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The mechanisms of nickel uptake by rat hepatocyte primary cultures: role of calcium channels

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Abstract

The present study was designed to clarify the mechanism of nickel uptake in primary cultures of rat hepatocytes. Exposure of the hepatocytes to Ni(2～50 μ M ; as $NiCl_2$) for up to 6 h was not cytotoxic, as assessed by the tetrazolium-based dye (MTT) assay. Hepatocytes were treated with 10 μ M $NiCl_2$ in the absence or presence of calcium (Ca) and magnesium (Mg) (1mM). Ni uptake was increased by 19% in medium lacking Mg or Ca and was increased by 37℃ in Ca and Mg-Free medium. The role of Ca channels on Ni uptake by the hepatocytes was investigated. Pretreatement with nicardipine of verapamil (200 μ M), potent inhibitor of Ca channels, decreased Ni uptake by 20%. This effect was only observed when the cells were incubated in the absence of Ca. Pretreatment with vasopressin (100 μ M), a well known Ca channel agonist, significantly increased Ni uptake by the hepatocytes (24%). To determine the involvement of carrier-mediated processes on Ni uptake, the effect of temperature was also investigated. At 4℃ the Ni uptake was decreased by 20% compared to uptake at 37℃. These results indicate that Ni uptake by the hepatocytes occurs, at least in part, through the Ca channel transport processes. Further study will be required to assess what other mechanisms are involved.

Key words : Hepatocytes; Nickel uptake; Calcium channel; Nicardipine; Vasopressin

1. Introduction

Nickel (Ni) is an important inorganic environmental pollutant and the potential for human exposure has generally risen with its increasing industrial usage[1]. The toxicity and carcinogenisity of Ni compounds in experimental animals and humans have been well documented[2,3]. Exposure to Ni can result in damage to various tissues, including liver, kidney, lung and testes[2-6]. Increased lipid peroxidation has been observed during Ni detoxication, and it is thought that Ni-induced oxidative damage may be an important mechanistic aspect of Ni toxicity[7,8]. Recently, Stinson et al. have demonstrated the occurrence of oxidative damage, specifically lipid peroxidation, in liver in response to $NiCl_2$ exposure[9].

The ability of Ni to enter target cells appears to be a major determinant of the toxic effects of the metal[10,11]. It has been reported that many metal ions, including zinc (Zn), cadmium (Cd), manganese (Mn) and Ni, inhibit calcium (Ca) inflow through the Ca channels[12]. Since Ca is taken up through specific channels, it would appear that these metals inhibit Ca uptake by

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competing with each other for the channels on the cellular membrane. Furthermore, since the ionic radius of Ni is smaller than that of Ca[13], Ni may also flow into cells through Ca channels. Indeed, in bullfrog ventricle strips and molluscan smooth muscles, Ni can cross the membrane through Ca channels and competes with Ca for specific receptors[14-17]. However, the mechanisms of Ni uptake by liver cells have not been defined. The transport pathways may vary in each cell type and may depend on the morphological and functional differences between the cell membranes.

In the present study, we examined the effects of Ca channel blockers, nicardipine and verapamil, and the agonist, vasopressin, on Ni uptake using primary cultures of rat hepatocytes. The effects of essential metals such as Ca and magnesium (Mg), and the effect of temperature on Ni uptake were also investigated.

2. Materials and Methods

2.1. Chemicals

Nickel chloride ($NiCl_2 \cdot 6H_2O$), was purchased from Nakarai Tesque, Inc. (Kyoto, Japan). Nicardipine and verapamil were obtained from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan) and Eisai pharmaceutical Co., Ltd.(Tokyo, Japan), respectively. Vasopressin was purchased from Peptide Institute, Inc. (Osaka, Japan). Radioisotopic nickel (^{63}Ni) was purchased from New England Nuclear Co. (Boston, MA). Other chemicals were of reagent grade.

2.2. Rat hepatocyte cultures

The hepatocytes were isolated from male Wistar rats (Kyudo Co., Ltd., Kumamoto Japan), weighing 150-200 g, by the method of Berry and Friend[18]. Cell viability, determined by the trypan blue exclusion method, was greater than 95%.

Primary cultures of hepatocytes were established by seeding 7 x 10^5 cells onto 6-well plates. The cells were cultured in Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 5% fetal bovine serum and 0.03% glutamine for 24 h before treatment. Cultures were maintained in a humidified atmosphere of 5%CO_2/95% air at 37℃.

2.3. Cytotoxicity

The hepatocytes were treated with various levels of $NiCl_2$ (0, 2, 5, 10, or 50 μM) for 0.5, 1, 2, 4 or 6 h. Cell viability was assessed by the 3-(4,5-dimethylthioazole-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay [19].

2.4. Determination of Ni uptake

Twenty-four hours after hepatocyte isolation, cells were washed with Hank's balanced salt solution (HBSS) (Sigma Chemical Co., St. Louis, MO) containing 1.26 mM Ca^{2+} and 0.9 mM Mg^{2+}, and then treated with 10, 30, or 50 μM $NiCl_2$ (4 kBq ^{63}Ni per well) in HBSS for up to 6 h. After the treatment, the cells were washed three times with phosphate-buffered saline containing 2 mM *O,O'*-bis (2-aminoethyl) - ethyleneglycol-*N,N,N',N'*-tetraacetic acid (EGTA) to remove free and loosely bound metal. The washed cells were scraped to remove them from the plate and suspended in 1ml of 1 N NaOH to dissolve the cells. Then an aliquot (100 μ *l*) of the solution was added to 1 ml of liquid scintillator (ACS II; Amersham International, UK). Cellular ^{63}Ni radioactivity was measured with an Aloka liquid scintillation counter (model LCS-3500).

2.5. Effects of Ca and Mg on Ni uptake

The cells were treated with 10 μM $^{63}NiCl_2$ in the presence or absence of CaCl2 and MgCl2 (1 mM) for up to 60 min. The amount of Ni uptake by the cells was determined.

2.6. Effects of Ca channel blockers and Ca agonist on Ni uptake

The cells were pretreated with 200 μM nicardipine or verapamil for 30 min, or 100 nM vasopressin for 3min. The amount of Ni uptake in the presence or absence of $CaCl_2$ and $MgCl_2$ (1 mM) was determined after 60 min incubation uptake10 μM $^{63}NiCl_2$.

2.7. Effect of temperature on Ni uptake

The cells were incubated in HBSS at either 4℃ or 37℃ for 30 min prior to treatment with $NiCl_2$. Then the cells were treated with 10 μM $^{63}NiCl_2$ in HBSS at either 4℃ or 37℃ for up to 60 min. The amount of Ni uptake by the cells was determined.

2.8. Statistical analysis

Data were analyzed by a one-way analysis of variance. When the analysis indicated significant difference, the treated groups were compared to the controls by Duncan's new multiple range test ($p<0.05$).

3. Results

3.1. Effect of Ni on cell viability

The effect of Ni on cell viability was assessed in hepatocytes using the MTT assay. Exposure to Ni (ranging from 2 to 50 μM) for up to 6 h did not induce detectable toxic effects on the cellular level (data not shown).

3.2. Effect of time and dose on Ni uptake

Time course of Ni uptake by hepatocytes is shown in Fig. 1. The uptake of Ni was increased rapidly in the first 30 min and then continued at a slower rate for the remainder of the 5.5 h observation period. Total accumulations after 6 h were 0.9, 1.8 and 3.4 nmol Ni per mg protein in the cells treated with 10, 30 and 50 μM Ni^{2+}, respectively.

3.3. Effects of Ca and Mg on Ni uptake

Hepatocytes were treated with Ni in the presence or absence of the essential metals, Ca and Mg (Fig. 2). Increases in the uptake of Ni was observed in the cells incubated in Ca-free, Mg-free, or Ca- and Mg-free HBSS as compared with the cells incubated in complete HBSS. Ni uptake after 60 min was significantly greater in Ca- and Mg-free HBSS (37%) than in HBSS lacking either Ca^{2+} or Mg^{2+} (19%)

3.4. Effects of Ca channel blockers and Ca agonist on Ni uptake by hepatocytes

The role of Ca channels on Ni uptake by hepatocytes was studied (Table 1). Pretreatment with either 200 μM nicardipine or verapamil, a potent inhibitor of Ca channels, significantly decreased Ni uptake by approximately 20%. This inhibitory effect was only observed when the cells were incubated in the absence of Ca.

Vasopressin was used to determine whether the stimulation of Ca channels would increase Ni uptake into hepatocytes (Table 1). Ni uptake was 24% greater in the presence of vasopessin, a known agonist of Ca channels, but had not effect on Ni uptake when Ca was present.

3.5. Effect of temperature on Ni uptake by hepatocyte

To determine the involvement carrier-mediated

transport processes on the uptake of Ni, the hepatocytes were treated with Ni at either 4℃or 37℃ (Fig. 3). The uptake of Ni at 4℃ was 80% of the level observed at 37℃.

4. Discussion

The results of the present study indicate that Ca channels are involved in Ni uptake in hepatocytes. This is consistent with previously reported studies in bullfrog ventricle strips[15-17]. Similar to the toxic metals, like Cd and mercury (Hg) [20,21], the uptake of Ni by hepatocytes is a rapid and biphasic process. A part of the Ni transport processes in hepatocytes was determined to occur through Ca channels. Two Ca channel blockers tested showed very similar inhibitory effects on Ni uptake with a maximum inhibition of 20%. Additionally, Ca channel agonist, vasopressin, enhanced the Ni uptake by hepatocytes to a 24%. These finding clearly indicate that Ni is taken up through Ca channels. However, other mechanisms responsible for Ni uptake by hepatocytes remain to be elucidated. Therefore, further study will be requited to assess what other mechanism are involved.

Three kinds of processes whereby Ca can cross the plasma membrane of animals include voltage-operated Ca channels[22,23], receptor -operated Ca channels[23] and facilitated diffusion[24]. It has been reported that Ca channels in hepatocytes are receptor-operated rater than voltage-operated and are inhibited by many metal ions such as Zn, Cd, Mn and Ni[12]. This is supported by our findings that, in the presence of Ca and Mg, Ni uptake by hepatocytes was significantly decreased. Combination of Ca and Mg had a greater inhibitory effect on Ni uptake than either metal alone. This is because the ionic radii of Ni (0.66 Å) and Mg (0.65 Å) are smaller than that of Ca (0.99 Å), enabling these metals to pass through the Ca channels. Thus, it can be deduced that Ni may compete with Ca and Mg for Ca channels.

Previously, Abbracchio et al. reported that treatment of Chinese hamster ovary cells with $NiCl_2$ at 4℃ resulted in 50% decrease of Ni uptake compared to the control cells treated at 37℃[25]. Similarly, in the present study, the uptake of Ni by hepatocytes was affected by low temperature (20% decrease). It has been reported that transport processes involving membrane carriers are temperature-dependent[26]. Recently, Blazka and Shaikh found that 80% of Cd uptaken by hepatocytes was inhibited by low temperature at 4℃[21], while verapamil pretreatment decreased the Cd uptake with a maximum inhibition of 31%[27]. These results suggest that the uptake of Cd by hepatocytes occurs not only through Ca channels but also through the other membrane carrier transport processes. However, the current data show that the percentage of the inhibitory effects of Ca channel blockers on uptake of Ni was actually the same as that of low temperature, indicating that inhibition by low temperature may correspond to inhibition of the Ca channels by the blockers. In addition, simple diffusion probably accounts for some of the Ni uptake as well. From these results, it can be concluded the uptake of Ni by hepatocytes occurs, at least in part, through Ca channels, and temperature -insensitive process may be involved in the uptake.

In conclusion, the results of the present study indicate that Ca channels are involved in the uptake of Ni by hepatocytes. However, other mechanisms responsible for Ni uptake by hepatocytes remains to be elucidated. Further

study will be required to assess what other mechanisms are involved.

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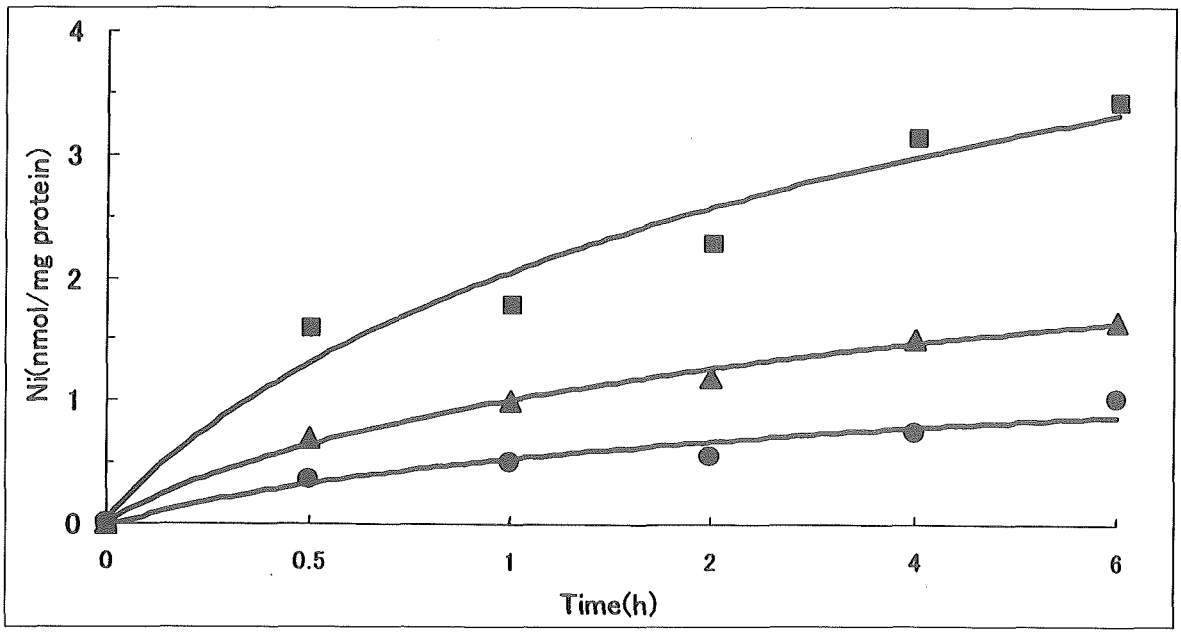

Fig. 1. Effects of time and dose on Ni uptake by hepatocytes.
Hepatocytos were treated with 10, 30, or 50 μ M $NiCl_2$ for up to 6 h. The data represent the mean ± S.D. for three independent cell preparations. ●, 10 μ M; ▲, 30 μ M; ■, 50 μ M.

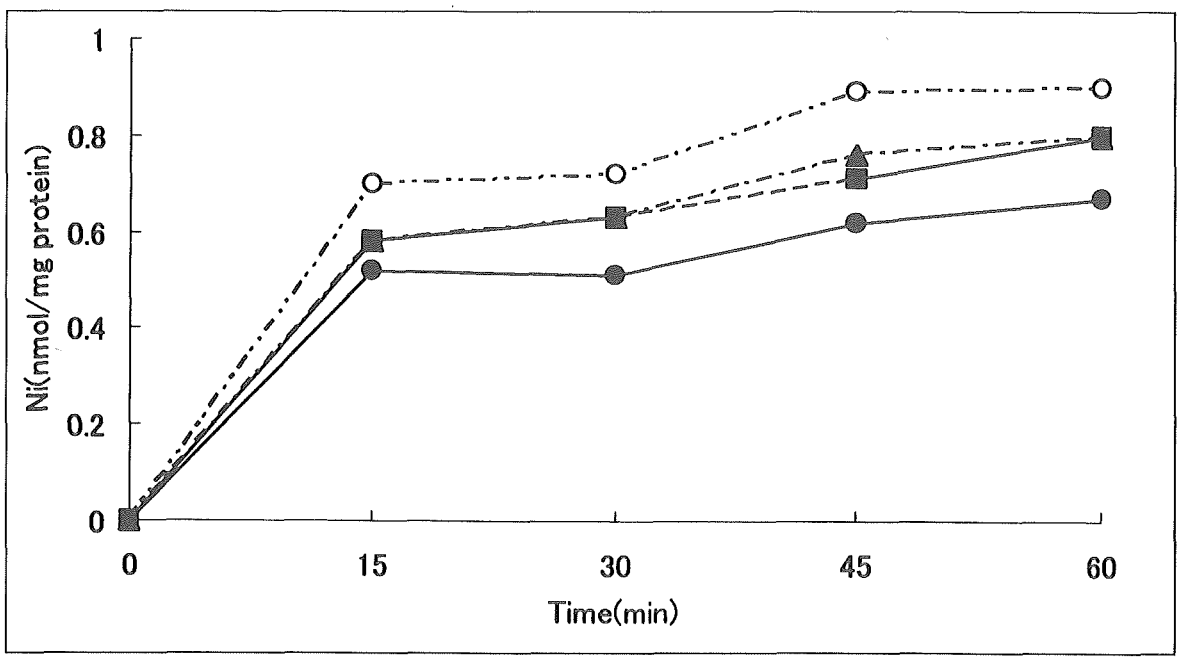

Fig. 2. Effects of Ca and Mg on Ni uptake by hepatocytes.
Hepatocytes were treated with 10 μ M $NiCl_2$ for up to 60 min in the presence or absence of Ca and Mg. The data represent the mean ± S.D. for three independent cell preparations. ●, HBSS containing 1.26 mM Ca and 0.9 mM Mg; □, Ca-free HBSS; △, Mg-free HBSS; ○, Ca- and Mg-free HBSS.

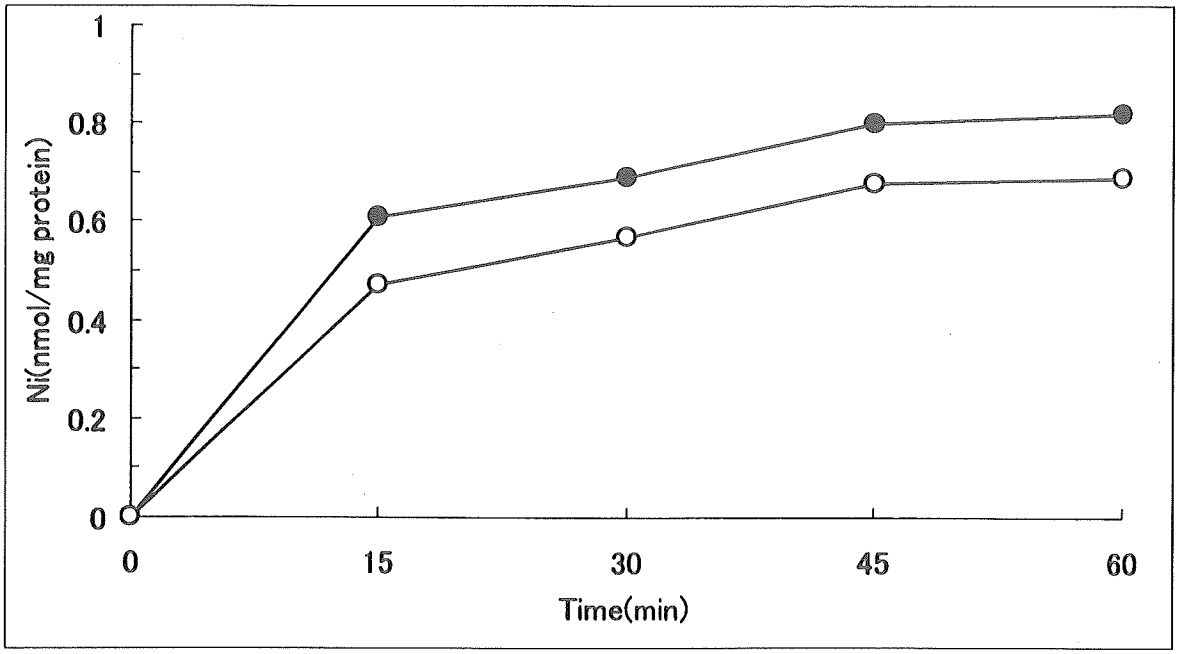

Fig. 3. Effect of temperatue on Ni uptake by hepatocytes.
Hepatocytes were treated with $NiCl_2$ (10 μ M) at 4 or 37℃ for up to 60 min. ●, 37℃; ○, 4℃.

Table 1
Effects of nicardipine, verapamil and vasopressin on Ni uptake by hepatocytes

Medium	Pretreatment			
	None	Nicardipine	Verapamil	Verapamil
HBSS	0.67±0.02	0.68±0.03	0.71±0.05	0.68±0.02
Ca-free HBSS	0.80±0.03	0.66±0.06*	0.72±0.01*	0.92±0.01*
Mg-freeHBSS	0.81±0.01	0.76±0.08	0.73±0.04	0.83±0.05
Ca- and Mg-free HBSS	0.92±0.02	0.76±0.02*	0.79±0.02*	1.10±0.06*

Hepatocytes were pretreated with nicardipine or verapamil (200 μ M) for 30 min, or vasopressin (100 nM) for 30 min, then treated with $NiCl_2$ (10 μ M) for an additional 60 min in the presence or absence of Ca and Mg (1 mM). The data represent the mean±S.D. (n=3).
*Significant difference from non-pretreatment ($p<0.05$).

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ラット初代培養肝細胞におけるニッケルの取り込み機構：カルシウムチャネルの役割

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要　旨

ニッケル（Ni）は生体内微量元素で、RNAの安定化や特定の酵素活性（Arginase, Amino acid decarboxylase, Phosphoglucomutase, Acetyl CoA synsetase 等々）の発現に必須である。欠乏症としては、生殖低下、肝脂質・リン脂質代謝異常、グリコーゲン代謝低下等がある。一方、Niは環境汚染物質の一つであり、鋼材、合金材料、電子機器、メッキ、触媒、電池、台所器具等に使用され、日常的に暴露されている。その経口毒性は比較的低く、Cu, Co, Zn 等必須金属と同程度であるが、化合物として気道・肺から吸入されると肺炎・肺うっ血・浮腫、肝・腎炎等の毒性が大きい。本研究では、ラット初代培養肝細胞を用いてNiの肝細胞内への取り込み機構について検討した。その結果、2～50μM Ni存在下で37℃、6時間培養しても細胞毒性は認められず、Ca, Mg非存在下ではNiの細胞内取り込み量が19%上昇した。また、Caチャネルの強力な阻害剤ニカルジピン及びベラパミルで前処理すると取り込み量は20%減少し、作動薬のバソプレシンで前処理すると24%上昇した。このことからNiの肝細胞内取り込みにCaチャネルの一部関与が推察された。

キーワード：培養肝細胞, ニッケル, カルシウムチャネル, ニカルジピン, バソプレシン