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Pargyline and Tryptophan Enhancement of Tonic Immobility: Paradoxical Attenuation with Combined Administration

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BOREN, J. L., G. G. GALLUP, JR., S. D. SUAREZ, L. B. WALLNAU AND G. J. GAGLIARDI. *Pargyline and tryptophan enhancement of tonic immobility: Paradoxical attenuation with combined administration.* PHARMAC. BIOCHEM. BEHAV. 11(1) 17–22, 1979.—Four experiments were conducted to examine the individual and combined effects of pargyline and tryptophan on the duration of tonic immobility in chickens. Injection of either compound alone produced a dose-dependent potentiation of tonic immobility. However, combined administration of pargyline and tryptophan resulted in a dramatic attenuation of the response and this effect was completely blocked by pretreatment with p-chlorophenylalanine. In addition to reducing the duration of tonic immobility, combined administration of pargyline and tryptophan produced a complex behavioral syndrome which may be analogous to that observed in mammals after similar drug treatment. These results suggest the need for a modification of the recently proposed serotonergic-raphe model of tonic immobility.

Pargyline Behavioral syndrome Raphe neurons Tryptophan PCPA Serotonergic model Tonic immobility Serotonin Chickens

TONIC immobility (TI) is a temporary state of extreme behavioral inhibition and loss of the righting reflex that occurs in a wide variety of species as the result of a brief period of physical restraint (see [18]). The response is typically produced in the laboratory by manually restraining an animal on its side or back. After a few seconds of such restraint, animals will usually stop struggling, instead assuming a frozen posture which persists in the absence of further restraint. The duration of TI is highly variable, even within species, and in domestic fowl may last from a few seconds to several hours.

Interest in the neuropharmacology of TI has recently focused on the role of brain serotonin (5-HT) since a variety of manipulations thought to effect changes in central serotonergic function have been found to modify the duration of TI in chickens (see [36]). Systemic administration or dietary deprivation of tryptophan, the essential amino acid precursor to 5-HT, results in respective increases or decreases in the duration of tonic immobility [19]. Drugs thought to increase synaptic concentrations of 5-HT either through inhibition of its catabolism (iproniazid, pargyline) or reuptake (intraventricular imipramine) exert corresponding increases in immobility durations [28,33]. Similarly, d-LSD, which mimics the action of 5-HT at receptor sites (e.g. [4,7]) also increases the duration of TI, as does BOL-148 [33], a non-hallucinogenic isomer of d-LSD having comparable, but weaker serotonergic effects. In addition, it has been shown that the duration of TI is enhanced by morphine [29,37], attenuated by amphetamine [11], and that both of these effects can be blocked [11, 29, 37] by pretreatment with p-chlorophenylalanine (PCPA), a relatively specific depletor of brain serotonin. Finally, intraventricular administration of 5-HT prolongs the response in chickens [28]. These and other drug effects on TI have recently been reviewed and a serotonergic-raphe model of TI proposed [36].

A number of investigators have reported that a complex behavioral syndrome can be elicited in rats by administration of the monoamine oxidase (MAO) inhibitors tranylcypromine or pargyline followed by tryptophan [20, 21, 22, 23, 30, 32], but not by any of these agents alone [15, 20, 32]. Typical components of the syndrome include hyperactivity, hyperthermia, resting tremor, rigidity, reciprocal forepaw treading, hindlimb abduction, and straub tail [31]. The syndrome can be blocked by prior 5-HT synthesis inhibition with PCPA or the 5-hydroxytryptophan decarboxylase inhibitor NSD-1055 [20, 30, 32] and is unaffected [30], or only temporarily affected [13], by catecholamine synthesis inhibition, with the exception of the hyperactivity component

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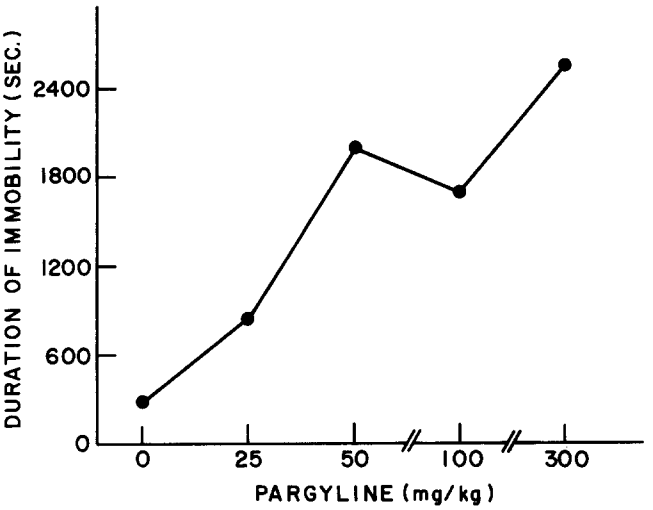

FIG. 1. Mean duration of tonic immobility as a function of pargyline dose.

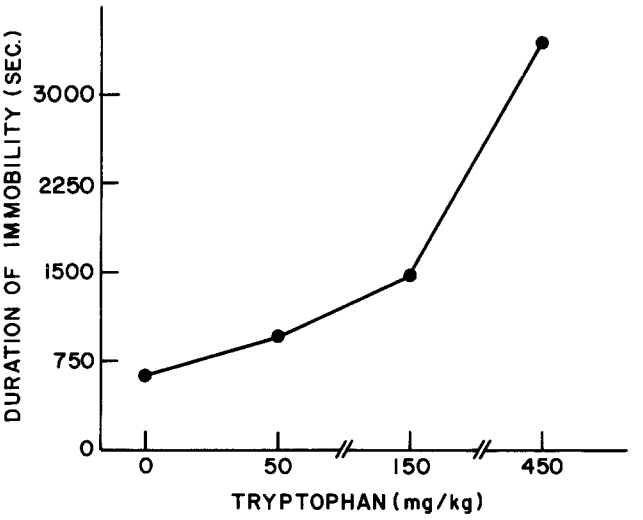

FIG. 2. Mean duration of tonic immobility as a function of tryptophan dose.

[13,22]. Similarly, the syndrome can be prevented by pretreatment with 5-HT receptor blockers [13,30], but not by several catecholamine receptor blockers [30,32], although again hyperactivity is affected [12]. Findings such as these and others (see [24,31]) have led Jacobs [31] to conclude that the syndrome, with the exception of the hyperactivity component, represents a "specific behavioral reflection of central serotonergic activity".

In light of the findings that combined MAO inhibition and tryptophan loading produce serotonergically-mediated behavioral changes not seen with either agent alone, and in view of the apparent serotonergic involvement in TI, the individual and combined effects of pargyline, tryptophan, and PCPA on TI were investigated.

EXPERIMENT 1

Although previous research [33] has shown that IP administration of pargyline increases the duration of TI, only one dose of approximately 100 mg/kg was investigated. The present experiment attempted to replicate the pargyline effect on TI and to provide a dose-response curve for pargyline.

METHOD

Animals

The animals were 30 straight-run Production Red chickens *(Gallus gallus)* obtained from a commercial supplier at one day of age. The birds were group-reared in commercial brooders and given continuous access to chick feed and water under a 14-hr photoperiod. Light onset occurred at 7:00 a.m. and lights went out at 9:00 p.m.

Procedure

Testing was conducted when animals were 25 days of age. Birds were randomly assigned to one of five groups of six animals each. Four groups received IP injections of 25, 50, 100, or 300 mg/kg of pargyline HCl (Sigma) dissolved in distilled water, and the remaining six animals received an equivalent volume (2.5 ml/kg) of the vehicle alone. Animals were then placed in individual cardboard boxes and 45 min later were transported to separate rooms where they were tested for tonic immobility. The reaction was induced by gently restraining the bird on its right side with both hands for 15 sec at which time the experimenter's hands were slowly withdrawn. Immobility durations were timed from the moment of release until the bird showed a righting response. Animals failing to exhibit tonic immobility after five induction attempts separated by 60 sec were given a duration score of zero sec. Testing was performed by four experimenters who were unaware of the treatments the birds received. To preclude any confounding effect of periodicity, testing was staggered between the hours of 10:00 a.m. and 5:00 p.m. with a comparable number of birds from each group tested at different times throughout the day.

RESULTS

Inspection of Fig. 1. reveals that pargyline produced a dose-dependent increase in the mean duration of TI, with reactions at the highest dose being almost 10 times as long as water controls. Due to heterogeneity of variance, the data were subjected to a square-root transformation and a trend analysis revealed that the duration of TI was a linear function of pargyline dose, $F(1,25)=5.45$, $p<0.025$. No other trend components were significant.

EXPERIMENT 2

Gallup *et al.* [19] found that IP injections of tryptophan led to dose-related increases in the duration of TI, with maximal effects being evident 30 min after administration and lasting at least until 60 min after injection. However, since the dose range used in that study was somewhat limited (0–139.5 mg/kg), the present study was conducted in order to extend the dose-response curve for tryptophan.

METHOD

Animals

Twenty-four straight-run Production Red chickens were obtained and maintained as described in the first experiment.

Procedure

Testing was conducted when the birds were 21 days of age. Injection volume, vehicle, and route of administration were the same as those used in Experiment 1. Four groups of six randomly-selected birds were given injections of either 0, 50, 150 or 450 mg/kg L-tryptophan methyl ester HCl (Sigma). All animals were placed in individual cardboard boxes immediately after injection and, 30 min later, were transported in the boxes to a separate room where they were tested for TI as described in the first experiment.

RESULTS

The dose-response curve for tryptophan is shown in Fig. 2. Due to experimenter error, data from one of the animals in the 50 mg/kg group had to be eliminated, reducing the number of animals in that group to five. Again, because of heterogeneity of variance a square-root transformation was performed and a trend analysis revealed a significant linear component, $F(1,19)=6.63$, $p<0.025$. No other trend components were significant.

EXPERIMENT 3

Grahame-Smith [20] reported that the so-called serotonergic syndrome could not be elicited either by MAO inhibition or tryptophan alone, even when tryptophan was given in doses as high as 1 g/kg. However, when tryptophan was given to an animal 30 min after administration of a MAO inhibitor, it produced dramatic behavioral changes which appeared to be maximal 30 to 60 min after tryptophan injection and which he interpreted as being due to a spillover of excess 5-HT onto receptor sites. Experiment 3 was conducted to examine the combined effect of pargyline and tryptophan on the duration of TI and to determine if a behavioral syndrome similar to that observed in rats also occurs in chickens.

METHOD

Animals

Twenty-six straight-run Production Red chickens were obtained and maintained as in previous experiments.

Procedure

Injection volume, vehicle, and route of administration were identical to those used in the preceding experiments. At 24 days of age 16 animals were randomly assigned to one of two groups of eight birds each. Animals in one group received an injection of 100 mg/kg pargyline followed 30 min later by an injection of 150 mg/kg tryptophan. These doses were chosen since they are comparable to those used in research on the syndrome (e.g. [15]) and pilot work in our laboratory indicated that combined administration of higher doses of these compounds produces toxic effects in chickens (cf [20]). Animals in the second group received two comparable injections of distilled water separated by 30 min. After each injection, birds were placed in individual cardboard boxes where they remained until testing. Sixty min after the second injection, animals were transported to a separate room where they were tested for TI as described in Experiment 1.

In order to observe gross behavioral changes which may occur in chickens as a result of combined pargyline and tryptophan administration, 10 additional 25 day old birds were randomly assigned to one of two groups of five birds each and given either pargyline followed by tryptophan or two vehicle injections. Doses and interinjection intervals were identical to those used for animals that were tested for tonic immobility. Immediately after the first injection, each bird was placed in a 25.4×18.4×17.8 cm steel cage with a wire mesh front and bottom. The behavior of each bird was then observed for six 1 min periods 15, 30, 45, 60, 75 and 90 min after the first injection.

RESULTS

In contrast to the increased immobility durations produced by either pargyline or tryptophan alone, combined administration of these compounds resulted in a dramatic attenuation of TI (mean=83.69, SE=29.59) relative to vehicle controls (mean=711.88, SE=214.14). To correct for heterogeneity of variance, data were subjected to a $\log_{10}(x+1)$ transformation, and an analysis of variance indicated a significant effect of drug treatment, $F(1,14)=12.11$, $p<0.005$. In addition to the effect on TI, combined administration of these compounds produced dramatic behavioral changes in chickens, beginning 30 min after tryptophan administration and reaching a maximum 30 min later. Animals initially exhibited brief periods of eye closure and sat with their wings held slightly away from the body (wing abduction). Forty-five min after tryptophan injection, a majority of animals appeared unresponsive to visual stimulation and had adopted a peculiar posture with the head held up or against the back. Finally, in addition to these behaviors, a majority of animals exhibited continuous partial opening of the mouth 60 min after tryptophan administration.

EXPERIMENT 4

A number of studies [20, 30, 32] have shown that PCPA pretreatment prevents the occurrence of the syndrome in animals treated with an MAO inhibitor and tryptophan. Experiment 4 was an attempt to block the combined effect of pargyline and tryptophan on TI by pretreatment with PCPA.

METHOD

Animals

Thirty-two straight-run Production Red chickens were obtained and maintained as described in the preceding experiments.

Procedure

Injection volume, vehicle, and route of administration were identical to those used in the preceding experiments.

At 14 days of age, birds were randomly assigned to one of two groups. One group of 16 birds received an injection of 300 mg/kg PCPA (Sigma), while the remaining group received the vehicle alone. Seventy-two hrs. later, half of the birds in each group were given 100 mg/kg of pargyline followed 30 min later by a second injection of 150 mg/kg tryptophan. The remaining birds in each group were given two vehicle injections at the same intervals after the initial PCPA or vehicle injection. Sixty min after the final injection, all birds were tested for TI as described in the first experiment.

TABLE 1

EFFECTS OF PARGYLINE (100 MG/KG) AND TRYPTOPHAN (150 MG/KG) ON THE DURATION OF TONIC IMMOBILITY (SEC) FOLLOWING PCPA (300 MG/KG) OR WATER PRETREATMENT

		Drug Conditions	
		Pargyline-Tryptophan	Water-Water
PCPA	Mean	790.0	302.9
	S.E.	(484.2)	(128.3)
Water	Mean	4.4	206.9
	S.E.	(4.4)	(90.0)

RESULTS

Table 1 shows the combined effect of pargyline and tryptophan on TI duration following PCPA pretreatment. The data were subjected to a $\log_{10}(x+1)$ transformation and an analysis of variance revealed a significant effect of drug treatment, $F(3,28)=5.05, p<0.01$. Subsequent analyses using Duncan's multiple range test ($p=0.05$, all comparisons) revealed that animals pretreated with water and given both pargyline and tryptophan on the test day showed significantly shorter durations of TI than animals in any other group. No other differences were statistically significant. Inspection of Table 1 reveals that PCPA pretreatment completely blocked the combined effect of pargyline and tryptophan, but by way of replicating previous findings (e.g. [11,37]) had no effect on controls given vehicle injections on the test day.

DISCUSSION

The results of the first two experiments corroborate previous research showing that both pargyline [33] and tryptophan [19] produce increases in the duration of TI, and extend these findings to include higher doses of both drugs. As support for the generality of the effect of MAO inhibitors on TI, iproniazid also increases the duration of immobility [33] and we have recently found (unpublished data) that tranylcypromine, a potent MAO inhibitor, produces a dose-dependent increase in TI duration.

The results of the present experiments also bear on the serotonergic-raphe model of drug effects on tonic immobility [36]. Briefly, as a basis for this model it was noted that an inverse, bidirectional relationship exists between drug effects on the firing rate of single raphe neurons (the principal 5-HT containing neurons in the brain) in rats and the effects of the same drugs on TI in chickens. That is, drugs which decrease raphe firing produce increases in the duration of TI, while those that increase raphe firing, decrease immobility durations. In addition, the concept of feedback regulation (see [1]) was invoked as a possible mechanism whereby 5-HT precursors, catabolism inhibitors, reuptake blockers, and receptor agonists all produce inhibition of raphe firing and corresponding increases in TI duration. Thus, the presence of excess 5-HT at postsynaptic receptor sites was presumed to result in inhibitory feedback to the raphe and a consequent decrease in firing rate. In support of this parallel, both pargyline and tryptophan produce increases in brain 5-HT (e.g. [5, 14, 15]), decreases in raphe firing [2, 5, 6, 17] and in the present experiments, enhanced the duration of immobility.

The results of the third experiment, however, are not easily interpretable within this framework. Combined MAO inhibition and tryptophan administration results in a marked increase in brain 5-HT [8] relative to both vehicle controls and animals treated with MAO inhibitor alone [15,20]. This excess 5-HT should then theoretically act on postsynaptic receptors and lead to an inhibition of raphe and an increase in tonic immobility. In Experiment 3, however, combined pargyline and tryptophan administration, in doses of each drug that were shown to be sub-maximal with respect to their individual effects on TI, decreased immobility durations to approximately one-tenth those of controls and produced a number of other overt behavioral changes. Although the effect of combined pargyline and tryptophan administration on raphe firing is unknown, these results certainly would not be predicted from the serotonergic-raphe model and seem paradoxical in terms of past studies in which manipulations thought to increase brain 5-HT levels have uniformly prolonged tonic immobility.

Although a definitive explanation of the present results must await further investigation, an intriguing possibility is suggested by recent evidence concerning autoregulatory modulation of neuronal activity within the serotonergic system (see [10]). Evidence from studies utilizing techniques of retrograde tracing [9,34] and 5,7-DHT-induced axonal degeneration (reported in [10]), as well as demonstrations of a high-affinity uptake of 5-HT in the region of the raphe nuclei [3,34], suggest a direct serotonergic innervation of the raphe, possibly via axon collaterals [9]. In addition, iontophoretic application of certain agents (e.g., LSD, 5-HT) onto raphe neurons produces immediate inhibition of firing [4,7], implying the existence of 5-HT-sensitive receptors on the raphe membrane. Thus, rather than, or in addition to feedback inhibition from postsynaptic neurons in other brain areas, increased 5-HT availability in the region of the raphe soma could produce a direct inhibition of neuronal activity through mechanisms intrinsic to the raphe. Based on these and other findings, Aghajanian and Wang [10] proposed that there may be a dissociation between drug effects on raphe firing and the "mechanisms and physiological consequences" of such drug effects. That is, drugs which directly depress raphe firing should inhibit serotonergic transmission and release postsynaptic neurons in other brain areas from tonic inhibition imposed by the raphe. Alternatively, drugs that facilitate serotonergic transmission, although also inhibiting raphe, would have the opposite physiological consequences. If the present experiments are viewed in this light, one could postulate that both pargyline and tryptophan, when administered alone, exert their behavioral effects via direct inhibition of raphe and consequent decreased 5-HT input to postsynaptic receptors. That this may be the case at least for tryptophan is suggested by the findings that (1) iontophoretically applied tryptophan depresses raphe activity [6], and (2) the decrease in raphe firing observed after peripheral injections of tryptophan is not blocked by pretreatment with p-chlorophenylalanine [2]. Since PCPA is much more effective in inhibiting tryptophan hydroxylase in raphe terminals than it is in the perikarya [8], this suggests that tryptophan is exerting its effects independent of raphe terminals. However, pargyline administration followed by tryptophan loading may cause, in accord with the suggestion of Grahame-Smith [20], 5-HT to be synthesized far in excess of the capacity for intraneuronal storage and, with its catabolism effectively inhibited, this excess 5-HT then spills over onto postsynaptic receptor sites. If this is the case, it could ac-

count for the opposed effects on TI of individual versus combined administration of these compounds. Since pretreatment with PCPA in Experiment 4 completely blocked the combined effect of pargyline and tryptophan on TI, this argues that the effect is indeed mediated by an increase in 5-HT in raphe terminals. Such an interpretation is consistent with the finding that the increase in fluorescence intensity of raphe terminals, but not perikarya, induced by combined pargyline and tryptophan administration is blocked by PCPA pretreatment [8].

The interpretation presented above ties predictions of drug effects on immobility durations not to their effects on the electrical activity of raphe per se, but rather to their effects on postsynaptic serotonergic receptors. Drug treatments resulting in increased stimulation of postsynaptic receptors should be associated with decreased immobility durations independent of raphe firing, while treatments which decrease postsynaptic receptor stimulation should enhance TI durations. This view is consistent with the findings that both d-amphetamine [16] and peripherally administered 5-HT [35] result in an increase in raphe firing rate, which indicates increased stimulation of postsynaptic receptors, and a decrease in TI duration [13,33]. Also consistent with this view is the finding that peripheral administration of LSD, a compound that directly [7] and preferentially [26] inhibits raphe, resulting in disinhibition of neurons in areas receiving a dense 5-HT input [26], greatly enhances the duration of tonic immobility [33]. If this analysis is correct, it would argue for an asymmetry in the serotonergic-raphe model. That is, drugs which produce an increase in raphe firing should always result in decreased immobility durations. However, drugs that decrease raphe activity should only be associated with enhanced durations of TI when such decreases are not accompanied by stimulation of postsynaptic receptors. Although the present data are clearly insufficient to confirm these predictions, this revision of the serotonergic-raphe model represents a plausible account of the present findings and is in accord with recent evidence concerning autoregulatory modulation of serotonergic activity.

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