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SHORT COMMUNICATIONS

Stimulation of iron absorption by various polyhalogenated aromatic hydrocarbon environmental contaminants

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Polyhalogenated aromatic hydrocarbons are ubiquitous contaminants of the environment [1, 2]. This group of compounds includes polychlorinated biphenyls and terphenyls (PCBs and PCTs), insecticides, herbicides, flame retardants and various other commercial materials [3]. The major biological hazards presently ascribed to these compounds include carcinogenicity, mutagenicity and teratogenicity [4, 5]. We now report the stimulation of iron absorption in the rat by several hydrocarbons, indicating that these environmental contaminants have additional biological effects.

Groups of male Sprague–Dawley rats (80–120 g) (Marland Farms, Wayne, NJ) were given 0.2 ml dioxane, with or without test doses of hydrocarbon, by gastric tube after an overnight fast. The rats were refed 1 hr later, then fasted overnight prior to operation or sacrifice 24 hr after intubation. The sequential steps in iron absorption, uptake at the mucosal surface and transfer to the blood stream (or serosal surface *in vitro*), were estimated using duodenal loops *in vivo* and everted gut sacs *in vitro* as described previously [6]. Iron absorption *in vivo* was studied with duodenal loops prepared in rats anesthetized with ether, by placing ligatures just distal to the pylorus and about 4–5 cm distal to the pylorus. The loop was filled with 0.5 ml of the following medium: 0.145 M NaCl; 10^{-4} M $CaCl_2$; 0.04 M D-mannose; 8×10^{-4} M sodium ascorbate, freshly prepared; 0.004 M Tris buffer [Tris(hydroxymethyl)aminomethane, Sigma Chemical Co., St. Louis, MO] pH 7.4; 6.4×10^{-4} M $FeSO_4$ and sufficient $^{59}FeSO_4$ to give approximately 10,000 cpm/ml in a well-type scintillation counter. The abdominal incision was sutured and the rat was allowed to awaken before killing by exsanguination after a blow on the head 30 min after filling the loop. The loop was excised and drained, and the ^{59}Fe in the luminal fluid and in the excised loop wall was estimated, as described previously [6]. The ^{59}Fe absorbed from the lumen into the mucosal surface was calculated as the difference between the initial and final quantities of isotope in the luminal fluid and is expressed as the mucosal uptake. The ^{59}Fe transferred to the blood stream or carcass was calculated as the difference between the ^{59}Fe absorbed at the mucosal surface (mucosal uptake) and the final quantity of isotope in the wall of the excised loop. The recovery of luminal fluid averaged 130 per cent (104–158 per cent) of the initial volume. The increment in volume is due in part to bile, and earlier experiments [6] have demonstrated that bile duct ligation results in a smaller increment in volume and no significant change in iron absorption.

Everted gut sacs were prepared from the duodenum of rats killed by exsanguination after a blow on the head, as described previously [6]. Each sac was about 3.5 cm long with a wet weight of 450–550 mg. The sacs were filled with 0.5 ml of medium similar to that described above but without $FeSO_4$ and placed in 2.5 ml of similar medium with 10^{-4} M $FeSO_4$ and $^{59}FeSO_4$. Sacs were incubated under 100% oxygen, at 37°, in a Dubnoff Incubation Apparatus and then removed, and the total inside (serosal) medium was drained into tubes. The ^{59}Fe in the final mucosal and serosal medium was estimated in a well-type scintillation counter and the mucosal uptake was calculated as the difference between the initial and the final content in the outside (mucosal) medium. Serosal transfer was calculated similarly for the inside (serosal) medium. The data for iron transfer calculated from the ^{59}Fe recoveries in the loop and gut sac experiments were converted to nmoles of iron with the specific activity value of the ^{59}Fe in the initial medium. Statistical analyses were by Student's *t*-test.

The results obtained, and expressed as nmoles of iron

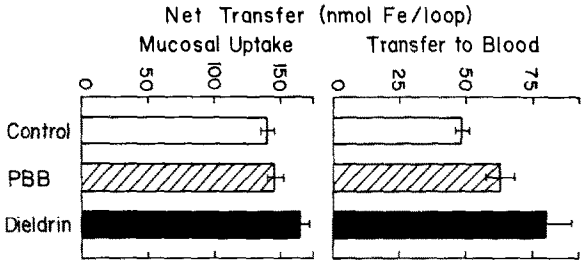

Fig. 1. Values are means ± S.E. Rats were given one dose of 0.2 ml dioxane with or without PBB (200 mg/kg) or dieldrin (500 mg/kg) by gastric tube after an overnight fast, refed 1 hr later and fasted overnight prior to operation 24 hr after the dose. Duodenal loops were tied with ligatures at the pylorus and 4 cm more distally, and filled with 0.5 ml of medium, as described previously [4], under ether anesthesia. The loop was excised and drained 30 min after filling the loop, suturing the abdomen and allowing the rat to awaken. Iron absorption was measured as described previously [4]. The number of rats in each group was: control = 27, PBB = 16 and dieldrin = 9. The differences of the means compared to controls were: $P < 0.001$ for both dieldrin values and PBB transfer to blood and $P < 0.02$ for PBB mucosal uptake (Student's *t*-test).

Table 1. Effects of various polyhalogenated hydrocarbons on iron transport by duodenal gut sacs*

Hydrocarbon	N	Dose (mg/kg)	Net iron transport (nmoles Fe/gut sac) Serosal Transfer Control	Serosal Transfer Chemical	Mucosal uptake Control	Mucosal uptake Chemical
PBB	19	200	7.81 ± 0.8	11.92 ± 1.1†	86.2 ± 1.1	89.7 ± 1.0†
Dieldrin	12	400	8.38 ± 0.8	13.44 ± 0.8†	88.3 ± 1.1	91.7 ± 0.4†
PCTs						
5032	8	400	8.42 ± 1.2	10.61 ± 1.5‡	86.3 ± 0.9	87.4 ± 1.7
5042	8	400	8.42 ± 1.2	10.63 ± 1.4‡	86.3 ± 0.9	88.3 ± 0.8§
5960	8	400	8.42 ± 1.2	9.24 ± 1.0	86.3 ± 0.9	86.9 ± 1.0
PCBs						
1232	26	500	7.10 ± 0.8	7.32 ± 0.5	86.2 ± 1.2	88.8 ± 0.9†
1242	26	500	7.10 ± 0.8	7.00 ± 0.5	86.2 ± 1.2	88.4 ± 0.6†
1254	23	500	7.10 ± 0.8	7.63 ± 0.6	86.2 ± 1.3	89.1 ± 0.8†
1268	20	400	7.10 ± 0.9	9.54 ± 0.7†	86.2 ± 1.4	88.4 ± 0.8†
Mirex	9	200	8.42 ± 1.1	5.84 ± 0.6†	86.3 ± 0.8	84.4 ± 1.4§
Aldrin	9	10	7.20 ± 1.3	7.76 ± 1.0	83.6 ± 2.4	86.6 ± 2.1
Kepone	8	10	7.20 ± 1.4	7.50 ± 1.1	83.6 ± 2.6	84.3 ± 1.4
2,4,5-T	9	100	7.20 ± 1.3	6.65 ± 1.5	83.6 ± 2.4	89.9 ± 1.4†
Tris-BP	9	100	7.20 ± 1.3	8.67 ± 1.2	83.6 ± 2.4	86.3 ± 1.0

* Values are means ± S.E. Rats were given 0.2 ml dioxane, with or without the indicated dose of hydrocarbon, by gastric tube after an overnight fast, refed 1 hr later and fasted overnight prior to sacrifice 24 hr after the dose. Everted duodenal gut sacs were prepared and incubated, as described previously [4]. N is the number of rats in each experimental group. Abbreviations: PBB, polybrominated biphenyl; PCT, polychlorinated terphenyl; PCB, polychlorinated biphenyl; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; and Tris-BP, Tris(2,3-dibromoisopropyl)phosphate.

† $P < 0.001$.

‡ $P < 0.02$.

§ $P < 0.01$.

transferred per loop or gut sac from rats treated with various polyhalogenated aromatic hydrocarbons, are shown in Fig. 1 and Table 1. The insecticide, dieldrin, and the flame retardant, polybrominated biphenyl (PBB), significantly increased both steps of the iron absorption mechanism in duodenal loops *in vivo* (Fig. 1). The greatest effect was on the transfer to the blood, which was increased 31 and 68 per cent, respectively, by PBB and dieldrin ($P < 0.001$). Correspondingly, mucosal uptake was increased 4 and 18 per cent, respectively ($P < 0.02$ and $P < 0.001$). Moreover, the absolute increase in iron taken up by the mucosa was less than the increase in iron transferred to the blood (5.9 and 14.8 nmoles/loop; 25.2 and 32.6 nmoles/loop, for PBB and dieldrin, respectively), suggesting that the predominant effect is on the second step of the absorptive mechanism.

These results were confirmed in experiments with everted duodenal gut sacs *in vitro* prepared from similarly treated rats (Table 1). Serosal transfer of iron was increased 53 and 60 per cent, respectively, by PBB ($P < 0.001$), and dieldrin ($P < 0.001$), whereas mucosal uptake was increased only 4 per cent by each hydrocarbon ($P < 0.001$). Table 1 lists twelve other hydrocarbons tested similarly for their effects on iron transport by everted duodenal gut sacs. Three significantly increased the serosal transfer of iron 26, 26 and 34 per cent, respectively [PCT 5032, PCT 5042 and PCB 1268 ($P < 0.02$, $P < 0.02$ and $P < 0.001$)]. In contrast, the insecticide, mirex, decreased serosal transfer 31 per cent ($P < 0.001$). The other PCT (5960), three PCBs, aldrin, kepone, 2,4,5-T* and Tris-BP did not affect the serosal transfer of iron.

All of the chemicals that increased the serosal transfer of iron also increased mucosal uptake, except PCT 5032. Similar to the effect of PBB and dieldrin, the increase was relatively small, 2–4 per cent, although statistically significant. In addition, the three PCBs that did not affect serosal transfer did increase mucosal uptake 3–4 per cent ($P < 0.001$), and 2,4,5-T increased this step 8 per cent ($P < 0.001$). Mirex, which decreased serosal transfer, decreased mucosal uptake 2 per cent ($P < 0.01$). In summary, all but three of the hydrocarbons tested increased one or both steps of the absorptive mechanism, while one hydrocarbon decreased both steps.

The nine polyhalogenated aromatic hydrocarbons and one straight chain polychlorinated hydrocarbon which affect one or both steps of the iron absorptive mechanism represent major groups of commercially and environmentally important materials. PCBs and PCTs are used as electrical and electronic insulators and in hydraulic and turbine fluids [3]. PBB is a flame retardant [7], dieldrin and mirex are insecticides [8], and 2,4,5-T is a herbicide [9]. Although the use of some of these has been banned or restricted, continued exposure can be anticipated due to the long environmental half-life of most of these compounds and to continued use of structurally related substances [1, 2, 10–12]. This report, in conjunction with similar findings that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) stimulate iron absorption in rats and mice [13, 14], suggests that exposure to polyhalogenated hydrocarbons may pose health hazards related to alterations in iron absorption and metabolism, in addition to the potential hazards of carcinogenicity, mutagenicity and teratogenicity.

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* Abbreviations: 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; and Tris-BP = Tris(2,3-dibromoisopropyl)phosphate.

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Inhibition of anaphylactic histamine release by complexes of lidocaine with zinc

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A protective effect of zinc ions in experimental allergy was shown earlier by us [1] and others [2]. *In vitro* experimental evidence indicates that zinc ions stabilize the membrane of rat peritoneal mast cells and significantly inhibit disruption of these cells by compound 48/80, lecithinase A or antigen–antibody interactions, to prevent histamine release [3, 4].

It has been reported that some local anaesthetics, among them lidocaine also exert an inhibitory effect on histamine release from mast cells [5]. Since lidocaine has ligand properties and can complex with metal ions, two complexes of zinc with lidocaine have been recently prepared [6] in order to combine the inhibitory action of both constituents. We showed that these complexes were more potent than lidocaine and zinc alone in inhibiting histamine release induced by compound 48/80 [7] and the ionophoreous antibiotics A 23187 and X 537A [8].

Here we present evidence that they are also potent inhibitors of anaphylactic histamine release.

Materials and methods. Female F1 hybrids of August and Wistar rats weighing 200–250 g were used for immunization. The antigen used was a dissociated hemocyanin according to White and Holm [9]. Aluminium hydroxide gel was used as an adjuvant/(60 mg $Al(OH)_3$ per ml).

Rats were injected subcutaneously into the back and the foot pads with 500 μg hemocyanin in 1 ml adjuvant [10]. 21 to 30 days after sensitization, mast cells from the abdominal and thoracic cavities were harvested and isolated in a Ficoll density gradient [11]. After isolation of the mast cells they were washed 3 times by centrifugation at 200 g for 5 min using a medium of following composition: 144mM-NaCl, 2.7mM-KCl, 1mM-$CaCl_2$, 2mM-glucose, 1 mg/ml of bovine serum albumin, buffered with Sörensen phosphate buffer 6.7 mM to pH 6.9–7.0. The same medium was used for incubation.

Cells were incubated for 5 min in 2 ml aliquots at 37° in the presence of tested compounds and then antigen (20 μl) was introduced and incubation continued for 10 min.

Following incubation the cells were centrifuged at 350 g for 10 min. The supernatants were decanted into new tubes and 2 ml 0.08 N HCl was added to the cell sediments to lyse the cells.

Histamine was determined fluorometrically according to Shore *et al.* [12] omitting the extraction procedure [13].

Histamine release was calculated as a percentage of the total histamine content of each cell sample. In all experiments triplicate samples were carried out and the mean values were used for calculations. The spontaneous release of histamine amounted to 2.9 ± 0.8 per cent; this has been deducted from all values presented.

The complexes of zinc with lidocaine tested were: a coordination complex in which lidocaine (Lid) is directly bound to metal by an oxygen atom ($ZnLidCl_2$) and an ionic complex in which metal is coordinated with chloride ions and lidocaine occupies an outer coordination sphere ($ZnCl_2HLid_2$, see ref. 6).

Giant keyhole limpet hemocyanin was obtained from Schwartz/Mann (Orangeburg, N.Y.)

Results and discussion. Antigen-induced histamine release in controls amounted to 23.9 per cent (Fig. 1). In the presence of tested agents at concentrations of 10^{-5} M histamine release was significantly inhibited by the ionic complex (14.7% release) whereas the coordination complex only slightly depressed the reaction (18.5% release); at 5×10^{-5} M both complexes markedly inhibited release (54% and 46% for ionic and coordination complex, respectively).

At 10^{-2} M ionic complex completely inhibited antigen-induced release and coordination complex gave 55 percent inhibition.

These results indicate that the ionic complex of lidocaine with zinc is more potent than the coordination complex in inhibition antigen induced histamine release from rat mast cells. From the best fitting pair of parallel regression lines the potency ratio is about 3. A similar pattern of the action of zinc

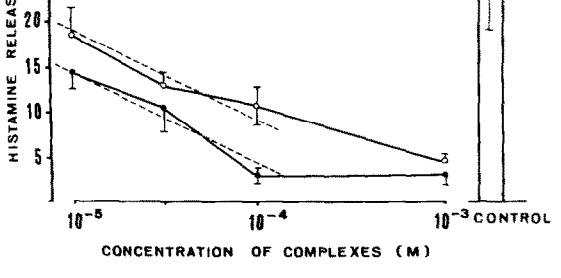

Fig. 1. The effect of zinc complexes with lidocaine on antigen-induced histamine release. O———O; coordination complex; ●———●: ionic complex. Each point represents mean ± S.E. of 4 experiments. Regression lines are marked with dotted line.