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Effect of neurotransmitter reuptake blockers on tonic immobility in chickens

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This research was undertaken to investigate the effects of two neurotransmitter reuptake blockers, nisoxetine and fluoxetine, on the tonic immobility (TI) response. In Experiment 1, nisoxetine was found to significantly diminish both the duration of the TI response and the number of inductions needed to elicit it. These results were discussed in terms of the drug's effects on dopaminergic and/or serotonergic systems with the implication of possible competing locomotor response effects. Fluoxetine, in Experiment 2, was found to have no significant effect on either the duration of TI or the number of inductions needed to elicit it. Additional research with fluoxetine was suggested as necessary, with the possible use of an intraventricular route for administration of the drug.

The tonic immobility (TI) response is an unlearned response found in a wide variety of species (Ratner, 1967). The reaction typically is induced by manually restraining an organism in an inverted position for a brief period of time; this position may then endure for a period of time varying from a few seconds to several hours after termination of restraint, even among members of the same species (Gallup, Nash, & Wagner, 1971). Response termination is distinguished by the organism's righting itself, and sometimes attempting to escape from the situation. The reaction in domestic fowl is characterized by tremors of the extremities, waxy flexibility, eye closure, decreased vocalizations, mydriasis, changes in heart and respiration rates, and decreased core temperature (for a review, see Gallup, 1974).

Recent research on the neuropharmacology of TI has implicated a serotonergic mechanism in the mediation of the response, since a variety of manipulations thought to effect changes in central serotonergic function have been found to modify the duration of the response in chickens (Wallnau & Gallup, 1977). For example, increasing brain levels of serotonin (5-HT) by peripheral injection of tryptophan, the essential amino acid precursor of 5-HT, significantly potentiates duration of TI (Gallup, Wallnau, Boren, Gagliardi, Maser, & Edson, 1977). Conversely, dietary depletion of tryptophan suppresses response duration (Gallup et al., 1977), theoretically due to a decrease in central levels of serotonin. In addition, a number of pharmacologic agents (e.g., iproniazid, pargyline, and imipramine) that increase synaptic levels of 5-HT exert corresponding increases in immobility duration (Harston, Sibley, Gallup, & Wallnau, 1976; Maser, Gallup, & Hicks, 1975). Also, intraventricular administration of 5-HT prolongs the duration of TI in chickens (Harston et al., 1976). As a result of these and other drug effects on immobility, a serotonergic-raphé model of TI has been proposed by Wallnau and Gallup (1977).

According to this model, activity of raphé nuclei (the principal 5-HT-containing neurons in the brain) is hypothesized to be inversely related to the duration of immobility. In addition, since the activity of these neurons is inhibited by an excess of 5-HT at postsynaptic receptor sites, due to an inhibitory feedback mechanism, manipulations designed to enhance central levels of 5-HT prolong the duration of TI, presumably due to a decrease in raphé firing.

However, recent evidence (Boren, Gallup, Suarez, Wallnau, & Gagliardi, 1979) suggests that the above model needs to be modified. It was found that injection of either pargyline or tryptophan potentiated the duration of TI, as would be predicted by the original serotonergic-raphé model of immobility. However, the combined administration of these drugs dramatically attenuated the response. As a result of this finding and research on serotonergic mechanisms by Aghajanian and Wang (1978), Boren et al. (1979) have suggested that drug effects on TI might not be due to their effects on raphé activity directly, but may result from their effects on postsynaptic serotonergic receptors. For example, if a particular drug treatment results in an excess of 5-HT relative to intraneuronal storage capacity, 5-HT could spill over onto postsynaptic serotonergic receptors, inhibiting them and thereby decreasing the duration of TI independent of raphé firing.

Since previous research with the 5-HT reuptake blocker imipramine (Maser et al., 1975) was found to diminish the duration of TI when injected peripherally but to potentiate the response when injected intraventricularly, the present study was undertaken to

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further investigate the effects of neurotransmitter reuptake blockers on the immobility response. Two drugs were used: nisoxetine, which affects several different neurotransmitters in addition to 5-HT, and fluoxetine, a very specific 5-HT reuptake blocker.

EXPERIMENT 1

In synaptosomes that have been isolated from the rat brain, nisoxetine is found to inhibit the uptake of norepinephrine (NE), dopamine (DA), and 5-HT. Nisoxetine also inhibits the uptake of NE into the rat heart (Fuller, Snoddy, & Molloy, 1975). In an attempt to assess the effects of this drug on TI, varying doses of nisoxetine were administered to domestic fowl.

Method

Subjects. The subjects were 72 straight-run Production Red chicks, obtained from a local hatchery at 2 days of age. The birds were housed in commercial brooders and given continuous access to Purina Chicken Chow (Growena) and water. The photoperiod in effect during rearing was 14-h of artificial light per day, from 0400 to 1800 h. Experimental procedures were carried out between the hours of 1300 and 1800.

Procedure. Testing was initiated when the birds were 10 days of age. A bird was randomly selected from the brooder, weighed, and injected intraperitoneally (IP) with nisoxetine dissolved in physiological saline at 0, 5, 10, 20, 25, or 30 mg/kg, resulting in 12 birds/group. The volume of saline injected for subjects in the control group (0 mg/kg) was equivalent to that administered to birds of the same weight receiving the 20-mg/kg dose of nisoxetine.

Immediately following injection, each subject was placed in a holding box located outside the testing room with a companion bird whose function was to minimize the stress resulting from isolation, and it was allowed to remain there for 15 min. At the end of this postinjection time period, the bird was removed from the box and taken into the testing room, where it was immediately restrained on its left side in a three-sided wooden induction box (.61 x .61 x .50 m), which was used to eliminate extraneous visual cues. Restraint continued for 15 sec, after which the experimenter slowly withdrew his hands and activated a Hunter Klockounter (Model 120c). While timing the duration of the response, the experimenter sat relatively motionless about .50 m away from the subject and avoided making direct eye contact. Termination of TI was judged to occur when the bird rose to its feet or had remained immobile for a maximum of 900 sec. If a subject failed to show the reaction after the first induction, it was given repeated 15-sec inductions, with a 15-sec intertrial interval, until immobility obtained or until five inductions had been administered, in which case a duration of 0 sec was recorded. As a control for possible experimenter bias, the person inducing the response was not informed of the dosage a given subject had received.

Results

As can be seen in Table 1, nisoxetine-treated subjects displayed greatly diminished response duration times. A between-groups analysis of variance computed on a $\log_{10}(X + 1)$ transformation of the duration data, used to reduce heterogeneity of variance, showed this effect to be significant [$F(5,66) = 6.97$, $p < .001$]. A Duncan's multiple-range test revealed that each of the dosage levels differed significantly ($p < .05$) from the control but not from each other.

Table 1
Means and Standard Deviations of TI Duration (sec) and the Number of Inductions Needed to Elicit the Response as a Function of Nisoxetine Dosage (mg/kg)

	Duration of Immobility		Number of Inductions	
Dose	Mean	SD	Mean	SD
0	385.92	276.64	1.58	1.16
5	191.78	354.34	3.42	1.98
10	135.12	256.61	3.67	1.67
20	39.50	97.21	4.33	1.56
25	12.33	24.65	4.08	1.68
30	10.38	17.23	4.17	1.59

The reduced susceptibility to TI, also seen in Table 1, was analyzed using a between-groups analysis of variance and was found to be significant [$F(5,66) = 4.72$, $p < .001$]. Based on Duncan's multiple-range test, a dosage effect was not found, but each of the dosage levels did differ significantly ($p < .05$) from the control.

Informal observations of nisoxetine-injected subjects following testing revealed hyperactivity and stereotyped behavior at the higher dosages. Birds intermittently displayed a squatting posture, frequently accompanied by wing abduction, with brief periods of trembling and swaying in a standing posture.

EXPERIMENT 2

The reduced susceptibility to and duration of TI found in nisoxetine-injected birds is difficult to interpret, since this drug blocks the uptake of DA and 5-HT, both of which may be involved in TI (Wallnau, Carnrike, & Dewey, 1979; Wallnau & Gallup, 1977). As a result, the second study was undertaken to investigate the effects of fluoxetine, an even more specific 5-HT uptake inhibitor than either imipramine or protryptyline (Fuller & Wong, 1977). Since pilot work in our laboratory suggested that this drug might prolong TI, as intraventricular injection of imipramine does (Maser et al., 1975), habituation training (Nash & Gallup, 1976) was carried out prior to injection so that a 900-sec response ceiling could still be used.

Method

Subjects. The subjects were 40 straight-run Production Red chicks, obtained from a local hatchery at 22 days of age. The birds were housed and maintained with the same photoperiod in effect as in the previous experiment.

Procedure. At 10 days of age, a habituation procedure was initiated for all of the birds. Each subject was given 2 consecutive days of training, which consisted of manually restraining a bird on its left side for 15 sec in the induction box. The duration of each resulting immobility episode was terminated after 15 sec by gentle prodding. Each subject was given five inductions per day, with a 15-sec intertrial interval.

On the day following the last habituation training day, a bird was randomly selected from the brooder, weighed, and injected IP with fluoxetine dissolved in physiological saline at 0, 20, 40, or 80 mg/kg, resulting in 10 birds/group. The volume of the vehicle injected for subjects in the control group (0 mg/kg) was equivalent to that administered to birds of the same weight

receiving the 40-mg/kg dose of fluoxetine. The postinjection testing procedure was the same as was followed in the first experiment.

Results

Due to heterogeneity of variance, a between-groups analysis of variance was computed on a $\log_{10}(X + 1)$ transformation of the raw duration data, and no significant drug effect was found [$F(3,36) = .97$]. Analysis of variance also showed that there was no significant effect of fluoxetine on the number of inductions needed to elicit TI [$F(3,36) = .83$].

DISCUSSION

Nisoxetine, a DA, NE, and 5-HT uptake blocker, greatly diminished susceptibility to TI and its duration. In light of research findings that the adrenergic system is not involved in TI (Thompson, Scuderi, & Boren, Note 1), the effect of this drug on TI is probably a result of its effects on dopaminergic and/or serotonergic systems. With respect to 5-HT involvement, previous research (Maser et al., 1975) with intraventricular injection of the 5-HT uptake blocker imipramine found potentiation of TI, in contrast to the reduced responsiveness found with nisoxetine. However, peripheral injection of imipramine did produce a similarly diminished duration of TI, but this finding may have been due to motoric confounding, as a result of the increased muscular activity that results from such a procedure (Maser et al., 1975).

Competing locomotor responses have also been suggested to mediate the effect of apomorphine, a DA receptor agonist, in producing shortened durations of immobility (Wallnau et al., 1979). Therefore, nisoxetine, by virtue of its central stimulating effect on dopaminergic systems involved in locomotor activity and its peripheral effect on serotonergic systems involved in muscle activity, may have affected a reduction in TI duration as a result of competing responses.

The failure in the second experiment to find an effect of serotonin uptake blockage on TI by fluoxetine is puzzling. However, it may be expected that 5-HT uptake blockage might reduce TI duration due to effects on postsynaptic serotonergic receptors (Boren et al., 1979) or as a result of competing motor responses produced through use of a peripheral injection procedure (Maser et al., 1975). Since the habituation procedure used in the second experiment might be expected to mask such a reduction in response duration, additional research in our laboratory was conducted using peripheral injection of fluoxetine, but without prior habituation training. Again, however, no significant effects of fluoxetine of TI duration were found. Additional research with fluoxetine and TI, possibly using an intraventricular route of administration, is obviously needed.

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