Pharmacology Biochemistry & Behavior, Vol. 9, pp. 153–156. Printed in the U.S.A.

Naloxone Interactions with Morphine- and Shock-Potentiated Tonic Immobility in Chickens[1]

RONALD H. PETERS AND RICHARD A. HUGHES

Department of Psychology, Iowa State University, Ames, IA 50010

(Received 14 March 1978)

PETERS, R. H. AND R. A. HUGHES. *Naloxone interactions with morphine- and shock-potentiated tonic immobility in chickens.* PHARMAC. BIOCHEM. BEHAV. 9(2) 153–156, 1978.—Opiate receptor involvement in tonic immobility was examined by administering various doses of the opiate antagonist naloxone before measuring morphine-potentiated, shock-potentiated or unpotentiated tonic immobility in chickens. Naloxone attenuated morphine-potentiated, but not shock-potentiated or unpotentiated tonic immobility. Morphine-potentiated tonic immobility appears to be opiate specific.

Naloxone Tonic immobility Chickens Morphine Shock

THE RECENT discovery of endogenous peptides with morphine-like properties (endorphins) has triggered a wide variety of neuropsychopharmacological research activities. Two simultaneous reports [1,12] demonstrated that central (cerebrospinal fluid or periaqueductal gray) injections of β-endorphin induced prolonged catatonic-like states in rats. These states were characterized by periods of muscular rigidity and immobility of often an hour or more duration. The animals would resume normal postures after the presentation of a sudden stimulus like a noise or a light. Further, intraperitoneal administration of naloxone, a morphine antagonist, fully reversed all of the behavioral effects induced by β-endorphin. Since these behavioral abnormalities were strikingly similar to the behavior sometimes displayed by schizophrenic patients, both research groups suggested that dysfunctions within brain endorphin systems may be an etiological factor in this behavioral disorder. Although the recent reports [5,16] that naloxone administration does not improve psychotic symptomatology are not consistent with this suggestion, the notion that disruption of endorphin homeostatic mechanisms may produce symptoms of mental illness remains viable.

We were impressed by at least the superficial similarity of these apparently abnormal states induced in rats by β-endorphin to the interesting phenomena termed tonic immobility (TI) that can be induced in a variety of species by physical restraint [4, 7, 14]. When restraint is terminated, animals often remain physically immobile for an hour or more and will resume normal postures following the presentation of sudden stimuli. Although various theories have been developed to account for this unusual animal behavior pattern, Gallup [8] views these catatonic-like states as fear-potentiated reactions and further suggests that TI may provide a useful laboratory model of catatonic schizophrenia [9].

Although the similarities between the immobility reactions induced by β-endorphin and physical restraint may indeed be only superficial, their neural substrates may also be at least partially overlapping. This speculation is enhanced by observations that morphine prolongs TI duration in chickens [11]. Since naloxone fully reversed the behavioral effects of central administration of β-endorphin in rats, Experiment 1 examined the possibility that TI duration in chickens may be attenuated or its induction blocked by systemic administration of naloxone.

EXPERIMENT 1

Method

Animals. White Rock cockerels were obtained from a local supplier (Welp, Inc., Bancroft, Iowa) at 1 day posthatch and were maintained in the same fashion for all experiments. They were housed in a commercial brooder with free access to food and water under artificial light on from 0600 to 1800 hr. Behavioral testing in all experiments occurred at 11 days of age.

Apparatus. Eight identical plywood chambers, each lined with 4.6 cm white polystyrene to form a cube of approximately 30 cm along each inside wall, were used. Access to the interior was provided by a 15 cm dia. hole in one wall which was covered with a white cloth flap. The chamber was diffusely illuminated by a 7 W white light bulb which extended through the ceiling and was covered by a white translucent Plexiglas panel. The chamber was vented by 6 holes (2

[1]Portions of this paper were presented at the annual meeting of the Midwestern Psychological Association, Chicago, May 1978. We thank Endo Laboratories for their contribution of naloxone hydrochloride.

cm) in the ceiling. An oval depression (18×10 cm) in the center of the floor was about 1 cm deep along the circumference and gradually sloped to a depth of 2.5 cm at the center. A photobeam was directed at a photosensor across the short axis of the oval approximately 4 cm from one end of the long axis. An automatic timer (1 sec increments), started by a silent manual switch located outside the chamber, stopped when the photobeam activated the photosensor as the chicken resumed an upright posture. Each test chamber was placed in a larger fan-ventilated polystyrene-lined (2.3 cm) plywood enclosure (inside dimensions 98×46×58 cm) to provide additional acoustic isolation during testing.

Procedure. A chicken was removed from the brooder and handcarried to an adjacent room where it was weighed and assigned by random block design to receive an IP injection of either a 0.0, 0.1, 0.5, 1.0, 5.0 or 10.0 mg/kg/2 ml naloxone in isotonic saline (N=16 per treatment). These doses extend both above and below those typically used in mammalian research. Since at least 8 hr were required to complete each experiment, the random block design was used in all experiments to minimize confounding of treatments with circadian influences on TI duration [15].

Following the injection each bird was placed in a wire-covered white plastic enclosure (inside dimensions 33×28×16 cm) containing wood shavings. The time between injection and TI induction was approximately 15 min. Near the end of this interval the bird was carried in the container from the treatment room to a test room where TI was induced. The bird was held in an upright position over the oval depression for 5 sec and was then inverted. It was held firmly on its back for 15 sec and was then released. At release the timer was started, the cloth flap was lowered over the opening, and the door of the large plywood enclosure was quietly closed. The timer continued until the photobeam activated the photosensor as TI terminated or until a criterion of 1800 sec elapsed. Birds that either turned over or stood up at release were given zero duration scores. This test procedure was used in all experiments. Further, immobility was induced by the same experimenter without treatment knowledge in all experiments.

Data analysis. Since treatment variances for raw score data were heterogeneous and some durations were zero, analysis of variance in this and all other experiments was performed on data transformed by $\log_{10}(X+1)$. One-tailed t tests were used to test the significance of the difference between treatment means because a priori directional predictions were made in each experiment.

Results

Systemic injections of naloxone at doses ranging between 0.1 and 10.0 mg/kg neither blocked the induction of TI nor attenuated its duration, $F(5,90)=1.34$, $p=0.25$. The only suggested effect of naloxone was a slight potentiation of TI duration, apparent in both raw and transformed scores, at the two lowest doses. Clearly, our initial expectations were not confirmed.

EXPERIMENT 2

Although naloxone failed to influence TI in Experiment 1, Hicks *et al.* [11] demonstrated that morphine substantially increased TI duration. If morphine potentiation is opiate specific, then naloxone should attenuate this morphine effect.

TABLE 1
NALOXONE REVERSAL OF MORPHINE-POTENTIATED TI

	$\bar{X}$ Sec	$\bar{X}$ log (Sec +1)
Sal-Sal	125.3	1.94
M-Sal	785.6	2.69*
M-0.1N	582.4	2.21†
M-1.0N	416.6	2.07†
M-10.0N	339.9	2.09†

* significantly different from Sal-Sal treatment.
† significantly different from M-Sal treatment.

Method

Thirty min before TI induction, each bird was weighed and received an IM injection of either 2.5 mg/kg/ml morphine sulfate (M) or saline (Sal) followed 15 min later by an IP injection of either saline or naloxone (N) at doses of either 0.1, 1.0, or 10.0 mg/kg/2 ml. The birds were placed in the white holding containers after each injection. Five treatments (N=19 per treatment) were formed by the following combinations of injections: Sal–Sal, M–Sal, M–0.1N, M–1.0N, and M–10.0N.

Results

Morphine injected 30 min prior to TI induction significantly increased TI duration (Table 1). Naloxone at concentrations of 0.1, 1.0 and 10.0 mg/kg significantly attenuated morphine potentiation when injected after morphine administration and before TI induction. These results suggest that morphine potentiation of TI duration is mediated by opiate receptor activity. The critical differences at the 0.05 and 0.01 levels of significance were 0.42 and 0.60, respectively.

EXPERIMENT 3

The results of Experiment 2 suggest that opiate receptor mechanisms are involved in the morphine potentiation effect. Interestingly, painful electric shock administered before physical restraint also potentiates TI [10,13]. Although superficially paradoxical, TI enhancement by morphine, an analgesic, and by shock, a painful stimulus, may have a common neural substrate. Shock stimulation may induce the release of endogenous opiate-like substances with behavioral effects similar to those produced by morphine administration. If shock potentiation is mediated by opiate receptor activity, then naloxone should attenuate this shock effect.

Method

Each bird was weighed and handcarried to an adjacent room where it received either shock or sham shock treatment. The shock apparatus was a constant current AC source set to deliver 3.0 mA through flattened miniature alligator clips. The alligator clips were attached to the bird's legs and three brief shocks were administered (3 sec duration separated by 1 sec intervals) while the bird was gently held by the experimenter. The alligator clips were not attached for the sham shock treatment. Each bird was then handcarried to a room acoustically isolated from the shock treatment where it immediately received an IP injection of either saline

TABLE 2

NALOXONE FAILURE TO REVERSE SHOCK-POTENTIATED TI

	$\bar{X}$ Sec	$\bar{X}$ log (Sec + 1)
Sham-Sal	119.5	1.64
Shock-Sal	379.6	2.09*
Shock-0.1N	634.9	2.64
Shock-1.0N	412.4	2.13
Shock-10.0N	566.7	2.45

* significantly different from Sham-Sal treatment.

TABLE 3

NALOXONE INTERACTIONS WITH SHAM, MORPHINE, AND SHOCK TREATMENTS

	Saline		10.0 Naloxone	
	$\bar{X}$ Sec	$\bar{X}$ log (Sec + 1)	$\bar{X}$ Sec	$\bar{X}$ log (Sec + 1)
Sham	155.2	1.45	131.5	1.60
Morphine	625.9	2.58*	288.2	2.04†
Shock	886.3	2.81*	1028.5	2.90

* significantly different from Sham-Sal treatment.
† significantly different from M-Sal treatment.

or 0.1, 1.0, or 10.0 mg/kg/2 ml naloxone. The birds were placed in the white holding containers after the injection and TI was induced 15 min later. Five treatments (N=20 per treatment) were formed by the following treatment combinations: Sham–Sal, Shock–Sal, Shock–0.1N, Shock–1.0N, and Shock–10.0N.

Results

Shock administered before TI induction significantly increased TI duration (Table 2). The critical difference at the 0.05 level of significance was 0.39. These data also clearly indicate that naloxone administered immediately after shock treatment and 15 min before TI induction did not attenuate the TI potentiating effects of shock. Mean TI durations for the three naloxone groups exceeded that of the group receiving saline after shock treatment. These observations suggest that shock potentiation of TI duration is not mediated by opiate receptor activity.

EXPERIMENT 4

Failure to obtain a drug effect may occur for various reasons. Obviously, the drug in question may not affect the mechanisms that mediate the particular behavior of interest. Naloxone, in Experiments 1 and 3, did not attenuate the duration of either unpotentiated or shock-potentiated TI. Thus we tentatively concluded, within the treatment parameters used in these experiments, that opiate receptor activity does not play a substantial role in the expression of these particular behaviors.

The absence of a drug effect may also occur because the specific drug sample was, for some unknown reason, biologically inactive. The pattern of results obtained in the present series of experiments argues against this interpretation because the naloxone which attenuated morphine-potentiated TI in Experiment 2 was prepared from the same naloxone stock used for the other two experiments. Experiment 4 was performed: a) to further discount the possibility that naloxone as prepared in Experiment 1 and 3 was biologically inactive, and b) to replicate the basic observations of the first three experiments.

Method

The procedures were factorial combinations of those used in the first three experiments except that only one dose of naloxone, 10.0 mg/kg/2 ml in isotonic saline, was used. After weighing, each bird received an IM injection of either 2.5 mg/kg/ml morphine sulfate or saline followed 15 min later by an IP injection of either naloxone or saline. Immediately before the second injection, each bird was handcarried to an adjacent room where it received either shock or sham shock treatment. TI was induced 15 min after the second injection. Six treatments (N=17 per treatment) were performed by the following treatment combinations: Sham–Sal, Sham–10.0N, M–Sal, M–10.0N, Shock–Sal, and Shock–10.0N.

Results

The data presented in Table 3 clearly replicate the pattern established in the first three experiments. The critical differences at the 0.05 and 0.005 levels of significance were 0.40 and 0.64, respectively. Both morphine and shock significantly increased TI duration. Naloxone significantly attenuated morphine- but not shock-potentiated TI. Finally, naloxone did not influence unpotentiated TI.

GENERAL DISCUSSION

Several factors contribute to our confidence in the reliability of the data reported here. Mean raw score TI durations were relatively stable for the control condition in the four experiments ranging between 119.5 and 166.9 sec. Further, blind TI induction and automated TI measurement procedures were used. Finally, the outcome pattern established in the first three experiments was fully replicated in Experiment 4.

Although behaviorally similar, the immobility reactions induced by β-endorphin in rats and by physical restraint in chickens are apparently mediated by neural substrates with little common overlap. The opiate antagonist naloxone, which reversed endorphin-induced catalepsy [1,12], did not influence TI or attenuate shock-potentiated TI. The only consistently observed effect of naloxone, obtained at all three doses used in Experiment 2 and replicated at the highest dose in Experiment 4, was attenuation of morphine-potentiated TI. Wallnau and Gallup [17] have also recently reported that naloxone does not affect TI in chickens. In contrast to the data reported here, however, naloxone did not attenuate morphine potentiation. While the basis for this discrepancy is not known, statistical power may be a relevant consideration since these researchers used only nine birds in each treatment condition.

Carli [2,3] has reported that morphine prolongs TI in rabbits. This potentiation was abolished by naloxone, an observation consistent with our data. Further, naloxone abolished the potentiation elicited by painful stimulation, an observation inconsistent with our data. While many procedural variables may account for this discrepancy, naloxone reversal in this instance occurred during a state of continuous nocicep-

tive stimulation induced by subcutaneous injection of Formalin.

An extremely wide variety of behavioral and pharmacological manipulations have been shown to affect TI. Although the present data provide no support for the hypothesis that restraint-induced TI is mediated by a neural system involving opiate receptor mechanisms, the data clearly demonstrate that TI potentiation induced by shock and morphine can be dissociated pharmacologically. Morphine potentiation appears to involve opiate receptor activity since such potentiation was attenuated by naloxone. The present data do not permit a characterization of the neural system mediating shock-potentiated TI. This system does not appear to involve opiate receptor mechanisms since naloxone did not attenuate shock-induced potentiation.

Although naloxone did attenuate morphine-potentiated TI, naloxone clearly did not attenuate either shock-potentiated or unpotentiated TI. In fact, naloxone at low doses appeared to increase both unpotentiated (Experiment 1) and shock-potentiated TI (Experiment 3). Considerable caution should be used in evaluating these potentially interesting latter observations. Since no a priori predictions were made with respect to these effects, two-tailed t tests were used to evaluate the significance of these differences. In Experiment 1, none of the differences between group means was significant at the 0.05 level (as suggested by the nonsignificant F value obtained using analysis of variance). With a critical difference of 0.47 at the 0.05 level of significance, naloxone at a concentration of 0.1 mg/kg was the only treatment that significantly increased TI duration above the mean of the Shock–Sal treatment (Experiment 3). Further, the mean of the Shock–Sal treatment (2.09) may have been unusually low (for unknown reasons) since the mean for this group (2.81) in the replication in Experiment 4 was substantially higher. Although there is some evidence that naloxone induces a hyperalgesic effect [6], naloxone in the present study was administered following shock treatment. Additional research is required to determine if the suggestive potentiation effects of naloxone represent substantive phenomena in opiate-naive animals.

Finally, Hicks *et al.* [11] demonstrated that morphine-induced enhancement of TI does not occur in chickens pretreated with *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase. Although serotonergic systems may participate in the morphine potentiation effect, this outcome does not necessarily imply that the morphine effect is nonnarcotic. Since morphine-potentiated TI is naloxone reversible, serotonergic participation may be induced by opiate receptor activation.

REFERENCES

1. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* **194:** 630–632, 1976.
2. Carli, G. Some evidence of analgesia during animal hypnosis. *Expl Brain Res.* Suppl. **23:** 67, 1975.
3. Carli, G., F. Farabollini and G. Fontani. Responses to painful stimuli during animal hypnosis. In: *Advances in Pain Research and Therapy,* Vol. 1, edited by J. J. Bonica and D. Albe-Fessard. New York: Raven Press, 1976, pp. 727–731.
4. Crawford, F. T. and A. M. Prestrude (Eds.). Animal hypnosis: research and theory. *Psychol. Rec.* **27:** 1–218, 1977.
5. Davis, G. C., W. E. Bunney, Jr., E. G. DeFraites, J. E. Kleinman, D. P. van Kammen, R. M. Post and R. J. Wyatt. Intravenous naloxone administration in schizophrenia and affective illness. *Science* **197:** 74–77, 1977.
6. Frederickson, R. C. A., V. Burgis and J. D. Edwards. Hyperalgesia induced by naloxone follows diurnal rhythm in responsivity to painful stimuli. *Science* **198:** 756–758, 1977.
7. Gallup, G. G., Jr. Animal hypnosis: factual status of a fictional concept. *Psychol. Bull.* **81:** 836–853, 1974.
8. Gallup, G. G., Jr. Tonic immobility: the role of fear and predation. *Psychol. Rec.* **27:** 41–61, 1977.
9. Gallup, G. G., Jr. and J. D. Maser. Tonic immobility: evolutionary underpinnings of human catalepsy and catatonia. Symposium paper presented at the Meeting of the American Psychological Association, New Orleans, 1974.
10. Gallup, G. G., Jr., R. F. Nash, R. J. Potter and N. H. Donegan. Effect of varying conditions of fear on immobility reactions in domestic chickens *(Gallus gallus). J. comp. physiol. Psychol.* **72:** 442–445, 1970.
11. Hicks, L. E., J. D. Maser, G. G. Gallup, Jr. and P. H. Edson. Possible serotonergic mediation of tonic immobility: effects of morphine and serotonin blockade. *Psychopharmacologia* **42:** 51–56, 1975.
12. Jacquet, Y. F. and N. Marks. The C-fragment of β-lipotropin: an endogenous neuroleptic or antipsychotogen? *Science* **194:** 632–635, 1976.
13. Nash, R. F. and G. G. Gallup, Jr. Effect of different parameters of shock on tonic immobility. *Behav. Res. Meth. Instru.* **7:** 361–364, 1975.
14. Ratner, S. C. Comparative aspects of hypnosis. In: *Handbook of Clinical and Experimental Hypnosis,* edited by J. E. Gordon. New York: Macmillan, 1967, pp. 550–587.
15. Rovee, C. K., L. W. Kaufman, G. H. Collier and G. C. Kent, Jr. Periodicity of death feigning by domestic fowl in response to stimulated predation. *Physiol. Behav.* **17:** 891–896, 1976.
16. Volavka, J., A. Mallya, S. Baig and J. Perez-Cruet. Naloxone in chronic schizophrenia. *Science* **196:** 1227–1228, 1977.
17. Wallnau, L. B. and G. G. Gallup, Jr. Morphine and tonic immobility: non-narcotic effects. Paper presented at the meeting of the Psychonomic Society, Washington, 1977.