Mechanism of Teratogenic Action of Hypoglycin-A

Hypoglycin-A[1] is a toxic non-proteinogenic amino acid of considerable biochemical interest[2,3]. It has been suggested that the toxicity of hypoglycin-A is due to the formation of a degradation product, methylenecyclopropane acetate, which inhibits the oxidation of long-chain fatty acids[4]. ENTMAN and BRESSLER[5] reported that L-carnitine, which promotes the intramitochondrial oxidation of long-chain fatty acids[6,7], antagonized the hypoglycaemic effects of hypoglycin-A. Hypoglycin-A inhibited the growth of sarcoma-180 cells in mice, but a simultaneous injection of leucine, a structural analogue of hypoglycin-A, prevented this inhibition of tumour growth[8].

In the pregnant rat, hypoglycin-A is highly teratogenic[9]. The present communication describes the effects on embryonic development, in pregnant rats, of administering hypoglycin-A simultaneously with riboflavin phosphate, L-leucine, or DL-carnitine.

Materials and methods. The methods used in the present studies were previously described[9]. Pregnant Wistar-derived rats (160–180 g) were divided into 4 groups. L-leucine (15 mg/kg) and hypoglycin-A (30 mg/kg) were administered to animals of Group I. Group II received riboflavin phosphate (3 mg/kg) and hypoglycin-A (30 mg/kg), Group III DL-carnitine (200 mg/kg) and hypoglycin-A (30 mg/kg), and Group IV animals received only hypoglycin-A (30 mg/kg) and served as a control. Hypoglycin-A, L-leucine, riboflavin phosphate, and DL-carnitine were dissolved in isotonic saline, the pH being adjusted to 7.4 with phosphate buffer, and administered i.p. from the first through the sixth day of gestation.

Results and discussion. The results are summarized in the Table. Simultaneous administration of leucine and hypoglycin-A to the pregnant rats did not counteract the teratogenic effect of hypoglycin-A, but resulted in a significant increase in the incidence of foetal resorption, compared with animals treated with only hypoglycin-A ($P < 0.01$). The simultaneous injection of carnitine did not prevent the teratogenic efects of hypoglycin-A, but the combined treatment caused marked embryotoxic and teratogenic effects which showed no significant difference compared with the hypoglycin-A treated animals. The incidence of foetal abnormalities was significantly reduced ($P < 0.001$) in the pregnant animals treated with both hypoglycin-A and riboflavin phosphate compared with the controls. However, the number of foetal resorptions was not significantly affected.

Inhibition of long-chain fatty acid oxidation is considered to be the primary effect of hypoglycin-A on intermediary metabolism[2–4]. Accumulation of fatty acids, uncoupling of oxidative phosphorylation, and the resulting block in the availability of energy for ATP synthesis may account for the teratogenicity of hypoglycin-A, since ATP is essential for normal embryonic growth and differentiation[10]. Reversal of hypoglycin-A induced teratogenic effects by riboflavin phosphate lends support to the suggestion of VON HOLT et al.[4] that the actual site of action of hypoglycin-A is inhibition of the acyl dehydrogenase flavin-dependent-oxidation reaction.

Influence of leucine, riboflavin phosphate and carnitine on hypoglycin-A induced teratogenicity

Groups (No. of animals)	Treatment	Total implantations	Resorptions	Malformed foetuses
I (6)	H + L	52	17 (32.7%)	35 (100%)
II (6)	H + R	51	3 (5.9%)	21 (43.7%)
III (6)	H + C	48	3 (6.3%)	36 (80%)
IV (6)	H	53	4 (7.5%)	40 (81.6%)

H, hypoglycin-A; L, leucine; C, carnitine; R, riboflavin phosphate.

Zusammenfassung. Simultane Verabreichung von Riboflavin und Hypoglycin-A reduzierte das Auftreten von foetalen Missbildungen in Ratten. Dagegen haben Carnitin und Leucin keinen Einfluss auf die teratogene Wirkung des Hypoglycins.

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Uptake of Aflatoxin B_1 by Plastic Materials[1]

Aflatoxins are a group of mycotoxins that have gained increasing interest as food contaminants during the past 8 years. Their hepatotoxic properties have led to extensive research on the chemical and physical characteristics of aflatoxins in an effort to determine their mode of action[2]. Since the carcinogenic properties of aflatoxins in animals, including different species of monkeys, have been well documented[3–6], these mycotoxins are suspected as being responsible for the high incidence of liver cancer in certain areas[7].

An investigation on aflatoxin B_1 was initiated in our laboratory: it included metabolism in perfused rat liver

Uptake of aflatoxin B_1 by plastic materials (30 μM aflatoxin B_1 in phosphate buffer 10 mM, pH 7.3)

Material	Shape	Size (cm)	Initial amount of aflatoxin in solution (μg)	Aflatoxin adsorbed (%)					
				15′	30′	45′	1 h	2 h	6 h
Silicone	Tube	0.40 × 100	117.6	9	15	22	32	40	53
Silicone	Tube	0.20 × 100	29.4	11	19	24	37	44	70
Tygon	Tube	0.40 × 100	117.6	31	42	52	59	70	75
Tygon	Tube	0.24 × 100	42.3	33	56	59	68	75	85
Tygon	Tube	0.16 × 100	23.5	60	73	76	77	80	88
PVC	Tube	0.40 × 100	117.6	22	28	36	42	62	70
PVC	Tube	0.10 × 100	7.4	34	40	43	53	67	75
Polyethylene	Tube	0.40 × 100	117.6	7	10	13	16	22	33
Plexiglass	Tube	0.80 × 36	169.2	0	0	0	0	1	1
Cellulose ester[a]	Filter	∅ 2.8	93.6	20	28	31	34	38	44
Cellulose ester[a]	Filter	∅ 2.4	93.6	14	22	26	28	32	36
Cellotate[a]	Filter	∅ 2.8	93.6	20	33	39	44	52	54
Cellotate[a]	Filter	∅ 2.4	93.6	13	24	30	35	44	47
Nylon	Filter	∅ 2.4	93.6	3	5	5	6	6	7
Regenerated cellulose[b]	Membrane	2.5 × 2.5	93.6	0	0	1	1	1	1
Teflon	Sheet	6.25 × 1.0	93.6	0	1	2	2	2	3
Parafilm[c]	Sheet	6.25 × 1.0	93.6	5	6	6	6	6	6

[a] Millipore Filter Corporation, Bedford (Mass., USA). [b] Bel-Art Products, Pequannock (N.Y., USA). [c] American Can Co., Neenah (Wisc., USA).

and in hepatic microsomal preparations, interactions with DNA and serum proteins, inhibition of DNA-directed RNA synthesis and DNA synthesis.

Preliminary experiments showed that aflatoxin B_1 could be rapidly taken up by soft plastic materials used in several analytical procedures. Research was started to ascertain the materials most suitable for circulating and filtering aqueous solutions of aflatoxin B_1.

The results, summarized in the Table, show that uptake of aflatoxin by soft plastic materials is a rapid and extensive phenomenon. It can be a very important source of error in most of the experimental work carried out with these lipophilic mycotoxins.

Zusammenfassung. Aflatoxin wird von einigen gebräuchlichen Kunststoffen innerhalb kurzer Zeit in verhältnismässig grosser Menge absorbiert. Die vorliegenden Ergebnisse erlauben es, analytische Fehler bei Verwendung ungeeigneten Materials zur Zirkulation oder Filtration von Aflatoxinlösungen zu vermeiden.

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[1] This is contribution No. 660 of the Euratom Biology Division.

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Relationship Between the Potentiation of Potassium-Induced Contracture of Cardiac Muscle by Four Cardenolides and their Inhibitory Effects on the Sodium Potassium Activated Adenosine Triphosphatase of Brain

In view of the current discussion of a close relationship between the positive inotropic action of cardiotonic steroids and their inhibitory action on the Na, K-activated ATP-ase[1], these two actions were measured and compared with digitoxigenin and 3 derivatives. A clear correlation was found as stated below.

In a previous paper, we reported that the cardiotonic activity of digitoxigenin was profoundly affected by introducing a hydroxy group or an oxo group in position 15. The order of the potency was: digitoxigenin (I) > 15β-hydroxydigitoxigenin (II) > 15-oxodigitoxigenin (III) > 15α-hydroxydigitoxigenin (IV), the last being practically inactive[2]. These 4 compounds were used in the present study. The compounds were kindly supplied by Dr. M. Okada of Tokyo Biochemical Research Institute, Tokyo.

Potentiation of potassium contracture of the cardiac muscle. The cardiotonic activity was measured by a new assay method which has been developed by Takeda et al.[3]. The method makes use of the potentiation of potassium contracture of the frog ventricular muscle by cardiotonic steroids.

Frogs, *Rana nigromaculata*, were used. A strip of the ventricular muscle was dissected and mounted in a bath which contained 3 ml of Ringer's solution (NaCl 111 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM, $NaHCO_3$ 1.2 mM, glucose 2.7 mM), which was aerated by oxygen. The muscle was stimulated electrically at a rate of 0.2 cps throughout