

ANTIANAPHYLACTIC AND ANTISEROTONIN ACTIVITY OF A COMPOUND OBTAINED FROM EGG YOLK, PEANUT OIL, AND SOYBEAN LECITHIN

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INTRODUCTION

SEVERAL investigators have indicated that extracts of egg yolk, peanut oil, and soybean lecithin have anti-inflammatory activity.¹⁻³ Purification of these anti-inflammatory extracts was achieved, and in each case the active agent was identified as N(2-hydroxyethyl) palmitamide.⁴ This compound was assayed in the guinea pig by measuring reduction of swelling induced in joints by an antigen-antibody reaction. Swelling was reduced about 60 per cent at low concentrations (3 γ per kilogram), and tuberculin reaction in the guinea pig was inhibited.^{3, 5} On the other hand, no activity was found by several other types of assays employed to measure anti-inflammatory activity.³ The findings in the guinea pig prompted us to investigate the effect of ethanolamine palmitamide and related compounds on systemic anaphylaxis and on serotonin and histamine toxicity in the mouse.

MATERIALS AND METHODS

Female mice (Swiss Webster mice from Tumblebrook Farms, Bryant Lake, New York), each weighing from 14 to 16 grams, were used. The mice were fed Purina Laboratory Pellets and had free access to water. All drugs used in this study were diluted in a phosphate buffer, pH 7.2.

Passive Anaphylaxis.—Mice were injected intraperitoneally with 2 billion killed smooth *Bordetella pertussis* cells suspended in 0.25 ml. saline. Four days later they received intraperitoneal injections of 0.15 to 0.2 ml. of a rabbit anti-egg albumin serum (250 γ antibody nitrogen). A half-hour later the drug to be tested was injected intraperitoneally, and one hour thereafter the mice were challenged intravenously with 0.5 mg. of commercial crystalline egg albumin in 0.2 ml. of saline. The number of survivors was recorded after sixteen to twenty hours.

Histamine Sensitivity.—Mice were sensitized to histamine by intraperitoneal injection of 2 billion *Bordetella pertussis* cells as described by Munoz.⁶ Four days later they were challenged by intraperitoneal injection of 0.5 mg. of histamine base. All drugs tested were given one hour before challenge. The number of surviving animals was recorded twenty hours later.

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Serotonin Sensitivity.—Using the procedures described by Munoz,⁷ we sensitized mice with *B. pertussis* and four days later challenged them intravenously with 10 mg. per kilogram of serotonin base. All treatments were given intraperitoneally one hour before challenge. The number of survivors was recorded after twenty hours.

RESULTS

Anaphylactic Shock.—The data presented in Table I indicate that ethanolamine palmitamide protects mice against fatal anaphylactic shock when given in a dose of 0.5 to 5 mg. per kilogram. Both ethanolamine hydrochloride and

TABLE I. EFFECT OF ETHANOLAMINE PALMITAMIDE, ETHANOLAMINE HCl AND FATTY ACIDS ON PASSIVE ANAPHYLAXIS IN THE MOUSE

DRUG	DOSE (MG./KG.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
N(2-hydroxyethyl) palmitamide	50	21/30	70
	25	5/8	62
	5	18/30	57
	0.5	19/30	63*
	0.05	10/20	50
	0.005	6/20	30
Ethanolamine HCl	50	18/29	62
	25	9/20	45
	5	20/29	69*
	0.5	13/30	43
	0.05	10/20	50
	0.005	7/20	35
Palmitic acid	50	19/30	63*
	5	14/30	47
	0.5	4/15	27
	0.05	4/15	27
	0.005	3/10	30
Hydrocortisone	100	19/25	76
Control	—	15/60	25

*The lowest active dose $P = <0.001$.

palmitic acid were also found to be effective in preventing death in this kind of experiment. When activity was obtained with any of the foregoing preparations, higher dosage did not increase the number of surviving animals. This appears, therefore, to be an "all-or-none" type of reaction. These experiments were repeated several times, and similar results were obtained. However, as the degree of sensitization increased and mortality of controls reached close to 100 per cent, higher doses were needed and fewer animals were saved by this drug. In this respect, it behaved similarly to hydrocortisone.

Antiserotonin Activity.—It has been known for some time that both histamine and serotonin are involved in anaphylaxis in the mouse. We felt, therefore, that it would be of interest to determine the effect of N(2-hydroxyethyl) palmitamide on serotonin and/or histamine sensitivity in the mouse. The data presented in Table II indicate that N(2-hydroxyethyl) palmitamide, 0.5 mg per kilogram, is highly effective in preventing death from serotonin

toxicity in the mouse. In order to protect the mice with ethanolamine hydrochloride, 500 times this amount was needed (250 mg. per kilograms). On the other hand, the palmitic acid moiety was found to be active at 0.5 mg. per kilogram, and the question of whether this was a specific property for palmitic

TABLE II. EFFECT OF N(2-HYDROXYETHYL) PALMITAMIDE AND ITS COMPONENTS ON SEROTONIN TOXICITY IN THE MOUSE

DRUG	DOSE (MG./KG.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
N(2-hydroxyethyl) palmitamide	50	72/85	84
	5	41/70	58
	0.5	15/35	42*†
	0.05	6/35	17
	0.005	1/14	7
	0.0005	2/10	20
Palmitic acid	5	16/25	64
	0.5	8/15	53*†
	0.05	5/10	50
	0.005	3/10	30
Ethanolamine HCl	250	16/30	53*§
	150	5/15	33
	100	4/10	40
	50	7/25	28
	5	3/15	20
Control	—	17/75	22

*The lowest active dose.

†P = 0.05.

‡P = <0.05.

§P = <0.01.

acid or a nonspecific one shared by other fatty acids and completely unrelated compounds was raised. Data presented in Table III indicate that a great variety of fatty acids are capable of protecting mice against serotonin toxicity. To test the specificity of this inhibitory reaction, we assayed a variety of other drugs for activity. At equivalent dose levels, ethanolamine hydrochloride and sarcosine were active whereas 1-amino-2-propanol was inactive. Similarly, urethane and pentobarbital sodium at 5 mg. per kilogram were inactive.

TABLE III. EFFECT OF FATTY ACIDS ON SEROTONIN TOXICITY IN THE MOUSE

COMPOUND	DOSE (MG./KG.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
Control	—	20/100	20
Palmitic acid	5	41/70	58
Lauric acid	5	35/55	63
Caproic acid	5	16/30	53
Butyric acid	5	20/30	67
Na acetate	5	10/30	33*
Oleic acid	5	27/40	68
Linoleic acid	5	27/50	54
Ethyl linolenate	5	24/40	60

*Not active, all others P = <0.001.

Antihistamine Effect.—The compounds that proved effective against serotonin toxicity were also tested for protective effect against histamine sensitivity and were found to be inactive at either 5 or 50 mg. per kilogram per dose.

DISCUSSION

All previous work with crude extracts of egg yolk, soybean lecithin, and peanut oil, and later with ethanolamine palmitamide, was performed in the guinea pig.¹⁻³ In this species, the foregoing materials were highly active in reducing swelling induced by antigen-antibody reaction and also in reducing tuberculin reaction. Since the guinea pig's physiologic behavior is frequently different from that of other species of animals, and since the methods of testing activity in the guinea pig were somewhat complicated, a search was made in other species for other biologic activities of this compound. Results presented here indicate that these compounds also are highly active in preventing passive anaphylaxis in the mouse. The number of survivors seen after treatment with 0.5 mg. per kilogram of palmitamide was about equal to that seen with 100 mg. per kilogram of hydrocortisone.

Furthermore, it has been shown that N(2-hydroxyethyl) palmitamide and variety of fatty acids can inhibit serotonin toxicity in the mouse without affecting histamine toxicity. This activity was found not only in fatty acids of high molecular weight but also in butyric acid with only four carbons. Similarly, the degree of saturation of these fatty acids did not seem to be important, since oleic acid, linoleic acid, and ethyl linolenate were equally active. It would be hard to believe that a fatty acid would act as a specific anti-serotonin compound, and it must therefore be assumed that its pharmacologic action is nonspecific.

Preliminary experiments in cats have also shown that palmitic acid given intraperitoneally in doses of 25 mg. per kilogram inhibits blood pressure changes induced by 3 to 5 γ per kilogram of serotonin while the responses to either histamine or epinephrine are not affected. Serotonin's mode of action in the cat is not very well understood at present, but it is believed to be due to a coronary chemoreflex and an inhibition of neurogenic vasoconstriction resulting in a depressor effect.⁸ As the mechanism of action of serotonin is not known, one can only speculate on the mode of action of the inhibitory effect of palmitic acid and hope that future studies will throw some light on this problem.

In recent years, the injection of *B. pertussis* vaccine into mice has been shown to make the mouse more sensitive to the action of histamine, serotonin, anaphylaxis, cold stress, and a variety of other forms of stress.⁹ These results suggest that some basic physiologic change is induced by *B. pertussis*, rather than a change which sensitizes the animal to particular noxious agents, such as histamine. Since serotonin but not histamine toxicity was affected, it must be assumed that these fundamental physiologic alterations brought about by *B. pertussis* in the mouse are not changed by either ethanolamine palmitamide or the fatty acids. At slightly higher dosage of the drug, adrenalectomized mice can also be protected against serotonin; this indicates that its action is independent of the adrenals. To substantiate this fact, it has been shown that this compound does not influence adrenal weight, corticosterone content of

adrenals, or thymus weight.¹⁰ The action of this compound cannot be attributed to anticomplementary activity or to changes in the clotting mechanism, as prothrombin time was unaffected at physiologic concentrations.¹¹

Little if any attention has been paid to the physiologic and pharmacologic action of fatty acids. The finding, therefore, that fatty acids could act as potent antiserotonin and antianaphylactic agents in the mouse was unexpected and opens a new area for study. No data are available on the amount of free fatty acids in the blood. It would be hard to conceive that a small amount of a particular fatty acid, given either orally or intraperitoneally in these experiments, is far in excess of that present in the normal diet. Woolley¹² believes serotonin acts by transporting calcium ions across a lipidlike cell membrane, suggesting that a fatty acid, possibly preferentially adsorbed or solubilized, could block this action.

SUMMARY

The effect of N(2-hydroxyethyl) palmitamide, ethanolamine hydrochloride, and a variety of fatty acids on passive anaphylaxis, serotonin, and histamine toxicity in the mouse was studied. The above compounds were found to be active in suppressing anaphylaxis and serotonin toxicity at low concentrations (about 5 mg. per kilogram), but they were shown to have no effect on histamine toxicity. Data presented indicate that fatty acids can act as potent pharmacologic agents.

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