poly(ADP-ribose) synthesis in intact cells versus permeable cells is unknown. Thus, achieving a concentration of exogenous 3AB sufficiently low to inhibit poly(ADP-ribose) synthesis, but to have no nonspecific effects in intact cells, may be difficult.

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References and Notes

1. H. Hilz and P. Stone, *Rev. Physiol. Biochem. Pharmacol.* **76**, 1 (1976); O. Hayaishi and K. Ueda, *Annu. Rev. Biochem.* **46**, 95 (1977); in *ADP-Ribosylation Reactions*, O. Hayaishi and K. Ueda, Eds. (Academic Press, New York, 1982), p. 3.
2. L. O. Burzio, S. S. Koide, P. Puigdomenech, A. Ruiz-Carrillo, in *Novel ADP-Ribosylations of Regulatory Enzymes and Proteins*, M. E. Smulson and T. Sugimura, Eds. (Elsevier/North-Holland, Amsterdam, 1980), p. 345; K. Yoshihara, Y. Tanigawa, L. Burzio, S. S. Koide, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 289 (1975); K. Yoshihara and S. S. Koide, *FEBS Lett.* **30**, 261 (1973); K. Yoshihara and Y. Tanaka, *J. Biol. Chem.* **256**, 6756 (1981); M. Nagao, M. Yamada, M. Miwa, T. Sugimura, *Biochem. Biophys. Res. Commun.* **48**, 219 (1972).
3. W. F. Morgan and J. E. Cleaver, *Mutat. Res.* **104**, 361 (1982); A. Oikawa, H. Tohda, M. Kanai, M. Miwa, T. Sugimura, *Biochem. Biophys. Res. Commun.* **97**, 1311 (1980); T.-A. Hori, *ibid.* **102**, 38 (1981).
4. F. Farzaneh, R. Zalin, D. Brill, S. Shall, *Nature (London)* **300**, 362 (1982).
5. F. R. Althaus, S. D. Lawrence, Y.-Z. He, G. L. Sattler, Y. Tsukada, H. C. Pitot, *ibid.*, p. 366; A. P. Johnstone and G. T. Williams, *ibid.*, p. 368.
6. S. Shall, in *ADP-Ribosylation Reactions*, O. Hayaishi and K. Ueda, Eds. (Academic Press, New York, 1982), p. 477.
7. G. G. Poirier, G. de Murcia, J. Jongstra-Bilen, C. Niedergang, P. Mandel, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3423 (1982).
8. D. Creissen and S. Shall, *Nature (London)* **296**, 271 (1982).
9. M. R. Purnell and W. J. D. Whish, *Biochem. J.* **185**, 775 (1980); S. Shall, *J. Biochem. (Tokyo)* **77**, 2p (1975).
10. J. L. Sims, G. W. Sikorski, D. M. Catino, S. J. Berger, N. A. Berger, *Biochemistry* **21**, 1813 (1982).
11. B. W. Durkacz, O. Omidiji, D. A. Gray, S. Shall, *Nature (London)* **283**, 593 (1980).
12. F. Wroblewski and J. S. LaDue, *Proc. Soc. Exp. Biol. Med.* **90**, 210 (1955).
13. B. R. Batchelor, J. S. Stern, P. R. Johnson, R. J. Mahler, *Metabolism* **24**, 77 (1975).
14. J. E. Cleaver, *Methods Cancer Res.* **11**, 123 (1975).
15. R. G. D. Steele and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960), p. 99.
16. A. T. Natarajan, I. Csukas, A. A. Van Zeeland, *Mutat. Res.* **84**, 125 (1981); R. Waters, F. Ramsay, I. Barrett, *Carcinogenesis* **3**, 1463 (1982); E. L. Jacobson, K. M. Antol, H. Juarez-Salinas, M. K. Jacobson, *J. Biol. Chem.* **258**, 103 (1983); B. W. Durkacz, J. Irwin, S. Shall, *Eur. J. Biochem.* **121**, 65 (1981); F. R. Althaus, S. D. Lawrence, G. L. Sattler, H. C. Pitot, *J. Biol. Chem.* **257**, 5528 (1982); K. Wielckens, A. Schmidt, E. George, R. Bredehorst, H. Hilz, *ibid.*, p. 12872; B. W. Durkacz *et al.*, *Biochem. Biophys. Res. Commun.* **101**, 1433 (1981).
17. N. L. Nolan and W. R. Kidwell, *Radiat. Res.* **90**, 187 (1982); M. R. James and A. R. Lehmann, *Biochemistry* **21**, 4007 (1982); L. A. Zwelling, D. Kerrigan, Y. Pommier, *Biochem. Biophys. Res. Commun.* **104**, 897 (1982).
18. N. A. Berger and G. W. Sikorski, *Biochem. Biophys. Res. Commun.* **95**, 67 (1980).
19. C. J. Skidmore *et al.*, *Eur. J. Biochem.* **101**, 135 (1979).
20. B. W. Durkacz, N. Nduka, O. Omidiji, S. Shall, A.-A. Zia'ee, in *Novel ADP-Ribosylations of Regulatory Enzymes and Proteins*, M. E. Smulson and T. Sugimura, Eds. (Elsevier/North-Holland, Amsterdam, 1980), p. 207.
21. N. Nduka, C. J. Skidmore, S. Shall, *Eur. J. Biochem.* **105**, 525 (1980).
22. C. Borek, W. F. Morgan, A. Ong, J. E. Cleaver, *Proc. Natl. Acad. Sci. U.S.A.*, in press; C. Borek, A. Ong, W. F. Morgan, J. E. Cleaver, *Radiat. Res.*, in press.
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Cerebellar Autonomic Function: Direct Hypothalamocerebellar Pathway

Abstract. *A direct hypothalamocerebellar projection in the cat was revealed by means of retrograde transport of wheat germ agglutinin–horseradish peroxidase complex. This appears to be the first demonstration of a significant autonomic input to the cerebellum. The projection has a widespread origin and is bilateral with an ipsilateral preponderance.*

Cerebellar influences on visceral functions, as described by Moruzzi (*1*) more than 30 years ago, have been recently confirmed (*2*). Although a cerebellohypothalamic pathway (from the fastigial nucleus) has been described (*3*), other projections participating in cerebellar autonomic control remain essentially unknown. In particular, information on input to the cerebellum from autonomic centers is lacking. A highly sensitive wheat germ agglutinin–horseradish peroxidase (WGA-HRP) complex, used as a retrograde tracer, revealed a projection from the hypothalamus to the cerebellar cortex.

Seven cats (2.1 to 3.1 kg) were anesthetized with pentobarbital sodium (Mebumal), and 0.2 μl of 1 percent WGA-HRP (Sigma) was injected into the cerebellar cortex through a 1-μl Hamilton syringe. Six cats each received two injections (altogether 0.4 μl of WGA-HRP), and the seventh received four injections in different cortical folia (altogether 0.8 μl of WGA-HRP). After 2 days, the cats, while under deep pentobarbital anesthesia, were killed by intracardiac perfusion with physiological saline; this was followed by perfusion with a solution containing 1.25 percent glutaraldehyde and 1 percent paraformaldehyde in 0.01*M* phosphate buffer at *p*H 7.4, and finally with a solution of 10 percent sucrose. The brain was immediately isolated, removed, and placed in 30 percent sucrose. The cerebellum was cut frontally or sagittally and the brainstem was cut transversally in serial sections at 50 μm on a freezing microtome. Two of five consecutive sections were mounted. The sections were treated with tetramethylbenzidine as a chromogen (*4*). One series was weakly stained with neutral red and the other was left unstained. Several normal animals that served as controls were processed in the same manner as the experimental animals. No labeled hypothalamic neurons were found in the controls.

The sections from the experimental animals (and the controls) were examined with bright-field and interference-contrast microscopy. In five cats the

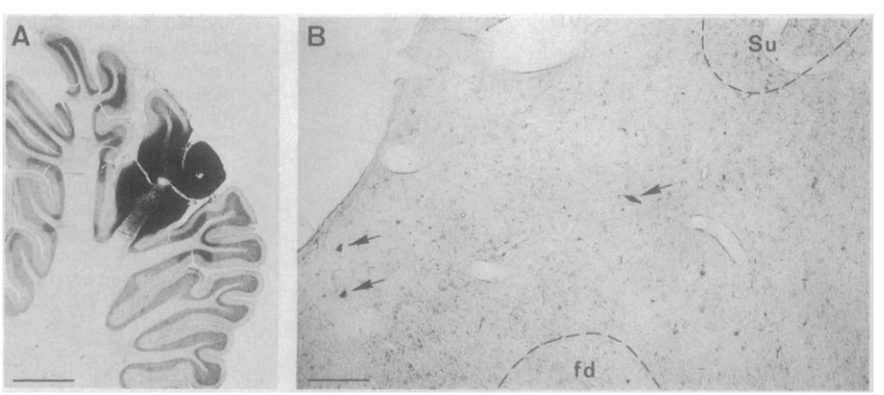

Fig. 1. (A) Photomicrograph showing the cerebellar cortical injection site in cat B.St.L.974 (injected in the anterior lobe). Scale bar, 1.5 mm. Patches of dark staining in the granular layer on both sides of the two injected folia are excess deposits of neutral red. (B) Photomicrograph showing three retrogradely labeled neurons (arrows) in the lateral hypothalamic area in cat B.St.L.977. Scale bar, 150 μm. For abbreviations, see Fig. 2.

injections were confined to the cerebellar cortex and the folial white matter (Fig. 1A). The other two animals had additional staining of adjacent parts of the central white matter, but the cerebellar nuclei were not involved. The size and distribution of retrogradely labeled neurons in the hypothalamus were mapped and later transferred to drawings representing equal intervals through the hypothalamus. The delineation of the various hypothalamic nuclei was based on the description by Bleier (*5*).

Retrogradely labeled hypothalamic neurons were found in all experimental animals [injections in the anterior lobe (vermal and intermediate cortex of lobules IV and V), the posterior lobe vermis (lobule VII), lobulus simplex, crus I, crus II, and the paramedian lobule]. In one animal (*6*), 222 retrogradely labeled hypothalamic neurons were counted. Most labeled cells were located within lateral hypothalamic areas, although several neurons were also found in dorsal and posterior hypothalamic areas and in periventricular and supraoptic nuclei. In addition, a few cells were located just inside the borders of the tuberomammillary, lateral mammillary, and ventromedial nuclei and tuber cinereum. The hypothalamocerebellar projection appears to be bilateral, although after unilateral injections most of the labeled cells were on the ipsilateral side. These labeled neurons usually had a maximum diameter between 20 and 30 μm. The hypothalamic localization of some retrogradely labeled cells is shown for cat B.St.L.977 (Figs. 1B and 2).

The absence of labeled cells in the controls shows that the stained hypothalamic neurons in the experimental animals are the result of retrogradely transported WGA-HRP and not endogenous peroxidase activity. Transneuronal transport of WGA-HRP could also explain these findings. However, only anterograde transneuronal transport has been described in mammals (*7*) and only after injections of large amounts of WGA-HRP in the visual system. Furthermore, experiments in our laboratory have failed to show such transport in various fiber systems at survival times and injected quantities comparable to those used in the present study.

Neuronal labeling as a result of vascular transport of WGA-HRP is not likely to have occurred during this study. The cerebellar injections were made superficially with visual guidance, and special care was taken to avoid larger vessels. Furthermore, none of the animals showed perivascular labeling in the hypothalamus as evidence for uptake of WGA-HRP from the vascular system. One additional control animal received a vascular injection of 0.4 μl of WGA-HRP. This cat showed no stained hypothalamic neurons. Uptake of WGA-HRP from the cerebrospinal fluid can also be excluded, since the staining at the injection sites was confined to the cerebellar cortex and did not approach the fourth ventricle.

Retrogradely labeled cells in the hypothalamus after cerebellar injections have been described in reptiles (*8, 9*). However, some of the authors of these studies considered the staining of hypothalamic neurons to result from the spread of tracer to the locus ceruleus (*8*). An involvement of this nucleus can be definitely excluded in the present study since the WGA-HRP injections were restricted to the cerebellar cortex.

The finding of a hypothalamocerebellar projection in the cat was unexpected, even though a cerebellohypothalamic projection from the fastigial nucleus had been described (*3*). A study of the latter projection with modern tracer techniques would provide information concerning its quantity, sites of termination, and potential interaction with the hypothalamocerebellar pathway (*10*). The hypothalamocerebellar projection and its potential reciprocal (*3*) provide circuits through which autonomic hypothalamic function could influence, and be influenced by, the cerebellum.

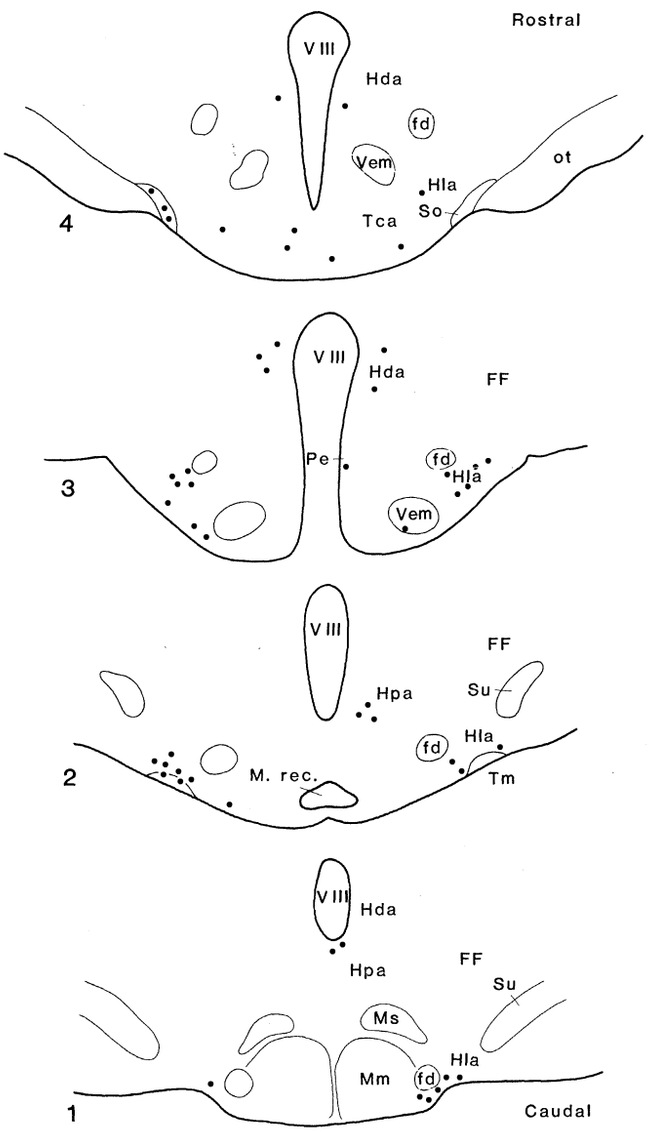

Fig. 2. Diagram showing the location of some of the retrogradely labeled neurons in cat B.St.L.977 as seen in four equidistant transversal sections through the hypothalamus. Abbreviations: *fd*, descending column of the fornix; *FF*, nucleus of the fields of Forel; *Hda*, dorsal hypothalamic area; *Hla*, lateral hypothalamic area; *Hpa*, posterior hypothalamic area; *Mm*, medial mammillary nucleus; *M.rec.*, mammillary recess of the third ventricle; *Ms*, supramammillary nucleus; *ot*, optic tract; *Pe*, periventricular nucleus; *So*, supraoptic nucleus; *Su*, subthalamic nucleus; *Tca*, area of the tuber cinereum; *Tm*, tuberomammillary nucleus; *Vem*, ventromedial nucleus; and *V III*, third ventricle.

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References and Notes

1. G. Moruzzi, *Problems in Cerebellar Physiology* (Thomas, Springfield, 1950), pp. 74–96.
2. See, for example, F. R. Calaresu, A. A. Faiers, G. J. Mogenson, *Prog. Neurobiol.* **5**, 1 (1975); J. Martner, *Acta Physiol. Scand. Suppl.* **425**, 1 (1975).
3. J. W. Harper and R. G. Heath, *Exp. Neurol.* **39**, 285 (1973).
4. M.-M. Mesulam, *J. Histochem. Cytochem.* **26**, 106 (1978).
5. R. Bleier, *The Hypothalamus of the Cat* (Johns Hopkins Univ. Press, Baltimore, 1961).

6. B.St.L.977; WGA-HRP (0.2 μl) was injected four times in the posterior lobe.
7. J. D. Coulter, M. C. Sullivan, M. A. Ruda, *Soc. Neurosci. Abstr.* **6**, 339 (1980); S. K. Itaya and G. W. van Hoesen, *Brain Res.* **236**, 199 (1982).
8. G. C. Bangma and H. J. ten Donkelaar, *J. Comp. Neurol.* **207**, 255 (1982).
9. H. Künzle, *Exp. Brain Res.* **49**, 1 (1983).
10. A potential reciprocal circuit between the ventromal cortex and hypothalamus may be completed by cerebellar corticonuclear fibers to the fastigial nucleus.
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Antigenic Similarity Between Cells Transformed by Ultraviolet Radiation in vitro and in vivo

Abstract. *Cells of the 10T 1/2 mouse fibroblast line transformed in vitro by ultraviolet radiation are antigenically similar to those from skin cancers produced in mice by repeated exposure to ultraviolet radiation. Both types of tumor cells grew preferentially in ultraviolet-irradiated syngeneic mice relative to untreated animals, and both were recognized by ultraviolet radiation–induced tumor-specific suppressor lymphocytes. These properties were not shared by 10T 1/2 cells transformed in vitro by x-rays or 3-methylcholanthrene.*

Skin cancers induced in mice by repeated exposure to ultraviolet (UV) radiation have two unusual immunological properties. First, these tumors are highly antigenic, in that most are rejected immunologically when transplanted into normal syngeneic mice. Second, they grow progressively in syngeneic mice that have been given a small number of exposures to UV radiation (*1*). The susceptibility of the UV-irradiated mice to transplanted tumors is caused, at least in part, by the suppressor T lymphocytes in their lymphoid organs, which prevent immunological rejection of the tumors (*2*). These lymphocytes are specific for UV radiation–induced tumors as a group, as evidenced by the finding that they do not decrease the resistance of the host to transplanted syngeneic tumors induced by other carcinogens (*3*). We investigated whether the antigenic determinant on UV radiation–induced tumor cells that is recognized by the UV radiation–induced suppressor lymphocytes arises as a consequence of exposing the cells to UV radiation or whether this determinant is present normally on cells of a particular lineage in the skin that are highly susceptible to transformation by UV radiation but less susceptible to transformation by other carcinogens.

We used tumor cell lines produced by in vitro transformation of cloned mouse fibroblasts by UV radiation, 3-methylcholanthrene (MCA), or x-rays. The development of these cell lines was compared in normal, UV-irradiated, and immunosuppressed mice. Only the tumor lines produced by transformation with UV radiation grew preferentially in UV-irradiated mice relative to untreated animals, and this preferential growth was attributable to the activity of the UV radiation–induced suppressor T lymphocytes. The results suggest that exposure of cells to UV radiation induces a carcinogen-associated determinant (or set of determinants) against which the UV radiation–induced suppressor T lymphocytes react, and that there is a similarity between UV radiation–induced transformation in vitro and UV carcinogenesis in vivo.

The mouse fibroblast cell line used was C3H 10T 1/2 clone 8 (*4*). Transformants were generated independently by a single exposure to x-rays, MCA, or 254-nm UV radiation (20 or 30 J/m^2 from a germicidal lamp) (*5*). Clones TU2 and TU3 were derived from type III foci, which are usually highly tumorigenic; clone TU4 was derived from a type II focus. Type II foci generally are composed of cells that are less tumorigenic than those in type III foci (*4*). Both of the cell lines obtained from x-ray–transformed clones were derived from type III foci generated by 600 R of x-radiation. The four MCA-transformed cell lines also originated from type III foci. Transformants, which grew as dense foci on top of a contact-inhibited monolayer of normal cells, were isolated with stainless steel cloning cylinders. These cells were propagated in culture to provide sufficient quantities for injection into mice. The minimum tumorigenic dose of

Table 1. Growth in syngeneic mice of C3H 10T 1/2 cells transformed in vitro by MCA, x-rays, or UV radiation.

Carcinogen	Cell line	Number of cells injected	Tumor incidence in syngeneic mice		
			Untreated	UV-irradiated*	Thymectomized and x-irradiated (450 R)
None	10T 1/2, clone 8	1×10^8	0/10	0/10	0/10
MCA	PT	1×10^3	3/10	3/10	9/10
	16	1×10^3	2/10	3/10	10/10
	609C-2	1×10^5	1/10	0/10	6/10
	5	1×10^4	4/10	3/10	10/10
Total (%)			10/40 (25)	9/40 (23)	35/40 (88)
X-radiation	F11	5×10^3	5/10	5/10	10/10
	F17	1×10^5	0/10	0/10	4/10
Total (%)			5/20 (25)	5/20 (25)	14/20 (70)
UV radiation	TU2	1×10^6	0/10	9/10	8/10
	TU3	1×10^4	1/10	6/10	10/10
	TU4	5×10^6	0/10	2/10	2/10
Total (%)			1/30 (3)	17/30 (57)	20/30 (67)

*Exposed to sunlamp radiation three times per week for at least 12 weeks.

Table 2. Effect of UV radiation–induced suppressor lymphocytes on the growth of C3H 10T 1/2 fibroblasts transformed in vitro.

Cells used to reconstitute lethally x-irradiated mice	Tumor incidence in mice challenged with		
	UV radiation–induced sarcoma cells (1×10^6)*	UV radiation–transformed 10T 1/2 cells (1×10^6)†	MCA-transformed 10T 1/2 cells (1×10^4)
Normal lymphoid cells (5×10^7)	0/10	0/10	4/10
UV-irradiated lymphoid cells (5×10^7)‡	10/10	8/10	4/10
Normal cells (5×10^7) plus UV-irradiated lymphoid cells (5×10^7)‡	5/10	5/10	5/10

*Derived from a tumor induced by repeated irradiation of a C3H mouse with FS40 sunlamps. †10T 1/2 cells transformed in vitro with 254-nm UV radiation. ‡Spleen and lymph node cells from mice exposed to sunlamp radiation four times per week for at least 12 weeks.