Transport and Subcellular Distribution of Nickel in the Olfactory System of Pikes and Rats

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Olfactory epithelium. The metal was then transported along the olfactory axons. The metal was transported from the olfactory epithelium along the axons of the primary olfactory neurons. In the olfactory region, the metal was transported from the olfactory epithelium along the axons of the primary olfactory neurons. The metal was transported to the brain. We have recently shown that soluble nickel compounds can also result in morphological changes of the respiratory epithelium, which may be preneoplastic events (Torjussen et al., 1978; Klein-Szanto, 1987). Cellular lesions and atrophy of the olfactory and respiratory epithelia have, in addition, been observed in rodents exposed to soluble or insoluble nickel compounds by inhalation (Benson et al., 1987, 1988; Evans et al., 1995). The level of camosine, which is a neurochemical marker of functional primary olfactory neurons, has been shown to decrease in the olfactory epithelium and the olfactory bulbs of rats following inhalation of soluble nickel, indicating injuries of these cells (Evans et al., 1995).

In the olfactory epithelium dendrites of the primary olfactory neurons are in contact with the environment in the nasal cavity, and via the axons these neurons are also connected with the olfactory bulbs of the brain. The olfactory neurons therefore provide a route by which materials taken up in the olfactory epithelium may be transported to the brain. We have recently shown that soluble nickel (NiCl₂) instilled intranasally in rats is taken up in the olfactory epithelium. The metal was then transported along the axons of the primary olfactory neurons to the glomeruli of the bulbs. Low levels of the metal were also found to migrate to the olfactory peduncle and tubercle and the anterior parts of the cerebral hemispheres (Henriksson et al., 1997).

The olfactory nerves of pike have been shown to be suitable
for studying axonal transport of proteins, amino acids, and metals (Gross and Kreutzberg, 1978; Gottofrey and Tjalve, 1991; Tjalve et al., 1995). In the present study we investigated the axonal transport and the subcellular distribution of nickel in the olfactory nerves of pikes after administration of the metal in the olfactory chambers. In addition, we examined the subcellular distribution of nickel in the olfactory system of rats after intranasal administration of the metal. The objective of the study was to bring insight into the mechanisms involved in the transport of nickel in the olfactory system.

MATERIALS AND METHODS

Chemicals

$^{63}$NiCl$_2$, specific activity 12.5 mCi/mg Ni$^{2+}$, dissolved in 0.1 M HCl, was purchased from Du Pont Scandinavia AB (Stockholm, Sweden). Sephadex G-75 Superfine and Superdex 30 Prepar grade were obtained from Pharmacia Biotech AB (Uppsala, Sweden). Dextran blue, bovine serum albumin, carbonic anhydrase, cytochrome c, aprotinin, neurotensin, cytidin, L-histidine, and L-carnosine were obtained from Sigma Chemical Company (St. Louis, MO). Other chemicals used were purchased from Kebo AB (Stockholm, Sweden).

Animals

Ten pikes (Esox lucius) of both sexes, weighing 2.5-3.5 kg, were caught by net fishing in Lake Mälaren (Sigtuna, Sweden). They were kept at our laboratory in 200-liter all-glass aquaria in aerated tap water at 10 ± 0.5°C and were not fed during the experimental period. Twelve male Sprague-Dawley rats, weighing about 150 g, were obtained from Baniin and Ringman (Sollentuna, Sweden). The animals were housed 4 and 4 in macronol cages at 22°C with a 12-h light/dark cycle, with free access to tap water and a standard pellet diet (R36; Lactamin AB, Vadstena, Sweden).

Experimental Procedures

Intranasal administration. The $^{63}$Ni$^{2+}$-solution was evaporated by N$_2$ gas and physiological saline was added to obtain 400 μg (5 mCi)/ml. Ten microliters (50 μCi) of the $^{63}$Ni$^{2+}$ solution was applied with a micropipette in the olfactory chambers of pikes, as described previously (Gottofrey and Tjalve, 1991). The nares were closed with latex and sealed with tissue glue (Histoacryl Chemicals et al., 1981). The rats were anesthetized by pentobarbital sodium (30 mg/kg body wt ip) and 10 μl (50 μCi) $^{63}$Ni$^{2+}$ solution was instilled in each nostril by means of a polyethylene tube connected to a Hamilton syringe, as described previously (Tjalve et al., 1996). The rats were killed after 1 day, 1 week, and 3 weeks by CO$_2$ asphyxiation. The olfactory epithelium, the olfactory bulbs, and a part designated basal hemisphere were removed. The latter comprised the olfactory peduncles and tubercles and the rostral parts of the frontal and cingulate cortices.

Cell fractionation. The tissues were homogenized in 20 vol 50 mM Tris-HCl (pH 7.4, 4°C) in Potter-Elvehjem homogenizers and subcellular fractions were separated by centrifugation at 4°C, according to Webb and Weinzierl (1972). The obtained 6000g pellet was considered to contain mainly nuclei and cell debris, the 12,000g pellet mainly mitochondria, and the 105,000g pellet mainly microsomes. The remaining 105,000g supernatant was considered to constitute the cytosol. The various pellets and aliquots of the supernatants were dissolved in 1 ml of 1 M NaOH for determination of $^{63}$Ni$^{2+}$ content by β-spectrometry in a Packard CA 1900 Tri-Carb liquid scintillation analyzer, using Hionic fluor (Packard) as scintillation fluid. The parts of the supernatants not used for the β-spectrometry were stored at -80°C until they were used for gel filtration.

Gel filtration. The supernatants were applied to either a Sephadex G-75 (70 × 1.5 cm) or a Superdex 30 column (30 × 0.6 cm), equilibrated at 4°C with 50 mM Tris-Cl (pH 7.4) containing 1 mM NiCl$_2$ × 6 H$_2$O and 0.02% Na-azide. The absorbance at 280 nm was continuously recorded by an Altex Model 150 Biochemical UV Monitor, with 5-mm light path, connected to a Kipp and Zonen BD 41-printer. Samples of 3 ml (Sephadex G-75) or 0.7 ml (Superdex 30) were collected at a flow rate of 0.7 ml/min (Sephadex G-75) or 0.4 ml/min (Superdex 30). The amount of $^{63}$Ni$^{2+}$ in the collected samples was determined by β-spectrometry, using Ultima-Gold XR (Packard) as scintillation fluid. The MW standards were applied on the columns under the eluent. The void volumes ($V_v$) of the columns were determined with dextran blue (MW 2 MD). The Sephadex G-75 column was calibrated with bovine serum albumin (MW 66 kDa), carbonic anhydrase (MW 29 kDa), cytochrome c (MW 12.4 kDa), and aprotinin (MW 6.5 kDa) and the Superdex 30 column with aprotinin, neurotensin (MW 1673 Da), and cytidine (MW 243 Da). The elution volume ($V_v$) for each standard was determined spectrophotometrically by measuring the volume of the effluent collected from the point of sample application to the center of the effluent peak. To obtain the standard curves the molecular weights vs the $V_v$/$V$ for each standard were plotted on a semilogarithmic scale. Molecular weights of unknown peaks were determined by using the equations of the obtained standard curves.

Determination of $^{63}$Ni$^{2+}$ transport rate. The transport rate of $^{63}$Ni$^{2+}$ in the olfactory nerves of pikes killed at different intervals after application of the metal in the olfactory chambers was determined as described previously (Gottofrey and Tjalve, 1991). In this procedure, the olfactory nerves were dissected and cut into 4-mm segments for determination of $^{63}$Ni$^{2+}$ contents by β-spectrometry using Soluene 350 (Packard) for dissolving the tissue and Hionic fluor as scintillation fluid. The $^{63}$Ni$^{2+}$ transport rate was calculated using linear regression analysis.

Radioluminography. One pike was given $^{63}$Ni$^{2+}$ (4 μg; 50 μCi) in the right nostril and killed after 8 days. The head was embedded in carboxymethyl cellulose and put in a hexane bath cooled to -78°C with CO$_2$-ice. Sections were taken on a ultratome (20 μm thick) in a Jung Cryocut microtome (Leica, Germany) at ~20°C, as described by Ullberg et al., (1982). The sections were used for radioluminography. In this procedure the imaging plates were exposed to the tape-fastened sections for 7 days. The imaging plates were then analyzed using a bioimaging analyzer system (BAS 2000, Fuji, Japan). In this system the radiation energy stored on the imaging plate is emitted as photo-stimulated luminescence (PSL), having an intensity that is proportional to the radiation energy stored (Ahr and Steinke, 1994). The PSL values are transformed into concentration units by means of $^{63}$Ni$^{2+}$ standards (see below). The obtained pictures were displayed on a monitor and—with the aid of a mouse function—the olfactory nerves and various regions of the CNS were delineated for calculation of average concentrations within the selected areas. In order to obtain a calibration curve known concentrations of $^{63}$Ni$^{2+}$ were applied in gelatin capsules, which were embedded in carboxymethyl cellulose and sectioned on a rotating wheel along with the head of the pike. A standard curve was prepared by plotting the concentrations of $^{63}$Ni$^{2+}$ in the capsules vs the obtained photoluminiscence values.

RESULTS

The cellular fractionation of the tissues of the rats killed 1 day, 1 week, and 3 weeks, respectively, after intranasal instillation of $^{63}$Ni$^{2+}$ showed that about 60-70% of the metal in the olfactory epithelium was present in the supernatant, whereas in the olfactory bulb and the basal hemisphere about 70-80% of the $^{63}$Ni$^{2+}$ was bound to particulate cellular constituents (Table
TABLE 1
Proportions of Nickel in Subcellular Fractions of the Olfactory Epithelium, Olfactory Bulb, and Basal Hemisphere of the Brain of Rats 1 Day, 1 Week, and 3 Weeks after Intranasal Instillation of $^{63}$Ni$^{2+}$ (4 μg/Nostril)

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>Olfactory epithelium</th>
<th>Olfactory bulb</th>
<th>Basal hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>1 week</td>
<td>3 weeks</td>
</tr>
<tr>
<td>600g pellet</td>
<td>11 ± 2</td>
<td>15 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>12,000g pellet</td>
<td>11 ± 2</td>
<td>18 ± 5</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>105,000g pellet</td>
<td>3 ± 1</td>
<td>6 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Supernatant</td>
<td>75 ± 4</td>
<td>61 ± 8</td>
<td>57 ± 4</td>
</tr>
</tbody>
</table>

$^a$Means ± SD, n = 4.

1). The highest proportion of the $^{63}$Ni$^{2+}$ in the olfactory bulb and the basal hemisphere was associated with the 600g pellet. The 105,000g pellet contained low levels in all tissues. In the olfactory epithelium of the pikes killed after 4 and 10 days, respectively, about 60% of the $^{63}$Ni$^{2+}$ was present in the supernatant and about 25% in the 600g pellet (Table 2). In the olfactory nerves the proportion of $^{63}$Ni$^{2+}$ in the supernatant decreased from 69% at 4 days to 43% at 10 days, whereas in the 600g pellet of the nerve the proportion increased from 7% at 4 days to 28% at 10 days. Also in the olfactory epithelium and the olfactory nerves of the pikes the smallest proportion of the $^{63}$Ni$^{2+}$ was present in the 600g pellet.

The Sephadex G-75 filtrations of the supernatants of the rat tissues showed that the highest levels of $^{63}$Ni$^{2+}$ were eluted in the total volume, containing materials with MW < 3000 (Fig. 2). Carnosine and a histidine mixture. When $^{63}$Ni$^{2+}$ and L-histidine were mixed in vitro and subsequently applied on the Superdex 30 column, $^{63}$Ni$^{2+}$ was eluted in association with the UV peak of this amino acid. The $\gamma/V_o$ ratio for L-histidine was the same as the dominating peak in the Superdex 30 filtrations of the tissue supernatants. When unbound $^{63}$Ni$^{2+}$ was applied on the Superdex 30 column it was eluted in the total volume MW < 3000 (Fig. 2). Carnosine applied on the Superdex 30 column was eluted at a $\gamma/V_o$ ratio which was lower than that for the peak of the tissue supernatants and the $^{63}$Ni$^{2+}$-histidine mixture.

The $\beta$-spectrometry showed a wave of transported $^{63}$Ni$^{2+}$ in the nerves of the pikes. The wave showed a profile which decreased in height with the distance along the nerve, and reached a point at which no more decrease was observed (Fig. 3). This point was designated wave front base. The wave front base moved toward the olfactory bulb at a constant rate. This rate was calculated by plotting the move-
FIG. 1. Sephadex G-75 filtrations of supernatants of rat olfactory epithelium, rat olfactory bulb, rat basal hemisphere, pike olfactory epithelium, and pike olfactory nerve in animals killed at different intervals after intranasal administration of $^{63}\text{Ni}^{2+}$ ($4 \mu\text{g}$). The results show the proportions of $^{63}\text{Ni}^{2+}$ eluted in the void volume (MW > 80000), in association with a component with an MW of 25000 ± 3000, and in the total volume (MW < 3000). In the gel filtrations the supernatants of tissues from four rats and two pikes, respectively, were pooled at each survival period and run twice on the Sephadex G-75 column. The curves denote the mean values of the two gel filtrations. The nominal means are also given with the individual values within brackets.
FIG. 2. Representative Superdex 30 filtration profiles of $^{63}$Ni$^{2+}$ in the supernatants of tissues of the olfactory system of pikes and rats. The profile of the pike olfactory nerve is from an animal killed 10 days after intranasal administration of the $^{63}$Ni$^{2+}$ (4 μg). The profile of the rat olfactory bulb is from an animal killed 1 week after intranasal administration of $^{63}$Ni$^{2+}$ (4 μg). The $^{63}$Ni$^{2+}$ in the supernatants was mainly eluted at $V/V_o = 1.7$, corresponding to an MW of about 250. The figure also shows the elution profile of $^{63}$Ni$^{2+}$ mixed with L-histidine and of $^{63}$Ni$^{2+}$ alone. $^{63}$Ni$^{2+}$ mixed with L-histidine was also eluted at $V/V_o = 1.7$. $^{63}$Ni$^{2+}$ alone was eluted at $V/V_o = 1.9$, corresponding to the total volume (MW < 100).

The radioluminographic images showed a high labeling of the right olfactory epithelium and right olfactory nerve in the pike killed 8 days after application of $^{63}$Ni$^{2+}$ in the right olfactory chamber (Fig. 5). The labeling of the left olfactory nerve was low. The level of $^{63}$Ni$^{2+}$ was also low in all parts of the brain (olfactory bulbs, telencephalon, optic lobes, and cerebellum). The level of $^{63}$Ni$^{2+}$ in the cerebrospinal fluid was higher than that in the rest of the brain (Fig. 5). The results of the quantitative measurements are shown in Table 3. It can be
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seen that the quantitative data correlate with the radioluminographic image described above.

DISCUSSION

The results of the present study have shown that nickel is transported slowly in the olfactory nerves of the pike. Thus, the data showed that there was a wave of nickel in the olfactory nerves which slowly moved toward the olfactory bulbs. The observation that the regression line related to the movement of this wave was close to zero of the time axis indicates that the $^{63}\text{Ni}^{2+}$ transport is not markedly delayed due to uptake or somal processes in the nerve cells prior to the transport of the metal in the axons. The transport of nickel in the olfactory neurons occurs in an anterograde direction. This mode of transport has been shown to exhibit several distinct rate classes, the major ones being slow, intermediate, and fast. The rate by which nickel is transported (about 3 mm/day) falls into the class of slow axonal transport ($\approx 0.1-4$ mm/day). Materials transported at this rate involve cytoskeletal proteins and various soluble enzymes, including cytoplasmic enzymes of intermediary metabolism (Vallee and Bloom, 1991; Nixon, 1992). It is possible that the nickel within the olfactory nerves will adhere to and move with components undergoing slow axoplasmic transport. The slow rate by which nickel was transported in the olfactory nerves implied that the metal did not reach the brain of the pikes within the longest survival period used in the present study (10 days).

There is also a possibility that the movement of nickel in the olfactory system might be explained by diffusion. However, the observation that the transport of the nickel occurred at a constant rate does not support this assumption. In addition, diffusion of the metal over the long distances of nickel transport observed in the present study seems less likely.

The cellular fractionations of the olfactory neurons and the olfactory epithelium of the pikes and also of the tissues of the olfactory system of the rats showed that the nickel was present both in the soluble cell fraction (cytosol) and in association with various particulate cell constituents. This pattern of subcellular nickel distribution has been observed also in other tissues of nickel-treated animals (Webb and Weinzierl, 1972; Oskarsson and Tjälve, 1979; Herlant-Peers et al., 1983). It should be noted that the cell fractionations include several steps, and there is a possibility that nickel loosely bound to particulate cellular constituents partly may dissociate during homogenization and centrifugation and consequently be released to the cytosol.

The gel filtration of the cytosols showed that the nickel mainly was eluted at a $V/V_c$ ratio corresponding to a MW of about 250. The same ratio was obtained in the gel filtrations performed with nickel mixed with l-histidine in vitro. It is well known that nickel has a strong affinity for histidine, and this amino acid appears to be a binding ligand for the metal in blood serum (Sarkar, 1984; Nieboer et al., 1984). It is possible, therefore, that the cytosolic nickel may be bound to histidine. However, it cannot be excluded that the nickel may also bind to other amino acids which are similar in size to histidine.

The dipeptide carnosine (β-alanyl-l-histidine), which is
partly in an association with a cytosolic component with a MW of about 25,000. The identity of this component is not known, but gel filtrations of other tissues have shown similar results (Oskarsson and Tjalve, 1979).

Our previous studies on the disposition of cadmium ($^{109}$Cd$^{2+}$) and manganese ($^{54}$Mn$^{2+}$) in the olfactory system of pikes have shown that these metals are transported in the olfactory nerves at a rate which is about 20 times higher as compared to nickel (Gottofrey and Tjalve, 1991; Tjalve et al., 1995). When cadmium reached the terminal parts of the axon in the glomerular layer of the bulbs it appeared unable to pass the synapses to the secondary olfactory neurons. Manganese, on the other hand, easily passed the synapses and reached large areas of the brain. Similar results were obtained in rats (Tjalve et al., 1996). Our previous study in rats indicated that nickel which reaches the axonal terminations of the primary olfactory neurons slowly passes to the interior of the bulbs and further to the olfactory peduncle and tubercle and the anterior parts of the cerebral hemispheres (Henriksson et al., 1997). It appears that nickel, cadmium, and manganese behave differently in the olfactory system. The reason for the differences is not known, but may be related to varying affinities of the metals for tissue constituents.

As mentioned introductorily, exposure to soluble nickel compounds in electrorefining factories has been shown to impair the olfactory sense (Tatarskaya, 1960; Kucharin, 1970) and abnormal olfactory function has also been observed in rats exposed experimentally to the metal (Benson et al., 1987, 1988; Evans et al., 1995). It is known that exposure to cadmium and nickel dust in alkaline battery factories is associated with anosmia (Friberg, 1950; Adams and Crabtree 1961). It has been assumed that cadmium is the critical causative agent (see Hastings, 1990). However, an experimental study in rats exposed to cadmium oxide by inhalation showed lack of negative effects on the olfactory function (Sun et al., 1996). It is thus
possible that nickel may contribute to the olfactory dysfunction in alkaline battery workers.

The mechanism by which nickel induces toxicity toward the olfactory system may involve increased intracellular free radicals. Thus, nickel can directly catalyze the reduction of hydrogen peroxide to hydroxyl radicals (Torreilles and Guérin, 1990). The metal can also inhibit free radical scavenging enzymes, such as catalase and superoxide dismutase (Rodriguez et al., 1990; Shainkin-Kestenbaum et al., 1991) and in addition alter glutathione metabolism (Athar et al., 1987). It has been shown that nickel can promote lipid peroxidation in the brain of rats (Hasan and Ali, 1981).

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