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## Activation of Tremorine by Liver.*† (26737)

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Tremorine (1,4-dipyrollidino-2-butyne) was reported by Everett(1) to produce sustained tremors and parasympathetic-like stimulation in several species of laboratory animals. Since it elicits tremors and other parkinsonian-like signs, Tremorine has been used in routine screening for potential antiparkisonian drugs. The report(2) that an hour elapses before the cat shows typical drug effects after administration of Tremorine (10-20 mg/kg) suggested that Tremorine may have to be biotransformed before it becomes pharmacologically active. Evidence presented here indicates that the liver converts Tremorine to an active form which has pharmacologic properties differing from those of the original product.

*Materials and methods.* Young adult male Swiss-Webster mice, hamsters weighing 100-150 g, 2-3 kg cats and 5-10 kg dogs were used. Tremorine dihydrochloride‡ was dissolved in pH 7.4 Krebs-phosphate buffer (2 mg/ml) prior to incubation with liver. Homogenates and tissue slices totaling 2-4 g wet weight from the livers of mice, hamsters, or rats were incubated at 37°C for 2 hours in a shaking incubator with 12 ml of buffered Tremorine solution in 250 ml flasks ($O_2$ gas phase). After incubation filtrates of the solution were injected into animals in doses expressed as equivalents of Tremorine present before incubation.

Time of onset of tremors was measured from injection to time of head and limb tremors of a fully developed intensity as judged by a single observer throughout the study. Five or more mice per group were used in each experiment, except as noted.

*Results.* Tremorine incubated with mouse liver slices produced tremors within 5 sec after intravenous injection into mice, whereas non-incubated Tremorine required more than 15 min to produce effects (Table I). It

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TABLE I. Effect of Incubation of Tremorine with Liver Slices and Route of Injection on Time of Onset of Tremors in Mice.

| Conditions of incubation | SKF-525A* pretreatment, mg/kg | Tremorine inj. route† | No. of animals | Onset of tremors ± S.D. |
|---|---|---|---|---|
| Not incubated | 0 | Intraper. | 12 | 6.3 ± 2.0 min. |
| *Idem* | 0 | Intrav. | 15 | 16.3 ± 3.0 " |
| " | 0 | Subcut. | 6 | 20.2 ± 4.1 " |
| " | 25 | Intrav. | 6 | None in 5 hr |
| Incubated without drug | 0 | " | 6 | No effects |
| Incubated with drug | 25 | " | 25 | Within 5 sec. |
| *Idem* | 0 | " | 6 | *Idem* |

* Injected intraper. 1 hr before Tremorine.
† Injected dose equivalent to 20 mg/kg Tremorine dihydrochloride.

was further noted that intraperitoneal administration of non-incubated Tremorine produced tremors sooner than intravenous or subcutaneous administration (Table I), suggesting that the liver activates the drug more rapidly after intraperitoneal injection. More rapid and shorter-lasting effects (1½ hr duration) were produced by 5 mg/kg of the activated product than by 20 mg/kg of non-incubated Tremorine (3-4 hr duration), although the 2 products evoked similar effects of approximately equal intensity. In 3 cats activated Tremorine produced vomiting, salivation, agitation, and hissing beginning 5 min following intraperitoneal injection of a dose equivalent to 8 mg/kg. Non-activated Tremorine preparations did not begin to produce these effects for 15-30 min following doses of 5 and 10 mg/kg, as seen in 4 cats.

SKF-525A (diethylaminoethyldiphenylpropylacetate), an inhibitor of liver microsomal activity(3), given to mice in a dose of 25 mg/kg 30 min prior to Tremorine (20 mg/kg) completely prevented the onset of tremors. Unlike atropine(4) SKF-525A did not abolish tremors already established. Liver-activated Tremorine, however, produced tremors within 5 sec following intravenous administration, whether or not the mice were pretreated with SKF-525A (Table I). Control solutions in which liver was incubated without Tremorine produced no effects in mice.

Two other reported inhibitors of liver microsomal activity(5) were also tested in doses of 25 mg/kg, as prophylactic agents against Tremorine in mice. Lilly 18947 (2,6-dichloro-6-phenylphenoxyethyldiethylamine hydrobromide) prevented the onset of tremors, but iproniazid did not. Four antihistamines (diphenhydramine, tripelennamine, pyrilamine, doxylamine) structurally related to SKF-525A did not prevent tremor development in mice when given at 25 mg/kg one hour prior to 20 mg/kg of Tremorine.

Although SKF-525A in doses of 25 mg/kg or higher, did not completely block the effects of large doses of Tremorine (80 mg/kg) in mice, the tremors and other signs were delayed in onset. SKF-525A also delayed the onset of tremors in rats given 10 mg/kg Tremorine.

The effect of Tremorine and its activated product on blood pressure was studied in 5 dogs anesthetized with pentobarbital. Intravenous injection of 100 μg/kg of the activated product produced bradycardia and an immediate fall in blood pressure of approximately 65 mm Hg, which was restored to control level within 65 sec after injection. Doses of the activated product as small as 1 μg/kg produced a significant fall in blood pressure of 8-10 mm Hg. Atropine (0.5 mg/kg) abolished these responses. Non-incubated Tremorine in doses up to 1 mg/kg had no observable effect on blood pressure and at 5 mg/kg the immediate effect varied from none to a slight fall of 5-10 mm Hg, although a gradual decline of approximately 50 mm Hg occurred over a period of about 1½ hours. This depressed blood pressure returned to normal after atropine (0.5 mg/kg).

Diarrhea is recognized as a common sign of the widespread cholinergic effects produced by Tremorine. Tremorine, however, in a concentration of $3.5 \times 10^{-5}$M had no

effect on the activity, as recorded kymographically, of strips of rabbit intestine suspended in Tyrode's solution, and at a level of $7 \times 10^{-5}$M even slightly depressed it. By contrast, activated Tremorine at a concentration equivalent to $3.5 \times 10^{-5}$M produced a marked stimulation of the rabbit intestinal strip, an effect antagonized by atropine (1:10,000,000). Interestingly, the activity of intestinal strips stimulated by activated Tremorine ($3.5 \times 10^{-5}$M) or acetylcholine (1:10,000,000) returned immediately to a normal pendular movement when non-incubated Tremorine ($1.4 \times 10^{-4}$M) was added.

Rat and hamster liver homogenates and hamster liver slices incubated with Tremorine also activated Tremorine, since filtrates from these preparations consistently produced typical symptoms within 5 sec in 20 mice injected intravenously with doses equivalent to 20 mg/kg of Tremorine. Urine was expressed from the bladders of 30 mice for 3 hr starting 15 min after they received 20 mg/kg of Tremorine. This urine, diluted with an equal volume of saline and injected intravenously into 8 mice in a dose of 0.1 ml, produced immediate signs of Tremorine poisoning of an intensity similar to that seen in the mice from which the urine was collected, but of shorter duration. Normal urine, similarly tested, had no effect.

The incubates of Tremorine with liver slices from hamsters, rats and mice, as well as urine from Tremorine-treated rats and mice were extracted with chloroform after adjusting to pH 11. The oily residue remaining after evaporating the combined extracts to dryness was dissolved in 0.01 N HCl, and tested for activity in normal and SKF-525A-treated (25 mg/kg) mice, on the blood pressure of 4 dogs anesthetized with pentobarbital and on the isolated rabbit intestine. It was found that 3 mg/kg of any of these residues when injected into mice intravenously immediately produced severe symptoms characteristic of Tremorine. Lower doses produced effects of variable duration and intensity, the residues from urine appearing to be more potent than those from liver slices.

The extracts from the various liver preparations also produced an immediate transient fall in the blood pressure of anesthetized dogs, ranging from 45 to 70 mm Hg when 100 $\mu$g/kg of any residue was injected intravenously, the response apparently varying with the efficiency of activation in different incubates. Extracts from the urine of Tremorine-treated rats and mice were more potent in depressing blood pressure, a fall of approximately 65 mm Hg resulting from 50 $\mu$g/kg.

Extracts of urine from Tremorine-treated rats and mice as well as Tremorine liver slice incubates from hamsters, rats and mice all produced a sustained tonic contraction of the isolated rabbit intestine when added to the bath to give a final concentration of 1 $\mu$g/ml in case of urine extract and a concentration equivalent to $3.5 \times 10^{-5}$M Tremorine in case of the liver incubates. Control liver incubates and extract residues produced no effect in mice, on blood pressure of dogs, or on the isolated intestine of rabbits.

*Discussion.* The above data indicate that the liver converts administered Tremorine to an active form having pharmacologic properties that sharply distinguish it from its original form. The effects of activated Tremorine in stimulating rabbit gut *in vitro* and in momentarily depressing blood pressure of the anesthetized dog are consistent with the diarrhea, bradycardia, prolonged depression of blood pressure and other cholinergic effects seen in the fully developed picture of Tremorine's action. Before it is activated, however, Tremorine does not stimulate the isolated rabbit gut, and it lowers the dog blood pressure only after an appreciable latent period. The rapidity with which activated Tremorine acts suggests that the characteristic delay in onset of action of Tremorine in most animals is the time required for the liver to activate it. Since Tremorine is presumably activated continuously as long as it persists in the body, it would be expected that the effects of administered Tremorine should last longer than those produced by single acute doses of the active form. Thus, the prolonged depression of blood pressure gradually induced by Tremorine over a period of 90 min is markedly dif-

ferent from the sharp evanescent fall in blood pressure produced by intravenous injection of activated Tremorine.

The fact that SKF-525A, an inhibitor of liver microsomal activity, blocks Tremorine but does not block activated Tremorine serves as a basis for determining the presence of the active form. It points, furthermore, to the liver microsomes as the site of the activation process.

Although the pharmacologic properties of activated Tremorine from different sources (urine of Tremorine-treated mice and rats, and incubates of Tremorine with preparations of liver from different species) are similar, it is clear that their chemical identity has not been established. Studies to determine the chemical nature of activated Tremorine are in progress.

*Summary.* Mouse liver slices converted Tremorine to an active form which differed from non-incubated Tremorine preparations (a) by its faster onset of action in untreated mice and cats, (b) by not being blocked by SKF-525A (diethylaminodiphenylpropylacetate), an inhibitor of liver microsomal activity, (c) by stimulating rabbit gut *in vitro* and (d) by producing an immediate slowing of the heart and fall in blood pressure on intravenous injection into anesthetized dogs. Material having the biological properties of activated Tremorine was also found in the urine of rats and mice given Tremorine and in solutions of Tremorine incubated with hamster liver slices and with hamster and rat liver homogenates.

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## Tissue and Serum Manganese Levels in Evaluation of Heart Muscle Damage. A Comparison with SGOT. (26738)

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The normal myocardium contains about 1800 μg of manganese per 100 g of dried tissue; this is roughly 2,000 times the manganese concentration present in the blood serum. Myocardial injury results in a sharp decrease in tissue manganese associated with a proportional rise in serum manganese content. Following myocardial infarction tissue manganese *decreases* to approximately 30% of its original value. Concomitantly, serum manganese *increases* approximately 2½ times. Thus, after infarction human serum contains more than 2.0 μg manganese per 100 ml, whereas prior to infarction, serum manganese content is about 1.0 μg manganese per 100 ml.

Because tissue manganese is lost very rapidly from the infarcted myocardium following cardiac injury, and a reciprocal rise in manganese concentration is observed in the serum, determination of serum manganese level should be of great value in detection of myocardial injury and, in equivocal cases, in confirmation of acute myocardial damage.

In a study at Los Angeles County Hospital, serial measures of serum manganese levels in 26 patients with unequivocal evidence of myocardial infarction were compared with serum samples from 25 patients of comparable age, sex and race admitted to the hospital with complaints other than myocardial injury. This report correlates clinical findings in our patients with serum manganese levels, as determined spectrographically from 80 blood samples taken from the patients with acute myocardial infarction, and