Biochemical changes produced by cocaine in rats receiving different types of nutrition

R. RAMOS-ALIAGA AND GUILLERMO ARROYAVE
Instituto de Nutrición de Centro América y Panamá (INCAP), Guatemala, C. A.
and
Puerto Rico Nuclear Center, San Juan, Puerto Rico [1]

SUMMARY

Some biochemical changes produced by cocaine fed to rats with either high protein diets (20% casein) or low protein diets (5% corn protein) were studied and the following results obtained:

With a high protein diet significant decrease in the GOT and GPT activities in the liver is observed and a marked increase of the fumarase and urea in the liver and serum, respectively, is noted.

No significant changes were observed in the concentration of protein, lipids, D-aminoacid oxidase activity in the liver, GOT and GPT, total protein, and beta and gamma globulin in the serum. The alpha globulin decrease notably.

With a low protein diet a significant decrease in the GPT activity of the liver is observed and there is no significant change in the activity of GOT, fumarase, and the concentration of serum urea.

There is no significant change in the concentration of proteins, D-amino acid oxidase activity in liver, GOT, and GPT activities, total proteins, and serum beta and gamma globulin. The alpha globulin level decreased significantly.

The liver lipids in the animal fed with corn diet decreases significantly due to the effect of cocaine an event of great importance in the undernourished situation in which the majority of the Andean population live and who chew coca leaves.

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INTRODUCTION

A series of investigations, many of them carried out in Perú (1, 13, 9, 6, 7, 8, 10, 11, 12, 17, 29) have demonstrated the neurostimulating and invigorating effect of the coca leaf which is chewed by the Indians of Perú, Bolivia, and other South American countries. Millions of people in the South Andean region have this habit which has existed since colonial times for various socio-economic reasons of which the malnutrition affecting them is very important (2, 26). The diet, incipient in quality and quantity, especially proteins of high biological value, has produced a condition of subnutrition (2) in the population which must be taken into consideration when studying the effects of chewing the coca leaf, upon various aspects of the protein metabolism.

The objective of this biochemical investigation is to study the effects of cocaine on rats fed with proteins of high biological value such as a 20% casein diet compared to those fed with low biological value protein such as a 5% corn protein diet. A series of parameters were selected and determined in two groups of animals. These include the enzymatic activities of GOT; GPT, fumarase, D-aminoacid oxidase and total protein, proteins fraction serum, urea, lipids in serum and liver.

MATERIALS AND METHODS

Two groups of 16 Wistar rats each of both sexes, were studied. One group received a diet based on a 20% casein and the other group was fed a 5% corn protein diet. In both cases the experimental diets were started as soon as the animals were removed from their mother (3 weeks). Each group was divided into two subgroups: one subgroup receiving cocaine (15 mg of cocaine chlorhydrate per 10 g of food) and the other subgroup receiving no cocaine.

TABLE 1

COMPOSITION OF DIET

Ingredients	Diet with high level of protein (casein 20%)		Diet with low level of protein (5% corn protein)	
	Without cocain	With cocain	Without cocain	With cocain
Corn Starch	69.70	69.55	41.00	40.85
Cotton Seed Oil	5.00	5.00	5.00	5.00
Casein	21.30*	21.30*	—	—
Minerals[1]	4.00	4.00	4.00	4.00
Vitamin (ml/100g)[2]	5.00	5.00	5.00	5.00
Corn Meal	—	—	50.00*	50.00
Cocain HCl	—	0.15	—	0.15
* Concentration of protein	20.00	20.00	5.00	5.00

[1] *Hegsted's Mineral Mixture.*

[2] Vitamin solution, according to the formula of Manna and Hange (J. Biol. Chem. 202: 91, 1953).

The average weight per rat at the beginning of the experiment was 46 g. Food and water were given *ad libitum.* At the end of the 4th week, during which the amount of food and body growth were determined, the rats were sacrificed. The liver was removed, serum samples were taken and the following test made: glutamic oxalacetic transaminase and glutamic pyruvic transaminase levels in liver and serum were determined according to the method of Reitman S. and Frankel S. (22). D-aminoacid oxidase activity in the liver was determined by measuring the amount of pyruvic acid formed from DL-alanine (19-25). Fumarase activity in liver was determined by the Massey V. method (3). Serum urea content was determined with acetyl monoxime using a Coleman Jr. spectrophotometer (15). Total protein content in serum was determined by using the Gornall, Bardawill, and David technique (5). Total protein content in liver was determined on supernatant aliquots (700 × g) from liver homogenate (1:10) by using the same method (5). Serum protein fractions were determined by electrophoresis in microzones using an EMF of 400-600 volts and veronal buffer of pH 8.6. Lipids were determined by means of the ether extraction from the tissue dried at 60°C in a vacuum.

RESULTS

Studies conducted with the four animal groups, produced the following results:

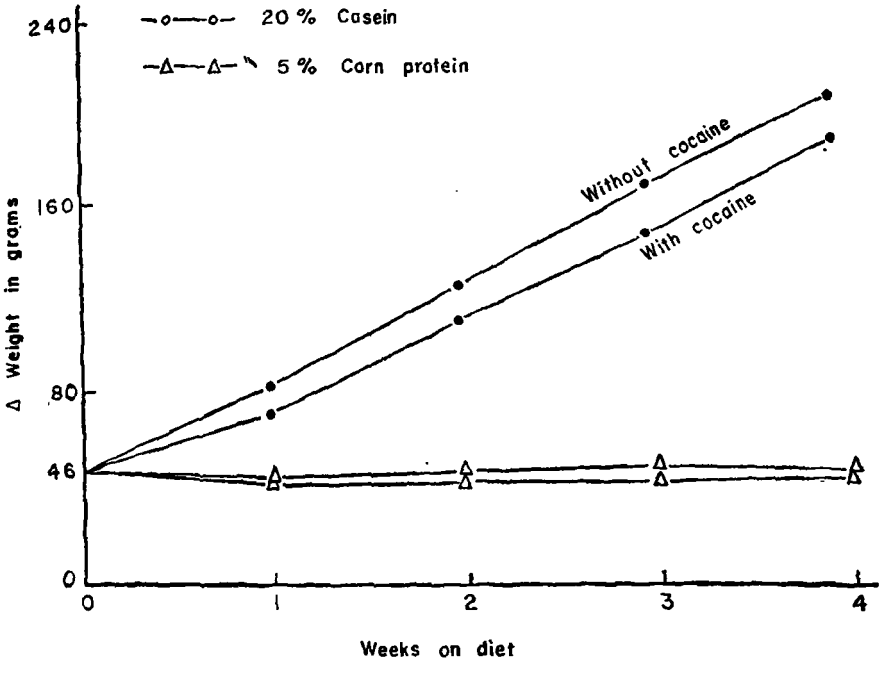
The consumption of food per day and the body development, as measured by increase in weight per week, was lowered more by the cocaine with the animals fed 20% casein than with the animals fed 5% corn protein.

Body growth in weight during 4 weeks varied from 161.3 g for the control group to 142.9 g for those fed with cocaine. For the animals eating a 5% corn protein diet plus cocaine, the change in weight was insignificant (+ 0.7 g for the control group to 2.57 g for the cocaine group, Graph I).

GRAPH I

GROWTH IN BODY WEIGTH IN GRAMS

Each point is an average value for eight animals



GOT and GPT activities decreased significantly in the presence of cocaine except in the animals fed with a 5% corn protein diet where there is a tendency to gain GOT activity (Table III). Fumarase and urea nitrogen levels in animals fed a 20% casein diet increased notably (Table II) although fumarase activity is only shown as a tendency to gain because of the wide standard deviation range. In the animals fed with corn plus cocaine there is no change in these values.

The total liver and serum protein levels, albumin fractions and the beta and gamma globulin do not show notable changes when cocaine was added to either of the two diets. However, the alpha globulin shows a decrease in value (Table III). Total lipids in the liver of rats fed with corn increased notably. Cocaine reduces these levels to normal (Graph II, Table II).

GRAPH II

CONCENTRATION OF LIPIDS IN LIVER. THE VALUES ARE AVERAGES FOR THE EIGHT ANIMALS IN EACH GROUP

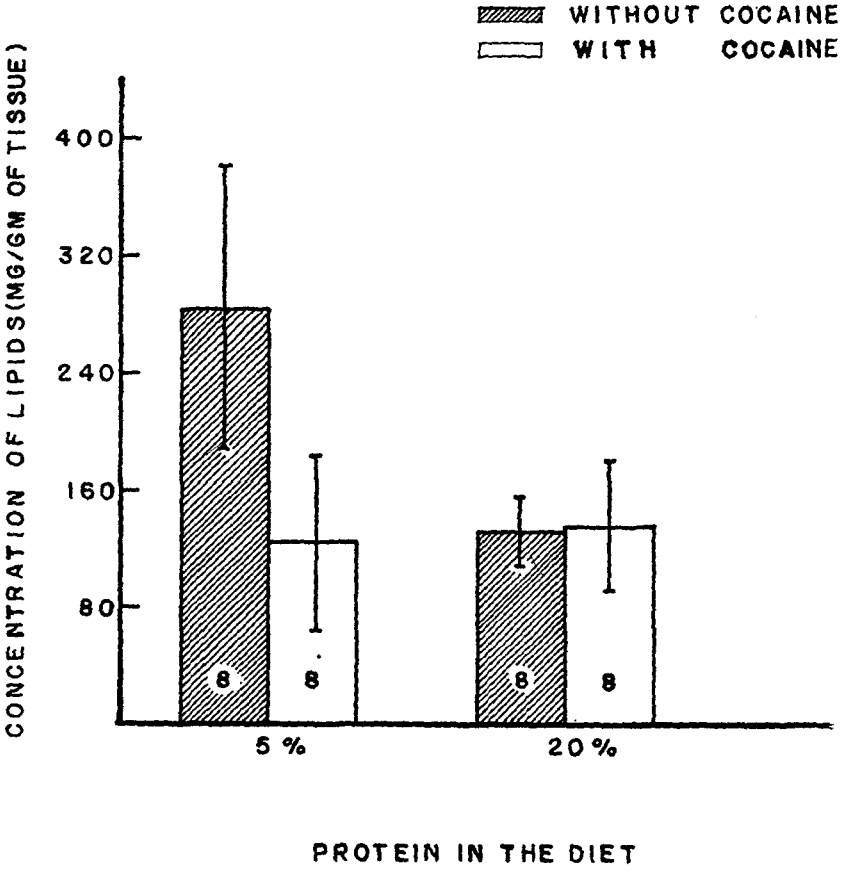


TABLE 2

BIOCHEMICAL CHANGES IN THE LIVER OF RATS FED DIETS WITH 20% CASEIN OR 5% CORN PROTEIN

	20% CASEIN				5% CORN PROTEIN			
	WITHOUT COCAINE		WITH COCAINE		WITHOUT COCAINE		WITH COCAINE	
	mg/g of tissue	S. D.	mg/g of tissue	S. D.	mg/g of tissue	S. D.	mg/g of tissue	S. D.
Proteins	112.30	± 20.920	106.470	± 19.400	65.500	± 12.680	64.800	± 9.540
Lipids	130.70	± 26.000	136.800	± 0.045	284.100	± 0.100	122.700	± 0.058**
Water	701.500	± 7.360	696.500	± 4.833	674.100	± 24.409	713.600	± 21.080
Fumarase	484.460[1]	± 133.600	699.070	± 385.850	319.690	± 121.000	328.600	± 126.800
GOT	43.230[1]	± 10.190	33.350	± 4.590*	34.070	± 16.190	39.940	± 11.080
GPT	25.700[1]	± 7.600	16.440	± 3.810**	15.150	± 2.320	9.730	± 2.560**
D-amino acid oxidase	0.085[1]	± 0.052	0.106	± 0.031	0.079	± 0.042	0.087	± 0.050

[1] Activity expresed in μM of substratum transformed per g of tissue per minute.

* Level of significance: $P<0.05$

** Level of significance: $P<0.01$

TABLE 3

BIOCHEMICAL CHANGES IN THE SERUM OF RATS FED DIET WITH 20% CASEIN OR 5% CORN PROTEIN

	20% CASEIN				5% CORN PROTEIN			
	WITHOUT COCAINE		WITH COCAINE		WITHOUT COCAINE		WITH COCAINE	
	g/100 ml	S. D.	g/100 ml	S. D.	g/100 ml	S. D.	g/100 ml	S. D.
Total Protein	5.790	± 0.461	5.380	± 0.552	3.480	± 0.586	3.590	± 0.387
Albumin	3.300	± 0.286	3.150	± 0.526	1.730	± 0.306	1.960	± 0.182
Alpha globulin	1.230	± 0.130	0.990	± 0.077**	0.840	± 0.095	0.640	± 0115***
Beta globulin	0.980	± 0.158	0.960	± 0.105	0.660	± 0.174	0.780	± 0.238
Gamma globulin	0.280	± 0.071	0.270	± 0.130	0.240	± 0.121	0.220	± 0.067
Urea nitrogen	0.01931	± 0.005	0.02894	± 0.006	0.01969	± 0.006	0.01796	± 0.005
GOT	0.718[1]	± 0.032	0.735	± 0.075	0.518	± 0.095	0.538	± 0.081
GPT	0.123[1]	± 0.036	0.152	± 0.090	0.109	± 0.060	0.102	± 0.019

[1] Activity expresed in μM of substratum transformed per ml of serum per minute.
** Level of significance: $P<0.01$
*** Level of significance: $0.05<P<0.10$

DISCUSSION

It has been confirmed by a series of laboratory experiment that cocaine has an inhibitory effect on certain aspects of cellular metabolism. Significant inhibition of the condensation reaction of active acetate with oxalacetate to form citrate (23), the formation of malate from glyxolic acid in *Eschericha coli* (14), as well as the decarboxylation of pyruvic acid in the anaerobic process of glucose utilization by yeast (24) have been described. In like manner the pharmacological work related to the stimulant action of cocaine on the sympathetic adrenal system in relation to a minor stimulus of the vagun insulinic system in rats and dogs (20), helps us to see that in these animals a reduction of glucose utilization by the cell occurs after an injection of cocaine, resulting in hyperglycemia. Notable changes have taken place in pigeons with vitamin B_1 deficiency in which symptoms of avitaminoses are aggravated by the ingestion of cocaine (10).

Studies related to the amination of ketoacids such as pyruvic and oxalacetic acid to form alanine and aspartic acid in the presence of cocaine indicate that there is a reduction in the value of these processes (4-17). However, the amination of the alpha ketoglutaric acid under these conditions seems not to be inhibited except in the case of a block in citrate formation that may lead to a reduction in the formation of ketoglutaric diminishes the quantity of glutamic acid available for the transamination of pyruvic and oxalacetic acids.

The background information related to our finding regarding the significant variations in hepatic enzyme levels of GOT and GPT and their relation to the utilization of the proteins by rats fed with casein and corn plus cocaine, leads us to suppose that *in vivo* also there is a depression in enzymatic activities of GOT and GPT with the casein diet and in the GPT activity but not the GOT activity in the group fed corn.

On the other hand the direct relation of GOT and GPT activities is well known, as well as the enzymes of the urea cycle at the protein level, and that a reduction in one may depress the others (28-27). In the case of the ingestion of cocaine by rats fed a 20% casein diet, there is a reduction of GOT and GPT activities which would be in agreement with the inhibitory effects *in vitro* of cocaine of the amination re-

actions already mentioned, even when the protein level remains unchanged. However, the possible increase of fumarase in the liver and the greater concentration of urea in the serum may be correlated to a greater utilization of proteins produced by cocaine in these animals.

In the animals fed corn protein, the decrease in GPT activity and the insignificant variation of the GOT (though there is a tendency to increase) of the fumarase and the urea under the effect of cocaine leads one to suppose that the inhibition in the GPT activity may be due to the same reasons as in the first case and that in these animals there is maximum economy of the small amount of protein fed to them in order to maintain a normal physiological process.

These conclusions appear to agree with the observed development of animals fed a diet with cocaine. Thus the development of rats fed with a good level of proteins would agree with other studies, in which a notable loss of weight was observed when animals were fed 200 mg of cocaine or more per kg of body weight (12). In this case, those fed casein, received an average of 220 mg/kg cocaine of body weight and those fed corn, 171 mg/kg. The most significant lost of weight occurs during the first two weeks in the first group which could be due both to the fact that the food consumption is less and to the metabolic action of the cocaine. However, in those fed corn protein and cocaine, the small loss of weight is due almost exclusively to the fact that the amount of food consumed was less and to a lesser extent to the effect of cocaine alone. The invariability of fumarase activity and urea concentration appear to be offsetting the diminished protein utilization by the poorly fed organism.

The liver lipids content of rats fed with corn and cocaine was less than that of the rats fed with corn only due to an alkaloid effect, which may involve a greater utilization of these lipids possible with an intermediate demethylation and transmethylation process to form phosphatylcoline. In other studies we are verifying that the demethylation process of cocaine (21) in the liver is important. In the same manner the alpha globuline fraction in the serum of rats receiving cocaine at one and another level decreased significantly. This fact related to the transport property of lipids by this fraction

could be indicating a greater utilization of the fatty acids by hepatic tissue (16).

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RESUMEN

Cambios bioquímicos producidos por la cocaína en ratas con diferente estado nutricional

Se han estudiado los cambios bioquímicos que produce la cocaína ingerida por ratas alimentadas con dieta a base de caseína al 20% y con dieta a base de proteína de maíz al 5%. Con dieta de caseína se observa un descenso significativo en las actividades de las transaminasas glutámico oxalacética y glutámico pirúvica en hígado y un aumento marcado de la fumarasa y urea en hígado y suero, respectivamente. No se observó cambios significativos en la concentración de proteínas, lípidos, actividad de la D-aminoácido oxidasa del hígado, transaminasas glutámico oxalacética y glutámico pirúvica, proteína total, beta y gamma globulina del suero. La alta globulina disminuyó significativamente. Con dieta de proteína de maíz hubo un descenso significativo en la actividad de la transaminasa glutámico pirúvica en el hígado, pero no hubo cambio en las actividades de la transaminasa glutánico oxalática y fumarasa del hígado y urea en suero. No hubo cambios significativos en la concentración de proteínas, actividad del D-aminoácido oxidasa del hígado, transaminasas glutámico oxalacética y glutámico pirúvica, proteínas totales, beta y gamma globulina del suero. La alfa globulina disminuyó significativamente.

Los lípidos acumulados en el hígado de ratas alimentadas con dieta de proteína de maíz disminuyeron significativamente por efecto de la cocaína, fenómeno éste de importancia fisiológica en el estado subnutricional en que vive la mayor parte de la población andina dedicada al cocaísmo.

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