Pituitary Microadenomas*, G. Faglia, M. A. Giovanelli, R. M. MacLeod, Eds. (Academic Press, New York, 1980), p. 211.
17. S. A. Fine and L. A. Frohman, *J. Clin. Invest.* **54**, 973 (1978).
18. D. S. Robinson *et al.*, *Lancet* **1972-I**, 290 (1972); P. L. McGeer and E. G. McGeer, *Adv. Exp. Med. Biol.* **113**, 41 (1978).
19. C. H. Cheng and G. F. Wooten, *J. Pharmacol. Methods* **3**, 333 (1980).
20. The illustrated sections of the arcuate nucleus and ME were removed 5.4 to 6.2 mm anterior to vertical plane [J. deGroot, *Trans. R. Neth. Acad. Sci.* **52**, 1 (1959)]. We studied changes of catecholamine fluorescence in all regions of the arcuate nucleus and ME and observed similar changes throughout.
21. Similar intensity of catecholamine fluorescence in the external layer of the ME was observed in 3-month-old diestrous Wistar Furth rats.
22. We are indebted to C. L. Chen for prolactin antibodies and to R. Echt for valuable technical advice. This work was supported by research grants CA10771 and AG00416 from the National Institutes of Health.

11 August 1982

Chromosomal Localization of the Human Homolog (*c-sis*) of the Simian Sarcoma Virus *onc* Gene

Abstract. *Nonrandom chromosome rearrangements of chromosome 22 have been identified in different human malignancies. As a result of Southern blot hybridization of a* c-sis *probe to DNA's from mouse-human somatic cell hybrids, the human homolog (*c-sis*) of the transforming gene of simian sarcoma virus was assigned to chromosome 22. Hybrids between thymidine kinase–deficient mouse cells and human fibroblasts carrying a translocation of the region q11-qter of chromosome 22 to chromosome 17 were also analyzed. These studies demonstrate that the human* c-sis *gene is on region 22q11>qter.*

The transforming genes of oncogenic retrovirus are derived from a set of cellular sequences known as cellular *onc* genes (*1*). These sequences are present in the genome of vertebrate species; their high degree of evolutionary conservation suggests that they code for proteins that are essential for cellular metabolism or for tissue differentiation (or both) (*1*). In one well-studied case it has been shown that the product of the cellular *onc* gene is very closely related to that of the viral *onc* gene (*2*). Moreover, the *onc* gene product is present at abnormally high levels in infected transformed cells (*2*). Thus, as an alternative to direct transformation by a viral *onc* gene, abnormal activation of a cellular *onc* gene may be involved in neoplastic transformation. At least three models can be proposed for such a mechanism. First, high levels of expression of a cellular *onc* gene may be caused by the insertion nearby of a viral promoter (*3*) or by alteration of the physiologic promoter by a mutagenic agent such as a chemical carcinogen. Second, a cellular *onc* gene may be expressed at high levels as a consequence of gene amplification (*4*). Third, a cellular *onc* gene may be relocated in a transcriptionally active region of the genome (*5*) as a consequence of chromosomal rearrangements. Since nonrandom chromosome rearrangements have been identified in several human malignancies (*6*), we can test the latter hypothesis by determining the chromosomal location of various human homologs of viral *onc* genes in normal and neoplastic cells. We now report on the chromosomal localization of the human homolog (*c-sis*) of the transforming gene (*v-sis*) of simian sarcoma virus (*7*). Knowledge of the chromosomal location of the *c-sis onc* gene should make it possible to determine whether the human *c-sis onc* gene can be activated by chromosome translocation or rearrangement in human malignancies.

We have shown that sequences homologous to the transforming gene of simian sarcoma virus are present in the

Table 1. Presence of the human *c-sis* gene in hybrid clones.

Human chromosome	Number of hybrid clones that are*			
	+/+	+/−	−/+	−/−
1	1	9	2	19
2	0	10	3	18
3	3	7	3	18
4	0	10	4	17
5	0	10	2	19
6	5	5	3	18
7	1	9	7	14
8	3	7	2	19
9	1	9	4	17
10	1	9	1	20
11	3	7	4	17
12	2	8	10	11
13	2	8	2	19
14	8	2	10	11
15	3	5	4	19
16	2	8	1	20
17	3	7	6	15
18	3	7	2	19
19	1	9	6	15
20	3	7	2	19
21	2	8	2	19
22	10	0	0	21
x	4	6	2	19

*+/+, Clones that contain the *c-sis* gene and the numbered chromosome; +/−, clones that contain the *c-sis* gene but not the numbered chromosome; −/+, clones that contain the numbered chromosome and do not contain the *c-sis* gene; −/−, clones that contain neither the numbered chromosome nor the *c-sis* gene.

0036-8075/82/1112-0686$01.00/0