Bull. Environ. Contam. Toxicol. (1986) 37:421-426

Bulletin of Environmental Contamination and Toxicology

Effects of Cadmium on the Uptake of Dopamine and Norepinephrine in Rat Brain Synaptosomes

Marvin Hobson, McDonald Milhouse, and Bettaiya Rajanna*

Division of Natural and Applied Sciences, Selma University, Selma, AL 36701

Cadmium (Cd) a known environmental contaminant is neurotoxic (Vallee and Ulmer 1972). It has direct and immediate effects on the activity of the central nervous system (Hall et al. 1985). Cadmium inhibits Na^+-K^+ ATPase involved in energy utilization, and oligomycin-sensitive Mg^{2+} ATPase responsible for energy synthesis in mitochondira (Rajanna et al. 1980). Kinetics of cadmium inhibition indicate that the metal may compete with ATP and Na^+ sites on Na^+-K^+ ATPase in rat brain synaptosomes (Rajanna et al. 1983).

Uptake and release processes of catecholamines into the central nervous system are dependent on membrane bound Na^+-K^+ ATPase (Rodriguez 1972). It is suggested that the uptake and release processes of dopamine (DA) and norepinephrine (NE) in neurons are energy utilizing and hence are dependent on active ion transport (Dengler et al. 1962). If the two aforementioned mechanisms are truly interdependent, then any alteration caused by a toxin to either of the above two mechanisms should also cause a parallel change in the other. The purpose of this study was to examine in-vitro effects of cadmium chloride on the uptake of DA and NE and the activity of ATPase in the rat brain synaptosome.

MATERIALS AND METHODS

All biochemicals used in this study were purchased from Sigma Chemical Co., (St. Louis, MO). The ^{3}H-dopamine (^{3}H-DA), ^{3}H-norepinephrine (^{3}H-NE) and Aquasol were purchased from New England Nuclear, Inc. All other chemicals were of analytical reagent grade (J.T. Baker Co.) and all solutions were made in deionized glass-distilled water. Male, Sprague-Dawley rats weighing 175-200g each, were obtained from Southern Animals Farm, Prattville, Ala. Upon arrival, the rats were maintained in our animal facility for one week before they were used in this study. Rats were housed in pairs in polycarbonated cages with stainless steel lids and were permitted free access to

*Correspondence and reprint requests

glass distilled deionized water and cadmiun-free purina rat chow. All precautions were taken to eliminate any contamination of cadmium. Whole brains of normal rats were removed after decapitation and placed in ice-cold sucrose solution containing 0.32M sucrose, 1 mM EDTA and 10 mM imidazole, pH 7.5. Brain synaptosomes were prepared according to the method of Cotman and Matthews (1971) and as reported by Rajanna et al. (1983). The brain tissues were homogenized in 9 vol. of sucrose solution in a ground-glass homogenizer. The homogenate was centrifuged at 750g for 10 min. to remove nuclei and cell debris. The supernatant was then centrifuged at 17,000xg for 10 min to obtain a crude synaptosomal fraction as pellet. This fraction was further purified on a discontinuous ficoll-sucrose density gradient. The synaptosomal fraction was resuspended in sucrose solution, layered on a gradient consisting of 7.5% and 13% ficoll in 0.32M sucrose (w/v) and centrifuged at 65,000xg for 45 min. The synaptosomal fraction was recovered at the interface of 7.5-13% ficoll-sucrose gradient, diluted with 9 vol of sucrose and then centrifuged at 17,000xg for 10 min. The synaptosomal pellet was suspended in 5 ml of sucrose solution, divided into small aliquots and quick-frozen in liquid N_2 and stored at -85°C until used for assays. For all assays, preparation from four different animals were used. Each brain sample was assayed in triplicate.

ATPase activities in rat brain synaptosomes were measured by the enzymatic method (Fritz and Hamrick 1966) with modifications as reported (Rajanna et al. 1983). A 3-ml reaction mixture contained: 5 mM ATP, 5 mM Mg^{2+}, 100 mM Na^{+}, 20 mM K^{+}, 135 mM imidazole-HCL buffer (pH 7.5), 0.2 mM NADH, 0.6 mM phosphoenol pyruvate, approximately 9 units of pyruvate kinase and 12 units of lactic acid dehydrogenase. A 50 µl synaptosomal preparation with a protein content of 20 to 30 µg was used. Absorbance changes in the reaction mixture were measured at 340 nM over a period of 10 minutes using a Gilford 250 UV spectrophotometer at 37°C. The absorbance values were used to calculate enzyme activities expressed as micromoles of Pi released per milligram of protein per hour. Total ATPase was measured in the presence of Na^{+}, K^{+} and Mg^{2+}. Mg^{2+} ATPase was measured by the addition of 1 mM ouabain, a specific inhibitor of Na^{+}-K^{+} ATPase, into the reaction mixture. Mg^{2+} ATPase was further differentiated into oligomycin-sensitive (mitochondrial) and insensitive Mg^{2+} ATPase by the addition of 1 µl (1×10^{-6}M) oligomycin in ethanol. Oligomycin has been shown to be a potent inhibitor of mitochondrial Mg^{2+} ATPase activity (Lardy et al. 1958). Protein was determined (Lowry et al. 1951) using bovine serum albumin as a standard.

The uptake of ^{3}H-DA and ^{3}H-NE was determined by procedures described earlier with slight modifications (Slotkin et al. 1978). A 2 ml reaction mixture contained: 50 ml trizma buffer pH 7.5; 5 mM $MgCl_2$ and 10 mM NaCl, 5 mM ATP. Synaptosomal samples 30-50 µg) with 1 µ Ci of ^{3}H-DA (specific activity=27.0 Ci/mmol) or ^{3}H-NE (specific activity=15.4 Ci/mmol) were incubated for 15 min.

at 37°C. A blank was also carried in parallel with similar reaction mixture except 0.1 ml of non-radioactive DA/NE was added and incubated for 5 min. at 37°C before the addition of ^{3}H-DA or ^{3}H-NE. Uptake was stopped by the addition of 1 ml of ice-cold buffer and immediately filtered through a 0.45 μm size cellulose acetate filter (Millipore Corp.). After washing three times more with 5 ml of ice-cold buffer each time, the filter paper was placed in a scintillation vial containing 10 ml of Aquasol and counted using a Beckman LS 6800 Liquid Scintillation Counter. Uptake was determined by substracting the non-radioactive blank from the radioactive sample and was expressed as counts per minutes (CPM) per mg of protein. For determination of Cd toxicity synaptosomal preparations were preincubated with $CdCl_2$ for 3 minutes before the addition of ^{3}H-DA or ^{3}H-NE.

Statistical analysis for the significance of differences among the treatment and control values were done by the Student t test for both ATPase and uptake studies.

RESULTS AND DISCUSSION

Table 1. Effect of in vitro cadmium chloride ($CdCl_2$) on ATPase in rat brain synaptosomes.

Treatment[a] $CdCl_2$ (uM)	ATPase ± S.D. (moles Pi/mg protein/h) Na^+-K^+	O-S-Mg^{2+}
0	28.17 ± 0.17[b]	15.37 ± 0.71
10	21.37 ± 0.09*	12.16 ± 0.13*
20	14.00 ± 0.11*	9.69 ± 0.38*
30	9.37 ± 0.27*	7.20 ± 0.01*
40	7.01 ± 0.07*	6.75 ± 0.28*
50	4.67 ± 0.27*	4.85 ± 0.21*
75	1.85 ± 0.21*	0.93 ± 0.57*

a) Different concentrations of $CdCl_2$ were added to the reaction mixture with synaptosomal protein and incubated for 3 min. before the reaction was initiated by the addition of ATP.
b) Mean ± S.D.
*Significantly different from control ($P < 0.01$).

The results presented in table 1 show a dose dependent decrease in the activities of Na^+-K^+ and oligomycine-sensitive Mg^{2+} (O-S-Mg^{2+} mitochondrial) ATPase in rat synaptosomes treated with $CdCl_2$. A 50% decrease in the activities of Na^+-K^+ and O-S-Mg^{2+} ATPases was observed at dose levels of 20 and 30 μM $CdCl_2$ respectively. We previously reported inhibitory effects of *in vivo* and *in vitro* Cadmium on Na^+-K^+ and O-S-Mg^{2+} ATPases in liver and kidney (Rajanna et al. 1980). Eventhough Cd is known to cause toxic effects in kidney and liver, toxic effects to central nervous

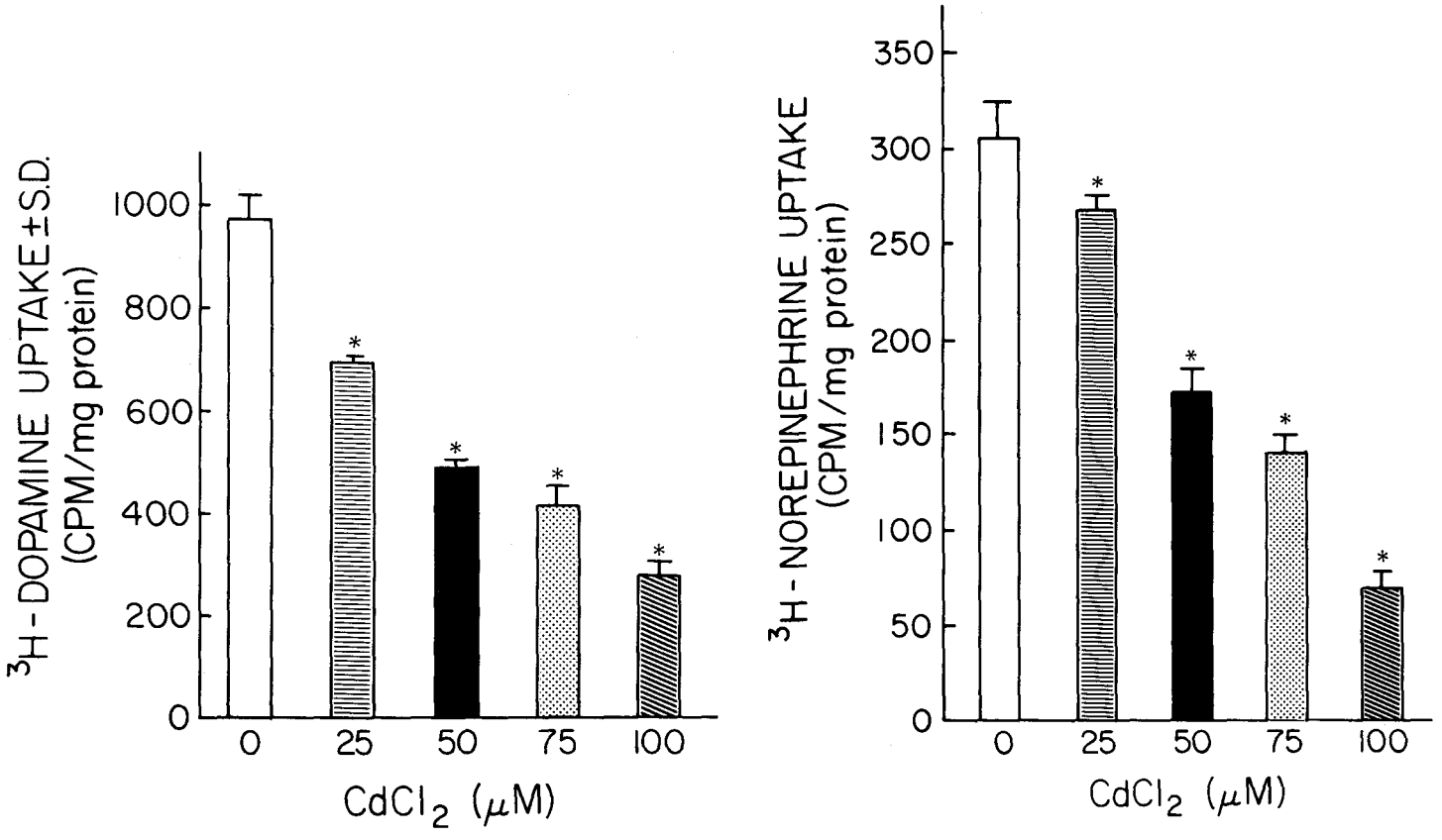

Figure 1 & 2. In vitro effect of $CdCl_2$ on the uptake of ^{3}H-DA and ^{3}H-NE in rat brain synaptosomes. Each bar represents the mean of synaptosomal preparations from 4 animals. Each preparation was assayed in triplicate. The T-bar on each bar represents SD. *Significantly different from control $P < 0.05$.

system have also been reported (Vallee and Ulmer 1972, Gabbiani et al. 1974.

The Na^+-K^+ ATPase in the brain synaptosome membrane plays a pivotal role in the active transport of cation across synaptosomal membrane in the CNS. It has been suggested that the energy available from the electrochemical gradient created by Na^+-K^+ ATPase may be responsible for synaptosomal uptake systems (Bogdanski 1976). If, Na^+-K^+ ATPase is involved in the uptake of catecholamines in the synaptosomal membrane, and if Cd inhibits Na^+-K^+ ATPase activity, then it is probable that Cd may also inhibit the uptake of catecholamine in the synaptosomal membrane. It has been reported that transitional metal ions such as Hg^{2+} and Cu^{2+} exert parallel inhibitory effects on Na^+-K^+ ATPase activity and the uptake of norepinephrine and choline in the rat brain synaptosomes (Prakash et al. 1973).

Effects of Cd on the uptake of DA and NE in the brain synaptosomes were determined by incubating the synaptosomal preparation with Cd for 3 minutes before the addition of radioactive DA and NE. The uptake was assayed at the incubation temp for 37°C for 15 min. Under these conditions, Cd significantly inhibited the synaptosomal uptake of ^{3}H-DA (figure 1) and ^{3}H-NE (figure 2) in a dose-dependent manner. A 50% inhibition occurred at 50 μM and 60 μM $CdCl_2$ for ^{3}H-DA and ^{3}H-NE respectively. The quantity of ^{3}H-DA uptake in the brain synaptosomes was 200% more in control treatments compared to ^{3}H-NE uptake. However, the inhibitory influences of Cd on the uptake of both ^{3}H-DA and ^{3}H-NE were significant at all treatment levels with relations to control.

The results suggest that Cd influences parallel inhibitory effects on Na^+-K^+ ATPase and uptake of ^{3}H-DA and ^{3}H-NE in rat brain synaptosomes. It is interesting to note, that O-S-Mg^{2+} ATPase which is responsible for ATP synthesis in mitochondria was also affected by cadmium. This inhibition is dose dependent and comparable to Cd effects on Na^+-K^+ ATPase and DA and NE uptake. These results verify that the electrochemical potential gradient resulting from the ionic gradient maintained by the Na^+-K^+ ATPase may be responsible for the uptake of DA and NE.

Acknowledgements. This study was supported by National Science Foundation grant RII-8215548 and by MBRS program of Division of Research Resources, NIH Grant RR-08169.

REFERENCES

Bogdanski BF (1976) Transport phenomena in the nervous system. Plenum Press, New York

Cotman CW, Matthews DA (1971) Synaptic plasma membranes from rat brain synaptosomes: isolation and partial characterization. Biochem Biophys Acta 249-380-394

Dengler HJ, Michaelson JA, Spieged HE, Titus EO (1962) The Uptake of labelled norepinephrine by isolated brain and other tissues of the cat. Int J Neuropharmacol 1:23-28
Fritz PJ, Hamrick ME (1966) Enzymatic analysis of adenosine triphosphatase. Enzymol Acta Biocat 30:57-64
Gabbiani G, Badonnel MC, Matthewson SM, Ryan GB (1974) Acute cadmium intoxication. Early selective lesions of endothelial clefts. Lab Invest 30:686-695
Hall CE, Nasseth D, Hungerford S (1985) Augmented depression and reduced excitability of the central nervous system (CNS) by cadmium in the rat. Pharma Biochem Behavior 22:619-621
Iversen LL (1963) The uptake of noradrenaline by the isolated perfused rat heart. Brit J Pharmac Chermother 25:34-49
Lardy HA, Witonsky P, McMurray WC (1958) Antibiotics as tools for metabolic studies. 1. A survey of toxic antibiotics in respiratory phosphorylative and glycolytic systems. Arch Biochem Biophys 78:587-597
Lowry OH, Rosebrough NT, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 162:421-428
Prakash NJ, Fantana J, Henkin, RI (1973) Effects of transitional metals ions on (Na^+-K^+) ATPase activity and the uptake of norepinephrine and choline by brain synaptosomes. Life Sci 12:249-259
Rajanna, B, Chapatwala KD, Vaishnav DD, Desaiah D (1980) Changes in ATPase activities in tissues of rats fed on cadmium. J Environ Biol 2:1-9
Rajanna, B, Hobson M, Bansal SK, Desaiah D (1983) Effect of cadmium chloride on rat brain synaptosomal ATPases. Toxicol Letters 18:331-336
Rodriquez de Lores AG, DeRoberts E (1972) Current topics in membranes and transport. Bonner F, Kleinzeller (eds) Academic Press, New York 3:237-272
Slotkin TA, Salvaggio M, Lau C, Kinsey DF (1978) ^{3}H-Dopamine uptake by synaptic storage vesicles of rat whole brain and brain regions. Life Sci 22:823-830
Vallee BL, Ulmer DD (1972) Biochemical effects of mercury, cadmium and lead. Ann Rev. Biochem 41:91-128
Received November 11, 1985; accepted December 15, 1985.