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Duration of Tonic Immobility in Chickens as a Function of Alpha-Adrenergic Receptor Stimulation and Blockade[1]

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HENNIG, C. W., J. K. FAZIO, C. A. HUGHES, W. R. CASTALDI AND B. D. SPENCER. *Duration of tonic immobility in chickens as a function of alpha-adrenergic receptor stimulation and blockade.* PHARMACOL BIOCHEM BEHAV **20**(5) 731–738, 1984.—Tonic immobility in chickens was influenced by a variety of drugs that act on the adrenergic neurochemical system of the body. Alpha$_1$ agonists such as methoxamine and phenylephrine produced decreases in the duration of immobility, although the former compound also caused a significant increase in the immobility response at high dosages. Alpha$_2$ agonists such as clonidine, naphazoline, and guanfacine enhanced the duration of immobility, but clonidine also produced an apparent reversal of this effect at high dosages. Subsequent experiments examined more fully the biphasic effects by methoxamine and clonidine on tonic immobility through interactions with alpha antagonists. Yohimbine, an alpha$_2$ blocker, attenuated the duration of immobility, either alone or in conjunction with various dosages of methoxamine. Prazosin, an alpha$_1$ blocker, had no direct effect on tonic immobility, but potentiated the duration of the response when given in conjunction with various dosages of methoxamine. When these antagonists were given in conjunction with clonidine, yohimbine reduced immobility durations, while prazosin had no apparent effect on this response. These results are discussed in terms of the relative contributions of the alpha$_1$ and alpha$_2$ adrenoceptors to the duration of the immobility response.

Tonic immobility Animal hypnosis Chickens Adrenoceptors Methoxamine Phenylephrine Clonidine Naphazoline Guanfacine Yohimbine Prazosin

IN response to physical restraint many animals will, upon subsequent release, remain in a catatonic-like state for periods of time ranging from a few seconds to several hours. This state of temporary paralysis has been given a variety of names, among which the most widely used are animal hypnosis, immobility reflex, and tonic immobility (for reviews see [9,24]). Although tonic immobility is an excellent preparation for studying the initiation and suppression of movement, there is not much agreement about the mechanisms that control this behavior. Some researchers have attempted to treat the immobility reflex as a model for human hypnosis [17] or compare it to sleep [6], but experimental evidence seems to suggest that tonic immobility actually represents an innate fear reaction (for a review see [9]). Under natural conditions, tonic immobility is thought to serve as the terminal defense reaction in a sequential series of distance-dependent antipredator responses (for reviews see [10, 24, 25]). Recently, it has also been suggested that tonic immobility may be related to other types of motor inhibition such as the retrieved reflex in young mammals [12] and the lordosis response in female rats [23]. Tonic immobility has even been compared to special states of behavioral inhibition in humans such as catatonic schizophrenia [11] and rape-induced paralysis [27]. Therefore, the immobility reflex may be of interest to a variety of researchers.

A number of studies have investigated the neuropharmacology of the immobility response. Several researchers [13, 18, 29, 37] have proposed a cholinergic inhibition sytsem as the control mechanism for tonic immobility based on findings with cholinergic agonists and antagonists. However, a number of studies have also suggested that the serotonergic system plays an active role in the immobility reflex based on findings with psychomimetic drugs, monoamine oxidase inhibitors, serotonin blockers, serotonin, and its precursors on both immobility and raphe electrical activity in chickens (for a review see [35]). More recently, the emphasis on serotonergic involvement with tonic immobility has shifted from the effects of serotonin on raphe activity in the brain to its action at postsynaptic serotonin receptors [3,33]. Several articles have even examined the possibility of an interaction between serotonergic and dopaminergic systems as the basis for tonic immobility [31, 32, 34]. However, results from all these studies were not totally predictable and many questions still remain about the neuropharmacology of the immobility reflex.

One of these unanswered questions involves the role of

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the adrenergic neurochemical system with tonic immobility. The adrenergic system has long been implicated with fear responses and as the basis for flight-or-fight reactions, so it would appear very anomolous if this system was not involved in some way with a putative antipredator defense such as tonic immobility. Therefore, it was not really a surprise when researchers found that injections of epinephrine produce large increases in the duration of tonic immobility in various species [4, 16, 20]. More recently, norepinephrine was also shown to increase immobility duration in chickens [28]. These studies demonstrated the general involvement of the adrenergic system with the immobility reflex, but actually explained little about the precise relationship between immobility and the adrenergic system.

The adrenergic neurochemical system is a complex network of neurons each of which release norepinephrine to excite or inhibit receptors in different parts of the nervous system and thereby produce various physiological changes in the body. There are several types of adrenoceptors and the specific bodily changes depend on which receptors are stimulated. Adrenergic receptors were initially classified as either alpha or beta according to their relative sensitivity to different catecholamines [1]. Subsequently, both alpha and beta receptors were further subdivided into two groups each, based on the ratio of their affinity for certain types of agonists [2,21]. A recent study [15] found that isoproterenol, a very potent beta agonist, had no apparent effect on tonic immobility in chickens. Thus, beta receptors are probably not very important to the immobility response in that species. However, the same study found that alpha-adrenergic agonists produced dramatic changes in the duration of tonic immobility in domestic fowl. $Alpha_1$ agonists depressed the duration of immobility, while $alpha_2$ agonists potentiated the response. The present study is an attempt to replicate and extend these findings in order to determine more precisely how the alpha adrenoceptors are involved with tonic immobility.

EXPERIMENT 1

$Alpha_1$ receptors are found on the postsynaptic membrane of adrenergic neurons and their function is generally excitatory in nature [2]. They tend to stimulate various smooth muscle in the body via the peripheral nervous system and can also act on selected brain areas via the central nervous system to control arousal [7,36]. Methoxamine and phenylephrine are two highly specific $alpha_1$ agonists which act predominantly on $alpha_1$ adrenoceptors in the mammalian nervous system [26], but not much is known about the effect of these drugs on the avian nervous system or the immobility response. One study [15] found that these drugs drastically depressed the duration of tonic immobility in chickens, but that work only examined the effects of one dosage level of those chemicals (0.3 mg/kg) on the immobility response. Moreover, other researchers reported that methoxamine increased the duration of immobility during the retrieved reflex in young rats and that large doses of this drug produced sedation in mice [7,12]. These inconsistent effects by methoxamine on various types of inhibitory behavior in both mammals and birds suggest the need for further study with $alpha_1$ agonists over a wide range of dosages in order to more carefully evaluate their involvement with the immobility reflex.

METHOD

Subjects

The subjects were 140 straight run Production Red chickens (*Gallus gallus*), 2 to 3 weeks of age, obtained from a commercial supplier at one day of age and group-reared in thermostatically regulated brooders. Food (Growena chick chow) and water were continually available. The photoperiod in effect during rearing consisted of 14 hr of light per day.

Apparatus and Procedure

The experiment consisted of two parts. In the first part, seventy chicks were randomly assigned to one of five groups of 14 animals each. These birds were weighed and given intraperitoneal (IP) injections of 1 ml/kg body weight of the following substances. The control group received only distilled water, while the other four groups received IP injections of 0.01, 0.1, 1 or 10 mg/kg of methoxamine hydrochloride (Burroughs Wellcome) dissolved in distilled water. In the second part, the other seventy chicks received equivalent dosages of phenylephrine hydrochloride (Sigma) dissolved in distilled water. The remaining procedure was the same for all subjects.

Immediately after the injection, each bird was placed in a cardboard box and transported to a separate testing room. Ten minutes after injection, the bird was removed from the box, placed on a table and quickly inverted on its right side, whereupon gentle restraint was maintained with both hands for 15 sec. Then the experimenter withdrew his hands 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 intertrial interval between attempts. If the subject did not show immobility for any of the five attempted inductions, a duration score of zero was recorded. For those birds that did become immobile, the duration of immobility was measured from the time of release until either the bird showed a spontaneous righting response and returned to its feet, or a maximum duration of 3600 sec had elapsed. Testing was performed by experimenters who were unaware of the treatments the birds received. To preclude any confounding effects of periodicity, testing was staggered over the day with a comparable number of birds from each group tested at different times throughout the day.

RESULTS AND DISCUSSION

Injections of methoxamine produced a biphasic effect on the duration of tonic immobility in chickens. As shown in Fig. 1, this drug attenuated immobility durations at low dosages, but potentiated the response at doses of 10 mg/kg. Most subjects in the latter group also showed decreased spontaneous motor activity even before restraint was imposed. Due to extreme skewness and variability in the data, a square-root transformation was applied to all duration scores prior to statistical analysis. Then Dunnett's test comparing each drug group with the control was performed. This analysis revealed that the mean duration of immobility for the 0.1 mg/kg methoxamine group was significantly shorter than the immobility duration for the control group, $t(65)=2.59$, $p<0.05$, while the mean duration of immobility for the 10 mg/kg group was significantly longer than the response duration for the control group, $t(65)=3.72$, $p<0.01$. In contrast, phenylephrine simply produced a dose-dependent decrease

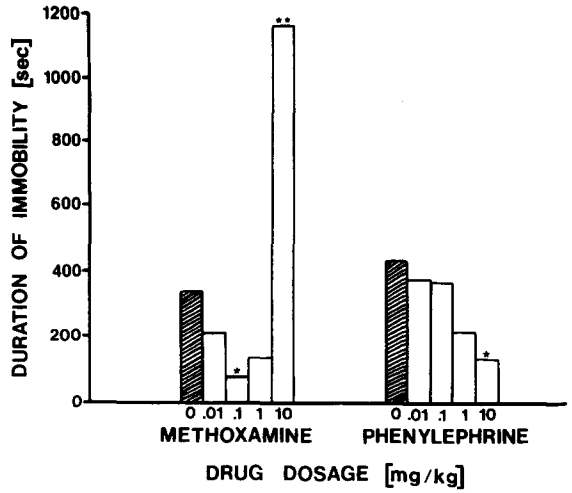

FIG. 1. Mean durations of tonic immobility as a function of the dosage of methoxamine or phenylephrine received. Differences of each drug group from the control were assessed statistically (*$p<0.05$; **$p<0.01$).

in the duration of tonic immobility (see Fig. 1). However, Dunnett's test on the transformed data revealed that only the mean duration of immobility for the 10 mg/kg phenylephrine group differed significantly from the response duration of the control group, $t(65)=2.80$, $p<0.05$.

The present data support the findings by past researchers [15] that alpha$_1$ agonists decrease immobility duration in domestic fowl. The first experiment showed that both methoxamine and phenylephrine attenuated the duration of tonic immobility in chickens, although methoxamine seemed to be the more potent drug. These compounds are known to have a preference for excitatory postsynaptic alpha$_1$ receptors in the mammalian nervous system, with methoxamine being the more potent agonist at these receptor sites [2], and so it seems likely that it is the activation of these same receptors that inhibits the duration of immobility in chickens. However, there was also a new finding which indicated that the relationship between alpha$_1$ agonists and their effects on immobility duration was not as clear cut as previously thought. Although both agonists attenuated the duration of immobility, methoxamine also produced a significant increase in immobility duration at dosages of 10 mg/kg. Since methoxamine is classified as an alpha$_1$ agonist and such drugs normally seem to inhibit immobility, one must ask why the opposite effect occurred with high doses of methoxamine. One answer may be that although this agonist has a strong affinity for alpha$_1$ receptors, it might also act on alpha$_2$ receptors at high enough dosages. This matter is examined in the third experiment of the present study.

EXPERIMENT 2

Alpha$_2$ receptors are considered inhibitory in nature and are located primarily on the presynaptic membrane of adrenergic neurons [2], although recent evidence suggests that some alpha$_2$ receptors may also be found on the postsynaptic membrane of these neurons [36]. These adrenoceptors mediate the inhibition of norepinephrine release from adrenergic neurons. There are numerous alpha$_2$ agonists, but one of the most well-known of these drugs is clonidine [2]. At fairly low doses this drug can produce hypotension, lowered body temperature, bradycardia, suppression of spontaneous motor activity, and marked sedation in both birds and mammals [5,8]. A recent study [15] also found that 0.3 mg/kg doses of clonidine substantially increased the duration of tonic immobility in chickens. However, the complete role of clonidine with sedation, and perhaps other forms of motor inhibition such as tonic immobility, is not entirely predictable. Clonidine-induced sedation in mammals seems to peak at dosages of 0.05 to 0.2 mg/kg [7] and doses greater than 10 mg/kg produce aggression instead of sedation [22]. These data suggest the need for further study on the dose-response effects of this agonist on various forms of motor inhibition. The present study attempts to do this with tonic immobility in chickens. There are also other alpha$_2$ agonists which may be evaluated in a similar manner. One such drug is guanfacine, which is at least 10 times less potent than clonidine in reducing blood pressure or producing sedation in rats [7,19]. Naphazoline is another fairly weak alpha$_2$ agonist which also reduces blood pressure and produces sedation in rodents, although not as effectively as clonidine [8]. However, neither drug has been tested for its effect on other forms of behavioral inhibition. Therefore, these two drugs should be good choices in an attempt to evaluate the role of other alpha$_2$ agonists with tonic immobility.

METHOD

Subjects

The subjects were 196 straight run Production Red chickens, 2 to 3 weeks of age, obtained and maintained as in the previous experiment.

Apparatus and Procedure

The experiment consisted of three parts. In the first part, eighty-four chicks were randomly assigned to one of six groups of 14 animals each. They were weighed and given IP injections of 1 ml/kg body weight. The control group received only distilled water, while the other five groups received IP injections of 0.01, 0.1, 1, 10 or 25 mg/kg of clonidine hydrochloride (Boehringer Ingelheim) dissolved in distilled water. The 25 mg/kg dose was included in order to approximate the large doses of clonidine that previous research [22] found were needed to produce aggression in mice. In the second part of this experiment, fifty-six chicks received either control injections or IP injections of 0.01, 0.1 or 1 mg/kg of naphazoline hydrochloride (Ciba) dissolved in distilled water. In the third part, the final 56 birds received equivalent amounts of distilled water or guanfacine hydrochloride (Sandoz) dissolved in distilled water. The latter parts of this experiment did not use larger doses of guanfacine or naphazoline because previous pilot studies showed that 10 mg/kg doses of these drugs produced severe ataxia and sedation, which interfered with the normal motor functions of the subjects. All other procedures were the same as in the previous experiment.

RESULTS AND DISCUSSION

All the chickens which received clonidine injections of doses greater than 0.01 mg/kg showed wing abduction, mild sedation, and lack of spontaneous motor behavior shortly after administration of the drug. Once restraint was imposed,

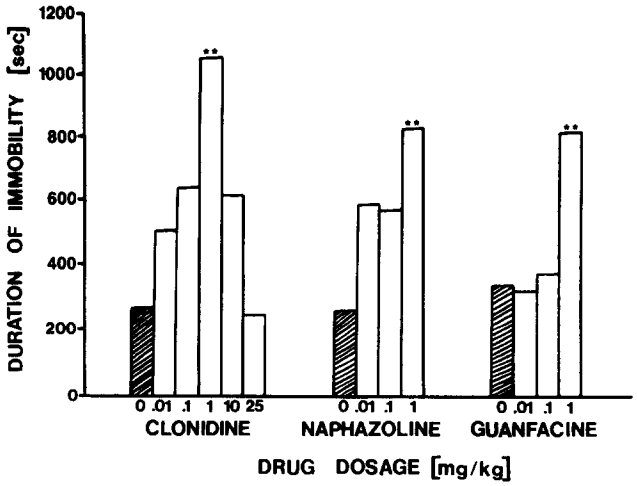

FIG. 2. Mean durations of tonic immobility as a function of the dosage of clonidine, naphazoline or guanfacine received. Differences of each drug group from the control were assessed statistically (*$p<0.05$; **$p<0.01$).

clonidine also produced dramatic changes in the duration of tonic immobility. As shown in Fig. 2, this drug caused dose-dependent increases in the duration of immobility for dosages up to 1 mg/kg and then produced decreases in immobility duration with progressively higher dosages. After a square-root transformation of the data in order to reduce variability, Dunnett's test revealed that only the mean duration of immobility for the 1 mg/kg clonidine group differed significantly from the control, $t(78)=4.22$, $p<0.01$. In contrast, naphazoline produced straightforward increases in the duration of tonic immobility (see Fig. 2). However, Dunnett's test on the transformed data revealed that only the mean duration of immobility for the 1 mg/kg naphazoline group was significantly longer than the mean immobility duration for the control group, $t(52)=3.16$, $p<0.01$. All the subjects in the 1 mg/kg naphazoline group also appeared to be more passive and sedate than chickens in the other groups shortly after administration of the drug and before restraint was imposed. Thus, the largest dose of naphazoline probably had several different effects on behavior in chickens. Injections of guanfacine seemed to produce the weakest effect on the immobility reflex by any of the alpha$_2$ agonists used in this study. As can be seen in Fig. 2, only the largest dose of guanfacine potentiated the duration of tonic immobility. This finding was supported by a Dunnett's test on the transformed data which revealed that only the mean duration of immobility for the 1 mg/kg guanfacine group was significantly greater than the mean duration for the control group, $t(52)=3.31$, $p<0.01$.

The present experiment found that each of the drugs clonidine, naphazoline, and guanfacine potentiated immobility duration in chickens, with clonidine as the most potent of the drugs. All three agonists have a preference for the presynaptic alpha$_2$ receptors in the mammalian nervous system which inhibit the release of norepinephrine [2,19], and so it seems likely that it is the stimulation of these same receptors that prolongs the duration of tonic immobility in chickens. These data support past research on adrenergic involvement with the immobility reflex which suggest that alpha$_2$ agonists increase the duration of immobility [15]. In addition, the relative potencies of these three drugs on the immobility response seem to parallel the strength of their affinities for alpha$_2$ receptors in the mammalian nervous system [2,26], with clonidine being the most potent of these drugs in both situations. However, the relationship between alpha$_2$ agonists and immobility duration was not as clear cut as previously thought. Clonidine also produced somewhat unusual biphasic effects on the duration of tonic immobility when several different dosages were utilized. Low to moderate doses of clonidine produced the expected increases in immobility duration, but the effect tended to vanish at larger doses until immobility duration returned to control levels with dosages of 25 mg/kg. Since clonidine is well-documented as an alpha$_2$ agonist [2] and such drugs normally seem to potentiate immobility duration [15], one must ask why such an effect failed to occur with very high doses of clonidine. The answer might revolve around the fact that this agonist has a strong affinity for alpha$_2$ receptors at low to moderate dosages which is the same range where clonidine potentiates immobility duration, but it might also activate some alpha$_1$ receptors at high dosages and this may result in the reduction of tonic immobility duration as found in the present study. This question is examined further in the fourth experiment.

EXPERIMENT 3

Previous research has suggested that adrenergic drugs such as methoxamine and phenylephrine act primarily to stimulate alpha$_1$ adrenoceptors in mammals [2,26] and produce a decrease in the duration of tonic immobility in chickens via the same neurochemical system [15]. However, the first experiment of this study found that although low doses of methoxamine attenuated the duration of immobility as expected, high doses of this chemical potentiated the immobility reflex. These findings raised the question of how a drug whose action was supposedly on alpha$_1$ receptors could have had two opposite effects on immobility duration. One possible answer was that while low doses of methoxamine stimulated alpha$_1$ receptors and caused a reduction in the duration of tonic immobility, very large doses of this drug may have also stimulated alpha$_2$ receptors, thereby potentiating the immobility response. The present experiment attempted to test this hypothesis by selectively blocking out the effects of these receptors on immobility with the administration of prazosin, an alpha$_1$ antagonist, and yohimbine, an alpha$_2$ antagonist [36]. This procedure should permit an assessment of which alpha adrenoceptors were involved with the attenuation of the immobility response and which receptors caused potentiation of tonic immobility.

METHOD

Subjects

The subjects were 108 straight run Production Red chickens, 2 to 3 weeks of age, obtained and maintained as in the previous experiments.

Apparatus and Procedure

On the day of the experiment, the subjects were randomly divided into nine groups of 12 chickens each. The subjects in three of these groups were weighed and given IP injections of

TABLE 1

EFFECTS OF INTERACTIONS BETWEEN ALPHA-ADRENERGIC ANTAGONISTS AND METHOXAMINE ON THE DURATION OF TONIC IMMOBILITY (SEC)

Antagonists	Control water	Methoxamine 0.1 mg/kg	Methoxamine 10 mg/kg
Control (water)	210.0	74.1	464.5
Prazosin (1 mg/kg)	173.7	307.0	601.2
Yohimbine (1 mg/kg)	93.5	94.8	61.5

1 ml/kg body weight of distilled water. The subjects in another three groups were weighed and given comparable injections of distilled water containing 0.1 mg/kg doses of methoxamine hydrochloride (Burroughs Wellcome). The last three groups of chickens received 10 mg/kg dosages of methoxamine. These dosage levels were chosen in order to optimize the effects of methoxamine on immobility duration found in the first experiment. Then each animal was placed in a cardboard holding box for 10 min. After this period, each subject received a second IP injection. One group of chickens that received distilled water, one group which received 0.1 mg/kg doses of methoxamine, and one group which received 10 mg/kg of methoxamine were all given 1 ml/kg injections of distilled water. A second set of these same three groups received 1 mg/kg doses of prazosin hydrochloride (Pfizer) dissolved in distilled water, while the final three such groups were given 1 mg/kg doses of yohimbine hydrochloride (Sigma) dissolved in distilled water. Previous research had shown these dosage levels produced the optimal effects by those drugs on the immobility response [14]. Following the second injection, each subject was placed in a holding box for another 10 min. Then each bird was tested for its duration of tonic immobility in the same manner used in the previous experiments.

RESULTS AND DISCUSSION

The mean durations of tonic immobility for the nine groups in this experiment can be seen in Table 1. As shown in this table, low doses of methoxamine attenuated immobility duration, while high doses of methoxamine potentiated the response. Prazosin, by itself, had no apparent effect on the duration of immobility, but it blocked the attenuation of immobility produced by low dosages of methoxamine and further potentiated the increase in tonic immobility duration caused by high doses of methoxamine. Yohimbine, either singly or in conjunction with various dosages of methoxamine, always produced drastic reductions in the duration of immobility. These results were supported by statistical analyses on the transformed (square-root) duration scores. A two-way factorial Analysis of Variance (ANOVA) indicated a significant main effect of methoxamine pretreatment, $F(2,99)=5.40$, $p<0.01$; a significant main effect of alpha antagonists, $F(2,99)=6.78$, $p<0.005$; and a significant interaction between these two factors, $F(4,99)=3.42$, $p<0.005$. Analyses of the simple effects also revealed that there were no statistical differences between the mean immobility durations of the three groups that received yohimbine, but there were highly significant differences between the three groups that received prazosin, $F(2,99)=5.89$, $p<0.005$, and between the three groups that received no alpha antagonists, $F(2,99)=6.13$, $p<0.005$. A Duncan's test for post hoc comparisons on the latter three groups showed that the effects of the 0.1 mg/kg dose of methoxamine were significantly different ($p<0.01$) from those of the 10 mg/kg dose of this drug and a similar analysis on the former three groups indicated that the effects of the 10 mg/kg dose of methoxamine in conjunction with prazosin were significantly greater than those of the control group ($p<0.01$), while other comparisons did not differ significantly from each other.

The present data support the findings of the first experiment which indicate that low doses of methoxamine attenuate the duration of immobility in chickens, while large doses of the same drug increase immobility duration. The results of the third experiment also demonstrated that yohimbine, an $alpha_2$ antagonist, reduced immobility duration when given singly and blocked the increase in tonic immobility produced by large doses of methoxamine, but had no apparent effect on the attenuation of immobility duration caused by low doses of this drug. In contrast, prazosin, a well-known $alpha_1$ antagonist, blocked the reduction in immobility duration which was normally produced by low doses of methoxamine and may have even further potentiated the increase in tonic immobility produced by high doses of this drug. Moreover, previous research has shown that large doses of methoxamine can produce sedation in rats, although most evidence suggested that sedation is caused by stimulation of $alpha_2$ adrenoceptors [7]. Taken together, all of these findings tend to support the hypothesis that low dosages of methoxamine act on $alpha_1$ receptors to produce arousal and attenuation of immobility duration in chickens, since these effects can be blocked by an $alpha_1$ antagonist such as prazosin, while high doses of methoxamine seem to act on $alpha_2$ receptors to produce sedation and a subsequent potentiation of the immobility response, since the latter effects could be blocked by an $alpha_2$ antagonist such as yohimbine.

EXPERIMENT 4

Past research has also indicated that certain drugs such as clonidine, naphazoline, and guanfacine act primarily on $alpha_2$ receptors in mammals [2,26] and potentiate the duration of tonic immobility in chickens via the same neurochemical system [15]. However, results from the second experiment of this current study indicated a biphasic effect by clonidine on the immobility response. This raised the question of what could produce such an effect, since clonidine should only have affected $alpha_2$ receptors and potentiated the duration of immobility. One possible answer may be that low and high doses of clonidine stimulated different alpha receptors, in a manner similar to that suggested for methoxamine in the previous experiment. The present research attempted to test this hypothesis by using prazosin and yohimbine to selectively block clonidine's effects on $alpha_1$ and $alpha_2$ receptors.

METHOD

Subjects

The subjects were 90 straight run Production Red chickens, 2 to 3 weeks of age, obtained and maintained as in the previous experiments.

TABLE 2

EFFECTS OF INTERACTIONS BETWEEN ALPHA-ADRENERGIC ANTAGONISTS AND CLONIDINE ON THE DURATION OF TONIC IMMOBILITY (SEC)

Antagonists	Control water	Clonidine 1 mg/kg	Clonidine 25 mg/kg
Control (water)	253.4	1038.2	447.7
Prazosin (1 mg/kg)	177.9	1248.0	583.5
Yohimbine (1 mg/kg)	62.8	302.3	672.6

Apparatus and Procedure

The procedure was the same as in the previous experiment with the following exceptions: there were only 10 subjects per group instead of twelve, 1 mg/kg doses of clonidine hydrochloride (Boehringer Ingelheim) were used instead of 0.1 mg/kg doses of methoxamine, and 25 mg/kg doses of clonidine were used instead of 10 mg/kg doses of methoxamine. These dosage levels were chosen in order to optimize the effects of clonidine on immobility duration found in the second experiment.

RESULTS AND DISCUSSION

The mean durations of immobility for the nine groups in this experiment can be seen in Table 2. As shown in this table, low doses of clonidine potentiated the immobility response, while high doses of this drug seemed to return immobility durations toward control levels. Moreover, the animals that received low doses of clonidine seemed slightly ataxic, but those that received high doses of the drug appeared more aggressive than normal when they came out of immobility. Prazosin seemed to have no real effect on immobility durations, either when used singly or in conjunction with either dose of clonidine. Yohimbine, on the other hand, attenuated the immobility duration when administered singly, blocked the increases in immobility produced by low doses of clonidine, and potentiated the duration of immobility when given in conjunction with high doses of clonidine. These results were supported by a two-way factorial ANOVA performed on the transformed (square-root) data which indicated a significant main effect of clonidine pretreatment, $F(2,81)=19.34$, $p<0.001$; a significant main effect of alpha antagonists, $F(2,81)=4.26$, $p<0.05$; and a significant interaction between these two factors, $F(4,81)=4.24$, $p<0.05$. Analyses of simple effects also revealed that there were highly significant differences between the three groups that received no alpha antagonists, $F(2,81)=7.66$, $p<0.005$; between the three groups that received prazosin, $F(2,81)=13.33$, $p<0.001$; and between the three groups that received yohimbine, $F(2,81)=6.83$, $p<0.005$. However, Duncan's test for post hoc comparisons on the latter three groups which received yohimbine found that the effects of the 25 mg/kg dose of clonidine were significantly different ($p<0.01$) from those of the control, while the effects of 1 mg/kg doses of clonidine were significantly different ($p<0.01$) from those of both the control solution and the 25 mg/kg dose of clonidine when they were given either alone or in combination with prazosin. The other comparisons did not differ significantly from each other.

The present data support the findings of the second experiment which indicate that clonidine has somewhat unusual biphasic effects on tonic immobility in chickens when several different dosages are utilized. Although low to moderate doses of this drug produced the increases in immobility duration expected with such a well-documented $alpha_2$ agonist, the effect tended to vanish at larger doses until the duration of immobility returned to control levels with 25 mg/kg dosages of clonidine. The present experiment also showed that yohimbine, an $alpha_2$ antagonist, blocked the increase in tonic immobility produced by moderate doses of clonidine, but failed to attenuate the duration of immobility produced by high doses of clonidine. This suggests that the potentiation of immobility duration which occurs with moderate doses of clonidine is due to that drug's action on $alpha_2$ receptors, since it can be blocked by yohimbine, while the failure of that same antagonist to attenuate immobility duration when it is given in conjunction with high doses of clonidine suggests that some other type of receptor is involved with that effect. Moreover, previous research demonstrated that low to moderate doses of clonidine induced sedation in rats [7], while high dosages of this drug caused aggression in rodents [22]. These data suggest that there may be a similar mechanism responsible for both the reduced immobility durations in chickens which occur with high dosages of clonidine in the present study and the increased aggression in rodents that can be produced by similar dosages of the same drug [22]. This other type of receptor might be an $alpha_1$ adrenoceptor, since it has already been shown that stimulation of $alpha_1$ receptors produce reductions in immobility duration. However, prazosin, an $alpha_1$ antagonist, had no apparent effect on the immobility response when given singly or in conjunction with various dosages of clonidine. This finding seems to preclude a simple explanation of the biphasic effects of clonidine based on initial $alpha_2$ receptor stimulation by low doses of this drug and subsequent $alpha_1$ activation by high doses. The final answer to this problem may center around the fact that high doses of clonidine only reduced immobility durations back to control levels. Such a result might indicate competition between $alpha_2$ and $alpha_1$ receptors, not merely the simple stimulation of one type of receptor or the other, and this may have caused the apparent lack of effect by prazosin in the present study.

GENERAL DISCUSSION

In recent years there has been a surge of interest in discovering the role of the adrenergic neurochemical system with tonic immobility. Some studies re-examined the actions of epinephrine and norepinephrine on tonic immobility and found that both substances potentiated the immobility response [28,30]. Other work [15] suggested that $alpha_1$ adrenergic agonists attenuated and $alpha_2$ agonists potentiated the duration of immobility in chickens. More recently, it has also been shown that yohimbine, an $alpha_2$ antagonist, and phentolamine, a non-specific alpha antagonist, diminshed the duration of tonic immobility, but that prazosin, an $alpha_1$ antagonist, had no apparent effect on the immobility response [14]. Thus, there seems to be ample evidence linking the immobility reflex with the adrenergic system.

The present study supported much of this past research on adrenergic involvement with tonic immobility, but it also

discovered some important new findings concerning the effects of alpha-adrenergic drugs on the immobility response. Contrary to previous beliefs [15], classification according to type of alpha agonist was not the definative determinant as to whether duration of tonic immobility was potentiated or attenuated by adrenergic drugs. Some agonists may affect several types of neurochemical receptors at different dosages and it seems to be the relative degree of $alpha_1$ and $alpha_2$ adrenoceptor stimulation which determines the ultimate effect on immobility duration by adrenergic drugs. Activation of $alpha_1$ receptors seemed to produce arousal and the attenuation of tonic immobility, while stimulation of $alpha_2$ receptors produced sedation and the potentiation of immobility duration. Moreover, since yohimbine, an $alpha_2$ antagonist, caused decreases in immobility duration when administered either alone or in conjunction with alpha agonists, but prazosin, an $alpha_1$ antagonist, only had an effect on tonic immobility through its blocking action on low doses of methoxamine; it appeared that activation of $alpha_2$ adrenoceptors produced the immobility response, while stimulation of $alpha_1$ receptors attenuated the duration of tonic immobility only as a type of rebound effect.

These results suggest that future research with drugs must utilize wider dosage ranges in order to compensate for the possibility of dual receptor stimulation by the same drug at different concentrations. This type of action may help explain behavioral dichotomies such as the flight-or-fight response which has already been linked to the catecholamines. There might also be similar dual receptor mechanisms operating within other neurochemical systems. Two other implications suggested by the present research are the potential use of tonic immobility as a simple behavioral screening device to test the $alpha_1$ and $alpha_2$ sensitivities of new adrenergic drugs and the possible generalization of the biphasic effects produced by alpha-adrenergic drugs on tonic immobility to other behaviors which are known to be influenced by alpha adrenoceptor stimulation.

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