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Anxiogenic Effects of a β-Carboline on Tonic Immobility and Open Field Behavior in Chickens (*Gallus gallus*)

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MORIARTY, D. D. *Anxiogenic effects of a β-carboline on tonic immobility and open field behavior in chickens (Gallus gallus).* PHARMACOL BIOCHEM BEHAV **51**(4) 795–798, 1995.—Tonic immobility (TI) is an innate form of active motor inhibition displayed by many species in response to restraint. It is strongly influenced by manipulations that affect fear, and is thought to be the last in a series of responses to attack by a predator. The suggestion that GABA systems may be involved in TI was investigated by assessing the effects of the α-$GABA_A$ inverse agonist β-CCM (β-carboline-3-carboxylic acid-*N*-methylamide), which is thought to have anxiogenic properties, on the immobility response and open field behavior in chickens (*Gallus gallus*). Birds given β-CCM displayed longer durations of TI than those given control injections. Although there was a tendency towards increased susceptibility in the groups given the drug, it was not significant. In the open field test, the drug reduced activity and vocalization relative to control levels. The results are discussed in terms of the involvement of GABA systems in TI and related behaviors, and the suggestion that α-$GABA_A$ inverse agonists are anxiogenic agents.

GABA β-Carboline Anxiogenic drug Fear Tonic immobility Open field behavior Chickens

TONIC immobility (TI), the response of many species to physical restraint, is an innate form of motor inhibition characterized by an immobile posture, muscular rigidity and tremors, eye closure, apparent loss of responsiveness to external stimuli, and physiological changes associated with strong emotion (4,5). It is augmented by manipulations producing fear, and attenuated by those that reduce fear. TI is the last in a series of defensive behaviors displayed in response to attack by a predator, and is thought to function by reducing stimuli leading to further attack (5,16).

Identification of neurochemical systems involved in TI has been of considerable interest. Cholinergic (9,10,24), adrenergic (8,23,25), serotonergic (7,27), and dopaminergic (2,26) contributions have been demonstrated.

γ-Aminobutyrate (GABA) has been described as the most common inhibitory transmitter (11), and it has been suggested that GABA might be involved in TI (12). GABAergic systems may be related to the emotional component of TI because the benzodiazepines used in the treatment of anxiety act by potentiating GABA transmission. GABA could also be more directly involved in the motor inhibition that characterizes TI. Klemm (12) proposed a model of immobility involving immobility and anti-immobility brain systems. In reviewing the pharmacological literature, he noted the interaction of different transmitter systems in producing active immobility responses such as TI, and attempted to relate them to limbic system involvement in the role of emotional states associated with TI, and suggested that active immobility was facilitated by GABA agonism.

GABA receptors have been divided into two categories on the basis of their pharmacological activities (1). In addition to being influenced by different agonists and antagonists, $GABA_A$ receptors are characterized as being coupled with Cl-channels, while $GABA_B$ receptors are thought to be associated with CA^{2+} channels. Also, $GABA_B$ sites seem to mediate presynaptic inhibition, whereas $GABA_A$ sites are involved in postsynaptic inhibitory effects.

Martin (13) described the $GABA_A$ receptor complex as being composed of two receptor sites (α and β) and a chloride ionophore. GABA and its analogues bind to the β site and open the chloride channel, whereas drugs that bind to the α site regulate the frequency of ionophore opening. Fanselow, Helmstetter, and Calcagnetti (3) reviewed the pharmacological and behavioral effects of drugs acting at the α site. They noted that the anxiolytic benzodiazepines are α-$GABA_A$ agonists, and increase the frequency of chloride channel opening, thus enhancing the inhibitory effect of GABA. On the other hand, drugs such as the β-carboline DMCM (6,7-dimethoxy-4-ethyl-

β-carboline-3-carboxylic acid methyl ester), which also bind at the α site, have the opposite effect in that they reduce the frequency of chloride ionophore opening, reduce the inhibitory effects of GABA, and are thus called inverse agonists. Fanselow et al. suggested that inverse agonists might potentiate or generate anxiety, and presented evidence of an anxiogenic effect in rats produced by DMCM. Their results are consistent with earlier observations (17,18) that among the β-carbolines were substances that could be either anxiogenic or anxiolytic depending upon their status as $GABA_A$ agonists, antagonists, or inverse agonists.

In the experiments reported here, the effects of a β-carboline, purported to be an anxiogenic agent, on tonic immobility and open field behavior of chickens were investigated.

EXPERIMENT 1

In the first experiment, the effects of β-CCM, a β-carboline known to have anxiogenic effects in mice (17), on TI were assessed. If the drug has anxiogenic effects in chicks, then birds given the drug should be more susceptible to, and display longer durations of, TI than those given control substances.

METHOD

Subjects

The subjects were 48 straight-run Production Red chickens (*Gallus gallus*) obtained 1 day posthatch from Cebe Hatchery in Ramona, CA. They were housed in a commercial brooder under a 12L : 12D cycle of artificial lighting. Purina Chick Lab Chow and water were always available. The birds were 25 days old at the time of testing.

Drugs

β-CCM (β-carboline-3-carboxylic acid-*N*-methylamide, Sigma) dissolved in DMSO (dimethyl sulfoxide, Sigma) was administered in doses of 0, 1, and 2.5 mg/kg of body weight to different groups of birds, as was distilled water. Injections were given intraperitoneally at a volume of 0.1 ml/100 g of body weight.

Procedure

Chicks were removed from the brooder, weighed, and randomly assigned to one of the four groups so that there were 12 birds in each group. After the injection, the bird was placed in a cardboard box and carried to a testing room where a Lafayette Instrument Company white noise generator (Model #15800) was used to provide a masking sound. Ten minutes after the injection, the bird was placed on a bare table, turned onto its right side facing away from the experimenter, and gently restrained for a 15-s induction period. At the end of the period, the chick was released, and a timer started. If the bird remained immobile for 15 s, it was left undisturbed until it righted itself, or until 1800 s had elapsed. The duration of TI was recorded. If the bird failed to meet the 15-s immobility criterion, it was returned to the box for 30 s and then the induction procedure was repeated. A maximum of five inductions was given, and birds failing to meet the criterion were assigned a duration of 0 s. An index of susceptibility to TI was generated by taking the reciprocal of the number of inductions required to produce immobility (1/number of inductions).

RESULTS AND DISCUSSION

As shown in Table 1, birds given the β-carboline remained immobile longer than those given only DMSO or distilled water. ANOVA revealed significant differences, $F(3, 44) = 4.04$, $p = 0.013$. Orthogonal comparisons indicated that the difference between the distilled water and DMSO control groups was not significant, nor was the difference between the two groups given the β-carboline. The difference between the two control groups and the two β-carboline groups was significant, $F(1, 44) = 12.12$, $p = 0.001$.

The two groups given the β-carboline were more susceptible to TI than the two control groups (see Table 1), but these differences were not significant.

The results show that DMSO had no effect on TI. They suggest that β-CCM had an anxiogenic effect, because birds given the drug displayed longer durations of TI than control subjects. Increased susceptibility to TI in birds given the drug would further support this conclusion, but few birds in any of the groups required more than two inductions, so that susceptibility to TI would not be sensitive to manipulations that reduce it. The differences in susceptibility were in the expected direction but were not significant. Thus, the results suggest a role for GABA systems in TI.

EXPERIMENT 2

The open field test has long been used as a measure of general emotionality in animals (28). Gallup and Suarez (6,19–22) proposed that open field behavior of chickens and some other species represents a compromise between the tendencies to avoid predation and to reinstate social contact. For example, socially isolated birds vocalize more than ones tested in the presence of conspecifics, but the presentation of fear-eliciting stimuli reduces vocalization. They noted that several fear-inducing manipulations that prolong TI also reduce vocalization and activity in an open field test, whereas fear-attenuating manipulations that shorten TI increase vocalization and activity in an open field. It has recently been shown that anticholinergic drugs that affected TI also influenced open field behavior of chicks (9,15).

In this experiment, the effects of β-CCM on the open field behavior of chicks was observed. If the drug has anxiogenic effects, as suggested by the results of Experiment 1, then chicks given the drug should be less active and less vocal than those given control substances.

TABLE 1

SUSCEPTIBILITIES (1/NUMBER OF INDUCTIONS) TO, AND DURATIONS (s) OF, TONIC IMMOBILITY IN CHICKENS GIVEN INJECTIONS OF DIFFERENT DOSES OF β-CCM OR OF CONTROL SUBSTANCES

Drug Group	Susceptibility to Immobility (1/Inductions)	Duration of Immobility (s)
Water	0.36 (0.56)	556.3 (633.8)
DMSO	0.37 (0.65)	572.2 (601.9)
β-CCM (1 mg/kg)	0.59 (0.55)	1230.5 (709.4)
β-CCM (2.5 mg/kg)	0.54 (0.74)	1246.2 (604.8)

Values are mean with SD in parentheses.

METHOD

Subjects

The subjects were 42 straight-run Production Red chickens (*Gallus gallus*) acquired and housed under the same conditions as the birds used in the first experiment. They were also 25 days old at the time of testing.

Apparatus

A 92.4-cm^2 open field activity monitor, with 60.9-cm high sides was used. It was made of particle board and was painted white. The floor of the activity monitor was marked off into 49 equal-sized squares. A video camera was suspended 3 m above the center of the activity monitor so that test sessions could be recorded.

Drugs

The drugs and injection procedures were the same as those used as in the first experiment, except that only three groups were involved, one given water, another given DMSO, and the third given 2.5 mg β-CCM/kg of body weight.

Procedure

Chicks were removed from the brooder, weighed, and randomly assigned to one of the three groups so that each group contained 14 birds. After the appropriate injection was given, the chick was placed in a cardboard box and carried to the testing room. Ten minutes after the injection, the chick was removed from the box and placed in the center square of the open field, and its behavior was videotaped for the next 10 min. Latency of vocalization, number of vocalizations, latency of activity (time elapsed before the bird left the center square), and activity level (number of squares entered) were measured for each bird by examination of the videotapes, as were the number of attempts to escape from the apparatus (jumps at the walls).

RESULTS AND DISCUSSION

Although fewer of the birds given β-CCM tried to escape from the open field (see Table 2), there were no significant differences among the groups in the mean numbers of attempted escapes.

Data on the remaining dependent variables (see Table 2) were analyzed using one-way ANOVAs. Prior to analysis, square-root transformation of the data was performed to correct for heterogeneity of variance. Newman-Keuls tests were used subsequent to the ANOVAs.

Birds given β-CCM displayed longer vocalization latencies than those given the vehicle or water, $F(2, 39) = 5.87$, $p = 0.006$. The birds given β-CCM differed significantly from those given water ($p = 0.004$), but not from those given DMSO ($p = 0.09$). The two control groups did not differ significantly ($p = 0.10$).

Birds given β-CCM emitted fewer vocalizations than those given DMSO or water, $F(2, 39) = 3.27$, $p = 0.048$. Those given β-CCM differed significantly from those given water ($p = 0.04$), but not from those given DMSO ($p = 0.21$). The difference between the two control groups was not significant ($p = 0.77$).

The mean activity latency for the birds given β-CCM was longer than that for those given DMSO or water, $F(2, 39) = 3.87$, $p = 0.029$. Those given β-CCM differed significantly from those given water ($p = 0.03$) and from those given DMSO ($p = 0.05$). The two control groups did not differ significantly ($p = 0.41$).

Birds given β-CCM were less active than birds given DMSO or water, $F(2, 39) = 4.73$, $p = 0.015$. Those given β-CCM differed significantly from both those given DMSO ($p = 0.03$) and those given water ($p = 0.02$). The two control groups did not differ significantly ($p = 0.56$).

The observation that chicks given β-CCM displayed longer vocalization latencies, fewer vocalizations, longer activity latencies, and lower activity levels than birds given water is consistent with the hypothesis that β-CCM has anxiogenic properties that influence open field behavior. However, the further observation that chicks given β-CCM differed from those given DMSO on only two of the four measures of open field behavior, activity latency and activity level, suggests that DMSO might have some effect on vocalization (latency and number). Yet there were no significant differences between the water and DMSO control groups on any of the dependent variables, and the DMSO group was more similar to that of the water group than the β-CCM group on all of those variables. Taken together with the observation in Experiment 1 that DMSO had no effect on the immobility response and the drug did, these findings clearly suggest that β-CCM has anxiogenic effects as assessed in the present studies.

GENERAL DISCUSSION

The results of both experiments suggest that β-CCM, an α-GABA$_A$ inverse agonist, had anxiogenic effects in chicks because the drug increased the duration of TI and influenced open field behavior as would a fear-inducing manipulation. The results also suggest that GABA systems are involved in TI

TABLE 2

LATENCIES OF VOCALIZATION (s), NUMBERS OF VOCALIZATIONS, LATENCIES OF ACTIVITY (s), ACTIVITY LEVELS (NUMBER OF SQUARES ENTERED), AND NUMBERS OF ATTEMPTS TO ESCAPE FROM THE APPARATUS IN CHICKENS GIVEN β-CCM AND CONTROL SUBSTANCES

Drug Group	Vocalization Latency (s)	Number of Vocalizations	Activity Latency (s)	Activity Level	Number of Escape Attempts
Water	9.7 (36.3)	184.0 (167.0)	212.4 (252.1)	10.8 (13.4)	1.1 (1.3)
DMSO	183.2 (332.4)	149.3 (227.5)	233.5 (188.9)	9.9 (19.5)	0.7 (0.6)
β-CCM	355.6 (423.7)	72.1 (93.2)	705.9 (320.6)	0.6 (1.6)	0.6 (1.2)

Values are mean with SD in parentheses.

and related behaviors, and that the effect of the drug is related to the anxiety level of the birds.

Because the drug's presumed mechanism of action involves reduction of the inhibitory effects of GABA, these results suggest that TI is facilitated by GABA antagonism rather than its agonism, as was suggested by Klemm (12). However, baclofen, a potent $GABA_B$ agonist, which is inactive at $GABA_A$ sites, increased the duration of TI (14). Thus, the role of GABA in TI is more complex than Klemm suggested.

The results also support the more general conclusion that GABA systems are involved in the behavioral expression and perhaps experience of fear and anxiety. Clarification of the mechanism of GABA involvement and of the interactions among GABA and other neurotransmitter systems await further research.

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REFERENCES

1. Bradford, H. F. Chemical neurobiology: An introduction to neurochemistry. New York: Freeman; 1986.
2. Ettinger, R. H.; Thompson, R. W. The role of dopaminergic systems in the mediation of tonic immobility (animal hypnosis). Bull. Psychon. Soc. 12:301-302; 1978.
3. Fanselow, M. S.; Helmstetter, F. J.; Calcagnetti, D. J. Parallels between the behavioral effects of dimethoxy-β-carboline (DMCM) and conditioned fear. In: Flaherty, C.; Dachowski, L., eds. Current topics in animal learning: Brain, emotion, and cognition. Hillsdale, NJ: Erlbaum; 1991:187-206.
4. Gallup, G. G., Jr. Animal hypnosis: Factual status of a fictional concept. Psychol. Bull. 81:835-853; 1974.
5. Gallup, G. G., Jr. Tonic immobility: The role of fear and predation. Psychol. Rec. 27:41-61; 1977.
6. Gallup G. G., Jr.; Suarez, S. D. An ethological analysis of open-field behavior in chickens. Anim. Behav. 28:368-378; 1980.
7. Hennig, C. W. Biphasic effects of serotonin on tonic immobility in domestic fowl. Pharmacol. Biochem. Behav. 12:519-523; 1980.
8. Hennig, C. W.; Dunlap, W. P.; Harston, C. T.; McPhee, A. A. Tonic immobility and the α-adrenergic system in chickens. Physiol. Behav. 24:21-25; 1980.
9. Hennig, C. W.; McIntyre, J. F.; Moriarty, D. D., Jr.; Picerno, J. M.; Allen, J. L. Differential cholinergic influences on the immobility response in various strains of domestic fowl. Pharmacol. Biochem. Behav. 30:625-634; 1988.
10. Hughes, R. A. Anticholinergic drugs, blood-brain barrier, and tonic immobility in chickens. Physiol. Behav. 29:67-71; 1982.
11. Iverson, S. D.; Iverson, L. L. Behavioral pharmacology. New York: Oxford University Press; 1981.
12. Klemm, W. R. Drug effects on active immobility responses: What they tell us about neurotransmitter systems and motor functions. Prog. Neurobiol. 32:403-442; 1989.
13. Martin, I. L. The benzodiazepines and their receptors: Twenty-five years of progress. Neuropharmacology 26:957-970; 1987.
14. Merrigan, M.; Moriarty, D. D., Jr. GABA and tonic immobility in chicks. Paper presented at the meeting of the Western Psychological Association, Los Angeles, CA; 1990.
15. Moriarty, D. D., Jr.; Roberts, K. A.; Allen, J. L.; Hennig, C. W. Anticholinergic drugs and open-field behavior in chickens. Bull. Psychon. Soc. 26:559-562; 1988.
16. Ratner, S. C. Comparative aspects of hypnosis. In: Gordon, J. E., ed. Handbook of clinical and experimental hypnosis. New York: Macmillan; 1967:335-351.
17. Stephens, D. N.; Kehr, W. β-carbolines can enhance or antagonize the effects of punishment in mice. Psychopharmacology (Berlin) 85:143-147; 1985.
18. Stephens, D. N.; Kehr, W.; Duka, T. Anxiolytic and anxiogenic β-carbolines: Tools for the study of anxiety mechanisms. In: Biggio, G.; Costa, E., eds. GABAergic transmission and anxiety. New York: Raven; 1986:91-106.
19. Suarez, S. D.; Gallup, G. G., Jr. An ethological analysis of open-field behavior in ducks, *Anas platrhynchos*. Bird Behav. 2:93-105; 1980.
20. Suarez, S. D.; Gallup, G. G., Jr. An ethological analysis of open-field behavior in rats and mice. Learn. Motiv. 12:342-363; 1981.
21. Suarez, S. D.; Gallup, G. G., Jr. Predatory overtones of open-field testing in chickens. Anim. Learn. Behav. 9:153-163; 1981.
22. Suarez, S. D.; Gallup, G. G., Jr. Open-field behavior in guinea pigs: Developmental and adaptive considerations. Behav. Proc. 7:267-274; 1982.
23. Thompson, R. W.; Joseph, S. The effect of norepinephrine on tonic immobility in chickens. Bull. Psychon. Soc. 12:123-124; 1978.
24. Thompson, R. W.; Piroch, J.; Fallen, D.; Hatton, D. A central cholinergic inhibitory system as a basis for tonic immobility (animal hypnosis) in chickens. J. Comp. Physiol. Psychol. 87:507-512; 1974.
25. Thompson, R. W.; Scuderi, R.; Boren, J. The effect of epinephrine on tonic immobility (animal hypnosis) in chickens. Bull. Psychon. Soc. 9:409-410; 1977.
26. Wallnau, L. B.; Carnrike, C. L.; Dewey, G. I. Tonic immobility: Effects of dopamine receptor blockade and stimulation. Pharmacol. Biochem. Behav. 10:177-180; 1979.
27. Wallnau, L. B.; Gallup, G. G., Jr. A serotonergic midbrain-raphe model of tonic immobility. Biobehav. Rev. 1:35-43; 1977.
28. Walsh, R. N.; Cummins, R. A. The open-field test: A critical review. Psychol. Bull. 83:482-504; 1976.