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Differential Effects of Alpha-Adrenergic Antagonists on Tonic Immobility in Domestic Fowl[1]

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HENNIG, C. W., E. B. CARL, S. ALDRICH, J. K. FAZIO AND C. A. HUGHES. *Differential effects of alpha-adrenergic antagonists on tonic immobility in domestic fowl.* PHARMAC. BIOCHEM. BEHAV. **15**(5) 739–742, 1981.—Groups of young chickens were injected with various dosages of several alpha-adrenergic antagonists and tested for the effects of these drugs on tonic immobility. Yohimbine, a very potent alpha$_2$ antagonist, produced a significant decrease in the duration of tonic immobility at doses of 1 mg/kg, while prazosin, a potent alpha$_1$ antagonist, had no apparent effect on immobility duration. Phentolamine, a non-specific alpha antagonist, produced a significant decrease in the duration of immobility at doses of 0.1 mg/kg, but had no effect at higher or lower doses. These results support the involvement of the alpha-adrenergic system with the duration of the tonic immobility reflex, but at the same time they also suggest that alpha$_2$ receptors are more closely involved with the immobility response than alpha$_1$ receptors. However, none of these drugs had any apparent effect on the initial susceptibility of subjects to the immobility reflex, as measured by the number of inductions required to produce tonic immobility.

Tonic immobility Animal hypnosis Chickens Yohimbine Phentolamine Prazosin Alpha-adrenergic antagonists

WHEN chickens and many other animals are physically restrained for a short period of time they will often, upon subsequent release, remain in a catatonic-like state which can last from a few seconds to several hours. This state of motor inhibition has been called by many names over the past three centuries [16], but the most commonly used terms for this phenomenon are animal hypnosis, death feigning, tonic immobility, and immobility reflex. The immobility response seems especially sensitive to maniuplations thought to affect fear and, under natural conditions, tonic immobility appears to function as the terminal reaction in a sequence of distance-dependent antipredator responses [10, 16].

In recent years there has been special interest in the neuropharmacology of the immobility response. Gallup and associates have suggested a serotonergic control system for tonic immobility [3, 15, 23], while Thompson and colleagues have favored a central cholinergic inhibition system as the basis for this response [17,19]. Involvement of these systems, however, does not preclude the possible involvement of other neurochemical systems such as those mediated by the catecholamines. These neurotransmitters have long been implicated as the basis for flight-or-fight reactions, and it would appear very anomolous if this system was not also involved in some way with a putative antipredator defense such as tonic immobility. Therefore, it was not too unexpected when experimenters found that injections of epinephrine produced increases in the duration of tonic immobility in anoles, frogs, and chickens [4, 12, 13, 21]. The increased durations of immobility produced by monoamine oxidase inhibitors such as pargyline and iproniazid [15] could also be explained in terms of the effects of catecholamines on the immobility response. More recently, studies have found

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that both L-DOPA and norepinephrine produced increases in the duration of immobility in chickens [8,18], and alpha-adrenergic agonists produced differential effects on the duration of tonic immobility, with $alpha_1$ agonists causing a decrease in the duration of immobility and $alpha_2$ agonists producing an increase in the response duration [11].

The present study further examines the differential effects of alpha-adrenergic receptors (adrenoceptors) on tonic immobility by assessing the influence of various alpha antagonists on the immobility response. If the adrenergic system is important in the control of immoiblity, then drugs which block the action at alpha adrenoceptors should have significant effects on the duration of the response. Moreover, there are some antagonists with very specific receptor affinity such as yohimbine, a highly selective blocker of $alpha_2$ adrenoceptors, or prazosin, which is almost exclusively an $alpha_1$ blocker; while other antagonists such as phentolamine are relatively non-specific [22,24]. Therefore, this experiment also attempts to evaluate the relative involvement of $alpha_1$ and $alpha_2$ adrenoceptors with tonic immobility by determining whether one type of antagonist has more influence on the immobility response than other antagonists.

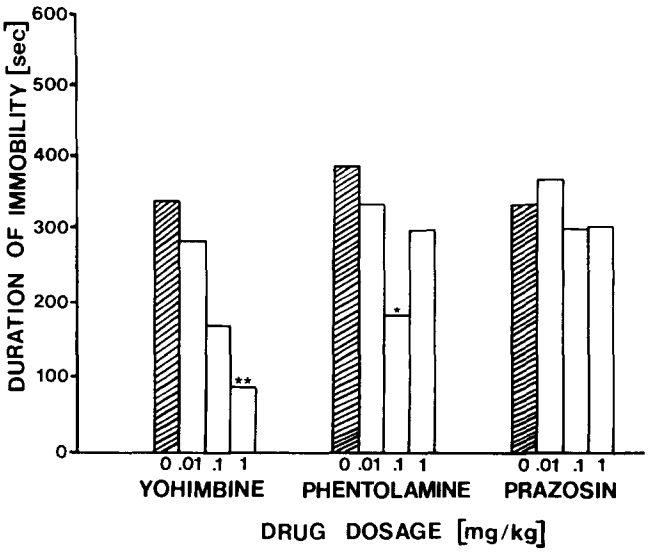

FIG. 1. Mean durations of tonic immobility as a function of the dosage of yohimbine, phentolamine or prazosin received. Differences from the control were assessed statistically using the Dunnett test (*$p<0.05$; **$p<0.01$).

METHOD

Animals

The subjects were 168 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. Chick feed and water were continually available. The photoperiod in effect during rearing consisted of 14 hr of light per day.

Procedure

The experiment consisted of three sections. In the first part, fifty-six chicks were randomly assigned to one of four 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 three groups received IP injections of 0.01, 0.1 or 1 mg/kg of yohimbine hydrochloride (Sigma) dissolved in distilled water. The second group of 56 chicks received the same dosages of prazosin hydrochloride (Pfizer) dissolved in distilled water and the final 56 birds received equivalent amounts of phentolamine hydrochloride (Ciba). 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 immmobility was measured from the time of release until either the bird showed a spontaneous righting response and returned to its feet, or a maximum duraiton of 3600 sec had elapsed. Testing was performed by experimenters who were unaware of the treatments the birds received. To preclude any confounding effect 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

Yohimbine produced a dose-dependent decrease in the mean duration of tonic immobility, with responses at the highest dosage being three times shorter than those for water controls (see Fig. 1). Due to high variability and extreme skewness in the raw data, a square-root transformation was applied to all duration scores prior to statistical analysis. An analysis of variance (ANOVA) performed on these transformed data revealed a significant difference between the mean durations of immobility for these groups, $F(3,52)=3.86$, $p<0.025$. Then Dunnett's test comparing each drug dosage group with the control was performed. This analysis revealed that the mean duration of immobility for the 1 mg/kg group was significantly shorter then the immobility duration for the control group, $t(52)=3.24$, $p<0.01$. Other comparisons were not statistically significant. The mean number of inductions required to produce immobility for the control and three yohimbine groups were 1.29, 1.00, 1.00, and 1.50, respectively. However, an ANOVA performed on these data failed to find any statistical difference between groups.

In contrast, prazosin produced no apparent effect on the duration of immobility, regardless of the dosage used (see Fig. 1), and neither ANOVA nor Dunnett's test on the transformed data revealed any significant differences between groups for this measure. The mean number of inductions required to produce immobility for the control and three prazosin groups were 1.21, 1.29, 1.64, and 1.79, respectively; but an ANOVA performed on these data failed to find any statistical difference between groups.

Phentolamine produced the most complex results of the

study. This drug decreased the duration of tonic immobility at 0.1 mg/kg doses, but had little effect at higher or lower dosages (see Fig. 1). After a square-root transformation of the data, an ANOVA revealed that the differences between mean durations for these groups only approached accepted levels of significance, $F(3,52)=2.33, p<0.10$. However, Dunnett's test comparing each drug dosage group with the control revealed that the mean duration of immobility for the 0.1 mg/kg phentolamine group differed significantly from that of the control group, $t(52)=2.56, p<0.05$, although other comparisons were not statistically significant. The mean number of inductions required to produce immobility for the control and three phentolamine groups were 1.36, 1.07, 1.14, and 1.36, respectively; but statistical analysis failed to find any signficant difference between groups for this measure.

DISCUSSION

Previous research concerning the effects of drugs on tonic immobility has for the most part ignored the involvement of the adrenergic neurochemical system with the immobility response [15, 19, 23]. Several studies have shown that epinephrine substantially prolonged the duration of immobility in several species [4, 12, 13, 21], but other attempts with norepinephrine and various NE-blocking agents to show the involvement of adrenergic drugs with immobility were inconclusive [17,20]. More recently, however, research has shown that L-DOPA, norepinephrine, and various adrenergic agonists can significantly affect the duration of tonic immobility in chickens [8, 11, 18]. Nevertheless, the relationship between the adrenergic system and the immobility response may not be as simple as one might think. There are a variety of different adrenoceptors upon which adrenergic drugs may act. Adrenergic receptors were initially classified as either alpha or beta [1]. Later, each type was further subdivided into two groups on the basis of their affinity for specific agonists [2,14]. Current research suggests that beta receptors are probably not involved with the immobility response since isoproterenol, a non-specific beta agonist, had no apparent effect on tonic immobility [11]. However, alpha-adrenergic receptors seem to be involved because both $alpha_1$ and $alpha_2$ agonists had significant effects on the immobility response. $Alpha_2$ agonists, which act on presynaptic inhibitory adrenoceptors, increased the duration of immobility, while $alpha_1$ agonists, which act on the postsynaptic adrenergic receptors, produced a decrease in the duration of the immobility response [11]. The present study attempted to confirm these findings by examining the role of alpha-adrenergic antagonists with tonic immobility. These drugs tend to block the action of alpha agonists at the adrenoceptors. Therefore, it seems likely that alpha antagonists should produce effects on immobility which are opposite to those caused by their respective agonists.

The first part of this study examined the effects of yohimbine, a highly specific $alpha_2$ antagonist [22,24], on the immobility response. Previous research found that yohimbine blocked the sedation effects produced by clonidine, an $alpha_2$ agonist, in chickens and several species of mammals [5, 7, 9]. However, virtually nothing is known about the individual effects by yohimbine on behavior. The present study remedied this deficiency by demonstrating that yohimbine significantly reduced the duration of tonic immobility in chickens. Thus, since a potent $alpha_2$ blocker could attenuate the duration of tonic immobility and $alpha_2$ agonists are known to potentiate this response [11], there is now substantial evidence to support the involvement of $alpha_2$ adrenoceptors with the control of immobility duration.

The second part of this study sought to examine the effects of prazoisin, a highly selective $alpha_1$ antagonist [22,24], on the immobility response. Past research found that $alpha_1$ agonists tended to decrease the duration of tonic immobility [11]. Therefore, if $alpha_1$ receptors are critical for the control of the immobility response, then $alpha_1$ blockers should increase the duration of tonic immobility. However, prazosin had no apparent effect on the duration of tonic immobility in the present study. One might suggest that this lack of effect by prazosin was due insufficient drug dosage, but previous work with this drug in mammals found that it was usually effective in blockade of $alpha_1$ receptors at much lower doses than were effective in blockade of $alpha_2$ receptors by phentolamine or yohimbine [7,22]; and the present study found that both these drugs attenuated the duration of immobility within the same dose parameters currently utilized for prazosin. Thus, an explanation for prazosin's lack of effect on immobility due insufficient drug dosage seems highly unlikely. Other research also found that phenoxybenzamine, another $alpha_1$ antagonist, had no substantial effect on tonic immobility [17]. Therefore, these results tend to suggest that $alpha_1$ adrenoceptors probably do not have a direct influence on the duration of the immobility response and that attenuation of immobility duration by $alpha_1$ agonists [11] was due to some secondary mechanism.

The third part of this study examined the effect of phentolamine, a non-specific alpha antagonist, on tonic immobility. This drug blocks both $alpha_1$ and $alpha_2$ receptors, although it seems slightly more effective at the latter sites [7,24]. This preference to act as an $alpha_2$ antagonist has been shown in several studies where phentolamine blocked the sedation effect produced by clonidine and naphazoline, two well-known $alpha_2$ agonists [5, 7, 9]. Moreover, this has even been accomplished at lower doses than those found effective for yohimbine, a highly specific $alpha_2$ antagonist [6]. The present study showed that phentolamine also significantly reduced the duration of tonic immobility at lower dosages than those required with yohimbine in the first part of this experiment. Since $alpha_2$ antagonists such as yohimbine tend to decrease duration of immobility, the attenuation of the immobility response with 0.1 mg/kg doses of phentolamine was most likely due this drug's action on $alpha_2$ receptors. In contrast, phentolamine's lack of effect on immobility duration at higher dosages may reflect its action as an $alpha_1$ antagonist, since $alpha_1$ antagonists such as phenoxybenzamine and prazosin seem to have no apparent effect on tonic immobility and phentolamine is known to act on $alpha_1$ adrenoceptors at high dosages [24]. Thus, phentolamine's biphasic effect on duration of tonic immobility may simply represent initial $alpha_2$ receptor blockade and then subsequent $alpha_1$ receptor blockade.

None of the alpha-adrenergic antagonists used in this study had any apparent effect on initial susceptibiltiy to tonic immobility, as measured by the number of inductions required to produce the response. Therefore, the adrenergic system does not seem to be involved with this aspect of the immobility reflex. However, the other results of the present study do support the hypothesis that the adrenergic system mediates control of the duration of the immobility response; although $alpha_1$ receptors only seem to have minimal involvement with tonic immobility, while $alpha_2$ receptors are the primary control mechanism. Previous research already indicated that $alpha_2$ adrenoceptor potencies were more

sensitive indicators of adrenergic agonist effects on the duration of tonic immobility than alpha$_1$ potencies [11]. Therefore, all of the available data seem to suggest that presynaptic inhibiton due alpha$_2$ receptor stimulation is the main adrenergic control mechanism for the state of motor inhibition known as tonic immobility.

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