Effects of Glutamate on the Potentiating Action of Certain Ataraxics. (23651)

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The potentiating activity of reserpine and of the phenothiazine group of ataraxics with barbiturates is known. These drugs probably act by different mechanisms and at different sites. The role of glutamate in cerebral metabolism, as well as its clinical usefulness, is controversial(1). Himwich has suggested that the discrepancy in the literature may be due partly to the use of the different forms of glutamate (glutamic acid, glutamic acid hydrochloride, and monosodium glutamate) and he believes that the monosodium salt is more readily absorbed(2).

Various combinations of hexobarbital, reserpine, proclorperazine, and monosodium glutamate were administered to mice in order to study possible antagonistic, additive or potentiating activity. Monosodium glutamate moderately increased the depressant action of hexobarbital and very markedly enhanced the potentiation of reserpine on hexobarbital. This effect of monosodium glutamate was absent in proclorperazine treated animals.

Materials and methods. Albino mice (Harlan strain) weighing 20-30 g were used only once. "Sleeping time" in the mice is reported as that time interval from the loss of, to the return of, the righting reflex following intraperitoneal injection of 100 mg/kg hexobarbital sodium (Evipal). Reserpine, proclorperazine and hexobarbital were used as commercial ampul-forms and monosodium glutamate (MSG) as the salt. Each was diluted with distilled water and all drugs were injected intraperitoneally. Monosodium glutamate was administered one hour before the hexobarbital. Reserpine or proclorperazine was given one-half hour before the barbiturate. A 2x3x4 factorial design was used. The 3 factors involved were: (a) sexual differences, 2 levels, ♂ and ♀; (b) ataraxics, 3 levels, 0, 5 mg/kg reserpine, 5 mg/kg proclorperazine; (c) monosodium glutamate, 4 levels, 0, 1 g/kg, 2 g/kg and 3 g/kg. Twenty mice were used per block; a total of 480 mice.

Results. Table I represents the results of the analysis of variance. All 3 factors (sex, ataraxics and glutamate) proved to be significant. A summary of the obtained results showing the P values of the t test is included in Table II. Administration of glutamate did not alter the effect of the barbiturate in females, but in males increased their sleeping time in higher doses (3 g/kg). Animals receiving reserpine only or glutamate and reserpine were depressed but did not lose their "righting reflex" and therefore are not included in the table. Reserpine increased the hypnotic effect of hexobarbital as has been reported by Brodie(3) and others, but pretreatment with glutamate *and* reserpine effected a sleeping time that is longer than seen in animals receiving reserpine only before hexobarbital. This is considered to be an increase in the potentiating or synergistic activity of reserpine since monosodium glutamate alone did not appreciably increase the activity of hexobarbital below doses of 3 g/kg. In female mice this increased effectiveness of reserpine was obtained only with larger doses of MSG. There was no prolongation of reserpine-barbiturate hypnosis until an amount of 3 g/kg of MSG was administered.

To determine if this unusual potentiating action of glutamate might be peculiar to reserpine or a non-specific type of synergism, proclorperazine (Compazine), which differs markedly from reserpine in chemical configuration and pharmacological action, was administered in like manner. When proclorperazine was substituted for reserpine there was no potentiation by glutamate.

TABLE I. F Ratios.

Source	F ratio value	n_1	n_2	P
Sex difference	155	1	473	<.001
Tranquilizers	302	2	"	"
M. S. G.	21.7	3	"	"

TABLE II. Effects of Glutamate-Reserpine Combinations on Duration of Hexobarbital-Hypnosis in Mice. 20 mice/series, 100 mg/kg of hexobarbital I.P.

Sex	MSG, g/kg 1 hr before hexob.	Reserpine, mg/kg ½ hr before hexob.	Compazine, mg/kg ½ hr before hexob.	Sleeping time, $\bar{X}$	P values of t tests: vs opposite sex	vs Ataraxic: Reserpine	vs Ataraxic: Compazine	vs Monosodium glutamate: 1 g/kg	vs Monosodium glutamate: 2 g/kg	vs Monosodium glutamate: 3 g/kg
♂	0	0	0	17.0	<.01	<.001	<.001	n.s.	n.s.	<.01
♂	1	0	0	17.7	<.05	"	"		"	n.s.
♂	2	0	0	20.7	<.1	"	"	"		"
♂	3	0	0	24.3	<.001	"	"	<.01	"	
♂	0	5	0	32.3	n.s.		"	"	<.001	<.001
♂	1	5	0	40.2	<.01		n.s.		"	"
♂	2	5	0	57.2	<.001		<.001	<.001		<.05
♂	3	5	0	51.0	<.1		"	"	<.05	
♂	0	0	5	36.2	<.001	n.s.		n.s.	<.02	n.s.
♂	1	0	5	38.2	"	"			<.01	"
♂	2	0	5	29.5	<.1	<.001		<.01		<.001
♂	3	0	5	40.0	<.001	"		n.s.	<.001	
♀	0	0	0	9.2	<.01	"	"	"	<.02	n.s.
♀	1	0	0	12.2	<.05	"	"		n.s.	"
♀	2	0	0	15.8	<.1	"	<.01	"		"
♀	3	0	0	13.7	<.001	"	<.001	"	"	
♀	0	5	0	31.9	n.s.		"	"	"	<.001
♀	1	5	0	32.4	<.01		<.01		"	"
♀	2	5	0	35.3	<.001		<.001	"		"
♀	3	5	0	45.7	<.1		"	<.001	<.001	
♀	0	0	5	22.2	<.001	"		n.s.	n.s.	<.1
♀	1	0	5	24.1	"	<.01			"	n.s.
♀	2	0	5	24.3	<.1	<.001		"		"
♀	3	0	5	27.1	<.001	"		"	"	

It is interesting that there was a predominant sex-difference in response throughout our experiments. This occurred with hexobarbital alone and in animals receiving hexobarbital but pretreated with reserpine and glutamate (1 and 2 g/kg). There was no significant difference in the sexes as to response to hexobarbital when pretreated with reserpine only. Such a triple combination as we have used may have an additive activity which is not measurable as sleeping time. The general trend of results certainly indicated that there is a response to the barbiturate alone and also to the barbiturate-glutamate-reserpine combination which is dependent upon the sex of the experimental animal. Holck reports that the duration of barbiturate-induced hypnosis is different sex-wise in rats but not in mice(4). Our results indicate that studies with mice in one sex only or in unseparated sexes may result in a diversity of findings.

This relationship of sex to the potentiating action of monosodium glutamate suggested the possibility that the toxicity might be greater in one sex. Fig. 1 is a graphic illustration of the toxicity of monosodium glutamate in mice (Winthrop Logarithmic-Probit paper). The LD_{50} in females was 5.4 g/kg and in male mice was 6.5 g/kg. The graph emphasizes that the overall response (mortality-rate) is lower in the males at every dosage level. This is of special interest because female mice were less sensitive to the central potentiating action than were the males.

Arnold(5,6) has reported that prior administration of glutamic acid to patients delayed the hallucinatory effects of LSD (Lysergic acid diethylamide) for several hours. LSD has been shown to have an *in vitro* antiserotonin action(7). This might suggest that glutamate may have a contributory antiserotonin action as an explanation for its hyperpotentiating activity. The release of 5-hydroxytryptamine (Serotonin) is an important factor in the mechanism of activity of reserpine but this neurohormone apparently is not

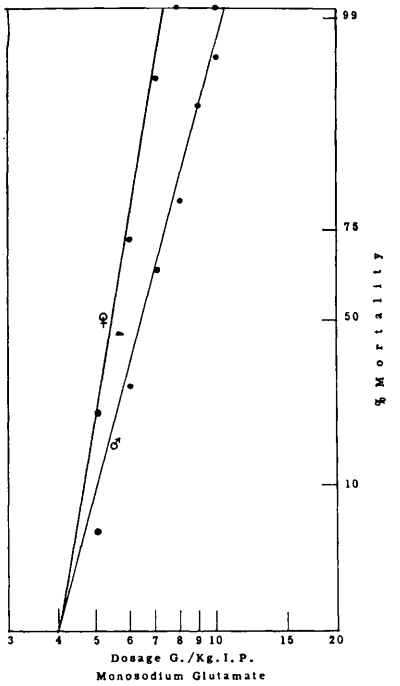

FIG. 1. Sex difference in toxicity of monosodium glutamate in mice.

implicated in the action of the phenothiazine-type of tranquilizer(8). Furthermore Lamson *et al.* has demonstrated that the administration of glutamate will reanesthetize an animal that has awakened from barbiturate hypnosis(9).

Summary. Glutamate moderately increased the mean sleeping time in mice that received hexobarbital. Reserpine markedly potentiated the action of the barbiturate. Administration of glutamate prior to reserpine-hexobarbital combinations caused a further and pronounced increase in the sleeping time. This "potentiation of the potentiation" by MSG was not observed when proclorperazine is substituted for the reserpine although proclorperazine also increased the duration of hexobarbital-hypnosis. This suggests that the specificity of this effect of monosodium glutamate is probably dependent upon the type of tranquilizer. Regardless of which of the 2 ataraxics was used there was an overall sex difference in response to the combination of these drugs, male mice being more sensitive to the depressant drugs.

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Received September 16, 1957. P.S.E.B.M., 1958, v97.

Teratogenic Effect of Trypan Blue on the Developing Chick.* (23652)

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Gillman, Gilbert and Gillman(1) first reported the teratogenic action of trypan blue after injections into pregnant rats. Several workers have since employed this method to produce a variety of anomalies in the offspring of mice(2,3,4,5), rabbits(6,7), as well as rats (8,9,10). Recently Waddington and Perry (11) have reported a teratogenic action of trypan blue on amphibian embryos. The malformations reported have primarily involved the neural axis, skeleton, and heart and major vessels. Despite these relatively extensive studies, little is known of the underlying mechanism or even the site of action of the dye. It has been postulated that one or more

* Supported by research Grant A-1090C from Natl. Inst. of Arthritis and Metabolic Diseases N.I.H., P.H.S.