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Neonatal Parathion Exposure and Interactions with a High-Fat Diet in Adulthood: Adenylyl Cyclase-Mediated Cell Signaling in Heart, Liver and Cerebellum

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Abstract

Organophosphates are developmental neurotoxicants but recent evidence points to additional adverse effects on metabolism and cardiovascular function. One common mechanism is disrupted cell signaling mediated through cyclic AMP, targeting neurohumoral receptors, G-proteins and adenylyl cyclase (AC) itself. Earlier, we showed that neonatal parathion evokes later upregulation of the hepatic AC pathway in adolescence but that the effect wanes by young adulthood; nevertheless metabolic changes resembling prediabetes persist. Here, we administered parathion to neonatal rats (postnatal days 1–4, 0.1 or 0.2 mg/kg/day), straddling the threshold for cholinesterase inhibition, but we extended the studies to much later, 5 months of age. In addition, we investigated whether metabolic challenge imposed by consuming a high-fat diet for 7 weeks would exacerbate neonatal parathion's effects. Parathion alone increased the expression or function of G_i , thus reducing AC responses to fluoride. Receptors controlling AC activity were also affected: β -adrenergic receptors (β ARs) in skeletal muscle were increased, whereas those in the heart were decreased, and the latter also showed an elevation of m_2 -muscarinic acetylcholine receptors, which inhibit AC. The high-fat diet also induced changes in AC signaling, enhancing the hepatic AC response to glucagon while impairing the cardiac response to fluoride or forskolin, and suppressing β ARs and m_2 -muscarinic receptors; the only change in the cerebellum was a decrease in β ARs. Although there were no significant interactions between neonatal parathion exposure and a high-fat diet, their convergent effects on the same signaling cascade indicate that early OP exposure, separately or combination with dietary factors, may contribute to the worldwide increase in the incidence of obesity and diabetes.

Keywords

Adenylyl Cyclase; β -Adrenergic receptor; Cyclic AMP; Heart development; High-fat diet; Liver development; Muscarinic receptor; Organophosphate insecticides; Parathion

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INTRODUCTION

Organophosphate (OP) pesticides represent nearly half of worldwide insecticide use [4] but are undergoing increased scrutiny because of their propensity to elicit developmental neurotoxicity at levels that are below the threshold for acute signs of exposure, and even doses lower than those required for cholinesterase inhibition, the mechanism that is typically used for biomonitoring [8,22,23,27]. Pregnant women and young children are typically exposed to OPs under these low-dose conditions [5,9,17] and recent findings confirm that such exposures can produce long-term neurobehavioral impairment [6,7,19,21]. Although the systemic toxicity and signs of OP intoxication reflect their shared ability to inhibit cholinesterase [16, 18], other important mechanisms of toxicity clearly exist. Recent data point to dysregulation of cell signaling cascades as one of the critical targets that contribute to the adverse outcomes seen at lower exposures [8,22,23,27]. Chief among these is the pathway that generates cyclic AMP, a critical second messenger that mediates numerous neurotransmitter and hormonal receptor signals; these are linked to the generation of cyclic AMP through G-proteins that regulate adenylyl cyclase (AC), the enzyme that synthesizes cyclic AMP from ATP.

The impact of OPs on AC signaling is critically important for effects outside the central nervous system, since cyclic AMP controls cell function in all the organs and tissues involved in metabolic and cardiovascular homeostasis. In adult rats, chlorpyrifos exposures exceeding the threshold for cholinesterase inhibition lead to enhanced weight gain [14] and diabetes-like changes in hepatic energy metabolism [1]. At lower doses administered during development, chlorpyrifos also produces excess weight gain and dysregulation of leptin [11], along with a metabolic profile resembling prediabetes [24]. Similarly, when we exposed neonatal rats to parathion at doses straddling the threshold for barely-detectable cholinesterase inhibition, we found later emergence of a prediabetes-like state, involving excessive weight gain, hyperglycemia, abnormalities of lipid metabolism and adipose tissue inflammation [12,13]. Further, many of the metabolic effects of early-life OP exposure were exacerbated when animals were switched to a high-fat diet in adulthood, including a much greater fat-induced weight gain than that with the equivalent dietary change in controls [12,13,20].

Although OPs are likely to disrupt metabolism and cardiovascular function at many different levels, one common feature is their ability to produce lasting changes in AC signaling, notably involving a net gain of pathway function, an effect noted for chlorpyrifos, diazinon and parathion [2,15]. For all three, early-life OP exposure produced heterologous sensitization of the AC pathway later in life, with parallel increases in all aspects of activity, ranging from receptor-mediated stimulation, through effects on G-protein activity, and on the expression or catalytic activity of AC itself. However, there was a difference in that the effects of parathion on AC signaling waned between adolescence and young adulthood, whereas those of diazinon and chlorpyrifos did not [2,15]. Nevertheless, the metabolic consequences of neonatal parathion exposure persisted [12,13], suggesting either that the earlier changes are sufficient to reprogram metabolic function, or alternatively that the AC changes are not critical to parathion's effects on metabolism. The latter seems highly unlikely, given that cyclic AMP is one of the primary mediators of hepatic gluconeogenesis, glycogenolysis and lipolysis. In the current study, we explored whether the additional metabolic stress imposed by consuming a high-fat diet in adulthood could unmask persistent effects of neonatal parathion exposure on AC signaling, an approach modeled after our earlier studies on metabolic effects of OPs [12, 13,20]. As before, we focused on parathion treatment regimens straddling the threshold for cholinesterase inhibition [26] and then in adulthood, we switched half the animals to a high-fat diet that more than doubles serum β -hydroxybutyrate concentrations [13]. To assess the impact on AC signaling, we evaluated function at each step in the cascade: basal AC activity, the responses mediated by β -adrenergic receptors (β AR) and glucagon receptors, the response to G-protein activation by fluoride, and activity with maximal activation of AC itself by

forskolin [10]. Further, we measured ligand binding for β ARs and for the inhibitory m_2 -muscarinic acetylcholine receptors (m_2 AChRs). We conducted our studies in peripheral tissues (heart, liver) and compared the effects to those seen in a brain region (cerebellum) that shows a similar AC response to β AR activation. Finally, we also assessed β AR binding in a skeletal muscle (gastrocnemius) to determine whether this prominent site of energy utilization was affected by parathion or the high-fat diet, separately or together.

MATERIALS AND METHODS

Animal treatments and diet

All experiments were carried out humanely and with regard for alleviation of suffering, with protocols approved by the Duke University Institutional Animal Care and Use Committee and in accordance with all federal and state guidelines. Timed-pregnant Sprague–Dawley rats were housed in breeding cages, with a 12 h light–dark cycle and free access to water and food (PMI LabDiet 5001). On the day after birth, all pups were randomized and redistributed to the dams with a litter size of 10 (5 males, 5 females) to maintain a standard nutritional status. Parathion was dissolved in dimethylsulfoxide to provide consistent absorption [26,28,30] and was injected subcutaneously in a volume of 1 ml/kg once daily on postnatal days 1–4; control animals received equivalent injections of the dimethylsulfoxide vehicle. Doses of 0.1 and 0.2 mg/kg/day were chosen because they straddle the threshold for barely-detectable cholinesterase inhibition and the first signs of reduced weight gain or impaired viability [26, 28]. To avoid the possibility that dams might distinguish between control and parathion-treated pups, all pups in a given litter received the same treatment. Randomization of pup litter assignments within treatment groups was repeated at intervals of several days up until weaning, and in addition, dams were rotated among litters to distribute any maternal caretaking differences randomly across litters and treatment groups. Offspring were weaned on postnatal day 21 and the final litter assignment for each rat was noted. Studies were conducted using one male and one female from each final litter, with 6 animals for each group, defining a group as a specific neonatal treatment, dietary condition and sex. After weaning, animals were separated by sex and housed in groups according to standard guidelines.

Beginning at 15 weeks of age, half the rats were switched to a high-fat diet (OpenSource D12330), providing 58% of total calories as fat; 93% of the fat is hydrogenated coconut oil. The remaining rats continued on the standard LabDiet 5001 diet, which provides 13.5% of total calories as fat; with this diet, 27% of the fat is saturated. Although the high-fat diet contains 37% more calories per gram, we found that animals on this diet reduce their food intake by approximately the same proportion [13], so that the total dietary intake is isocaloric; nevertheless, animals gain excess weight when fed a diet with a higher fat content [13]. During the 24th postnatal week, animals from each of the finally-assigned litters were decapitated and the heart, one liver lobe (the same lobe from each animal), cerebellum and gastrocnemius muscle were dissected, blotted, frozen in liquid nitrogen and maintained at -45°C .

Assays

Tissues were thawed and homogenized (Polytron; Brinkmann Instruments, Westbury, NY) in buffer containing 145 mM sodium chloride, 2 mM magnesium chloride, and 20 mM Tris (pH 7.5), strained through several layers of cheesecloth to remove connective tissue, and the homogenates were then sedimented at $40,000 \times g$ for 15 min. The pellets were washed twice and then resuspended in 250 mM sucrose, 2 mM MgCl_2 , and 50 mM Tris. For determinations of AC activity, aliquots of the membrane preparation were incubated for 30 min (heart, liver) or 10 min (cerebellum) at 30°C with final concentrations of 100 mM Tris-HCl (pH 7.4), 10 mM theophylline, 1 mM ATP, 2 mM MgCl_2 , 10 μM GTP, 1 mg/ml bovine serum albumin, and a creatine phosphokinase–ATP–regenerating system consisting of 10 mM sodium

phosphocreatine and 8 IU/ml phosphocreatine kinase. The enzymatic reaction was stopped by heating and sedimentation, and the supernatant solution was then assayed for cyclic AMP using commercial radioimmunoassay or immunoassay kits; the two types of kits gave equivalent results. In addition to assessing basal AC activity, we evaluated responses to 100 μ M isoproterenol, 3 μ M glucagon, 10 mM NaF and 100 μ M forskolin. These concentrations produce maximal responses to each stimulant as assessed in earlier studies [3,31,32].

To evaluate β AR binding, aliquots of the same membrane preparation were incubated with 67 pM [125 I]-iodopindolol in 145 mM NaCl, 2 mM MgCl_2 , 1 mM sodium ascorbate, 20 mM Tris (pH 7.5), for 20 min at room temperature; samples were evaluated with and without 100 μ M isoproterenol to displace specific binding. Incubations were stopped by addition of 3 ml ice-cold buffer, and the labeled membranes were trapped by rapid vacuum filtration onto glass fiber filters, which were washed with additional buffer and counted by liquid scintillation spectrometry. For cardiac m_2 AChR binding, the membrane suspension was reconstituted in 10 mM sodium-potassium phosphate buffer (pH 7.4) and incubated with 1 nM [3 H] AFDX384, with or without 1 μ M atropine to displace specific binding.

We did not assess m_2 AChR binding in the liver, cerebellum or gastrocnemius muscle because of the sparsity of the receptors in these tissues. We conducted preliminary studies of AC activity in the gastrocnemius muscle and found extremely variable results; accordingly, these determinations also were not carried out in the present work. Similarly, we did not evaluate the effects of glucagon in the cerebellum, a tissue in which this metabolic hormone has no known biologic role.

Data analysis

Data were compiled as means and standard errors. The experimental design was identical to that in two previous papers using animals from the same treatment cohorts [13,25], so the overall statistical procedures were the same and will be described only briefly here. Each set of determinations began with a global ANOVA incorporating all variables in a single test: neonatal treatment, diet, sex, tissue and the multiple dependent measures made for each class of variables, with the latter regarded as repeated measures (since multiple measurements were made from the same tissue sample). Data were log-transformed because of heterogeneous variance contributed by tissue, diet and sex. When this initial test showed main effects of the contributing variables as well as significant interactions among the variables, we then conducted a series of nested, lower-order ANOVAs separating the measurements according to the interactive variables. Where permitted, the lower-order tests were followed by Fisher's Protected Least Significant Difference to establish individual groups that differ from the corresponding control. For all tests, significance was assumed at $p < 0.05$. However, for interactions at $p < 0.1$, we also examined whether lower-order main effects were detectable after subdivision of the interactive variables [29]. The criterion for interaction terms was not used to assign significance to the effects but rather to identify interactive variables requiring subdivision for lower-order tests of main effects of parathion or the high-fat diet, the variables of chief interest. Where treatment effects were not interactive with other variables, we report only the main treatment effects without performing lower-order analyses of individual values.

To ensure that treatment and diet effects could be compared across all groups, all assays were conducted simultaneously on all samples for a given tissue and sex, but technical limitations dictated that each tissue and sex had to be performed in divided runs. Accordingly, the control values for tissue vs. tissue or for males vs. females should not be compared directly, since each tissue was assayed separately, as was each sex. However, treatment and diet effects and their interactions with tissue and sex *can* be interpreted, since these depend solely on the internal comparison to the matched control groups that were run together. Because of the large range of AC activities for different tissues and stimulants, the scales for each graph were adjusted to

give an equivalent visual presentation of the treatment- and diet-related differences, irrespective of the differences in absolute values.

Materials

Animals were purchased from Charles River (Raleigh, NC). LabDiet 5001 came from PMI Nutrition (St. Louis, MO) and the OpenSource D12330 diet came from Research Diets Inc. (New Brunswick, NJ). Parathion (99.2% purity) was obtained from Chem Service (West Chester, PA). [125 I]Iodopindolol (specific activity, 2200 Ci/mmol) and [3 H]AFDX384 (115 Ci/mmol) both came from PerkinElmer Life Sciences (Boston, MA), and cyclic AMP radioimmunoassay and enzyme immunoassay kits were purchased from GE Healthcare Biosciences (Piscataway, NJ). All other chemicals were bought from Sigma Chemical Company (St. Louis, MO).

RESULTS

Body and tissue weights (data not shown)

Global ANOVA incorporating all variables (treatment, diet, sex) and the five dependent measures (body weight, heart weight, liver lobe weight, cerebellum weight, gastrocnemius weight) identified significant main effects of diet ($p < 0.0001$), sex ($p < 0.0001$) and tissue ($p < 0.0001$), along with interactions among these three variables: $p < 0.0004$ for diet $\times$ sex, $p < 0.0001$ for diet $\times$ tissue, $p < 0.0001$ for sex $\times$ tissue, and $p < 0.02$ for diet $\times$ sex $\times$ tissue. Since there was no main treatment effect of parathion or interaction of treatment with the other variables, we separated the values for the individual tissues and still obtained a diet $\times$ sex interaction for each peripheral measure ($p < 0.04$ for body weight, $p < 0.02$ for heart, $p < 0.009$ for liver, $p < 0.02$ for gastrocnemius) but not for the cerebellum. For body weights, the main effect of diet was significant in both males ($p < 0.0003$) and females ($p < 0.0001$), with the sex difference reflecting a greater effect of the high-fat diet in females, as reported earlier [13]. Similarly, the high-fat diet evoked significant weight increases of 15-20% in heart ($p < 0.0002$), liver ($p < 0.003$) and gastrocnemius ($p < 0.0001$) in females but not males.

The weight results represent the values from the animals used in the current study. Earlier, we reported on the longitudinal effects of neonatal parathion treatments and dietary manipulations on body weights in a much larger group from the same cohort of animals [13], which revealed further effects not evident from the smaller group. Parathion had no initial effect on body weight during or immediately after the exposure period but after weaning, the parathion group displayed a small (2-3%), significant elevation in weight at the low dose in males, and reductions of about 4% at either dose in females. Regardless of the neonatal treatment, the high fat diet increased body weights by about 10% in males and 30% in females, with the low dose of parathion augmenting the effect of the high-fat diet on weight gain.

Adenylyl cyclase

The global ANOVA (factors of treatment, diet, sex, tissue; dependent measures for each AC stimulant condition) indicated interactions of sex $\times$ measure ($p < 0.0001$), tissue $\times$ measure ($p < 0.0001$), diet $\times$ tissue $\times$ measure ($p < 0.02$), sex $\times$ tissue $\times$ measure ($p < 0.0001$), and diet $\times$ tissue $\times$ measure ($p < 0.02$). Accordingly, we separated the values for the individual tissues and then performed lower-order analyses. The global test had to omit the response to glucagon, since this stimulant was not tested in the cerebellum; this measure was reinstated for the separate analyses of heart and liver, which then uncovered significant treatment effects of parathion. Additionally, the repeated measures analysis of the different AC stimulants confirmed significant responses to each of the agents ($p < 0.0001$ collectively as well as individually in each tissue). However, the stimulant responses differed among the three tissues (stimulant $\times$ tissue interaction, $p < 0.0001$). In the heart, the rank order was forskolin $>$ fluoride

> isoproterenol > glucagon > basal AC, pointing out the predominant role of β AR input as compared to glucagon. In the liver, the sequence was forskolin > glucagon > fluoride > isoproterenol > basal AC, reflecting the greater physiological importance of glucagon in the metabolic response; indeed, glucagon stimulation in the liver was greater than that of fluoride, which maximally activates both excitatory (G_s -related) and inhibitory (G_i -related) G-protein responses. In the cerebellum, the rank order of responses was forskolin > isoproterenol $\approx$ fluoride > basal, indicating that β AR stimulation is highly coupled to G-proteins in this brain region.

In the heart, the global ANOVA identified a significant interaction of diet $\times$ measure $p < 0.0001$, necessitating a separate consideration for each AC measurement; however, there were no parathion-related effects, nor was there a significant interaction of parathion $\times$ diet or parathion $\times$ diet $\times$ other variables. The high-fat diet did not have any net effect on basal AC (Fig. 1A), isoproterenol-stimulated AC (Fig. 1B) or glucagon-stimulated AC (Fig. 1C), but evoked a significant decrement in the response to fluoride (Fig. 1D) and forskolin (Fig. 1E). To illustrate the differences in the main effect of diet across the three treatment groups and both sexes, we collapsed all the interactive variables and determined the average effect (geometric mean) on each AC parameter (Fig. 1F). This procedure dilutes any specific decreases by averaging them with smaller effects, so that the absolute magnitude is reduced; nevertheless there was a clear hierarchy of effects reflecting the conclusions reached in the multivariate ANOVA, namely a net decrease evoked by the high-fat diet for the fluoride and forskolin responses.

In the liver, the global test indicated sex-dependent effects of both parathion treatment and diet: $p < 0.05$ for treatment $\times$ sex and $p < 0.05$ for diet $\times$ sex $\times$ measure. Accordingly separate evaluations of each measure were conducted for males and females. Again, there were no parathion or diet-related effects on basal AC (Fig. 2A) or on the AC response to isoproterenol (Fig. 2B). However, there were sex-selective effects on the responses to other stimulants that targeted males: the high-fat diet increased the response to glucagon (Fig. 2C) and the low-dose parathion treatment suppressed the fluoride response (Fig. 2D). Unlike the heart, the liver showed no changes in the forskolin response as a result of dietary manipulation or neonatal parathion exposure, separately or together (Fig. 2E). The main effects for the two significant changes are shown in Fig. 2F, collapsed across the interactive variables; this clearly shows the selective increase in the glucagon response evoked by high-fat diet in males and the decrease in the fluoride response evoked by the low dose of parathion.

In contrast to the peripheral tissues, the global ANOVA for the cerebellum revealed no statistically significant AC signaling effects of either parathion treatment or diet, separately or together, nor any significant interactions of these factors with the other variables (Table 1).

Receptor binding

For β AR binding, the global test (parathion treatment, diet, sex, tissue) identified a main effect of parathion ($p < 0.006$) that also depended on sex (treatment $\times$ sex, $p < 0.06$) and tissue (treatment $\times$ tissue, $p < 0.02$); there was also a tissue-selective effect of diet (diet $\times$ tissue, $p < 0.02$). Accordingly, we again separated the tissues for examination of lower-order main effects. In the heart, neonatal parathion exposure evoked a significant reduction in β ARs at the lower dose but not at the higher dose (Fig. 3A); the high-fat diet by itself also produced a decrement. In contrast, there were no effects of either parathion or dietary manipulation on β AR binding in the liver (Fig. 3B). Like the heart, the cerebellum displayed a significant decrease in receptor binding from the high-fat diet (Fig. 3C); although there was a significant interaction of parathion $\times$ diet $\times$ sex, none of the differences was statistically significant from control values after separation by the interactive variables. Uniquely, the gastrocnemius muscle showed β AR upregulation in response to parathion exposure but was unaffected by the high-fat diet (Fig. 3D).

For cardiac m_2 AChRs (Fig. 3E), we identified a main effect of treatment ($p < 0.03$) that was significant at either dose of parathion ($p < 0.008$ individually vs. control) and that displayed sex-selectivity (treatment $\times$ sex, $p < 0.04$). After subdivision of the results, males were significantly affected by parathion ($p < 0.003$ for the main treatment effect, $p < 0.006$ at 0.1 mg/kg, $p < 0.002$ at 0.2 mg/kg), whereas females showed no significant effect. In addition, the high-fat diet by itself caused a significant decrease (main effect of diet, $p < 0.0001$).

Figure 3F shows the main effects of diet and parathion exposure on receptor binding, collapsed across the remaining interactive variables: the diet-induced reduction in β ARs in the heart and cerebellum, and in cardiac m_2 AChRs; the parathion-induced decrement in heart β AR binding at the low dose and the increase in the gastrocnemius at the higher dose, as well as increases in cardiac m_2 AChR binding at either dose.

DISCUSSION

In our earlier studies, we found that early-life OP exposure results in sensitized hepatic AC signaling in response to neuronal and hormonal signals that promote gluconeogenesis, glycolysis and lipolysis that are evident later in life [2,15]. The consequent metabolic dysfunction resembles prediabetes, although different OPs vary in their specific pattern of effects [11-13,24]. With chlorpyrifos, serum glucose is maintained within normal limits, but only because of insulin hypersecretion, and the animals display hyperlipidemia, leptin dysregulation and excessive weight gain [11,24]. Parathion produces hyperglycemia, loss of lipid homeostasis and adipose inflammation, all without incurring hyperinsulinemia [12,13]. These differences are also reflected in disparities in OP effects on hepatic and cardiac AC signaling. Whereas sensitization of the pathway is maintained throughout the lifespan with chlorpyrifos and diazinon, the effects of parathion are prominent only through adolescence and then wane in young adulthood [2,15]. Here, we explored whether parathion's effects might emerge even later, in full adulthood, but still did not find the global upregulation of the AC pathway that we saw for chlorpyrifos and diazinon. However, there were focal abnormalities involving G-protein function, exemplified by a decreased hepatic AC response to fluoride; because the effects was not shared by stimulants that activate G_s -coupled receptors (isoproterenol, glucagon), this result implies that parathion exposure enhances the expression or function of the inhibitory G-protein, G_i . Interestingly, the effect was nonmonotonic, since the reduction was significant in the group receiving 0.1 mg/kg parathion but not the group given the higher dose. This is in keeping with the effects on body weight [13] and thus implies that the metabolic consequences of early-life parathion exposure are distinctly different once the levels exceed the threshold for cholinesterase inhibition and the emergence of systemic toxicity.

Similarly, although there were no significant effects of parathion on heart AC parameters, we did see a reduction in β AR expression with the same, nonmonotonic dose-effect relationship. Since parathion also evoked upregulation of m_2 AChRs, which produce opposing physiological effects to the actions of β ARs, our result point to the potential for sympathetic/parasympathetic imbalance that could ultimately contribute to cardiovascular disorders. Clearly, future work should address this possibility. In contrast, we found the opposite effect, increased β AR binding, in the gastrocnemius muscle, reinforcing the fact that the changes are related to specific metabolic functions rather than reflecting a global effect on β AR expression; this interpretation is reinforced by the different sensitivity of the skeletal muscle, which required exposure to the high dose in order to show effects (Fig. 3D), whereas effects in the heart (Fig. 3A) or liver (Fig. 2) were seen at the lower dose of parathion. Since skeletal muscle is a major site for glucose and lipid utilization, the changes seen here are likely to be involved in the general prediabetic profile seen with early-life OP exposure [12,24]. Finally, none of these effects were seen for

AC signaling or β AR expression in the cerebellum, pointing again to specific targeting of peripheral organ function, unrelated to the developmental neurotoxicity of OPs.

Despite their differences in effects on AC signaling and metabolic profiles, diazinon and parathion both share a common response to a high fat diet, in that animals exposed to either agent gain more weight than do controls [13,20]. Here, we did not observe any interaction between parathion and the high-fat diet at the level of AC signaling or receptors, implying that the augmented weight gain represents separate actions. It is therefore notable that we found effects of the high-fat diet on signaling that coincide with some of those obtained with OP exposure. In the liver, the high-fat diet enhanced the AC response to glucagon in males without producing a corresponding increase in the response to isoproterenol, fluoride or forskolin. Accordingly, this points to a specific enhancement of glucagon receptor expression or coupling to G_s ; the sex-selectivity seen here for signaling effects is paralleled by differences in several metabolic parameters [12,13]. Increased responsiveness to gluconeogenic signals later in life is also a hallmark of neonatal OP exposure [2,15], so the dietary effect seen here represents a endpoint that converges with that of OPs.

The effects of the diet in the heart again point out the targeting of specific organ function as opposed to global changes in cell signaling. Unlike the liver, the high-fat diet reduced the cardiac AC responses to fluoride and forskolin, implying heterologous changes in the expression or function of G-proteins and AC, downstream from the receptors. Notably, though this should have produced a parallel reduction in the responses to receptor stimulants but instead, these were unchanged; in turn, that implies that there are compensatory increases in receptor coupling that maintain the response to isoproterenol and glucagon in the face of downstream impairment. These adaptations are not at the level of receptor expression, since cardiac β ARs were decreased, not increased. Although the cerebellum also showed reduced β ARs in response to the high-fat diet, there were no corresponding changes in AC signaling, implying that this effect is functionally unimportant.

In conclusion, unlike chlorpyrifos or diazinon, neonatal parathion exposure does not produce global upregulation of hepatic AC cell signaling that is sustained throughout the life span. Rather, it elicits heterologous sensitization only through adolescence [2]; although this effect then wanes in young adulthood, more subtle changes in hepatic signaling then emerge even later, involving the expression or function of G_i , accompanied by changes in the concentration of β ARs in skeletal muscle, which govern glucose and lipid utilization. The disparities among the various OPs in their impact on cell signaling are likely to contribute to the differences in their effects on metabolic function [11-13,24]. Although addition of a high-fat diet did not augment the effect of neonatal parathion exposure on signaling, the diet by itself affected the AC pathway in both liver and heart, indicating that OP exposure produces changes in the same signaling events that are targeted by excessive fat consumption. In that fashion, early OP exposure, separately or combination with dietary factors, may contribute to the worldwide increase in the incidence of obesity and diabetes.

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Abbreviations

AC	adenylyl cyclase
ANOVA	analysis of variance
β AR	β -adrenergic receptor
m ₂ AChR	m ₂ -muscarinic acetylcholine receptor
OP	organophosphate

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Figure 1.

Effects of neonatal parathion exposure and subsequent adult consumption of a high-fat diet on cardiac AC activity: (A) basal AC, (B) isoproterenol-stimulated AC, (C) glucagon-stimulated AC, (D) fluoride-stimulated AC, (E) forskolin-stimulated AC. Data represent means and standard errors obtained from 6 animals in each group. Significant ANOVAs appear at the top of the corresponding panels. Panel (F) shows the simple main effect of diet, collapsed across all the other variables.

Figure 2.

Effects of neonatal parathion exposure and subsequent adult consumption of a high-fat diet on hepatic AC activity: (A) basal AC, (B) isoproterenol-stimulated AC, (C) glucagon-stimulated AC, (D) fluoride-stimulated AC, (E) forskolin-stimulated AC. Data represent means and standard errors obtained from 6 animals in each group. Significant ANOVAs appear at the top of the corresponding panels. and, where justified by the interactions, lower-order tests are shown within panels. Panel (F) shows the simple main effects for the two measures showing significant changes, collapsed across all the other variables.

Figure 3.

Effects of neonatal parathion exposure and subsequent adult consumption of a high-fat diet on receptor binding: (A) heart β ARs, (B) liver β ARs, (C) cerebellum β ARs, (D) gastrocnemius β ARs, (E) heart m_2 AChRs. Data represent means and standard errors obtained from 6 animals in each group. Significant ANOVAs appear at the top of the corresponding panels, and, where justified by the interactions, lower-order tests are shown within panels.. Panel (F) shows the simple main effects for the measures showing significant changes, collapsed across all the other variables.

TABLE 1

Adenylyl Cyclase Activities in the Cerebellum*

Treatment	Diet	Basal	Isoproterenol	Fluoride	Forskolin
Male					
Control	Normal	215 ± 11	252 ± 15	246 ± 15	828 ± 37
	High-Fat	177 ± 19	232 ± 8	258 ± 13	780 ± 32
Parathion 0.1 mg/kg	Normal	201 ± 14	233 ± 13	249 ± 14	809 ± 60
	High-Fat	178 ± 19	235 ± 8	256 ± 11	807 ± 30
Parathion 0.2 mg/kg	Normal	199 ± 15	239 ± 12	242 ± 14	807 ± 40
	High-Fat	200 ± 22	239 ± 6	235 ± 6	772 ± 39
Female					
Control	Normal	192 ± 9	245 ± 10	242 ± 7	901 ± 50
	High-Fat	177 ± 19	216 ± 19	212 ± 14	840 ± 49
Parathion 0.1 mg/kg	Normal	174 ± 22	210 ± 14	235 ± 14	856 ± 69
	High-Fat	178 ± 19	251 ± 28	233 ± 16	930 ± 75
Parathion 0.2 mg/kg	Normal	209 ± 21	278 ± 30	275 ± 18	960 ± 86
	High-Fat	200 ± 22	245 ± 21	232 ± 10	896 ± 58

* pmol/min per mg protein