tion correlated better with the PGE content of the intestinal fluids or washes than to the tissue concentration of cAMP.

We performed preliminary kinetic experiments to determine the effects of dibutyryl cAMP on fluid accumulation at earlier time intervals with two rabbits per time point. After 4.5 hours, 100 m*M* dibutyryl cAMP caused 0.2 ml of fluid accumulation per centimeter, whereas cholera toxin (350 ng/ml) caused 0.4 ml/cm. After 9 hours, the fluid in the dibutyryl cAMP–treated loop was completely absorbed, whereas fluid in loops treated with cholera toxin, $PGE_1$, or $PGE_2$ progressively increased (to $\geq$1 ml/cm). The stimulatory effects of dibutyryl cAMP on early secretory events in vivo is known (*21, 22*), but the above reversal to net absorption did not coincide with the sustained secretory effects of cholera toxin.

We question whether the magnitude and duration of the effect of cAMP alone can account for the massive loss of water and electrolytes in cholera. If cAMP were the sole intracellular mediator of the diarrhea elicited by cholera toxin, then it might be expected that a sustained fluid accumulation response would have been observed with cAMP derivatives and adenylate cyclase stimulators. Instead, only a small, transient secretory effect was seen with dibutyryl cAMP after 4.5 hours. Neither membrane-permeable derivatives of cAMP nor forskolin caused fluid accumulation in rabbit intestinal loops after overnight exposure despite increased mucosal tissue concentrations of cAMP equal to that of tissues exposed to cholera toxin.

Although one possible interpretation of our data questions the role of cAMP in the cholera toxin–mediated secretory response, an alternative explanation is that the cAMP derivatives or forskolin may not have reached the appropriate secretory cells, presumably the crypt cells. To address this latter possibility, we used inordinately large concentrations of these substances to maximize their diffusion into the crypts. We did not attempt to isolate and measure the cAMP content of the crypt cells because of technical problems (for example, diffusion of dibutyryl cAMP from isolated crypt cells). However, in these experiments, cholera toxin, $PGE_1$, and $PGE_2$ reached the appropriate cells and elicited a net secretory response.

We conclude that enhanced production of both cAMP and PGE occurred in cholera toxin–treated cells and that cAMP did not cause PGE release. The mechanism leading to alterations in prostaglandin synthesis remains to be elucidated. The hypothesis that cAMP is the sole mediator of intestinal water and electrolyte transport in cholera should be reexamined. We observed a poor correlation between tissue cAMP concentrations and net fluid secretion in this model. In contrast, PGE release from cholera toxin–treated mucosal cells correlated with cholera toxin–induced fluid accumulation and increased in a dose-related manner. Thus, cAMP alone might not be responsible for cholera toxin–mediated fluid accumulation in this in vivo model. Cholera toxin–induced fluid secretion does not parallel tissue cAMP concentrations in rat jejunum, ileum, and colon (*23*). We propose that PGE could be an important factor in the secretory process leading to the loss of water and electrolytes during cholera, which could explain the effectiveness of several prostaglandin synthesis inhibitory drugs in reducing cholera toxin–mediated fluid accumulation (*8–10*).

REFERENCES AND NOTES


1. R. A. Finkelstein, in *Handbook of Natural Toxins*, vol. 4, *Bacterial Toxins*, M. C. Hardegree and A. T. Tu, Eds. (Dekker, New York, 1988), pp. 1–38.
2. J. Moss and M. Vaughan, *ibid.*, pp. 39–87.
3. R. L. Guerrant, L. L. Brunton, T. C. Schnaitman, L. I. Rebhun, A. G. Gilman, *Infect. Immun.* **10**, 320 (1974).
4. M. Field, *Am. J. Physiol.* **221**, 992 (1971).
5. ———, D. Fromm, Q. Al-Awqati, W. B. Greenough III, *J. Clin. Invest.* **51**, 796 (1972).
6. A. Bennett, *Nature* **231**, 536 (1971).
7. D. V. Kimberg, M. Field, J. Johnson, A. Henderson, E. Gershon, *J. Clin. Invest.* **50**, 1218 (1971).
8. H. I. Jacoby and C. H. Marshall, *Nature* **235**, 163 (1972).
9. A. D. Finck and R. L. Katz, *ibid.* **238**, 273 (1972).
10. R. E. Gots, S. B. Formal, R. A. Giannella, *J. Infect. Dis.* **130**, 280 (1974).
11. J. W. Peterson, L. G. Ochoa, W. D. Berg, *Fed. Eur. Microbiol. Soc. Microbiol. Lett.* **56**, 139 (1988).
12. P. Speelman, G. H. Rabbani, K. Bukhave, J. Rask-Madsen, *Gut* **26**, 188 (1985).
13. I. E. Duebbert and J. W. Peterson, *Toxicon* **23**, 157 (1985).
14. E. Beubler, G. Kollar, A. Saria, K. Bukhave, J. Rask-Madsen, *J. Physiol. (London)* **382**, 46P (1987).
15. ———, *Gastroenterology* **96**, 368 (1989).
16. R. M. Burch, C. Jelsema, J. Axelrod, *J. Pharmacol. Exp. Ther.* **244**, 765 (1988).
17. S. N. De and D. N. Chatterjee, *J. Pathol. Bacteriol.* **66**, 559 (1953).
18. New Zealand White rabbits (2 to 2.5 kg) were anesthetized by intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine (5 mg/kg) before surgery (*19*). Intestinal segments (5 to 10 cm long) were ligated, beginning approximately 20 cm proximal to the cecum-appendix, and 2-cm interspaces were tied between the loops. After 16 hours, all animals were again anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg) and killed by the injection of 10 ml of air into the marginal ear vein. The cAMP content of mucosal tissue extracts was measured by a protein kinase competitive binding assay (*19*). The PGE concentration of intestinal fluids or phosphate-buffered saline (PBS) washes of negative loops was measured with a PGE radioimmunoassay kit (Dade Baxter Travenol Diagnostics, Miami, FL) (*13*). All chemicals were purchased from Sigma (St. Louis, MO) except cholera toxin, which was acquired from List Biological Laboratories (Campbell, CA).
19. J. W. Peterson, N. C. Molina, C. W. Houston, R. C. Fader, *Toxicon* **21**, 761 (1983).
20. C. J. Hawkey and D. S. Rampton, *Gastroenterology* **89**, 1162 (1985).
21. S. Eklund, J. Cassuto, M. Jodal, O. Lundgren, *Acta Physiol. Scand.* **120**, 311 (1984).
22. I. Lonnroth and S. Lange, *J. Cyclic Nucleotide Res.* 7, 247 (1981).
23. U. M. Farack, R. Gerzer, T. M. Keravis, K. Loeschke, *Dig. Dis. Sci.* **33**, 1153 (1988).


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# Buprenorphine Suppresses Cocaine Self-Administration by Rhesus Monkeys


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**Cocaine abuse has reached epidemic proportions in the United States, and the search for an effective pharmacotherapy continues. Because primates self-administer most of the drugs abused by humans, they can be used to predict the abuse liability of new drugs and for preclinical evaluation of new pharmacotherapies for drug abuse treatment. Daily administration of buprenorphine (an opioid mixed agonist-antagonist) significantly suppressed cocaine self-administration by rhesus monkeys for 30 consecutive days. The effects of buprenorphine were dose-dependent. The suppression of cocaine self-administration by buprenorphine did not reflect a generalized suppression of behavior. These data suggest that buprenorphine would be a useful pharmacotherapy for treatment of cocaine abuse. Because buprenorphine is a safe and effective pharmacotherapy for heroin dependence, buprenorphine treatment may also attenuate dual abuse of cocaine and heroin.**


COCAINE ABUSE IS WIDESPREAD IN the general population (*1*) and has also increased among heroin-dependent persons, including those in methadone maintenance treatment programs (*2*). The many adverse medical consequences of cocaine abuse (*3*) are augmented by the combined use of cocaine and heroin (*4*). For example, dual addiction to intravenous


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cocaine and heroin may increase the risk of acquired immunodeficiency syndrome (AIDS), both through needle sharing and through the combined immunosuppressive effects of both drugs (*5*). Intravenous drug abuse was estimated to account for more than 30% of AIDS victims in the United States in 1988 (*1*).

At present, there is no uniformly effective pharmacotherapy for cocaine abuse (*6*), and the dual abuse of cocaine plus heroin is an even more difficult treatment challenge. Heroin abuse can be treated with opiate agonists [methadone and α-*l*-acetylmethadol (LAAM)] (*7*) and the opiate antagonist naltrexone (*8*), but these pharmacotherapies are not useful for combined cocaine and heroin abuse (*9*). Although desipramine (a tricyclic antidepressant) reduces cocaine abuse in some patients (*6, 10*), it can stimulate relapse to cocaine abuse in abstinent patients (*11*). Treatment with methadone and desipramine has yielded inconsistent effects on cocaine use by heroin abusers (*12*).

An ideal pharmacotherapy would be one that antagonized the reinforcing effects of cocaine and that had minimal adverse side effects or potential for abuse liability. The opioid mixed agonist-antagonist buprenorphine (*13*) meets these criteria for the treatment of opiate abuse. Buprenorphine effectively suppressed heroin self-administration by heroin-dependent men during inpatient studies (*14*) and blocked opiate effects for more than 24 hours (*15*). Cessation of buprenorphine treatment does not produce severe and protracted withdrawal signs and symptoms in man (*14, 16, 17*). Buprenorphine is safer than methadone because its antagonist component appears to prevent lethal overdose, even at approximately ten times the analgesic therapeutic dose (*18*). Buprenorphine is also effective for the outpatient detoxification of heroin-dependent persons (*19*). The opioid agonist effects of buprenorphine make it acceptable to heroin abusers (*14, 16*), but illicit diversion has been minimal in comparison to heroin (*20*). Preclinical studies indicate that buprenorphine is less reinforcing than other opioids in rhesus monkey and baboon (*21, 22*).

Here we describe the effect of buprenorphine treatment on cocaine self-administration by rhesus monkeys. Cocaine effectively maintains operant responding, leading to its intravenous administration in primates, and it is well established that primates self-administer most drugs abused by man (*23*). The primate model of drug self-administration is a useful method for the prediction of drug abuse liability and can be used to evaluate new pharmacotherapies for drug abuse disorders (*24*).

Two male and three female adult rhesus monkeys (*Macaca mulatta*) (*25*) with a 262 ± 79 day history of cocaine self-administration were studied. Each monkey was implanted with a double-lumen silicone rubber intravenous catheter under aseptic conditions to permit administration of buprenorphine or saline during cocaine self-administration. The intravenous catheter was protected by a custom-designed tether system (Spaulding Medical Products) that permits monkeys to move freely. Monkeys worked for food (1-g banana pellets) and for intravenous cocaine (0.05 or 0.10 mg per kilogram of body weight per injection) on the same operant schedule of reinforcement. An average of 64 responses was required for each food pellet or cocaine injection under a second-order schedule of reinforcement (*26*). Food and cocaine each were available during four 1-hour sessions each day. Food sessions began at 11 a.m., 3 p.m., 7 p.m., and 7 a.m.; cocaine sessions at 12 noon, 4 p.m., 8 p.m., and 8 a.m. Each food or drug session lasted for 1 hour or until 20 drug injections or 65 food pellets were delivered. The total number of cocaine injections was limited to 80 per day to minimize the possibility of adverse drug effects. The nutritionally fortified diet of banana pellets was supplemented with fresh fruit, vegetables, biscuits, and multiple vitamins each day.

Buprenorphine treatment was administered at two doses (0.40 and 0.70 mg/kg per day) that effectively suppressed opiate self-administration in our previous studies in primates (*24*). Buprenorphine (or an equal volume of saline solution) was administered daily beginning at 9:30 a.m. Buprenorphine and saline were gradually infused at a rate of 1 ml of solution every 12 min and flushed through the catheter with sterile saline in a volume that exceeded the estimated catheter dead space. Each dose of buprenorphine and saline was studied for 15 consecutive days (60 sessions). After 30 days of treatment, buprenorphine was abruptly discontinued and daily saline treatment was resumed.

We measured cocaine and food self-administration during 15 days of saline treatment and six successive 5-day periods of buprenorphine treatment (Fig. 1). During base-line saline treatment, each of the five monkeys self-administered 2.1 to 4 mg/kg per day of cocaine [group average (± SEM) of 3.07 ± 0.17 mg/kg per day]. This level of cocaine self-administration corresponds to that commonly reported by cocaine abusers (1 to 2 g per week is equivalent to 2.04 to 4.08 mg/kg per day in man) (*27*). All animals reduced their cocaine self-administration significantly during buprenorphine treatment ($P < 0.0001$) (Fig. 1). On the first day of buprenorphine treatment, cocaine self-administration decreased by 50% or more in four of the five subjects (range 50 to 67%). Average cocaine self-administration decreased by 49 ± 15.5% to 1.60 ± 0.25 mg/kg per day during the first 5 days of buprenorphine treatment ($P < 0.01$). Average cocaine self-administration then decreased to 77 ± 7.4% and 83 ± 8.2% below base-line levels during buprenorphine treatment days 6 to 10 and 11 to 15, respectively. Cocaine self-administration averaged 0.98 ± 0.11 mg/kg per day over the first 15 days of buprenorphine treatment at 0.40 mg/kg per day (Fig. 1).

During the second 15 days of buprenorphine treatment at 0.70 mg/kg per day, cocaine self-administration decreased to between 91 ± 2.7% and 97 ± 0.9% below base-line levels (Fig. 1). Monkeys self-administered an average of 0.19 ± 0.03 mg/kg per day of cocaine. Analysis of individual subject data showed that the rate and degree of suppression by buprenorphine of cocaine-maintained responding was equivalent

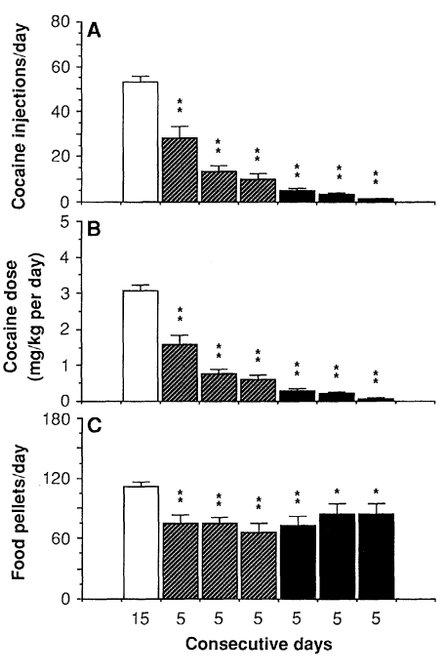


**Fig. 1.** The effects of single daily infusions of buprenorphine or a control saline solution on cocaine and food self-administration. Saline treatment is shown as an open bar and buprenorphine treatment as a striped bar (0.40 mg/kg per day) and a solid bar (0.70 mg/kg per day). The number of days that each treatment condition was in effect is shown on the abscissa. Each data point is the mean ± SEM of five subjects. (**A**) The average number of cocaine injections self-administered; (**B**) the average dose of cocaine (milligrams per kilogram per day) self-administered; (**C**) the average number of food pellets self-administered. The statistical significance of each change from the saline treatment base line as determined by analysis of variance for repeated measures and Dunnett's tests for multiple comparisons (*39*) is shown ($*P < 0.05$; $**P < 0.01$).

in animals that self-administered relatively high (4 mg/kg per day) and low (2.1 mg/kg per day) doses of cocaine during the saline base-line treatment period. Cocaine-maintained operant responding remained suppressed for at least 15 days after cessation of buprenorphine treatment. This time course is similar to clinical reports of abstinence signs and symptoms 15 to 21 days after abrupt withdrawal of buprenorphine (*16*) and probably reflects the slow dissociation of buprenorphine from the opiate receptor (*13*). Individual monkeys returned to base-line levels of cocaine self-administration at different rates ranging from 15 to 58 days (mean, 30.5 ± 10 days).

In contrast to its dose-dependent effects on cocaine self-administration, buprenorphine administration (0.40 mg/kg per day) suppressed food-maintained responding by 31 ± 8.3% during the first 15 days of treatment. Then food self-administration gradually recovered to average 20 ± 12.5% below base line during the second 15 days of treatment with a higher dose of buprenorphine (Fig. 1). Although these changes were statistically significant ($P < 0.05$ to 0.01), it is unlikely that they were biologically significant. There were no correlated changes in body weight, and animals continued to eat daily fruit and vegetable supplements. Moreover, food self-administration during the first daily session after buprenorphine treatment was not suppressed in comparison to saline treatment. The distribution of food intake across the four daily food sessions was equivalent during saline and buprenorphine treatment conditions. Four of five animals returned to base-line levels of food-maintained operant responding within 3 to 17 days after cessation of buprenorphine treatment (8.5 ± 2.9 days). Animals were not sedated during buprenorphine treatment and activity levels appeared normal. These data suggest that buprenorphine treatment suppressed cocaine-maintained responding but did not produce a generalized suppression of behavior.

These data are consistent with our previous observations that chronic buprenorphine self-administration (0.01 to 1.0 mg/kg per injection) did not significantly suppress food self-administration by rhesus monkeys (*28, 29*). Although administration of single doses of buprenorphine (0.10 to 0.30 mg/kg) significantly suppressed food-maintained responding (*28*) during chronic buprenorphine administration (1.0 mg/kg), recovery of food-maintained responding occurred rapidly (*29*). After an initial suppression, food self-administration increased significantly above control levels during 25 days of buprenorphine treatment (1.0 mg/kg) (*29*). The fact that buprenorphine is not an appetite suppressant in primates (*28, 29*) or in man (*14*) is another indicator of its relative safety during chronic use.

These preclinical data suggest that buprenorphine may be an effective pharmacotherapy for the treatment of cocaine abuse. However, clinical evaluation of buprenorphine treatment will require double-blind (buprenorphine versus placebo) trials with randomized patient assignment and independent indices of compliance with the treatment regimen (for example, buprenorphine blood levels) and objective measures of drug use (frequent drug urine screens). One advantage of the primate model for preclinical evaluation of pharmacotherapies is that compliance and multiple drug use are not at issue. It is important to emphasize that if buprenorphine treatment of cocaine abuse were to prove clinically efficacious, this would not be a "substitute addiction" with a less toxic cocaine-like stimulant drug analogous to methadone treatment of heroin dependence. Buprenorphine is an opioid mixed agonist-antagonist (*13*), whereas cocaine is a stimulant drug (*3*). Moreover, buprenorphine does not substitute for cocaine in primate drug self-administration studies (*21*).

We do not yet understand the mechanisms accounting for the suppression of cocaine self-administration by buprenorphine. The relative contribution of buprenorphine's opioid agonist and antagonist components to its effects on the reinforcing properties of cocaine are unknown. However, since opioid antagonists such as naloxone and naltrexone do not suppress cocaine self-administration in primates (*30*) or in rodents (*31*), we postulate that either the opioid agonist component or the opioid agonist-antagonist combination is critical for the effects of buprenorphine on cocaine self-administration. Clinical and primate studies of opioid agonist effects on cocaine self-administration are inconsistent. Methadone treatment did not reduce the incidence of cocaine-positive urines in heroin-dependent patients (*9*), but morphine treatment suppressed cocaine self-administration in a dose-dependent manner in squirrel monkeys (*32*).

There is a consensus that dopaminergic neural systems play a critical role in cocaine reinforcement (*33*), and our data suggest that buprenorphine modifies the reinforcing properties of cocaine. This interpretation is consistent with several lines of evidence indicating comodulatory interactions between endogenous opioid and dopaminergic systems in brain (*34–36*). Neuroendocrine (*34*), neuropharmacological (*35*), and behavioral studies (*36*) suggest that dopaminergic systems modulate endogenous opioid system activity and the converse. Attenuation of cocaine self-administration by buprenorphine further illustrates an interrelationship between opioid and dopamine systems. Our findings also suggest the importance of examining commonalities in the way in which abused drugs maintain behavior leading to their self-administration (*37*).

Buprenorphine is potentially valuable for the treatment of dual addiction to cocaine and heroin because it suppresses heroin use by heroin addicts (*14*). Empirical support for this prospect comes from a report of an open clinical trial (*38*). Opioid-dependent patients treated with methadone had a significantly higher incidence of cocaine-positive urines than patients treated for 1 month with daily sublingual doses of buprenorphine (average 3.2 mg/day; range 2 to 8 mg) (*38*). If buprenorphine reduces cocaine abuse, as well as dual cocaine and heroin abuse, it could be very beneficial to society in reducing drug abuse problems and the associated risks for human immunodeficiency virus infection.

## REFERENCES AND NOTES


1. N. J. Kozel and E. H. Adams, *Science* **234**, 970 (1986); *National Institute on Drug Abuse, ADAMHA* (Alcohol, Drug Abuse, and Mental Health Administration), *Request for Applications DA-89-01* (December 1988).
2. T. R. Kosten, B. J. Rounsaville, F. H. Gawin, H. D. Kleber, *Am. J. Drug Alcohol Abuse* **12**, 1 (1986); B. Kaul and B. Davidow, *ibid.* **8**, 27 (1981).
3. L. L. Cregler and H. Mark, *N. Engl. J. Med.* **315**, 1495 (1986); J. H. Mendelson and N. K. Mello, in *Harrison's Principles of Internal Medicine*, E. Braunwald *et al.*, Eds. (McGraw-Hill, New York, ed. 11, 1986), pp. 2115–2118.
4. M. J. Kreek, in *Psychopharmacology, The Third Generation of Progress*, H. Y. Meltzer, Ed. (Raven, New York, 1987), pp. 1597–1604.
5. R. M. Donahoe and A. Falek, in *Psychological, Neuropsychiatric and Substance Abuse Aspects of AIDS*, T. P. Bridge *et al.*, Eds. (Raven, New York, 1988), pp. 145–158; T. W. Klein, C. A. Newton, H. Friedman, *ibid.*, pp. 139–143.
6. H. D. Kleber and F. H. Gawin, *J. Clin. Psychiatry* **45**, 18 (1984); F. H. Gawin and E. H. Ellinwood, *N. Engl. J. Med.* **318**, 1173 (1988).
7. V. P. Dole and M. Nyswander, *J. Am. Med. Assoc.* **193**, 646 (1965); J. B. Blaine *et al.*, *Ann. N.Y. Acad. Sci.* **311**, 214 (1978); J. B. Blaine *et al.*, *ibid.* **362**, 101 (1981).
8. R. E. Meyer and S. M. Mirin, *The Heroin Stimulus* (Plenum, New York, 1979); W. R. Martin *et al.*, *Arch. Gen. Psychiatry* **28**, 784 (1973); N. K. Mello *et al.*, *J. Pharmacol. Exp. Ther.* **216**, 45 (1981).
9. T. R. Kosten, B. J. Rounsaville, H. D. Kleber, *Arch. Gen. Psychiatry* **44**, 281 (1987).
10. F. H. Gawin and H. E. Kleber, *ibid.* **41**, 903 (1984); F. S. Tennant, Jr., and R. A. Rawson, in *Problems of Drug Dependence, 1982*, L. S. Harris, Ed. (Committee on Problems of Drug Dependence, Washington, DC, 1983).
11. R. E. Weiss, *J. Am. Med. Assoc.* **260**, 2545 (1988).
12. C. P. O'Brien *et al.*, *J. Clin. Psychiatry* **49**, 17 (1988); T. R. Kosten *et al.*, *ibid.* **48**, 442 (1987).
13. The opioid mixed agonist-antagonist buprenorphine is an oripavine derivative of thebaine with partial μ opioid agonist activity. It is a congener of a potent opioid agonist, etorphine, and an opioid antagonist, diprenorphine. The structure and chemical derivation of this opioid mixed agonist-antagonist have been described by J. W. Lewis, in *Narcotic*

*Antagonists: Advances in Biochemical Pharmacology*, M. Braude *et al.*, Eds. (Raven, New York, 1974), vol. 8, pp. 123–136. The pharmacology of buprenorphine has been described by J. W. Lewis, M. J. Rance, and D. J. Sanger [in *Advances in Substance Abuse, Behavioral and Biological Research*, N. K. Mello, Ed. (JAI, Greenwich, CT, 1983), vol. 3, pp. 103–154].
14. N. K. Mello and J. H. Mendelson, *Science* **207**, 657 (1980); ———, J. C. Kuehnle, *J. Pharmacol. Exp. Ther.* **223**, 30 (1982); N. K. Mello and J. H. Mendelson, *Drug Alcohol Depend.* **14**, 282 (1985).
15. W. K. Bickel *et al.*, *J. Pharmacol. Exp. Ther.* **247**, 47 (1988).
16. D. R. Jasinski, J. S. Pevnick, J. D. Griffith, *Arch. Gen. Psychiatry* **35**, 601 (1978).
17. S. E. Lukas, D. R. Jasinski, R. E. Johnson, *Clin. Pharmacol. Ther.* **36**, 127 (1984); P. J. Fudala, R. E. Johnson, E. Bunker, *ibid.* **45**, 186 (1989).
18. C. D. Banks, *N.Z. Med. J.* **89**, 255 (1979).
19. W. K. Bickel *et al.*, *Clin. Pharmacol. Ther.* **43**, 72 (1988); T. R. Kosten and H. D. Kleber, *Life Sci.* **42**, 635 (1988).
20. J. J. O'Connor *et al.*, *Br. J. Addict.* **83**, 1085 (1988).
21. S. E. Lukas, J. V. Brady, R. R. Griffiths, *J. Pharmacol. Exp. Ther.* **238**, 924 (1986).
22. N. K. Mello, S. E. Lukas, M. P. Bree, *Drug Alcohol Depend.* **21**, 81 (1988); J. H. Woods, in *Proceedings* (Committee on Problems of Drug Dependence, Washington, DC, 1977); A. M. Young *et al.*, *J. Pharmacol. Exp. Ther.* **229**, 118 (1984).
23. T. Thompson and K. R. Unna, Eds., *Predicting Dependence Liability of Stimulant and Depressant Drugs* (University Park Press, Baltimore, 1977); R. R. Griffiths, G. E. Bigelow, J. E. Henningfield, in *Advances in Substance Abuse, Behavioral and Biological Research*, N. K. Mello, Ed. (JAI, Greenwich, CT, 1980), vol. 1, pp. 1–90; R. R. Griffiths and R. L. Balster, *Clin. Pharmacol. Ther.* **25**, 611 (1979).
24. N. K. Mello, M. P. Bree, J. H. Mendelson, *J. Pharmacol. Exp. Ther.* **225**, 378 (1983).
25. Animal maintenance and research were conducted in accordance with guidelines provided by the Committee on Laboratory Animal Resources. The facility is licensed by the U.S. Department of Agriculture and protocols were approved by the Institutional Animal Care and Use Committee. The health of the animals was periodically monitored by a consultant veterinarian from the New England Regional Primate Research Center. Surgical implantation of an intravenous catheter for drug infusion was performed under aseptic conditions. A surgical level of anesthesia was induced with ketamine (25 mg/kg, intramuscular) or pentobarbital (30 mg/kg, intravenous). Because the procedure usually takes 25 min, supplemental doses of anesthetic were seldom required. A mild analgesic (Tylenol) was administered every 4 to 6 hours for the first 24 hours after surgery.
26. Completion of a fixed ratio (FR) of four consecutive variable ratio (VR) components, in which an average of 16 responses produced a brief stimulus light (S+), was required for cocaine or food delivery. This is a second-order FR 4 schedule with VR 16 components [FR 4 (VR 16:S)].
27. J. H. Mendelson *et al.*, *Am. J. Psychiatry* **145**, 1094 (1988); J. H. Mendelson *et al.*, in preparation.
28. N. K. Mello *et al.*, *Pharmacol. Biochem. Behav.* **23**, 1037 (1985).
29. S. E. Lukas *et al.*, *ibid.* **30**, 977 (1988).
30. J. H. Woods and C. R. Schuster, in *Stimulus Properties of Drugs*, T. Thompson and R. Pickens, Eds. (Appleton-Century-Crofts, New York, 1971), pp. 163–175; A. K. Killian *et al.*, *Drug Alcohol Depend.* **3**, 243 (1978); S. R. Goldberg *et al.*, *Pharmacol. Exp. Ther.* **176**, 464 (1971).
31. A. Ettenberg, H. O. Pettit, F. E. Bloom, G. F. Koob, *Psychopharmacol. Ser. (Berlin)* **78**, 204 (1982); M. E. Carroll, S. T. Lac, M. J. Walker, R. Kragh, T. Newman, *J. Pharmacol. Exp. Ther.* **238**, 1 (1986).
32. R. Stretch, *Can. J. Physiol. Pharmacol.* **55**, 778 (1977).
33. M. W. Fischman, in *Psychopharmacology: The Third Generation of Progress*, H. Y. Meltzer, Ed. (Raven, New York, 1987), pp. 1543–1553; C. A. Dackis and M. S. Gold, *Neurosci. Biobehav. Rev.* **9**, 469 (1985); M. J. Kuhar, M. C. Ritz, J. Sharkey, *Natl. Inst. Drug Abuse Research Monogr. Ser. 88* (1988), pp. 14–22; M. C. Ritz, R. J. Lamb, S. R. Goldberg, M. J. Kuhar, *Science* **237**, 1219 (1987); W. L. Woolverton, L. I. Goldberg, J. Z. Ginos, *J. Pharmacol. Exp. Ther.* **230**, 118 (1984); W. L. Woolverton, *Pharmacol. Biochem. Behav.* **24**, 531 (1986).
34. J. H. Mendelson, N. K. Mello, P. Cristofaro, A. Skupny, J. Ellingboe, *Pharmacol. Biochem. Behav.* **24**, 309 (1986); R. O. Kuljis and J. P. Advis, *Endocrinology* **124**, 1579 (1989); N. K. Mello, J. H. Mendelson, M. P. Bree, M. Kelly, in *Problems of Drug Dependence 1989*, L. S. Harris, Ed. (Committee on Problems of Drug Dependence, Washington, DC, 1989).
35. Y. Ishizuka *et al.*, *Life Sci.* **43**, 2275 (1988); G. Di Chiara and A. Imperato, *J. Pharmacol. Exp. Ther.* **244**, 1067 (1988).
36. M. A. Bozarth and R. A. Wise, *Life Sci.* **19**, 1881 (1981); H. Blumberg and C. Ikeda, *J. Pharmacol. Exp. Ther.* **206**, 303 (1978); T. S. Shippenberg and A. Herz, *Brain Res.* **436**, 169 (1987).
37. N. K. Mello, in *The Pathogenesis of Alcoholism, Biological Factors*, B. Kissin and H. Begleiter, Eds. (Plenum, New York, 1983), vol. 7, pp. 133–198; G. Di Chiara and A. Imperato, *Ann. N. Y. Acad. Sci.* **473**, 367 (1986).
38. T. R. Kosten *et al.*, *Life Sci.* **44**, 887 (1989).
39. B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1971).
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# Inhibition of Postsynaptic PKC or CaMKII Blocks Induction But Not Expression of LTP

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**Long-term potentiation (LTP) of synaptic transmission is a widely studied cellular example of synaptic plasticity. However, the identity, localization, and interplay among the biochemical signals underlying LTP remain unclear. Intracellular microelectrodes have been used to record synaptic potentials and deliver protein kinase inhibitors to postsynaptic CA1 pyramidal cells. Induction of LTP is blocked by intracellular delivery of H-7, a general protein kinase inhibitor, or PKC(19–31), a selective protein kinase C (PKC) inhibitor, or CaMKII(273–302), a selective inhibitor of the multifunctional $Ca^{2+}$-calmodulin–dependent protein kinase (CaMKII). After its establishment, LTP appears unresponsive to postsynaptic H-7, although it remains sensitive to externally applied H-7. Thus both postsynaptic PKC and CaMKII are required for the induction of LTP and a presynaptic protein kinase appears to be necessary for the expression of LTP.**

LONG-TERM POTENTIATION (LTP) of synaptic transmission results from tetanic stimulation of afferent fibers in the hippocampus (*1*) and is widely studied as a cellular model of learning and memory (*2*). However, despite much effort, the mechanisms responsible for LTP are incompletely understood (*2*). Pharmacological experiments have identified different aspects of LTP, referred to as induction, maintenance, and expression (*2–4*). *N*-Methyl-D-aspartate (NMDA) receptor channels are involved in inducing the enhanced transmission, but not in maintaining or expressing it (*2*, *4*). Maintenance and expression are distinguished (*3*) by the protein kinase inhibitor H-7 (*5*), which suppresses potentiated transmission in a reversible manner when applied in the bath. Thus, a persistent signal responsible for the enhanced transmission can be maintained, even though its expression is interrupted (*3*). Recently, we and others have attempted to localize these aspects of LTP to postsynaptic or presynaptic structures and to determine their molecular basis. NMDA receptor activation produces a $Ca^{2+}$ entry into the postsynaptic cell (*6*) that is critical in inducing LTP (*7*). What happens next to achieve a persistent signal is unclear. In particular, the site of this persistent modification is not known. Some evidence supports primarily a postsynaptic locus (*8*), whereas other data point to a presynaptic change (*9*, *10*). Involvement of a protein kinase has been repeatedly suggested, with protein kinase C (PKC) (*10–12*) and $Ca^{2+}$-calmodulin–dependent protein kinase (CaMKII) (*13*) as the leading candidates. However, the evidence to date does not establish either of these as necessary participants in LTP (*14*).

To learn more about the role of the postsynaptic kinases in LTP, we used intracellular microelectrodes to deliver protein kinase inhibitors to postsynaptic cells. Our experiments focused on two central questions: (i) is activity of postsynaptic PKC or CaMKII required for LTP? and (ii) does the postsynaptic cell contain persistent protein

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