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Differential Cholinergic Influences on the Immobility Response in Various Strains of Domestic Fowl[1,2]

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HENNIG, C. W., J. F. McINTYRE, D. D. MORIARTY, JR., J. M. PICERNO AND J. L. ALLEN. *Differential cholinergic influences on the immobility response in various strains of domestic fowl.* PHARMACOL BIOCHEM BEHAV **30**(3) 625–634, 1988.—A series of five experiments examined the effects of two anticholinergic drugs, atropine and scopolamine, on the duration of tonic immobility (TI) and susceptibility to the TI response in both Production Red and White Leghorn chickens (*Gallus gallus*), in an attempt to resolve previous contradictory findings about the effects of cholinergic manipulations on tonic immobility. These two anticholinergic drugs significantly reduced the duration of TI and, therefore, supported the conclusion that cholinergic systems are involved with the immobility response. However, the effects of these drugs on TI differed depending on the age, strain, local population, and handling experience of the individual birds.

Scopolamine Atropine Tonic immobility Strain-related differences Age-related differences Anticholinergics Habituation Chickens

TONIC immobility (TI), or animal hypnosis, is a prolonged but reversible state of motor paralysis that is typically produced by manual restraint. This response is shown by a variety of animals and can be an excellent preparation for studying the initiation and suppression of movement. Tonic immobility has proven especially sensitive to manipulations designed to affect fear [11], and is thought by many researchers to have evolved as the terminal defensive reaction in a sequential series of antipredator responses [32]. It has also been proposed as an animal model for human catalepsy and catatonic schizophrenia [13].

Much of the recent TI research has centered on the pharmacology of the immobility response and on the role of various neurotransmitters that may influence the behavior. However, the task of summarizing TI pharmacology is not easy. Although serotonergic participation appears critical [3, 15, 40, 43], adrenergic [17,18], dopaminergic [42], and cholinergic [19, 39, 44] systems are also implicated. There are further complications due to partially overlapping neurochemical systems. Interactions have been reported between the serotonergic and dopaminergic systems [41] and between the cholinergic and dopaminergic systems [34] in the control of tonic immobility. Moreover, research on cholinergic involvement in TI has provided additional contradictory results.

The two major problems found in the cholinergic studies of TI have been a suggested avian-mammalian reversal of cholinergic drug effects and failures to obtain consistent effects of anticholinergic drugs on the immobility response. Several studies have reported that the cholinergic antagonist scopolamine attenuated TI duration in chickens [10, 19, 20, 34, 39] and ducks [44], whereas cholinergic agonists such as physostigmine and pilocarpine potentiated duration of TI in

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chickens and ducks [34, 39, 44]. However, scopolamine increased TI durations in guinea pigs and rabbits, while physostigmine attenuated the immobility durations in the same species [14,44]. This apparent avian-mammalian reversal of cholinergic effects on TI has yet to be fully explained, other than by attributing it to neurochemical differences in the brains of birds and mammals. In addition, there are exceptions to this proposed avian-mammalian reversal. Atropine, a cholinergic antagonist, decreased restraint-induced catalepsy in mice [21,22]. There are also some paradoxical reversals of catalepsy and locomotor activity in young rats when scopolamine and atropine are injected at different ages [1, 4, 35]. Further complicating the situation, Ksir [23] found no apparent effect of scopolamine on TI in chickens, while other researchers [19,28] reported problems obtaining effects of atropine on immobility durations in similar animals. Thus, there are inconsistencies in the action of cholinergic drugs on TI and related behaviors. Hughes [20] attempted to resolve the latter problem and found that both scopolamine and atropine produced dose-dependent decreases in TI duration in White Leghorn chickens. His results with these drugs and their methyl analogs suggest the involvement of central cholinergic systems in the immobility response, but do not actually explain what caused the previous contradictory findings. The present study attempted to provide specific data on strain differences and the effects of anticholinergic drugs on TI in domestic chickens as the basis for a theoretical explanation of the role of cholinergic systems in the TI response.

EXPERIMENT 1

The first experiment was designed as a partial replication of the work done by Hughes [20]. It examined the effects of scopolamine and atropine, two cholinergic competitive antagonists, on the immobility response in 10-, 18-, and 25-day-old White Leghorn chickens, which were obtained from the same supplier as those used by Hughes.

METHOD

Subjects

The subjects were 108 straight run White Leghorn chickens (*Gallus gallus*) obtained from Welp, Inc. (Bancroft, IA) at one day posthatch. They were group-reared in commercial brooders under normal daylight. Food (Purina chick starter) and water were continually available. Treatment and testing occurred when the birds were either 10, 18, or 25 days old.

Drugs

Scopolamine hydrochloride (Sigma) and atropine sulfate (Sigma) were dissolved in distilled water and injected intraperitoneally (IP) in a volume of 1 ml/kg body weight. Drug solutions were made fresh each day and coded prior to use. The doses of the drugs were chosen because they were similar to those previously employed [19,20]. Ten-fold larger doses of atropine than scopolamine were used because the latter drug is thought to be 10 to 20 times more potent than the former [36].

Procedure

The experiment was divided into three phases. In the first, 36 chicks were removed from their brooder at 10 days posthatch and were randomly assigned to one of three groups (n=12 chickens per group). The birds were weighed and given either distilled water, 2 mg/kg of scopolamine, or 20 mg/kg of atropine. Another 36 chicks were injected in the same manner at 18 days posthatch, and the final 36 birds were treated with the same drugs at 25 days of age. The birds were tested at these different ages in order to permit comparisons between the present data and past research, and to assess any age-related differences in the effects of these anticholinergic drugs on TI in chickens.

Immediately after the injection, each bird was placed in a cardboard box and transported to a separate testing room. Ten min after injection, the subject was removed from the box, placed on a flat table, and quickly inverted on its right side. Gentle restraint was maintained with both hands for 15 sec, then the experimenter released the chick and activated a stopwatch. Any subject failing to remain immobile for at least 5 sec was given up to five successive 15-sec inductions in an attempt to elicit immobility, with a 30-sec interval between attempted inductions. The number of inductions required to meet this criterion was recorded. If the subject did not show immobility after any of the five attempted inductions, then a duration score of zero was recorded. For those birds that did become immobile, the duration of TI was measured from the time of release until either the chicken showed a spontaneous righting response and returned to its feet, or a maximum duration of 900 sec elapsed. Testing was performed by experimenters who were unaware of the treatment that each bird received. To preclude any confounding effects of periodicity, testing was staggered over the day with comparable numbers of birds from each group tested at different times.

Statistics

Two-way factorial analyses of variance (ANOVAs) involving three levels of age (10, 18, and 25 days posthatch) and three drug conditions (water, scopolamine, atropine) were used to analyze the TI induction and duration data.

TABLE 1

MEAN NUMBER OF INDUCTIONS REQUIRED TO PRODUCE IMMOBILITY AND MEAN DURATIONS OF TONIC IMMOBILITY EPISODES IN VARIOUS AGE WHITE LEGHORN CHICKENS AFTER INJECTIONS OF ANTICHOLINERGIC DRUGS

	Age of Subjects		
Drug Group[a]	10 Days	18 Days	25 Days
	Number of Inductions		
Water (control)	1.00	1.00	1.25
Atropine (20 mg/kg)	1.08	1.42	1.83
Scopolamine (2 mg/kg)	1.25	1.42	1.58
	Durations of Tonic Immobility (in sec)		
Water (control)	881.9	559.1	712.8
Atropine (20 mg/kg)	312.4†	272.2†	343.8†
Scopolamine (2 mg/kg)	153.8†	130.8†	226.1†

Note. Maximum number of inductions=5. Maximum durations of tonic immobility episodes=900 seconds.

[a]n=36 for each group, with 12 subjects per cell.

Dunnett's Test: vs. control, *$p<0.05$; †$p<0.01$.

TABLE 2

MEAN NUMBER OF INDUCTIONS REQUIRED TO PRODUCE IMMOBILITY AND MEAN DURATIONS OF TONIC IMMOBILITY EPISODES IN VARIOUS AGE PRODUCTION RED CHICKENS AFTER INJECTIONS OF ANTICHOLINERGIC DRUGS

Drug Group[a]	Age of Subjects 10 Days	18 Days	25 Days
	Number of Inductions		
Water (control)	1.17	1.50	1.50
Atropine (20 mg/kg)	2.75	3.08	2.42
Scopolamine (2 mg/kg)	2.00	2.17	2.25
	Durations of Tonic Immobility (in sec)		
Water (control)	177.1	275.6	301.3
Atropine (20 mg/kg)	46.6	69.6*	88.2†
Scopolamine (2 mg/kg)	146.1	280.5	57.5†

Note. Maximum number of inductions=5. Maximum durations of tonic immobility episodes=900 seconds.

[a]n=36 for each group, with 12 subjects per cell.

Dunnett's Test: vs. control, *$p<0.05$; †$p<0.01$.

Separate analyses were used to test the significance of drug effects in the different age groups, and Dunnett's test was used to determine the significance of any differences between the control group and each experimental drug group. Due to extreme skewness and variability in the duration data, a square-root transformation was performed on all duration scores prior to statistical analysis.

RESULTS AND DISCUSSION

Susceptibility to TI (as measured by the number of inductions required to produce the immobility response) decreased as a function of both age and exposure to the anticholinergic drugs. As shown in Table 1, both scopolamine and atropine increased the number of inductions required to produce TI. Susceptibility also seemed to decrease with age since more inductions were required to produce immobility in the older birds. These results were supported by significant main effects of age, $F(2,99)=4.10$, $p<0.05$, and of drug condition, $F(2,99)=3.29$, $p<0.05$. In addition, Dunnett's tests on the overall means for the drug groups revealed that the susceptibility of control subjects was significantly lower than that of birds in the scopolamine ($p<0.05$) or the atropine ($p<0.05$) groups. Such findings were unexpected because Hughes [20] reported no effects on TI susceptibility as a function of either age or anticholinergic drugs. Other researchers [19, 34, 39, 44] did not examine cholinergic influences on this variable. The present data are further proof that TI is influenced by cholinergic systems.

Mean TI durations for each treatment group are presented in the lower part of Table 1. As can be seen, all the scopolamine and atropine groups displayed much lower mean durations than the control groups, regardless of the chicken's age. This was supported by a significant main effect of drug condition, $F(2,99)=51.70$, $p<0.001$. There was no apparent effect due to age or the age × drug interaction. Dunnett's tests on the overall means for the drug groups revealed that the control birds showed significantly longer TI durations than those of chickens in either the scopolamine ($p<0.01$) or the atropine ($p<0.01$) groups. Separate analyses performed on each of the different age categories and followed by Dunnett's tests on drug groups further supported these results at similar confidence levels (see Table 1). The present data support the findings of Hughes [20] in that both scopolamine and atropine attenuated TI duration in young chickens. This clearly demonstrates the influence of the cholinergic systems on the duration of the immobility response and suggests large differences in the potency of these two cholinergic antagonists.

EXPERIMENT 2

The first experiment demonstrated that anticholinergic drugs such as scopolamine and atropine reduce TI duration in White Leghorn chickens, but there are still some unanswered questions. Two previous studies [19,28] reported no apparent effect of atropine on TI duration in chicks and a third study [23] found no effect of scopolamine. What could have caused these discrepancies? Thompson [38] and Ksir [24] discussed possible answers including different testing procedures, experimenter bias, and strain differences among chickens. Hughes [20] and the first experiment of this study used a standard testing procedure and controlled for experimenter bias, but still found that both cholinergic antagonists reduced TI duration. However, no researchers have actually examined the possibility of strain differences in response to injections of scopolamine and atropine, although previous studies [12, 27, 29] have shown dramatic strain differences in TI duration among chickens and rats when no drugs were given, and each of the failures with cholinergic antagonists [19, 23, 28] used different strains of chickens. The current experiment utilized the same procedure as in the previous experiment and attempted to show that there are substantial strain differences in the reactions of chickens to anticholinergic drugs.

METHOD

The same drugs, procedures, and statistics were used as in the previous experiment. The only difference between the two experiments was that 108 straight run Production Red chickens were used as subjects instead of White Leghorns.

RESULTS AND DISCUSSION

As can be seen in Table 2, both scopolamine and atropine increased the number of inductions required to produce immobility in Production Red chickens. These results were supported by a significant main effect of drug condition, $F(2,99)=8.30$, $p<0.001$. Dunnett's tests on the overall means for the drug groups also revealed that the susceptibility of control subjects was significantly lower than that of birds in the scopolamine ($p<0.05$) or the atropine ($p<0.01$) groups. No other differences were significant. These results support the findings of the previous experiment and demonstrate that cholinergic drugs influence not only the duration of TI, but also the susceptibility to the response. The reductions in susceptibility produced by scopolamine and atropine also appear to be greater in Production Red chickens than in White Leghorns.

Mean durations of immobility for each treatment group are presented in the lower part of Table 2. As can be seen, all the atropine groups displayed much lower mean durations than the control groups, regardless of the subject's age, but scopolamine seemed to reduce TI duration only in the 25-

day-old chicks. A two-way factorial ANOVA on these data revealed a significant main effect of drug condition, $F(2,99)=9.95$, $p<0.001$, but failed to demonstrate any other significant differences. However, separate analyses on the data from 10-, 18-, and 25-day-old birds and subsequent Dunnett's tests comparing controls with drug groups revealed some interesting results. Although the differences between drug treatment groups did not reach accepted levels of significance in chicks tested at 10 days of age, $F(2,33)=2.71$, $p<0.10$, the differences between drug groups were significant in 18-day-old, $F(2,33)=4.04$, $p<0.05$, and 25-day-old chickens, $F(2,33)=7.99$, $p<0.005$. Dunnett's tests further demonstrated that atropine groups showed significantly shorter TI durations than control groups at 25 and 18 days of age ($p<0.01$ and $p<0.05$, respectively) and even approached significance in 10-day-old chicks ($p<0.10$); however, the scopolamine group only differed significantly from the control in 25-day-old subjects ($p<0.01$).

These effects of both scopolamine and atropine on TI duration in the Production Red chicks were unexpected. Maser and associates [28] reported no apparent effect of atropine on TI in this strain of chicken, and previous researchers [19, 20, 39] have never suggested age-dependent effects of scopolamine on tonic immobility in domestic fowl. However, there have been reports of age-dependent differences in susceptibility to TI and effects of scopolamine on catalepsy in rats [1,26]. In addition, Ksir [23] used only 14-day-old mixed-strain chickens when he failed to find scopolamine effect on TI, suggesting that his subjects might have been too young to respond to the anticholinergic drug. But this explanation would have to include a consideration of strain differences because scopolamine reduced TI durations in all ages of White Leghorn chickens ([20], Experiment 1 of the present study). Thus, there seem to be important strain differences in the effects of atropine and scopolamine on the duration of TI in domestic fowl.

TABLE 3

MEAN NUMBER OF INDUCTIONS REQUIRED TO PRODUCE IMMOBILITY AND MEAN DURATIONS OF TONIC IMMOBILITY EPISODES IN WHITE LEGHORN CHICKENS AFTER VARIOUS DOSAGE INJECTIONS OF TWO ANTICHOLINERGIC DRUGS

	Drug	
Dosage (mg/kg)[a]	Scopolamine	Atropine
	Number of Inductions	
0	1.00	1.00
2	1.29	1.07
5	1.14	1.14
10	1.14	1.21
20	1.14	1.36
	Durations of Tonic Immobility (in sec)	
0	857.6	857.6
2	591.6	669.6
5	589.6	716.6
10	622.6	668.0
20	536.0*	535.3*

Note. Maximum number of inductions=5. Maximum durations of tonic immobility episodes=900 seconds.

[a]n=14 for each dosage of a drug.

Dunnett's Test: vs. control, *$p<0.05$; †$p<0.01$.

EXPERIMENT 3

Although the two previous experiments demonstrated that there are strain differences in the immobility responses of chickens after injections of anticholinergic drugs, these differences cannot explain all the negative findings. Hicks [19] reported that scopolamine decreased TI durations in White Leghorn chickens, but that atropine did not. This is inconsistent with both past [20] and present findings which indicated that both scopolamine and atropine attenuated TI duration in the same strain of chickens. Moreover, Maser and associates [28] found no effect of atropine on TI in Production Reds, whereas the previous experiment demonstrated a strong attenuation of TI duration in that strain. How can these discrepancies be explained? One answer might be that there are within-strain differences in TI duration after administration of anticholinergic drugs. The third experiment was designed to test this hypothesis using a different genetic population of White Leghorn chickens.

METHOD

Subjects

The subjects were 126 straight run White Leghorn chickens obtained at one day posthatch from McWilliams Hatchery (Bloomingdale, CA). This was a different hatchery from the one which supplied White Leghorns for the previous experiment. The chickens were group-reared in commerical brooders under artificial light (14 hr light/10 hr dark) until 18 days of age. Food and water were continually available.

Drugs and Procedure

The same drugs were used as in the previous two experiments. At 18 days posthatch the subjects were randomly divided into nine equal groups (n=14 chickens per group). The birds were weighed and given IP injections of the following substances. A control group received only distilled water while the other eight groups received 2, 5, 10, or 20 mg/kg of scopolamine or atropine. All other aspects of immobility testing were the same as in the two previous experiments.

Statistics

Separate one-way ANOVAs were used to analyze TI duration and susceptibility data for the various dosages of scopolamine and atropine. Then, Dunnett's tests were used to test the significance of differences between the control and each drug dosage group of scopolamine and atropine. A square-root transformation was performed on all duration scores prior to statistical analysis.

RESULTS AND DISCUSSION

Although both scopolamine and atropine slightly increased the number of inductions required to produce TI (see Table 3), the differences were not significant. These findings are contrary to the results in our first two experiments, but consistent with Hughes [20].

The mean durations of TI for the various scopolamine and atropine groups are shown in the lower part of Table 3. As can be seen, both drugs reduced TI duration by about 200

sec, regardless of the dosage used. Scopolamine seemed slightly more effective than atropine. Differences between the scopolamine groups, $F(4,65)=2.21$, $p<0.10$, and the atropine groups, $F(4,65)=2.00$, $p<0.10$, failed to reach accepted levels of significance; however, Dunnett's tests revealed that the TI durations of the 20 mg/kg groups of both scopolamine and atropine differed significantly from those of the control group ($p<0.05$). None of the other drug dosage groups was significantly different from the control. These drugs seem to have only marginal effects on TI duration in this population of White Leghorns, even at fairly high dosages.

The limited effects by scopolamine and atropine on TI duration and susceptibility in these chickens, compared to the results obtained in the first two experiments, showed that there were not only strain differences in response to anticholinergic drugs, but also differences within the same strain. Since all the chicks employed in these studies were incubator-hatched, arrived in the lab within two days of hatching, and were housed and treated comparably thereafter, it is reasonable to suppose that the observed within-strain differences in response to anticholinergic drugs might reflect genetic differences among the birds obtained from different suppliers. This may help explain some of the contradictory results relating immobility and the cholinergic system, but one major problem still remains. What causes the differential reactions to these drugs? We attempted to answer this question in the next two experiments.

EXPERIMENT 4

Previous research has demonstrated species differences in the effects of cholinergic drugs on tonic immobility [14, 20, 39, 44] and the present study has shown that there were also between- and within-strain differences in the effects of some of these drugs on TI duration and susceptibility in domestic fowl. In addition, data from Experiment 2 indicated that the age of the subject could influence the action by these anticholinergic drugs on TI duration in the Production Red strain of chickens. But, what causes the differential reactions to these drugs? Previous studies on cholinergic modulation of TI have often differed in the handling procedures used with subjects. Some researchers utilized composite duration scores based on several trials [10, 14, 39, 44], whereas others used scores from single trials [19, 20, 23, 28, 34]. Prior handling and repeated immobilization have been shown to drastically influence TI duration in chickens [29, 30, 33]. Therefore, the different handling procedures used in previous research on cholinergic modulation of TI may have interacted with the drugs to alter their effects on immobility reactions in some instances. Moreover, some strain differences in response handling and habituation to TI in domestic fowl have been observed [29]. The present experiment attempted to explain previous failures of some anticholinergic drugs to attenuate TI duration in domestic chickens [19, 23, 28] by examining whether drugs such as scopolamine and atropine interact with prior handling and/or previous immobilization to influence TI duration and susceptibility in White Leghorn chickens.

METHOD

Subjects

The subjects were 108 straight run White Leghorn chickens obtained at one day posthatch from McWilliams Hatchery and maintained as in the previous experiment until 18 days of age. The birds were undisturbed except for feeding and cleaning.

Drugs and Procedure

The same drugs were used as in the previous experiments. At 15 days posthatch the chicks were randomly divided into three equal groups and given separate pretest treatments. The animals in the Immobilized Group were carried individually to the testing room in a cardboard box. The chick was removed from the box and placed on a flat table. The bird was seized, inverted, and restrained on its right side for a 15-sec induction interval. Then it was released and the duration of TI was measured from the time of release until either the chick righted itself or 300 sec had elapsed. In the latter case the bird was gently prodded into righting. If the bird failed to remain immobile for at least 5 sec after release, it was returned to the cardboard box for 30 sec. Then it was restrained again. A maximum of five such inductions was given. Almost all the subjects stayed immobile for the full time. The experimenter sat quietly nearby and observed the bird with an indirect gaze. This procedure was designed to habituate the chicks to immobilization and was repeated on each of the next two days. The animals in the Handled Group were treated in a similar manner except that instead of being immobilized, they were held in both hands by the experimenter for 300 sec. The chicks in the Undisturbed Group were not handled during the three-day pretesting period. The order for pretest manipulations was randomized each day.

On Day 18, each pretest group was randomly divided into three subgroups and the birds received IP injections of either distilled water, 2 mg/kg of scopolamine, or 20 mg/kg of atropine. Ten min after the injection, the subject was tested for TI in the same manner as the previous experiments.

Statistics

Susceptibility and duration data were subjected to two-way factorial ANOVAs involving three levels of pretreatment (immobilized, handled, and undisturbed) and three drug conditions (water, scopolamine, and atropine). Separate analyses were used to test the significance of drug effects in the various pretreatment groups, Dunnett's tests were used to determine the significance of any differences between the control group and each experimental drug group, and orthogonal contrasts were used to test the significance of differences between pretreatment conditions. A square-root transformation was performed on all duration scores prior to statistical analysis.

RESULTS AND DISCUSSION

As shown in the upper part of Table 4, the undisturbed birds required fewer inductions to produce TI than either the immobilized or handled chickens. This was supported by a significant main effect of pretest treatment, $F(2,99)=3.06$, $p<0.05$. Even though the handled birds did not appreciably differ from the undisturbed birds in the drug control group, orthogonal contrasts indicated that overall the undisturbed animals required significantly fewer inductions than the pooled immobilized and handled birds, $F(1,99)=5.88$, $p<0.05$. The immobilized birds did not differ from the handled subjects. These results indicate that undisturbed chicks are more susceptible to TI than previously handled or immobilized birds after injections of anticholinergic drugs. Handling or immobilization prior to testing seemed

TABLE 4

MEAN NUMBER OF INDUCTIONS REQUIRED TO PRODUCE IMMOBILITY AND MEAN DURATIONS OF TONIC IMMOBILITY EPISODES IN WHITE LEGHORN CHICKENS AFTER PRETEST MANIPULATIONS AND INJECTIONS OF ANTICHOLINERGIC DRUGS

Drug Group[a]	Pretest Conditions: Undisturbed	Handled	Immobilized
	Number of Inductions		
Water (control)	1.00	1.08	1.75
Atropine (20 mg/kg)	1.50	1.75	1.67
Scopolamine (2 mg/kg)	1.08	2.00	1.75
	Durations of Tonic Immobility (in sec)		
Water (control)	850.6	642.8	482.8
Atropine (20 mg/kg)	563.6	435.6	277.5
Scopolamine (2 mg/kg)	569.5	123.7†	147.0*

Note. Maximum number of inductions=5. Maximum durations of tonic immobility episodes=900 seconds.

[a]n=36 for each group, with 12 subjects per cell.

Dunnett's Test: vs. control, *$p<0.05$; †$p<0.01$.

to make chickens harder to immobilize, although specific habituation to the TI response did not seem to occur. Neither anticholinergic drug had an apparent effect on susceptibility. This was similar to the findings of the previous experiment which used the same population of White Leghorns, but contrary to the results with other birds in the first two experiments of this study. Whether or not anticholinergic drugs act on susceptibility to TI may depend on the initial susceptibility of the animals. The chickens in this experiment may have been somewhat easier to immobilize than the birds in the first two experiments and this may influence whether the anticholinergic drugs can alter susceptibility to TI.

The mean durations of TI for each group are shown in Table 4. The chickens given injections of water displayed the longest durations, while those given scopolamine showed the shortest durations and atropine produced an intermediate effect. This was supported by a significant main effect of drug condition, $F(2,99)=14.85$, $p<0.001$. Dunett's tests on the overall means for the drug groups revealed that the control birds remained immobile significantly longer than chickens in either scopolamine ($p<0.01$) or atropine ($p<0.01$) groups. Separate analyses performed on each of the different pretreatment conditions supported these findings, but at reduced confidence levels ($p<0.05$) for the undisturbed and immobilized birds. Moreover, Dunnett's tests comparing drug groups with controls revealed that scopolamine significantly reduced TI duration only in handled ($p<0.01$) and previously immobilized birds ($p<0.05$), and that atropine produced no significant effects in these animals (see Table 4). The differences caused by both these drugs in the undisturbed chickens only approached accepted levels of significance ($p<0.10$). Chicks which were immobilized or handled during the pretest phase displayed shorter TI durations than undisturbed birds, as indicated by the significant main effect of pretest treatment, $F(2,99)=14.75$, $p<0.001$, and by orthogonal comparisons which revealed that the undisturbed birds remained immobile longer than the pooled combination of handled and immobilized birds, $F(1,99)=27.01$, $p<0.001$, while the two latter groups were not different from each other.

Both anticholinergic drugs attenuated TI duration, but scopolamine reduced immobility duration more than atropine, even though much lower doses of the former drug were used. The strength of these effects varied depending on the pretest condition. The anticholinergic drugs produced only nonsignificant reductions of TI duration in undisturbed birds, and the effects of the two drugs did not differ appreciably from each other. This was very similar to findings with that same type of White Leghorn chicken in the previous experiment. In contrast, the anticholinergic drugs significantly reduced TI duration in previously handled and immobilized birds, with scopolamine having a much stronger effect than atropine.

EXPERIMENT 5

The previous experiment demonstrated that handling manipulations interacted with injections of anticholinergic drugs to determine whether scopolamine and/or atropine attenuated TI duration in White Leghorn chickens. However, previous research [29] has suggested that Production Reds show greater reduction of TI duration than White Leghorns after prior handling or habituation. The present experiment examined whether handling manipulations and anticholinergic drugs interacted in the same or a different manner to influence TI duration in Production Red chickens. The existence of differing forms of interactions between these two variables in different strains of chickens might help explain some previous failures of anticholinergic drugs to attenuate TI duration ([19, 23, 28], Experiment 2 and 3 of the present study).

METHOD

The subjects were 81 straight run Production Red chickens obtained from McWilliams Hatchery at one day posthatch. They were maintained as in the previous experiment, but were not tested until 25 days of age because Experiment 2 showed that scopolamine did not affect TI duration in younger birds of this strain. The drugs and procedures were exactly the same as in Experiment 4, except that pretest treatments began when the birds were 22 days old and there were nine subjects per group. Statistical methods were also the same.

RESULTS AND DISCUSSION

The mean number of inductions required to produce TI in each group is shown in Table 5. The undisturbed birds needed fewer inductions than handled or immobilized animals, as indicated by the significant main effect of pretest condition, $F(2,72)=3.47$, $p<0.05$. Orthogonal comparisons showed that while the undisturbed birds differed significantly from the others, $F(1,72)=6.25$, $p<0.05$, there were no differences between immobilized and handled animals. Neither the main effect of drug condition, nor the interaction between factors was significant. These results were similar to those with White Leghorns in the previous experiment, except that the Production Reds seemed less susceptible to TI than White Leghorns. The anticholinergic drugs had no apparent effect on this behavior.

TABLE 5

MEAN NUMBER OF INDUCTIONS REQUIRED TO PRODUCE IMMOBILITY AND MEAN DURATIONS OF TONIC IMMOBILITY EPISODES IN PRODUCTION RED CHICKENS AFTER PRETEST MANIPULATIONS AND INJECTIONS OF ANTICHOLINERGIC DRUGS

Drug Group[a]	Pretest Conditions: Undisturbed	Handled	Immobilized
	Number of Inductions		
Water (control)	1.56	2.67	3.00
Atropine (20 mg/kg)	1.89	3.00	2.44
Scopolamine (2 mg/kg)	1.67	2.56	1.78
	Durations of Tonic Immobility (in sec)		
Water (control)	648.1	252.3	149.4
Atropine (20 mg/kg)	440.7	190.6	256.7
Scopolamine (2 mg/kg)	168.7†	195.3	96.6

Note. Maximum number of inductions=5. Maximum durations of tonic immobility episodes=900 seconds.

[a]n=27 for each group, with 9 subjects per cell.

Dunnett's Test: vs. control, *$p<0.05$; †$p<0.01$.

The mean durations of TI for each group are shown in the lower part of Table 5. A two-way factorial ANOVA revealed a significant main effect of pretest treatment, $F(2,72)=6.36$, $p<0.005$, with the undisturbed chicks showing much longer durations than the handled and immobilized animals, $F(1,72)=12.15$, $p<0.001$, but no differences between the two latter groups. The main effect of drug condition was not significant, but the pretest treatment × drug condition interaction approached accepted levels of significance, $F(4,72)=2.07$, $p<0.10$. Separate analyses revealed that the drug groups in the undisturbed condition differed significantly, $F(2,24)=5.28$, $p<0.025$, but there were no apparent differences between drug groups in either the handled or immobilized conditions. Dunnett's tests on the undisturbed birds indicated that those animals given scopolamine displayed significantly shorter TI durations than the control subjects ($p<0.01$), but that atropine had no significant effect on immobility duration (see Table 5). The apparent lack of effect of scopolamine and atropine on TI duration in handled and immobilized Production Red chickens might indicate that those procedures somehow reduced the strength of the immobility response to a point where anticholinergic drugs could produce no further attenuation. If TI duration in these birds can be influenced by such manipulations, then it could explain differential immobility reactions in experiments that used this strain of chicken as subjects.

GENERAL DISCUSSION

The present series of experiments clearly demonstrated the involvement of the cholinergic system in the control of tonic immobility since scopolamine and atropine, two anticholinergic agents, were shown to attenuate TI duration in chickens. These results are consistent with a number of previous reports [10, 19, 20, 34, 39]. In addition, at least two studies [20,39] have demonstrated that these anticholinergic drugs act on TI through some central, rather than a peripheral mechanism. Scopolamine and atropine attenuated TI duration in chickens, regardless of the animal's age, but the methyl analogs of these drugs only reduced immobility duration in very young chickens whose blood-brain barrier had not yet fully formed. However, the present results indicate that anticholinergics seem to vary in their effect on TI depending on the strain of chicken involved, and may even differ across populations within the same strain. Furthermore, these differences may depend on the age and prior handling experience of the subjects.

Most previous research has not examined the effects of anticholinergic drugs on susceptibility to TI, and the one article that studied this variable found no effect of either scopolamine or atropine on the number of inductions required to produce immobility [20]. The final three experiments in the present study also found no effects of these drugs on TI susceptibility, although White Leghorns were easier to immobilize than Production Reds and prior handling or immobilization reduced the susceptibility of subjects to TI. The latter result partially supports previous research which indicated that any extra handling made TI more difficult to induce [30], but it did not demonstrate the significant difference in susceptibility between handled and immobilized birds that earlier research had suggested. This may have been due to procedural differences in the habituation process. In contrast to these final three experiments, both White Leghorn and Production Red chickens in the first two experiments required more inductions to elicit TI after injections of anticholinergic drugs than after injections of the control solution. The latter strain of chicken also seemed more resistant to immobilization than the former. Therefore, TI susceptibility can be reduced by excess handling or the administration of anticholinergic drugs, but it can also vary due to differences in the genetic makeup of the subjects that are used in the experiments. This may help explain some of the inconsistencies found when TI susceptibility is used to measure the influence of cholinergic systems on the immobility response. Experiments utilizing several of these interacting variables might require much larger populations of subjects before the effect of the anticholinergic drugs on TI susceptibility could reach statistical significance. This may be what happened in the present study. Experiments 1 and 2 used a total of 36 subjects in each drug condition, with only age as a second variable, and obtained significant effects of anticholinergics on TI suceptibility. The other three experiments employed designs in which manipulations involving drug dosages or handling conditions could interact to lessen the influence of the anticholinergic drugs. These procedures resulted in fewer subjects per drug group and the number of available subjects may not have been great enough to demonstrate the direct action of anticholinergic drugs on TI susceptibility. Thus, susceptibility to tonic immobility is influenced by the cholinergic system, although perhaps weakly, and it may require optimal experimental conditions for the cholinergic influences to be clearly demonstrated.

The duration of TI appears much more responsive than susceptibility to the action of anticholinergic drugs. In Experiment 1, both scopolamine and atropine significantly attenuated TI duration in White Leghorn chickens, but scopolamine was much more effective than atropine, even though the latter drug was used at ten-fold larger doses than the former. This supports previous research on the dose-response characteristics of these drugs in this strain of domestic fowl [20,36]. However, in Experiment 2, scopol-

amine only attenuated TI duration in Production Reds after 25 days of age, whereas atropine reduced duration in subjects of various ages. In addition, durations of TI in this strain of chickens were less than half the length of those found in White Leghorns. There appear to be strain differences in both the basic length of TI duration and the subject's response to anticholinergic drugs. There also seem to be qualitative as well as quantitative differences in the effects of scopolamine and atropine on immobility duration. Experiment 3 demonstrated even more inconsistencies with these drugs. Another population of White Leghorn chickens showed no apparent differences in their response to injections of scopolamine or atropine. There seemed to be only minimal effects of either of these drugs on TI duration in the White Leghorns. The four dosages of these drugs (2, 5, 10, and 20 mg/kg) used in Experiment 3 reduced TI duration by approximately 200–300 sec, but only the largest dose of each drug significantly attenuated durations of TI in these animals. The apparent lack of dose-related effects of these drugs on TI was not entirely unexpected because previous research had often failed to find significant differences in TI duration between various dosages of scopolamine and atropine [19, 20, 39]. However, there were two unanticipated results. Scopolamine and atropine only proved effective in significantly reducing TI duration at dosages of 20 mg/kg and the relative potency of the drugs seemed about equal. Both drugs usually attenuated TI duration at much lower dosages than the 20 mg/kg dose shown effective in this experiment [10, 14, 19, 20, 39, 44] and scopolamine was normally more potent than atropine ([19,20], Experiment 1 of the present study). There was something different about this population of White Leghorn chickens that caused their atypical reaction to the anticholinergic drugs. There were no direct strain comparisons performed in the present experiments because it was believed that the wide variability in the basic length of immobility episodes might obscure the more important effects of the cholinergic system on immobility and previous research [27, 29, 30] had already shown strain differences in response to immobility. However, indirect comparisons of the chickens in the present study showed substantial between- and within-strain differences in both the basic length of TI duration and response to the anticholinergic drugs. This could explain some previous failures of these drugs to reduce TI duration in past research [19, 23, 28]. Experiments 4 and 5 further demonstrated that different strains of domestic chickens reacted in distinctive ways to anticholinergic drugs based on individual past experiences. Undisturbed White Leghorns showed quite long durations of tonic immobility and the anticholinergics only had minimal effects on them. This finding was very similar to the results in Experiment 3 with the same population of chickens. However, if the subjects had been previously handled or immobilized, then the cholinergic antagonists attenuated TI duration. In addition, scopolamine reduced TI duration more than atropine. The former drug produced a significant attenuation of tonic immobility, whereas the latter only reduced TI duration to a limited degree. In contrast to these results with White Leghorns, the Production Reds in Experiment 5 reacted to the anticholinergic drugs when the birds had been undisturbed prior to testing, but failed to respond if the animals had received prior handling or immobilization. The duration scores of undisturbed Production Reds were very similar to those of the handled White Leghorns in Experiment 4 and scopolamine was once again more potent than atropine. Thus, prior handling experience seems to interact quite strongly with the subject's genetic background to determine exactly how the cholinergic system influences TI duration in domestic fowl. This may explain some of the variable results with anticholinergic drugs reported in the past ([19, 23, 28], present study).

Even though the present study has shown that the effects of anticholinergic drugs on TI can differ depending on the age, strain, local population, and handling experience of the individual birds; there are still three important unanswered questions remaining about the involvement of the cholinergic system with tonic immobility. What causes the differential immobility reactions to anticholinergic drugs based on age, genetic background, and handling experience? Why do the effects of scopolamine on TI differ from those of atropine? Why should there be an avian-mammalian reversal of cholinergic effects on TI? None of these questions can be answered conclusively based on our present knowledge, but tentative hypotheses can be proposed that might tie together all these problems and provide suggestions for future research.

Both casual observations and experimental manipulations have indicated that white Leghorn chickens are more emotional than Production Reds [12]. Numerous studies have shown that fear-producing stimuli play an important role in the induction and maintenance of the immobility response [11]. Several studies have also suggested that anticholinergic drugs can affect the neural system mediating fear [5, 9, 31, 37]. Therefore, any cholinergic effects on TI which differ across age, strain, population, or handling experience in chickens might be due to differences in initial fear levels. However, there are alternatives to this hypothesis. Carlton [6,7] suggested that cholinergic effects on motor behaviors could be accounted for by a central inhibition process and that anticholinergic drugs act on this mechanism to disinhibit responses which are normally suppressed. In support of this belief, anticholinergic drugs such as scopolamine and atropine have been shown to block various forms of behavioral inhibition (e.g., passive avoidance, extinction of operant responses, habituation) [8]. Thus, anticholinergic drugs might attenuate the immobility response, another type of behavioral inhibition, through some form of disinhibition process. There may even be a dual mechanism, involving both fear reduction and disinhibition, through which anticholinergic drugs exert influence on the immobility response. If such a mechanism exists, then it could explain why there are differences in the effects of scopolamine and atropine on TI, even though both drugs are cholinergic blockers. Scopolamine has often been shown to lessen fear in animals [5, 31, 37], whereas atropine has not been found to possess that ability [9]. This suggests that perhaps scopolamine acts to block both fear and behavioral inhibition, while atropine might only act to block the latter process. However, more direct research is needed before other alternative explanations can be eliminated and any definite conclusions can be made.

In light of the differences in TI duration between and within strains of chickens after injections of anticholinergics, it may also be easier to understand the reported reversal in the effects of cholinergic drugs on TI in birds and mammals [14, 34, 39, 44]. There should be even greater differences in behavior between such widely divergent species. But, why should there be a reversal of the cholinergic effects on TI in birds as compared to mammals? This proposed reversal has usually been attributed to neuroanatomical and neurochemical differences between birds and mammals [14,34]. However, there are also differences in response to cholinergic

drugs across species of mammals [14, 21, 22, 44] and even within the same species at different ages [1, 4, 35]. In addition, Experiments 4 and 5 of the present study showed that two different strains of domestic chickens reacted in disparate ways when tested for TI under varied handling conditions after injections of scopolamine and atropine. Habituation to human presence through handling or immobilization increased the effect of these drugs on TI in White Leghorns, but decreased the effect in Production Reds. How can all of these differences be explained? One plausible explanation is based on the fact that earlier research on the suggested avian-mammalian reversal of cholinergic effects on TI employed composite scores obtained from three separate immobility episodes [10, 14, 39, 44]. Chickens are known to habituate to TI after repeated immobilizations [29,30], whereas mammals such as guinea pigs and rats show increases in TI duration after several immobility tests [2, 16, 25]. The composite score technique may have produced the increases in TI duration in guinea pigs and rabbits after injections of anticholinergics [14,44] and the decrease in immobility found with chickens and ducks [10, 39, 44]. But, even this is probably not the entire answer. Repeated testing for TI without intertrial intervals potentiated duration scores in chickens [30] and different strains of domestic fowl in the present study reacted differently to habituation caused by prior handling or immobilization. The final answer may depend on an interaction between several factors such as the animal's level of fear, its hierarchy of defenses, and the testing procedure that has been used. If a relatively helpless animal such as the chicken is seized, then it may be very frightened and easy to immobilize because that behavior is high on its hierarchy of defenses. The duration of TI is proably related to the level of fear and strains of chickens would differ in their immobility reactions because of different levels of emotionality. Anticholinergics could attenuate TI duration by reducing fear levels and/or inhibiting the predominant response. In contrast, when mammals are seized they are usually less helpless than birds and more likely to fight back. They are probably more difficult to immobilize because that behavior is lower on their hierarchy of defenses. In that case, the anticholinergic drugs may have increased TI duration by inhibiting the more predominant response of fighting. The interaction between these factors could explain the contradictory effects of cholinergic manipulations on TI in different species and within various strains of the same species. However, this hypothesis is still very speculative and requires further testing.

The present study demonstrated that there was a significant cholinergic influence on the immobility response in domestic fowl. Indirect comparisons of chicken strains also provided abundant circumstantial evidence that there are strain differences in TI duration after injections of anticholinergic drugs. The divergent nature of this relationship between TI and cholinergic drugs across species, strains, and even populations provides an excellent model for comparative research into the function of cholinergic neurochemical systems. However, it is clear that other systems are also involved and partially overlap in TI phenomena [34,41]. The exact ways in which such systems interact are matters for future research.

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