Effects of Dibutyryl Cyclic AMP on Restoration of Function of Damaged Sciatic Nerve in Rats

Abstract. *In two experiments, the sciatic nerve of rats was either crushed or hemisected, and* N^6,O^2*-dibutyryl adenosine 3′,5′-monophosphate or saline was injected intramuscularly near the site of the lesion. In both types of nerve damage, the sensorimotor functions of animals treated with* N^6,O^2*-dibutyryl adenosine 3′,5′-monophosphate returned earlier than did those of saline-treated control animals.*

Numerous studies of nerve regeneration have dealt with the stimulatory effects of nerve growth factor on peripheral, sympathetic, and sensory neurons (*1*). Adenosine 3′,5′-monophosphate (cyclic AMP) and N^6,O^2-dibutyryl cyclic AMP (dibutyryl cyclic AMP) were reported to enhance the in vitro growth of axons in sensory ganglia (*2*). It was suggested (*2*) that the action of nerve growth factor was mediated by cyclic AMP and that both substances might, therefore, be functionally similar in stimulating regeneration (*2a*). It was of interest, therefore, to determine whether dibutyryl cyclic AMP would enhance in vivo nerve regeneration and thus facilitate the return of nerve function.

In the first experiment, nine male Holtzman rats (200 to 220 g) were anesthetized with Equithesin (chloral hydrate and pentobarbital); then dorsal incisions were made in the skin overlying the biceps muscles of both hindlimbs. The muscles were protracted to expose each sciatic nerve, and one of the nerves was clamped with a hemostat for 2 seconds (*3*). The jaws of the hemostat were taped to limit damage done by the serrated edges. The nerve was crushed at similar sites in all animals. After 24 hours the animals were injected with dibutyryl cyclic AMP. Six animals received a daily injection of dibutyryl cyclic AMP (50 mg/kg) (*4*) into the biceps muscle just above the site of the lesion. Three rats in a control group were injected daily with 0.9 percent saline solution (1.0 ml/kg).

All injured animals were tested daily for sensorimotor functioning, before the injection of either dibutyryl cyclic AMP or saline. The two persons who tested the animals did not know which animals had been treated with dibutyryl cyclic AMP. Animals were coded, and their cages were changed at random. In the foot-flick procedure for testing sensorimotor function [modification of that described in (*5*)], the rat's hindpaw was held over a hot beam of light until the animal withdrew it; the time to withdrawal was recorded. Each animal was tested independently by two observers and was given at least two trials per day for each hindpaw by each observer. If the rat did not respond within 30 seconds, the trial was terminated and the response latency was defined as 30 seconds. All animals had been tested for the foot-flick response prior to surgery, and the mean value obtained at that time was defined as the normal response latency.

Rats in the saline-treated control group did not show a full recovery of nerve function until day 35 (Fig. 1), a finding consistent with previous reports for spontaneous regeneration after nerve crush (*1*). By day 7 the group that was treated with dibutyryl cyclic AMP differed significantly in response latency from the saline-treated group (Mann-Whitney U test, $P < .05$), and these animals had a full return of nerve function by day 12.

Foot-flick latencies for rats with sham-operated hindlimbs were essentially the same as normal response times, in both the dibutyryl cyclic AMP– and saline-treated groups.

In the second experiment, 17 rats were prepared surgically as described, except that the nerves were hemisected by clamping them in the serrated jaws of a hemostat for 20 seconds. The rats were injected twice daily intramuscularly. Nine were injected with 0.9 percent saline solution, and eight others with dibutyryl cyclic AMP (50 mg/kg). Three of the dibutyryl cyclic AMP–treated animals died during the experiment (*6*). Foot-flick latencies were measured daily by two independent observers (three observations by each) who were unaware of the treatment each rat had received. The mean value for the normal response was obtained as described above.

Rats with hemisections of the sciatic nerve that were treated with saline required approximately 75 days (extrapolated) for complete return of function (Fig. 2), a finding consistent with previous reports of recovery from nerve hemisection (*1*). Rats treated with dibutyryl cyclic AMP showed a foot-flick latency significantly shorter than that of saline-treated rats by day 12 ($U = 5.5$, $P < .01$) and had a full recovery of nerve function by day 38.

The return of the toe-spreading reflex and of normal use of the impaired limb coincided with the return of normal foot-flick latency. When rats with a crushed sciatic nerve were treated with dibutyryl cyclic AMP, four of six animals had regained the toe-spreading reflex by day 10; and all six had regained this reflex by day 12. There was some noticeable improvement in toe spreading every second or third day. Saline-treated rats with crushed sciatic nerves required two to three times as long as dibutyryl cyclic AMP–treated rats for full return of the toe-spreading reflex. When rats with a hemisected sciatic nerve were treated with dibutyryl cyclic AMP, all nine animals showed gradual return of the toe-spreading reflex, which had been regained completely by day 44. The toe-spreading reflex of saline-treated rats with crushed sciatic nerves did not fully return during the testing period.

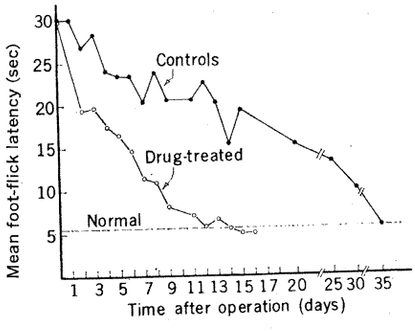

Fig. 1. The effects of daily intramuscular administration of dibutyryl cyclic AMP (50 mg/kg) on the return of sensorimotor function after crushing of the sciatic nerve.

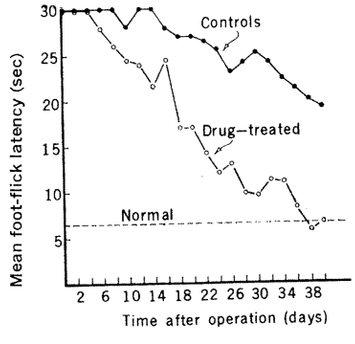

Fig. 2. The effects of twice daily intramuscular administration of dibutyryl cyclic AMP (50 mg/kg) on the return of sensorimotor function after hemisection of the sciatic nerve.

The results of the two experiments demonstrate that treatment with dibutyryl cyclic AMP hastened the return of sensorimotor functioning in rats after nerve damage.

Others have reported effects of nerve growth factor on the regeneration of neurons. Scott and Liu (*7*) reported evidence for enhanced regenerative growth of afferent neurons in kittens after the administration of this material. Our report here is the first to show that a derivative of a cyclic AMP nucleotide may affect the return of function after peripheral nerve injury. It has been shown that there is a large increase in the concentration of cyclic AMP below the site of crushing of a sciatic nerve within 6 hours after surgery (*8*). Such an increase in concentration of cyclic AMP might be the mechanism for the normal regenerative process.

Our finding raises some interesting possibilities. Dibutyryl cyclic AMP or other nucleotides may have clinical utility in accelerating reinnervation by peripheral nerves of sensory and motor receptor sites. Recent reports (*9*) that nerve growth factor may mediate regenerative processes in the central nervous system open the possibility that cyclic AMP might affect as well the return of function after injury to central neurons.

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References and Notes

1. L. Guth, *Physiol. Rev.* **36**, 441 (1956); R. Levi-Montalcini and P. Angeletti, *ibid.* **48**, 534 (1968).
2. F. Roisen, R. Murphy, M. Pichichero, W. Braden, *Science* **175**, 73 (1972).
2a. *Note added in proof*: Two recent reports have questioned the mediation of the in vitro effects of nerve growth factor by cyclic AMP [D. B. Hier, B. G. W. Arnason, M. Young, *Science* **183**, 79 (19[illegible] W. A. Frazier, C. E. Ohlendorf, L. F. Boyd, L. Aloe, E. M. Johnson, J. A. Ferrendelli, R. A. Bradshaw, *Proc. Nat. Acad. Sci. U.S.A.* **70**, 2448 (1973)].
3. Lesions made by crushing and hemisection rather than transection afford better alignment for reinnervation. Crushing resulted in complete axonal separation, as demonstrated in nontreated rats by lack of muscular contraction after electrical stimulation of the nerve fibers above the lesion immediately after surgery. In histological studies, we observed clear Wallerian degeneration of the nerve distal to the site of either kind of lesion.
4. A 50 mg/kg dose of dibutyryl cyclic AMP was selected because it did not produce any behavioral effects when tested in rats in a general observational screen. Because the animals with hemisected nerves were expected to take longer to recover than those with crushed nerves, dibutyryl cyclic AMP (50 mg/kg) was injected twice daily. It is unlikely that the foot-flick results were due to any stimulating properties of dibutyryl cyclic AMP because dibutyryl cyclic AMP decreases motor activity and abdominal tone in rats when administered as a single dose (100 to 400 mg/kg) intraperitoneally.
5. F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.* **72**, 74 (1941).
6. These animals treated with dibutyryl cyclic AMP developed foot infections after considerable self-mutilation. Two of these animals were killed. The death of the third animal was possibly due to the infection. All surviving animals appeared to be healthy during the prolonged daily administration of dibutyryl cyclic AMP.
7. D. Scott, Jr., and C. N. Liu, *Exp. Neurol.* **8**, 279 (1963).
8. O. Appenzeller and G. Palmer, *Brain Res.* **42**, 521 (1972).
9. A. Bjorkland and U. Stenevi, *Science* **175**, 1251 (1972); B. D. Berger, L. Stein, C. D. Wise, *ibid.* **180**, 506 (1973).
10. We thank I. Miller for technical assistance. Request reprints from B.B., Department of Pharmacology, Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540.

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Lead Metabolism in the Normal Human: Stable Isotope Studies

Abstract. *Kinetic and metabolic balance studies in a healthy man fed a diet normal in lead content and labeled with lead-204 indicated that approximately two-thirds of his assimilated lead was dietary in origin; the remainder was inhaled. Kinetic analysis shows that the isotopic data can be interpreted by a three-compartment model.*

There has been considerable discussion in the scientific and general literature concerning environmental contamination by lead. Evaluation of possible dangers from lead contamination has been impeded by the relative lack of data concerning normal human metabolism of lead during typical urban exposure, and the relative contributions to the total lead intake of various sources of environmental lead. Previous methods of studying lead metabolism in human subjects have involved either abnormally high exposures to lead (*1*) or the use of radioisotopes with half-lives that are short compared to the transfer times for lead within body tissues (*2*).

These limitations were overcome by using stable isotopes of lead as tracers. This permits a prolonged metabolic study without the introduction of long-lived radioisotopes into the body. Moreover, with modern methods of mass spectrometry (*3*) the concentration and isotopic composition of microgram quantities of lead can be accurately measured (standard error less than $\pm$ 1 percent). This report presents the use of this technique in one subject to assess (i) the kinetics of lead metabolism under steady-state conditions; (ii) the relative contributions of respired and dietary lead to the total lead intake; and (iii) possible homeostatic responses to an abrupt decrease in lead intake to subnormal quantities.

A healthy 53-year-old white male weighing 70 kg was fed a constant diet with a low lead content (156 μg/day), for 160 days while he lived in a metabolic unit and underwent metabolic studies by standard balance techniques (*4*). The diet provided 2500 kcal/day and abundant quantities of protein, vitamins, and the required minerals. During the first 104 days of the experiment, each meal was supplemented with lead-204 nitrate to increase the total dietary lead intake to 367 μg/day (the subject's approximate intake before the study) so that a metabolic steady-state condition was maintained. Then lead-204 nitrate was replaced with lead-207 nitrate for 10 days. For the next 46 days, the subject received only the diet low in lead.

The concentration and isotopic composition of lead were determined serially in the diet, feces, blood, urine, facial hair, and atmosphere by mass spectrometric isotope dilution analysis. During the course of study, two samples of bile and gastric secretions, one sample of sweat, and a skeletal biopsy containing both cortices of the ilium, from a point 2 cm below the iliac crest, were analyzed for the concentrations of the four stable isotopes of lead.

The kinetics and distribution of lead in the body were analyzed in terms of a three-compartment model (Fig. 1). Compartment 1 can be considered to be primarily blood [principally erythrocytes (*5*)] and possibly some soft tissues which exchange rapidly with blood. This compartment receives isotopically labeled lead from the gut and unlabeled lead from the atmosphere and exchanges lead with compartments 2 and 3. Lead is excreted from compartment 1 into the urine. Compartment 2 includes primarily soft tissue and possibly the more actively exchanging parts of the skeleton. Compartment 3 includes the skeleton, which contains most of the lead in the body (*6*), and the remainder of the soft tissue. Because of the