Enhancement of Intestinal Glucose Transport following Chronic Parathion Poisoning

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Abstract ☐ Chronic administration of parathion to male rats stimulated glucose ($p < 0.05$) but not calcium ($p > 0.05$) transport in the everted duodenal gut sac preparation. Chronic parathion stimulation was not reduced by concurrent administration of atropine. Acutely applied parathion or paraoxon, its active metabolite, did not increase glucose transport in this preparation.

Keyphrases ☐ Parathion—chronic administration, effect on intestinal glucose and calcium transport, rats ☐ Paraoxon—chronic administration, effect on intestinal glucose and calcium transport, rats ☐ Glucose transport, intestinal—effect of chronic administration of parathion or paraoxon, rats ☐ Calcium transport, intestinal—effect of chronic administration of parathion or paraoxon, rats ☐ Insecticides—parathion and paraoxon, chronic administration, effect on intestinal glucose and calcium transport, rats

Parathion (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate), a widely used insecticide, is toxic to both insects and mammals only after conversion to the active metabolite paraoxon (*O,O*-diethyl *O-p*-nitrophenyl phosphate), which is a potent inhibitor of cholinesterases. The toxicity observed after parathion poisoning results from an accumulation of acetylcholine at certain critical sites subsequent to cholinesterase inhibition. Other effects not clearly associated with cholinesterase inhibition also have been reported for organophosphates. Two organophosphate cholinesterase-inhibiting insecticides (azinphosmethyl and dimethoate) reduced glucose penetration across the isolated mouse intestine (1). Dimethoate resembles parathion in that it requires activation to an active metabolite before becoming a cholinesterase inhibitor; azinphosmethyl does not require activation. The reduction of glucose penetration observed (1) in the presence of parathion was not significant ($p > 0.05$).

It is unlikely that a large portion of an organophosphate that requires activation would be activated in the isolated intestine. Thus, any change in intestinal glucose transport occurring after the addition of such an organophosphate would probably result from a direct effect of the unactivated organophosphate or from an effect of a very small quantity of active metabolite. Therefore, it was of interest to determine the effects of acute exposure to parathion and its active metabolite paraoxon and of chronic parathion poisoning on intestinal glucose transport. The mechanism and specificity of these effects on glucose transport were also examined.

EXPERIMENTAL

Two experiments were undertaken to examine the effects of parathion or paraoxon on intestinal transport of glucose and calcium. One experiment concerned the effects of the acute addition of the insecticides *in vitro*. Male Holtzman rats were initially placed on a diet of ground rat chow[1] and tap water for 7 days and then on a ground, low calcium diet[2] (0.02% calcium) and distilled water for 7 days. Each animal was given vitamin D (200 units po) every 3rd day. The mean weight of the rats at the time of sacrifice was 81 ± 10 g. The low calcium diet and vitamin D were used to optimize calcium transport.

Rats were fasted overnight before the intestinal segments were removed. Upon removal of the segments, they were placed into one of three types of incubation media: with parathion (0.1 m*M*), with paraoxon (0.1 m*M*), or with no insecticide. Each of the three solutions contained polysorbate 80[3] (0.03%), used to solubilize the insecticides. The organophosphate was in the media both inside and outside the gut sacs.

In the other experiment, the effects of chronic parathion poisoning on intestinal transport were investigated. Rats, 60–80 g (mean of 69 g), were randomly divided into four groups. One group received rat chow containing 20 ppm of parathion, a second group received rat chow containing 20 ppm each of parathion and atropine, and a third group was fed rat chow containing no parathion as a control. Each group was allowed free access to food. In another control group, rats were pair-fed with the parathion-poisoned rats.

Rats were poisoned for 14 days. Vitamin D was administered to these rats as described in the previous experiment; during the last 7 days of the poisoning period, the poisons were administered in the low calcium diet previously described. Animals were fasted overnight before removal of the intestinal segments. Thus, poisoning was discontinued 15–18 hr before removal of the intestinal segments. The mean weight of the control rats at the time of sacrifice was 108 ± 10 g.

Glucose and calcium transport were measured by a modification of the everted gut sac technique of Wiseman (2). The animals were lightly anesthetized with ether, bled by cardiac puncture, and killed by cervical fracture. Blood was collected in heparinized syringes, and the plasma was separated immediately. The upper intestine was quickly removed, everted, and washed in saline. Duodenal segments, 5 cm in length, were filled with 0.5 ml of an oxygenated phosphate buffer and placed into 25-ml erlenmeyer flasks containing 3 ml of the same solution, which was sufficient to cover the gut sac.

The phosphate buffer contained 2.4 m*M* dibasic sodium phosphate, 1.6 m*M* monobasic sodium phosphate, 150 m*M* sodium chloride, 5.6 m*M* glucose, and 0.045 μCi/ml of calcium-45. The buffer pH was adjusted to 7.4. The duodenal segments were incubated under an atmosphere of 95% O_2–5% CO_2 for 1 hr at 37° on a gyrorotary metabolic shaker[4]. The ratio of the final concentration of glucose and calcium-45 in the serosal medium (solution inside the gut sac) to the concentration in the mucosal medium (solution bathing the gut sac) was used as an index of transport.

Calcium-45 in the mucosal and serosal media was measured by counting 0.1-ml aliquots of the media on a liquid scintillation counter[5]. Plasma calcium was determined by the method of Baginski *et al.* (3). Plasma and incubation medium glucose was measured by the method of Feteris (4).

In the parathion and control groups of the chronic study, a 2-cm section of intestine immediately adjacent to that used in each transport study was removed, washed, blotted dry, weighed, and frozen for the later determination of cholinesterase activity. This assay was accomplished by the automated colorimetric method of Fowler and McKenzie (5).

Statistical comparisons were made using the Student t test for unpaired data.

RESULTS

Both glucose and calcium were actively transported in the rat everted duodenal sac from calcium-deprived rats (Table I). Neither parathion nor paraoxon affected ($p > 0.05$) the transport of either of these substances when the toxins were added to the incubation media.

Rats chronically poisoned with parathion had diarrhea and other signs of parasympathetic stimulation. These signs tended to become less severe

[1] Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.
[2] General Biochemicals, Chagrin Falls, Ohio.
[3] Tween 80, Atlas Chemical Co., Wilmington, Del.
[4] New Brunswick Scientific, New Brunswick, N.J.
[5] Beckman model LS-100, Beckman Instruments, Fullerton, Calif.

Table I—Effects of Acute Exposure to Parathion or Paraoxon on Glucose and Calcium Transport in Duodenal Segments

Treatment	Transport, Serosal/Mucosal[a]	
	Glucose	Calcium
Control	3.9 ± 0.80	4.2 ± 0.42
Parathion, 0.1 m*M*	3.4 ± 0.48	4.2 ± 0.44
Paraoxon, 0.1 m*M*	4.2 ± 1.02	4.8 ± 0.88

[a] After 60-min incubation. Each value is the mean ± *SEM* of one segment from each of five or six rats.

with time and, in some cases, disappeared toward the end of the 14-day poisoning period. Rats receiving atropine and parathion exhibited few, if any, signs of parasympathetic stimulation.

Cholinesterase activities were lower ($p < 0.05$) in segments from parathion-poisoned rats (7.16 ± 0.95 m*M* of acetylcholine hydrolyzed/min/g of tissue) than in segments from control rats (10.49 ± 0.89).

Chronic parathion poisoning enhanced intestinal glucose transport ($p < 0.05$) without affecting calcium transport in calcium-deprived rats (Table II). Glucose and calcium transport in the control group was lower than in the acute experiment. At the time of sacrifice, these rats averaged 27 g more than those in the acute experiment. A decreasing rate of active intestinal transport with maturing may have accounted for some, or perhaps all, of the differences in control transport rates between the two experiments. Rats weighing less than 100 g have a faster rate of intestinal glucose transport than heavier rats (6), and the rate of intestinal transport of calcium in rats decreases with maturing (7). However, treatment-induced changes in transport should not be influenced by differences in baseline values in the control groups.

Addition of the muscarinic blocking drug atropine did not decrease the parathion-induced enhancement of glucose transport. Thus, if increased glucose transport results from acetylcholine accumulation following cholinesterase inhibition, it is unlikely that it is mediated through stimulation of muscarinic receptors.

Reduction in food intake by parathion-poisoned rats apparently was not involved in the enhanced glucose absorption, judging from the glucose transport values observed in intestinal segments from control rats pair-fed with poisoned rats and from control rats allowed to eat *ad libitum* (Table II).

Neither plasma glucose nor plasma calcium levels were different ($p > 0.05$) in chronically parathion-poisoned rats (glucose, 125.4 ± 17.2 mg %; calcium, 11.7 ± 0.5 mg %) than in control rats (glucose, 128.3 ± 11.4 mg %; calcium, 11.3 ± 0.4 mg %).

DISCUSSION

Although acute *in vitro* exposure of duodenal segments to parathion or its active metabolite paraoxon did not alter glucose transport, chronic poisoning with parathion enhanced it. Concurrent administration of atropine did not alter the parathion effect. However, since both atropine and parathion were discontinued 15 or more hr prior to obtaining the duodenal segments, neither atropine, free paraoxon, nor parathion should have been present in the duodenum at the time of the transport study. However, partial inhibition of cholinesterase (30%) was still observable at this time. Thus, if the enhancemnt of glucose transport results from the accumulation of acetylcholine, either it does not involve chronic stimulation of muscarinic receptors or the period of time after the bioremoval of atropine was sufficient to allow this effect to develop.

One model of glucose transport across the intestinal epithelial cell has glucose transferred across the brush border coupled with a sodium ion (8, 9) by a membrane carrier system allowing coupled facilitated diffusion. The model requires that the sodium concentration be lower inside the cell than in the lumen. This concentration gradient is maintained by a sodium pump (10). Thus, stimulation of movement of sodium ions into the cells should increase the rate of glucose transport. One means of accomplishing this is by stimulating the extrusion of sodium from epithelial cells. Phenytoin stimulated extrusion of sodium ions in brain (11) and muscle (12) cells and also enhanced intestinal glucose absorption in the rat isolated jejunal loop (13). Sodium transport by the acetylcholine receptor in cultured muscle cells has been observed (14). If acetylcholine stimulates a receptor to enhance coupled sodium and glucose transport, it should probably also do so on acute exposure. However, enhanced glucose transport on acute exposure to cholinesterase inhibitors was not observed. Also, acute applications of acetylcholine in isolated human ileal strips did not affect sodium transport (15).

Table II—Effects of Chronic Poisoning with Parathion or Parathion and Atropine on Glucose and Calcium Transport in Duodenal Segments

Treatment[a]	Transport, Serosal/Mucosal[b]	
	Glucose	Calcium
Control	2.6 ± 0.69	2.4 ± 0.50
Control (paired with parathion)	2.6 ± 0.57	2.1 ± 0.37
Parathion, 20 ppm	4.4[c] ± 0.38	3.1 ± 0.37
Parathion, 20 ppm, and atropine, 20 ppm	4.5 ± 0.97	3.2 ± 0.60

[a] In rat chow for 14 days. [b] After 60-min incubation. Each value is the mean ± *SEM* of one segment from each of four or five rats. [c] Significantly different from control-paired ($p < 0.05$) and control ($p = 0.05$).

This effect may be unrelated to cholinesterase inhibition. Whatever the mechanism, the results of this study indicate that sublethal chronic exposure to parathion stimulates glucose transport in the rat duodenum. Furthermore, this enhancement shows some specificity, since the rate of calcium transport was not significantly altered.

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