Environmental Health Perspectives
Vol. 78, pp. 47–51, 1988

Influence of Membrane Sodium Transport upon the Relation Between Blood Lead and Blood Pressure in a General Male Population

by Thierry Moreau,* Patrick Hannaert,† Genevieve Orssaud,‡ Guy Huel,* Ricardo P. Garay,* Jean R. Claude,‡ Bernard Juguet,§ Bernard Festy,§ and Joseph Lellouch*

Five red blood cell cation transport systems (RBCTS), together with blood lead level and blood pressure, were measured in 129 male adult subjects who were not occupationally exposed to lead or subsequent to a course of treatment for hypertension. Blood lead was positively related with systolic blood pressure, and to a lesser degree with diastolic blood pressure. Blood lead was found significantly negatively related to one of the RBCTS, Na^+,K^+ cotransport, and in addition, Na^+,K^+ cotransport appeared negatively related to blood pressure. Final results showed that blood lead no longer accounts for an increase in systolic blood pressure when Na^+,K^+ cotransport was taken into account; the same trend was observed with diastolic blood pressure. These findings suggest that a blood lead-related Na^+,K^+ cotransport impairment could explain the blood pressure increase observed to parallel the blood lead increase.

Introduction

The relationships of blood lead to blood pressure have been investigated in several general populations, with somewhat divergent published results (*1,2*).

None of these studies attempted to explain the mechanisms possibly involved in these relationships; however, several works have shown that blood lead could inhibit one of the red blood cell cation transport systems (RBCTS): the Na^+,K^+ pump (*3–5*), and other reports suggest that one or more RBCTS, including Na^+,K^+ pump, could be impaired in hypertensive subjects (*6–8*).

Therefore, it was logical to test the hypothesis that the increase of blood pressure with blood lead could be explained at least partly by RBCTS impairment due to an effect of lead exposure as reflected in blood lead concentration. It is the aim of the present study to ininvestigate in a sample of not occupationally lead-exposed men the possible role of five RBCTS, including the Na^+,K^+ pump, and the role of red blood cell Na^+ and K^+ content in the blood lead-blood pressure relationship.

Materials and Methods

Population

The study population consisted of 129 adult men belonging to the Paris Police Administration, who were not occupationally exposed to lead, who were not following a course of treatment for hypertension, and who were aged 23 to 57 years (mean, 36.2 years). Each

*INSERM, Unité 169, 16 Ave P.V. Couturier, 94807, Villejuif Cedex, France.
†INSERM, Unité 7, 161 rue de Sèvres, 75730, Paris Cedex 15, France.
‡Laboratoire DASES, 88 rue de La Jonquière, 75017, Paris, France.
§Laboratoire LHVP, 1 bis rue des Hospitalièeres St. Gervais, 75004, Paris, France.

subject was given a standardized questionnaire asking for detailed alcohol and tobacco consumption, among other information. Several physical parameters were recorded and a fasting blood sample was taken for biochemical measurements.

Alcohol Consumption and Smoking Habits

For each subject, the daily consumption of wine, beer, aperitif, and spirits was recorded and converted in grams of alcohol per day. The smoking status was also noted: nonsmokers, 45 subjects; exsmokers, 24 subjects; current smokers, 57 subjects. For current smokers, the quantity of tobacco smoked per day was expressed in grams.

Physical Examination

Systolic and diastolic blood pressures were measured using a mercury apparatus. Three measurements were performed: the first after 10 min resting, the second 10 min later (both in supine position), and the third in the standing position. This protocol followed the recommendations for blood pressure measurements of the British Hypertension Society (*9*). Individual blood pressure values reported (systolic or diastolic) are the means of these three measurements. Height and weight were also recorded.

Biochemical Measurements

The following parameters were measured on each blood sample.

Blood Lead. Whole heparinized blood was diluted (1/10) into an ammonium nitrate solution; the dilution was injected into a tantalum-coated graphite furnace of a Jarrel-Ash 850 AA spectrometer (dry 100°C, ash 750°C, atomized 2,450°C). Measurements were performed at 283.3 nm wavelength with background correction and calibration by standard additions. Special precautions were taken to avoid contamination by ambient lead, reagents, and materials (heparinized collection tubes, Vacutainer, BD 06527).

RBCTS and Red Blood Cell Na^+ and K^+ Contents. Five RBCTS were considered in fresh red cells: Na^+,K^+ pump; Na^+,K^+ cotransport; Na^+,Li^+ countertransport; and Na^+ and K^+ passive permeabilities. Their mechanisms of action are outlined in Figure 1. Methods of measurement of each RBCTS are described in detail elsewhere (*10*) and can be summarized as follows. Blood samples were stored at 4°C and used within 3 hr. After removal of plasma and buffy coat, red cells were washed free of Na^+. The activity of the different transport systems was assessed by spectrophotometrically measuring Na^+ efflux in four different Na^+-free media containing: a) K^+; b) ouabain; c) ouabain and bumetanide; and d) ouabain, bumetanide, and Li^+. Red blood cell Na^+ and K^+ contents were expressed

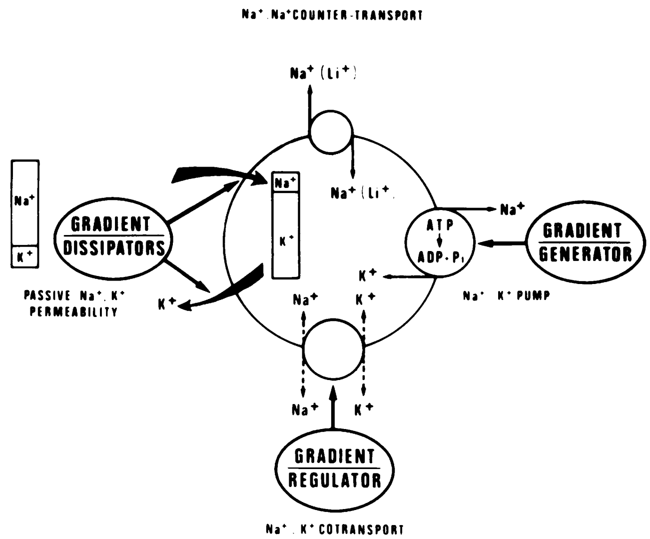

FIGURE 1. Red blood cell sodium (Na^+) and potassium (K^+) transport systems (RBCTS).

in µmole/L·cells. Na^+ and K^+ passive permeabilities were expressed in hr^{-1}. Activity of Na^+,K^+ pump, Na^+,K^+ cotransport, and Na^+,Li^+ countertransport were expressed in µmole/L·cells·hr.

Results

Blood Lead and Blood Pressure

Blood lead levels and systolic blood pressure values had log-normal distributions, so that log transformations were used in statistical analysis.

Among information obtained from the questionnaires, daily alcohol consumption appeared to be strongly positively related to blood lead (correlation coefficient $r = 0.29$, $p < 0.01$); the blood lead means ± SEM in nondrinkers (19 subjects), in subjects who drank between 1 and 50 g/day (64 subjects), in subjects who drank more than 50 g/day (35 subjects) were, respectively: 142.1 ± 8.6 µg/L, 160.8 ± 5.2 µg/L, and 188.3 ± 8.8 µg/L. Age was weakly positively associated with blood lead ($r = 0.14$, $p = 0.13$). Daily tobacco consumption was not related to blood lead in current smokers ($r = 0.01$); the blood lead means ± SEM in current smokers and in exsmokers were 175.4 ± 5.9 µg/L and 178.5 ± 9.9 µg/L, significantly higher than the mean in nonsmokers (146.8 ± 6.2 µg/L).

Blood lead was found to be significantly positively related to systolic blood pressure ($r = 0.19$, $p = 0.05$) and to a lesser degree to diastolic blood pressure ($r = 0.17$, $p = 0.07$) Taking into account alcohol consumption and age yielded the partial correlation coefficients 0.17 ($p = 0.09$) and 0.14 ($p = 0.15$), respectively; taking further into account body mass index did not change these latter results. The means of systolic blood pressure according to blood lead levels are shown in Table 1; they appear to increase about 8 mm Hg through the three lowest blood lead classes (lower than 120 μg/L, between 120 to 160 μg/L, and those between 161 to 200 μg/L) and then, at ≥ 200 μg/L, to reach a plateau in blood pressure values. As shown in Table 1, similar results were obtained after adjusting systolic blood pressure values for alcohol consumption, age, and body mass index. Table 1 also shows that the same trend is observed concerning diastolic blood pressure.

RBCTS, Red Blood Cell Na^+ and K^+ Contents, and Blood Pressure

The distribution of Na^+,K^+ pump appeared log-normal, so that statistical tests were performed after log transformation.

Among RBCTS and red blood cell Na^+ and K^+ contents, only Na^+,K^+ cotransport was significantly inversely related to either systolic or diastolic blood pressure ($r = -0.20$, $p < 0.05$; $r = -0.19$, $p < 0.05$, respectively). Figure 2 shows the systolic and diastolic blood pressure means according to Na^+,K^+ cotransport activity values. The systolic or, respectively, diastolic blood pressure mean increase is about 6 mm Hg or 5 mm Hg from the highest Na^+,K^+ cotransport class (271 μmole/L·cells·hr and over) where the means are 126.0 mm Hg for systolic (83.7 mm Hg for diastolic) until the two lowest classes (131–200 μmole/L·cells·hr and lower than 130 μmole/L·cells·hr), in which the mean pressures are nearly equal: 131.9 mm Hg and 131.1 mm Hg (respectively, diastolic 88.6 mm Hg and 88.1 mm Hg).

After adjustment for alcohol consumption, age, and body mass index (whose correlations with Na^+,K^+ cotransport were: $r = -0.20$, $p < 0.05$; $r = -0.21$, $p < 0.05$; $r = 0.13$, $p = 0.15$; respectively), the partial correlation coefficients between Na^+,K^+ cotransport and either systolic or diastolic blood pressure were $r = -0.28$ ($p < 0.01$).

RBCTS, Red Blood Cell Na^+ and K^+ Contents, and Blood Lead

The correlation coefficients of RBCTS and red blood cell Na^+ and K^+ contents with blood lead are listed in Table 2. It appears that only a decrease in Na^+,K^+ cotransport is significantly related to an increase in blood lead ($r = -0.23$, $p < 0.05$). The corresponding regression coefficient indicates a 39.5 μmole/L·cells·hr Na^+,K^+ cotransport decrease for a 100 μg/L blood lead increase. This negative relationship remains significant after allowance for alcohol consumption, age, and body mass index (partial correlation coefficient, $r = 0.19$, $p < 0.05$).

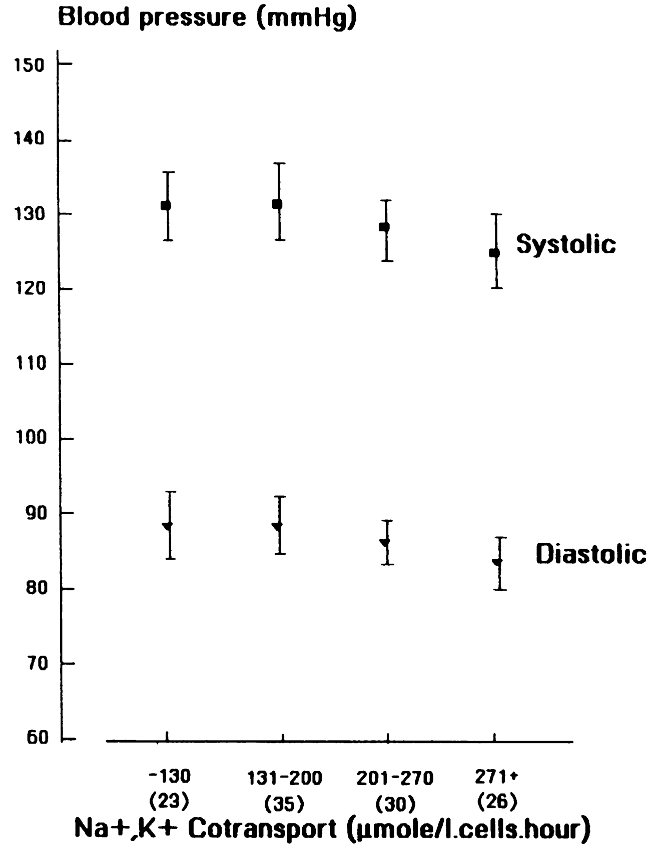

FIGURE 2. Means of systolic (■) and diastolic (▼) blood pressure according to Na^+,K^+ cotransport activity. Means are represented together with their 95% confidence limits. Numbers of subjects in each Na^+,K^+ cotransport class are in parentheses.

Table 1. Systolic and diastolic blood pressure means according to blood lead values.

Blood lead, μg/L	Systolic blood pressure, mm Hg: Mean ± SEM	Systolic: Adjusted mean[a]	Diastolic blood pressure, mm Hg: Mean ± SEM	Diastolic: Adjusted mean[a]
< 121	124.7 ± 1.9 (18)[b]	124.6 (17)	82.9 ± 1.9 (18)	83.8 (17)
121–160	126.7 ± 1.7 (44)	126.3 (40)	85.7 ± 1.4 (44)	86.4 (40)
161–200	133.4 ± 2.2 (31)	132.0 (27)	89.8 ± 2.0 (31)	88.9 (27)
> 200	130.9 ± 2.4 (20)	129.0 (20)	88.0 ± 2.8 (20)	87.4 (20)

[a]Systolic and diastolic blood pressure values were adjusted for alcohol consumption, age, and body mass index.
[b]Numbers of subjects are in parentheses.

Table 2. Correlation coefficients of blood lead levels with RBCTS and red blood cell Na^+ and K^+ contents.

Parameter	Correlation coefficient	Significance
Na^+,K^+ pump	0.05 (124)[a]	NS[b]
Na^+,K^+ cotransport	− 0.23 (124)	0.02
Na^+,Li^+ countertransport	− 0.04 (124)	NS
Na^+ passive permeability	0.01 (124)	NS
K^+ passive permeability	0.09 (119)	NS
Red blood cell Na^+ content	0.11 (124)	NS
Red blood cell K^+ content	− 0.09 (124)	NS

[a]Numbers of subjects are in parentheses.
[b]NS, not significant at the 10% level.

Na^+,K^+ Cotransport, Blood Lead, and Blood Pressure

The effect of blood lead upon blood pressure independent of Na^+,K^+ cotransport was studied by the means of multiple regressions with either systolic or diastolic blood pressure as the dependent variable.

Results concerning systolic blood pressure are summarized in Table 3, showing the decrease of the blood lead regression coefficients as a) blood lead alone considered as an independent variable (regression coefficient, 3.7 mm Hg/100 µg Pb/L, $p = 0.05$); b) alcohol consumption, age, and body mass index added as independent variables (regression coefficient, 3.0 mm Hg/100 µg Pb/L, $p = 0.09$); c) Na^+,K^+ cotransport included further in the regression equation (regression coefficient, 2.1 mm Hg/100 µg Pb/L, $p = 0.21$). In this latter case, the Na^+,K^+ cotransport regression coefficient is −3.5 mm Hg/100 µmole/L·cells·hr. ($p < 0.01$).

Table 4 shows the results of the regression analyses performed as described with diastolic blood pressure as a dependent variable. The blood lead regression coefficients are: 3.0 mm Hg/100 µg Pb/L, ($p = 0.07$); 2.1 mm Hg/100 µg Pb/L, ($p = 0.15$); 1.4 mm Hg/100 µg Pb/L, ($p = 0.32$). In the latter regression equation, the Na^+,K^+ cotransport regression coefficient is −3.2 mm Hg/100 µmole/L·cells·hr ($p < 0.01$).

Discussion

The overall blood lead mean observed in this study (165.8 µg Pb/L) is in agreement with blood lead levels found in other general populations (*11–13*). The factors contributing to variation of blood lead found in this study are the same as those found in other studies: alcohol consumption is positively related to blood lead; age and blood lead are weakly positively correlated. The association between blood lead and tobacco consumption is of less importance, since the amount of tobacco smoked by current smokers is not correlated with blood lead.

Table 3. Multiple regressions of systolic blood pressure on blood lead, Na^+,K^+ cotransport, age, body mass index, and alcohol consumption.

	Regression coefficients × 100 ± SEM[b]	
Independent variables[a]	Blood lead	Na^+,K^+ cotransport
Blood lead alone (113)[c]	3.0 ± 0.22 $p = 0.05$	
Blood lead and A, B, C (104)	3.0 ± 0.22 $p = 0.09$	
Blood lead, Na^+,K^+ cotransport, and A, B, C (102)	2.1 ± 0.22 $p = 0.21$	−3.5 ± 0.13 $p < 0.01$

[a]Independent variables: A, age; B, body mass index; C alcohol consumption.
[b]Regression coefficient × 100 indicates the systolic blood pressure increase in mm Hg for a 100 µg Pb/L increase and for a 100 µmole/L·cells·hr Na^+,K^+ cotransport increase.
[c]Numbers of subjects are in parentheses.

Table 4. Multiple regressions of diastolic blood pressure on blood lead, Na^+,K^+ cotransport, age, body mass index, and alcohol consumption.

	Regression coefficients × 100 ± SEM[b]	
Independent variables[a]	Blood lead	Na^+,K^+ cotransport
Blood lead alone (113)[c]	3.0 ± 0.20 $p = 0.07$	
Blood lead and A, B, C (104)	2.1 ± 0.20 $p = 0.15$	
Blood lead, Na^+,K^+ cotransport, and A, B, C (102)	1.4 ± 0.20 $p = 0.32$	−3.2 ± 0.12 $p < 0.01$

[a]Independent variables: A, age; B, body mass index; C, alcohol consumption.
[b]Regression coefficient × 100 indicates the systolic blood pressure increase in mm Hg for a 100 µg Pb/L increase and for a 100 µmole/L cells·hr Na^+,K^+ cotransport increase.
[c]Numbers of subjects are in parentheses.

The association of blood lead to systolic blood pressure was found to be significant ($r = 0.19$, $p = 0.05$). The correlation of blood lead with diastolic blood pressure is slightly lower ($r = 0.17$, $p = 0.07$), as already noted in our earlier study (*14,15*). Taking into account alcohol consumption and age as confounding variables slightly decreases these correlations (systolic, $r = 0.17$, $p = 0.09$; diastolic, $r = 0.14$, $p = 0.15$); adjusting further for body mass index does not change these values.

A significant negative relationship was observed between Na^+,K^+ cotransport and both systolic and diastolic blood pressure ($r = -0.20$, and $r = -0.19$, respectively). Adjusting for alcohol consumption, age, and body mass index appears to strengthen these relationships ($r = -0.28$). This result confirms those obtained in a previous study where hypertensive and normotensive subjects were compared (*16*). Although the interpretation of Na^+,K^+ cotransport remains controversial as far as red blood cells are concerned, it must be stressed that the role of Na^+,K^+ cotransport in regulating Na^+ cell content appears to be of greater importance in other cells, including vascular smooth muscle cells (*17–19*). Thus, an impaired Na^+,K^+ cotransport activity in these cells could increase Na^+ content. This in turn may increase Ca^{2+} content and blood pressure, via a Na^+, Ca^{2+} exchange mechanism.

Results presented above show that Na^+,K^+ cotransport is significantly negatively related to blood lead. No other association was found between blood lead and either other RBCTS or red blood cell Na^+ and K^+ contents. In particular, the lack of correlation observed between Na^+, K^+ pump and blood lead level is not in agreement with other results (*3–5*). However, it must be noted that, in these latter studies, Na^+,K^+ ATPase activity was measured on red blood cell hemolysates; instead, we measured Na^+,K^+ pump activity. This particular point deserves further confirmation.

Finally, considering the influence of both Na^+,K^+ cotransport and blood lead upon systolic blood pressure, after adjustment for alcohol consumption age and body mass index, the blood lead regression coefficient is very far from significance (p = 0.21), while Na^+,K^+ cotransport is highly significantly negative ($p < 0.01$) (Table 3). By comparison, when Na^+,K^+ cotransport is no longer taken into account, the blood lead regression coefficient is either nearly significant (p = 0.09) when adjusting for alcohol consumption, age, and body mass index, or significant (p = 0.05) without any adjustment. Put together, these results show that Na^+,K^+ cotransport impairment explains the relationship between blood lead and systolic blood pressure to a greater extent than do the confounder variables alcohol consumption and age. This trend is also observed, to a lesser degree, for diastolic blood pressure (Table 4).

In conclusion, these results are in agreement with the hypothesis that blood lead could impair Na^+,K^+ cotransport activity, and this Na^+,K^+ cotransport alteration could increase blood pressure. Further studies are needed to confirm this finding.

The authors gratefully acknowledge the work of Josianne Sahuquillo and Gisèle Salomon in providing valuable technical assistance. This work was supported by the Programme Interdisciplinaire de Recherche sur l'Environment (PIREN) under contract number 034598.

REFERENCES

1. Shaper, A. G., and Pocock, S. J. Blood lead and blood pressure. Br. Med. J. 291: 1147–1149 (1985).
2. Sharp, D. S., Becker, C. E., and Smith, A. H. Chronic low-level lead exposure: Its role in the pathogenesis of hypertension. Med. Toxicol. 2: 210–232 (1987).
3. Hasan, J., Vikho, V., and Hernberg, S. Deficient red cell membrane Na^+,K^+ ATPase in lead poisoning. Arch. Environ. Health 14: 313–318 (1967).
4. Hernberg, S., Vikho, V., and Hasan, J. Red cell membrane ATPases in workers exposed to inorganic lead. Arch. Environ. Health 14: 319–324 (1967).
5. Secchi, G. C., Alessio, L., and Cambiaghi, G. Na^+,K^+ ATPase activity of erythrocyte membranes in urban populations not occupationally exposed to lead. Arch. Environ. Health 27: 399–400 (1973).
6. Hilton, P. J. Cellular sodium transport in essential hypertension. N. Engl. J. Med. 314: 222–229 (1986).
7. Postnov, Y. V., and Orlov, S. N. Ion transport across plasma membrane in primary hypertension. Physiol. Rev. 65: 904–945 (1985).
8. Diez, J., Hannaert, P., and Garay, R. P. Kinetic study of Na^+,K^+ pump in erythrocytes from essential hypertensive patients. Am. J. Physiol. 252: H1–H6 (1987).
9. Petrie, J. C., O'Brien, E. T., Littler, W. A., and De Swiet, M. Recommendations on blood pressure measurement. Br. Med. J. 293: 611–615 (1986).
10. Garay, R. P., Nazaret, C., Diez, J., Etienne, A., Bourgain, R., Braquet, P., and Esanu, A. Stimulation of K fluxes by diuretic drugs in human red cells. Biochem. Pharmacol. 33: 2013–2020 (1984).
11. Harlan, W. R., Landis, J. R., Schmouder, R. L., Goldstein, N. G., and Harlan, L. C. Blood lead and blood pressure relationship in the adolescent and adult US population. JAMA 253: 530–534 (1985).
12. Pocock, S. J., Shaper, A. G., Ashby, D., Delves, T., and Whitehead, T. P. Blood lead concentrations, blood pressure, and renal function. Br. Med. J. 289: 872–874 (1984).
13. Huel, G., Boudène, C., Jouan, M., and Lazar, P. Assessment of exposure to lead of the general population in the French community through biological monitoring. Int. Arch. Occup. Environ. Health 58: 131–139 (1986).
14. Orssaud, G., Claude, J. R., Moreau, T., Lellouch, J., Juguet, B., and Festy, B. Blood lead concentration and blood pressure. Br. Med. J. 290: 244 (1985).
15. Moreau, T., Orssaud, G., Juguet, B., and Busquet, G. Plombémie et pression artérielle: Premiers résultats d'une enquéte transversale de 431 sujets de sexe masculin. Rev. Epidemiol. Sante Publique 30: 395–397 (1982).
16. Garay, R. P., Nazaret, C., Hannaert, P., and Price, M. Abnormal sodium potassium cotransport function in a group of patients with essential hypertension. Eur. J. Clinical Invest. 113: 311–320 (1983).
17. Price, M., Hannaert, P., Dagher, G., and Garay, R. P. Interaction of internal Na^+ and external K^+ with the erythrocyte Na^+,K^+ cotransport system in essential hypertension. Hypertension 6: 352–359 (1984).
18. Tuck, M. L., Garay, R. P., and Meyer, P. Identification of the Na^+,K^+ cotransport system in vascular smooth muscle cells: Effects of cathecholamines on cation transport. Clin. Res. 30: 340A (1982).
19. Hannaert, P., Thormann, B., and Garay, R. P. Effect of this canrenone on the disturbances of cation handling in macrophages and vascular smooth muscle cells. J. Pharmacol. Exp. Ther. 239: 867–871 (1986).