Bull. Environm. Contam. Toxicol. 28, 611–616 (1982)

Elevation of Brain Cyclic Nucleotides During Acute Dieldrin Exposure

Robert M. Joy, Shri N. Giri, and Mary J. Schiedt

Department of Physiological Sciences, School of Veterinary Medicine
University of California, Davis, CA 95616

Exposures to situations that evoke convulsive seizures change brain cyclic nucleotide levels. After electroshock, which produces immediate tonic-clonic convulsions, brain adenosine 3', 5'-monophosphate (cAMP) and guanosine 3', 5'-monophosphate (cGMP) levels are elevated (LUST et al. 1976). In cortex cAMP levels increase throughout the period of convulsions and reach maximal values (approximately 500% of control values) in 3-5 min. In mice pentylenetetrazol (PTZ) increases levels of both cAMP and cGMP with different temporal patterns (FERRENDELLI 1980; FERRENDELLI et al. 1980). cGMP increases during the preconvulsant and convulsant periods whereas cAMP only increases during clinically evident seizures. A similar temporal patterning has been described in mice following ip bicuculline (WASTERLAIN & CSISZAR 1980). With the onset of paroxysmal EEG activity, cGMP, but not cAMP, was elevated. When convulsive movements appeared, cGMP was further elevated, and cAMP began to increase. Levels of both nucleotides became maximal during overt seizures. Regional differences in basal and convulsant-induced changes in cyclic nucleotide levels have also been demonstrated (FERRENDELLI et al. 1980; FERRENDELLI & KINSCHERF 1977; OPMEER et al. 1976).

These data suggest a correlation between cyclic nucleotide levels and events induced by exposures leading to convulsions. cGMP appears to increase during the preconvulsive stage of heightened CNS excitability and remains elevated or increases further when convulsions appear. cAMP, on the other hand, rises only after overt convulsive activity develops. This correlation, if correct, has important implications as to the role of these nucleotides in epileptogenesis (FERRENDELLI 1980). It also has important implications as to the role of cyclic nucleotides in the expression of neurotoxic actions of certain of the organochlorine insecticides, such as dieldrin, endrin and lindane (JOY et al. 1981).

The duration of preconvulsive exposure in the studies described above is short, and in most instances only convulsant exposures were examined. As such it remains uncertain how well changes in brain cyclic nucleotides, particularly cGMP, correlate over time with changes in the level of CNS excitability. We have examined the effect on cyclic nucleotides of exposures to dieldrin, an organochlorine insecticide producing CNS stimulation similar to PTZ (JOY 1974; 1975; 1978) but having a much longer duration of action. We were primarily interested in determining: 1) does acute dieldrin exposure change cyclic nucleotide levels, 2) if so, is the degree of change dependant

0007-4861/82/0028-0611 $01.20

upon level of exposure, 3) do convulsant and subconvulsant exposures produce different changes in cyclic nucleotide levels, and 4) are changes transient in nature or sustained over the period of time that CNS excitability is elevated.

MATERIALS AND METHODS

Male Swiss-Webster mice, 25g, were subjects. To determine time- and dose-related changes in brain cyclic nucleotide levels, groups (N=30) were given 0, 5, 10 or 15 mg/kg dieldrin ip in DMSO, and subgroups (N=6) were killed at 15, 30, 60, 120 and 240 min after injection. Mice were decapitated, and the heads were allowed to drop into liquid nitrogen. The frozen brains were stored at -20° C until assayed. Additional groups (N=6) were used to provide comparative data. These included: 1) a non-injected control group, 2) a group given 40 mg/kg dieldrin ip and killed at the onset of tonic convulsions, 3) a group exposed to maximal electroshock (MES) through pinnal electrodes and sacrificed at the end of the tonic phase of the convulsion and 4) a sham-electroshock group that was not shocked but was killed after placement of electrodes at equivalent time points as the MES mice.

For cAMP and cGMP determinations the frozen heads were split on dry ice, and the frozen brain tissue was removed and dropped into ice cold 10% trichloroacetic acid (TCA). Each sample was immediately homogenized using a polytron (Brinkman) with a PT10ST generator. After centrifugation at 10,000g the supernatent volume was measured and stored frozen for radioimmunoassay (RIA). The pellet was used to measure protein by the method of LOWRY et al. (1951) as modified by MILLER (1959). For RIA, the TCA supernatents were neutralized with solid $CaCO_3$ (TIHON et al. 1977), and the RIAs were performed using the method of FRANDSEN & KRISHNA (1976). Antigens and cGMP antiserum were obtained from Collaborative Research Inc. The cAMP antiserum was a gift from Dr. Krishna at NIH. $CaCO_3$-neutralized TCA was added to the standards in the RIA. Values are expressed as picomoles/mg protein for cAMP and femtomoles/mg protein for cGMP or as differences in percent from control.

Cyclic nucleotide levels in treated versus control groups were compared using Student's T-test. As no significant differences were observed between non-injected and DMSO injected controls, or between controls sacrificed at different time points, they were combined. cAMP/cGMP ratios between treated and control subjects were compared using the Mann-Whitney U-test.

RESULTS

Changes in whole brain cyclic nucleotides due to convulsions are shown in Table 1. Maximal electroshock convulsions (tonic phase) were associated with increased levels of both nucleotides, but particularly

cGMP. The ratio: cAMP/cGMP was decreased by more than 50% at the end of the tonic convulsion. The stress associated with the placement of the pinnal electrodes did not significantly affect cAMP, cGMP or the ratio: cAMP/cGMP. Animals killed during the tonic phase of the convulsion produced by dieldrin also had significantly higher levels of cAMP and cGMP. The ratio: cAMP/cGMP was decreased, but not to the degree seen with MES.

TABLE 1. Effects of convulsions on brain cyclic nucleotide levels.

	cAMP[a] % of control	cGMP[b] % of control	cAMP/cGMP % of control
Controls[c]	100 ± 4.3[d]	100 ± 7.2	100
Dieldrin[e] (40 mg/kg ip)	152 ± 9.5**	191 ± 12.8**	80
Electroshock[f] controls	120 ± 10.0	121 ± 21.6	99
Electroshock[g]	153 ± 16.3*	324 ± 41.7**	47*

a. Control value was 40.1 ± 1.7 picomoles/mg protein.
b. Control value was 234 ± 17 femtomoles/mg protein.
c. Controls receiving nothing or 0.1 ml/100g DMSO did not differ in cyclic nucleotide levels. They were combined.
d. Values are means ± SEM.
e. Mice sacrificed 5 sec after the onset of the tonic phase of the seizure.
f. Pinna electrodes were placed but the subjects were not stimulated. They were sacrificed at the same time points as subjects in the electroshock group.
g. Mice sacrificed at the end of the tonic phase of the seizure.
* $p < 0.05$ as compared to the appropriate control group.
** $p < 0.01$ as compared to the appropriate control group.

Subconvulsive exposures of dieldrin led to the changes in cyclic nucleotides shown in figure 1. Moderate increases in cAMP were observed. The magnitude of these changes was related to exposure level, with peak changes occurring at 1-2 h. The effects on cGMP were

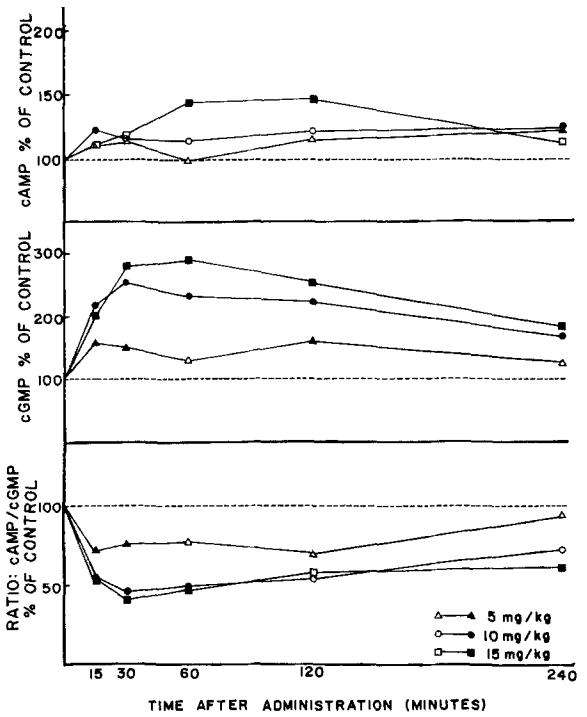

Figure 1. Effects of acute dieldrin administration on whole brain cAMP and cGMP levels and their ratio: cAMP/cGMP. Values shown as solid symbols are significant at the 0.05 level. Combined control value for cAMP was 40.1 + 1.7 picomoles/mg protein. Combined control value for cGMP was 234 ± 17 femtomoles/mg protein.

more pronounced and more consistent. The magnitude of change was clearly related to exposure. Levels of cGMP remained elevated throughout the 4 h period. Peak changes were observed between 30-60 min. cGMP elevations peaked more rapidly and persisted longer than cAMP elevations. The ratio: cAMP/cGMP was also proportionately affected by exposure. This ratio was maximally depressed at 30 min, the period of time at which overt signs of excitability were maximal. Significant depression of the ratio was still evident 4 h after administration.

DISCUSSION

It is clear from these data that dieldrin, like other excitatory substances, increases whole brain cyclic nucleotide levels. Convulsions are not necessary for this effect as increases are seen over a wide range of nonconvulsant as well as convulsant exposures. The ratio:

cAMP/cGMP is reduced over these same exposures. This is primarily a reflection of the changes occurring in cGMP levels.

There are two points that merit emphasis. First, there is a clear, positive correlation between cGMP levels and the degree of exposure to dieldrin. cGMP increases in direct proportion to dose over the range of nonconvulsive exposures. These same exposures also lead to proportional increases in CNS excitability (JOY 1974; 1975). Second, the changes in cGMP levels are not transiently altered as excitability is modulated, but rather appear to follow closely the ongoing level of CNS activity.

These findings support the view that cGMP levels fluctuate with the level of excitability of the nervous system. This has been previously suggested by others (FERRENDELLI et al. 1980; OPMEER et al. 1976). The present study demonstrates that this correlation holds well even over long periods of observation (4 h). As such it suggests that, in general, convulsant-induced increases in cGMP reflect the capacity of these substances to excite the CNS. Changes in cGMP levels would appear to be a reflection of that action. These data do not suggest that cGMP has a specific action as a precipitant of convulsive behavior.

With nonconvulsive exposures to dieldrin whole brain cAMP levels are marginally elevated. These changes occur later and are not as sustained as the changes in cGMP. It is probable that these changes are related to the increased release of catecholamines that occurs during the period of elevated CNS excitability. This has been suggested also for other convulsant substances (FERRENDELLI 1980; FERRENDELLI et al. 1980; GROSS & FERRENDELLI 1979; WASTERLAIN & CSISZAR 1980).

The changes demonstrated here are changes in whole brain cyclic nucleotide levels. It is of great interest to examine regional cyclic nucleotide levels over long time intervals as well. If cGMP levels in a neuron bear a close relationship to its ongoing state of activity and/or intrinsic excitability, then regional differences would indicate specific loci at which neurotoxic substances, such as dieldrin, produce greater or lesser effects.

ACKNOWLEDGEMENTS

This work was supported in part by NIH Grant No. RRO-5457 and by a UCD Faculty Research Grant.

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