cent remained in tyramine), areas showing intense labeling were similarly localized within dense granule-containing efferent fibers. Sections parallel to the cornea were surveyed for efferent profiles within ommatidia near rhabdoms. All efferent profiles in this region were labeled. We conclude that efferent fibers in both ventral and lateral eyes synthesize and store octopamine.

The ability of efferent fibers to release newly synthesized octopamine was tested in experiments such as that illustrated in Fig. 3. Ventral eyes were incubated overnight in [^{3}H]tyramine, rinsed with normal saline, and then depolarized with 200 m*M* KCl in the presence of saline containing either normal Ca^{2+} or zero Ca^{2+} plus 2 m*M* $CoCl_2$. Depolarization dramatically increased the rate of efflux of radioactivity from ventral eyes, and approximately 60 percent of this radioactivity was identified by high-voltage electrophoresis as octopamine. No octopamine was found in the rinses with normal saline. Octopamine release was reversibly blocked in saline containing zero Ca^{2+} plus $CoCl_2$; thus release is dependent on extracellular calcium. Potassium depolarization also stimulated octopamine release in the lateral eye.

Many observations now support the idea that octopamine, a known neurotransmitter of invertebrates (*12*), is a neurotransmitter in *Limulus* retinal efferents. We have shown that *Limulus* eyes contain and synthesize octopamine, that new synthesized octopamine is located exclusively in efferent fibers in both ventral and lateral eyes, and that octopamine is released from efferent fibers with depolarization. Octopamine-stimulated increases in adenosine 3′,5′-monophosphate have been measured in both ventral photoreceptor cells (*13*) and in lateral eyes (*14*), suggesting that octopamine receptors are present in both types of eyes. Low concentrations of octopamine injected into lateral eyes also mimic electrophysiological and anatomical effects of natural efferent activity (*15*).

Functions of efferent innervation to *Limulus* ventral eyes are not known. However, the frequent direct contacts between octopamine-containing efferent fibers and ventral photoreceptor rhabdoms suggest that octopamine may be involved in regulating sensitivity, rhabdom turnover, or other metabolic functions of the photoreceptor cell. The identification of octopamine as a likely neurotransmitter in efferents to lateral eyes opens up new possibilities for investigations of the mechanisms of known efferent effects. A thorough understanding of the effects of octopamine and efferent innervation in the *Limulus* visual system, a preparation that is already well characterized, may provide a basis for investigating efferent function in other species and give new information on basic sensory mechanisms.

B.-A. BATTELLE
J. A. EVANS
Laboratory of Vision Research, National Eye Institute, Bethesda, Maryland 20205
S. C. CHAMBERLAIN
Institute for Sensory Research, Syracuse University, Syracuse, New York 13210

References and Notes

1. For example, *Aplysia* [A. Eskin, *Z. Vgl. Physiol.* **74**, 353 (1971)]; birds [W. H. Cowan and T. P. S. Powell, *Proc. R. Soc. London Ser. B* **158**, 232 (1963); F. A. Miles, *Science* **170**, 992 (1970)]; lizard [G. A. Engbretson and C. H. Lent, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 654 (1976)]; and rat [S. K. Itaya, *Brain Res.* **195**, 436 (1980); P. S. Teirstein, A. I. Goldman, P. J. O'Brien, *Invest. Ophthalmol.* **19**, 1268 (1980)].
2. A. W. Clark, R. Millecchia, A. Mauro, *J. Gen. Physiol.* **54**, 289 (1969); W. H. Farenbach, *Z. Zellforsch.* **144**, 153 (1973); *Int. Rev. Cytol.* **41**, 285 (1975); *Cell Tissue Res.* **216**, 655 (1981).
3. H. K. Hartline and C. H. Graham, *J. Cell. Comp. Physiol.* **1**, 277 (1932); H. K. Hartline and F. Ratliff, *J. Gen. Physiol.* **40**, 357 (1957); R. Milleachia and A. Mauro, *ibid.* **54**, 310 and 331 (1969); J. E. Lisman and J. E. Brown, *ibid.* **59**, 701 (1972).
4. R. B. Barlow, Jr., S. J. Bolanowski, Jr., M. L. Brachman, *Science* **197**, 86 (1977).
5. E. Kaplan and R. B. Barlow, Jr., *Nature (London)* **286**, 393 (1980).
6. R. B. Barlow, Jr., S. C. Chamberlain, J. Z. Levinson, *Science* **210**, 1037 (1980).
7. S. C. Chamberlain and R. B. Barlow, Jr., *ibid.* **206**, 361 (1979); *Invest. Ophthalmol. Vis. Sci.* **20** (Suppl.), 75 (1981).
8. B.-A. Battelle, *Vision Res.* **20**, 911 (1980); ____, E. A. Kravitz, H. Stieve, *Experientia* **35**, 778 (1979).
9. The radioactive products formed during incubation with [^{3}H]tyramine were analyzed by high-voltage paper electrophoresis (*8*).
10. P. D. Evans, B. R. Talamo, E. A. Kravitz, *Brain Res.* **90**, 340 (1975); M. S. Livingstone, S. F. Schaeffer, E. A. Kravitz, *J. Neurobiol.* **12**, 27 (1981).
11. Ventral photoreceptors have two segments [J. Bacigalupo, J. Stern, J. Lisman, *Invest. Ophthalmol. Vis. Sci.* **20** (Suppl.), 181 (1981)] only one of which contains rhabdom (B. Calman and S. C. Chamberlain, unpublished data).
12. M. S. Livingstone, R. M. Harris-Warrick, E. A. Kravitz, *Science* **208**, 76 (1980); M. O'Shea and P. D. Evans, *J. Exp. Biol.* **79**, 169 (1979); J. A. Nathanson, *Science* **203**, 65 (1979); B.-A. Battelle and E. A. Kravitz, *J. Pharmacol. Exp. Ther.* **205**, 438 (1978).
13. U. B. Kaupp, C. C. Malbon, B.-A. Battelle, J. E. Brown, *Vision Res.*, in press.
14. B.-A. Battelle, unpublished data.
15. L. Kass and R. B. Barlow, Jr., *Biol. Bull. (Woods Hole, Mass.)* **159**, 487 (1980); *ibid.* **161**, 348 (1981).
16. B. M. Kopriwa, *Histochemistry* **44**, 201 (1975).
17. B.-A.B. and J.A.E. thank J. V. Maizel and R. P. Lenk of the National Institute of Child Health and Human Development for the generous use of their electron micrographic facilities. Computer-assisted reconstructions were made at the Department of Anatomy, State University of New York, Upstate Medical Center. This work was supported in part by a postdoctoral fellowship to J.A.E. and research grants, both from the National Eye Institute.

3 February 1982

Naloxone Reverses Neonatal Depression Caused by Fetal Asphyxia

Abstract. *Pregnant near-term rabbits were given an intravenous dose of saline or the opiate antagonist naloxone and then asphyxiated. The fetuses were delivered by cesarean section and evaluated for respiration, color, muscle tone, response to stimulation, and general activity at 1, 3, 5, 10, 15, and 30 minutes of age. The naloxone-treated pups had significantly better scores during the first 15 minutes after birth than the saline-treated pups. Naloxone did not adversely affect the scores of nonasphyxiated pups. These data suggest that endogenous opiates worsen the neonatal depression caused by intrauterine asphyxia and that this effect can be reversed by naloxone.*

Endogenous opiates appear to play a role in the ventilatory response of neonates to asphyxia or hypoxia (*1–3*). In the neonatal rabbit the primary apnea induced by asphyxia is nearly abolished by naloxone, an opiate antagonist (*1*). The characteristic neonatal response to hypoxia—respiratory stimulation followed by respiratory depression—is also abolished by naloxone (*3*). However, naloxone does not affect the ventilatory response to hypercapnia or hypoxemia in the adult rabbit or human (*3, 4*). Thus, endogenous opiates appear to influence the ventilatory response to chemical stimuli only early in life.

Many of the characteristics of the neonate that has suffered intrauterine asphyxia mimic the effects of exogenously administered opiates: ventilatory depression, hypotonia or flaccidity, and depressed reflex responses and spontaneous activity (*5*). We therefore postulated that endogenous opiates might be implicated in the neonatal depression seen following intrauterine asphyxia.

On day 30 of pregnancy (full term is 31 days), rabbits were injected intravenously with naloxone (1 mg/kg) or saline (2.5 ml). Five minutes later, each doe was placed in a 2-liter chamber into which flowed nitrogen with 7 percent CO_2 at the rate of 20 liters per minute, rapidly replacing the air. Within 3 or 4 minutes the animal died. Eight minutes after being placed in the chamber, the doe was removed from the chamber and her uterus was exposed by laparotomy. At 10

0036-8075/82/0611-1252$01.00/0

minutes the fetuses were delivered as rapidly as possible by cesarean section. Delivery took approximately 30 to 40 seconds. All the fetuses in the control and treated groups survived the asphyxial insult. The pups (six to eight per litter) were placed in numbered bins and kept warm by an overhead heating lamp. An observer (R.J.C.) who did not know whether the doe had received saline or naloxone assessed the condition of the pups 1, 3, 5, 10, 15, and 30 minutes after their delivery. The parameters were respiration (regular, shallow, or gasping or absent), color (pink, dusky, or cyanotic), muscle tone (good, fair, or absent), response to stimulation (good, fair, or absent), and general activity (good, fair, or absent). For each parameter, 2, 1, or 0 points were scored, giving a maximum score of 10 in a normal, healthy pup.

The 10-minute period of asphyxia was chosen because preliminary experiments revealed that after an 8-minute asphyxiation all fetuses had scores of 9 or 10. Prolonging the period of asphyxia to 15 minutes killed half the fetuses.

Pups whose mothers received naloxone ($N = 27$) had significantly higher scores 1, 3, 5, 10, and 15 minutes after birth than did pups whose mothers received saline ($N = 24$) (Fig. 1). At 30 minutes of age, naloxone-treated pups had a slightly higher score than control pups, but the difference was not statistically significant.

In a control experiment, pregnant does, injected as before, were placed in the chamber with air instead of the anoxic mixture. They were removed after 10 minutes and killed by cervical dislocation. There were 12 fetuses in the saline group and 16 in the naloxone group. All had scores of 9 to 10 at 1 minute after birth, and this score did not change with time.

Depression of cardiorespiratory function, muscle tone, skeletal motor activity, and reflexes immediately after birth are signs of antepartum asphyxia (*5*). The present study suggests that the poor condition of neonates asphyxiated in utero is due at least in part to the release of endogenous opiates. The study also indicates that naloxone might be effective in resuscitating neonates that have suffered asphyxia during delivery.

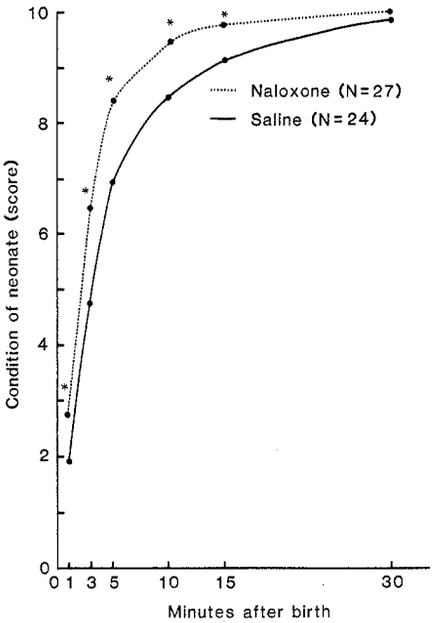

Fig. 1. Mean postnatal scores for pups treated with naloxone or saline and asphyxiated in utero. Asterisks indicate significant differences ($P < .05$, Spearman's rank correlation test). All pups in both groups survived to 30 minutes.

However, these conclusions must be tempered with recent information suggesting that naloxone may not be as selective an antagonist of endogenous opiates as previously thought (*6*). For example, high doses of naloxone can reverse diazepam-induced respiratory depression, suggesting that naloxone competes with diazepam receptors (*7*). Alternatively, the respiratory depression associated with diazepam might be induced by endogenous opiates.

The American Academy of Pediatrics has recommended that naloxone be reserved for adjunctive therapy in human infants who are depressed at birth and who are at high risk for ventilatory depression secondary to narcotic exposure (*8*). The academy has suggested, on theoretical grounds, that the routine use of naloxone may interfere with important endogenous opiate–mediated stress responses. We agree that naloxone should not be used clinically until an appropriately controlled trial is performed to test for potential adverse as well as beneficial effects of naloxone in the depressed newborn infant not exposed to exogenous narcotics. In our study, naloxone did not affect the parameters we measured in the nonasphyxiated newborn rabbits.

A study by Goodlin (*9*) indicated that morphine protects against the effects of intrauterine asphyxia and that naloxone interferes with the ability to resuscitate asphyxiated rabbit neonates. However, the method of resuscitation Goodlin used, hyperbaric oxygenation, is not in general clinical use. The mechanism for the decreased survival of asphyxiated pups given naloxone and then resuscitated with hyperbaric oxygen is not known.

Wardlaw *et al.* (*10*) reported high concentrations of circulating β-endorphin and β-lipotropin in the human fetus at term. There was an excellent negative correlation between the concentration of β-endorphin and the *p*H of umbilical arterial blood, suggesting that hypoxia and secondary acidosis stimulate the release of endogenous opiates. Our data are consistent with the findings of Wardlaw *et al.* and suggest that endogenous opiates have adverse physiological effects in the neonate which are reversible with naloxone.

VICTOR CHERNICK
RANDY J. CRAIG
Perinatal Physiology Laboratory, Department of Pediatrics, Children's Hospital, University of Manitoba, Winnipeg, Manitoba R3E 0W1, Canada

References and Notes

1. V. Chernick, D. L. Madansky, E. E. Lawson, *Pediatr. Res.* **14**, 357 (1980).
2. V. Chernick, *N. Engl. J. Med.* **304**, 1227 (1981).
3. M. M. Grunstein, T. A. Hazinski, M. A. Schlueter, *J. Appl. Physiol.*, in press.
4. J. A. Fleetham, H. Clarke, S. Dhingra, V. Chernick, N. R. Anthonisen, *Am. Rev. Respir. Dis.* **121**, 1045 (1980).
5. V. Apgar, *Anesth. Analg. (Cleveland)* **32**, 260 (1953); L. S. James, J. M. Weisbrot, C. E. Prince, D. A. Holaday, V. Apgar, *J. Pediatr.* **52**, 379 (1958); M. L. Kirby, *Exp. Neurol.* **730**, 430 (1981).
6. J. Sawynok, C. Pinsky, F. S. Labella, *Life Sci.* **25**, 1621 (1979).
7. C. J. Tech, J. R. Lehane, J. G. Jones, *Anesthesiology* **53**, 293 (1980).
8. American Academy of Pediatrics Committee on Drugs, *Pediatrics* **65**, 667 (1980).
9. R. C. Goodlin, *Am. J. Obstet. Gynecol.* **140**, 340 (1981).
10. S. L. Wardlaw, R. I. Stark, L. Baxi, A. G. Frantz, *J. Clin. Endocrinol. Metab.* **79**, 888 (1979).
11. Naloxone was kindly supplied by N. Gupta, Endo Laboratories, Garden City, N.Y. Supported by the Medical Research Council of Canada, the Hospital for Sick Children Foundation, Toronto, and the Children's Hospital of Winnipeg Research Foundation, Inc.

30 September 1981; revised 25 February 1982