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BRIEF COMMUNICATION

Chlorpromazine Dosage and Duration of Tonic Immobility: Biphasic Effects

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MASER, J. D., G. G. GALLUP, JR., L. E. HICKS AND P. H. EDSON. *Chlorpromazine dosage and duration of tonic immobility: biphasic effects.* PHARMAC. BIOCHEM. BEHAV. 2(1) 119–121, 1974. – Five groups of 2-1/2 to 3 week old chickens were injected with an average of 3.4, 7.4, 18.2, 46.3, 89.4 mg/kg of chlorpromazine (CPZ). Low doses of the drug produced a significant enhancement of tonic immobility but high doses depressed the reaction relative to control subjects. Other investigators have found only enhanced immobility with CPZ, creating a paradox of a tranquilizer potentiating what is thought to be a fear reaction. This report extends the dose-response curve and resolves the paradox.

Chlorpromazine dosage Tonic immobility

WHEN chickens and many other animals are placed in manual restraint for a few seconds they will, upon subsequent release, remain in a cataleptic, hypnotic-like state, often times also exhibiting waxy-flexibility and leg tremors. Eye closure gives the impression of sleep, but EEG records contradict this behavioral observation [10]. Although onset of the response appears innate, i.e., non-associative, the duration of tonic immobility, commonly referred to as the immobility response (IR) may be profoundly influenced by Pavlovian fear and safety signals [6,14], habituation [3], proximity of a predator [5], and administration of exogenous drugs [1, 2, 4, 6, 8, 9, 10, 13, 15, 16, 17, 18, 19]. Concerning the latter factor, 4 studies using adrenalin have reported an increase in IR duration [1, 8, 11, 19], while one cited by Ratner [16] failed to find an effect. Metoserpate HCl (Pacitran), a tranquilizing agent specifically designed for use with domestic fowl, produced a decrease in the duration of IR [4,6]. The adrenalin and metoserpate HCl studies are in agreement with the fear hypothesis of IR, which states that the basis of the behavior is predation-induced fear, and that manipulation of fear-related stimuli will modify the response in predictable directions.

At variance with this hypothesis is the literature on chlorpromazine (CPZ). As a tranquilizer Chlorpromazine should produce a reduction in fear and a corresponding decrease in the duration of immobility. In fact, the duration increases. Schaeppi and Rubin [17] report that 2 mg/kg increased IR in the rabbit. Davis [2] used a 5 mg/kg dose to achieve a 5-1/2 fold increase in the duration of the same species, and Klemm [9] obtained similar results with 10 mg/kg. Liberson, Smith and Stern [13] reported that 4 mg/kg increased duration in the guinea pig, while Gallup, Nash and Brown [4] noted a substantial increase in the chicken (up to 2 hr), but their dose was unreported.

The present study describes a dose-response curve for CPZ and IR in the chicken for concentrations of the drug higher than previous reports, and resolves the paradox of high duration of IR following administration of a drug known to reduce fear related behaviors.

METHOD

Animals

The subjects were 95 straight run Production Red chickens *(Gallus gallus)* 2-1/2 to 3 weeks of age, obtained from a local hatchery at one day of age and raised in commercial brooders under a 14 hr light cycle. Food (Purina Chick Chow) and water were continually available. On the day of the experiment the mean weight of the chicks in all drug groups was 139.7 g with a standard deviation of 21.6.

Procedure

The following concentrations of CPZ mixed with distilled water were selected: 0.5, 1.0, 2.5, 6.25, and 12.5 mg. Since handling modifies the IR in a profound and lasting

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manner [3] it was decided to administer a constant volume (0.5 cc), test for IR, and determine weight following IR testing. The values reported, therefore, are an average mg/kg dosage, based on the mean weights of all animals given a particular concentration. Using this procedure, the birds in the first group received an average dose of 3.4 mg/kg with successively higher concentrations of 7.4, 18.2, 46.3, and 89.4 mg/kg in the remaining groups. Two control procedures were used: needle puncture alone and a 0.5 cc injection of distilled water. Subjects were randomly assigned to either one of the two control groups or one of the five drug groups. There were 15 birds in each drug group, and 10 in each control group.

The injection was administered intramuscularly in the thigh, and the subject was then placed individually in a holding chamber and carried to a sound attenuated room. Ten minutes following injection, the bird was removed from the chamber and manually restrained on a table. The chick was held with both hands and gently placed on his right side. Restraint was maintained for about 15 sec. The experimenter's hands were removed slowly and a stopwatch activated. If an animal failed to become tonically immobile, restraint was imposed for a maximum of 5 successive inductions, and if a bird failed to show the reaction, a score of 0 sec was recorded. The duration of IR was scored as having terminated when the subject rose to his feet. The animal was returned to the animal room weighed, and replaced in a brooder. Although research assistants collecting data knew what drug was given, they were unaware of the concentration differences and of the expectations of the investigators.

RESULTS

Figure 1 depicts the dose response curve for CPZ and tonic immobility. Only at the lowest dose was CPZ found to enhance IR time. In order to normalize the data, square root transformations were performed on all scores. No statistically significant difference was found between the needle puncture and distilled water control groups, so these data were pooled, and in order to achieve an equal number of birds in each group, five birds were randomly excluded from the combined control group. An analysis of variance performed on the six groups revealed a significant overall effect ($F = 5.51$, $df = 5/84$, $p<0.001$). A Duncan's test for post hoc comparisons showed the 3.4 mg/kg dose to be significantly different from the control group and all other drug dosage groups. The 3.4 and 7.4 mg/kg doses were also significantly different from each other ($p<0.05$) but the 7.4 mg/kg concentration did not reliably produce durations different from the control animals. The 18.2, 46.3, and 89.4 mg/kg levels of the drug were not significantly dif-

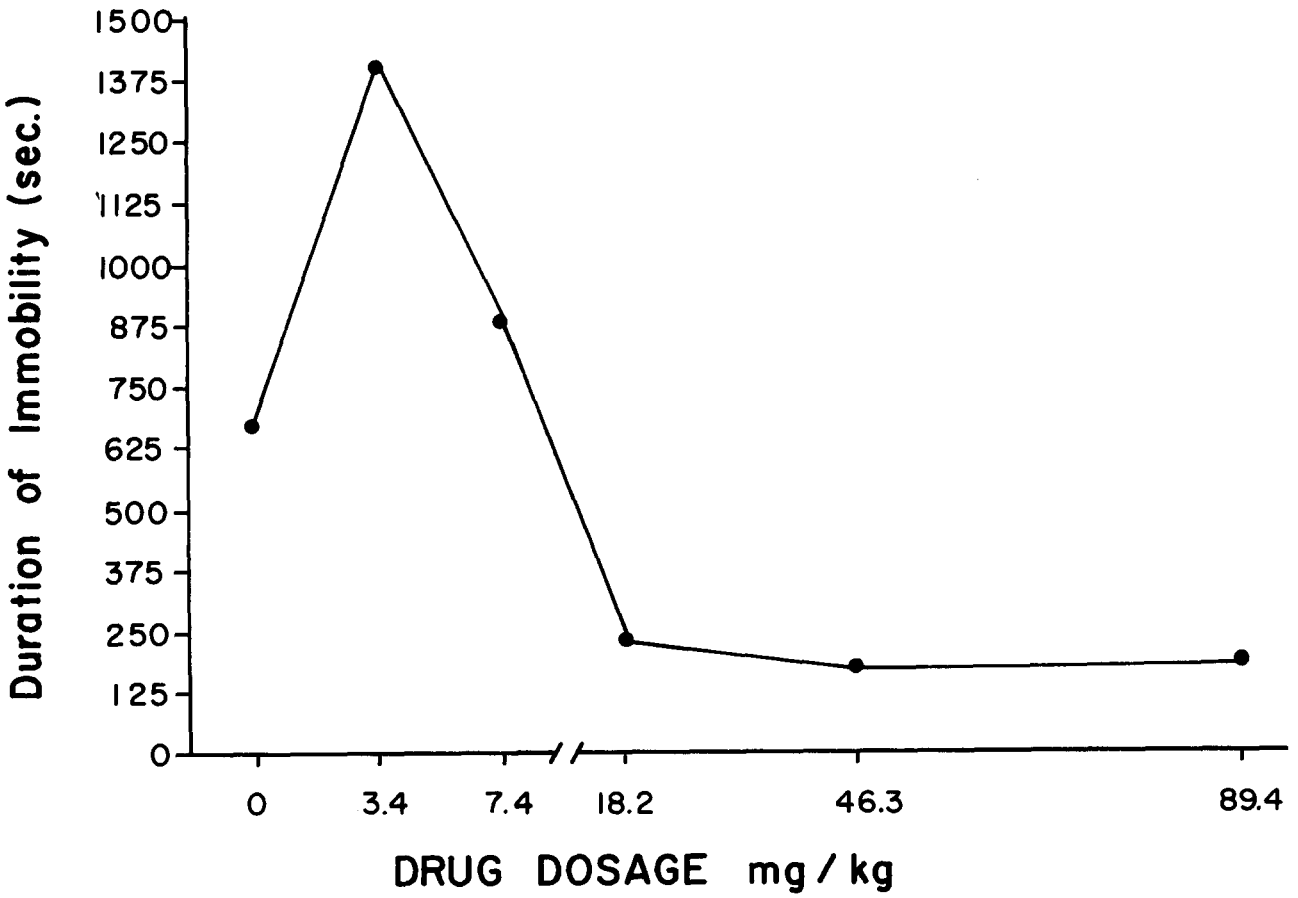

FIG. 1. Mean duration of tonic immobility as a function of average CPZ dosage. The control group (0 dosage) contains 20 birds, while there are 15 birds in each of the remaining groups.

ferent from each other, but each was significantly lower than the control group ($p<0.05$).

DISCUSSION

In previous investigations using doses of CPZ ranging from 2–10 mg/kg increases in immobility have been consistently found. Our lowest dose was within this range and replicated previously observed IR potentiation. However, as dosage increased to 18.2 mg/kg and beyond, it became clear the CPZ does attenuate immobility time, as would follow from the fear hypothesis. These data further emphasize the importance of using a wide range of doses in studies of drug related behavior.

The question remains as to why low doses of CPZ produce extended durations of IR. Given the wide-spread biochemical effects of CPZ on the central nervous system [7], it is difficult to discern a simple pharmacological rationale for behavioral reversal with increasing dosage. Guth and Spirtes [7] reviewed over 15 studies showing reversal of CPZ effects with high and low doses, but none of the effects cited was behavioral. More recently, however, Lewis and Evans [12] found that low doses of CPZ increase REM sleep in humans, whereas higher levels decreased the proportion of REM sleep. Although REM sleep and IR are probably qualitatively different phenomena, they seem to be influenced by similar neurological structures in the brainstem [10].

Thompson's laboratory has provided tentative evidence that at least one neurotransmitter involved in IR may be acetylcholine [18,19], and our laboratory has collected data suggesting 5-hydroxytryptamine as another possible transmitter [15]. Chlorpromazine is known to effect a variety of systems including both acetylcholine and 5-hydroxytryptamine and it is possible the reversal represents a disruption of balance among two or more transmitters.

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