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Cadmium and Membrane Ion Transport in a French Urban Male Population

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Toxic effects of cadmium upon cell membranes structure and function have been well documented (Sorensen et al. 1985, Kunimoto et al. 1986). In particular, experimental studies have shown that cadmium is a potent Na^+-K^+ ATPase inhibitor (Tokushige et al. 1984, Kramer et al. 1986, Ahammad Sahib et al. 1987). One report has confirmed these findings with the human Na^+-K^+ ATPase (Nechay and Saunders 1978).

Based on the above observations, several authors have suggested that a Na^+-K^+ ATPase-inhibitory action could be involved in health related cadmium toxicity (Tokushige et al. 1984, Kramer et al. 1986, Mustapha et al. 1971). However, to our knowledge, this hypothesis has not been tested in the general population. Moreover, we previously reported that Na^+-K^+ pump activity is inversely correlated with hair lead in such a general population (Hajem et al. 1990).

Therefore, we measured hair cadmium and red blood cell Na^+-K^+ pump (physiological counterpart of the Na^+-K^+ ATPase) in 129 urban caucasian males, without known occupational exposure to cadmium. In addition, four other ion transport pathways (Na^+-K^+ cotransport system, Na^+-Li^+ countertransport, Na^+ and K^+ passive permeabilities) were measured in red blood cells. Hair cadmium was considered, as it is known, as an indicator of cadmium body burden (Huel et al. 1984, Laker 1982). A particular emphasis was placed in defining the smoking habits of the subjects since tobacco is known to be the main source of cadmium exposure in the general population.

MATERIALS AND METHODS

The population study consisted of 166 caucasian men recruited among active employees of the Paris Police Administration with no occupational exposure to cadmium. Subjects were invited to participate on the occasion of their routine professional physical examination. Among them, 37 men with personal history of hypertension, or undergoing treatment for other diseases, or who had taken any medication the day before the examination were not included in the study. The 129 subjects of the present report ranged in age from 24 to 55 years (mean=36.2, SEM=7.8).

One observer measured blood pressure, height and weight for all the subjects. Systolic and diastolic blood pressures were measured using a

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mercury sphygmomanometer and recorded in mm Hg. Three measurements were performed: the first after a 10 min resting period, the second 5 min later (both in supine position), and the third at standing up.
The value used in the calculations is the mean of these three measurements. Using the mean of these three measurements offers two advantages: i) it improves the precision of the calculations for each subject, and ii) it reduces the inter-individual variability.
From weight, expressed in Kgs, and height, expressed in cms, body mass index (BMI) was computed as weight/height^2.
Data regarding age, smoking history and alcohol consumption were obtained through the use of a standardized questionnaire.
For each subject, the daily consumption of wine, beer, aperitif, and spirits was recorded, converted in grams of alcohol and summed up to give a total of grams of alcohol drunk per day.
Concerning smoking habits, subjects were divided into three groups: nonsmokers, if they had never smoked (n=45); exsmokers, if they had stopped smoking at least one month before the visit (n=25); and current smokers (n=57). For current smokers, the quantity of tobacco smoked per day was expressed in grams (1 cigarette= 1g, 1 cigarillo= 2g, 1 cigar=4g; for the pipe smokers, the weight of tobacco was used).
Fasting venous blood samples were drawn from subjects between 8.00 and 10.00 A.M. and used for the determination, among others, of five red blood cell cation transport pathways (Na^+-K^+ pump, Na^+-K^+ cotransport, Na^+-Li^+ contertransport and Na^+ and K^+ permeabilities). Head hair samples were taken in a standardized way for the determination of cadmium.
Methods of measurement of the five red blood cell cation transport pathways are described in detail elsewhere (Hannaert et al. 1988), and can be summarized as follows. Peripheral blood was drawn in heparinized tubes. After centrifugation at 1750 g for 10 min at 4 °C, plasma and buffy coat were removed. The red blood cells were washed with $MgCl_2$ (110 mmol/l) and then used for determination of red blood cell cation transport pathways activities. The activity of the different transport pathways was assessed by spectrophotometrically measuring Na efflux in four different Na-free media containing: a) K^+; b) ouabain; c) ouabain and bumetanide; d) ouabain, bumetanide, and Li^+. The Na^+ and K^+ fluxes were expressed in moles (l.cells x h)$^{-1}$. The experimental procedure used for the determination of cadmium in hair was previously described (Huel et al. 1981).
This procedure includes a careful washing technique; cadmium concentration was measured by graphite furnace atomic absorption. Hair cadmium was expressed in µg/g.
Standard linear regression and analysis of variance techniques were performed. Hair cadmium distribution was divided approximatively in tertiles in order to illustrate the correlation between this variable and Na^+-K^+ pump by a quantitative visualization.
The following variables were observed to have an approximately log-normal distribution: Na^+-K^+ pump, hair cadmium and Na^+ passive permeability, so that they were transformed to their logarithms prior to statistical testing.

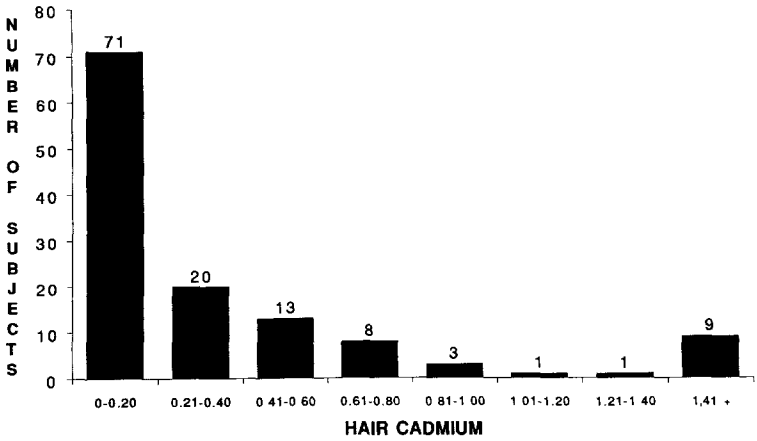

Figure 1. Distribution of hair cadmium in µg/g (range: 0.02-15.06, n=126)

RESULTS AND DISCUSSION

Figure 1 shows the distribution of hair cadmium, which appears skewed. Table 1 shows the observed means ± SEM of the parameters of interest, including hair cadmium level (0.53 ± 0.14 µgrams/g) and Na^+-K^+ pump activity (2106.8 ± 31.3 µmoles per l.cells per h).

Table 1. Means and samples sizes for study variables.

Variables	Mean ± Sem	N
Na^+/K^+ pump	2106.8 ± 31.3	127
Na^+/K^+ cotransport	206.8 ± 7.8	127
Na^+/K^+ countertransport	87.5 ± 6.0	127
Na^+ Passive permeability	175.2 ± 4.3	127
K^+ Passive permeability	90.3 ± 2.3	122
Hair cadmium (µg/g)	0.53 ± 0.14	126
Systolic blood pressure (mm Hg)	129.5 ± 1.2	116
Diastolic blood pressure (mm Hg)	86.9 ± 1.0	116

Na and K fluxes are expressed in mol.(l.cells x h)$^{-1}$

Table 2 shows that the mean values of hair cadmium differ significantly according to the smoking status ($p<0.05$). In addition, the means of hair cadmium are significantly higher in ex- and current-smokers than in non-smokers ($p<0.05$) but ex-smokers have approximatively the same mean as current-smokers.

Age, body mass index, and alcohol consumption were investigated as possible factors of variations of red blood cell cation transport pathways activities and hair cadmium.

Table 2. Mean values of hair cadmium levels according to smoking status of subjects.

	NON SMOKERS	EX SMOKERS	CURRENT SMOKERS	P*
hair cadmium (µg/g)	0.21 ± 0.03 (45)	0.78 ± 0.34 (25)	0.67 ± 0.27 (56)	< 0.05

* indicates that the three means differ in their whole at the p=0.05 level. Values are given as mean ± SEM. Numbers of subjects are given in parentheses.

Age was significantly associated only with Na^+-K^+ cotransport (r=-0.21, p<0.05). Blood pressure and BMI were not significantly correlated with hair cadmium or Na^+-K^+ pump activity. Regarding alcohol consumption, a positive trend was found with Na^+-K^+ pump: in heavy drinkers, the Na^+-K^+ pump mean ± SEM is significantly higher (2264.8 ± 72.1) than in moderate- (2039.5 ± 37.2) or non-drinkers (2076.0 ± 74.4). The overall correlation coefficients between Na^+-K^+ pump and alcohol intake in the total sample and in drinkers were r=0.13 (p=0.17) and r=0.23 (p=0.02) respectively. Alcohol consumption was not significantly correlated with other ion transport pathways or with hair cadmium.

Table 3. Correlation coefficients between hair cadmium and red cell cation transport pathways.

	WHOLE POPULATION	NON SMOKERS	EX SMOKERS	CORRENT SMOKERS
Na^+,K^+ pump	- 0.15* (126)	+ 0.02 (45)	- 0.11 (25)	- 0.28** (56)
Na^+,K^+ Cotransport	- 0.06 (126)	- 0.33 (45)	-0.009 (25)	0.04 (56)
Sodium-Lithium Countertransport	- 0.04 (126)	-0.04 (45)	- 0.17 (25)	-0.006 (56)
Na^+ Passive Permeability	- -0.06 (126)	-0.17 (45)	0.16 (25)	0.11 (56)
K^+ Passive Permeability	- -0.02 (121)	-0.04 (44)	0.14 (25)	0.13 (52)

* p<0.10
** p<0.05
Numbers of subjects are given in parentheses.

Table 3 gives the correlation coefficients between hair cadmium and the red blood cell cation transport pathways. It can be observed that among the five transport pathways, only the Na^+-K^+ pump showed a decrease with hair

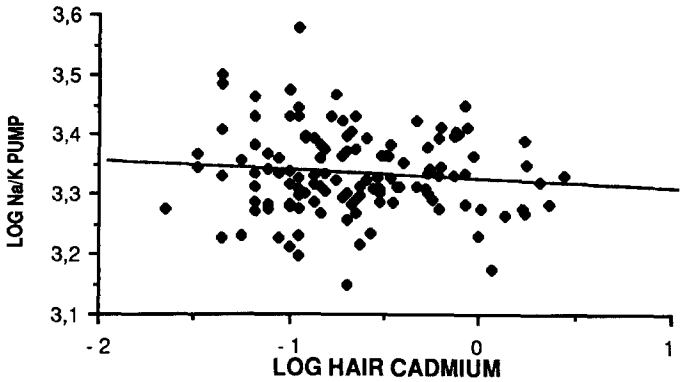

Fig.2. Relationship between Na/K pump and hair cadmium in current smokers. Correlation coefficient: r = -0.28, p = 0.03, n = 56.

cadmium (r=-0.15, p<0.10). However, when dividing the sample in "non-smokers", "ex-smokers" and "current-smokers"; the Na^+-K^+ pump activity was significantly inversely related to hair cadmium at the 0.05 level (r=-0.28, p=0.03) in the group of current smokers. This negative relationship remained significant after adjusting for age, alcohol and tobacco consumptions (r=-0.24, p<0.05). Figure 2 shows individual values for the relationship between Na^+-K^+ pump activity and hair cadmium level in current-smokers, and makes appearent the negative relationship between both parameters. The relationship between hair cadmium and Na^+-K^+ pump activity was not significant in non- and ex-smokers (r=0.02, p=0.86; r=-0.12, p=0.50 respectively).

The purpose of this study was to investigate potential relationships between cadmium and membrane ion transport (particularly the Na^+-K^+ pump).

Hair cadmium was previously used as indicator of cadmium body burden (Manson et Zlotkin 1985). As a sample material hair offers several advantages; cadmium is bioconcentrated in hair and hair cadmium contents can provide integrated measures of chronic cadmium exposure (Laker 1982). Conversely, blood is not a suitable material for cadmium analysis because of a rapid turnover leading to extremely low blood cadmium levels (Petering et al. 1973).

We found a mean hair cadmium content of 0.53 µgrams/g. This agrees with previous observations by Huel et al. (1984) and Medeiros et Pellum (1985) who reported that the means of hair cadmium in the general population not occupationally exposed were 0.59 and 0.70 µgrams/g respectively. Conversely, in Ellis et al's study (1983), mean (± SEM) hair cadmium in nonexposed men was 1.6 ± 0.46 µgrams/g. One possible source for the discrepancy might be the differences in the analytical methods. Another explanation might be the smoking habits of the study populations.

We confirmed earlier reports showing a correlation between hair cadmium and tobacco consumption (Whanger 1979). Indeed, it is known that tobacco is an important source of cadmium exposure (Menden et al. 1972). Conversely, we found no correlation of hair cadmium with age, in agreement with Boiteau et al. (1983) but not with others (Wilhelm et al. 1988). Similarly, we found no significant correlation with blood pressure. This is in

agreement with most epidemiologic studies (Beevers et al. 1980, Lauwerys et al. 1979) but not with other human studies (Fontana et Boulos 1988).
Since smoking constitutes an important source of cadmium for the general population (not occupationally exposed to cadmium), we investigated the relationship between this metal and red cell cation transport pathways activities separately in the non-smoker, the ex-smoker and the smoker groups.
The major finding of our study was that Na^+-K^+ pump activity was significantly and inversely correlated to hair cadmium in current-smokers ($r=-0.28$, $p<0.05$). This finding extend to smokers previous in vitro studies showing that cadmium is a potent inhibitor of the Na^+-K^+ ATPase.
To explain the Na^+-K^+ ATPase inhibitory action of cadmium, several authors have suggested binding of this metal to sulfhydryl groups of the enzyme (Tuker et Matte 1980, Jacobson et Turner 1980). Indeed, SH-groups in the Na^+-K^+ pump are necessary for activity as suggested by the pump inhibition seen with sulfhydryl reagents like N-ethylmaleimide (Fahn et al. 1966). Moreover, Rajanna et al. (1983) have shown that cadmium may compete with Na^+ and ATP sites on the Na^+-K^+ ATPase.
On the other hand, experimental studies have shown that the inhibition of the Na^+-K^+ pump by cadmium is dose-dependent beyond a threshold (Nechay et Saunders 1978). This can explain the lack of significant correlation between Na^+-K^+ pump and cadmium in the non-smoker subjects (who have very low hair cadmium levels). In ex-smokers the similar lack of correlation may result from the fact that red cell Na^+-K^+ pump activity is inhibited by the most recent cadmium exposure. Such is the case for smokers, but not for ex-smokers, whose hair cadmium contents reflect mainly their past exposure.
It should be also noted that cadmium may induce various and serious health hazards by inhibition of the Na^+-K^+ ATPase activity (Tokushige et al. 1984, Kramer et al. 1986). In particular, it was suggested that cadmium could play a role in the pathogeny of arterial hypertension by affecting the Na^+-K^+ ATPase activity (Kramer et al. 1985). Also, the depression of cardiac function and metabolism observed by Kopp et al. (1980) in the experimental cadmium exposure might be due to the inhibition of the Na^+-K^+ ATPase, which affects the myocardial function. Finally, Mustapha et al. (1971) have suggested that cadmium-induced pulmonary effect could be mediated via inhibition of the Na^+-K^+ ATPase activity of pulmonary alveolar macrophage cells.
It is not known by which mechanism cadmium can inhibit Na^+-K^+ pump in vivo. Thus, further studies are required in order to elucidate this mechanism and its potential implications in cadmium-induced health disorders.
In conclusion, our results show that the Na^+-K^+ pump can be altered by cadmium in human red cells, even at environmental exposure, far from toxic levels. This could be one of the factors involved in the high cardiovascular risk of smoking. These findings appear to extend, to the general population of smokers, previous experimental results showing that cadmium is a potent inhibitor of the Na^+-K^+ ATPase and enhance the quality of hair cadmium as a good biological index of cadmium body burden.

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REFERENCES

Ahammad Sahib KL, Moorthy KS, Desaiah D (1987) Effects of methyl mercury and cadmium on the kinetics of substrate activation of K^+-paranitrophenyl phosphatase. J Appl Toxicol 7: 221-226.

Beevers DG, Cruickshank JK, Yeoman WB, Carter GF, Goldberg A, Moore MR (1980) Blood lead and cadmium in human hypertension. J Environ Pathol Toxicol 4: 251-260.

Boiteau HL, Stoklov M, Remond D, Buffet H, Metayer C, Vincent F, Corneteau H, Faure J (1983) Levels of lead, cadmium and mercury in the hair of inhabitants of the Nantes and Grenoble areas. Toxicol Eur Res 5: 281-291.

Ellis KJ, Yasumura S, Vartsky D, Cohn SH (1983) Evaluation of biological indicators of body burden of cadmium in humans. Fundam Appl Toxicol 3:169-174.

Fahn S, Hurly MR, Koval GJ, Albers RW (1966) Sodium-potassium-activated adenosine triphosphatase of Electrophorus electric organ. II. Effects of N-ethylmaleimide and other sulfhydryl reagents. J Biol Chem 241: 1890-1895.

Fontana SA, Boulos BM (1988) Blood cadmium levels as affected by hypertension, smoking, occupation, and body mass. Am J Hypertension 1: 158S-160S.

Hannaert P, Moreau T, Huel G, Orssaud G, Lellouch J, Claude JR, Sahuquillo J, Garay RP (1988) Blood pressure and erythrocyte Na^+ transport systems in a French urban male population. J Hypertension 6: 905-911.

Huel G, Boudene C, Ibrahim MA (1981) Cadmium and lead content of maternal and newborn hair: relationship to parity, birth weight, and hypertension. Arch Environ Hlth 36: 221-227.

Huel G, Everson RB, Menger I (1984) Increased hair cadmium in newborns of women occupationally exposed to heavy metals. Environ Res 35: 115-121.

Jacobson KB, Turner JE (1980) The interaction of cadmium and certain other metal ions with proteins and nucleic acids. Toxicology 16: 1-37.

Kopp SJ, Perry HM Jr, Glonek T, Erlanger M, Perry EF, Barany M, D'agrosa LS (1980) Cardiac physiologic-metabolic changes after chronic low-level heavy metal feeding. Am J Physiol 239: H22.

Kramer HJ, Gonick HC, Lu E (1986) In vitro inhibition of Na^+-K^+ ATPase by trace metals: relation to renal and cardiovascular damage. Nephron 44: 329-336.

Kramer HJ, Glanzer K, Sorger M (1985) The role of endogenous inhibition of Na^+-K^+ ATPase in human hypertension-sodium pump activity as a determinant of peripheral vascular resistance. Clin Exp Hypertension A7: 769.

Kunimoto M, Miyasaka K, Miura T (1986) Changes in membrane properties of rat red blood cells by cadmium accumulating in the membrane fraction. J Biochem 99: 397-406.

Laker M (1982) On determining trace element levels in man: The uses of blood and hair. Lancet 2: 260-262.

Lauwerys RR, Roels HA, Buchet JP, Bernard A, Stanescu D (1979) Investigations on the lung and kidney function in workers exposed to cadmium. Environ Hlth Perspect 28: 137-145.

Manson P, Zlotkin S (1985) Hair analysis-a critical review. Can Med Assoc J 133: 186-188.

Medeiros DM, Pellum LK (1985) Blood pressure and hair cadmium, lead, copper, and zinc concentrations in Mississipi adolescents. Bull Environ Contam Toxicol 34: 163-169.

Menden EE, Elia VJ, Michael LW, Petering HG (1972) Distribution of cadmium and nickel of tobacco during smoking. Environ Sci Technol 6: 830-832.

Mustapha MG, Cross CE, Munn RJ, Hardie JA (1971) Effects of divalent metal ions on alveolar macrophage membrane adenosine triphosphatase activity. J Lab Clin Med 77: 563-571.

Nechay BR, Saunders JP (1978) Inhibitory characteristics of cadmium, lead, and mercury in human sodium and potassium dependent adenosinetriphosphatase preparations. J Environ Pathol Toxicol 2: 283-290.

Petering HG, Yeager DW, Witherup SO (1973) Trace metal content of hair. II. Cadmium and lead of human hair in relation to age and sex. Arch Environ Hlth 27: 327-330.

Rajanna B, Hobson M, Bansal SK, Desaiah D (1983) Effect of cadmium chloride on rat brain synaptosomal ATPases. Toxicol. Lett. 18: 331-336.

Sorensen EM, Acosta D, Nealon DG (1985) Effects of cadmium and calcium on the fluidity of plasma membranes. Toxicol Lett 25: 319-326.

Tokushige A, Higashino H, Searle BM, Tamura H, Kino M, Bogden JD, Aviv A (1984) Cadmium effect on the Na^+-K^+ ATPase system in cultured vascular smooth muscle cells. Hypertension 6: 20-26.

Tuker RK, Matte A (1980) In vitro effects of cadmium and lead on ATPases in the gill of the rock crab, cancer irroratus. Bull Environ Contam Toxicol 24: 847-852.

Wilhelm M, Hafner D, Lombeck I, Ohnesorge FK (1988) Variables influencing cadmium concentrations in hair of pre-school children living in different areas on the Federal Republic of Germany. Int Arch Occup Environ Hlth 60: 43-50.

Whanger PD (1979) Cadmium effects in rats on tissue iron, selenium, and blood pressure; blood and hair cadmium in some Oregon residents. Environ Hlth Persp 28: 115-121.

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