Tremorine-Induced Rage and Its Antagonism by Atropine.* (25783)

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Tremorine (1,4 - dipyrrolidino - 2 - butyne) was reported to produce a marked parasympathetic stimulation and a sustained tremor(1, 2). Extensive use has been made of its tremorgenic effects to evaluate compounds for antiparkinsonian activity(3,4). The tremorgenic(5), analgesic(6), hypothermic(7), and stimulatory properties(8) of Tremorine were compared with those of other centrally active agents which possess these actions. We were interested in supplementing this work, most of which had been done in rodents, with Tremorine studies in the cat. During the investigation we noted that Tremorine, in addition to its previously described actions, produced a rage response which was abolished by atropine. An attempt was made to relate this response to parallel changes in cortical and subcortical electrograms and to gain an understanding of the underlying central mechanisms.

Materials and methods. Over 40 cats (2.0-3.0 kg) were used to study changes in overt behavior and brain electrical activity produced by Tremorine hydrochloride.‡ The agents were administered parenterally at following doses: Tremorine (5-20 mg/kg), atropine sulfate (2 mg/kg), and atropine methylnitrate (2 mg/kg). *Overt effects.* Tremorine (10-20 mg/kg) was studied in conscious, unrestrained animals. Atropine sulfate or atropine methylnitrate was injected before Tremorine or at peak of rage response. Postmortem observations were made, and brain and other tissues were removed for histologic study. *Electrographic studies.* These experiments were performed under anesthesia with pentobarbital (35 mg/kg), Dial-urethane (0.7 ml/kg), or while the cat was immobilized with succinylcholine chloride. A small series of encéphale isolé preparations was also studied. Spontaneous electrical activity from several cortical and subcortical areas was recorded bipolarly on a Grass polygraph using silver ball and stainless steel concentric electrodes, respectively. Doses of Tremorine slightly smaller (5-10 mg/kg) than those used to observe behavior were employed. Atropine was given either before or after the Tremorine-induced cortical activations. Blood pressure was monitored from the femoral artery during some experiments. Measures also were taken to maintain body temperature (rectal) to offset hypothermia produced by Tremorine.

Results. Overt responses and antagonism by atropine. In cats which were conscious and unrestrained different phases could be recognized during action of Tremorine (10-20 mg/kg). No signs were evident during the first hour (latency). Then there was a period of intense parasympathetic stimulation (vomiting, defecation, salivation, etc.). At this time some of the cats developed fine tremors of head and limbs. As these effects subsided, the animals became agitated and easily excited. During the next several hours the rage response which included hissing, clawing, and biting mounted in intensity (Fig. 1). The animals reacted violently to various external stimuli at which they directed their attack. This agitation, which persisted for several hours, was fatal within 24 hours if unchecked. Atropine abolished the rage response and quieted the animal within a few minutes after injection (Fig. 2). When given before Tremorine, it inhibited both the rage and parasympathetic discharge. In contrast, methylatropine at the same dose antagonized only the peripheral parasympathetic effects. At smaller, sublethal doses (less than 5 mg/kg) Tremorine did not produce rage; at higher doses (greater than 20 mg/kg) it produced ataxia, and death shortly thereafter.

Pathology. At this time only a preliminary

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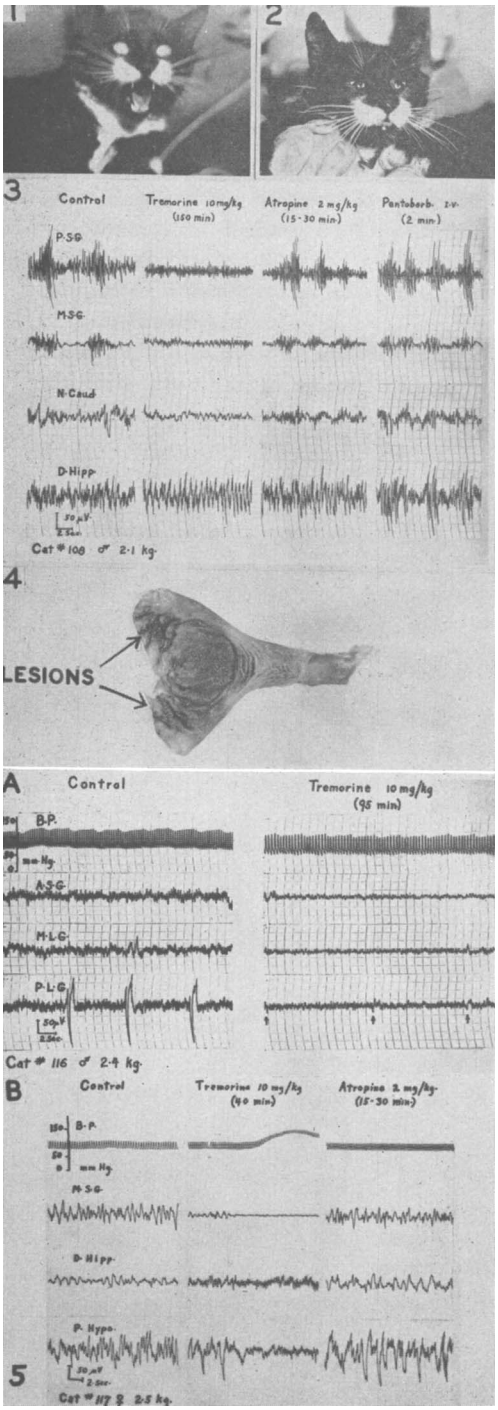

FIG. 1-4. Actions of Tremorine in cat and their antagonism by atropine. 1. Rage response. 2. Antagonism of rage by atropine. 3. Cortical and subcortical electrical changes in cat anesthetized with pentobarbital. Leads: P.S.G.—posterior suprasylvian gyrus; M.S.G.—middle suprasylvian gyrus; N. Caud.—caudate nucleus; D. Hipp.—dorsal hippocampus. 4. Gastric lesions. Time after administration of the agent indicated in parentheses.

FIG. 5. Electrographic changes produced by Tremorine in succinylcholine-immobilized cats. A. Diffuse cortical activation. B. Increased brain activity accompanied by phasic rise in blood pressure. Leads: B.P.—femoral artery blood pressure; A.S.G. — anterior suprasylvian gyrus; M.L.G. — middle lateral gyrus; P.L.G. — posterior lateral gyrus; M.S.G. — middle suprasylvian gyrus; D. Hipp. — dorsal hippocampus; P.Hypo. — posterior hypothalamus. Time after administration of agent indicated in parentheses.

report can be made of the pathologic effects produced by Tremorine. In enraged animals pertinent lesions were found in gastrointestinal tract, lungs, and brain. Gastric lesions were most consistent, but varied in severity from mucosal hyperemia to ulceration with hemorrhage (Fig. 4). Some of the animals evidenced hyperemia of rectal mucosa. The lungs, when involved, were congested, hemorrhagic, and often contained excessive bronchiolar secretions. Histologic sections of the brain revealed acute congestion with endothelial proliferation, edema, and various forms of neuronal degeneration in cerebrum, cerebellum, and brain stem. Necropsy of animals pretreated or treated at the height of rage with atropine did not reveal similar changes.

Cortical and subcortical electrograms. EEG changes produced by Tremorine were similar in anesthetized (Fig. 3), succinylcholine-immobilized (Fig. 5), and encéphale isolé animals and followed a comparable time sequence. The first EEG effects appeared shortly after onset of salivation (parasympathetic discharge), usually an hour after injection. Quite consistently, the records showed diffuse cortical activation which persisted during entire experimental period (Fig. 5). Onset of activation was particularly apparent when pentobarbital spindling was converted to low amplitude, high frequency activity (Fig. 3). In one of the posterior cortical leads (Fig. 5), a spontaneously occurring spike-like potential similarly disappeared during activation. Atropine consistently terminated EEG arousal and restored the control or spindling (Fig. 3) pattern within 15 minutes. Supplementary doses of pentobarbital further increased frequency of spindling.

None of the subcortical areas surveyed were as consistently involved as the cortex. Of the former, the posterior hypothalamic nuclei and dorsal hippocampus most often exhibited increased electrical activity (Fig. 5B). Caudate, anterior hypothalamic, and thalamic intralaminar nuclei seemed least affected. In animals anesthetized with pentobarbital Tremorine produced a steady drop in blood pressure which could be readily offset by small doses of methylatropine. Abrupt and phasic rises in blood pressure occurred in some of the succinylcholine preparations (Fig. 5B). These coincided with a further activation of cortex and increased activity in hippocampus and posterior hypothalamus.

Discussion. A central cholinergic mechanism appears to be involved in Tremorine-induced rage, since atropine, itself lacking a C.N.S. depressant action, rapidly abolishes the rage. This is further suggested by the atropine reversal of electrographic changes produced by Tremorine, thus paralleling the changes in behavior. The efficacy of atropine, in contrast to its quaternary derivative, methylatropine, is in agreement with the finding of P'an *et al.*(3) that tertiary congeners are much more effective against the central actions of Tremorine in mice.

Our results also support an interpretation which links the activated cortical EEG with the rage phase and identifies the reversal of this electrical pattern by atropine with subdued behavior. Kaelber and Correll(9) were prompted in their studies to relate the increased cortical activity directly to the tremorgenic actions of Tremorine.

The relationship between a central cholinergic action and the accompanying changes in EEG and behavior were variously interpreted. Evidence obtained by Bradley and Elkes(10) indicates that cortical activation *per se*, such as produced through the central cholinergic actions of physostigmine, need not be accompanied by any significant behavioral arousal. Nor is atropine reversal necessarily associated with sedation since atropine previously has been demonstrated to result in a pharmacologic dissociation of behavior and the EEG(11). It is significant in our investigation that such a dissociation with atropine is no longer evident after Tremorine since both EEG and behavior are in accord with sedation.

Perhaps more significance should be attached to the subcortical actions of Tremorine. Thus, the antagonism by atropine in our studies might be explained by its effectiveness in blocking an upward cortical activation resulting from hypothalamic stimulation(12). Some of the results suggest such a posterior hypothalamic involvement during rage. Also in accord with this was the increased electrical activity of the hypothalamus which preceded cortical activation and the phasic rises in blood pressure (autonomic manifestation of rage) in the succinylcholine-immobilized preparation. Ingram *et al.*(13) related changes in hypothalamic activity to EEG and behavior arousal. Prolonged stimulation of hypothalamic areas was also shown to produce gastric lesions(14) and might contribute to the lesions seen after Tremorine-induced rage.

The link between subcortical actions and central cholinergic mechanisms requires further study and is being investigated. Still unexplained are the latency, progressive nature, and long duration of Tremorine rage. These could result from a slow biochemical transformation of Tremorine to an active compound which produces rage and is then slowly metabolized.

Summary. In the cat Tremorine produced a directed, sustained rage and gastric lesions. Electrographic changes paralleling rage response included diffuse cortical activation and increased activity in posterior hypothalamus. The rage and electrical changes are antagonized by atropine and appear to involve a central cholinergic mechanism of subcortical origin.

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Effect of Zinc Deficiency in Hens on Hatchability and Embryonic Development.*† (25784)

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Following demonstration of zinc deficiency in chicks(1,2) and poults(3), an investigation of the effect of low zinc intake by the laying hen was undertaken in this laboratory. A preliminary study revealed that hens receiving a purified diet containing 6 ppm zinc laid eggs which hatched considerably poorer than those of hens fed a similar diet to which 96 ppm zinc had been added(4). Subsequently, Turk *et al.*(5) reported that hatchability was decreased when hens were fed a soy protein diet of low zinc content (8-9 ppm) and that the chicks which hatched were weak, feathered poorly and usually died within a few days.

Methods. Babcock strain White Leghorn pullets were housed in individual stainless steel cages and fed the basal diet described in Table I, estimated to contain 6 ppm zinc. Feed and distilled water were provided *ad libitum* in stainless steel containers. Fresh diet was prepared at 3-week intervals. Following a 6-week preliminary feeding period, 12 hens were fed the basal diet with 120 ppm zinc added as zinc chloride and 12 hens were continued on the unsupplemented basal diet. The hens were inseminated at weekly intervals with 0.1 cc of pooled semen from a group of flightless strain males which were maintained

TABLE I. Composition of Basal Ration.

	%		%
Isolated soybean protein*	21.7	Corn oil	4.6
		Antioxidant†	.02
Sucrose	61.63	Choline chloride	.15
DL-methionine	.6	Mineral mixture‡	10.45
Glycine	.5	Vitamin " §"	.35

* ADM assay protein (Archer Daniels-Midland Co.) washed 3 times with distilled water and dried at approximately 55°C.

† 1,2-dihydro-6-ethoxy, 2,2,4-trimethylquinoline (Santoquin).

‡ C.P. or reagent grade, supplied (% of diet): 1.53 $CaCO_3$, 6.80 $Ca_3(PO_4)_2$, 0.80 K_2HPO_4, 0.27 KCl, 0.30 $MgSO_4 \cdot 7H_2O$, 0.65 NaCl, 0.05 $FeSO_4 \cdot 7H_2O$, 0.002 $CuSO_4 \cdot 5H_2O$, 0.03 $MnSO_4 \cdot H_2O$, 0.0002 $CoSO_4 \cdot 7H_2O$, 0.001 KI, 0.01 $AlK(SO_4)_2 \cdot 12H_2O$, 0.001 $Na_2MoO_4 \cdot 2H_2O$, 0.005 $Na_2SiO_3 \cdot 9H_2O$, 0.001 H_3BO_3, 0.00007 Na_2SeO_3.

§ Supplied/100 g of diet: mg, 6.6 alpha-tocopheryl acetate, .003 cyanocobalamin, 1.0 menadione, 100 inositol, .5 para-aminobenzoic acid, .08 biotin, .2 folacin, 1.8 pyridoxine HCl, 10.0 niacin, 3.4 calcium pantothenate, 2.0 riboflavin, 2.0 thiamine HCl, 3.0 ascorbic acid; also 1170 I.U. vit. A and 150 I.C.U. vit. D_3.

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