Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1271–1274, 1982. Printed in the U.S.A.

Hibernation "Trigger": Opioid-Like Inhibitory Action on Brain Function of the Monkey

P. R. OELTGEN

Department of Pathology, University of Kentucky, College of Medicine and Lexington Veterans Administration Medical Center, Pathology Service, Lexington, KY 40511

J. W. WALSH

Lexington Veterans Administration Medical Center and Division of Neurosurgery University of Kentucky, College of Medicine, Lexington, KY 40536

S. R. HAMANN

Lexington Veterans Administration Medical Center and Graduate Center for Toxicology University of Kentucky, College of Medicine, Lexington, KY 40536

D. C. RANDALL

Department of Physiology and Biophysics, University of Kentucky, Lexington, KY 40536

W. A. SPURRIER

Division of Neurosurgery, Loyola University Medical Center, Maywood, IL 60153

R. D. MYERS

University of North Carolina School of Medicine Departments of Psychiatry and Pharmacology and Center for Alcohol Studies, Chapel Hill, NC 27514

Received 23 September 1982

OELTGEN, P. R., J. W. WALSH, S. R. HAMANN, D. C. RANDALL, W. A. SPURRIER AND R. D. MYERS. *Hibernation "trigger": Opioid-like inhibitory action on brain function of the monkey.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1271–1274, 1982.—A hibernation "trigger" factor derived from the blood of the hibernating woodchuck acts to suppress vital physiological processes in the primate. When infused into the cerebral ventricle of the conscious monkey, the factor induced hypothermia, behavioral depression, bradycardia and aphagia. The opiate antagonists, naloxone and naltrexone, either reverse or retard these behavioral and physiological signs. We hypothesize that the "trigger" molecule is an endogenous opioid-like peptide which may be unique to the hibernator. Moreover, the non-hibernating primate apparently possesses receptor sites in the brain that are capable of responding to this potent molecule.

Hibernation "trigger" Plasma albumin fraction Hypothermia Bradycardia Hypophagia
Endogenous opioid-like peptide Behavioral depression Opioid antagonists

IT has been over a decade since Dawe and Spurrier [3] presented the first evidence for the presence of a hibernation "trigger" (HT) factor in the blood of the hibernating woodchuck and ground squirrel. They demonstrated that whole plasma withdrawn from the animal during the phase of winter hibernation induces a similar state of torpor when the plasma is injected in an active squirrel or woodchuck during the summer months. Attempts to biochemically characterize the HT molecule clearly show that the factor is bound to, or closely associated with, the plasma albumin fraction [11], is a protein [13], and exerts a profound effect on blood constituents [12,15]. Recently it was discovered that HT infused into the cerebral ventricle (ICV) of the conscious monkey produces a transitory fall in body temperature and a long term reduction in the animal's intake of food [10].

We now report that in the primate an ICV infusion of the

isolated HT-containing albumin fraction obtained from the hibernating woodchuck not only produces profound physiological alterations including hypothermia, bradycardia and hypophagia, but also induces an opiate-like modification in behavior [1]. Moreover, treatment with an opiate antagonist either entirely abolishes the effects of HT or retards its onset.

METHOD

Three adult male rhesus monkeys, *Macaca mulatta*, weighing 6–8 kg were accustomed to primate restraining chairs. The experimental room was maintained at an ambient temperature of 22–24°C and an illumination cycle of 15 hr light and 9 hr dark. Each monkey was provided with an ad lib supply of water and was trained to press a lever to activate an automatic feeder which delivered specially formulated 0.3 g banana pellets (P. J. Noyes Co., Precision Food Pellets).

Neurosurgical Procedures

After each animal was sedated with 15 mg/kg ketamine and subsequently anesthetized with halothane, aseptic surgical procedures were used as follows. Cardiac electrodes for monitoring heart rate were implanted as described previously [14]. A YSI thermistor bead for measuring cranial temperature was positioned 1.0 cm beneath the surface of the dura mater. This was accomplished by drilling a 5 mm craniotomy hole in the parietal bone and then stainless steel screws were inserted around the opening. After the thermistor was lowered in place, cranioplastic cement was used to affix the thermistor to the anchor screws and bone [8]. An intracerebroventricular (ICV) cannula of the Collison design was implanted stereotaxically in either or both lateral ventricles according to rigid aseptic procedures described previously [6,9]. For this procedure, two holes were drilled equidistantly 6.0 to 7.0 mm from the midsagittal suture at an AP coordinate of 12.0 to 14.0 mm anterior to stereotaxic 0. After each hole was threaded by a tap, the 18 gauge stainless steel guide cannula was screwed into the skull so that the tip rested 10.0 to 12.0 mm below the surface of the dura. Retaining screws were then positioned in the cranium around the base of each cannula and cranioplastic cement was packed around the entire array. To verify patency with the ventricle, a 20 gauge inner cannula, connected to a length of PE tubing, was first filled with artificial CSF [6]. After the cannula was passed through the diaphragm of the Collison cap, it was lowered until the pressure head of CSF was observable as reflected by rhythmic pulsations of the fluid within the PE tubing [9].

The animals were permitted two weeks to recover from the surgical procedures. Then, all overt primate behavioral patterns were monitored by a television camera and recorded on videotape both before and after ICV infusions of control or HT-containing albumin fractions.

Preparation of Woodchuck and Primate Plasma

Plasma which was fractionated and assayed for opioid activity following ICV infusion in primates was obtained from hibernating woodchucks. Blood was drawn aseptically by cardiac puncture while these animals were in deep hibernation as evidenced by having core temperatures of approximately 4–6°C and heart rates of one or two beats per minute.

An affinity chromatography technique, utilizing Affi-Gel Blue (Bio-Rad Industries, Richmond, CA) as the chromatography matrix was utilized to obtain nearly homogeneous

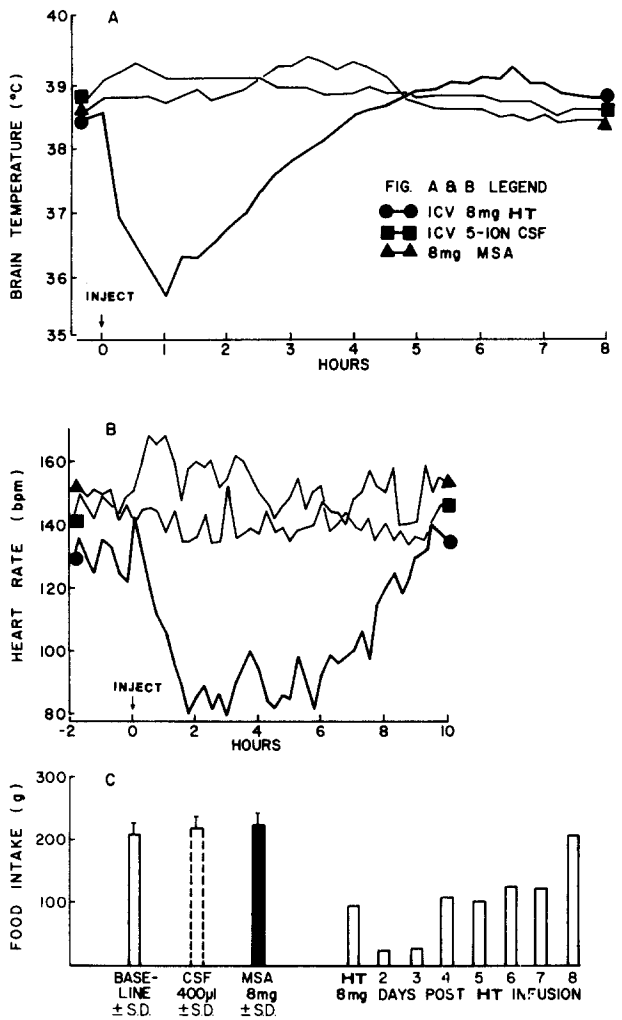

FIG. 1. (A) Brain temperature of a representative monkey following ICV injection of 8.0 mg HT in 400 µl of CSF; 400 µl of control CSF; or 8.0 mg MSA in 400 µl CSF. Abscissa is time in hours. (B) Heart rate (15 min computer averages) of a representative monkey. An interval of 48 hr or longer between each ICV injection of 400 µl CSF, 8.0 mg MSA, or 8.0 mg HT. Abscissa is time in hours. (C) Baseline intake of banana pellets of a representative monkey over five days (1st bar). Food intakes at interval of 48 hr or longer after ICV infusion of 400 µl CSF vehicle (2nd bar); 8.0 mg MSA (3rd bar); of 8.0 mg of HT (4th bar). Recovery from hypophagia post-HT infusion is depicted by the 5th through 11th bars.

HT-containing albumin fractions for ICV infusions in primates. This technique was also utilized for fractionating primate plasma albumin fractions. This technique is highly effective in selectively absorbing albumin from the plasma and has been described previously [11].

ICV Injections

Before an experiment began each animal's baseline temperature and heart rate was monitored for at least 2 hours while food intake was monitored for 5 days. Prior to ICV infusion the hibernating woodchuck albumin or monkey serum albumin fractions were reconstituted in iced artificial CSF [6] at a concentration of 2.0 mg/100 µl and rendered

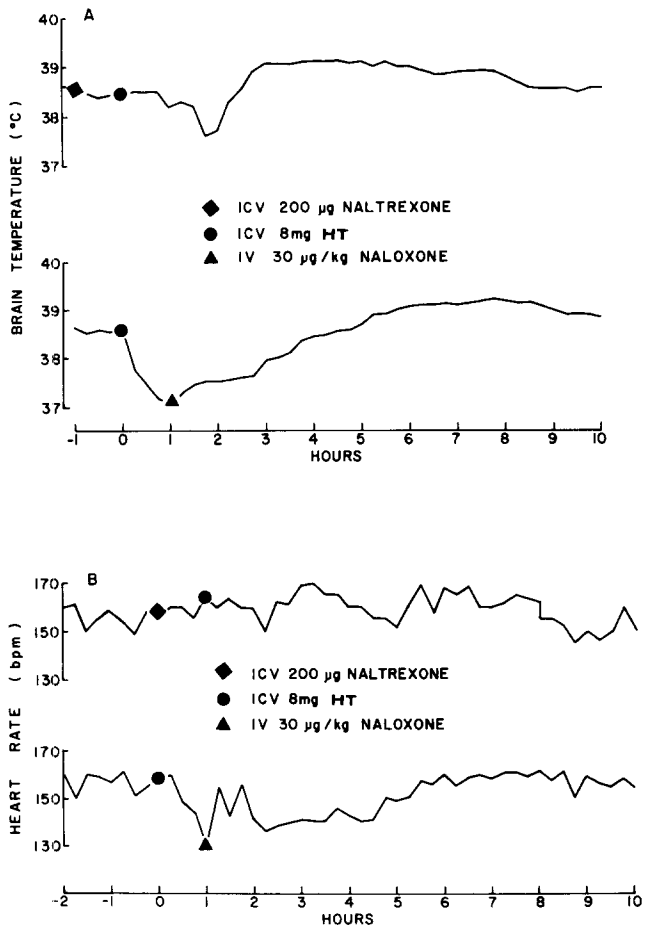

FIG. 2. The effects of opiate antagonists, naloxone (▲) and naltrexone (◆), on brain temperature and heart rate of a representative monkey either 1 hr before or after injection of HT (●). (A) Delay (top) of the hypothermic response produced by 200 μg of naltrexone ICV 1 hr prior to injection of 8.0 mg HT; reversal (bottom) of hypothermic response after intravenous injection of 30 μg/kg naloxone. (B) Abolition of bradycardia (top) by ICV injection of 200 μg naltrexone 1 hr prior to HT injection; attenuation of bradycardia (bottom) after intravenous injection of 30 μg/kg naloxone 1 hr after 8.0 mg HT.

pyrogen-free by passing them through a 0.21 micron Amicon Sterilet (Amicon, Danvers, MA). The dose of HT selected was twice that used in previous experiments [10] with the overall set of responses being essentially double in intensity. The protein concentration of the HT material was 0.93 mg/ml [4]. Serum immunoglobulin and CSF protein and glucose levels were determined before and at the end of each experiment. No significant change in these parameters were noted following HT or MSA injections nor were any signs of an untoward immunological response to the purified HT albumin fraction observed in these experiments.

RESULTS

As depicted in Fig. 1, an infusion over a 2 min interval of 8.0 mg of the HT-containing albumin fraction [10] in 400 μl of artificial CSF into the lateral ventricle of a representative animal exerted a profound effect on the behavioral and physiological parameters. The sequence of behavioral changes which ensued usually within 10–15 min following an infusion were retching, yawning, mouth gaping and marked lethargy. These responses were followed by closure of the eyes, slumping of the head and the overt appearance of an anesthetized state which persisted for 3 to 5 hrs. Physiological changes included a marked hypothermia of varying intensity, onset and duration which ranged from 1.5 to 3.0°C below the baseline with the mean fall in brain temperature of 2.6°C. A concomitant bradycardia persisted for up to 8 hrs after infusion with a maximal decrease of 43% to 50% below the baseline heart rate of 150 b.p.m. The period of hypophagia lasted for 5 to 7 days. Especially notable is the fact that the monkeys ate no food during the first 12 to 18 hr period after the ICV infusion of HT. In the three animals studied, we noted that there was virtually no weight loss over the period of hypophagia possibly because the metabolic rate was transiently suppressed during this interval. Following parallel ICV infusions of 400 μl of either a 5-ion artificial CSF vehicle or 8.0 mg of monkey serum albumin (MSA), none of the afore-mentioned behavioral or physiological modifications were observed. This corresponds to the absence of any effect of the albumin fraction obtained from the summer-active, non-hibernating woodchuck [10].

To determine whether opiate receptor blockers would alter the responses to HT, we pretreated the brain with ICV naltrexone, a longer lasting antagonist; to examine the more short acting antagonism, we gave naloxone intravenously following HT infusion. The intravenous infusion of the opiate antagonist, naloxone (30 μg/kg), one hr following ICV infusion of HT exerted a remarkable effect in all three animals. Within minutes they became alert and then oriented to their environment. In fact, naloxone completely abolished the long-term hypophagic response and in some animals immediate feeding followed the drug injection. Moreover, naloxone attenuated or reversed the hypothermia as well as the extent and duration of the accompanying bradycardia. As shown in Fig. 2A, an ICV infusion of 200 μg of the long-acting opiate antagonist, naltrexone, 1 hr prior to HT infusion not only delayed the onset of the hypothermic response by over an hour but also attenuated the magnitude of the fall to only 1°C. Naloxone given after temperature had fallen 1.5°C, one hr after HT, not only stopped a further decline but reversed it as well (Fig. 2A). Naltrexone's effect differed from that of naloxone's in that it also abolished the bradycardia. Further, the animals exhibited only brief episodes of lethargy and eye closure and no long-term hypophagia.

DISCUSSION

Previously the hibernation "trigger" factor had been shown to exert a highly specific effect in an environmentally adapted hibernating species. The present experiments extend our initial finding [10] that the HT molecule induces a sequence of profound physiological and behavioral effects in the primate species that is not able to undergo hibernation, at least as we know it. Thus, the broad range of biological activity of the HT demonstrates that the factor is not species-specific. Further, the experiments with naloxone and naltrexone provide the first evidence that the HT fraction from the hibernating woodchuck may serve as a carrier for a potent opiate-like substance. Possibly synthesized in the brain of the hibernator, the substance is apparently bound subsequently to a circulating albumin component and thus may be responsible for an animal's entry into natural hibernation.

Two findings bear on this deduction. First, Margules *et al.* [5] found that naloxone but not saline increases the heart rate of the hibernating Turkish hamster and can precipitate arousal, but has no cardio-acceleratory nor arousing action in a non-hibernating hamster. Second, the hibernating ground squirrel, but not the active animal, is resistant to the induction of physical dependence on morphine [2]. This could be due to a blockade of the brain's opiate receptor sites by an endogenous opioid-peptide factor, namely the HT molecule.

In any event, the clinical potential for a molecule such as HT is quite extraordinary. It could be used to depress metabolism, lower body temperature, manage cardiovascular function or reduce food intake, all in a pharmacologically controlled manner. In this context, the conscious primate may constitute a most suitable species for the further examination and analysis of HT activity of resolved plasma fractions.

ACKNOWLEDGEMENTS

This study was supported by the Veterans Administration to P. R. Oeltgen and NIH Grant HL-19343 to D. C. Randall and NSF Grant BMS 75-18441 to R. D. Myers. The authors also acknowledge the contribution of Mr. T. Skinner.

REFERENCES

1. Adler, M. W. Opioid peptides. *Life Sci.* **26:** 497–510, 1980.
2. Beckman, A. L., C. Llados-Eckman, T. L. Stanton and M. W. Adler. Physical dependence on morphine fails to develop during the hibernating state. *Science* **212:** 1527–1529, 1981.
3. Dawe, A. R. and W. A. Spurrier. Hibernation induced in ground squirrels by blood transfusion. *Science* **163:** 298–299, 1969.
4. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
5. Margules, D. L., B. Goldman and A. Finck. Hibernation: An opioid-dependent state? *Brain Res. Bull.* **4:** 721–724, 1977.
6. Myers, R. D. *Methods in Psychobiology,* vol. 1. London: Academic Press, 1971, Chapters 2 and 8.
7. Myers, R. D. Chronic methods: Intraventricular infusion, cerebrospinal fluid sampling, and push-pull perfusion. In: *Methods in Psychobiology,* vol. 3, edited by R. D. Myers. New York: Academic Press, 1977, pp. 281–315.
8. Myers, R. D., T. L. Yaksh, G. H. Hall and W. L. Veale. A method for perfusion of cerebral ventricles of the conscious monkey. *J. appl. Physiol.* **30:** 589–592, 1971.
9. Myers, R. D., W. L. Veale and T. L. Yaksh. Changes in body temperature of the unanaesthetized monkey produced by sodium and calcium ions perfused through the cerebral ventricles. *J. Physiol.* **217:** 381–392, 1971.
10. Myers, R. D., P. R. Oeltgen and W. A. Spurrier. Hibernation "trigger" injected in brain induces hypothermia and hypophagia in the monkey. *Brain Res. Bull.* **7:** 691–695, 1981.
11. Oeltgen, P. R., W. A. Spurrier, L. C. Bergmann and S. B. Jones. Isolation of a hibernation inducing trigger(s) from the plasma of hibernating woodchucks. *Prep. Biochem.* **8:** 171–188, 1978.
12. Oeltgen, P. R., W. A. Spurrier and L. C. Bergmann. Hemoglobin alterations of the 13-lined ground squirrel in various activity states. *Comp. Biochem. Physiol.* **64B:** 207–211, 1979.
13. Oeltgen, P. R. and W. A. Spurrier. Characterization of a hibernation induction trigger. In: *Survival in the Cold, Hibernation and Other Adaptations,* Chapter 8, edited by X. J. Musacchia and L. Jansky. New York: Elsevier/North Holland, Inc., 1981, pp. 139–157.
14. Randall, D. C., J. V. Brady and K. H. Martin. Cardiovascular dynamics during classical apetitive and aversive conditioning in laboratory primates. *Pavlov. J. biol. Sci.* **10:** 66–75, 1975.
15. Spurrier, W. A. and A. R. Dawe. Several blood and circulatory changes in the hibernation of the 13-lined squirrel. *Comp. Biochem. Physiol.* **44A:** 267–282, 1973.