

β -ENDORPHIN SUPPRESSION OF ACUTE MORPHINE ABSTINENCE IN MORPHINE
DEPENDENT MONKEYS: EFFECTIVE GIVEN INTRAVENTRICULARLY BUT
INEFFECTIVE GIVEN INTRAVENOUSLY

Edward F. Domino, Department of Pharmacology, University
of Michigan, Ann Arbor, MI 48109, and Choh Hao Li
Laboratory of Molecular Endocrinology, University of
California, San Francisco, CA 94143
(reprint requests to EFD)

Abstract

Six female adult Macaca mulatta monkeys were made dependent upon morphine sulfate and were implanted with a chronic indwelling needle in the lateral ventricle of the brain for sterile intraventricular injections. Both β -endorphin and morphine, in a dose dependent manner given intraventricularly suppressed the signs of 14 hour acute morphine abstinence. On a molar basis, β -endorphin was more active than morphine in suppressing the signs of morphine abstinence. When given intravenously in much larger doses, β -endorphin was ineffective in contrast to morphine which was effective in suppressing abstinence.

Introduction

The evidence that β -endorphin is a narcotic agonist when given intraventricularly to rodents is very impressive. This untriakontapeptide, β -endorphin, of camel and human pituitary glands, isolated and synthesized by Li and colleagues (1-4), is a potent narcotic analgesic. When compared to morphine in mice, β -endorphin is 18-33 times more potent when given intraventricularly (5) but 3-4 times more potent when given intravenously (6). As is typical of a narcotic agonist, tolerance to and physical dependence on β -endorphin and naloxone antagonism of its acute effects have been demonstrated in rodents (7,8,9). When given intravenously to narcotic dependent humans, β -endorphin has been reported to suppress the acute abstinence syndrome (10,11). In cats, β -endorphin given intraventricularly also produces narcotic-like manic behavior (12,13). However, intravenous injection of β -endorphin to humans does not produce a typical narcotic-like state (14). In fact, the evidence that intravenous β -endorphin has a central opioid action in primates is very poor. Additional studies of β -endorphin compared to morphine in morphine dependent monkeys seem imperative. We report here the effectiveness of β -endorphin in suppressing acute withdrawal in chronic morphine-dependent monkeys, but only when this peptide is given intraventricularly.

Materials and Methods

Six adult female monkeys, weighing from 3 to 5 kg were made dependent upon morphine with daily injections of 3.0 mg/kg of morphine sulfate subcutaneously every 6 hours at 7:00 a.m., 1:00 p.m., 7:00 p.m., and 1:00 a.m. for periods up to 2 years. They were fed a commercial monkey chow dusted with isoniazid for an estimated isoniazid dose of 10 mg/kg. All six animals were housed in one large gang cage. Each animal was prepared surgically under ketamine anesthesia with a special chronic indwelling stainless steel needle which contained a rubber septum covered with silastic for sterility. Evidence that the needle was in the lateral ventricle was obtained by withdrawal of ventricular fluid and via skull x-rays obtained after injection of 0.2 and 1.0 ml of the radiocontrast media metrizamide (250 mg of iodine/ml). The distribution of 0.2 ml of metrizamide given intraventricularly is illustrated in one monkey, M900 in Fig. 1.

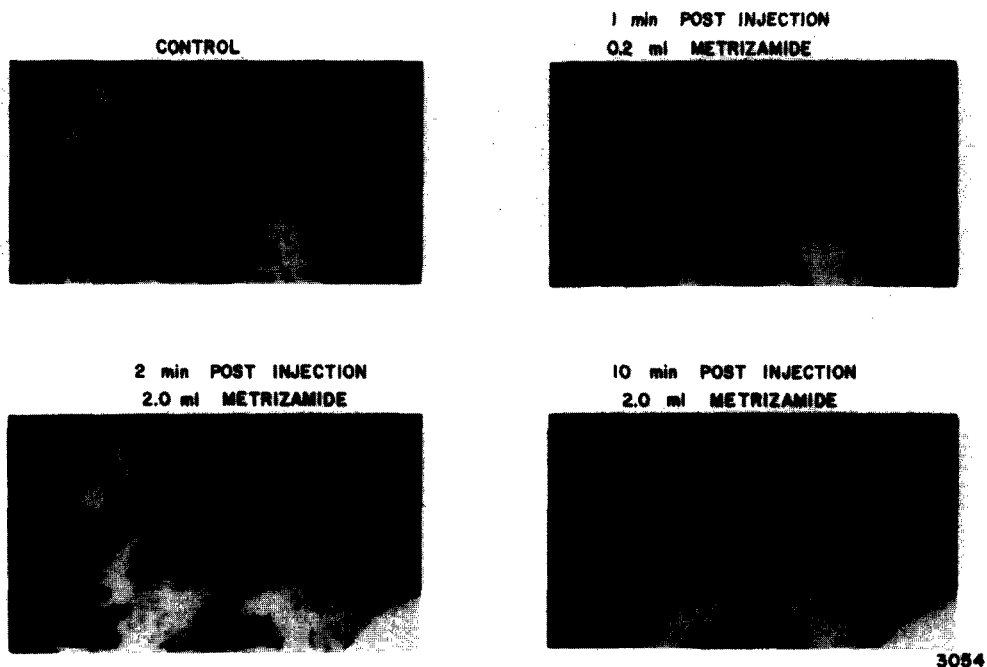


Fig. 1. Brain distribution of metrizamide given intravenously to a monkey with a chronic indwelling needle. Lateral view of four different x-ray photographs of M900 taken at different times after metrizamide.

At approximately 1-4 week intervals the animals were subjected to 14 hour abstinence in which the 1:00 and 7:00 a.m. doses of morphine were omitted. Each experiment began at about 9:00 a.m. or 14 hours after the previous 7:00 p.m. dose of morphine. Abstinence signs were scored before drug injection and at various times thereafter. Morphine and synthetic human β -endorphin (4) were dissolved in artificial cerebrospinal fluid (15). To maintain sterility, all solutions were passed through separate SLG 5025 OS Millex disposable millipore filters prior to injection. Each drug was given intraventricularly or intravenously in doses of 10, 32, 100, 320, 570

and sometimes 1000 μg in a random fashion over an experimental design which lasted one year. Not all six animals completed all of the doses scheduled for various reasons but all animals received most of the doses of all drugs. The drug dosages were compared with an equal volume of artificial cerebrospinal fluid. During the experimental period over 1 to 2 years, two of the animals died from acute cerebral ventriculitis due to faulty sterile procedures. Abstinence scores were obtained using an 8 point scale as previously described (16-20). These signs are grouped in the following categories: general behavior in the home cage, facial, trunk and extremity characteristics, autonomic signs, and those signs elicited by a handling routine. The handling routine was by far the most sensitive means of measuring abstinence. When abstinence was not terminated with an injection of the scheduled substance, it was then routinely terminated with 10 mg/kg morphine subcutaneously and continuation of morphine injection every 6 hours beginning at 1:00 a.m. the night after the day's experiment.

Results and Discussion

The administration of artificial cerebrospinal fluid (0.2 ml), either intraventricularly or intravenously, had no effect on morphine abstinence (see Fig. 2). In contrast, 100 μg of either morphine or β -endorphin given

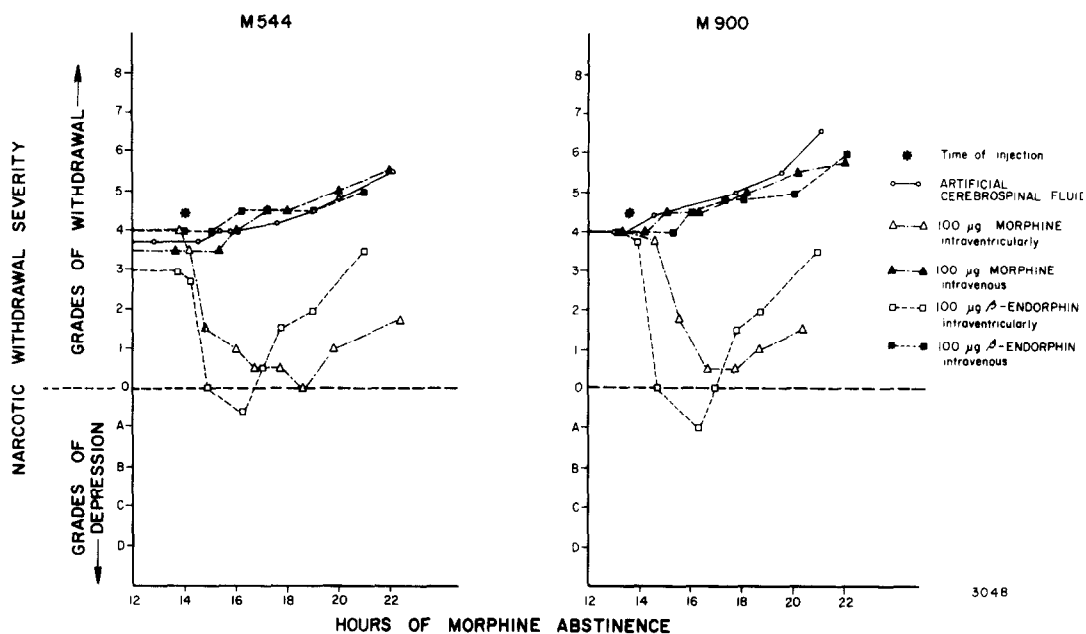


Fig. 2. Comparative effects of artificial cerebrospinal fluid, morphine and β -endorphin on abstinence severity in two morphine dependent monkeys.

intraventricularly was very effective in suppressing morphine abstinence. However, when given intravenously in a dose of 100 μg , neither morphine nor β -endorphin had any significant effect, indicating that at this dose both substances have only a central nervous system site of action to suppress withdrawal.

The effects of increasing doses of β -endorphin given intraventricularly on abstinence severity in two morphine dependent monkeys is shown in Fig. 3.

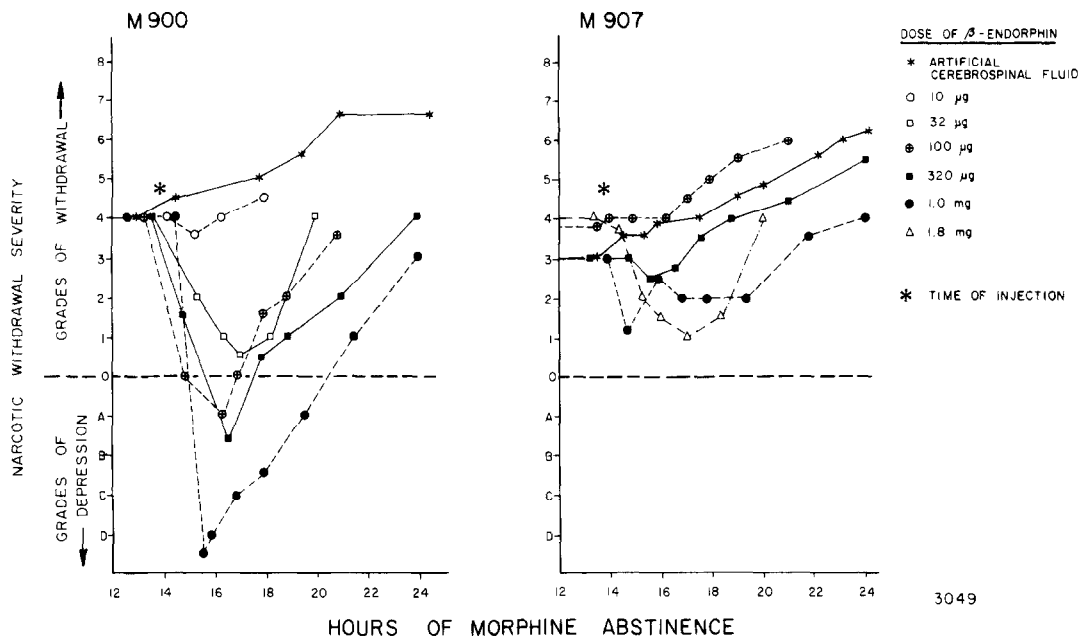


Fig. 3. Effects of intraventricular β -endorphin on abstinence severity in two monkeys, M900 and M907.

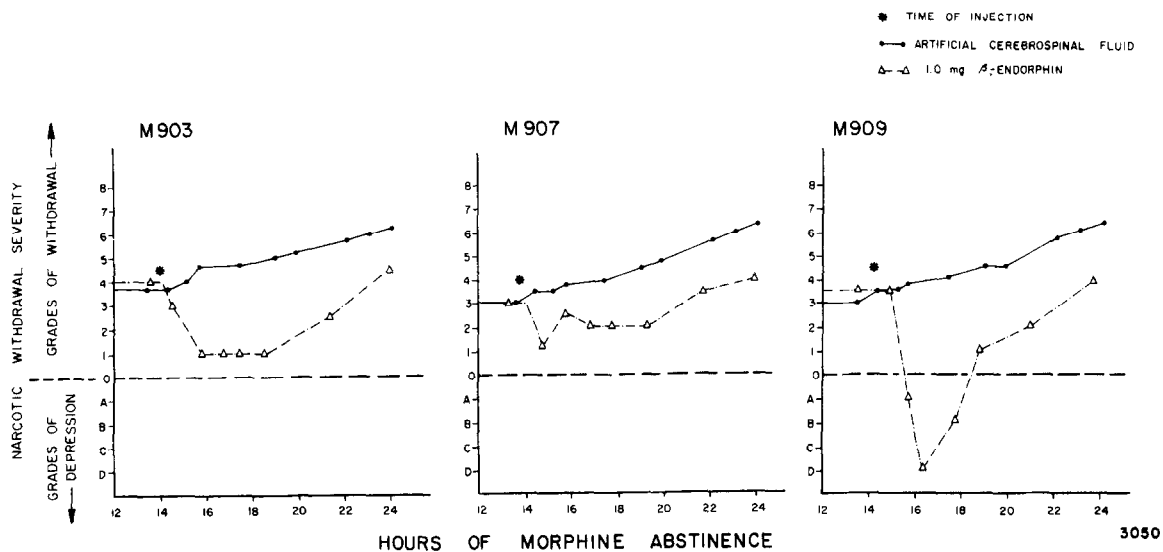


Fig. 4. Variability in the effects of β -endorphin given intraventricularly on abstinence severity in three morphine dependent monkeys. The effects of 1.0 mg of β -endorphin are compared to those of artificial cerebrospinal fluid given in equal volumes (0.2 ml) on two different occasions 14 hours after the last dose of morphine. Abstinence scores are as per Seever and associates (17-21).

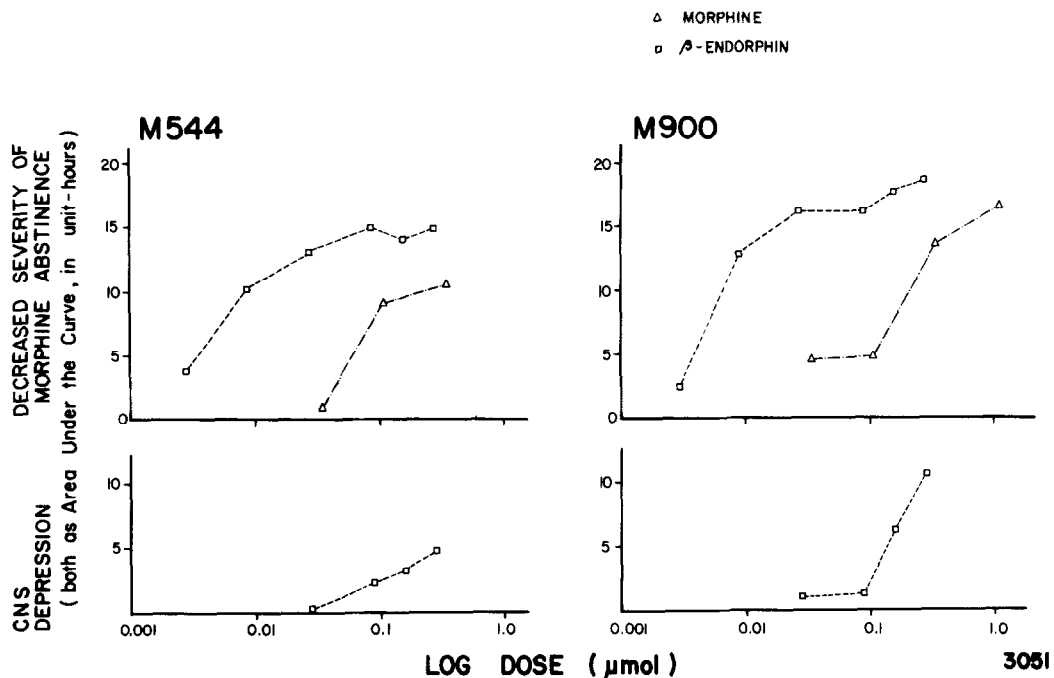
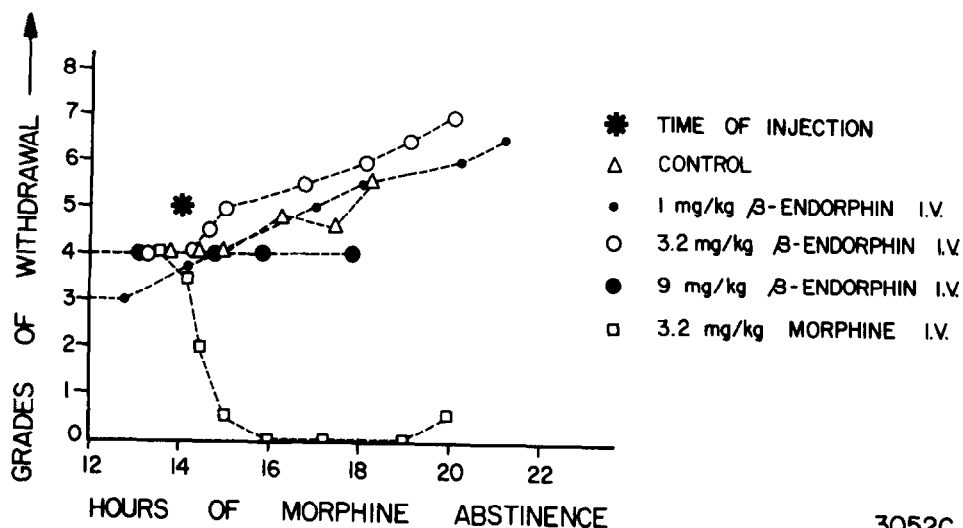


Fig. 5. Dose-effect relations of morphine and β -endorphin on abstinence severity in two morphine dependent monkeys. The data are expressed as the area under the curve in decreased abstinence and central nervous system depression in unit hours. Both drugs were given intraventricularly. Note that β -endorphin was more effective than morphine in reducing abstinence syndromes.

There was considerable variability in the effectiveness of both morphine and β -endorphin in suppressing the severity of morphine abstinence as shown in the data from another three monkeys. This is illustrated in Fig. 4.

The dose (μmol)-area of the response relations of β -endorphin and morphine, given intraventricularly, are shown in Fig. 5 for two morphine-dependent monkeys undergoing abstinence. Both substances suppress withdrawal signs but β -endorphin is much more potent on a molar basis (about 35X) and has a greater central nervous system depressant component. A larger dose of morphine (1000 μg) given intraventricularly to one monkey caused grand mal seizures. In contrast, no gross seizures were observed with β -endorphin.

As described above, doses of both β -endorphin and morphine which were effective in suppressing morphine withdrawal when given intraventricularly were ineffective when given intravenously. This indicates that morphine withdrawal and its suppression occurs in the brain and not at peripheral sites. Furthermore, in all six monkeys morphine in doses up to 10 mg/kg given either subcutaneously or intravenously was extremely effective in suppressing morphine withdrawal. In contrast, β -endorphin given intravenously



3052C

Fig. 6. Lack of a significant effect of intravenous β -endorphin on abstinence severity in a morphine dependent monkey.

in large doses of 1, 3.2 and 9 mg/kg was totally ineffective as shown in the data for monkey M893 in Fig. 6.

Acknowledgement

The authors would like to acknowledge the efforts of Messrs. Clifton Fly (deceased) and Fred Adams and their staff who maintained the monkeys' dependence and for teaching one of the authors (EFD) the subtleties of grading morphine abstinence. The authors would also like to thank Messrs. Cyrus and Peter Pourzan and Ms. Mary Ann Werz for assisting with various aspects of the project. This work was supported by the Psychopharmacology Research Fund (Domino) and the National Institute of Drug Abuse grant DA-03434 (Li). The housing and dependence of the animals were supported by NIDA grant DA-00254-06 (Swain). Metrizamide was obtained as the lyophilized sterile product through the courtesy of Dr. W.P. Blackmore, Sterling-Winthrop, Rensselaer, New York.

References

1. Li, C.H. and Chung, D. (1976). Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. Proc. Nat. Acad. Sci. (USA) 73: 1145-1148.
2. Li, C.H., Lemaire, S., Yamashiro, D. and Doneen, B.A. (1976). The synthesis and opiate activity of beta-endorphin. Biochem. Biophys. Res. Commun. 71: 19-25.

3. Li, C.H., Chung, D. and Doneen, B.A. (1976). Isolation, characterization and opiate activity of beta-endorphin from human pituitary glands. *Biochem. Biophys. Res. Commun.* 72: 1542-1547.
4. Li, C.H., Yamashiro, D., Tseng, L.F. and Loh, H.H. (1977). Synthesis and analgesic activity of human beta-endorphin. *J. Med. Chem.* 20: 325-328.
5. Loh, H.H., Tseng, L.F., Wei, E. and Li, C.H. (1976). Beta-endorphin is a potent analgesic agent. *Proc. Nat. Acad. Sci. (USA)*. 73: 2895-2898.
6. Tseng, L.F., Loh, H.H. and Li, C.H. (1976). Beta-endorphin as a potent analgesic by intravenous injection. *Nature* 263: 239-240.
7. Tseng, T.F., Loh, H.H. and Li, C.H. (1976). Beta-endorphin: cross tolerance and cross physical dependence on morphine. *Proc. Nat. Acad. Sci. (USA)*. 73: 4187-4189.
8. Wei, E. and Loh, H.H. (1976). Physical dependence on opiate-like peptides. *Science* 193: 1262-1263.
9. Tseng, T.F., Loh, H.H. and Li, C.H. (1977). Human beta-endorphin: development of tolerance and behavioral activity in rats. *Biochem. Biophys. Res. Commun.* 74: 390-396.
10. Catlin, D.H., Hui, K.K., Loh, H.H. and Li, C.H. (1977). Pharmacologic activity of beta-endorphin in man. *Comm. Psychopharmacol.* 1: 493-500.
11. Su, C.Y., Lin, S.H., Wang, Y.T., Li, C.H., Hung, L.H., Lin, C.S. and Lin, B.C. (1978). Effects of beta-endorphin on narcotic abstinence syndrome in man. *Taiwan I Hsueh Hui Tsa Chih. J. Formosan Med. Assoc.* 77: 133-141.
12. Meglio, M., Hosobuchi, Y., Loh, H.H., Adams, J.E. and Li, C.H. (1977). Beta-endorphin: behavioral and analgesic activity in cats. *Proc. Nat. Acad. Sci. (USA)*. 74: 774-776.
13. Hosobuchi, Y., Meglio, M., Adams, J.E. and Li, C.H. (1977). Beta-endorphin: development of tolerance and its reversal by 5-hydroxytryptophan in cats. *Proc. Nat. Acad. Sci. (USA)*. 74: 4107-4019.
14. Kline, N.S., Li, C.H., Lehmann, N.H., Lajtha, A., Laski, E. and Cooper, T. (1977). Beta-endorphin--induced changes in schizophrenic and depressed patients. *Arch. Gen. Psychiat.* 34: 1111-1113.
15. Myers, R.D. (ed.) (1971). Appendix C. Physiological Solutions. *Methods in Psychobiology*, Academic Press, Vol. 1, London, p. 65.
16. Deneau, G.A. (1956). An analysis of factors influencing the development of physical dependence to narcotic analgesics in the rhesus monkey with methods for predicting physical dependence liability in man. *Doctoral Dissertation*, University of Michigan.
17. Seevers, M.H. (1936). Opiate addictions in the monkey: I. Methods of study. *J. Pharmacol. Exp. Ther.* 56: 147-156.
18. Irwin, S. (1953). Characteristics of depression, antagonism and development of tolerance, physical dependence and neuropathology to morphine and morphine-like agents in the monkey. *Doctoral Dissertation*, University of Michigan, 1-186.

19. Villarreal, J.E. (1969). Pharmacologic characterization of morphine-antagonists in the rhesus monkey. Doctoral Dissertation, University of Michigan.
20. Villarreal, J.A. and Karbowski, M.G. (1974). The actions of narcotic antagonists in morphine-dependent rhesus monkeys. Narcotic Antagonists, Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. and Villarreal, J.E. (eds.), Adv. Biochem. Psychopharmacol. 8: 273-289.

Accepted 7/5/85