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EFFECT OF A SYNTHETIC CHOCOLATE COLOURANT AND A FLAVOURANT ON SOME GLYCOLYTIC ENZYME SYSTEMS OF ALBINO RATS

FERIAL M. ABU SALEM AND SAFIA S. MOHAMMED
Department of Food Technology, National Research Centre, Dokki, Cairo, Egypt

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The effect of administering a synthetic chocolate colourant, a chocolate flavourant and a mixture of both for 28 days period on some glycolytic enzyme activities and of albino rats, their metabolite contents in some subcellular fraction of liver, brain and kidneys were studied. The activities of cytoplasmic and mitochondrial hexakinase and pyruvate kinase in liver, brain and kidneys increased by the synthetic food additives but the acitivity of lactate dehyrogenase was decreased. A significant decrease in pyruvate and lactate contents of the organ homogenates was seen. Administration of these food additives was accompanied by an increase in blood glucose level and decrease in liver glycogen content. Mixture of the two food additives produced more pronounced effects than the individual ones.

Key words: Synthetic chocolate colourant and a flavourant, Glycolytic enzyme.

Introduction

Food additives added to products with specific motives, are considered to be one of the most important classes of chemicals effecting human health. Hence an intensive studies including chemical and physical specification, acute toxicity carcinogenicity and metabolic studies are being carried out before recomending a food additive for use (FAO/WHO, [1]]). Both food colourants and flavourants appeared to have genotoxic effect also keeping this in veiw , the present study has been under taken limiting to metabolic studies only (Kasamaki *et al.* [2].

Materials and Methods

(a) Both synthetic chocolate colourant (Index No.20285) and flavourant were obtained by Cairo Food Flavour and Essence Company, Giza, Egypt.

(b) Fourty healthy adult male albino rats, weighing about 100 g were used in the present study.

The animals were fed in its diet consisted of casein 18.8%, methionine 0.2%, cotton seed oil 10%, salt mixture 4.0% (Hegested *et al.*, [3]), vitamins mixture 1.0% (Campbell, [4] cellulose 5.0% and rice starch 60.0 % two weeks prior to the study. Rats were allowed free excess of water. They were then divided into four groups (each of 10 rats). The first group was fed on normal basal diet only and served as a control group . The other three groups of animals were fed on basal diets supplemented with synthetic chocolate colourant (0.8 g/kg basal diet) synthetic chocolate flavourant (0.8 g/kg basal diet) and their mixture (0.8 g colour/kg diet and 0.8 g flavour /kg basal diet) respectivity for a period of 28 days. Diet and water were supplied *ad libitum*. At the end of the experiments, the animals were killed by decapitation and liver (L), brain (B) and kidneys (K) were dissected out and homogenated. Blood samples were also collected from the animals. All were kept at a temperature below 4° till the analysis started.

Analysis. (a) From the blood samples estimation of glucose was carried out according to the Trinder method [5] enzymatically and given in Table 2.

(b)Determination of pyruvate in B,K and L homogenates was carried out by the method of CZOK and Lampercht [6] and given in Table 2.

(c) Determination of lactate in rat B, K and L homogenates was carried out by the method of Bergmeyer [7] and given in Table 2.

(d) Glycogen was determined in the rat liver homogenates as per the method of Rerup and Lundquist [8] and given in Table 2.

(e) Mitochondrial and cytoplasmic fractions of brain, kidney and liver of the animals were prepared according to the method of Astawrov [9]. From these tractions hexokinase (HK) pyruvate kinase (PK) and lactate dehydrogenase (LDH) activities were carried out as per the method of Bergmeyer [7] spectrophotometerically and given in Table 1.

(f) Statistical analysis of data were evaluated by method of the analysis of variance (T-test).

Result and Discussion

The data in Table 1 clearly show that the activities of cytoplasmic and mitochondrial PK and HK in L,B and K of test group rats were increased securing highest with mixture treated group followed by colourant and flavourant treated group of rats as compared to control groups. The data in Table 1 also demonstrate that the acitivity of LDH in the mitochondrial and cytoplasmic cell emphasized that the effect of feeding a mixture of chocolate colourant and flavourant on the decreased LHD activity was greater than that of colourant

matter or flavourant matter alone. Of L,B,K of test group rats were decreased in the order of mixture treated group followed by flavourant treated group and colourant treated group as compared to control group rats. Data in Table 2 show that, there was a significant decrease in pyruvate and lactate contents of the organ homogenates of the test group as com-

TABLE 1. EFFECT OF A SYNTHETIC CHOCOLATE COLOURANT A FLAVOURANT AND THEIR MIXTURE ON GLYCOLYTIC ENZYME ACTIVITIES OF RATS.

	Fraction	Enzyme activities	Control group Mean*	%	Colourant group Mean*	%	Flavourant group Mean*	%	Mixture treated group Mean*	%
LIVER	Mitochondria									
		PK	2.03±0.02	100	2.19±0.30	108	2.11±0.22	104	2.19±0.30	108
		HK	0.023±.002	100	0.026±.003	112	0.024±.002	106	0.27±.003	120
		LDH	0.040±.005	100	0.033±.003	82	0.034±.003	85	0.032±.003	80
	Cytoplasm									
		PK	2.70±0.30	100	3.13±0.32	116	3.05±0.31	113	3.19±0.32	118
		HK	0.23±0.02	100	0.271±.03	118	0.26±0.03	115	0.28±0.03	120
		LDH	0.50±0.05	100	0.033±.003	66	0.035±.004	70	0.03±.003	60
BRAIN	Mitochondria									
		PK	6.50±0.71	100	6.87±0.70	106	6.76±0.70	104	7.02±0.71	108
		HK	0.021±.002	100	0.023±.002	109	0.022±.002	105	0.025±.003	117
		LDH	0.090±0.01	100	0.081±0.01	90	0.083±0.01	92	0.079±0.01	88
	Cytoplasm									
		PK	7.31±0.80	100	8.41±0.82	115	8.18±0.10	112	8.41±0.09	115
		HK	0.021±.003	100	0.24±.003	114	0.024±.003	115	0.25±.003	117
		LDH	0.100±0.01	100	0.071±.08	71	0.080±0.01	80	0.007±.007	67
KIDNEYS	Mitochondria									
		PK	3.60±0.40	100	4.03±0.41	112	3.96±0.40	110	4.14±0.42	115
		HK	0.016±.002	100	0.018±.002	114	0.017±.002	105	0.019±.002	118
		LDH	0.030±.002	100	0.027±.003	91	0.028±.003	94	0.026±.003	87
	Cytoplasm									
		PK	3.86±0.4	100	4.55±0.50	118	4.32±0.42	112	4.63±0.44	120
		HK	0.016±.002	100	0.019±.002	118	0.018±.002	114	0.019±.003	118
		LDH	0.040±.004	100	0.032±.008	81	0.033±.003	82	0.031±.005	72

*Average values of 10 rats. % Relative to control group. P<0.05. PK: Pyruvate kinase, HK: Hexokinase, LDH: Lactate dehydrogenase.

TABLE 2. EFFECT OF A SYNTHETIC CHOCOLATE COLOURANT AND FLAVOURANT AND THEIR MIXTURE ON GLYCOLYTIC METABOLITES ON BLOOD GLUCOSE LEVEL AND ON LIVER GLYCOGEN IN RATS.

	Metabolites	Control group Mean	%	Colourant group Mean	%	Flavourant group Mean	%	Mixture treated group Mean	%
LIVER									
	Lactate	1.30±0.11	100	1.12±0.10	86.2	1.17±0.12	90.0	1.04±0.11	80.4
	Pyruvate	0.75±0.08	100	0.449±0.05	59.9	0.465±0.04	62.0	0.45±0.05	60.0
	Glycogen	5.11±0.39	100	4.15±0.36	81.2	4.32±0.41	84.5	3.84±0.31	75.1
BRAIN									
	Lactate	2.84±0.30	100	2.58±0.26	91.1	2.64±0.27	93.0	2.52±0.24	89.0
	Pyruvate	0.73±0.07	100	0.416±0.04	57.1	0.438±0.05	60.0	0.395±0.04	54.0
KIDNEYS									
	Lactate	1.61±0.15	100	1.38±0.14	86.0	1.47±0.15	91.0	1.32±0.11	82.0
	Pyruvate	0.23±0.02	100	0.184±0.02	80.1	0.193±0.02	84.0	0.177±0.02	77.0
BLOOD									
	Glucose	102.22±8.1	100	129.82 ±9.23	127	113.31±10.1	110.8	133.3 ±11.3	130.3

*Average values of 10 rats. % Relative to control group. P<0.05.

pared with control group rats. Our results are in good agreement with those of Salah [10] who showed that synthetic food colourant are capable of stimulating the activity of pyruvate kinase and hexokinase and inhibited the activity of lactate dehydrogenase in animals. Table 2 also show that administering the synthetic chocolate colourant, the flavourant and their mixtrue could increase the blood glucose level in rats. This elevation was also accompanied by a remarkable reduction in liver glycogen content relative to control group. Mixture of both colourant and flavourant agents had greater effect than individual food additives. The contrasted results of liver glycogen and blood glucose could be attributed to the glucose production after food additives induction by glycolysis and glucogenesis (Abd-el-Rahim *et al.*, [11]) and to the increase of both glycogen breakdown enzyme acitivity and glycolytic processes to proudce energy requirements for animal system processes and the exprimental conductions(Salah, [10]).

The results obtained were confirmed from the altered activities of pyruvate kinase and lactate dehydrogenase by administration of chocolate colourant, the flavourant and their mixture.

The pyruvate and lactate levels in the different organs were dependent on the glycolysis , in particular the pyruvate kinase and lactate dehydrogenase activities. This might explain the decrease in pyruvate and lactate levels than the control.

The present finding could suggest that synthetic chocolate colourant and flavourant may stimulate the glycolytic process in different organs to produce pyruvate. LDH activities were stimulated to reformation of pyruvate from lactate. The observed increase in PK acitivity (convert phosphoenol pyruvate to pyruvate and ATP) in both mitochondria and cytoplasm in which the utilization of pyruvate occur. Pyruvate produced was metabolized through Kreb's cycle to obtain large amount of energy (ATP) in order to get rid of these agents. On the other hand, several reports have been recorded of an interactions between certain food additives and protein molecules.

Furthermore, it was found that food grade samples of Brown FK (chocolate colourant) and two of its consitituents were mutagenic (Gangolli *et al.* [12] and Haveland Smith and Combes, [13].

Accordingly the present finding also may be due to the fed back mechanism toward toxic compounds, whereas pyruvate was required as carbon skeletons of amino acids in order to compensate the damaged protein as well as nucleic acids with food additives.

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