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ALTERATION OF CENTRAL CHOLINERGIC FUNCTION BY CHRONIC LEAD ACETATE EXPOSURE

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Abstract—Mice chronically exposed to lead during initial periods of development demonstrate increased levels of spontaneous motor activity. Their behavioral responses to a number of drugs indicate a decrease in central cholinergic activity. Studies utilizing peripheral nervous tissue have shown a decreased evoked and an elevated spontaneous release of ACh by lead. The possibility was examined, therefore, that the evoked and spontaneous ACh release in brain tissue might be similarly altered by chronic lead treatment *in vivo*. The results indicate that chronic lead administration inhibits the potassium-induced release of both choline and ACh from cortical minces. Potassium-induced release of labeled ACh synthesized from labeled choline is also significantly impaired in the lead-treated animals. Administration of methylphenidate to lead-treated animals, previously reported to suppress lead-induced hyperactivity, reverses the inhibition of potassium-induced choline and ACh release. Spontaneous release of ACh in lead-treated animals is significantly increased. Omission of calcium significantly inhibits the potassium-induced release of ACh without significantly altering choline release. No changes were found in the steady state levels of choline and ACh nor in the activities of choline acetyltransferease, choline phosphokinase, and acetylcholinesterase in the brains of lead-treated animals during development. The results suggest that the inhibition of potassium-induced release of ACh by lead may occur by two different mechanisms: (1) lead may reduce the availability of choline for ACh synthesis, and (2) lead may interfere with the role of calcium in the evoked release of ACh. The present work indicates that chronic lead exposure, at doses previously shown in mice to elicit hyperactivity, also disrupts central ACh function. Also, the results indicate that lead may be a valuable tool in elucidating the dynamic processes involved in central ACh metabolism.

Low levels of inorganic lead chronically administered from birth through the termination of the experiment increase the spontaneous motor activity of mice [1–3], rats [4] and monkeys [5]. Previously, it has been suggested that lead-induced hyperactivity in mice is associated, at least in part, with a decrement in central cholinergic function [2, 3]. This suggestion is predicated on studies dealing with the influence of lead on both central and peripheral cholinergic systems. Administration of several drugs to the lead-induced hyperactive mice influences both the hyperactive state and central ACh metabolism. For example, the muscarinic blocking agents atropine and benztropine, exacerbate the hyperactive state and block the effects of ACh at post-synaptic receptor sites. Conversely, the anticholinesterase agent, physostigmine, suppresses the hyperactive state probably by causing an accumulation of ACh at post-synaptic receptor sites.

In the peripheral nervous system, lead *in vitro* has been shown to impair cholinergic function in several different experimental models. Lead inhibits the release of ACh from the superior cervical ganglion of the cat during stimulation of the preganglionic fiber; the addition of extracellular calcium reverses this inhibition [6]. Lead decreases the size of the end plate potential (EPP) and increases the frequency of miniature end plate potentials (MEPP) in the sartorious muscle of the frog [7]. Also, it reduces the force of diaphragmatic contraction during stimulation of the phrenic nerve of the mouse [8, 9]. That lead reduces both the EPP and diaphragmatic contraction cannot be explained by a post-synaptic inhibition since it does not alter the postsynaptic sensitivity to ACh [7–9].

Many studies have suggested that extracellular calcium plays an important role in both the peripheral and central release of ACh and lead toxicity [6, 10]. In the peripheral nervous system, extracellular calcium has been shown to reverse the inhibition of ACh release by lead [6]. Calcium administration has also been shown to protect against central lead toxicity whereas a calcium-free diet has been shown to potentiate central lead toxicity [11]. Thus, the effect of chronic lead administration on various parameters of central ACh metabolism has been compared with calcium omission.

The pharmacological evidence obtained with whole animal studies and the direct measurements of ACh release indicate that the release of ACh in the central nervous system (CNS) of lead-induced hyperactive

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mice is impaired. In this study, the spontaneous and potassium-induced release of both choline and ACh, the transport of choline, the activity of enzymes, and the levels of substrates associated with cholinergic function were examined using brain preparations of lead-treated and coetaneous control mice.

MATERIALS AND METHODS

Pregnant CD 1 mice weighing between 25 and 35 g were obtained from Charles River Laboratories 15–17 days after impregnation. The method of administering lead to the offspring has been reported previously [1]. Briefly, lead is administered to the mothers and via her milk to the nursing off-spring which are the subjects of study. Three concentrations of lead solutions which replaced the drinking water were used: 2, 5 and 10 mg/ml as lead acetate. In most of the studies, the 5 mg/ml exposure level was used unless otherwise stated. Litters were normalized to six animals within 24 hr after birth. After weaning, the offspring were exposed to the same concentrations of lead as their mothers until the termination of the experiment. Control and lead-treated animals were handled identically with the exception that control animals were not exposed to solutions of lead acetate. The behavior of animals used in this study was not measured.

Choline and ACh release. Coetaneous control and lead-treated mice 30–70 days of age were utilized for ACh and choline release studies. The animals were decapitated, and the cerebral cortex was quickly removed and washed with several hundred ml of ice-cold Krebs–Ringer–HCO_3 buffer of the following compositions: NaCl, 6.83; KCl, 0.261; $CaCl_2$, 0.277; KH_2PO_4, 0.163; $MgSO_4 7H_2O$, 0.295; $NaHCO_3$, 2.35 and glucose, 2.0 g/liter. The cortex was removed from the washing medium and minced, and a weighed portion (25–50 mg) transferred to 2 ml of ice-cold Krebs containing the acetylcholinesterase inhibitor paraoxon (0.1 μM). In the studies reported below, paired coetaneous samples were always run and the experiments were repeated at least once. The release of choline and ACh from brain tissue was accomplished by changing the potassium concentration from 5 to 35 mM and reducing the sodium concentration from 145 to 115 mM; the pH of the medium was maintained at 7.4.

The release of choline and ACh from brain cortical minces was determined in both Krebs and 35 mM K^+ Krebs solution (elevated K^+ Krebs). The minces were agitated in Erlenmeyer flasks (25 ml) at 90 cycles/min in a Dubnoff metabolic shaker under an atmosphere of 95% O_2–5% CO_2 at 37° for varying time periods. Samples incubated at 0–2° for the same periods of time were used as tissue blanks. These samples release a small amount of choline and ACh which was subtracted from the total amount of choline or ACh released at 37°. At the end of the incubation period, the samples were chilled and centrifuged for 10,000 *g*-min, and an aliquot of the supernatant was used for the determination of choline and ACh. The supernatant (100 μl) was extracted with 300 μl of 5 mg/ml of tetraphenylboron dissolved in 3-heptanone (TPB/3-heptanone). After a brief centrifugation, 200 μl of the organic phase was transferred to a tube containing 300 μl of 0.4 N HCl. After thorough mixing and a 10,000 *g*-min centrifugation, the organic layer was removed and a 50-μl aliquot of the aqueous layer transferred to a new tube and lyophilized. These samples were then analyzed for choline and ACh by the method of Goldberg and McCaman [12, 13]. In the present experiments, the conversion of choline to phosphoryl-choline catalyzed by choline kinase was complete for at least 1000 pmoles choline. Choline kinase (EC 2.7.1.32.) was initially prepared by the method of McCaman *et al.* [14] and also purchased from Sigma Chemical Corp. when it became commercially available.

In some experiments, brain cortical minces were incubated with 100 μM [^{14}C]choline in Krebs buffer containing 35 mM K^+ for 1 hr and the amount of [^{14}C]ACh released into the media was then determined. Identification and quantification of labeled ACh released from minces were determined by two different methods: (1) the conversion of labeled choline to labeled phosphorylcholine and its subsequent separation from labeled ACh by liquid cation exchange chromatography (TPB/3 heptanone) as described above, and (2) paper chromatography. In the paper chromatographic separation, the samples, after conversion of choline to phosphorylcholine, were streaked on Whatman 3M chromatogram paper and developed for 22 hr in a descending system of butanol–ethanol–acetic acid–H_2O (8:2:1:3, v/v) at room temperature. Standards (unlabeled phosphorylcholine and ACh) were co-chromatographed and their positions detected by exposure to iodine vapor [15]. The developed chromatograms were cut into 1-cm strips, eluted with 1 ml H_2O and counted in Aquasol by liquid scintillation spectrometry. There was no overlap between labeled phosphorylcholine and labeled acetylcholine. Additionally, no labeled choline was detected on the chromatograms.

Choline transport. The effect of chronic lead treatment on the accumulation of choline by non-depolarized cortical minces, a process having "high" and "low" affinity components [10, 16], was studied using the brains of lead-treated and coetaneous control mice. For comparative purposes, the influence of calcium omission on high and low affinity transport into cortical minces was also examined. Previously, Michaelis constants of 6 and 50 μM have been determined, respectively, for high and low affinity choline transport into non-depolarized cortical minces [10]. To avoid a possible overlap between the two processes, a [^{3}H]choline concentration of 0.6 μM (sp. act. 16.6 Ci/m-mole, source: New England Nuclear) was used to study high affinity choline transport, whereas a [^{14}C]choline concentration of 100 μM (sp. act. 21 mCi/m-mole, source: New England Nuclear) was used to study low affinity transport. Minces were incubated for 4 min at 37° under an atmosphere of 95% O_2–5% CO_2. Zero degree samples were routinely used as blanks. Incubation was terminated by the addition of 5 ml of ice-cold 0.32 sucrose. The minces were centrifuged, washed with 5 ml of 0.32 sucrose, and the radioactivity was extracted using 1 N formic acid–acetone (FA–A, 15:85, v/v) according to the method of Toru and Aprison [17].

Endogenous ACh, choline and enzyme assays. The amounts of ACh and choline and the activities of choline acetyltransferase, choline phosphokinase and ace-

tylcholinesterase were determined in the forebrains of lead-treated and coetaneous control mice from 3 to 100 days of age. Mice were killed by cervical dislocation and the brains immediately removed and sectioned through the central sulcus, and each half was weighed. One half of the brain (right side) was homogenized in 1 ml FA/A (150 mg/ml for ACh and choline); the contralateral half was homogenized in 1 ml of distilled water for the enzyme assays. The time between sacrifice and homogenization in FA/A was less than 1 min. Formic acid–acetone homogenates were allowed to stand for 30–60 min in the cold (0–2°) to allow for complete extraction of ACh. Samples were homogenized by hand in ground glass homogenizers, at 0–2°, and all assays performed in duplicate. Choline and ACh were assayed by the radio-enzymatic assay of Goldberg and McCaman [12, 13]. Choline acetyltransferase and acetylcholinesterase were assayed by the method of McCaman and Hunt [18] as modified by Fonnum [19] and described by Spyker *et al.* [20]. Choline phosphokinase activity was measured by the method of Goldberg and McCaman [12]. All enzyme assays were standardized for all age groups so that product formation was linear with both the amount of tissue and the time of incubation. In each assay, less than 10 per cent of the substrate was utilized during the incubation period chosen. Protein was analyzed by the method of Lowry *et al.* [21]. All values reported represent the mean and the standard error of the mean and are expressed as nmoles/g of tissue wet weight except in the case of the enzyme assays, which are expressed as μmoles/g of protein/hr. Statistical analysis of data was done using Student's *t*-test or analysis of variance, randomized block design [22].

RESULTS

Potassium-induced ACh release. In these studies, the potassium-induced release of ACh was found to be linear for at least 1 hr (Fig. 1), calcium dependent (Table 1) and inhibited by hemicholinium at a concentration of 100 μM [10].

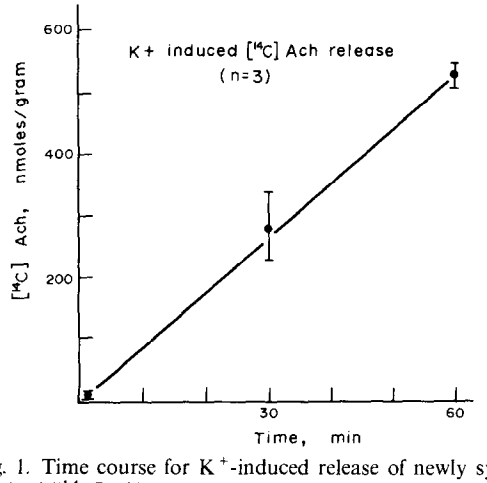

Fig. 1. Time course for K^+-induced release of newly synthesized [^{14}C]ACh. Cortical minces prepared from mouse brains were incubated for 2, 30 and 60 min with 100 μM [^{14}C]choline in 35 mM K^+ Krebs buffer containing paraoxon (0.1 μM), and the release of [^{14}C]ACh was determined.

Effect of lead treatment on potassium-induced ACh release. To determine if all three chronic doses of lead acetate (2,5 and 10 mg/ml) inhibit the potassium-induced release of ACh, minces of cortex prepared from the brains of these animals and their coetaneous controls were incubated in an elevated potassium Krebs buffer for 1 hr and the amount of ACh released into the incubation media was determined. The results presented in Table 1 show that all three dose levels *in vivo* of lead significantly reduce the potassium-induced release of ACh from mouse brain cortical minces. However, there is no increase in the magnitude of the effect as the exposure to inorganic lead is increased.

Previous reports from the laboratory demonstrated that the administration of methylphenidate (40 mg/kg, i.p.) suppresses the hyperactive state of lead-treated animals 2 hr after administration [3]. To determine if methylphenidate reverses the inhibition of potassium-induced ACh release in the lead-treated animals, the potassium-induced ACh release was determined in minces from lead-treated animals given the same dose of methylphenidate and killed 2 hr later (see Table 1). The results indicate that methylphenidate eliminates the lead inhibition of potassium-induced ACh release at the same time that it reverses the behavioral effect. Additionally, methylphenidate augments the release of ACh approximately 15 per cent from control animals (P. T. Carroll, unpublished observations).

To test if chronic lead treatment significantly inhibits the release of newly synthesized ACh, control and lead-treated cortical minces were incubated in 100 μM [^{14}C]choline for 1 hr and the release of [^{14}C]ACh was determined. The results show that the release of labeled ACh during continuous exposure of minces to elevated potassium is significantly impaired in the lead-treated animals (Fig. 2).

Effect of lead treatment on choline release from cortical minces during exposure to elevated potassium. Several groups of investigators have established that brain tissue produces and releases a net amount of choline when incubated and that this choline can be

Table 1. Effect of chronic lead treatment and calcium omission on potassium-induced release of ACh and choline from mouse brain cortical minces

Treatment	ACh (nmoles/g/hr)	N	Choline (nmoles/g/hr)	N
Control	249 ± 22	9	361 ± 68	9
Pb^{2+} (2 mg/ml)*	208 ± 23†		279 ± 46	
Control	290 ± 32	11	297 ± 37	11
Pb^{2+} (5 mg/ml)	201 ± 21†		128 ± 21†	
Control	282 ± 17	5	355 ± 31	5
Pb^{2+} (10 mg/ml)	225 ± 17†		233 ± 30†	
Control	302 ± 42	4	315 ± 36	4
Ca^{2+} free	95 ± 18†		259 ± 17	
Pb^{2+} (5 mg/ml)	158 ± 21	5	187 ± 14	7
Pb^{2+} (5 mg/ml) and methylphenidate (40 mg/kg, i.p.)	311 ± 51†	5	295 ± 20†	7

* Pb^{2+} as lead acetate.

† Mean ± S.E.M. Treated significantly differ from control ($P < 0.05$).

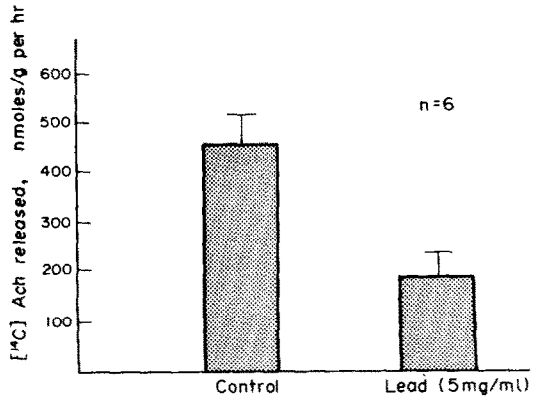

Fig. 2. Effect of chronic lead treatment on the K^+-induced release of [^{14}C]ACh from mouse brain cortical minces. Minces from control or lead acetate-treated (5 mg/ml) mice were incubated with 100 μM [^{14}C]choline in 35 mM K^+ Krebs buffer, and the release of [^{14}C]ACh was determined.

taken up by cholinergic nerve endings for the formation and release of ACh during continuous exposure to elevated potassium [10, 23]. Thus, the effect of chronic lead administration on choline efflux from cortical minces was determined. Two doses of lead (5 and 10 mg/ml) inhibit the release of choline; methylphenidate administration completely reverses this inhibitory effect of the 5 mg/ml dose of lead (see Table 1). The magnitude of the inhibition of choline efflux due to lead is twice the magnitude of its inhibition of ACh release (see Table 1).

Effects of lead treatment on enzymes involved in central ACh metabolism. Since the alteration of choline and ACh release from minces due to chronic lead treatment could reflect changes in central ACh metabolism, the activities of choline acetyltransferase, acetylcholinesterase and choline phosphokinase were determined in the brains of lead-treated and control animals from day 3 to day 100 of age. Also, the levels of choline and ACh were determined in these animals. As shown in Figs. 3–5, the activities of these enzymes are not significantly altered. It should be mentioned, however, that at day 60 of age, choline phosphokinase activity is significantly elevated. Additionally, chronic lead treatment fails to significantly alter the steady

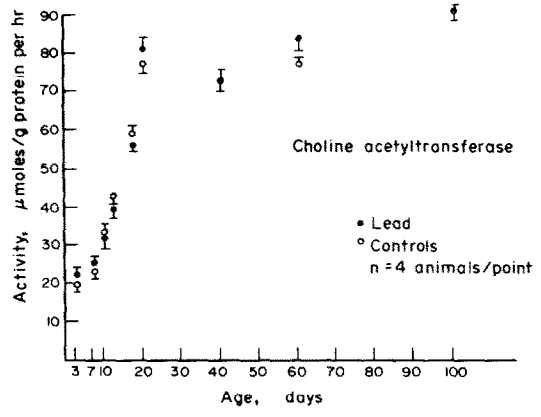

Fig. 3. Choline acetyltransferase activity in the forebrains of control and lead-treated mice between the ages of 3 and 100 days.

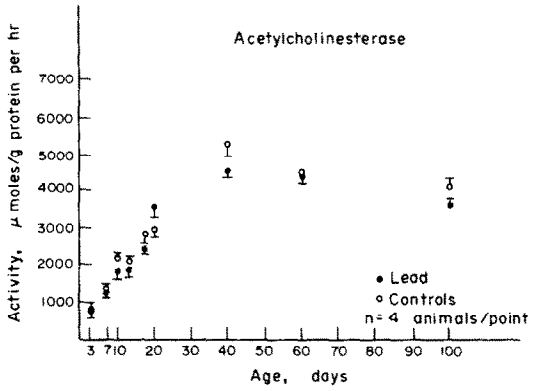

Fig. 4. Acetylcholinesterase activity in the forebrains of control and lead-treated mice between 3 and 100 days.

state levels of choline and ACh over the time periods studied (Figs. 6 and 7).

Effect of lead treatment on the spontaneous release of choline and ACh. The effect of chronic lead treatment on the spontaneous release of ACh from mouse brain cortical minces was measured (Table 2). The results show that lead acetate (5 mg/ml) significantly elevates the spontaneous release of ACh without significantly altering choline release.

Effect of lead treatment on choline transport. The effect of lead acetate pretreatment (5 mg/ml) on high and low affinity choline transport was determined. The results show that neither high nor low affinity choline transport by cortical minces is significantly inhibited in the lead-treated mice (Table 3).

Effect of calcium omission on central ACh metabolism. In many of these studies, the influence of calcium omission on various parameters of central ACh metabolism was compared with chronic lead treatment. The results indicate that calcium omission significantly inhibits the potassium-induced release of ACh without significantly altering the release of choline. Conversely, lead treatment significantly inhibits both choline and ACh release (see Table 1). Calcium omission fails to alter the spontaneous release of ACh (see Table 2). Calcium omission significantly inhibits both high and low affinity choline transport whereas lead treatment fails to inhibit either (see Table 3).

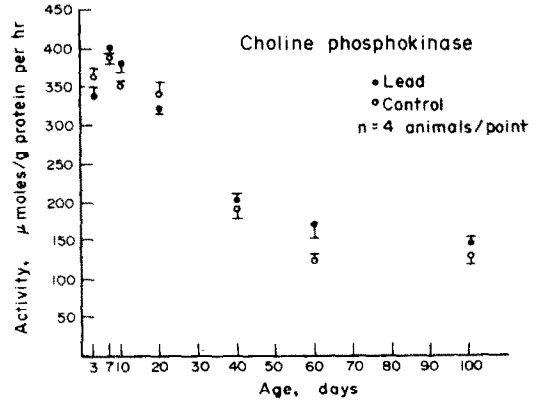

Fig. 5. Choline phosphokinase activity in the forebrains of control and lead-treated mice between 3 and 100 days. At 60 days of age, the two groups are statistically difficult.

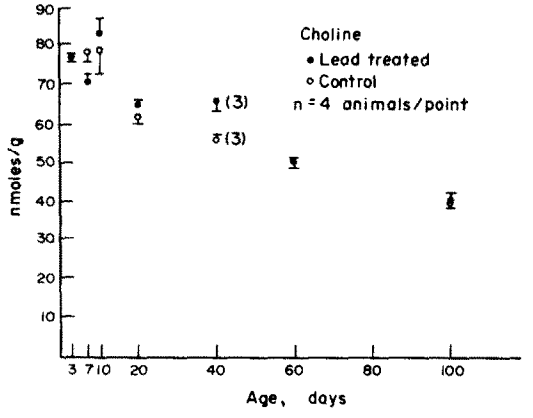

Fig. 6. Choline level in the forebrains of control and lead-treated mice between 3 and 100 days.

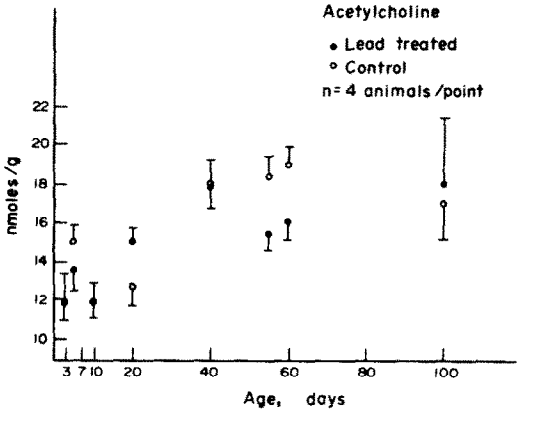

Fig. 7. ACh level in the forebrains of control and lead-treated mice between 3 and 100 days.

Table 2. Effect of chronic lead treatment and calcium omission on the spontaneous release of ACh and choline from mouse brain cortical minces

Treatment	N	ACh (nmoles/g/hr)	Choline (nmoles/g/hr)
Control	8	69.1 ± 14.8	486 ± 61
Lead acetate (5 mg/ml)	8	97.0 ± 10.0*	497 ± 50
Control	4	79.7 ± 4.8	—
Ca^{2+} free	4	74.9 ± 4.9	—

* Mean ± S.E.M. Results significantly differ from control ($P < 0.05$).

Table 3. Effect of chronic lead treatment and calcium omission on high and low affinity choline transport by mouse brain cortical minces

Treatment	[^{3}H]choline (0.6 μM)	[^{14}C]choline (100 μM)
Control*	0.242 ± 0.014 (9)	39.3 ± 3.6 (4)
Lead acetate (5 mg/ml)	0.232 ± 0.024 (9)	40.0 ± 3.8 (4)
Control*	0.238 ± 0.031 (4)	35.7 ± 4.0 (7)
Ca^{2+} free	0.163 ± 0.010† (4)	25.7 ± 4.7† (7)

* Mean ± S.E.M. Results are expressed as nmoles/g/4 min.
† Results significantly differ from control ($P < 0.05$).

DISCUSSION

The results indicate that chronic inorganic lead (as lead acetate) administered during critical periods of development and continued throughout life inhibits the release *in vitro* of ACh induced by potassium. The identical route and exposure of lead have been shown to produce an increase in spontaneous motor activity [1–3]. Like the induction of hyperactivity by lead, the inhibition of potassium-induced ACh release is not dose dependent over the exposure range studied. Administration of methylphenidate, at a dose and time that reverses the lead-induced hyperactivity in mice [3], also reverses the inhibition of ACh release due to lead. We suggest that the lead-induced hyperactivity is, at least in part, due to a defect in the central release of ACh. However, these results do not exclude the possibility that lead may be altering other neurotransmitter systems during its production of hyperactivity.

Chronic lead treatment appears to inhibit the potassium-induced release of ACh by at least two mechanisms: (1) the inhibition of choline efflux during potassium stimulation, and (2) by interfering with the role of calcium in the potassium-induced release of ACh. The inhibition of choline efflux caused by lead during potassium stimulation could decrease the amount of extracellular choline available for transport, acetylation and subsequent release. Since methylphenidate not only reverses the inhibition of potassium-induced release of ACh produced by lead but also reverses the inhibition of choline release, it appears that choline release and the subsequent acetylation and release of ACh are linked. The source of the choline being released is not known; however, it would appear to be originating from a source other than ACh, since the release of ACh is calcium dependent whereas the release of choline is not. When brain minces from lead-treated mice are incubated with a high extracellular labeled choline concentration (100 μM), the potassium-induced release of labeled ACh is still significantly impaired. Under these conditions, the inhibition of endogenous choline efflux by lead, which approaches a final concentration of 15 μM, could not account for the inhibition of potassium-induced release of labeled ACh.

Lead may interfere with the role of calcium in the evoked release of ACh [6–9]. Calcium omission *in vitro*, like chronic lead treatment, significantly reduces the potassium-induced release of ACh. However, calcium omission, unlike lead, does not significantly inhibit the release of choline during potassium stimulation. Thus, the inhibition of choline release by lead cannot be totally attributed to a competition between lead and calcium. The role of calcium in the potassium-induced release of ACh and the antagonism by lead could involve choline transport, conversion to ACh, available stores of ACh or enzyme systems involved in the metabolism of choline and ACh. Calcium omission significantly inhibits both high and low affinity choline transport by cortical minces whereas chronic lead treatment fails to affect either. This latter result appears to conflict with the finding that chronic lead treatment inhibits high affinity transport by synaptosomes prepared from the brains of hyperactive mice [3]. This disparity may be due

to differences in the transport characteristics of the two preparations. Recently, it has been suggested that glucose transport into synaptosomes differs from that of minces [24].

Lead-induced decreases of potasssium-induced ACh release cannot be attributed to a decrease in available stores of choline or ACh or to the enzymes involved in choline metabolism or ACh hydrolysis, since none of these parameters of ACh metabolism are significantly altered by chronic lead administration. These results differ from those reported for the rat in that lead administration significantly alters the activity of both acetylcholinesterase [25, 26] and choline acetyltransferase in certain brain regions [25].

That the spontaneous release of ACh is elevated in the brains of lead-treated animals is not surprising, since the addition of lead to a sciatic nerve-sartorious muscle preparation of the frog has been reported to elevate the MEPP frequency and to inhibit the EPP frequency [7]. Two experimental results suggest that the spontaneous and potassium-induced release of ACh differ: (1) lead elevates the spontaneous but inhibits the potassium-induced release of ACh, and (2) lead significantly reduces the release of choline during potassium-induced ACh release but fails to significantly alter the spontaneous release of choline. It should be noted that lead is not unique in its ability to elevate the spontaneous release of ACh while inhibiting the potassium-induced release of ACh. Black widow spider venom also elevates the spontaneous and inhibits the potassium-induced release of ACh from brain tissue [27].

Chronic lead treatment fails to alter the amount of choline and ACh in the CNS while altering both the spontaneous and potassium-induced release of ACh. These results suggest that measurement of steady state levels of ACh and or choline does not provide complete information on changes in central cholinergic metabolism.

Throughout this study, animals born at the same time were used. However, lead-treated animals are somewhat smaller. Thus, as previously reported [1], lead treatment impairs growth during early stages of life. By adulthood the animals weigh at least as much as controls. Since lead-treated animals exhibit different growth patterns as compared to identically handled control animals, it is conceivable that the early impairment of growth, caused by the lead treatment, may alter central neurochemical processes. The decrease in acetylcholine release seen in lead-exposed animals is consistent with the effects *in vitro* of lead on peripheral nervous tissue. Since the effects *in vitro* are not dependent upon the nutritional status of the animals, it is unlikely that the early under-nutrition is the cause of the altered neurochemistry observed in this study.

In summary, the present work indicates that chronic lead exposure, at doses previously shown in mice to elicit hyperactivity, disrupts central ACh metabolism. Methylphenidate, a drug which reverses the behavioral state of the animals, also reverses the inhibition of choline and ACh release due to lead. Several experimental results suggest that lead may be a valuable tool in elucidating the dynamic processes involved in central ACh metabolism. Specifically, the results obtained with lead may suggest that the mechanisms underlying the spontaneous and evoked release of ACh in the CNS may differ.

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REFERENCES

1. E. K. Silbergeld and A. M. Goldberg, *Life Sci.* **13**, 1275 (1973).
2. E. K. Silbergeld and A. M. Goldberg, *Expl Neurol.* **42**, 146 (1974).
3. E. K. Silbergeld and A. M. Goldberg, *Neuropharmacology* **14**, 431 (1975).
4. M. W. Sauerhoff and I. A. Michaelson, *Science, N.Y.* **182**, 1022 (1973).
5. J. Allen, P. J. McWay and S. J. Suomi, *Envir. Hlth Perspect.* **7**, 239 (1974).
6. K. Kostial and V. B. Vouk, *Br. J. Pharmac. Chemother.* **12**, 219 (1957).
7. R. S. Manalis and G. P. Cooper, *Nature, Lond.* **243**, 354 (1973).
8. E. K. Silbergeld, J. T. Fales and A. M. Goldberg, *Nature, Lond.* **241**, 49 (1974).
9. E. K. Silbergeld, J. T. Fales and A. M. Goldberg, *Neuropharmacology* **13**, 795 (1974).
10. P. T. Carroll and A. M. Goldberg, *J. Neurochem.* **25**, 523 (1975).
11. K. R. Mahaffey, R. Goyer and J. K. Haseman, *J. Lab. clin. Med.* **82**, 92 (1973).
12. A. M. Goldberg and R. E. McCaman, *J. Neurochem.* **20**, 1 (1973).
13. A. M. Goldberg and R. E. McCaman, in *Chemical Methods for the Determination of Choline and Acetylcholine* (Ed. I. Hanin), p. 47. Raven Press, New York (1974).
14. R. E. McCaman, S. A. Dewhurst and A. M. Goldberg, *Analyt. Biochem.* **42**, 171 (1971).
15. G. Brante, *Nature, Lond.* **163**, 651 (1949).
16. H. I. Yamamura and S. H. Snyder, *J. Neurochem.* **21**, 1355 (1973).
17. M. Toru and M. H. Aprison, *J. Neurochem.* **13**, 1533 (1966).
18. R. E. McCaman and J. M. Hunt, *J. Neurochem.* **12**, 253 (1965).
19. F. Fonnum, *Biochem. J.* **115**, 465 (1969).
20. J. M. Spyker, S. B. Sparber and A. M. Goldberg, *Science, N.Y.* **177**, 621 (1972).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
22. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics with Special Reference to the Biological Sciences*, p. 1. McGraw-Hill, New York (1960).
23. B. Collier, P. Poon and S. Salehmoghaddam, *J. Neurochem.* **19**, 51 (1972).
24. J. Elbrink and I. Bihler, *Science, N.Y.* **188**, 1177 (1975).
25. A. T. Modak, S. T. Weintraub and W. B. Stavinoha, *Toxic. appl. Pharmac.* **34**, 340 (1975).
26. T. J. Sobotka, R. E. Brodie and M. P. Cook, *Toxicology* **5**, 175 (1975).
27. N. Frontali, F. Granata and P. Parisi, *Biochem. Pharmac.* **21**, 969 (1972).