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Augmented Depression and Reduced Excitability of the Central Nervous System (CNS) by Cadmium in the Rat[1,2]

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HALL, C. E., D. NASSETH AND S. HUNGERFORD. *Augmented depression and reduced excitability of the central nervous system (CNS) by cadmium in the rat.* PHARMACOL BIOCHEM BEHAV **22**(4) 619–621, 1985.—Reports indicating that low doses of cadmium caused vasodilation, but that larger quantities elicited a pressor response, apparently mediated by a CNS reflex, prompted an examination of cadmium-induced changes in CNS responsiveness and activity. Rats were injected intraperitoneally with either 2 mg/kg or 4 mg/kg of $CdCl_2$ solution, after which the CNS was either depressed by pentobarbital or excited by strychnine at different dose levels. Cadmium treatment, administered before pentobarbital, decreased the time required for sleep induction and prolonged sleep duration at doses of either 20 mg/kg or 30 mg/kg: at 40 mg/kg only induction was affected and at 60 mg/kg neither was influenced. At a dosage of 60 μg/kg, strychnine caused convulsions in all control animals, but in none pretreated with $CdCl_2$. When either 75 or 120 μg/kg of strychnine was used, cadmium at either dosage failed to prevent convulsions, although the onset was delayed and duration curtailed. The rapidity with which Cd modified CNS activity indicated that the effect can not depend upon cadmium-induced synthesis of metallothionine, but represents a direct effect of Cd on the CNS. Cadmium treatment did not substantially improve the survival of rats that convulsed when treated with strychnine.

Central nervous system Cadmium Depression Drug interaction

THE increasing industrial use of cadmium has led to progressively higher levels in the environment. There is belief that the metal might cause hypertension in man [10, 14, 21], although the evidence linking the two has been questioned [1, 13, 18]. Similarly, there is argument as to whether it does [12, 15, 22] or does not [2, 7, 9, 19, 23] cause chronic hypertension experimentally in rats. The importance of resolving this dispute has been editorially emphasized [3].

Recent investigations revealed that when rats were given Cd intraperitoneally in the dose usually employed for acute pressor effects (2 mg/kg), then anesthetized with 40 mg/kg of sodium pentobarbital (Pb), recipients slept longer than controls (unpublished observations). This prompted examination of the interaction between cadmium and pentobarbital on the central nervous system (CNS). Parallel experiments assessed possible interaction between cadmium and strychnine-induced CNS excitation.

METHOD

Female Sprague-Dawley rats (weighing 200–230 g) were obtained (Laboratory Supply Co., Indianapolis, IN), and individually caged in windowless, temperature and humidity-controlled quarters lighted at 8 a.m. to 8 p.m. Purina Laboratory Chow and tap water were continuously available. The cages and drinking spouts were of stainless steel. All experiments were begun between 8 and 9 a.m.

Rats were injected intraperitoneally with 2 mg/kg of a $CdCl_2$ solution (2 mg/ml). The cadmium salt was dissolved in 0.9% NaCl solution. Controls received only saline. In these experiments, Pb was injected intraperitoneally 5–10 minutes after cadmium administration, the applicable dose being contained in a volume of 0.2 ml/100 g body wt. In strychnine experiments, strychnine sulfate (in 0.9% NaCl solution) was injected into the jugular vein under light ether anesthesia at various doses (each dose contained in a volume of 0.1 mg/100 g body weight). Controls received only this injection: experimental animals had been given 2 mg/kg or 4 mg/kg of $CdCl_2$ intraperitoneally 20 minutes earlier.

In Pb experiments, the intervals between injection of the drug and loss of the righting reflexes (induction-time), and between then and their recovery (sleep-time), was measured. When strychnine was given, the periods intervening between its rapid injection (within 4 sec), and the beginning of convulsions (induction-time), and from then until the animal either died or the seizures ceased (convulsion-time), were measured. A digital stopwatch measuring to 0.01 sec was

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[2]This paper was accepted by the *Journal of Experimental Pathology and Toxicology* in September 1979, for publication in Vol. 4, No. 5, 1980. The Journal failed and the paper was transferred to its successor, the *Journal of Environmental Pathology, Toxicology and Oncology*, to be published in Vol. 6, No. 1, in late 1982. It never appeared. The data are the same although the discussion has been altered to accommodate [5, 8, 25], added to update the article.

used to time the onset of strychnine convulsions, and a "Time It" laboratory timer (Precision Scientific Co.) measuring to 0.01 min was used to time sleep and convulsion durations. Statistical comparisons employed Student's *t*-test.

RESULTS

At either 20 or 30 mg/kg of Pb, induction-time was shortened and sleep-time significantly prolonged by cadmium pretreatment. At 40 mg/kg induction-time was curtailed, but sleep-time was unaffected; at 60 mg/kg the two periods were identical in both groups. The results are summarized in Table 1.

Similarly with strychnine, the lowest dose of the drug (60 μg/100 g), which uniformly induced convulsions in controls, failed to do so in any animal pretreated with cadmium. At a dose of 75 μg/100 g, induction-time was prolonged and convulsion-time shortened by pretreatment with $CdCl_2$ but all treated animals convulsed at 2 mg/kg of $CdCl_2$, although variability was great and the differences were not significant statistically. When the dosage of $CdCl_2$ was doubled, variability was reduced, and the differences in both measurements attained statistical significance. At this dosage of strychnine, all rats convulsed and deaths occurred, cadmium having no effect on mortality.

When the dose of strychnine was raised to 120 μg/100 g, cadmium at 2 mg/kg significantly reduced the duration of convulsions, but did not lengthen the induction-time. However, a dose of 4 mg/kg both reduced duration and lengthened the induction-time. Mortality was not prevented by cadmium pretreatment, and indeed did not appear to be greatly reduced, certainly not in a dose dependent manner. The data are given in Table 2.

DISCUSSION

Cadmium treatment potentiated the depressive activity of Pb on the CNS and antagonized excitation caused by strychnine. These effects were more striking at the lower dosages of either pentobarbital or strychnine. Thus when pentobarbital-induced depression was slight to moderate it could be enhanced, and when strychnine stimulation was minimal it could be overcome. However, in the case of strychnine, where two different cadmium dosages were evaluated, it was apparent, both at the 75 μg and at the 120 μg/100 g doses, that the higher cadmium dosage was more efficacious than the lower, indicating that the effect was, to a degree, dose dependent. This agrees with the findings of Schnell *et al.* [20] who noted that while 2 mg/kg of Cd acetate increased hexobarbital-induced sleep, 1.5 mg/kg was ineffective in doing so.

The induction of anesthesia was progressively shortened and its duration prolonged by increasing dosages of pentobarbital. However, at the highest dosage used, 60 mg/kg, induction was very rapid in all rats, individual variation was minuscule, and Cd pretreatment had no discernible influ-

TABLE 1

POTENTIATION OF PENTOBARBITAL (Pb)-INDUCED CNS DEPRESSION BY CADMIUM CHLORIDE ($CdCl_2$) TREATMENT

No.	$CdCl_2$ (mg/kg)	Pb IP	Induction Time Min	Sleep Time Min
10	—	20	6.56 ± 1.06	56.56 ± 6.84
10	2	20	3.30 ± 0.26†	90.50 ± 4.13‡
10	—	30	2.68 ± 0.07	126.78 ± 5.17
10	2 (IV)	30	2.42 ± 0.09*	154.36 ± 6.74†
10	—	40	2.72 ± 0.12	319.22 ± 28.67
10	2	40	2.05 ± 0.14†	317.45 ± 13.44
10	—	60	1.50 ± 0.08	489.90 ± 31.10
10	2	60	1.60 ± 0.10	545.82 ± 34.26

Times given are Means ± SEM.
*Significantly different from control value ($p<0.05$).
†Significantly different from control value ($p<0.01$).
‡Significantly different from control value ($p<0.001$).
IP=intraperitoneal administration.
IV=intravenous administration.

TABLE 2

EFFECT OF CADMIUM PRETREATMENT ON CNS EXCITATION BY STRYCHNINE

No.	Strychnine μg/100 g IV	Cd mg/kg IP	% Convulsed	% Survived	Convulsions Onset sec	Convulsions Duration min
10	60	0	100	100	35.28 ± 7.46	3.68 ± 0.24
10	60	2	0‡	100	—	—
20	75	0	100	95	22.88 ± 6.87	7.21 ± 1.14
10	75	2	100	80	99.61 ± 48.34*	5.54 ± 1.65
10	75	4	100	90	90.75 ± 17.18‡	3.13 ± 0.23†
10	120	0	100	40	8.09 ± 1.04	20.04 ± 2.63
10	120	2	100	90	6.00 ± 1.50	7.68 ± 2.55†
10	120	4	100	60	48.55 ± 13.17†	9.16 ± 1.12‡

Times given are Means ± SEM.
*Statistically different from controls ($p<0.05$).
†Statistically different from controls ($p<0.01$).
‡Statistically different from controls ($p<0.001$).

ence. The effect of cadmium pretreatment on both responses to the drug were clear-cut at doses below 30 mg/kg. At 40 mg/kg only induction time was affected and at 60 mg/kg neither was influenced. Higher cadmium doses, as in the case of strychnine, might have proved efficacious at the highest dosage of pentobarbital, but they were not tested.

Antagonism to strychnine was indisputable at a dosage of 60 μg/100 g, one near the convulsive threshold, because complete protection was afforded cadmium-pretreated rats, whereas controls uniformly convulsed. At higher dosages of strychnine, however, convulsions could not be prevented with either of the cadmium dosages used, although the CNS depression was still manifested as a delayed onset and shorter duration of convulsions.

Strychnine and the barbiturates act at many levels of the CNS. The convulsive effect of the former is believed to reflect a reduced activity of inhibitory spinal cord neurons, and barbiturates are thought primarily to inhibit synaptic activity. These generalizations do not permit identification of the precise locus or loci at which Cd acts to inhibit the first or potentiate the second.

It has been reported [6,20] that 2 mg/kg of cadmium prolonged sleep time when hexobarbital was given 1 to 10 days later. The former noted that although sleep was prolonged by cadmium, the plasma concentration of hexobarbital was no different from that of controls at the time of awakening. They did not study earlier responses or induction time, and suggested that the effect might be due to cadmium-induced hepatic synthesis of metallothionine. The present findings, however, indicate that the cadmium effect is virtually immediate, modifies induction-time, and is too rapid to be ascribed to hepatic enzyme synthesis. Because sensitivity to strychnine is similarly reduced, a direct effect of cadmium on the CNS is suggested.

Intracerebroventricular injections of Ca^{2+}, Mn^{2+}, Zn^{2+} or Cd^{2+} (but not Mg^{2+}) increase the sleep-time of rats or mice given ethanol, t-butanol or chloral hydrate [4,5] although whether pentobarbital-induced sleep is [8] or is not [5] so influenced is debated. Cd^{2+} has been reported to increase ethanol-induced sleep in a dose-dependent manner [24]. The present findings show that Cd^{2+} prolongs pentobarbital-induced sleep in rats when the barbiturate is given in low, but not in high dosage. They show equally clearly that the speed with which righting reflexes are lost following pentobarbital administration, or the onset of convulsions is delayed following IV injection of strychnine, are at least as reliable (and more rapidly assessable) indices of central CNS depression by Cd^{2+} as the duration of the respective responses.

Findings presented herewith provide evidence for effects of cadmium on the CNS. These may be related to its acute pressor [15,17] and other [11] effects on the cardiovascular system.

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