

# Effects of MIF-I, Sex, and Weight on Tonic Immobility in Lizards (*Anolis carolinensis*)<sup>1</sup>

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CASHNER, F. M., S. W. DELATTE, T. K. VON ALMEN, G. A. OLSON AND R. D. OLSON. *Effects of MIF-I, sex, and weight on tonic immobility in lizards (Anolis carolinensis)*. PHARMAC. BIOCHEM. BEHAV. 16(6) 1017-1019, 1982.— Three experiments were done with the lizard, *Anolis carolinensis*, as a follow-up on our previous work which showed that MIF-I reduced tonic immobility (TI) during the breeding season and that females had longer TI durations than males in the non-breeding season. In June, during the breeding season, 60 male and 60 female lizards were injected with 0.1 mg/kg of MIF-I or naloxone or the diluent vehicle and placed in small aquaria for ten minutes. TI was then induced in the small aquaria. Similar experiments were conducted in September and October (non-breeding season). There was a reduction in TI durations in MIF-I- and naloxone-treated lizards of both sexes in June, but the differences between drug treatment groups and controls were not significant. In September and October, MIF-I treatment resulted in TI durations similar to controls but naloxone treatment resulted in slightly shorter durations. Increased TI duration in females as compared to males was seen during both seasons, but diminished during the breeding season. Weight was found to be a factor in male lizards, with males  $\geq 4.0$  g showing significantly longer TI durations. The lack of a significant effect of MIF-I on TI durations during the breeding season is possibly due to changes in the experimental design from the earlier report.

MIF-I    Naloxone    Tonic immobility    Sex differences    Lizards

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THE most recent works on the neural mechanisms of the catatonic-like state termed tonic immobility (TI) suggest that a serotonergic feedback to the midbrain raphe nuclei is involved [9] and that TI is mediated by an effect on postsynaptic serotonergic receptors of raphe neurons rather than by a direct effect of raphe activity [1,5].

Opiate peptides have been shown to increase TI durations [2], and the effect appears to be a non-narcotic action [6]. This corresponds well with the fact that hyperalgesia occurs during TI, suggesting a relation of opiate peptides with serotonergic receptors in the raphe [5]. MIF-I (Pro-Leu-Gly-NH<sub>2</sub>), which has some opiate antagonist effects [4,7], has been shown to mildly [8] or significantly reduce TI durations in lizards, *Anolis carolinensis*, during the breeding season but not at other times [3]. A difference in TI durations between the sexes [3] also suggests an interaction with endogenous hormones affecting TI mechanisms mediated through either reproductive hormones or behavioral mechanisms. The following experiments were undertaken to further explore the seasonal differences in MIF-I and gender influences on TI duration.

## METHOD

### Animals

Wild adult lizards (*Anolis carolinensis*) were obtained lo-

cally from the Snake Farm, La Place, LA. They were housed in unisexual groups of about 50 animals in 30 gallon aquaria with vegetation, 24 hour light, and sprinkled with water twice a day. The lizards were tested one to four days after arrival and released after testing. Three shipments of animals were tested. In the first shipment (tested June 5-7, 1981), females (n=60) averaged 2.6 g (range 1.9-3.7 g) and 83% had palpable eggs. The males (n=60) averaged 2.7 g (range 1.6-4.1 g). In the second shipment (tested September 25-26, 1981), females (n=60) averaged 2.4 g (range 1.4-4.1 g) and none had palpable eggs. The males (n=60) averaged 2.4 g (range 1.2-4.8 g). In the third shipment (tested October 7, 1981), females (n=16) averaged 3.1 g (range 2.3-3.8 g) and none had palpable eggs. The males (n=16) averaged 3.8 g (range 1.8-5.6 g).

### Drugs

MIF-I and naloxone were dissolved in a vehicle consisting of 0.9% saline made with acetic acid to 0.01 M. The drug was given by IP injection in a volume of 1  $\mu$ l/g at a dose of 0.1 mg/kg since this had previously been shown to be the optimal dose [3].

### Procedure

Animals were captured from the holding aquaria and in-

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jected IP at a point on the left side of the abdomen near the groin. Each was then placed in a 2<sup>1</sup>/<sub>2</sub> gallon aquarium made of thick glass which caused great visual distortion, affording effective hiding from direct view of the experimenter. After a 10 minute wait, TI was induced inside the aquarium by holding the lizard on its back, pressing one finger on the ventral surface of the head and another at the middle of the tail for 20 seconds. Another experimenter, sitting about 2 to 4 m away, activated a stopwatch and watched the lizard at an oblique angle. If an animal did not remain immobile for at least 10 sec, it was immediately reinverted and TI was reinduced. The maximum number of induction attempts necessary to meet criterion was 4. Six aquaria were set up in the test room on separate lab tables approximately 2 m apart. Because of the different locations of the aquaria in relation to the location of the experimenters, all drug-gender combinations were counterbalanced to receive the same number of trials in each aquarium.

Animals were weighed and measured after termination of TI to prevent effects of excessive handling before the test session. Doses were determined by estimating weights of the lizards which resulted in dosage errors of only -3% to +6%. To prevent experimenter bias, the solutions were coded and the codes unbroken until after analysis of the data.

In the October experiment TI was induced on open lab tables instead of in aquaria. All other procedural details were unchanged except that naloxone was not given.

#### RESULTS

Although there was a reduction in TI durations in MIF-I-treated lizards in June, particularly in females, the difference was not significant (mean TI durations: 93 sec for MIF-I-treated and 201 sec for control females; 79 sec for MIF-I-treated and 91 sec for control males). In September there was no difference seen between MIF-I and diluent treatment in males, but females again showed shorter TI durations when treated with MIF-I rather than with diluent but the difference was not significant (mean TI durations: 103 sec for MIF-I-treated and 128 sec for control females; 82 and 84 sec for the respective treatments of males). Naloxone reduced TI durations slightly in both males and females compared to controls in both June and September. In October the differences were also not significant.

An unexpected effect on TI duration was found in the October experiment, which could not be examined in the previous experiments. Males  $\geq 4.0$  g (many of which were used in the October experiment due to the lack of availability of smaller lizards) showed significantly longer TI durations (mean: 256 sec) than smaller males (mean: 126 sec),  $F(1,14) = 4.904$ ,  $p < 0.05$ . The opportunity to observe this phenomenon had not occurred before because very few animals  $\geq 4.0$  g were used in previous experiments. No females were  $\geq 4.0$  g in the October experiment, so a breakdown was made at  $\geq 3.0$  g but did not indicate larger females to have longer TI durations.

The difference in TI durations with gender as previously reported for nonbreeding-season lizards [3] was repeated across drug treatments in the September experiments (mean female TI duration: 115 sec, mean male duration: 74 sec,  $F(1,118) = 13.739$ ,  $p < 0.0005$ ). Females had longer TI durations in both the June and October experiments, but the differences were not significant (mean female duration: 159 sec and mean male duration: 86 sec in June,  $p = 0.06$ ; mean

female duration: 223 sec and mean male duration: 191 sec in October,  $p = 0.55$ ). It is interesting to note that secondary analyses, combining the data from experiments conducted in September, 1980, October, 1980 and September, 1981 yielded a highly significant difference between the sexes (mean female duration: 132 sec and mean male duration: 83 sec,  $F(1,58) = 36.517$ ,  $p < 0.00001$ ). The inclusion of so many large males with longer TI durations explains the reduction of the effect in October, 1981.

#### DISCUSSION

The significant effect of MIF-I on TI durations during the breeding season reported previously [3] was not substantiated by these experiments. The difference in procedures between the present experiments and the earlier one could explain this. The original experiment was carried out on an open lab table with the experimenter approximately 0.75 m from the animals. Long average TI durations were accompanied by extreme variability. To reduce variability in subsequent experiments the animals were induced into TI in small aquaria and the experimenters were placed farther away. The variability was reduced, but the average durations of all treatment groups, including the control, were reduced to approximately the duration of the MIF-I-treated groups in the original experiment. The effect of the MIF-I may be expressed only under the more stressful experimental condition.

MIF-I-treated lizards did exhibit shorter average TI durations than the diluent-treated controls in every experiment done during the breeding season both previously [3] and in the current report. Naloxone was given in 2 of the experiments and both times resulted in slightly, but not statistically significantly, shorter TI durations than controls. The effects of naloxone were generally similar to those of MIF-I.

The effect of the opiate antagonist, naloxone, on TI durations was neither strong nor consistently found, as previously reported [6]. This is not surprising since the effect of opiate agonists, such as morphine, on TI durations is evidently non-narcotic [6]. The effect that MIF-I, and possibly naloxone, may have on TI durations during the breeding season is apparently through some mechanism which is not mediated by narcotic actions.

Longer TI durations were observed in female lizards when compared with males of the same size during the nonbreeding season, and is in agreement with our previous work [3]. Larger males also have longer TI durations than smaller males during this season. The use of large males may explain why other experimenters have not previously reported the gender difference in TI durations. During the breeding season the females had longer TI durations, but the difference was not significant. There may be a difference in the degree of the gender effect depending upon endogenous reproductive hormones.

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