Biochemical Pharmacology, 1963, Vol. 12, pp. 1201–1205. Pergamon Press Ltd., Printed in Great Britain.

A COMPARATIVE STUDY OF THE MODES OF ACTION OF FLUOROACETAMIDE AND FLUOROACETATE IN THE MOUSE AND AMERICAN COCKROACH*

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(*Received* 8 *January* 1963; *accepted* 3 *May* 1963)

Abstract—In the American cockroach and mouse, both fluoroacetate and fluoroacetamide poisoning led to large citrate concentrations in the body. In the cockroach poisoned with fluoroacetamide, fluoroacetate and fluorocitrate were found in muscle. It thus seems probable that the first step in fluoroacetamide poisoning is hydrolysis to fluoroacetate. This hydrolysis was more rapid in cockroach homogenates than in mouse homogenates, which may account for the selective toxicity of fluoroacetamide to the cockroach.

SODIUM FLUOROACETATE is one of the few poisons whose biochemical mode of action in vertebrates is precisely known. It is clear that the main lesion is inhibition of aconitase caused by the fluorocitrate which is a metabolite of the fluoroacetate; the aconitase inhibition leads to characteristic large citrate accumulations in certain tissues.[1] Sodium fluoroacetate is known to have good insecticidal action against mustard beetles,[2] aphids,[3] and cabbage-white caterpillars,[4] but because of its hazard it has not found commercial use as an insecticide. In 1958, David[5] reported that fluoroacetamide had aphicidal activity and was less hazardous than fluoroacetate. Fluoroacetamide is now used as a commercial insecticide in Japan.

We are interested in the biochemical basis of selective toxicity. The purposes of the present paper are to obtain data for insect and mammalian toxicity which would permit a proper evaluation of the selectivity of fluoroacetate and fluoroacetamide; to establish whether fluoroacetate kills insects by the same action that kills mammals; to determine whether fluoroacetamide and fluoroacetate have the same toxic mechanism; and to determine whether fluoroacetamide is hydrolyzed to fluoroacetate *in vivo*, and whether variation in the rate of this hydrolysis in different animals determines selectivity.

METHODS

Toxicity

For the American cockroach, each replicate consisted of five male and five female adults. Injection of water solutions, volume 5 μl/g body weight, was made through the fifth abdominal sternum. For the housefly, each replicate had twenty adult female houseflies, 3 days old. The strain was the Wilson susceptible; injection was made into

*This work was supported in part by Grants RG 7804 and E 2320 of the National Institutes of Health, U.S. Public Health Service.

the thoracic muscle. For mice, white females of about 20 g were used from Rolfsmeyer Farms, Madison, Wis. Injection was intraperitoneal in 0·9% sodium chloride. In each case a minimum of two replicates per dose level was used. All observations were made 24 hr after treatment.

Amidase activity

The method used was essentially that of Van Slyke and Archibald,[6] who measured urease activity, as further elaborated by Sobel *et al.*[7] for microanalysis. To a test tube was added 1 ml homogenate containing 250 mg of tissue in 0·067 M phosphate buffer, pH 7·4, and 1 ml substrate (prepared by dissolving 500 mg of sample in 15·8 ml 0·067 M phosphate buffer, pH 7·4). The system was allowed to stand for exactly 30 min at room temperature (24°). At the end of this incubation period 10 ml saturated K_2CO_3 was added. The tube was immediately closed by a rubber stopper, and air which was cleaned by 3·5 N NaOH and 2·2% H_3BO_3 was passed through. The NH_3 was collected in another test tube containing 1·5 ml indicator–H_3BO_3 mixture, and the final solution was titrated back to the original color by means of a standard acid solution; 20 min bubbling was enough to collect all NH_3 gas formed. A calibration curve was made with ammonium sulfate.

Conversion of fluoroacetamide to fluoroacetic acid in vivo

The muscle from 40 American cockroaches poisoned 24 hr earlier with 1 mg each of fluoroacetamide was collected, and homogenized with a large amount of distilled water. The debris was removed by brief centrifugation at 5000 rev/min for 15 to 20 min and the supernatant fluid poured through an IRA-400 Amberlite column (CO_3 type) at the rate of 1 to 1·5 ml/min. From this column the organic acids were eluted as sodium salts by 10% $NaHCO_3$. To remove the sodium and other ions the eluant was passed through an Amberlite IR-20 (H^+ type) column. The resulting solution, containing mainly carboxylic acids, was concentrated and chromatographed on paper according to Buffa *et al.*[8]

Assay of chromatographic products

Disks from the paper chromatograms were cut off, and the acids were extracted with 1·9% KCl. The toxicity of these extracts were assessed by the Thunberg-tube method as follows: 500 mg cockroach muscle (from unpoisoned roaches) was homogenized with 5 ml 0·067 M phosphate buffer (pH 7·4). The debris was removed through double cheesecloth, and 1 ml of this brei and 0·7 ml of the phosphate buffer were added to the main chamber of a Thunberg tube. To the side arm were added 0·5 ml 0·5 M succinic acid and 0·5 ml methylene blue, which was adjusted to a convenient concentration to judge its color, and 0·3 ml of the KCl extract. The time needed for complete decoloration by reduction of methylene blue was compared with that of a control.

Accumulation of citric acid in the cockroach and white mouse

The animals were injected with fluoroacetic acid or fluoroacetamide at a dose of 30 μg/g as in the toxicity work. At the end of the indicated period the animal was killed, and homogenized with 5 ml 5% trichloroacetic acid/g in a Waring blender for mice or a Servall Omni-mixer for roaches. The homogenate obtained was centrifuged at 8500 rev/min for 10 min to remove the debris. Five milliliters of this supernatant was analyzed for citric acid, as described by Pucher *et al.*[9]

RESULTS

Fluoroacetate and fluoroacetamide were convulsants for the cockroach as well as the mouse, the effects being most prominent at high doses, which caused hyperexcitability in about 3 hr, then violent convulsions followed by prostration with tremoring. At doses near the LD_{50}, however, death occurred with no such prominent symptoms. With mice, the familiar tonic convulsion was usual at periods of about 1 hr after poisoning.

Table 1 shows that fluoroacetate is more toxic to mice than to cockroaches, the ratio LD_{50} insect: LD_{50} mouse being 2·4. The housefly is about as susceptible as the mouse,

TABLE 1. TOXICITY OF FLUORO COMPOUNDS

LD_{50} in mg/kg

	Mouse	American cockroach	Housefly
Sodium fluoroacetate	18	43	21
Fluoroacetamide	85	32	9·5
p-Ethoxyfluoroacetanilide	28		45
p-Chlorofluoroacetanilide	25		130

TABLE 2. CITRIC ACID ACCUMULATION IN MICE AND COCKROACHES POISONED WITH SODIUM FLUOROACETATE AND FLUOROACETAMIDE*

Animals	Compound	Time (hr)			Control
		2	5	24	
Cockroaches	Fluoroacetamide	0·067	0·092	0·167	
	Sodium fluoroacetate	0·067	0·095	0·140	
					0·030
Mice	Fluoroacetamide	0·079	0·085	0·136	
	Sodium fluoroacetate	0·074	0·101*	0·166†	
					0·048

* Data are for milligrams citric acid per gram body weight, after poisoning with 30 mg/kg. Each figure is an average of two experiments.

† These animals were dead.

the corresponding ratio being 1·1. Fluoroacetamide by contrast is more toxic to insects than to mice, the corresponding ratios being 0·38 for the cockroach and 0·11 for the housefly. Other fluoroamides were, however, more toxic to mice than to houseflies.

It seemed probable that fluoroacetamide was converted to fluoroacetate by amidase action. This hypothesis was confirmed in the case of the cockroach. Extraction and paper chromatography (see Methods) of muscle from poisoned cockroaches gave three spots; comparisons with standards suggested that these were tricarboxylic acids, dicarboxylic acids, and fluoroacetate. The R_f's were 0·21, 0·32, and 0·57 respectively. Assay of the eluted spots (see Methods) showed that the tricarboxylic acid spot and the fluoroacetate spot inhibited succinate oxidation by muscle 53 per cent and 63 per cent, respectively, whereas the dicarboxylic acid spot was without activity. This is evidence that fluorocitrate and fluoroacetate were in fact produced.

It was next shown that poisoning by fluoroacetate and by fluoroacetamide causes large increases in the citrate concentrations in the whole bodies of mice and cockroaches (Table 2).

Finally the question was considered: Is the relatively low toxicity of fluoroacetamide to the mouse due to its relatively slow hydrolysis to fluoroacetate? Table 3 shows the amidase activity of various tissues toward acetamide and fluoroacetamide. In spite of the fact that hydrolysis of acetamide is far more rapid in the mouse than the cockroach, the opposite is true for fluoroacetamide, which is hydrolyzed about four times faster in cockroaches than in mice.

TABLE 3. AMIDASE ACTIVITY IN WHITE MOUSE AND COCKROACH*

	Cockroach			White mouse		
	Whole body homogenate	Fat body	Thoracic muscle	Whole body homogenate	Liver	Femoral muscle
Acetamide	1·40 ± 0·11	28·14	0·22	13·90 ± 0·22	1·29	0·76
Fluoroacetamide	2·46 ± 0·18	19·35	0·70	0·68 ± 0·18	2·11	1·23

* The data show milligrams of substrate degraded in 30 min by 250 mg of tissue. Each figure represents at least 3 experiments.

DISCUSSION

The findings on citrate accumulation in Table 2 indicate that in insects as in mammals, fluoroacetate kills by aconitase inhibition, with consequent accumulation of citrate.

In the cockroach the citrate accumulations found with fluoroacetamide poisoning, and the evidence for fluoroacetate and fluorocitrate in muscle of poisoned insects, give strong evidence that fluoroacetamide requires conversion to fluoroacetate in order to exert its effect. This conversion is more rapid in the cockroach than the mouse, a finding that may account for the greater toxicity to the cockroach. The conversion appears not to be catalyzed by the amidase that hydrolyzes the closely related substrate acetamide. This finding is reminiscent of the evidence that fluoroacetate and acetate are activated by different enzymes in pigeon liver,[10, 11] rabbit kidney,[11] and yeast.[11]

Two phenomena remain unexplained. If fluoroacetamide is hydrolyzed to fluoroacetate somewhat slowly in the mouse, why does it produce as great a citrate accumulation in whole body as does fluoroacetate? And if differences in rates of fluoroacetamide hydrolysis are important in cockroach and mouse, why does a given fluoroacetamide dose produce roughly comparable citrate levels in the two organisms? The explanation for the latter may be that these whole-body levels do not accurately reflect the concentration at some vital site. Until such evidence is obtained, our hypothesis for selective toxicity must remain tentative.

The findings tend to suggest an essential similarity in the tricarboxylic acid cycle of the mouse and in the cockroach and housefly. The fact that mitochondria of insects differ from those of mammals in being slow to oxidize added cycle intermediates but fast to oxidize α-glycerophosphate[12] is not in conflict with our findings. The relative slowness in oxidizing cycle intermediates probably reflects a poor permeability to added substrates rather than inactive enzymes; the rapid oxidation of α-glycerophosphate probably reflects the excellent permeability to this substrate which arises from its participation in the α-glycerophosphate shuttle. Sacktor and Dick[13] have recently

shown that in the housefly this shuttle is of greater activity than in vertebrates, which appear also to have additional shuttles for passing reducing equivalents into mitochondria.

It seemed at first surprising that mouse liver was less active per unit weight than whole mouse. However, the amidase activity of liver is exceptionally low toward 2-carbon amides in the case of the rabbit;[14, p. 296] and in the rat, amidases such as leucine aminopeptidase are low in liver, being only 13 per cent as concentrated as in kidney and 22 per cent as concentrated as in small intestine, and only a little more concentrated than in muscle.[14, p. 642] Our findings are therefore not unexpected.

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