Biol Metals (1991) 4:136–140

BIOLOGY OF METALS

Response to acute nickel toxicity in rats as a function of sex

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Received October 13, 1989

Summary. The effects of different nickel chloride doses upon blood and plasma glucose and essential metal homeostasis were studied in male and female rats. A definite sex-dependent response to injections of nickel has been observed for both the increase in plasma and blood glucose levels and the time at which these levels peak. Males showed a fast recovery from the rise in glucose levels and were much less affected by changes in the other parameters studied. In females, an extended rise in glucose levels was observed. All these effects are clearly nickel dose-dependent. Plasma, liver and kidney copper levels rose significantly in females while only a small decrease was observed in male kidneys. Zinc levels rose in all organs studied but males recovered to basal levels after the study period, whereas females maintained maximum levels at the end of the same period. An increase in urinary excretion of iron was observed. The present results show that the sex differences to acute nickel toxicity can be a helpful way to study metal interaction and discriminate between specific toxicity due to nickel or that induced by the associated hyperglucagonemia.

Key words: Sex differences – Nickel – Zinc – Copper – Glucose

Introduction

Although nickel is considered an essential element (Schnegg and Kirchgessner 1975), the requirements are extremely low (Schroeder et al. 1974). Its toxicity may have more impact on mammals than other species. Nickel toxicity is known to produce a dose-dependent hyperglycemia (Kadota and Kurita 1955; Clary 1975; Horak and Sunderman 1975a, b) by increasing glucagon levels (Horak and Sunderman 1975b; Mas et al. 1986a). A sex-dependent response to acute doses of nickel was observed in our laboratory in which female rats displayed higher levels of glucose, although the LD_{50} values were similar for both sexes (Mas and Peligero, unpublished results). The main mechanism for metal toxicity is by metal substitution. It is well known that some of the so-called toxic metals compete with essential metals for the same binding sites in metalloproteins; probably the best known mechanism is the interaction at the metallothionein level. Metallothionein is known to be strongly induced by non-essential metals such as cadmium or mercury and essential ones such as copper and zinc; it is also induced by hormones such as glucagon, glucocorticoids and epinephrine (for a review see Hamer 1986). Nickel is not considered a strong metallothionein inducer *per se*, yet some metallothionein increase has been reported in cases of acute nickel toxicity (Sunderman and Fraser 1983). However, the mechanism of nickel induction of metallothionein has not yet been established.

Acute nickel toxicity can be lethal to rats during the first few hours after injection (Mas et al. 1985a). In this study, acute nickel toxicity will be considered during the initial period when the nickel concentration, which has a short biological half-life (Parker and Sunderman 1974; Mas et al. 1986c), is at its maximum.

The aim of this work was to establish the sex-specific responses to nickel toxicity. These sex-related differences, produced as a response to acute doses of nickel (Peligero et al. 1985) on plasma glucose and metal homeostasis, may be a helpful way to study the biochemical mechanism(s) of nickel toxicity.

Materials and methods

Animals. Albino Wistar rats (180–220 g, 7–8 weeks old) were obtained from an inbred laboratory colony. Rats were housed under a 12-h light/dark cycle and allowed free access to water and food (Panlab A03, Barcelona).

Chemicals. $NiCl_2 \cdot 6H_2O$ and nitric acid of high purity and low metal content were obtained from Merck Co. (Darmstadt, FRG). A hexokinase kit from Boehringer (Mannheim, FRG) was used for the determination of glucose. The other chemicals were obtained from Sigma (St. Louis, MO).

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Experimental groups. Animals were separated by sex and randomly divided into six groups of five animals each. The animals were caged separately during the course of the experiment. Animals kept for the longest time interval in the study (4 h) were placed in metabolic cages for collection of urine. Experiments were always initiated at the beginning of the light cycle and animals were allowed free access to water and food. They were injected intraperitoneally with a constant volume (1 ml/kg body mass) of $NiCl_2$ (2, 4 or 8 mg Ni/ml, LD_{50}, 11 mg/kg) or saline, using saline solution as a vehicle. At intervals of 0.5, 1, 2 or 4 h after injection, the animals were sacrified by decapitation and the blood collected from the bleeding trunk into a beaker containing heparin to avoid coagulation. After centrifugation, blood and plasma samples were assayed for protein (Lowry et al. 1951) and glucose (Schmidt 1961). No differences were observed in the values obtained by decapitation or from the bleeding tip tail as previously reported (Peligero et al. 1985).

Metal analysis. The excised liver and kidneys, as well as plasma and urine were digested in nitric acid at 100° C during a 12-h period and dried at 150° C. The precipitate was redissolved in 5% nitric acid in Milli Q water and brought up to 10 ml. Analysis of zinc, iron and copper was carried out by using an IL 551 atomic absorption spectrophotometer. Recoveries and methods were as described previously (Mas et al. 1985b). Results were corrected for sample size and dilution.

Statistics. Statistical analyses of the significance of the differences between groups were carried out by the Student's t method. The acceptable level of significance was set at $P<0.05$. Comparisons were carried out at each time point between nickel- and saline-injected rats.

Results

As can be seen in Table 1, blood and plasma glucose showed a similar and immediate increase after nickel injection. However, a significant sex-related difference was observed for both the increase of glucose levels and recovery after the hyperglycemic response. Hyperglycemia peaked at 1 h after the injection in males, whereas in females the maximum was at 2 h. Males showed better tolerance to higher doses of nickel with increases in glucose levels that were half those of females.

As seen in Table 2, a significant sex-dependent difference was found in the plasma levels of copper as a response to nickel injection. Female plasma levels of copper rose significantly throughout the study period and at all doses tested, whereas a small increase was observed at the two higher doses of nickel for males. Liver copper levels showed a similar response to that of plasma with a very significant increase in females and no change in males. Following a similar pattern, kidney copper was significantly increased in females while the levels were slightly but significantly reduced in males. The copper levels in liver and kidneys of females was found to be nickel dose-dependent.

Similar differences due to sex were observed for plasma zinc (Table 3). Plasma zinc levels increased significantly in females for all doses of nickel, whereas male plasma zinc remained at the basal levels. A very different pattern was evident between males and females for hepatic zinc. Although the nickel effect in both sexes increased zinc levels, in males the maximum was reached at 30 min, recovering gradually afterwards, while in females the maximum was reached at the end of the study period after a continuous increase. The renal zinc levels followed a similar pattern to that of liver.

No difference whatsoever was found in the levels of plasma, kidney or liver iron (results not shown).

Short-term urinary excretion of the three essential metals was affected by nickel (Table 4). A similar reduction in urinary excretion of zinc was observed in both males and females, which was very similar to the reduction in the volume of urinary excretion. Unlike zinc, copper showed a significant difference due to sex. Whereas the female excretion of copper decreases in a

Table 1. Blood (B) and plasma (P) glucose concentrations after nickel injection

Time (h)	Sample	Glucose concentration (mmol/l) in							
		females receiving Ni (mg)				males receiving Ni (mg)			
		0	2	4	8	0	2	4	8
0	B	5.4±0.4				5.4±0.2			
	P	5.8±0.1				6.2±0.1			
0.5	B	5.2±0.3	7.0±0.6*	9.2±0.2*	11.5±0.3*	5.0±0.4	6.1±0.4	6.8±0.1*	7.2±0.4*
	P	7.3±0.4	10.2±0.9*	11.8±0.4*	13.0±0.2*	7.5±0.4	8.4±0.3	9.1±0.5*	11.7±0.6*
1	B	5.0±0.4	6.9±0.2*	9.6±0.6*	11.8±0.3*	5.2±0.2	7.0±0.3*	8.0±0.3*	9.6±0.2*
	P	7.7±0.4	9.0±1.0	12.5±0.9*	15.7±0.7*	7.4±0.1	8.7±0.6	10.7±0.5*	14.7±0.8*
2	B	5.2±0.2	7.0±0.2*	15.0±0.2*	19.0±0.4*	5.5±0.4	5.6±0.3	6.0±0.2*	8.2±0.2*
	P	7.4±0.1	8.5±0.1*	17.8±0.8*	21.5±1.0*	7.7±0.2	7.4±0.3	8.2±0.2	11.2±1.3*
4	B	4.8±0.2	6.2±0.3*	7.5±0.4*	8.0±0.3*	4.4±0.3	4.3±0.4	4.9±0.4	5.0±0.1*
	P	6.2±0.2	6.6±0.4	12.4±0.5*	15.1±0.6*	6.5±0.2	6.8±0.5	7.1±0.5	9.1±0.2*

The results are expressed ±SD of 5 animals per group. Doses of Ni were 2, 4 and 8 mg Ni (as $NiCl_2$), control animals were injected with saline solution. Statistically significant differences versus control values, * = $P<0.05$

Table 2. Ni effect upon copper levels in kidneys (K), liver (L) and plasma (P)

Time (h)	Tissue	Copper levels (μg/g wet tissue) in							
		females receiving Ni (mg)				males receiving Ni (mg)			
		0	2	4	8	0	2	4	8
0	K	19.2±0.7				13.2±0.2			
	L	4.3±0.1				4.1±0.2			
	P	1.3±0.1				1.0±0.0			
0.5	K	18.9±0.1	19.9±0.1*	23.2±0.2*	26.3±0.1*	14.1±0.9	12.5±0.3	11.9±0.4	10.9±0.8*
	L	3.8±0.2	4.8±0.3*	4.8±0.3*	4.3±0.4	3.6±0.2	3.7±0.3	3.8±0.3	4.0±0.6
	P	1.3±0.1	1.9±0.1*	2.6±0.1*	2.1±0.1*	1.0±0.1	1.0±0.0	1.0±0.1	1.1±0.3
1	K	19.1±0.2	19.2±0.1	22.1±0.2*	25.2±0.1*	15.8±1.6	14.9±0.1	13.9±0.7	11.7±1.2
	L	4.7±0.3	5.3±0.2*	5.3±0.3*	5.9±0.5*	4.5±0.6	3.9±0.3	4.0±0.1	4.1±0.3
	P	1.2±0.1	1.9±0.1*	2.5±0.3*	n.d.	1.1±0.1	1.0±0.2	1.1±0.0	1.2±0.0
2	K	19.2±0.1	23.1±0.2*	29.7±0.3*	35.7±0.4*	14.4±0.1	12.7±0.8*	11.4±0.9*	10.6±0.6*
	L	4.3±0.2	5.7±0.6*	5.9±0.9*	6.5±0.6*	3.7±0.3	3.7±0.3	3.8±0.3	4.2±0.8
	P	1.1±0.1	2.0±0.1*	2.6±0.1*	2.4±0.3*	1.0±0.1	1.0±0.0	1.1±0.1	1.3±0.0*
4	K	19.5±0.2	18.3±0.1*	21.4±0.1*	21.3±0.3*	13.4±0.5	12.6±0.7	10.8±0.8*	10.3±0.3*
	L	4.2±0.2	5.2±0.4*	5.3±0.4*	4.3±0.2	4.5±0.3	3.8±0.0*	3.9±0.2	4.1±0.7
	P	1.2±0.1	1.9±0.1*	2.0±0.3*	2.3±0.1*	1.0±0.2	1.1±0.1	1.2±0.1	1.5±0.1

Conditions, groups and symbols as in Fig. 1, n.d. = not determined

Table 3. Ni effect upon zinc levels in kidneys (K), liver (L) and plasma (P)

Time (h)	Tissue	Zinc levels (μg/g wet tissue) in							
		females receiving Ni (mg)				males receiving Ni (mg)			
		0	2	4	8	0	2	4	8
0	K	26.3±0.6				24.4±0.4			
	L	25.6±1.2				23.4±0.9			
	P	1.4±0.1				1.8±0.2			
0.5	K	25.7±1.2	24.4±0.2	25.4±0.6	30.0±1.6*	23.9±0.4	30.0±0.8*	28.8±0.4*	32.4±1.0*
	L	26.5±0.9	25.3±0.5	26.9±0.8	27.3±0.4	24.3±1.2	30.2±0.7*	39.5±1.9*	49.3±2.5*
	P	1.5±0.3	1.9±0.6	1.9±0.2	2.0±0.1	1.7±0.1	2.0±0.2	1.7±0.1	2.1±0.7
1	K	26.6±0.6	25.6±0.8	27.9±1.5	31.0±1.3*	25.2±0.3	29.4±1.1*	30.4±1.2*	32.4±1.0*
	L	25.8±0.4	25.9±1.1	28.4±1.5	30.5±0.9*	23.7±0.7	28.9±0.5*	35.7±0.8*	42.1±1.2*
	P	1.4±0.1	1.7±0.3	2.0±0.1*	2.3±0.2*	1.2±0.0	1.8±0.3	1.8±0.1*	2.4±0.2*
2	K	26.3±0.3	24.5±0.7	26.6±0.4	37.4±2.2*	21.1±0.1	25.0±0.4	24.0±0.4	26.4±1.0
	L	25.1±0.3	27.3±0.8	33.4±0.7*	39.5±1.3*	23.3±0.2	25.1±0.9	28.3±0.5*	31.2±1.0*
	P	1.5±0.3	2.0±0.1*	2.5±0.1*	3.0±0.8*	1.6±0.1	1.6±0.2	1.8±0.1	2.1±0.1
4	K	25.9±0.7	29.6±0.6*	37.7±1.1*	44.7±1.3*	25.7±0.8	25.6±0.5	24.1±0.5	25.1±1.0
	L	24.9±0.9	28.1±0.7*	38.9±1.1*	43.9±2.1*	25.3±1.5	24.1±1.2	25.9±1.3	28.3±1.1
	P	1.4±0.2	1.9±0.1*	2.4±0.2*	2.8±0.1*	2.1±0.6	1.6±0.3	1.2±0.1	1.8±0.6

Values are expressed as in Fig. 2

similar way as the total excreted volume of urine, a nickel dose-dependent increase was observed in males. Urinary excretion of iron is stimulated in both sexes, although females are more affected as seen for the larger number of statistically different values.

Discussion

The well-known hyperglycemic response to nickel administration has undergone detailed study (Kadota and Kurita 1955; Clemons and Garcia 1973; Clary 1975; Horak and Sunderman 1975a, b; Horak et al. 1978; Mas et al. 1985a and 1986a; Peligero et al. 1985). A

Table 4. Urinary excretion of trace elements 4 h after Ni injections

Ni dose (mg)	Urine of females				Urine of males			
	volume (ml)	Cu (μg/ml)	Fe	Zn	volume (ml)	Cu (μg/ml)	Fe	Zn
0	2.19±0.49	0.8±0.1	3.4±0.6	6.1±0.1	2.59±0.52	0.8±0.1	2.4±1.0	6.0±0.1
2	1.95±0.68	0.6±0.2	6.6±0.1*	5.2±0.1*	2.35±0.60	0.9±0.1	4.5±0.4	5.0±0.1*
4	1.71±0.60	0.4±0.1*	7.8±0.9*	4.8±0.1*	2.05±0.53	1.1±0.1*	5.1±0.8	4.4±0.2*
8	0.64±0.04*	0.2±0.3*	8.2±0.7*	3.0±0.2*	0.84±0.34*	1.5±0.1*	4.0±0.9	3.2±0.2*

Conditions, groups and symbols as in Fig. 2

transient rise in glucagon levels may explain this hyperglycemic response (Horak and Sunderman 1975b; Mas et al. 1986a). However, it is well known that the hyperglycemic effect of glucagon in vivo is rapidly reduced by a subsequent release of insulin, since the hyperglycemia is a result of hormone imbalance (McGarry and Foster 1980). After acute doses of nickel, it seems that insulin is not as effectively released. In the present study a striking sex-dependent dose response is evident for glucose levels and the time at which these levels peak, confirming preliminary results from this laboratory (Peligero et al. 1985). The observed difference between the sexes is probably due to a fast and effective response in males to hormone imbalance, because no sex-related differences were observed in nickel clearance (Mas et al. 1986c). A higher sensitivity to hormone imbalance is observed in females as indicated by the significant increase of glucose levels, whereas no such effect is observed in males. A different sex-dependent response to glucose tolerance tests was reported when rats were pretreated with caffeine (Barta-Bedö and Gaál 1977). In that report, females showed a slow recovery of basal levels of glucose in what was defined as a diabetic-like blood-glucose curve. Males probably recover more efficiently from the effect of hormone imbalance.

Previous reports on nickel-essential metal interaction have focused on long-term dietary interaction. Therefore, those results are not comparable with the interactions reported in this paper (Whanger 1973; Schnegg and Kirchgessner 1976; Nielsen et al. 1979, 1982; Nielsen 1980; Kirchgessner et al. 1982). With regard to the observed sex differences, limited information is available (Mas et al. 1986b) and it is important to consider that there is no reported sex polymorphism in metalloproteins.

The interaction of nickel with copper metabolism has been characterized at the molecular level as being the competition for the same sites in some molecules, such as plasma albumin (Glennon and Sarkar 1982), a protein that plays an important role in their metabolism. However, the interaction at the organism level is far from being completely understood. A slight increase in serum copper (Bordas et al. 1980) and copper accumulation in kidney as a result of dietary nickel overloading have been described (Schnegg and Kirchgessner 1976). No reference to sex-related differences have been described to date, the preliminary work from our laboratory (Mas et al. 1986b) being the first of this type. The effect of nickel on copper levels in females raises the question as to the origin of that endogenous copper. Liver is considered to be the main storage organ for copper and the levels increase in this organ as well as in plasma or kidneys. It is noteworthy that the changes in copper metabolism are clearly nickel-dose-dependent. However, males showed better regulation of copper metabolism and, in some cases, the effects are opposite to those in females. The differences in the urinary excretion of copper can not account for the observed differences in the renal levels of this element.

Zinc levels in the organs studied have been previously reported to increase as a response to dietary nickel (Clary 1975; Schnegg and Kirchgessner 1976). Metallothionein plays a very important role in zinc (and copper) homeostasis; both glucagon (Kuipers and Cousins 1984; Hamer 1986) and nickel (Sunderman and Fraser 1983) can induce it. This induction can account for the increase of copper and zinc, but the time course studied is obviously too short to explain the sudden rise of hepatic and renal zinc levels in males. However, that induction could be the reason for the gradually increasing zinc levels in female kidneys and liver which reach a maximum at 4 h, as this may be enough time for some newly synthesised metallothionein to appear (Etzel and Cousins 1981; Bracken and Klaassen 1987; Lehman-McKeeman and Klaassen 1987). It is evident that males are less affected than females with respect to the changes of copper and zinc homeostasis and that they recover sooner, in a similar way to that found with blood and plasma glucose.

Iron is known to be affected by nickel at the intestinal absorption level (Nielsen 1980) but this does not have to be taken into account in the present study because of the short time period considered. The lack of interaction contrasts with the profound changes observed for the other two essential elements. The increase of iron excretion due to nickel is more relevant if we consider the reduction of urinary volume. This could be added to the reported interaction for the absorption at the intestinal level and explain the nickel-induced anemic status (Nielsen 1980).

Although hyperglycemia and hyperglucagonemia were described earlier as being due to the effects of acute nickel toxicity (Horak and Sunderman 1975a, b), other metabolic alterations were also reported. The biochemical mechanisms that may explain these altera-

tions are still poorly understood. Also, some of the sudden changes observed in metal homeostasis are difficult to explain on the basis of the mechanisms known so far. Further study of the nickel-dependent induced changes in both males and females can be a useful way of assessing short-term changes in trace element homeostasis and the mechanism of nickel toxicity itself, as opposed to the changes induced by hormonal imbalance.

Acknowledgements. The present work was supported by the Grant PB87-0814 from the *Dirección General de Investigación Científica y Técnica* (DGICYT) of the Government of Spain.

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