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EFFECTS OF DDT AND DIELDRIN ON INTESTINAL GLUCOSE TRANSPORT AND BRUSH BORDER HYDROLASES

A COMPARISON WITH PHENOBARBITAL AND METHYLCHOLANTHRENE

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Abstract—The enhancement of *in vitro* small intestinal transcellular glucose transport in NMRI mice after oral administration of the organic pesticides 2,4-DDT and dieldrin can be shown to be due to an increased active transport at the site of the brush border membrane. Intestinal disaccharidase activities were concomitantly elevated in the dieldrin group, while DDT produced no effects with intestinal hydrolases. The classic enzyme inducing agents phenobarbital and methylcholanthrene failed to stimulate intestinal glucose transport, although both increased intestinal disaccharidase activities considerably, thus questioning a close relation between these digestive and absorptive functions in the translocation of glucose. Intestinal alkaline phospatase activity was enhanced after DDT, dieldrin and methylcholanthrene treatment, but not with phenobarbital. It is suggested that DDT and dieldrin exert their stimulating effect on intestinal glucose transport by a mechanism different from general induction of metabolic pathways.

Organic pesticides, including 2,4-DDT [1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)-ethane] and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4,5,6,7,8,8-octahydro-endo-exo-1,4:5,8-dimethanonaphtalene) still exhibit a growing environmental impact on world population. During many years of research numerous toxic effects of these compounds, which enter the human body almost entirely on an oral route [1], have been described. Mahmood and his co-workers reported several changes in the digestive absorptive surface of the small intestine due to DDT and dieldrin ingestion [2, 3], e.g. an increase in the rates of uptake for glucose and some amino acids in rhesus monkey intestine, but were able to identify similar effects with rat intestine [4], where only amino acid transport appeared to be enhanced. Together with data presented on the specific activity of brush border digestive enzymes, the stimulatory effect of the pesticides could be attributed to their enzyme inducing properties, commonly understood to be of the phenobarbital subtype [5].

The objective of this paper is to describe transport changes at the mucosal cell after pesticide treatment more precisely, to find out whether these changes could also be produced with other enzyme inducers and to establish some more data on the possible relation of intestinal disaccharidases and hexose transport as suggested earlier [6].

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MATERIALS AND METHODS

Animals. Male NMRI mice (22–25 g) received one of the following pretreatments: One oral dose of 2,4-DDT (250 mg/kg b.w.) suspended in corn oil 72 hr before experiments; dieldrin (40 mg/kg b.w.) in corn oil 24 hr before experiments; four oral applications of methylcholanthrene (30 mg/kg b.w. per day) in corn oil; phenobarbital–sodium/saline (80 mg/kg b.w.) as i.p. injections over 3 days before experiments. Control groups received treatment with the appropriate vehicle.

Transport measurements. Animals were killed by cervical dislocation, the jejunum was quickly removed, everted and rinsed in ice-cold Krebs–Ringer solution (mM: NaCl 120.0; KCl 4.69; $CaCl_2$ 3.33; $MgSO_4$ 2.16; $NaHCO_3$ 24.88; KH_2PO_4 1.18; D-glucose 5.5) gassed with 95% O_2/5% CO_2 at pH 7.4. A segment of 5 cm length was mounted in a perfusion system, the remainder of the tissue used for enzyme assays. Both compartments of the perfusion system held 50 ml of continuously gassed Krebs–Ringer solution at 37°, containing traces of [^{14}C]D-glucose on the mucosal and of [^{3}H]D-glucose on the serosal side. Perfusion of the serosal compartment was maintained with an infusion pump at 2 ml/min and a hydrostatic pressure of less than 3 cm H_2O. After one hour of incubation the tissue was rinsed in saline saturated with "cold" glucose, blotted carefully on filter paper and mucosal tissue gently scraped off with a glass slide. Samples of incubation fluids, taken at 20 min intervals, and tissue samples dissolved in Protosol were examined for ^{3}H- and ^{14}C-activity by liquid scintillation counting. Incubation fluids were controlled photometrically for

Table 1. Mouse intestinal glucose transport *in vitro* after pretreatment with DDT, dieldrin, phenobarbital and methylcholanthrene

Group	Mucosal to serosal (μmoles/hr·cm^2) J_{ms}	Serosal to mucosal (μmoles/hr·cm^2) J_{sm}
Control	3.90 ± 0.35	0.152 ± 0.030
DDT	5.92 ± 0.61†	0.240 ± 0.027 (n.s.)
Dieldrin	5.13 ± 0.27†	0.232 ± 0.019*
Phenobarbital	3.72 ± 0.26 (n.s.)	0.138 ± 0.023 (n.s.)
Methylcholanthrene	3.99 ± 0.47 (n.s.)	0.186 ± 0.024 (n.s.)
Control/corn oil p.o.	3.92 ± 0.26	0.174 ± 0.016
Control/saline i.p.	3.41 ± 0.49	0.174 ± 0.020

Each value represents the mean ± S.E.M. of 10 (methylcholanthrene: 9) experiments, flux values are given for the time 40–60 min, when flux differences were most prominent.
† $P < 0.01$; * $P < 0.05$; (n.s.) not significant according to Student's *t*-test for unpaired observations.

specific glucose activity by the hexokinase method before and after experiments.

Flux calculations. Unidirectional glucose fluxes were determined by monitoring the movements of ^{3}H- and ^{14}C-labelled glucose following the method of Naftalin and Curran [7], according to which the transport rates across the intestine (J_{ms}, J_{sm}) were measured directly as rates of transport of the two markers [^{3}H]D-glucose and [^{14}C]D-glucose into their *trans* solution. Fluxes across the borders of mucosal cells, i.e. transport across the brush border membrane in both directions (J_{mc}, J_{cm}) and across the basolateral membrane (J_{cs}, J_{sc}) were calculated from mucosal tissue isotope distribution ratio and transport rates across the intestine; for further discussion see [7, 8].

Enzyme assays. Intestinal disaccharidase activities, i.e. sucrase (EC 3.2.1.48), maltase (EC 3.2.1.20), trehalase (EC 3.2.1.28) and lactase (EC 3.2.1.23) as well as alkaline phosphatase (EC 3.1.3.1) activity were determined according to Dahlquist (9) from mucosal homogenate. Protein was estimated by the method of Bradford (10) with bovine serum albumin as a standard.

Chemicals. 2,4-DDT was obtained from Serva (Heidelberg, F.R.G.), dieldrin and methylcholanthrene from Fluka (Neu-Ulm, F.R.G.), D-[^{14}C](U)-glucose (360 mCie/mmol) and D-[6-^{3}H(N)]-glucose (31.1 Ci/mmole) from NEN (Dreieich, F.R.G.), enzyme reagents were purchased from Boehringer (Mannheim, F.R.G.), all other chemicals were of analytical purity.

RESULTS

Mucosal to serosal glucose transfer was measured to be about twenty times faster than movement of glucose in the opposite direction (see J_{ms}–J_{sm}, Table 1). Both dieldrin and DDT enhanced mucosal to serosal transport of glucose in mouse intestine *in vitro*. A further elevation of transport activity could not be achieved when doses were increased twofold.

In mucosal scrapings, 85–90% of measured radioactive tracer represented ^{14}C-activity from the mucosal solution; from the total amount of tracer measured in our tissue samples, it can be estimated that glucose accumulated inside the mucosal cells five to eight times the concentration of either incubation fluid, thus locating the site of active transport at the mucosal brush border membrane.

While there were no changes in glucose fluxes from the serosal solution into mucosal cells (see J_{sc}, Table 2) after pesticide treatment, glucose uptake from the mucosal solution (J_{mc}) was markedly enhanced in both the DDT and the dieldrin groups, as well as glucose fluxes from mucosal tissue into both incubation media (J_{cm} and J_{cs}, Table 2).

Administration of both enzyme inducing agents

Table 2. Calculated unidirectional glucose fluxes across the borders of mouse intestinal mucosal cells after administration of DDT and dieldrin

Group	Mucosal solution to cell J_{mc}	Cell to mucosal solution (μmoles/hr·cm^2) J_{cm}	Cell to serosal solution J_{cs}	Serosal solution to cell J_{sc}
Control	5.83 ± 0.67	2.08 ± 0.54	4.23 ± 0.36	0.48 ± 0.05
Control/corn oil	6.37 ± 0.43	2.63 ± 0.33	4.27 ± 0.30	0.53 ± 0.09
DDT	9.74 ± 0.57‡	4.06 ± 0.53*	6.32 ± 0.66*	0.64 ± 0.05 (n.s.)
Dieldrin	9.01 ± 0.52‡	4.12 ± 0.43*	5.46 ± 0.30*	0.56 ± 0.09 (n.s.)

All values are mean ± S.E.M. of ten experiments, significance of differences of means are given vs control/corn oil according to Student's *t*-test for unpaired observations.
‡ $P < 0.001$; * $P < 0.05$; (n.s.) not significant.

phenobarbital and methylcholanthrene produced no effect on mucosal to serosal or oppositely directed glucose transport (Table 1). No changes in glucose fluxes across the borders of mucosal cells were found. Several other dosage schemes for methylcholanthrene (20 mg/kg, 30 mg/kg or 40 mg/kg, single doses applied 24 hr before experiments) and phenobarbital (120 mg/kg, three doses in three days) also failed to enhance mouse intestinal glucose transport *in vitro*.

The effects of our pretreatments on intestinal disaccharidase activities are shown in Table 3. Sucrase and maltase specific activities were increased in the dieldrin group while DDT failed to produce significant effects on specific brush border hydrolase activities.

In contrast to our transport measurements, both enzyme inducing agents phenobarbital and methylcholanthrene elevated specific sucrase and maltase activities, trehalase was only slightly enhanced in the methylcholanthrene group.

Lineweaver–Burk plots of sucrase activity were done in all four experimental groups (see Fig. 1). While there was only a slight increase in V_{max} after DDT treatment compared to controls (79.4 ± 10.1 vs 65.2 ± 4.3 μmoles/min·g protein) with an unchanged K_m (9.6 ± 1.5 vs 10.0 ± 1.2 mM), considerable changes in sucrase kinetics indicating an increase in net enzyme content could be observed in the dieldrin group (V_{max} 107.3 ± 6.9 μmoles/min g protein, K_m 11.2 ± 1.0 mM), and after Phenobarbital treatment (V_{max} 99.0 ± 6.7 μmoles/min g protein, K_m 10.9 ± 1.2 mM).

Methylcholanthrene action on small intestinal sucrase activity (V_{max} 115.5 ± 11.2 μmoles/min g protein, K_m 16.3 mM) seems to be of a more complex nature.

As shown in Table 4 jejunal alkaline phosphatase activity was increased more than twofold after administration of dieldrin and methylcholanthrene, DDT produced a moderate enhancement of specific activity and phenobarbital treatment had no effect.

Table 3. Effect of DDT, dieldrin, phenobarbital and methylcholanthrene pretreatment on intestinal disaccharidase activity

Group	Sucrase	Maltase	Trehalase	Lactase
Control	46.7 ± 2.99	319.15 ± 18.1	147.29 ± 9.77	8.03 ± 0.80
DDT	53.9 ± 7.1 (n.s.)	330.9 ± 32.5 (n.s.)	164.7 ± 26.9 (n.s.)	13.5 ± 4.4 (n.s.)
Dieldrin	76.2 ± 2.0‡	433.6 ± 34.9†	127.6 ± 8.7 (n.s.)	9.50 ± 2.2 (n.s.)
Phenobarbital	72.5 ± 6.2‡	508.8 ± 49.2†	186.6 ± 32.7 (n.s.)	10.15 ± 1.16 (n.s.)
Methylcholanthrene	77.13 ± 6.46‡	432.2 ± 48.0*	173.6 ± 8.9*	10.30 ± 1.40 (n.s.)

Enzyme activities are expressed as μmoles glucose liberated per minute and g protein. Values are mean ± S.E.M. of four to eight determinations done in triplicate,
‡ $P < 0.001$; † $P < 0.01$; * $P < 0.05$; n.s. not significant, according to Student's *t*-test for unpaired observations.

DISCUSSION

The data presented in this paper verify the effects of pesticide pretreatment on intestinal digestive absorptive functions previously described by Mahmood *et al.* [2, 3] in adult rhesus monkeys. Mahmood's group found an increase in mucosal cell glucose uptake and an elevation of intestinal hydrolase, alkaline phosphatase and leucaminopeptidase activity, after DDT and dieldrin pretreatment, but was unable to confirm these findings in uptake studies with rat intestine.

In our studies of transmural intestinal glucose fluxes and of transport rates across the borders of mouse intestinal mucosal cells *in vitro*, an increase of intestinal glucose transport after DDT and dieldrin pretreatment has been demonstrated. Our data indicate, that these transport changes can be attributed to an elevated "uphill" movement of glucose from the mucosal solution into the mucosal tissue (J_{mc}), thus ruling out a mere increase of permeability due to toxic effects of the applied agents. Furthermore, we found no changes in the rate, at which

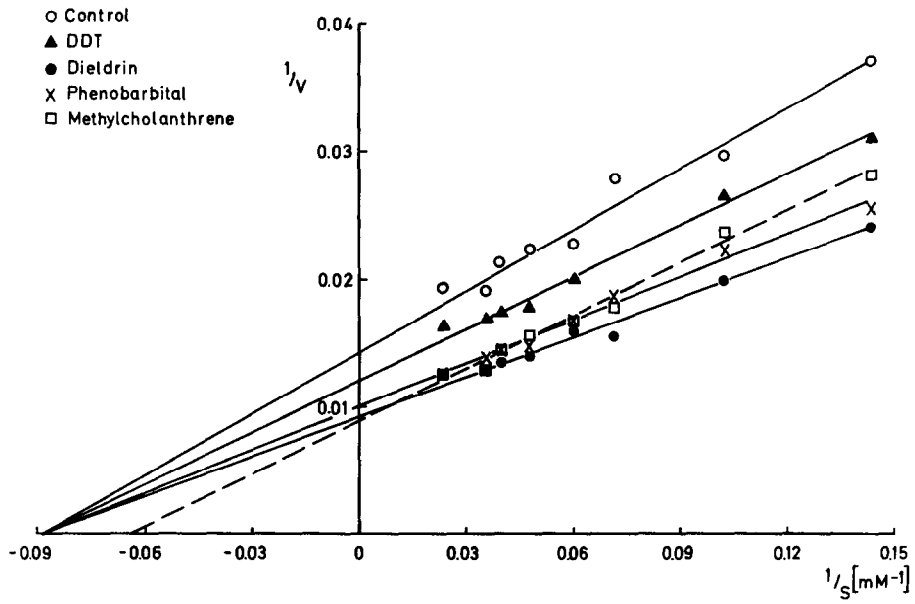

Fig. 1. Lineweaver–Burk plot of mouse intestinal sucrase activity after treatment with DDT, dieldrin, phenobarbital and methylcholanthrene (unweighted least squares, all correlation coefficients $r > 0.99$). Calculated values for V_{max} and K_m are given in the text. Sucrase activity is expressed as μmoles glucose liberated per min and g protein.

glucose entered the mucosal cells from the serosal side.

No conclusions can presently be drawn regarding the elevation of the basolateral flux J_{cs}, commonly understood to represent a "weak active transport system" [11]. This increase may reflect the higher glucose uptake at the luminal side of the cell or may as well be completely independent of it. As active glucose transport is known to be Na^+-dependent [12], alterations in intestinal Na^+-kinetics can be assumed; Na^+-fluxes have not been investigated in this work.

Changes in small intestinal hydrolase activities were less pronounced in our enzyme assays compared to Mahmood's findings; dieldrin pretreatment produced increases only with sucrase and maltase whereas no significant changes could be obtained in DDT treated mice. In a report on the chlorinated hydrocarbon lindane [13], a marked decrease in rat jejunal disaccharidase activities was found after a 90-day treatment, but no data regarding the first days of application were available.

The stimulating effect of both chlorinated hydrocarbons DDT and dieldrin on the intestinal glucose carrier system is most probably not attributable to any mechanisms, which assign both agents to the group of enzyme inducers of the phenobarbital type [5] or to the group of inducers in general; it seems that a more specific action is required to produce the stimulating effects on intestinal glucose transport systems referred to in this paper.

In several reports on the subject of enhanced intestinal glucose transport due to various pretreatments [14–17], in none of which the underlying mechanism could actually be verified, close correlations of intestinal hydrolase activity and transport kinetics have been described, implying some functional relation between carbohydrate digestion and absorption [6].

Our results show, that treatment with Phenobarbital induces jejunal disaccharidases with similar kinetic parameters as both pesticides but does not affect intestinal glucose transport; enhancement of disaccharidase activity with methylcholanthrene also

Table 4. Alkaline phosphatase activity in mucosal homogenates of mice treated with DDT, dieldrin, phenobarbital and methylcholanthrene

Group	Specific alkaline phosphatase activity (μmoles/min · g protein)
Control	807.0 ± 92.44
DDT	1409.6 ± 223.1*
Dieldrin	2123.0 ± 110.6‡
Phenobarbital	756.3 ± 147.4 (n.s.)
Methylcholanthrene	2005.1 ± 331.0*

Values are mean ± S.E.M. of four to eight determinations, the significance of differences is indicated as in Table 2.

bears no effect on sugar transfer, indicating that the functional relation between disaccharidase activity and glucose transport is not compulsory.

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