12. S. M. Hollenberg *et al.*, *Nature (London)* **318**, 635 (1985).
13. P. Walter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7889 (1985); S. Green *et al.*, *Nature (London)* **320**, 134 (1986); G. L. Greene *et al.*, *Science* **231**, 1150 (1986).
14. B. DeBuire *et al.*, *Science* **224**, 1456 (1984).
15. A. Krust, S. Green, P. Argos, V. Kumar, P. Walter, S. M. Bornert, P. Chambon, *EMBO J.* **5**, 891 (1986).
16. N. L. Weigel *et al.*, *J. Biol. Chem.*, in press.
17. C. Weinberger, S. M. Hollenberg, M. G. Rosenfeld, R. M. Evans, *Nature (London)* **318**, 670 (1985).
18. D. A. Melton *et al.*, *Nucleic Acids Res.* **12**, 7035 (1984).
19. P. E. Elder, R. A. Pickett, II, J. Hampton, R. A. Lerner, *J. Biol. Chem.* **252**, 6510 (1977).
20. We thank T. G. Beito and C. J. Krco for their participation in the preparation of antibodies.

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Distinct Pathways of Viral Spread in the Host Determined by Reovirus S1 Gene Segment

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The genetic and molecular mechanisms that determine the capacity of a virus to utilize distinct pathways of spread in an infected host were examined by using reoviruses. Both reovirus type 1 and reovirus type 3 spread to the spinal cord following inoculation into the hindlimb or forelimb footpad of newborn mice. For type 3 this spread is through nerves and occurs via the microtubule-associated system of fast axonal transport. By contrast, type 1 spreads to the spinal cord through the bloodstream. With the use of reassortant viruses containing various combinations of double-stranded RNA segments (genes) derived from type 1 and type 3, the viral S1 double-stranded RNA segment was shown to be responsible for determining the capacity of reoviruses to spread to the central nervous system through these distinct pathways.

FOR A VIRUS TO PRODUCE SYSTEMIC illness, it must first spread from its site of initial entry and primary replication in the host to distant target tissues (*1*). This aspect of viral pathogenesis is particularly well exemplified by neurotropic viruses which, after entering the host through a number of divergent portals (for example, respiratory, gastrointestinal, or venereal), subsequently spread to reach the central nervous system (CNS) (*2*). Two principal pathways of spread to the CNS have been identified for neurotropic viruses. Most neurotropic viruses (for example, arboviruses, enteroviruses, measles virus, mumps virus, and lymphocytic choriomeningitis virus) spread through the bloodstream to reach the CNS (hematogenous spread), but several viruses, including rabies virus, herpes simplex virus, herpesvirus simiae, and poliovirus can reach the CNS by traveling through nerves (*2, 3*). Almost nothing is known about the viral genes and proteins responsible for determining the capacity of viruses to spread through specific pathways within the infected host. Similarly, the precise cellular mechanisms utilized by viruses as they spread have not been identified.

The mammalian reoviruses are neurotropic viruses that have been useful in identifying genetic and molecular mechanisms of viral pathogenesis (*4*). For example, the reovirus hemagglutinin has been identified as the viral cell attachment protein that determines the capacity of reoviruses to bind to red blood cells, lymphocytes, and specific populations of cells within the CNS (*5–7*). The viral hemagglutinin has also been shown to mediate binding of reoviruses to isolated microtubule preparations in vitro (*8*). Our prior success in studying viral pathogenesis by using reoviruses encouraged us to investigate viral-host interactions involved in viral spread.

We report that two serotypes of reovirus spread by different pathways to reach the CNS after peripheral (forelimb or hindlimb footpad) inoculation into newborn mice. Reovirus type 3 spreads through nerves to reach the spinal cord after footpad inoculation, and this spread is mediated by the microtubule-associated system of fast axonal transport. In contrast, reovirus type 1 spreads to the spinal cord through hematogenous pathways. By using reassortant reoviruses containing specific combinations of double-stranded RNA (dsRNA) segments (genes) derived from type 1 and type 3, we have been able to show that the reovirus S1 dsRNA segment, which encodes the viral hemagglutinin, is responsible for determining the capacity of these viruses to spread by different routes to the CNS.

Reovirus type 3 is a neurotropic virus that infects neurons and produces a lethal necrotizing encephalitis after intracerebral inoculation into newborn mice (*6, 7, 9*). Following intracerebral inoculation, type 3 is able to spread to the retina, where it selectively infects and injures retinal ganglion cells (*10*). The axons of retinal ganglion cells, which form the optic nerve, synapse directly on intracerebral neurons. These facts suggested that type 3 is capable of being transported via nerves within the CNS and that a similar type of transport could be used by type 3 to spread to the CNS after its entry at a peripheral site. Type 1, in distinction to type 3, does not infect neurons or retinal ganglion cells after intracerebral inoculation, but instead infects ependymal cells and produces hydrocephalus (*6, 7, 10, 11*). The failure of type 1 to infect neurons suggested that this reovirus would utilize nonneural pathways to spread to the CNS.

To investigate our hypothesis that types 1 and 3 use different pathways of spread to the CNS, we took advantage of the fact that the motor and sensory neurons innervating the hindlimb and forelimb footpads are located in different regions of the spinal cord. If type 3 spreads via nerves, it should appear preferentially in the region of the spinal cord containing the neurons innervating the skin and musculature at the site of viral inoculation. If type 1 spreads through the bloodstream, it should appear in all regions of the spinal cord in equivalent amounts and with similar kinetics. Therefore, we injected type 3 and type 1 into the forelimb and hindlimb footpads of neonatal mice and studied the pattern of spread of these two viruses to the spinal cord. We found that after either hindlimb or forelimb inoculation, type 3 appeared first, and in up to 1000-fold higher titer, in the region of the spinal cord innervating the injected limb (Fig. 1, A and B). In contrast, type 1, after either hindlimb or forelimb inoculation, appeared at essentially the same time and in equivalent titer in all regions of the spinal cord (Fig. 2, A and B). These results support our hypothesis that type 3 spreads through nerves, and type 1 via the bloodstream, to reach the CNS.

To confirm these results, we studied the spread of type 1 and 3 from the hindlimb to the spinal cord in neonatal mice after section of the sciatic nerve. Since the sciatic nerve is the principal neural pathway from the hindlimb to the spinal cord, its section should completely prevent spread of virus from the hindlimb through nerves but should not affect its capacity to spread through the bloodstream. As predicted, sciatic nerve section completely inhibited spread of type 3 to the spinal cord. Nerve section contralateral to the injected limb and sham operation had no significant effect on the spread of type 3 (Fig. 1C). Section of the sciatic nerve had

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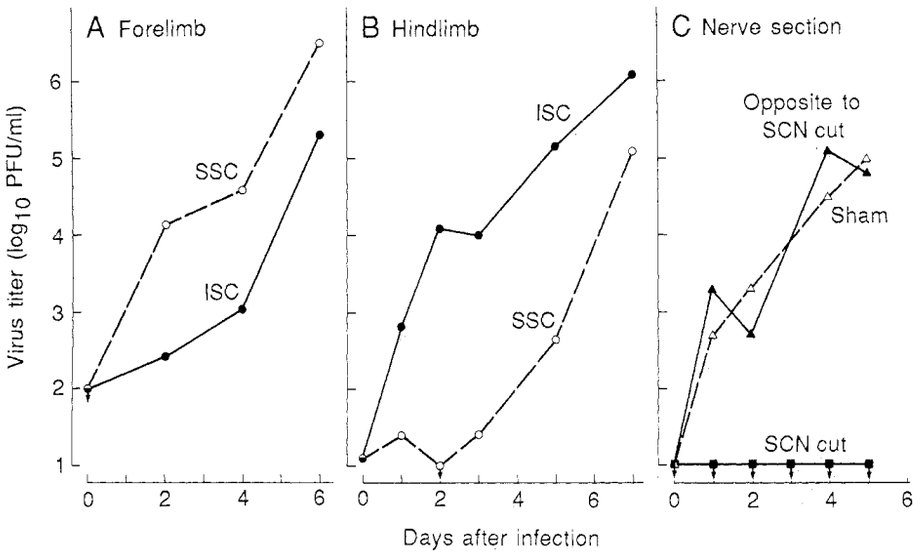

Fig. 1. Pattern of spread of reovirus type 3 to the spinal cord of neonatal mice after inoculation of the virus into either the forelimb (A) or hindlimb (B) footpad. (○) Superior spinal cord (SSC); (●) inferior spinal cord (ISC). (C) Sciatic nerve section in limb ipsilateral (■) or contralateral (▲) to virus inoculation. (△) Sham operation. A downward-pointing arrow indicates that no virus was detected at the lowest dilution shown. (A–C) A Hamilton syringe equipped with a 30-gauge needle was used to inoculate 1-day-old NIH Swiss mice with 2.2×10^5 to 4.5×10^5 PFU of virus in a volume of 0.010 ml. Spinal cord blocks were removed by microdissection with the aid of a stereomicroscope. Superior spinal cord blocks (SSC) included the cervical cord segments, and inferior spinal cord blocks (ISC) included the thoracic and lumbosacral cord segments. Virus titers were determined by plaque assay on mouse L cells (*10*). Specimens were titered in duplicate at serial tenfold dilutions. Plotted points represent the average of the log_{10} titer (measured in plaque-forming units per milliliter) of 9 to 14 specimens (days 0, 1, 3, and 5) or of 4 to 7 specimens (days 2 and 7). The standard error of the mean (SEM) is less than 0.3 for ISC and less than 0.5 for SSC at all time points. (C) Animals, method, and dose of virus and procedure for titration of tissue blocks were as described above. Sciatic nerve section was performed on anesthetized (methoxyflurane, Pitman-Moore) 1-day-old mice by microsurgical techniques. Adequacy of section was demonstrated by the postoperative finding of hindlimb analgesia and paresis. Virus was inoculated 24 hours after nerve section or sham operation. Sham operation was identical to the operation for nerve section except the nerve was not cut. Each plotted point represents the log_{10} titer of duplicate assays of the ISC tissue block from a single animal. The SEM was less than 0.5 for controls at all time points.

no significant effect on the spread of type 1 (Fig. 2C). Thus, these experiments establish that type 3 spreads to the CNS through nerves and that type 1 uses nonneural, hematogenous pathways.

Although our experiments indicated that type 3 spread to the CNS via nerves, they did not identify the transport mechanism involved. The time of initial appearance of type 3 in the spinal cord (<24 hours), and the distance between the site of viral inoculation and the spinal cord (approximately 14 mm), enabled us to calculate that the minimum transport velocity of type 3 was >14 mm/day. This suggested that its movement was via fast axonal transport. We therefore studied the spread of type 3 in neonatal mice treated with selective inhibitors of axonal transport. Colchicine interrupts fast axonal transport by causing a reversible dissociation of microtubules into their component tubulin monomers (*12*). We treated 1-day-old mice with a single intramuscular injection of colchicine (2×10^{-7} g per gram of body weight). These mice frequently (50% to 80%) developed paresis and analgesia of the injected hindlimb. Paresis and analgesia were maximal 48 hours after injection and had completely disappeared in all animals within 5 to 7 days, indicating that the clinical effect of a single dose of colchicine was transient and fully reversible. Twenty-four hours after colchicine treatment, we injected type 3 into the hindlimb footpad and studied the spread of virus to the spinal cord. In colchicine-treated mice, inhibition of the spread of virus to the spinal cord lasted for at least 3 days (Fig. 3A). By day 5, viral titers in the spinal cord were similar in colchicine-treated and untreated control animals. Multiple doses of colchicine produced a more profound inhibition of spread of type 3 (Fig. 3B). Thus, colchicine was capable of inhibiting the neural spread of type 3, and when only a single dose of colchicine was administered, this effect was fully reversible.

We tested the possibility that colchicine might be inhibiting the capacity of type 3 to spread through nerves by a mechanism other than inhibition of fast axonal transport. To ensure that colchicine was not inhibiting the growth of virus at its site of primary replication in skeletal muscle, we measured viral titers in hindlimb muscles from colchicine-treated and control animals. There was no significant difference between the growth of type 3 in the hindlimb muscles of colchicine-treated [mean viral titer, 4.7 to 5.7 log_{10} plaque-forming units (PFU) per milliliter; $n = 24$] and untreated control (4.5 to 5.0 log_{10} PFU/ml; $n = 47$) animals. To further establish the specificity of action of colchicine on neural spread, we also studied its effect on the spread of reovirus type 1 to the spinal cord. Colchicine had no significant effect on the spread of type 1 (Fig. 3C). In vitro studies have shown that colchicine does not affect the infectivity of reoviruses in cell-free fluids (*13*), or in a variety of cul-

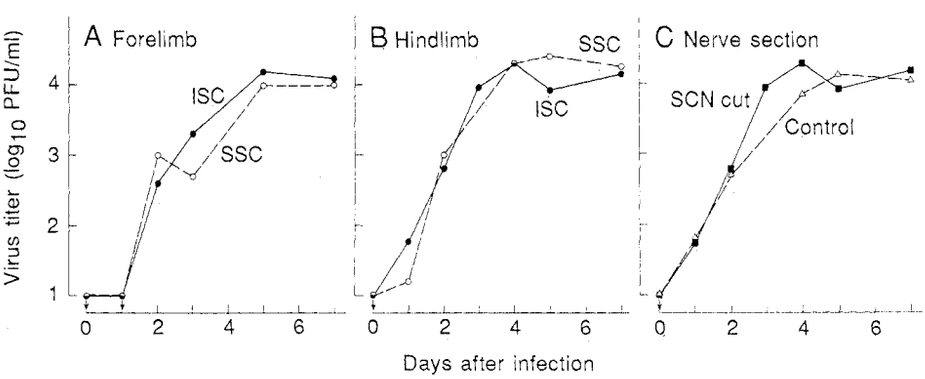

Fig. 2. Pattern of spread of reovirus type 1 to the spinal cord of neonatal mice after inoculation of the virus into the forelimb (A) or hindlimb (B) footpad. (○) Superior spinal cord (SSC); (●) inferior spinal cord (ISC). Each plotted point represents the average log_{10} titer of five to seven specimens. The SEM is less than 0.6 for SSC and ISC at all time points. (C) Spread of type 1 to the ISC after sectioning of the sciatic nerve (■) is compared with spread in control animals with intact nerves (△). A downward-pointing arrow below a data point indicates that no virus was detected at the lowest dilution. Dose of virus, method of inoculation, animals, and details of procedure are identical to those described for Fig. 1.

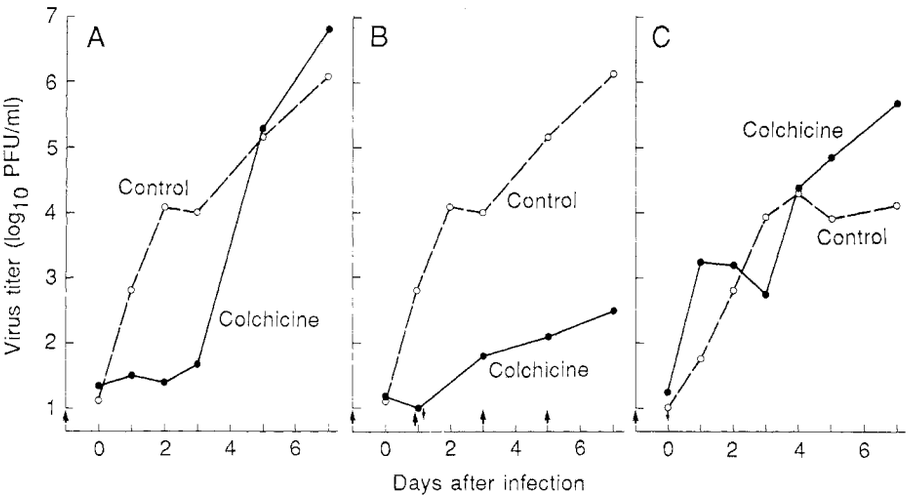

Fig. 3. Pattern of spread of type 3 (A and B) or type 1 (C) to the inferior spinal cord in colchicine-treated (●) and untreated control (○) mice. Upward-pointing arrows above the time scale indicate time of colchicine administration. Colchicine (J. T. Baker)-treated mice received 2×10^{-7} g of colchicine per gram of body weight. For studies of the effect of a single dose of colchicine (A and C), 1-day-old mice were given 0.010 ml of a $5 \times 10^{-5}M$ (20×10^{-6} g/ml) solution of colchicine into a hindlimb muscle by means of a 30-gauge needle and a Hamilton microsyringe. In (B) animals received 2×10^{-7} g of colchicine per gram of body weight; either a $5 \times 10^{-5}M$ colchicine solution (ages 1 and 3 days) or a $1 \times 10^{-4}M$ solution (ages 5 and 7 days) was used. Only mice showing evidence of hindlimb analgesia and paresis were used for the colchicine multidose experiments. Virus was inoculated in a 0.010- to 0.015-ml volume into the hindlimb footpad 24 hours after the first colchicine dose or at age 2 days (controls). The dose of virus was 3.8×10^5 to 4.5×10^5 PFU. Tissue specimens were collected and assayed as described in Fig. 1. For colchicine-treated animals, each plotted point represents the average of the log_{10} titer (plaque-forming units per milliliter) of ISC specimens from two to four animals (A and C) or five to seven animals (B). Controls for (A) and (B) are identical to those in Fig. 1. Each plotted point in (C) represents the average of two to four specimens. For colchicine-treated animals, the SEM was less than 0.3 at all time points in (A) and less than 0.5 for all time points in (B) and (C).

tured cell lines (*8, 13, 14*), and that it does not affect the binding of type 3 to its receptor on T lymphocytes (*15*). Thus, these studies indicate that colchicine is not acting by inhibiting the capacity of type 3 to infect cells or its primary replication in muscle; its effect occurs specifically on the spread of type 3 through nerves.

The nature of the colchicine-induced inhibition of axoplasmic transport in vivo varies with the dose and method of administration. The doses and concentrations of colchicine used in our experiments were in the range required for selective and reversible inhibition of fast axonal transport (*16, 17*) and were far below the threshold for degeneration of nerves (*18*). The complete reversibility of the inhibition of spread of type 3 caused by a single dose of colchicine (Fig. 3A), and the complete and rapid reversibility of its clinical effects (hindlimb analgesia and paresis), provide direct evidence that in our studies colchicine did not induce nerve degeneration. Our results indicate that the inhibitory effect of colchicine was due to its selective action on fast axonal transport.

To evaluate the role of slow axonal transport in the spread of type 3, we studied the effect of a selective inhibitor of slow axonal transport, β-β′-iminodipropronitrile (IDPN) (*19*), on the spread of type 3 to the spinal cord. The dose of IDPN selected for these experiments with neonatal mice (1×10^{-3} g per gram of body weight) was substantially higher than that shown to be effective in inhibiting slow axonal transport in adult mice (*19*), and was close to the median lethal dose (LD_{50}) of IDPN (1.3×10^{-3} to 2×10^{-3} g per gram of body weight) for neonatal mice. IDPN had no significant effect on the spread of type 3 to the spinal cord (Fig. 4).

To identify the role of specific viral

Table 1. Pattern of spread of type 1 (T1), type 3 (T3), and reovirus reassortants to the CNS. Genome segments originating from type 1 (or type 3) are designated simply as 1 (or 3).

Virus	Origin of genome segment										Differences (log_{10}) in viral titer in inferior and superior spinal cord after injection			Pattern of spread
	Outer capsid			Core					Nonstructural		F*	H†	Mean	
	M2	S1	S4	L1	L2	L3	M1	S2	M3	S3				
EB144	3	1	1	1	1	1	1	1	3	3	−0.80	0.48	−0.16	Hematogenous
EB126	1	1	1	3	3	3	3	3	1	3	0.10	0.10	0.10	Hematogenous
H24	1	1	3	1	1	1	1	1	1	1	0.17	0.21	0.19	Hematogenous
3HA1	3	1	3	3	3	3	3	3	3	3	0.22	0.40	0.31	Hematogenous
T1(Lang)	1	1	1	1	1	1	1	1	1	1	0.60	0.30	0.45	Hematogenous
H17	3	1	1	3	3	3	1	3	3	3	0.38	0.56	0.47	Hematogenous
EB145	3	1	3	3	3	3	3	3	1	3	0.57	0.62	0.60	Hematogenous
E3	1	3	3	3	3	3	3	3	3	3	1.40	2.82	2.11	Neural
H30	3	3	3	3	3	3	3	3	3	1	2.26	2.22	2.24	Neural
T3(Dearing)	3	3	3	3	3	3	3	3	3	3	1.75	3.03	2.39	Neural
EB143	1	3	1	3	1	1	1	1	1	1	3.06	2.13	2.60	Neural
1HA3	1	3	1	1	1	1	1	1	1	1	1.92	3.35	2.64	Neural
G2	1	3	1	1	3	1	1	1	1	1	3.21	2.42	2.82	Neural
T1 total	7	7	7	5	5	6	7	6	7	6				
T3 total	6	6	6	8	8	7	6	7	6	7				

*Maximum difference in log_{10} of the mean viral titer in the superior spinal cord and log_{10} of the mean viral titer in the inferior spinal cord at day 2 or 3 after forelimb inoculation of virus. Mean viral titer is measured as plaque-forming units per milliliter. †Maximum difference in log_{10} of the mean viral titer in the inferior spinal cord and log_{10} of the mean viral titer in the superior spinal cord at day 2 or 3 after hindlimb inoculation of virus.

dsRNA segments in determining the capacity of type 1 and type 3 to spread by different routes to the CNS, we studied the pattern of spread of 11 reassortant viruses containing various combinations of genes derived from type 1 and type 3. Earlier experiments with type 1 and type 3 showed that when virus spread via neural routes to the spinal cord after hindlimb or forelimb footpad inoculation, the maximum difference in viral titer between the inferior and superior spinal cord occurred 48 to 72 hours after viral inoculation. Therefore, we inoculated each reassortant virus into the hindlimb and forelimb footpads of separate litters of neonatal mice and assayed viral titers in the superior and inferior spinal cord 48 and 72 hours after viral inoculation. A large difference in mean titer (more than 100-fold) between the superior and inferior spinal cord would be indicative of neural spread, whereas a small difference (less than fourfold) would be indicative of hematogenous spread. All reassortant viruses containing a type 3 S1 dsRNA segment showed a neural pattern of spread to the spinal cord, whereas all reassortants containing a type 1 S1 dsRNA segment showed a hematogenous pattern of spread to the spinal cord (Table 1). The other nine reovirus dsRNA segments are equally represented in the neurally and hematogenously spreading reassortants (Table 1). If the parental origin (type 1 or type 3) of each gene segment for all the reassortants is analyzed, there is an equal representation of both parental genotypes, indicating that the genetic mapping was not the result of any preexisting bias favoring the selection of specific genes.

The S1 dsRNA segment of reovirus type 1 and type 3 has been cloned and sequenced (*20*). The messenger RNA derived from this dsRNA segment is functionally dicistronic and directs the synthesis of two distinct reovirus proteins (*21*). A long open reading frame encodes the 418– to 433–amino acid viral hemagglutinin, sigma 1. An overlapping short open reading frame encodes a second 119– to 120–amino acid basic protein referred to as σ_{1bNS} or as p12 (type 1) or p14 (type 3). This small protein is not a structural component of the virion but it is found in infected cells in vivo (*21*).

Although our genetic analysis does not permit a definitive conclusion about which of the two S1 encoded proteins is responsible for determining the pattern of spread of reoviruses, the viral hemagglutinin appears to be the logical candidate. The hemagglutinin is the viral cell attachment protein that determines the tropism of reovirus type 3 for neurons and retinal ganglion cells and the tropism of reovirus type 1 for ependymal and anterior pituitary cells (*6, 7, 10, 22*). The hemagglutinin also mediates the binding of purified reovirions to isolated microtubule preparations in vitro (*8*). Passive immunization of mice with monoclonal antibodies (mAb's) directed against the type 3 hemagglutinin, but not with mAb's to type 1 hemagglutinin, inhibits the neural spread of type 3 (*23*).

Our results indicating that type 3 uses the microtubule-associated system of fast axonal transport provide an explanation for the functional significance of the close association between reovirus particles and microtubules in infected cells (*24*). The form in which type 3 is transported within nerves and the mechanism of transsynaptic transfer of virus remain important unanswered questions.

We have shown that type 1 spreads to the CNS through the bloodstream. This conclusion is in accord with our previous data concerning spread of this virus to extraintestinal organs following peroral inoculation in adult mice (*25*). Type 1 could be transported through the bloodstream either free in the plasma or in association with cellular elements. After type 1 has spread to the CNS via the bloodstream, it must still cross the blood-brain barrier in order to reach ependymal cells. The form in which type 1 is carried in the blood and the exact pathway used by type 1 to travel from the bloodstream to reach ependymal cell targets in the CNS are unknown.

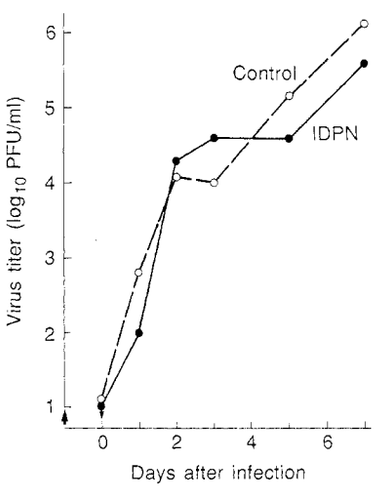

Fig. 4. Spread of type 3 to the inferior spinal cord (ISC) in IDPN-treated (●) and untreated control (○) animals. The downward-pointing arrow below the first time point indicates that no virus was detected at the lowest dilution shown. An upward-pointing arrow above the time scale indicates the time of IDPN administration. One-day-old mice received 1 mg of IDPN (Eastman Kodak) per gram of body weight. The IDPN was diluted in phosphate-buffered saline (PBS) and adjusted to *p*H 7.3 and was given in a 0.0075-ml volume by intramuscular injection into the hindlimb. Tissue collection and viral assay were as described in Fig. 1. For the IDPN experiment, each plotted point represents the average of the $\log_{10}$ titer (in plaque-forming units per milliliter) of ISC specimens obtained from three to six animals. Controls are identical to those in Fig. 1. The SEM is less than 0.5 at all time points.

In conclusion, our results provide evidence that two serotypes of the same virus use different pathways to spread to the CNS. Reovirus type 3 spreads to the CNS via microtubule-associated fast axonal transport, whereas reovirus type 1 spreads to the CNS via the bloodstream. Using reassortant viruses, we showed that the reovirus S1 dsRNA segment is responsible for determining the capacity of reoviruses to utilize discrete pathways to spread in the infected host, and we suggest that this is mediated via the viral hemagglutinin.

REFERENCES AND NOTES

1. C. A. Mims and D. O. White, *Viral Pathogenesis and Immunology* (Blackwell, Oxford, 1984).
2. R. T. Johnson and C. A. Mims, *N. Engl. J. Med.* **278**, 23 and 84 (1968); R. T. Johnson, in *Infections of the Nervous System (Part II): Handbook of Clinical Neurology, Vol. 34*, P. J. Vinken and G. W. Bruyn, Eds. (North-Holland, Amsterdam, 1978), p. 15; R. T. Johnson, *Viral Infections of the Nervous System* (Raven, New York, 1982).
3. K. Kristensson, in *Axoplasmic Transport in Physiology and Pathology*, D. G. Weiss and A. Gorio (Springer-Verlag, Berlin, 1982), p. 153.
4. B. N. Fields and M. I. Greene, *Nature (London)* **300**, 19 (1982).
5. L. Rosen, *Am. J. Hyg.* **71**, 242 (1960); H. J. Eggers, P. J. Gomatos, I. Tamm, *Proc. Soc. Exp. Biol. Med.* **110**, 879 (1962); A. M. Lerner, J. D. Cherry, M. Finland, *Virology* **19**, 58 (1963); H. L. Weiner, R. F. Ramig, T. A. Mustoe, B. N. Fields, *ibid.* **86**, 581 (1978); H. L. Weiner, K. A. Ault, B. N. Fields, *J. Immunol.* **124**, 2143 (1980).
6. H. L. Weiner, D. Drayna, D. R. Averill, Jr., B. N. Fields, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5744 (1977).
7. H. L. Weiner, M. L. Powers, B. N. Fields, *J. Infect. Dis.* **141**, 609 (1980).
8. L. E. Babiss *et al.*, *J. Virol.* **30**, 863 (1979).
9. G. Margolis, L. Kilham, N. Gonatos, *Lab. Invest.* **24**, 91 (1971); C. S. Raine and B. N. Fields, *J. Neuropathol. Exp. Neurol.* **32**, 19 (1973).
10. K. L. Tyler, R. T. Bronson, K. B. Byers, B. N. Fields, *Neurology* **35**, 88 (1985).
11. G. Margolis and L. Kilham, *Lab. Invest.* **21**, 189 (1969); P. A. Phillips, M. P. Alpers, N. F. Stanley, *Science* **168**, 858 (1970); S. L. Nielsen and J. R. Baringer, *Lab. Invest.* **27**, 531 (1972); S. L. Nielsen and G. E. Gauger, *ibid.* **30**, 618 (1974); C. Masters, M. Alpers, B. Kakulas, *Arch. Neurol.* **34**, 18 (1977).
12. J. C. Paulson and W. O. McClure, *Ann. N.Y. Acad. Sci.* **253**, 517 (1975).
13. R. S. Spendlove, E. H. Lennette, J. N. Chin, C. O. Knight, *Cancer Res.* **24**, 1826 (1964).
14. S. Dales, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 268 (1963).
15. R. L. Epstein, M. L. Powers, H. L. Weiner, *J. Immunol.* **127**, 1800 (1981).
16. J.-O. Karlsson and J. Sjostrand, *Brain Res.* **13**, 617 (1969); J. Sjostrand, M. Frizell, P.-O. Hasselgren, *J. Neurochem.* **17**, 1563 (1970); J.-O. Karlsson, H.-A. Hansson, J. Sjostrand, *Z. Zellforsch. Mikrosk. Anat.* **115**, 265 (1971).
17. B. T. Pickering, in *Axoplasmic Transport*, D. G. Weiss, Ed. (Springer-Verlag, Berlin, 1982), p. 139.
18. J. B. Angevine, *J. Exp. Zool.* **136**, 363 (1957); K. Kristensson, E. Lycke, J. Sjostrand, *Acta Neuropathol.* **17**, 44 (1971).
19. J. W. Griffin *et al.*, *Science* **202**, 633 (1978); J. W. Griffin, P. N. Hoffman, D. L. Price, in *Axoplasmic Transport in Physiology and Pathology*, D. G. Weiss, and A. Gorio, Eds. (Springer-Verlag, Berlin, 1982), p. 109; J. W. Griffin, K. E. Fahnestock, D. L. Price, P. N. Hoffman, *J. Neurosci.* **3**, 557 (1983).
20. L. Nagata, S. A. Masri, D. C. W. Mah, P. W. K. Lee, *Nucleic Acids Res.* **12**, 8699 (1984); L. W.

Cashdollar, R. A. Chmelo, J. R. Wiener, W. K. Joklik, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 24 (1985); R. Bassel-Duby *et al.*, *Nature (London)* **315**, 421 (1985).
21. H. Ernst and A. J. Shatkin, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 48 (1985); B. L Jacobs and C. E. Samuel, *Virology* **143**, 63 (1985); G. Sarkar *et al.*, *J. Virol.* **54**, 720 (1985); B. L. Jacobs, J. A. Atwater, S. M. Munemitsu, C. E. Samuel, *Virology* **147**, 9 (1985).
22. P. W. K. Lee, E. C. Hayes, W. K. Joklik, *Virology* **108**, 156 (1981); T. Onodera *et al.*, *J Exp. Med.* **153**, 1457 (1981).
23. K. L. Tyler, R. Duby, B. N. Fields, unpublished data.
24. R. S. Spendlove, E. H. Lennette, A. C. John, *J. Immunol.* **90**, 554 (1963); S. Dales, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 268 (1963); R. S. Spendlove, E. H. Lennette, J. N. Chin, C. O. Knight, *Cancer Res.* **24**, 1826 (1964) S. Dales, *Ann. N.Y. Acad. Sci.* **253**, 440 (1975); A. S. Sharpe, L. B. Chen, B. N. Fields, *Virology* **120**, 399 (1982).
25. R. S. Kauffman *et al.*, *Virology* **124**, 403 (1983).
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Psychotomimesis Mediated by κ Opiate Receptors

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The κ opioid agonists are analgesics that seem to be free of undesired morphine-like effects. Their dysphoric actions observed with the κ agonist cyclazocine are thought to be mediated by an action at σ-phencyclidine receptors. The benzomorphan κ agonist MR 2033 is inactive at σ-phencyclidine receptors. In male subjects, the opiate-active (−)-isomer, but not the (+)-isomer, elicited dose-dependent dysphoric and psychotomimetic effects that were antagonized by naloxone. Thus, κ opiate receptors seem to mediate psychotomimetic effects. In view of the euphorigenic properties of μ agonists, our results imply the existence of opposed opioid systems affecting emotional and perceptual experiences.

THE OBSERVATION THAT BENZOMORPHAN κ opioid agonists have analgesic properties but do not produce the drug-seeking behavior or cardiovascular and respiratory depression seen with morphine and other agonists at μ opiate receptors has raised the possibility that such compounds could be developed for use by humans (*1, 2*).

Detailed clinical studies of the actions of κ agonists have been performed with the benzomorphan derivative cyclazocine (*3*). This opioid, in addition to its κ agonistic activity, has antagonistic properties at μ opiate receptors (*1–3*). Moreover, in humans, cyclazocine and another benzomorphan, *N*-allylnormetazocine (Smith Kline & French 10047), elicit psychotomimetic and dysphoric effects similar to those of the dissociative anesthetics phencyclidine (PCP) and ketamine (*4*). Pharmacological studies suggested that these effects of the κ agonists are mediated by σ-PCP receptors (*1, 5, 6*). These latter receptors were distinguished from μ, δ, and κ opiate receptors by their resistance to the opiate antagonist naloxone, which classifies them as nonopiate receptors (*7*). In addition, PCP receptors displayed preference for (+)-isomers, whereas opiate receptors show stereospecificity for (−)-isomers (*6, 8*). During experiments designed to evaluate endocrine actions (*9*) of the potent benzomorphan κ agonist MR 2033 (*10*), which is inactive at σ-PCP receptors (*6*), we observed aversive and psychotomimetic effects. Because this observation contradicted the view that σ-PCP receptors mediate psychotomimetic effects of κ agonists, we undertook the present studies to characterize the type of receptor involved. Our results suggest that κ receptors elicit aversive and psychotomimetic actions in humans.

Thirty healthy male volunteers (24 to 62 years old) participated in the experiments. An intravenous cannula was inserted 60 minutes before the drug was administered and the subjects remained in a recumbent position throughout the experiment. Fifteen minutes before intravenous drug injection, the subjects completed five evaluation scales related to mood and physical experiences (*11*). Observer rating scales were used to assess psychotic symptoms and abnormal behavior (*12*). The same evaluation scales were again completed 30 and 90 minutes after drug treatment. Each subject first received either saline or 10 mg of naloxone followed immediately by MR 2033 (the racemic mixture), MR 2034 [the opiate-active (−)-isomer], or MR 2035 [the opiate-inactive (+)-isomer]. Subjects were unaware of which treatment they received.

In a first set of experiments, five subjects received MR 2033 (3.5 μg/kg) after being treated with either saline or naloxone. This relatively low dose was followed by an increase in nonspecific bodily complaints such as weakness, sweating, vertigo, and dizziness as well as an increase in anxiety (as assessed by the psychopathological evaluation scales). Three of five subjects experienced, respectively, racing thoughts, feelings of body distortion, and severe discomfort. These effects were blocked by prior treatment with naloxone (10 mg).

These experiments indicated that MR 2033–induced changes in subjective experience which were sensitive to naloxone and thus probably related to opiate receptors. To further characterize these subjective effects, six men in each of three groups were treated with the opiate-active (−)-isomer (MR 2034) (1.9 or 3.8 μg/kg) or with 10 mg of naloxone prior to injection of the dose of MR 2034 (3.8 μg/kg).

The lower dose of MR 2034 slightly increased and the higher dose considerably increased abnormal behavior; these symptoms were prevented by prior treatment with naloxone (Fig. 1). All subjects showed psychotomimetic symptoms after treatment with MR 2034. In particular, the high dose resulted in somesthetic changes and disturbances in the perception of space and time. Abnormal visual experiences reported by most subjects consisted of moving lines or walls or color phenomena. There were symptoms of depersonalization, derealization, and loss of self-control. For example, one subject had frequent episodes of unmotivated and uncontrolled laughter during a 90-minute period. Two subjects became unaware of the experimental situation for periods of 20 to 30 minutes and later described their experiences as dreamlike. Although pseudohallucinations were reported, true hallucinations did not seem to occur. Most subjects became intermittently disoriented, but remembered the experimental situation when asked by the observers. This disorientation was probably due to preoccupation with perceptual and cognitive alterations

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