Effect of a single oral dose of 250 μg dioxin/kg on body weight, liver weight and liver constituents of male C57 BL/6 mice

Days after dosing		Change in BW since dosing (%)	Liver wet weight (g/100 g BW)	Liver water content (% w/w)	Esterified fatty acids (μequiv/g wet liver)	Cholesterol (mg/g wet liver)	Protein (mg/g wet liver)	DNA (mg/g wet liver)
5	Controls	+ 5.5 ± 0.59 (16)	5.18 ± 0.20	67.2 ± 0.2	112 ± 2.9	2.95 ± 0.24	161 ± 2.3	2.15 ± 0.11
	Treated	+ 2.4 ± 0.54[c] (20)	6.45 ± 0.18[b]	68.9 ± 0.4[b]	156 ± 12.4[a]	5.02 ± 0.30[b]	154 ± 2.6	2.19 ± 0.03
8	Controls	+ 5.8 ± 1.98 (12)	4.24 ± 0.23	68.4 ± 0.2	115 ± 5.0	3.40 ± 0.04	174 ± 1.8	2.52 ± 0.21
	Treated	− 1.5 ± 1.16[b] (16)	4.56 ± 0.23	69.1 ± 0.4	263 ± 22.7[c]	7.33 ± 0.57[c]	156 ± 2.2[c]	2.77 ± 0.18
12	Controls	+ 3.8 ± 1.40 (8)	4.45 ± 0.25	67.6 ± 0.3	89 ± 6.8	3.47 ± 0.37	163 ± 2.8	2.71 ± 0.11
	Treated	− 8.3 ± 2.52[c] (12)	5.33 ± 0.13[a]	61.9 ± 2.9	540 ± 93[b]	10.55 ± 1.35[b]	113 ± 11.1[b]	2.58 ± 0.23
16	Controls	+ 7.2 ± 1.51 (4)	5.01 ± 0.14	68.3 ± 0.7	99 ± 4.7	3.46 ± 0.14	167 ± 5.0	2.45 ± 0.10
	Treated	−16.9 ± 3.82[c] (6)	8.07 ± 0.87[b] (6)	55.5 ± 4.3[a] (7)	742 ± 14.6[b] (7)	11.19 ± 0.72[c] (7)	107 ± 7.4[c] (7)	2.29 ± 0.24 (7)
2[d]	Controls	−18.9 ± 0.71 (7)	4.69 ± 0.08 (7)	—	348 ± 40.8 (7)	4.73 ± 0.19 (7)	—	—
	Treated	−17.7 ± 0.51 (7)	5.57 ± 0.11[c] (7)	—	402 ± 60.7 (7)	5.64 ± 0.11[b] (7)	—	—

Dioxin was dissolved in arachis oil (50 μg/ml), controls received an equivalent volume of arachis oil. Values are means ± SEM for groups of 4 mice (unless number of mice is otherwise indicated in brackets). Those marked differ significantly from the controls: [a] $p < 0.025$; [b] $p < 0.01$; [c] $p < 0.001$. [d] Food was withheld from both groups immediately following dosing.

of the liver wet weight as compared with 3% in the control mice. At later periods these increased lipid levels of the treated mice were associated with decreased liver protein and water contents. The fact that the DNA content of the liver was unaltered despite the increase in liver size may have been due to the inflammatory infiltration of the liver. Starvation of mice following dosing with either dioxin or oil resulted in elevated liver esterified fatty acid levels in both groups. However the dioxin treatment had caused significant increases in mean liver weight and cholesterol content (Table).

The pathological findings in the liver are similar to those reported by GOLDSTEIN et al.[10], and differ in many respects from the liver lesion induced in rats by dioxin[3, 11]. Extensive centrilobular necrosis, bile duct proliferation and lipid accumulation are not features of the lesion in rats and multinucleate hepatocytes were not seen in mice. In surviving animals of both species, the liver lesion regressed with little change in the basic liver architecture.

Although these results clearly indicate that dioxin induced a fatty liver in C57BL/6 mice the mechanism of this induction is possibly indirect. Mice are known to develop fatty livers following starvation and the loss of body weight following dioxin treatment would suggest that the lipid accumulation was, at least partially, a consequence of a depression of food intake. The increased cholesterol in the livers of dioxin treated mice may be associated with the synthesis of new membrane-bound enzymes[5, 12].

Summary. In C57BL/6 mice a single oral dose of 2, 3, 7, 8-tetrachlorodibenzodioxin (LD_{50} 126 μg/kg) results in loss of body weight and death with an enlarged fatty liver after ca. 21 days. A progressive necrotic centrilobular liver lesion is also seen.

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Quaternary Isoarecoline: a Potent Muscarine-Like and Nicotine-Like Acting Agonist

In our studies of cyclic and semi-rigid analogs of acetylcholine, we have synthesized and tested derivatives of isoarecaidine (1-methyl-1, 2, 5, 6-tetrahydroisonicotinic acid)[1, 2].

In several types of preparation (rat blood pressure, smoth muscle, heart muscle) the quaternary methylester (isoarecoline) shows a high muscarinic activity. On the guinea-pig ileum, it has the same intrinsic activity and affinity ($pD_2 = 8.0$) as acetylcholine (Figure 3).

It has been reported that arecoline (methyl 1-methyl-1, 2, 5, 6-tetrahydronicotinate) acts as a potent muscarinic agonist through the release of endogenous acetyl-

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choline from nerve terminals[3]. Because of the great structural analogy between arecoline and isoarecoline, we have investigated the site of action of the quaternary isoarecoline, and whether it acts directly by stimulation of the muscarinic receptor or indirectly by mobilizing endogenous acetylcholine.

Methods. The experiments were made on strips of isolated guinea-pig ileum, suspended in Tyrode solution of 34 °C, gassed with 95% oxygen and 5% carbon dioxide in a bath of 10 ml capacity. The responses of the gut were recorded using a transducer and a Hellige recorder. Solutions of the compounds were prepared in distilled water and the concentrations of the two agonists used caused a contraction of the isolated ileum of 100% of the maximal contraction possible with the drug concerned:

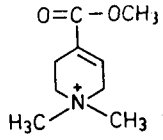

Fig. 1. Quaternary isoarecoline.

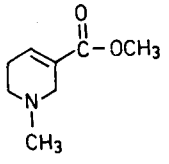

Fig. 2. Arecoline.

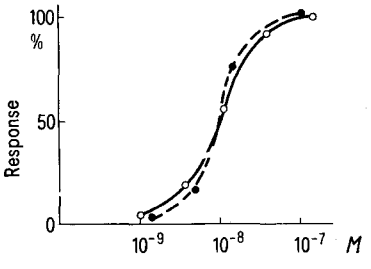

Fig. 3. Dose-response curves of acetylcholine (○) and of the quaternary isoarecoline (●) on the guinea-pig ileum. Each point is the mean of 4 experiments.

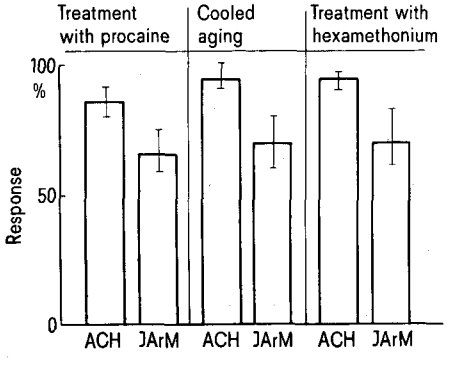

Fig. 4. The responses to single doses of acetylcholine (ACH, 1×10^{-7} *M*) and isoarecoline methoiodide (IArM, 1×10^{-7} *M*) on the guinea-pig ileum, applied in the presence of hexamethonium, after treatment with procaine and after cooling the gut. The contractions are represented as percentages of the responses under control conditions. The bars represent standard errors.

acetylcholine, 1×10^{-7} *M*; quaternary isoarecoline, 1×10^{-7} *M*. The maximal volume of drug solutions used was 0.2 ml.

Procaine causes a reduction of the output of acetylcholine at the nerve terminals[4]. After incubation of the strips for 40 min with 3×10^{-3} *M* procaine and washing 3 times with fresh Tyrode solution, the responses to indirectly acting cholinergic agonists are strongly reduced, whilst the activity of directly acting cholinergic drugs is little affected. The nerve tissue in the guinea-pig ileum is irreversibly damaged when it is stored in Tyrode solution without oxygen at 2 °C for 20 h[5,6]. After this treatment, the isolated gut is still responsive to exogenous acetylcholine, whilst the output of endogenous acetylcholine from the tissue decreases. In the presence of the ganglionic blocker hexamethonium (3×10^{-4} *M*), the action of nicotine-like ganglionic stimulants is blocked, whilst the activity of other indirectly acting cholinergic and directly acting muscarinic agents is not reduced. Because of its dependence on endogenous acetylcholine, the nicotinic action is classified as an indirect cholinergic action[5].

Results. Atropine sulphate (1×10^{-7} *M*) completely antagonized the spasmogenic effect of isoarecoline. Preliminary treatment of the isolated organ with physostigmine sulphate (4×10^{-7} *M*) does not intensify the activity of isoarecoline. These experiments show that isoarecoline is a cholinergic agonist and it is not a substrate for acetylcholine esterase.

The ganglionic blocking agent hexamethonium, procaine treatment and cooled aging blocked the responses to acetylcholine and to quaternary isoarecoline to a certain degree (Figure 4). The inhibition by hexamethonium indicates that a part of the cholinergic action of these two spasmogens is based on a nicotine-like action at the level of the ganglionic synapse resulting in a liberation of endogenous acetylcholine at the nerve terminals. The nicotine-like activity of the quaternary isoarecoline (30% blockade by hexamethonium) is greater than that of acetylcholine (5% blockade by hexamethonium). Because of the same respectively nearly the same extent of the decrease of the response to the quaternary isoarecoline in all 3 preparations, the cholinergic action of this drug is not based on increased liberation of acetylcholine at the nerve terminals by the action on these nerve terminals.

The quaternary isoarecoline possesses, like acetylcholine, a mixed cholinergic action, a combination of a direct stimulation of the muscarinic receptor and an indirect cholinergic action by stimulation of the nicotinic receptor at the level of the ganglionic synapse.

Summary. Quaternary isoarecoline (methyl 1-methyl-1,2,5,6-tetrahydroisonicotinate methoiodide) is a muscarine-like and nicotine-like agonist. Its action is not based on liberation of acetylcholine at the nerve terminals by the action on these nerve terminals.

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