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Brain development   mitochondria
cytochrome oxidase   NAD-linked respiration
inorganic lead   oxidative phosphorylation

Early Effects of Inorganic Lead on Immature Rat Brain Mitochondrial Respiration

D. HOLTZMAN[44] AND J. SHEN HSU

Departments of Neurology and Pediatrics, Stanford University School of Medicine, Stanford, California, USA

Extract

Inorganic lead, added to the diet of the suckling rat in high doses, produces an encephalopathy similar to that seen in the immature human. Pathologic changes of edema and hemorrhage are seen earliest and are most prominent in the cerebellum. In this study, we measured respiration in cerebral hemisphere and cerebellar mitochondria isolated from led-fed and age-matched normal rat pups. Lactating mothers were begun on *ad libitum* feedings containing 4% lead carbonate when their pups were 2 weeks old. Mitchondria were isolated by differential centrifugation. Oxygen consumption was measured polarographically. NAD-linked respiration was measured with oxidation of the substrate pair, glutamate and malate. Cytochrome oxidase (cytochrome *c* oxidase, EC. 1.9.3.1) activity was measured in the presence of tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD) and ascorbate. Within 2 days of starting lead feedings, rat pups showed a significant loss in body weight ($P < 0.02$) and, after 1 week, a significant loss in cerebral hemisphere wet weight ($P < 0.01$) compared with controls. Overt encephalopathy appeared in pups from two of nine litters receiving lead feedings for 1 week and in half of the litters after 2 weeks of feedings. None of the lead-fed mothers developed encephalopathic signs. With oxidation of the NAD-linked substrate pair, there was a progressive decrease, relative to controls, in ADP/O ratios in both cerebellar

and cerebral mitochondria from lead-fed animals. After 2 weeks these differences were significant in mitochondria from both regions (cerebellum, $P < 0.02$; cerebrum, $P < 0.005$). Respiratory control ratios were significantly lower in cerebellar mitochondria from lead-fed rats within 2 days of beginning feedings ($P < 0.02$) and in mitochondria from both regions after 2 weeks of lead feedings (cerebellum, $P < 0.01$; cerebrum, $P < 0.05$). The decrease in control ratios in cerebellar mitochondria from animals receiving lead feedings for 1 week or less was due to a small decrease in state 3 respiration and a large, but inconsistent, increase in state 4 respiration. The decrease in control ratios in both cerebellar and cerebral hemisphere mitochondria after 2 weeks of lead feedings was due to a marked inhibition of state 3 respiration, relative to controls (cerebellum, $P < 0.01$; cerebral hemisphere, $P < 0.05$). In cerebellar mitochondria from lead-fed animals, cytochrome oxidase activity showed similar changes compared with controls: a highly significant ($P < 0.001$) increase within 2 days of beginning feedings and a significant ($P < 0.01$) decrease after 2 weeks of feedings.

Speculation

The effects of inorganic lead feedings were greater in mitochondria from cerebellum than from cerebral hemispheres. The changes in respiratory chain function, which appeared sooner after beginning lead feedings than any previously reported biochemical or morphologic effect on rat cerebellum, may constitute an early step in the pathogenesis of an encephalopathy which is predominant in this region of the suckling rat brain. In cerebellar mitochondria, inorganic lead may be acting as an uncoupling agent, such as dinitrophenol, affecting both NAD-linked respiration and cytochrome oxidase activity. The maturing brain may be most sensitive to the encephalopathic effects of lead at a critical period of rapid synthesis and increasing activity of respiratory enzymes. The greater effects on cerebellar mitochondria may be related to regional differences in mitochondria respiratory chain properties during this maturational period.

Inorganic lead, added in large doses to the diet of the suckling rat, produces an encephalopathy similar to that seen in the immature human (6, 27, 32). The encephalopathy, manifested by obtundation and paraparesis, appears in the 4th or 5th week of life, if lead exposure is begun by 2 weeks. The mature rat brain, like that of the mature human, is not sensitive to these effects of inorganic lead. In the rat pups, pathologic changes of edema and hemorrhage are most prominent in the cerebellum and basal ganglia, but white matter edema does appear in the cerebral hemispheres (6). When the earliest signs of encephalopathy appear, chemical evidence of edema has been shown only in the cerebellum (11). An increase in capillary permeability has been demonstrated in many regions of the central nervous system at this stage (27). The regional edema and increase in capillary permeability have been interpreted as being secondary to a primary capillary endothelial cell dysfunction (11) or to a dysfunction in parenchymal cellular energy metabolism which may affect, either directly or indirectly, properties of the capillary endothelium (27). Recent studies suggest that inorganic lead feedings result in a retardation in maturation of brain glucose metabolism and metabolic compartmentation (25, 26).

We have studied respiratory chain function in cerebral hemisphere and cerebellar mitochondria isolated from lead-fed and age-matched normal rat pups. The rationale for study of the respiratory chain as the possible site of action of inorganic lead on the immature brain was based on the following observations. First, in other tissues, the primary effect of inorganic lead appears to be on oxidative phosphorylation (8, 13, 29, 31). Second, in mature rodent brains, organic lead compounds have been shown to affect glucose and oxygen consumption (2) and to decrease cerebellar and cerebral concentrations of the cytochrome heavy metal cofactors: copper, zinc, and iron (23). Third, the period of sensitivity to

inorganic lead is that in which the concentrations and activities of respiratory enzymes are rapidly increasing in the developing brain (14, 16, 17, 28, 33).

MATERIALS AND METHODS

Sprague-Dawley albino rats of both sexes were used. Pregnant females arrived before the end of gestation to allow accurate age-matching of experimental and control litters. Litters were reduced to eight pups on the first day of life and maintained in one room with one litter and mother per cage. When litters were 2 weeks old, mothers were begun on ad libitum feedings of powdered laboratory chow (34) containing 4% lead carbonate. This chow is comparable with the common laboratory rat pellets, containing calcium 1.0%, phosphorus 0.74% and vitamin D 3.3 IU/g. Control animals received powdered chow without the added lead. Pups also has free access to the powdered chows. All feedings were prepared by the laboratory staff.

Mitochondria were isolated from the cerebral hemispheres and cerebellum by differential centrifugation as described in previous publications (15, 22). After decapitation the brain was immersed in ice-cold isolation medium consisting of 225 mM mannitol, 75 mM sucrose, 0.2 mM EDTA, 5.0 mM Tris-HCl (pH 7.4). Cerebral hemispheres and cerebellums were separated and pooled from whole litters at 2 weeks of age and from two to four animals at older ages. The cerebral hemispheres were separated by cutting the peduncles above the mesencephalon. The tissues were then homogenized and 0.1 mg bacillus proteinase, 1 mg bovine serum albumin (BSA), and 1 mg $KHCO_3$ were added for each gram of brain tissue. This mixture was allowed to stand for 2 min before the centrifugation steps (15). The myelin layer overlying the final mitochondrial pellet was removed by hand and the pellet was washed and resuspended in a small volume of medium without EDTA. An aliquot of this suspension was frozen for protein determination (20).

Oxygen consumption was measured polarographically using the Gilson oxygraph with a 2-ml reaction chamber and Clarke platinum electrode polarized to 0.8 V (10). The system was calibrated for O_2 content as described previously (15). Chamber temperature was maintained at 20°. ADP concentrations were measured biweekly (1). All studies were performed on freshly isolated mitochondria and were completed within 3 hr.

NAD-linked respiration was measured with oxidation of an excess of the substrate pair, glutamate and malate (1.25 mM each). Respiratory rates were measured in the presence of a limiting quantity of ADP, state 3 respiration, and after consumption of the added ADP, state 4 respiration (4). The respiratory control ratio was defined as the ratio of state 3 to state 4 rates (4). ADP/O ratios were calculated by the usual method (5).

Cytochrome oxidase (cytochrome c oxidase, EC 1.9.3.1) activity was measured in the presence of TMPD (100 μM) and ascorbate (1 mM) with antimycin A (5 μg/ml) in the reaction mixture (30). Rates were determined with and without ADP, but measurable control ratios were not usually obtained, as noted by other investigators for the polarographic assay of cytochrome oxidase (30). The rates presented here are those measured in the absence of added ADP.

The results obtained with mitochondria from lead-fed animals were compared with those from age-matched control animals as matched samples. Mitochondrial samples were isolated from control and experimental litters at the same time. The significance of the difference of the mean results with matched samples compared with zero was calculated by the standard t-test for nonindependent samples (7).

CHEMICALS

Mannitol, sucrose, EDTA, Trizma base, ADP, substrates, and the bacillus proteinase were obtained from Sigma Chemical Co. (35). Defatted BSA was obtained from Miles Laboratories (36),

Table 1. *Brain and body weights of lead-fed and control rats*[1]

Age, days	Body weight, g		Cerebral hemisphere weight, g	
	Control	Lead	Control	Lead
15	41.47 ± 1.64	35.59 ± 1.05	1.06 ± 0.01	1.01 ± 0.02
	$P < 0.02$		$P < 0.1$	
21	56.72 ± 2.52	39.00 ± 1.05	1.06 ± 0.01	0.99 ± 0.01
	$P < 0.001$		$P < 0.01$	
28	84.17 ± 2.85	39.22 ± 2.90	1.10 ± 0.03	0.97 ± 0.02
	$P < 0.001$		$P < 0.005$	

[1] Each result is the mean ± SEM body or cerebral hemisphere wet weight of rats from at least nine litters. Experimental animals were fed 4% lead carbonate from 2 weeks of age and compared with normal, age-matched control litters. *P* values, for the significance of the mean difference between results with matched litters, were calculated by the standard *t*-test for nonindependent samples.

ascorbate from J. T. Baker Chemical (37), TMPD from Eastman Kodak (38), and antimycin A from CalBiochem (39). All inorganic chemicals were reagent grade.

RESULTS

Table 1 shows the effects of maternal inorganic lead feedings on body weight and cerebral hemisphere wet weight in the suckling rat. Within 2 days, the pups showed a significant loss in body weight compared with controls. None of these animals appeared to be sick. After 1 and 2 weeks of lead feedings, mean losses in body and brain weight were highly significant. Pups from two of nine litters receiving lead feedings for 1 week developed the encephalopathy, manifested by paretic hind legs and hemorrhagic cerebellums. After 2 weeks of feedings, pups from half of the litters were affected. None of the lead-fed mothers developed any encephalopathic signs.

ADP/O ratios, measured in isolated cerebral hemisphere and cerebellar mitochondria with oxidation of glutamate and malate, are shown in Table 2. Cerebral hemisphere mitochondria from control animals showed the age-dependent increase in ADP/O ratios reported previously for this age period (16), whereas cerebral hemisphere mitochondria from lead-fed animals did not. As in this earlier study (16), cerebellar mitochondria did not show this age-dependent increase in ADP/O ratios after 2 weeks of age. During the period of lead feedings, there was a progressive decrease in ADP/O ratios in cerebellar mitochondria from lead-fed animals. After 2 weeks, the difference between lead-fed and control animals was significant in mitochondria from both brain regions. The degree of significance was greater than shown in Table 2, since, if no clear *state 3-4* transition occurred, ADP/O ratios could not be measured and were excluded. This degree of uncoupling of respiratory control was found in 2 of 11 experiments at 3 weeks and 2 of 7 experiments at 4 weeks of age with cerebellar mitochondria from lead-fed animals. One of 7 experiments with cerebral hemisphere mitochondria from 4-week-old lead-fed animals showed complete loss of respiratory control.

The effects of lead feedings on respiratory control ratios with oxidation of glutamate and malate are shown in Figure 1. The range of mean control ratios in both cerebral hemisphere and cerebellar mitochondria from normal animals was 3.5-5.0. As in previous studies (16, 17), these values did not change as a function of age in either brain region. Control ratios were significantly lower in cerebellar mitochondria from experimental animals within 2 days of beginning lead feedings. After 2 weeks of lead feedings, control ratios were significantly lower in both cerebral and cerebellar mitochondria.

Mean *state 3* and *state 4* respiratory rates, which comprise the respiratory control ratio, are shown in Figure 2 for cerebellar

mitochondria. The decrease in respiratory control ratios in cerebellar mitochondria from animals receiving lead feedings for 1 week or less was due to a small decrease in *state 3* respiration and a large, but inconsistent, increase in *state 4* respiration. The significant decrease in control ratios in these mitochondria after 2 weeks of lead feedings was due to a marked inhibition of *state 3* respiration compared with controls. Cerebral hemisphere mitochondria from lead-fed animals showed no change in *state 4* rates. These mitochondria did show a relative decrease in *state 3* respiration which was similar to, but less significant ($P < 0.05$) than, that seen in cerebellar mitochondria. The age-dependent increase in *state 3* respiration, observed in normal rat cerebral

Table 2. *ADP/O ratios with oxidation of glutamate and malate in brain mitochondria from lead-fed and control rats*[1]

Age, days	Cerebral hemispheres		Cerebellum	
	Controls	Lead	Controls	Lead
15	2.06 ± 0.14	2.11 ± 0.19	2.37 ± 0.17	2.12 ± 0.13
	$P > 0.9$		$P < 0.25$	
21	2.38 ± 0.10	2.21 ± 0.08	2.20 ± 0.11	2.08 ± 0.12[2]
	$P < 0.2$		$P < 0.2$	
28	2.57 ± 0.15	2.14 ± 0.12[3]	2.27 ± 0.17	2.04 ± 0.18[4]
	$P < 0.005$		$P < 0.02$	

[1] Oxygen consumption was measured polarographically after adding 0.15-0.25 μmol ADP to the reaction mixture containing 1.25 mM each of glutamate and malate, fresh bovine serum albumin 0.2 mg/ml, mannitol 225 mM, sucrose 75 mM, EDTA 0.2 mM, Tris-HCl (pH 7.4) 5.0 mM, Tris PO_4 (pH 7.4) 10 mM, KCl 5.0 mM, and 0.5-1.0 mg mitochondrial protein. All measurements were at 20°. ADP/O ratios are expressed as micromoles of ADP added per microatom O_2 consumed during the rapid state of respiration in the presence of ADP. Each result is the mean ± SEM of 5-11 experiments at each age. ADP/O ratios are not included for experiments in which there was no clear *state 3-4* transition (*i.e.*, uncoupled). *P* values, for the significance of the mean difference between results with matched litters, were calculated by the standard *t*-test for independent samples.

[2] Two of 11 experiments in series were uncoupled.

[3] One of seven experiments in series was uncoupled.

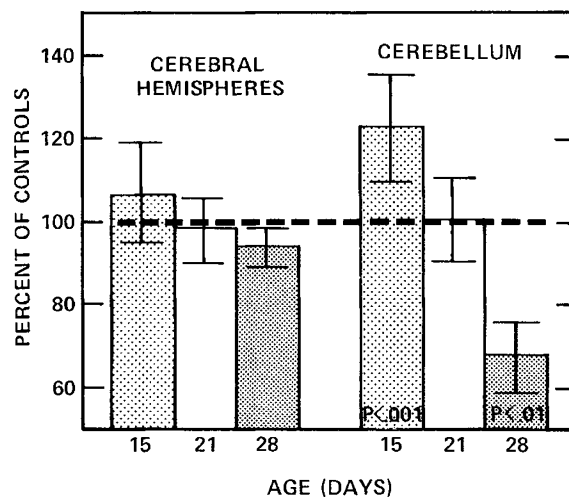[4] Two of seven experiments in series were uncoupled.

Fig. 1. Respiratory control ratios with oxidation of glutamate and malate in brain mitochondria from rats fed lead from 2 weeks of age. The reaction mixture and conditions are given in Table 2. The results are the means ± SEM of 7-12 experiments at each age expressed as a percentage of the results with simultaneously assayed mitochondria from age-matched control litters. *P* values, for the significance of the mean difference between results with matched litters, were calculated by the standard *t*-test for nonindependent samples.
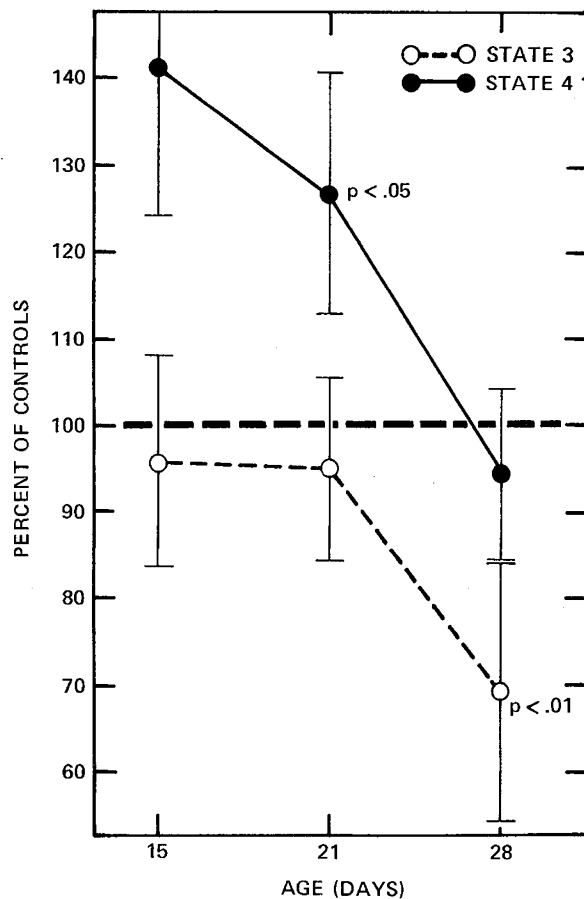
Fig. 2. *State 3* and *state 4* respiratory rates with oxidation of glutamate and malate in cerebellar mitochondria from rats fed lead from 2 weeks of age. The reaction mixture and conditions are given in Table 2. Respiratory rates were measured after adding ADP to the reaction mixture (*state 3*) and after consumption of the added ADP (*state 4*). The results are the means ± SEM of 7-12 experiments at each age expressed as a percentage of the results with simultaneously assayed mitochondria from age-matched control litters. *P* values, for the significance of the mean difference between results with matched litters, were calculated by the standard *t*-test for nonindependent samples.

of dependence of respiration upon the presence of phosphate acceptor, shown by the increase in ADP-independent (*state 4*) respiration and the resulting decrease in control ratios. Cytochrome oxidase activity in cerebellar mitochondria was significantly increased within 2 days of lead feedings. This increased respiratory rate probably also reflected a release of respiratory control but control ratios could not be measured polarographically (30). With continued lead feedings, mitochondria from cerebellum and from cerebral hemispheres showed a progressive inhibition of ADP-dependent (*state 3*) respiration, control ratios, and ADP/O ratios with oxidation of the NAD-linked substrates. After 2 weeks of lead feedings, cytochrome oxidase activity was comparably decreased in cerebellar mitochondria. Thus, the effects of inorganic lead feedings were greater in cerebellar mitochondria, where there was, initially, a loss of respiratory control with little change in ADP/O ratios and, later, a decrease in both respiratory control and ADP/O ratios with inhibition of ADP-dependent respiration.

These two phases of inorganic lead effects on mitochondrial respiration may be related to the increasing concentration of lead in the brain of the suckling rat during this period of feeding, as reported by Goldstein et al. (11). In the first week, when we observed only an uncoupling of controlled respiration in cerebellar mitochondria, there is little measurable increase in brain lead content. Lead concentrations rise sharply in both cerebellum and cerebrum in the second week of feedings, when we observed significant inhibition of coupled respiration in both regions. Assuming the tissue lead concentrations reflect the mitochondrial environment (3), inorganic lead may be acting like an uncoupling agent, such as dinitrophenol or dicoumarol. These agents cause a much greater drop in respiratory control than ADP/O ratios at low concentrations and inhibit respiration and oxidative phosphorylation at higher concentrations (4).

Inorganic and organic lead compounds produce similar concentration-dependent effects on mitochondrial respiration in mature brain and in other tissues. In mature rat brain and liver slices, organic lead compounds increase respiration at low concentrations and inhibit glucose and oxygen consumption at higher concentrations (2). If mature rats are fed inorganic lead for a period

mitochondria (17) was markedly attenuated in mitochondria from lead-fed animals.

Cytochrome oxidase activities in mitochondria from lead-fed and control animals are shown in Figure 3. The age-dependent increase in activity, reported in rat brain mitochondria during this period (14, 28), was seen in cerebral and cerebellar mitochondria from normal animals, but not lead-fed animals. Within 2 days of starting lead feedings, the cytochrome oxidase activity was significantly increased compared with controls in cerebellar mitochondria from lead-fed animals. With continued lead feedings, this activity progressively decreased, relative to controls, with the difference being significant after 2 weeks. Smaller changes, which were not significant, were observed in cerebral hemisphere mitochondrial cytochrome oxidase activity.

DISCUSSION

The effects of inorganic lead feedings on suckling rat brain mitochondrial respiration, both with NAD-linked substrates and with cytochrome oxidase activity, occurred in two phases temporally related to the onset of lead feedings. The early changes appeared in cerebellar mitochondrial respiration within 2 days of starting inorganic lead feedings to lactating mothers. With the NAD-linked substrate pair, glutamate and malate, there was a loss
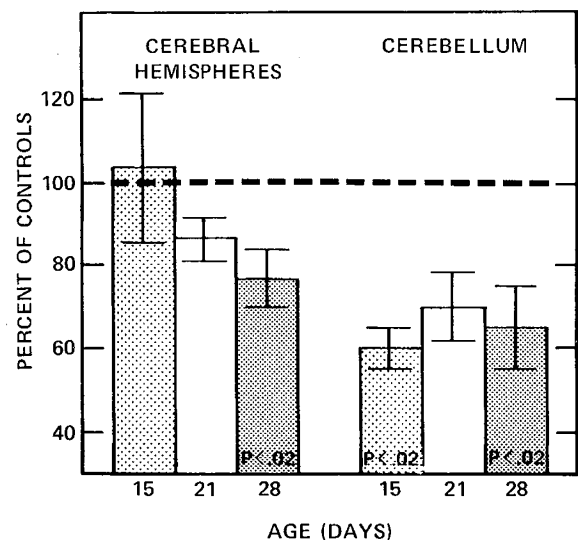

Fig. 3. Cytochrome oxidase activity in brain mitochondria from rats fed lead from 2 weeks of age. Respiratory rates were measured polarographically with oxidation of ascorbate (1 mM) in the presence of tetramethyl-*p*-phenylenediamine dihydrochloride (100 μM), antimycin A (5 μg/ml), and the reaction mixture described in Table 2. All measurements were at 20°. The results are the means ± SEM of 7-12 experiments at each age expressed as a percentage of the results with simultaneously assayed mitochondria from age-matched control litters. *P* values, for the significance of the mean difference between results with matched litters, were calculated by the standard *t*-test for nonindependent samples.

sufficient to produce renal tubular swelling and amino aciduria, isolated renal cortical mitochondria show decreased respiratory rates, ADP/O ratios, and respiratory control with NAD-linked substrates (13). Similar inhibition of the respiratory chain occurs with *in vitro* exposure of rabbit renal cortical mitochondria to high concentrations of lead acetate (18), whereas lower concentrations of inorganic lead produce increased cytochrome oxidase activity in human placental mitochondria (8).

The loss in brain and body weights and the incidence of the hemorrhagic encephalopathy, which we observed, were comparable with these reported previously in suckling rats receiving inorganic lead by maternal feeding (21). A recent report suggests that the degree of weight loss and the severity of the encephalopathy may be causally related, since both are decreased if lead is fed directly to the pups (12). Brain lead concentrations are reportedly similar with the two methods of administration, but specific concentrations and ages of measurement are not stated (12). Malnutrition alone, during the suckling period, does not result in the encephalopathic changes observed with lead feedings (9). However, systemic effects, such as malnutrition and anemia, resulting from maternal lead feedings may enhance the pathologic sequelae or the biochemical effects of inorganic lead on the suckling rat brain.

The age at which the rat brain is sensitive to the encephalopathic effects of lead is relatively constant if lead feedings are begun any time in the first 2 weeks of life (11, 21, 27). The critical period for exposure to lead appears to be the 3rd and 4th weeks of life. This is also a period of rapid increase in the activity and concentration of components of the rat brain mitochondrial respiratory chain (14, 16, 17, 28). The greater effects of lead on the cerebellum than the cerebrum, seen in morphologic studies (6, 11, 27, 32), as well as in our study of respiratory chain function, may be related, in part, to regional differences in mitochondrial respiratory chain function during this maturational period (16, 33). Differences in the effects of lead on cerebellar and cerebral hemispheres apparently are not due to differences in whole tissue lead concentrations, since, at the ages we have studied, the lead concentrations in these two regions are reportedly the same (11). The absence of encephalopathic effects of lead at older ages also may be related to the lower quantity of lead taken up by the mature brain (11).

We propose that the effects of inorganic lead on mitochondrial respiration, which appear more immediately after beginning feedings than any reported biochemical or morphologic effect on the rat cerebellum (11, 27, 32), constitute an early step in the pathogenesis of an encephalopathy predominant in this region of the suckling rat brain. These effects, which we are reporting, may be part of, or may result in, a more general effect on glucose metabolism in the developing brain (25, 26). Pentschew and Garro (27) postulated that lead encephalopathy results from a deficiency of a necessary blood-born factor, in analogy with the pathologic effects of thiamine deficiency. We propose a similar hypothesis, that lead, as a blood-borne agent, produces a primary abnormality in oxidative energy metabolism in the immature brain. Whether the pathologic sequelae are the results of effects upon one cell population, such as the capillary endothelium (11), or due to a general metabolic dysfunction, we cannot say from this study. Our protocol, using high dose lead carbonate feedings, was designed to study the pathogenesis of a model for the clinically overt lead encephalopathy. Similar effects on respiratory chain function, with lower dose lead feedings, at developmental stages other than that which we have studied may be the basis for the teratogenic effects and changes in behavior and intelligence attributed to subclinical lead poisoning in the developing human (19).

SUMMARY

The effects of high dose inorganic lead feedings on brain mitochondrial respiration were studied in the suckling rat as an animal model for the pathogenesis of lead encephalopathy. Lead carbonate (4%) was added to the diet of nursing rat mothers when litters were 2 weeks old. Cytochrome oxidase activity and oxidative phosphorylation with the substrate pair, glutamate and malate, were measured polarographically in isolated cerebral hemisphere and cerebellar mitochondria from experimental and control litters. Within 2 days of beginning feedings, cerebellar mitochondria from lead-fed animals showed significantly lower respiratory control ratios with glutamate and malate oxidation, due primarily to an increase in the rate of ADP-independent respiration, compared with controls. Cytochrome oxidase activity was significantly increased in these mitochondria. During the subsequent 2 weeks, cerebellar mitochondria from lead-fed animals showed a progressive inhibition of ADP-dependent respiration and efficiency of oxidative phosphorylation (ADP/O ratio) and a comparable inhibition of cytochrome oxidase activity. Cerebral hemisphere mitochondria showed similar, but smaller, changes. The loss of respiratory control with glutamate and malate oxidation and the increased cytochrome oxidase activity in cerebellar mitochondria appeared earlier than any previously reported biochemical or morphologic effect of inorganic lead on the cerebellum. The effects on cerebellar respiratory energy metabolism may represent an early step in the pathogenesis of an encephalopathy which is predominant in this region of the developing rat brain.

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Cell-mediated immunity   lymphocyte transformation
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Cell-mediated Immunity to Respiratory Syncytial Virus Induced by Inactivated Vaccine or by Infection

HYUN WHA KIM, SANFORD L. LEIKIN, JULITA ARROBIO, CARL D. BRANDT, ROBERT M. CHANOCK, AND ROBERT H. PARROTT[13]

George Washington University, Research Foundation of Children's Hospital Medical Center, Washington, D.C. and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Extract

Transformation and increased mitotic activity in donor lymphocytes exposed to specific antigens is considered by many to be a manifestation of cell-mediated immunity. In attempts to understand the apparent "sensitization" of individuals to respiratory syncytial virus (RSV) as a result of receiving inactivated RSV vaccine, *in vitro* lymphocyte transformation studies were carried out on infants who had received inactivated RSV vaccine and on infants who had received a similarly prepared inactivated African green monkey kidney (AGMK) cell-grown parainfluenza type 1 virus vaccine or a trivalent parainfluenza vaccine prepared in hen's eggs. Each group included some infants who had, and others who had not, undergone natural RSV infection under our observation before the lymphocyte studies.

Lymphocytes were studied from 21 infants and young children who had received the inactivated RSV vaccine, 14 who received a similarly prepared inactivated parainfluenza 1 vaccine, and 5 who received a trivalent parainfluenza vaccine. Twelve of the RSV vaccinees and 14 of the parainfluenza vaccinees had been naturally infected with RSV as indicated by virus recovery and/or antibody rise between the time of vaccination and the lymphocyte studies. In comparing the arithmetic mean for RSV-specific transformation and mitotic activity there was a significant difference between RSV vaccinees and parainfluenza vaccinees whether one compared those who had undergone natural RSV infection or those who had not undergone natural infection. The difference between RSV vaccinees who had not undergone natural RSV infection and RSV-infected parainfluenza vaccinees also was significant. There was a greater level of transformation and mitotic activity in those who had experienced natural infection than those who had not among both RSV vaccinees and parainfluenza vaccinees, but these differences were not significant statistically.

Speculation

We take these findings to mean that natural RSV infection probably stimulates a systemic cell-mediated immunity response and that such a response is definitely induced after administration of killed RSV antigen. These findings are consistent with the hypothesis that cell-mediated sensitization may in some way contribute to the altered response to natural infection which occurred after use of inactivated RSV vaccine. Our findings do not support the hypothesis that systemic cell-mediated immunity *per se* is important in protecting against RSV infection. The findings also suggest the possibility that transplacentally conferred RSV lymphocyte sensitization might play a part in the pathogenesis of nonvaccine related RSV bronchiolitis which characteristically occurs during early infancy, frequently in the presence of measurable serum antibody.

The first potential vaccines against RSV in infants were inactivated virus preparations. One of these was a 100 times concentrated virus which had been grown in AGMK cell cultures