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Lead concentration in skeletal muscle in amyotrophic lateral sclerosis patients and control subjects

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SUMMARY The concentration of lead in the vastus lateralis muscle was determined in patients with amyotrophic lateral sclerosis and control subjects by flameless atomic absorption spectrophotometry. No statistically significant differences were found between these groups, and in both the figures were of the same magnitude as those earlier reported for normal individuals. Increased plasma lead concentrations do not seem to cause a significant deposition of lead in skeletal muscle. Therefore, plasma rather than skeletal muscle is the more likely source if pathological amounts of lead enter the motoneurones via the retrograde axoplasmic flow in amyotrophic lateral sclerosis.

A number of different substances have been found to be taken up by the motor endplates and transported by retrograde axoplasmic flow to the cell bodies of lower motor neurones in the spinal cord and brainstem (Kristensson, 1975; Stöckel *et al.*, 1975). Since this transport route bypasses the blood-brain barrier it has gained interest as a possible way for noxious substances to reach the central nervous system, and an uptake of such substances through the retrograde axoplasmic flow would also offer a possible explanation for a selective damage to the motoneurones. When considering the possible significance of this mechanism in the pathogenesis of motor neurone diseases, the presence of potentially noxious substances in skeletal muscle thus appears to be of interest.

In a series of studies we are investigating the possible role of the retrograde axoplasmic flow of the lower motor neurones and an abnormality in the tissue distribution and turnover of lead in amyotrophic lateral sclerosis. We have recently demonstrated increased concentrations of lead in cerebrospinal fluid and plasma in patients with amyotrophic lateral sclerosis as compared with control subjects (Conradi *et al.*, 1976, 1978a). In the present investigation, the lead concentration was determined in muscle biopsies from amyotrophic lateral sclerosis patients and control subjects by flameless atomic absorption spectrophotometry according to a technique developed especially for this purpose (Vesterberg and Nise, personal communication.

Lead is considered to be stored in three compartments in the organism, each having a different turnover rate of the metal (Rabinowitz *et al.*, 1976). Thus, lead in blood has the shortest half-life (days) whereas soft tissues, including skeletal muscle, form an intermediate compartment with a half-life of weeks. The greatest lead compartment is the skeleton where the metal is firmly bound. The half-life here is months or years. Earlier estimations of the concentration of lead in muscle in normal individuals (mostly using the dithizone method) have given figures of 5–25 μg of lead per 100 g tissue wet weight (Schroeder and Balassa, 1961; Barry and Mossman, 1970; Gross *et al.*, 1975; Sumino *et al.*, 1975), and there is evidence for a decreasing concentration with age (Gross *et al.*, 1975). There is one earlier report on the concentration of lead in skeletal muscle of patients with amyotrophic lateral sclerosis as compared with control subjects (Petkau *et al.*, 1974). These authors reported 10–20 times higher concentrations in patients than in control subjects.

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Patients and methods

Twelve patients with amyotrophic lateral sclerosis and 14 control subjects were investigated (Table). The amyotrophic lateral sclerosis patients were diagnosed on conventional clinical grounds. They showed a varying degree of atrophy of the lower limb muscles but were all still able to walk. The degree of atrophy was not quantified. The control group consisted of routine necropsy cases at the Department of Forensic Medicine, Karolinska Institutet, Stockholm.

In the amyotrophic lateral sclerosis group, open muscle biopsies were performed on vastus lateralis, and about 2 g of muscle was removed. A similar procedure was undertaken on the control cases, within 24 hours of death. We tried to obtain samples with a minimum of connective tissue. The samples were immediately frozen in lead-free test tubes.

Before analysis, the samples were weighed, and the haemoglobin content was determined with an immunodiffusion technique. The samples were then dissolved in Soluene 350 at 40°C. The lead content was determined with an atomic absorption spectrophotometer model AA-6 (Varian Tectron, Melbourne, Australia) with a carbon rod CRA-63 and a temperature control unit measuring the temperature of the carbon rod with a photodiode. A hollow cathode lamp (Varian) was used and operated at 5 mA with use of the lead 217.0 nm resonance line in the determinations. The slit width was 1.0 nm. A deuterium lamp was used for background correction. A standard deviation of ±10% for within day runs and ±15% for runs between days was estimated. For each sample the lead concentration per 100 g wet weight and ash weight was calculated. The mean figures obtained for the two groups were compared using a t test.

Results

The muscle lead concentrations found in the two groups are shown in the Table. In the amyotrophic lateral sclerosis group, the mean wet weight concentration was 14.6 μg/100 g of muscle and the mean ash weight concentration was 51.9 μg/100 g. The corresponding figures for the control group were 11.9 and 40.6 μg/100 g respectively. The differences between the two groups were not statistically significant ($P<0.05$). The range of variation within the two groups was the same for the wet weight concentrations (4–25 μg/100 g) and nearly the same for the ash weight concentrations (17–95 and 13–87 μg/100 g respectively). In both groups, the ratio ash weight concentration/wet weight concentration was found to vary considerably (2.8–4.3 for the amyotrophic lateral sclerosis group and 2.6–5.0 for the control group). The mean ratio for the two groups was, however, close to the same (3.6 and 3.4 respectively). Neither in the group with amyotrophic lateral sclerosis nor in the control group was the variation in muscle lead concentration correlated with the age of the patient.

Discussion

The analytical method showed a good reproducibility and was considered suitable for this kind of work. The haemoglobin content of the specimens was always less than 5%, and the wet weight

Table *Year of birth and muscle lead concentration in the two groups studied*

Amyotrophic lateral sclerosis				*Control subjects*			
Number	*Year of birth*	*Muscle lead concentration*		*Number*	*Year of birth*	*Muscle lead concentration*	
		Wet weight (μg/100 g)	*Dry weight (μg/100 g)*			*Wet weight (μg/100 g)*	*Dry weight (μg/100 g)*
A 1	1915	12	45	N 1	1924	5	13
A 2	1922	19	62	N 2	1922	14	53
A 3	1943	16	50	N 3	1925	19	66
A 4	1928	20	79	N 4	1912	4	17
A 5	1900	9	37	N 5	1891	6	24
A 6	1913	14	39	N 6	1931	12	60
A 7	1903	17	49	N 7	1942	25	87
A 8	1932	8	24	N 8	1907	10	34
A 9	1919	17	66	N 9	1898	17	24
A10	1914	25	95	N10	1913	14	42
A11	1910	16	60	N11		9	32
A12	1917	4	17	N12	1916	8	35
				N13	1900	15	55
Mean	1918	14.6 s=5.77	51.9 s=22.16	N14	1905	9	27
				Mean	1914	11.9 s=5.86	40.6 s=21.02

concentrations of lead, as found here in both groups, were of the same magnitude as that of whole blood in both amyotrophic lateral sclerosis patients and control specimens (see Conradi *et al.*, 1976). Thus, contamination with blood seems to be a small source of error in the determinations, which makes possible comparisons between muscle tissue taken in vivo and at necropsy, respectively. When interpreting the figures obtained it must also be borne in mind that the specimens were very small. It is difficult to determine whether or not such a specimen is representative of the whole muscle or of the skeletal muscle tissue as a whole. No information is currently available on possible variations in lead concentration between different muscles or between different regions in the same muscle. In the present study the samples were taken from vastus lateralis since this muscle is commonly used for biopsies in routine clinical work.

When comparing the figures obtained for the two groups it should be noted that the mean age of the amyotrophic lateral sclerosis patients was slightly lower than that of the control subjects. There is some earlier evidence for decreasing lead concentrations in skeletal muscle with age (Gross *et al.*, 1975), but there was no obvious correlation between muscle lead concentration and age in any of the samples in the present study. It should also be emphasised that the patients with amyotrophic lateral sclerosis showed a varying degree of muscle atrophy caused by the disease. Nothing is known about a possible redistribution of the metal in the muscle tissue in atrophy. However, the mean ratio ash weight concentration/wet weight concentration was close to the same in both groups although there was a considerable variation within groups. In spite of the possible sources of error associated with the present analysis and the difficulties in comparing the figures obtained for the two groups, our results did not suggest that the lead concentration in skeletal muscle would be significantly increased in amyotrophic lateral sclerosis patients as compared with control subjects. Thus, the results of Petkau *et al.* (1974) indicating such an increase were not confirmed.

This study is part of a series dealing with the question as to whether lead might be pathologically available to lower motor neurones through uptake in motor endplates and subsequent transport via retrograde axoplasmic flow of motor axons in amyotrophic lateral sclerosis. Earlier findings of raised lead levels in CSF and plasma in amyotrophic lateral sclerosis (Conradi *et al.*, 1976, 1978a) support the hypothesis of an abnormal turnover and tissue distribution of this metal. Judging from the present results, the increased plasma lead concentrations do not seem to cause a significant deposition of lead in skeletal muscle. Therefore, plasma rather than skeletal muscle is the more likely source if pathological amounts of lead enter the motoneurones via the retrograde axoplasmic flow in amyotrophic lateral sclerosis. There is earlier experimental evidence for a passage of material from plasma to the cell bodies of lower motor neurones through retrograde axoplasmic flow (Broadwell and Brightman, 1976). Since most of the substances so far demonstrated to be transported via the retrograde flow are macromolecules (Kristensson, 1975; Stöckel *et al.*, 1975), a plausible source of lead immediately available for uptake by the endplates is plasma protein-bound lead in the extracellular fluid in the skeletal muscles. The plasma protein binding of lead has recently been studied in vitro on patients with amyotrophic lateral sclerosis and control subjects in our laboratory (Conradi *et al.*, 1978c), and a preferential binding of lead to orosomucoid (acid $alpha_1$-glycoprotein) seemed to occur in both groups. As to the occurrence of plasma proteins in extracellular fluid in amyotrophic lateral sclerosis, we have earlier found no substantial difference in capillary permeability of albumin between amyotrophic lateral sclerosis patients and control subjects (Conradi *et al.*, 1978b). The size of orosomucoid is similar to that of albumin (Schmidt, 1975) and the distribution of the two proteins in extracellular fluid might thus be similar. Consequently, the amount of protein-bound lead in the extracellular fluid in skeletal muscle in amyotrophic lateral sclerosis would be a reflection of the concentration in plasma. Retrograde axoplasmic transport of plasma protein-bound lead in lower motor neurones is now being studied experimentally in our laboratory.

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