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by

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Running Head

Neuroendocrine Effects of Nickel Chloride

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Abstract

Neuroendocrine Effects of Acute Nickel Chloride Administration in Rats. Gisela K. Clemons and Joseph F. Garcia ( ). Toxicol. Appl. Pharmacol.

A subcutaneous injection of nickel chloride (20 and 10 mg/kg) leads to a profound and consistent increase of circulating prolactin (PRL) levels after one day and lasts up to 4 days ($p < 0.001$) in male rats. Increases in insulin levels occurred 1 and 2 days post injection. The nickel-induced PRL rise could be abolished by a simultaneous administration of 2-Bromo-α-ergocryptine (CB154). In vitro incubation of pituitaries from rats that received 20 mg/kg of nickel chloride 48 hrs prior to sacrifice released more PRL into the culture medium, as well as contained more PRL in the final tissue than did the pituitaries from control animals. The hypothalamic extracts (HE) obtained from the hypothalami of nickel-injected rats was tested also in vitro on normal rat pituitaries and the results showed that the HE from such rats released more PRL and therefore had less prolactin-inhibiting factor (PIF) than the HE obtained from the control rats. The results show that nickel chloride has effects on the endocrine system that a) last considerably longer than previously investigated, b) are mediated through the neuroendocrine system, and c) instead of specifically inhibiting PRL secretion from the pituitary rather promotes high circulating PRL levels lasting from 1 to 4 days.

INTRODUCTION

Administration of certain metal compounds to laboratory animals has been associated with certain alterations within the endocrine system. Acute administrations of Ni(II) salts cause prompt hyperglycemia, hypergluconemia and subsequent hyperinsulinemia in the rat, rabbit and guinea pig (Kadota and Kurita, 1955; Horak and Sunderman, 1975a; Horak and Sunderman, 1975b; Clary, 1975; Horak et al., 1978). Neither somatostatin nor a combination of α and β adrenergic blocking agents can prevent this nickel-induced hyperglycemia (Horak and Sunderman, 1975; Horak et al., 1978). Administration of cobalt salts has also been associated with prompt hyperglycemia (Ellis, et al., 1953).

The effect of nickel on the pituitary and hypothalamic function has been investigated *in vitro* and *in vivo*. La Bella et al. (1973a) reported that nickel added to bovine pituitary slices *in vitro* for two hours specifically inhibited prolactin release but secretion of other pituitary hormones was not affected. The authors also showed *in vivo* supression of circulating prolactin levels in rats but only after induced prolactinemia following chlorpromazine pretreatment (LaBella et al., 1978b).

For the most part these studies have focussed on the short-term effects on these endocrine parameters; i.e., up to four hours. The present study was undertaken to explore the possibility of more prolonged effects of nickel administration within the endocrine system.

METHODS

Young adult male Sprague-Dawley rats (300-350g) were obtained from Simonson Laboratory, Gilroy, CA, and housed in our colony at least one week before the experiment with free access to food and water. The animals were injected subcutaneously with aqueous solutions of nickel chloride hexa-hydrate under light Metafane anaesthesia. Two doses of nickel chloride were administered: 20 mg/kg and 10 mg/kg which corresponds to 84 and 42 μmole/kg, respectively. Groups of 8 animals were killed by decapitation at 3 and 6 hrs and 1, 2, 4, and 7 days after the injection. Two groups of control rats received saline and were sacrificed at the beginning and the end of the experiment. Blood was collected from the trunk and allowed to clot at room temperature, refrigerated for one hour and the serum was separated by centrifugation for 30 minutes at 2000 rpm in a refrigerated Beckman J-6B centrifuge. The serum was stored at -20 C until assayed.

In vitro studies of the functional integrity of the pituitary and hypothalamus after acute nickel administration were done in a follow-up experiment in which again the control group of eight rats received normal saline and another group of eight rats received 20 mg/kg of nickel chloride. These animals were killed after 48 hrs, the blood was collected as before and the serum separated. The pituitaries of each group were rapidly excised, cut into eight pieces and randomized such that each incubation flask contained one piece from each pituitary of the same group. The eight randomized pieces were weighed and preincubated for one hour in 0.5 ml Medium 199 in a shaker bath at 37 C under 95 percent O_2 and 5 percent CO_2. After one hour the culture medium

was replaced with 0.5 ml of the same culture medium and incubated for another three hours. The incubation was terminated by separating the culture medium from the pituitary pieces. The pituitary fragments were subsequently homogenized in 0.05 M phosphate buffer in a glass homogenizer at a concentration of 10 mg/ml. The culture media and pituitary homogenates were kept frozen until radioimmunoassay (RIA) analysis. Hypothalami from these animals were saved for the preparation of hypothalamic extracts (HE). The hypothalami were obtained from the region immediately caudal to the optic chiasm including the stalk median eminence area, were pooled and stored on dry ice during the collection period and then transferred to -70 C. The pooled hypothalamic tissues were thawed and homogenized in a glass homogenizer in 0.5 ml cold 0.1 N HCl. The homogenates of both groups were spun at 35,000 x g in a refrigerated Sorvall centrifuge for 45 minutes. The acid supernatants were rinsed three times with petroleum ether (boiling point 40-60 C) in order to eliminate lipids and the organic phase was discarded. Large and small hypothalamic peptides in the acid extracts were separated by column chromatography on Sephadex G-10 (bed volume 6 ml) in 0.05 M phosphate buffer. The limits of the columns were set by elution of I^{125}-labeled rat prolactin (MW 22,000) and I^{125}-labeled thyroxine (MW 777) and the region of small hypothalamic peptides were pooled and lyophylized. The effect of these extracts was tested <u>in vitro</u> on normal rat pituitaries as described above. After a preincubation of one hour in Medium 199 the hypothalamic extract, also dissolved in Medium 199, was added at a concentration of one hypothalamus per flask (equivalent to one hypothalamus per pituitary).

Additional in vivo studies concerned with the inhibition and enhancement of pituitary PRL release involved use of the drug 2-Bromo-α-ergocryptine (CB154), an α-drenergenic PRL inhibitor. In these studies it was administered at 1 mg/kg (s.c.) with or without nickel chloride.

The hormone levels in the sera, culture media and pituitary homogenates were determined by radioimmunoasssy (RIA). Serum hormone concentrations were tested in duplicate while the appropriate dilutions of the culture media and pituitary homogenates were run in five halving dilutions. Growth Hormone (GH), Prolactin (PRL), Thyrotropin (TSH), Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) were measured using reagents provided by the NIAMDD Rat Pituitary Distribution Program of the National Institute of Arthritis and Metabolic Diseases, Bethesda, MD. The antibodies to thyroxine were purchased from Antibodies, Inc., Davis, CA, and the thyroxine reference preparation from Sigma Co., St. Louis, MO. ^{125}I-labeled thyroxine and carrier-free ^{125}-I for iodination was obtained from New England Nuclear, Boston, MA. Serum glucose concentrations were measured in duplicate in 25 μl samples by a glucose oxidase technique, using the YSI Model 23A Glucose Analyzer.

The analysis of the RIA results was done with the sigmoid computer program developed by Rodbard and Hutt (1974) on the LBL CDC 7600 computer. Duncan's multiple range test and Student's t-test were used for statistical analysis.

RESULTS

The results confirm La Bella's finding that nickel produces an acute fall in prolactin levels. This can be seen in Table 1 after three hours of 20 mg/kg of nickel chloride administration. However, the most significant effect observed was a profound increase in serum PRL concentrations to three and fourfold increases between one and four days. Also confirmed in Table 1 is the early nickel-induced hyperglycemia followed by a subsequent significant rise in insulin levels as reported by Horak and Sunderman (1975). Although none of the serum GH concentrations following nickel administration were significantly increased, it is interesting that the highest GH serum levels coincided with the highest glucose levels observed. The other pituitary hormones, FSH, LH, and TSH and thryroxin were measured but showed no patterns of change that could be attributed to the nickel injections.

As can be seen in Table 2, the profound increases of serum PRL levels observed 48 hrs after nickel administration can be depressed by CB 154 which is known to decrease pituitary PRL release *in vivo* and *in vitro*. CB 154 had no effect on insulin levels in normal rats or the elevated serum insulin measured 48 hrs after nickel administration but all animals were rendered slightly hypoglycemic after CB 154 whether they received nickel or not.

In Table 3 are presented the *in vitro* results of PRL and TSH pituitary release into the culture medium and final hormone concentrations in pituitary tissue fragments. Experiment A compares these

parameters in pituitaries obtained from control and nickel-injected rats. The same parameters were measured in Experiment B in which normal rat pituitary tissue was exposed to the hypothalamic extracts obtained from normal and nickel-injected rats. As can be seen from Experiment A, the incubation of pituitaries from rats that had received nickel chloride 48 hrs previously showed that the high circulating PRL levels were not due to a depletion of pituitary PRL. In fact, nickel increased PRL release and synthesis because at the end of the incubation period the pituitary tissues and culture media contained significantly more PRL than the tissues and culture media from the control group.

A comparison of the control groups in Experient A and B shows that the addition of normal hypothalamic extract caused a 64 percent reduction in PRL release (0.501 vs. 1.147 μg/mg) and a 30 percent reduction in pituitary content (2.105 vs. 3.003 μg/mg). These results support the fact that pituitary PRL release is mainly under inhibitory control by the hypothalamus through release of a prolactin-inhibiting factor (PIF). Simultaneous TSH measurements in both control groups showed a doubling of TSH release in the presence of hypothalamic extract due to its known thyrotropin releasing hormone (TRH) content (3.495 vs. 1.881 mU/mg).

The fact that the PRL released in the presence of HE from nickel-injected animals is greater than that in the presence of HE from control animals indicates that the PIF content must be reduced in the nickel-injected rats. Further evidence supporting a reduction in PIF, rather than an increase in TRH, a known PRL releasing neurosecretory

peptide, as being operational here is demonstrated by the fact that HE from nickel-injected rats reduced the TSH release into the culture medium.

DISCUSSION

The results of the present study show that endocrine effects following an acute injection of nickel chloride are not limited to a short period of time as has been reported (La Bella et al., 1973a/b; Horak and Sunderman, 1975a/b; Clary, 1975; Horak et al., 1978).

Thus, the rise in circulating PRL levels after 24 hrs and their long persistence was an unexpected finding in the present investigation since it had been thought that nickel only inhibits PRL (La Bella et al., 1973a/b). The secretion of PRL from the pituitary is not controlled by a classical negative feedback-loop from a target gland but rather by neurosecretory messengers from the hypothalamus which is the integral link between the central nervous and endocrine system. Therefore, the short inhibition of PRL release after nickel chloride may be a rather toxic effect on the CNS in general rather than be specific for PRL inhibition. The nickel-induced onset of deep sleep which has been reported (Clary, 1975) is another indication of direct effects of nickel on the CNS.

Also, the possibility of nickel involvement with pituitary function has been implicated by the preferential uptake of radioactive nickel by pituitary tissue (only second to renal tissue) (Smith and Hackley, 1968; Parker and Sunderman, 1974; Clary, 1975).

The simultaneous administration of nickel chloride and CB 154 abolished the nickel-induced PRL rise. CB 154 is an ergot alkaloid that at low doses (3 mg/kg) specifically lowers serum PRL without affecting other pituitary hormones (Flueckiger et al., 1976). The

mechanism of action of CB 154 seems to be a stimulation of dopamine receptors. McLeod and Lehmeyer (1974) demonstrated that the action of dopamine, apomorphine and CB 154 on PRL secretion from isolated pituitaries can be quantitatively antagonized by haloperidol and perphenazine. The interaction of the two types of drugs on the pituitary PRL cell strongly suggests the existence of dopamine receptors on the cell membrane and the CB 154 acts as a dopamine-receptor agonist. These findings may be supporting evidence that nickel(II) may have a higher affinity for the dopamine receptors on the pituitary PRL cell membrane than the endogenous PIF and thus prevent the action of dopamine and its inhibitory effects. This is also in accord with the results reported by Parker and Sunderman (1974) that in the rabbit ^{63}Ni accumulated in the pituitary to a large extent but not in the hypothalamus. Therefore, at the pituitary level nickel can account for the high plasma PRL by blocking the PIF receptors on the PRL cell membrane.

However, at the hypothalamic level the hypothalamic extract from the nickel-treated group had considerably less PIF activity than that from the control rats. Whether this is due to increased dopamine turnover and shorter disappearance rate or actual decrease in dopamine production cannot be answered at this time. It is unlikely that Ni(II) caused an increase in prolactin releasing factor (PRF) because the increase seen in the pituitary and culture medium was not higher than the levels seen for normal pituitaries which were neither stimulated nor inhibited. Since more and more evidence implicates TRH as PRF (Clemons et al., 1979) and in light of the fact that TSH was reduced

in the culture medium after addition of hypothalamic extract from the nickel-treated rats, we can assume that we are dealing with a reduction of PIF and not an increase in PRF.

The antidiuretic nickel effect observed by Nielsen (1971) and Clary (1975) can be attributed to either the hypothalamus by possibly releasing more antidiuretic hormone, or to an osmoregulatory effect on the kidney by increased circulating PRL levels, or both.

The rise in insulin observed after 24 and 48 hrs after the nickel administration could still be a result of the compensatory mechanism to the initial hyperglycemia, as suggested by Clary (1975) and Horak and Sunderman (1975) and may be delayed here due to the different route of administration. But the fact remains that in our studies neither the insulin nor the glucose levels return to resting levels within hours after nickel chloride injection.

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REFERENCES

CLARY, J. J. (1975). Nickel chloride-induced metabolic changes in the rat and guinea pig. Toxical. Appl. Pharmacol., 31: 55-65.

CLARY, J. J., and VIGNATI, L. (1972). Nickel chloride-induced changes in glucose metabolism in the rat. Toxicol. Appl. Pharmacol., 25: 467-468.

CLEMONS, G. K., RUSSEL, S. M., and NICOLL, C. S. (1979). Effect of mammalian thyrotropin releasing hormone on prolactin secretion by bullfrog adenohypophysis in vitro. Gen. Comp. Endocrinol. 38: 62-67.

ELLIS, S., ANDERSON, H. L., JR., and COLIINS, M. C. (1953). Pharmacologic differentiation between epinephrine and HGF-hyperglycemia: Application in analysis of cobalt-hyperglycemia. Proc. Soc. Exp. Biol. Med. 84: 383-388.

FLUCKIGER, E., DOEPFNER, W., MARKO, M., and NIEDERER, W. (1975). Effects of ergot alkaloids on the hypothalamic-pituitary axis. Postgraduate Medical Journal 52 (Suppl. 1), 57-61.

HORAK, E., and SUNDERMAN, F. W., JR. (1975a). Effect of Ni(II) upon plasma glucagon and glucose in rats. Toxicol. Appl. Pharmacol., 33: 388-391.

HORAK, E., and SUNDERMAN, F. W., JR. (1975b). Effect of Ni(II), other divalent metal ions, and glucagon upon plasma glucose concentrations in normal, adrenalectomized and hypophysectomized rats. Toxicol. Appl. Pharmacol., 32: 316-329.

HORAK, E., ZYGOWICZ, E. R., TARABISHY, R., MITCHELL, J. M., and SUNDERMAN, F. W., JR. (1978). Effect of nickel chloride and nickel carbonyl upon glucose metabolism in rats. Ann. Clin. Lab. Science 8 (No. 6), 476-482.

KADOTA, I., and KURITA, M. (1955). Hyperglycemia and islet cell damage caused by nickelous chloride. Metab. 4: 337-342.

LABELLA, F. S., DULAR, R., LEMONS, P., VIVIAN, S., and QUEEN, M. (1973a). Prolactin secretion is specifically inhibited by nickel. Nature 245: No. 5424: 330-332.

LABELLA, F. S., DULAR, R., VIVIAN, S., and QUEEN, G. (1973b). Pituitary hormone releasing activity of metal ions present in hypothalamic extracts. Biochem. Biophys. Res. Comm. 52: 786-791.

MCLEOD, R. M., and LEHMEYER, J. E. (1974). Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. Endocrinol. 94: 1077.

NIELSEN, F. H. (1971). Studies on the essentiality of nickel. In: Newer Trace Elements in Nutrition (W. Mertz and W. E. Cornatzer, Eds.), Marcel Decker, New York, 215-253.

RODBARD, D., and HUTT, D. Statistical analysis of radioimmunoassays and immunoradiometric (labelled antibody) assays: a generalized, weighted, interactive, least square method for logistic curve fitting. In: RIA and Related Procedures in Medicine, International Atomic Energy Agency, Vienna, 1974, Unipub., N.Y. 1974, 1965-192.

SMITH, J. C., and HACKLEY, B. (1968). Distribution and excretion of nickel 63 administered intravenously to rats. J. Nutr. 95: 541-546.

Table I. Serum Levels of Prolactin, Insulin, Glucose and Growth Hormone in Ni(II)-treated Rats

		SERUM CONCENTRATIONS			
	Time after $NiCl_2$	Prolactin (mg/ml)	Insulin (uU/ml)	Glucose (mg/dl)	Growth Hormone (ng/ml)
Control	3 h	45.7 ± 6.5[b]	35.6 ± 2.3	117. ± 3	18.9 ± 3.9
	7 d	48.9 ± 3.2	38.7 ± 2.1	119. ± 3	19.5 ± 2.7
$NiCl_2$ $6H_2O$ (10 mg/kg)	3 h	32.7 ± 7.0	30.4 ± 2.0	144. ± 4[e]	16.0 ± 2.2
	6 h	81.0 ± 16.0	34.6 ± 2.8	160. ± 9[e]	58.1 ± 30.5
	1 d	122.9 ± 17.3[e]	45.2 ± 2.7[e]	119. ± 6	15.4 ± 2.2
	2 d	130.2 ± 16.5[e]	45.2 ± 2.7[e]	134. ± 5	12.2 ± 2.2
	4 d	105.2 ± 11.3[e]	35.7 ± 1.4	137. ± 5[d]	37.2 ± 13.3
	7 d	85.8 ± 15.0[d]	29.5 ± 1.5	144. ± 4[e]	37.6 ± 20.7
$NiCl_2$ $6H_2O$ (20 mg/kg)	3 h	21.8 ± 3.6[c]	30.5 ± 2.0	140. ± 11[e]	9.3 ± 0.24
	6 h	32.1 ± 8.2	34.3 ± 2.3	154. ± 4[e]	61.7 ± 20.0
	1 d	189.9 ± 28.5[e]	47.1 ± 2.2[e]	124. ± 5	25.9 ± 4.0
	2 d	175.1 ± 18.5[e]	48.5 ± 3.9[e]	129. ± 9	29.3 ± 11.0
	4 d	174.5 ± 14.4[e]	35.8 ± 1.2	124. ± 5	57.5 ± 29.2
	7 d	91.7 ± 15.1[d]	32.1 ± 1.5	154. ± 11[e]	58.0 ± 15.9

a) Injection s. c.
b) Mean ± SEM
c) $p < 0.02$
d) $p < 0.005$
e) $p < 0.001$

Table II. Serum Levels of Prolactin, Insulin, and Glucose 48 hrs After Nickel Chloride Injection with and without CB154 (Bromocriptine). (n=8).

		SERUM CONCENTRATIONS		
Dosage of $NiCl_2$ (mg/kg)[a]	Dosage of CB 154 (mg/kg)	Prolactin (ng/ml)	Insulin (μU/ml)	Glucose (mg/dl)
0	0	47.9 ± 4.7[b]	40.0 ± 2.8	119 ± 3
10	0	158.9 ± 31[e]	48.6 ± 2.0[c]	134 ± 4
20	0	161.6 ± 25[e]	48.7 ± 3.3[c]	129 ± 7
0	1	0.7 ± 0.2[e]	38.3 ± 2.4	102 ± 3[d]
10	1	2.6 ± 0.6[e]	39.3 ± 2.4	102 ± 3[d]
20	1	2.5 ± 0.4[e]	49.0 ± 3.1[c]	94 ± 4[e]

a Injection s.c.
b Mean ± SEM
c $p < 0.05$
d $p < 0.01$
e $p < 0.001$

Table III. In Vitro Pituitary Prolactin and TSH Release and Final Pituitary Hormone Content.

A. 48 hrs After Nickel Administration (20 mg/kg).
B. Effect of Hypothalamic Extract (HE) Obtained from Rats in A on Normal Rat Pituitaries.

	CULTURE MEDIUM		PITUITARY CONTENT	
	Prolactin (ug/mg)	TSH (mU/mg)	Prolactin (ug/mg)	TSH (mU/mg)
Experiment A				
Control	1.147 ± 0.06[a]	1.881 ± 0.14[a]	3.003 ± 0.12[a]	27.43 ± 3.3[a]
$NiCl_2$	1.743 ± 0.13[b]	3.014 ± 0.21[b]	3.810 ± 0.18[b]	39.43 ± 1.5[b]
Experiment B				
HE-Control	0.501 ± 0.02[c]	3.495 ± 0.36[c]	2.105 ± 0.12[c]	36.87 ± 5.1[c]
HE-$NiCl_2$	0.978 ± 0.06[d]	2.269 ± 0.17[d]	2.765 ± 0.15[d]	43.98 ± 3.5[d]
Statistical Analysis and Protection level (%)	a – d NS a – b,c (1%) b – c,d (1%) c – d (1%)	a – d NS b – c NS a – b,c (1%) c – d (1%) b – d (5%)	a – d NS a – b,c (1%) d – b,c (1%) b – c (1%)	All values are not significantly different

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