Pharmacology Biochemistry & Behavior, Vol. 11, pp. 705–708. Printed in the U.S.A.

Possible Non-Narcotic Component to Action of Opiate Peptides on Tonic Immobility[1]

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Received 13 August 1979

OLSON, R. D., A. J. KASTIN, G. J. LAHOSTE, G. A. OLSON AND D. H. COY. *Possible non-narcotic component to action of opiate peptides on tonic immobility*. PHARMAC. BIOCHEM. BEHAV. 11(6) 705–708, 1979.—Chickens were tested in a tonic immobility paradigm after a single intraperitoneal injection of either 0.0, 0.1, 1.0, 10.0, 100.0, or 1000.0 μg/kg of the potent opiate analog, (D-Ala^2, F$_5$Phe^4)-Met-enkephalin-NH$_2$. An inverted-U relationship was obtained, with 100 μg/kg being the most effective in prolonging immobility. This dose was used in subsequent studies involving pretreatment with naloxone or diluent followed by treatment with diluent, (D-Ala^2, F$_5$Phe^4)-Met-enkephalin-NH$_2$ (a strong opiate), or (D-Phe^4)-Met-enkephalin (a weak opiate). The results indicated that although naloxone had mixed effects in attenuating the duration of tonic immobility, even the analog with negligible opiate activity reliably potentiated the response. Therefore, a component of the effect of opiate peptides on tonic immobility could be due to a non-narcotic action.

Enkephalin Naloxone Tonic Immobility

TONIC immobility (TI), a catatonic-like state produced by brief restraint, has been reported for insects, crustaceans, fish, amphibians, reptiles, birds, and even mammals [7]. The effect is produced by holding the animal on a flat surface for about 15 seconds, waiting for the struggling to cease, and then removing all restraint. This procedure normally leaves the animal in a relatively motionless, catatonic-like condition which may last from a few seconds to well over an hour. Because of the obvious superficial similarities, this reponse has been proposed as a laboratory-evolutionary model of catatonic schizophrenia [8].

Although TI is easily produced in a variety of species, the physiological basis for the effect is not clear. Wallnau and Gallup [20] have proposed a serotonergic, midbrain-raphe explanation for TI suggesting that neuronal mechanisms related to serotonin are involved in modulating the effect. Considerable support exists for this possibility (e.g., [10,14]).

At the same time, however, drug-induced catatonia in rats after central injections of β-endorphin produced effects very similar to TI [1,11], and caused some speculation that the endogenous opiates might be involved in the physiological control of TI [2,3]. Studies showing that morphine increased the duration of TI in both chickens [10] and rabbits [5], and also that the opiate antagonist naloxone reversed β-endorphine-induced catatonia [1,11] further supported this position.

However, several recent studies examining the effect of naloxone on the duration of TI found no effect [5,6,18,21], suggesting that any possible role of endorphin in TI might be non-narcotic in character. Non-narcotic effects of the brain opiates have been demonstrated in other paradigms and are reviewed elsewhere [12]. One study [2] did report a reduction in the duration of TI in rabbits with 15 to 25 mg/kg of naloxone, but the high doses made it difficult to interpret the effect.

Accordingly, the present studies examined the effects of a potent enkephalin analog on increasing the duration of TI and compared it with an analog of enkephalin having negligible direct opiate activity in order to evaluate the non-narcotic effect of the opiate peptides. The effects of pretreatment with naloxone were also examined.

EXPERIMENT 1

Based on increased durations of TI after injections of morphine [10] and the fact that the catatonia induced in rats [1,11] and, to a lesser degree, in fish [16] by β-endorphin greatly resembles the TI response, the first experiment evaluated varying doses of (D-Ala^2, F$_4$Phe^4)-Met-enkephalin-NH$_2$. This analog was chosen because it produced the greatest behavioral effects in an earlier comparative study of 20 peptides [17] and is a potent opiate [4].

[1]The authors wish to thank C. P. Carpenter, D. L. Hargis and M. D. Mauk for their assistance in data collection. This study was supported in part by the Medical Research Service of the VA. Please address reprint requests to Richard D. Olson, Department of Psychology, University of New Orleans, New Orleans, LA 70122.

0091-3057/79/120705-04$00.90/0

METHOD

Animals

Ninety straight-run Production Red chickens (*Gallus gallus*) were obtained from a local vendor at one day of age. The animals were housed in brooders with constant illumination to minimize any effects of circadian periodicity and maintained on Purina Startena feed.

Drugs

A pentafluorinated enkephalin analog, (D-Ala^2, F_5Phe^4)-Met-enkephalin-NH_2, was synthesized by solid phase methods [4] and dissolved in a vehicle consisting of 0.9% saline acidified with acetic acid to 0.01 M, with a pH of 4.1. The vehicle solution also served as the diluent control condition. In addition to the diluent condition, concentrations of 0.1, 1.0, 10.0, 100.0, and 1000.0 μg/kg were used as coded solutions.

Procedure

Animals were tested at 8–9 days of age because the blood-brain barrier is not fully formed until about 3–4 weeks, thus optimizing the probability that the analog would reach the brain [13,19]. All chickens were randomly assigned to one of the six dose groups and administered an intraperitoneal (IP) injection of the coded solution. After injection, animals were placed for 10 min in individual holding baskets in which they were subsequently carried to a test room. The animal was then placed on a table where it was physically restrained on its right side for 15 sec. The duration of TI was timed from the instant the experimenter released his hands from the animal until it rose to its feet. If the initial attempt at induction failed to generate TI, the experimenter waited 60 sec and repeated the procedure. No animals required more than two trials.

RESULTS

The results are presented in Fig. 1. After an $x=\log_{10}(x+1)$ transformation was performed to reduce skewness and variability, analysis of variance revealed a main effect for dose, $F(5,84)=2.42$, $p<0.05$. Duncan's Multiple Range Test further showed that the control was significantly different ($p<0.05$) from each of the experimental groups. Although the dose groups did not differ reliably from one another, the groups receiving 10 and 100 μg/kg had the longest durations.

EXPERIMENT 2

Having demonstrated that a potent opiate analog reliably increased the duration of the TI response, and that an effective dose was 100μg/kg, we now considered the nature of the effect. Conflicting opinions exist about TI. Carli [2,3] suggested that the opiate receptor function might be the mechanism for the induction and maintenance of TI. Wallnau and Gallup [21], on the other hand, proposed that morphine-potentiated TI might occur because of a non-narcotic action. Finally, Peters and Hughes [18] stated that morphine-potentiated TI was naloxone reversible but could be blocked by an inhibitor of serotonin synthesis, suggesting that serotonergic participation might be induced by opiate receptor activation.

The second experiment was designed to examine the possibility that TI might be modulated by the non-narcotic action of opiate peptides. Therefore, the effects of naloxone on analogs with potent and negligible opiate activity were studied.

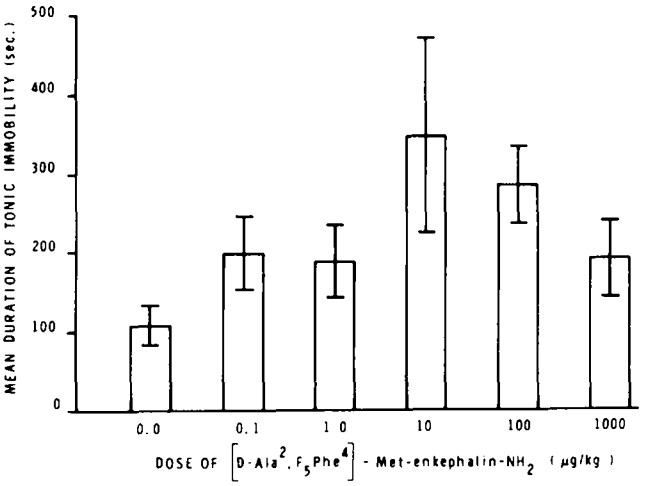

FIG. 1. Mean duration of tonic immobility in seconds for chickens 8–9 days old as a function of dose. Bars indicate the standard error of the mean at each point.

METHOD

Animals

Ninety straight-run Production Red chickens were housed and maintained as described in the first experiment.

Drugs

In addition to the pentafluorinated enkephalin analog described in the first experiment, an enkephalin analog with negligible opiate activity at the dose used, [D-Phe^4]-Met-enkephalin, was synthesized in a similar fashion and suspended in the diluent.

Procedure

All animals were again tested at 8–9 days of age. Chickens were randomly assigned to one of six groups, receiving a pretreatment injection of either diluent or naloxone (1.02 mg/kg) followed 10 min later by an injection of (D-Ala, F_5Phe^4)-Met-enkephalin-NH_2 (100 μg/kg), (D-Phe^4)-Met-enkephalin (100 μg/kg), or diluent. Each injection was administered IP in a volume of 0.5 ml. Testing began 10 min after the second injection using the procedures described in the first experiment. All solutions were administered without knowledge of contents. The parameters of this study essentially replicate those used in previous TI work with morphine [21].

RESULTS

Figure 2 presents the results. Analysis of variance was performed after an $x=\log_{10}(x+1)$ transformation and yielded a significant main effect for treatment, $F(2,84)=4.014$, $p<0.05$. Duncan's Multiple Range Test showed the animals receiving (D-Phe^4)-Met-enkephalin had reliably longer TI durations than the controls ($p<0.05$), but that neither group differed significantly from animals injected with (D-Ala^2, F_5Phe^4)-Met-enkephalin-NH_2. Comparisons using Scheffe's Test showed that both peptide groups taken together differed reli-

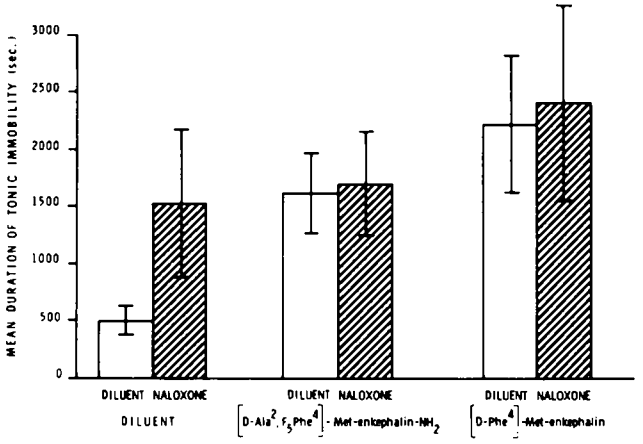

FIG. 2. Mean duration of tonic immobility in seconds for chickens 8–9 days old as a function of treatment. Bars indicate the standard error of the mean at each point.

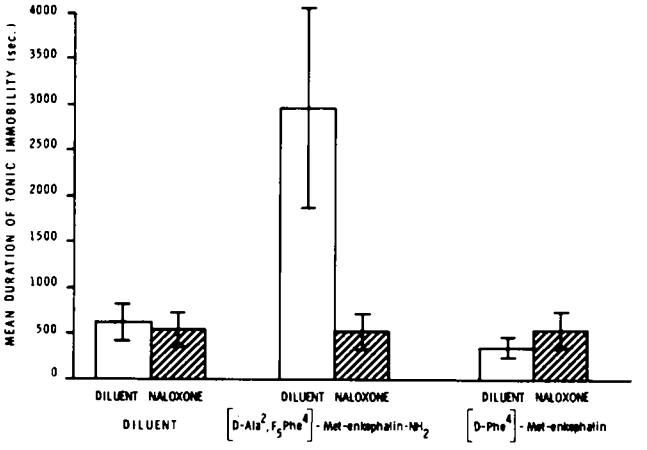

FIG. 3 Mean duration of tonic immobility in seconds for chickens 18–20 days old as a function of treatment. Bars indicate the standard error of the mean at each point.

ably from the control, $F(2,84)=7.477$, $p<0.05$. Naloxone effects were not significant, either as a main effect or in an interaction.

EXPERIMENT 3

The analog with neglibible direct opiate activity had the greatest effect in potentiating TI duration; the analgesically more potent analog also increased the response. Although times for both groups were not attenuated by naloxone, no effects associated with naloxone pretreatment were statistically significant. Experiment 3 was designed to replicate the previous results with older chickens in which the blood-brain barrier was likely to be better established.

METHOD

Animals

Sixty straight-run Production Red chickens were housed and maintained as described in the first experiment.

Drugs

All substances were identical to those used in Experiment 2.

Procedure

All animals were tested at 18–20 days of age using procedures identical to those used in the second experiment.

RESULTS

The results are shown in Fig. 3. Analysis of variance after an $x=\log_{10}(x+1)$ transformation yielded a significant treatment effect, $F(2,54)=3.943$, $p<0.01$, and a significant interaction of pretreatment with treatment conditions, $F(2,54)=3.943$, $p<0.05$.

Both Duncan's Multiple Range Test and Scheffe's Test showed that the pentaflourinated analog had significantly longer TI durations than the weaker enkephalin analog ($p<0.05$) and diluent ($p<0.05$). but that no reliable difference existed between the latter conditions. A test of simple effects showed that the treatment effect was significant when pretreatment was with diluent, $F(2,54)=8.867$, $p<0.01$, but not when pretreatment was with naloxone, $F<1.000$.

DISCUSSION

The potentiation of TI after injections of the potent opiate analog, (D-Ala^2, F$_5$Phe^4)-Met-enkephalin-NH$_2$, is in agreement with previous findings that administration of morphine enhanced the response [10,21]. The effect was highly reliable and was observed in each experiment. That a dose of 100 μg/kg produced a reliable behavioral effect is consistent with our results with this analog in learning paradigms involving fish (Olson, Kastin, Mauk, Olson, and Coy, unpublished observations) and monkeys [15]. In all studies, dose-response curves assume the general form of inverted U-shaped function, with 100 μg/kg as the apogee. Similar dose-response relationships have been observed with other brain peptides[12].

Experiment 2 was conducted with chickens 8–9 days of age to assure that the blood-brain barrier was not developed, allowing the injected substances a maximum probability of reaching the brain [13,19]. Although (D-Ala^2, F$_5$Phe^4)-Met-enkephalin-NH$_2$, an active opiate analog, again increased TI duration, it is important to note that the analog with negligible direct opiate activity, (D-Phe^4)-Met-enkephalin, generated the greatest potentiation of TI. This result suggests that a narcotic action is not required to induce and maintain the TI response. It also suggests that the primary role of both morphine as well as the endogenous opiates in TI may be non-narcotic in function. This finding is consistent with the work of Wallnau and Gallup [20,21] but does not exclude explanations based on the need for opiate receptor activation [2,3,18]. Naloxone pretreatment did not attenuate the duration of TI in any of the groups in Experiment 2. This essentially replicates previous findings by several other groups [5,6,18,21], and adds further support to the findings that a narcotic action is not essential for the behavioral actions of the opiate peptides or for TI.

The results of Experiment 3, involving chickens 18–20 days old, were similar to those of Experiment 2 with two important exceptions. First, the analog with no analgesic activity at the dose used, did not reliably potentiate the duration of TI. A likely explanation is that it had more difficulty penetrating the phospholipid-containing blood-

brain barrier than did the potent analog which was designed with a fluorinated side-chain for increased lipophilicity. The raw data were also consistent with this because the times of immobility were distributed bi-modally, perhaps as a function of the variation in the degree of development of an individual chicken's blood-brain barrier toward the time of its full maturation. The second difference between the two experiments was that naloxone reliably attenuated the effects of the peptides on TI in the latter experiment. Although other studies have not found that doses of naloxone similar to those used by us block the TI induced by morphine, larger doses of naloxone have been shown to be effective [21]. Our results suggest that there may also be non-narcotic components to the actions of the brain opiates on TI.

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