Cyclic GMP concentrations in cerebellum following organophosphate administration

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Organophosphorus cholinesterase inhibitors such as soman (*o*-pinacolyl-methyl-phosphono-fluoridate) cause muscle fasciculations, tremors, convulsions and death in experimental animals. The toxic effects of these compounds are believed to be mediated by a marked increase in acetylcholine concentrations subsequent to cholinesterase inhibition centrally and peripherally, Du Bois (1963). It is not well established, however, whether all of the toxic symptoms are due to alterations in cholinergic function or if other neurochemical changes might be in part responsible. For example, no clear relation has been demonstrated between protection by atropine-like drugs against poisoning by organophosphorus cholinesterase inhibitors and their central or peripheral antimuscarinic activity (Faff, Borkowska & Bak, 1976; Green, Muir & others, 1977). In the course of a study concerning the effects of organophosphates on other transmitter agents such as γ-aminobutyric acid (GABA) which is important in the production of convulsions (Wood, 1975), we have found that cyclic GMP (cGMP) which has a function in GABA-ergic transmission in the cerebellum (Mao, Guidotti & Costa, 1974a, b) was greatly elevated at the onset of soman-induced seizures. Certain anticonvulsants blocked the soman-induced convulsions and the increase in cGMP, whereas high doses of atropine failed to do either, suggesting that organophosphates may produce changes in other non-cholinergic systems in brain which may be important in producing their toxicity.

Male Sprague-Dawley rats (200–230 g) were treated with soman and the LD50 value was calculated by probit analysis. One LD50 of soman produced reproducible convulsions in rats in a relatively short time (mean 8·6 min). Groups of animals were treated with diazepam, clonazepam, atropine sulphate (30 min) or aminooxyacetic acid hemi-hydrochloride (AOAA) (90 min) before one LD50 of soman. At the onset of soman-induced convulsions, or at the average time taken to cause convulsions in control animals, the animals were killed by a microwave beam focused on the skull (3·5 kw for 2·95 s) to prevent post-mortem changes in cGMP concentrations. cGMP was measured by the radioimmunoassay (RIA) method outlined by Steiner, Pagliari & others (1972) using Schwartz-Mann RIA kits with the modification of Tihon, Goren & others (1977).

Table 1 shows the effects on cGMP concentrations in the cerebellum of the three anticonvulsants when used alone. All three lowered cGMP concentrations at doses that blocked soman-induced convulsions. The benzodiazepines were effective in low doses, but high doses of AOAA were needed to control the seizure activity.

Table 2 shows that diazepam, clonazepam and AOAA, at doses sufficient to control convulsions, blocked the increase in cGMP concentrations normally seen following soman at the onset of seizures. In no case did the cGMP values following soman in combination with either benzodiazepine reach control values. Atropine had no significant effect on the soman-induced increase in cGMP concentrations or on convulsive activity.

Studies from several laboratories have indicated that the benzodiazepines are effective in blocking organophosphate induced seizure activity and also offer some protection against their lethal effects (Lipp, 1973; Rump, Grudzinska & Edelwjn, 1973). Furthermore, a GABA-ergic link expressed as changes in cGMP may underly the mechanism of action of the benzodiazepines (Costa, Guidotti & Mao, 1975). AOAA probably inhibits convulsions by increasing GABA concentrations subsequent to inhibition of GABA transaminase (Wallach, 1961).

Previous reports have indicated the cGMP concentrations are altered by changes in neurotransmitter substances and that alterations in GABA metabolism markedly affect cGMP values (Mao & others, 1974a, b;

Table 1. *The effect of soman, atropine and some anticonvulsants on cerebellar cGMP content (p mol mg^{-1} microwaved tissue (number of animals)).* Soman was administered subcutaneously and animals were killed at first signs of convulsions (mean 8·6 min post injection). Atropine, diazepam and clonazepam were all given intraperitoneally 30 min and AOAA was given interperitoneally 90 min before death.

Treatment	Dose μmol kg^{-1}	cGMP
Saline		1·66 ± 0·20 (9)
Soman	1 LD50 0·74	3·29 ± 0·26** (11)
Atropine	43·17	1·81 ± 0·26 n.s. (6)
Diazepam	8·78	1·20 ± 0·21* (6)
Clonazepam	4·24	0·73 ± 0·06** (4)
Clonazepam	1·06	1·27 ± 0·11* (6)
AOAA	457·5	1·25 ± 0·09* (5)

* Significance at $P < 0.05$. ** $P < 0.01$.
n.s., not significantly different from soman alone.

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Table 2. *The effect of atropine and three anticonvulsants on the cerebellar cGMP content (p mol mg^{-1} microwaved tissue (number of animals)).* Atropine (i.p.) was given 30 min before soman (s.c.) and the animals killed at first signs of convulsions. Diazepam and clonazepam (i.p.) were given 30 min before soman and AOAA (i.p.) was given 90 min before. The animals were killed at the average time to convulse following soman.

Treatment	Dose μmol kg^{-1}	cGMP
Soman	0·74	3·29 ± 0·26 (11)
Soman + atropine	43·17	3·01 ± 0·40 n.s. (6)
Soman + diazepam	17·56	1·13 ± 0·10* (6)
Soman + diazepam	8·78	1·18 ± 0·18* (5)
Soman + clonazepam	4·24	1·17 ± 0·10* (6)
Soman + clonazepam	1·06	1·45 ± 0·14* (6)
Soman + AOAA	457·5	1·65 ± 0·16* (6)

* Significance at $P < 0.05$ (at least).
n.s., not significantly different from soman alone.

Costa & others, 1975). Lee, Kuo & Greengard (1972) have shown that muscarinic cholinergic stimulation can raise the cGMP content in various animal tissues, although recent results have failed to confirm these findings in brain (Kinscherf, Chang & others, 1976). It is unlikely that muscarinic stimulation is responsible for the increased cGMP concentrations in the cerebellum, because very small amounts of the benzodiazepines totally abolish organophosphate-induced convulsions, whereas, even high doses of the antimuscarinic compound atropine, had no effect. Furthermore, atropine is known to abolish organophosphate-induced increase in acetylcholine concentrations (Wecker, Mobley & Dettbarn, 1977; and Stavinoha, Modak & Weintraub (1977) have shown that the cerebellum is unique in that organophosphates produce only a slight increase in the rate of accumulation of acetylcholine in this area of the brain.

Thus it is possible that organophosphate cholinesterase inhibitors may produce convulsions and elevation of cGMP concentrations via alteration in GABA metabolism or via some other non-cholinergic transmitter candidate. On the other hand, the convulsions may result from central nicotinic effects of soman although acetylcholine concentrations do not increase in atropine pre-treated organophosphate poisoned animals (Wecker & others, 1977), and atropine has been reported to block convulsions induced by nicotinic stimulation, although some controversy exists on this point (Brimblecombe, 1974). The change in cGMP concentrations may be secondary to a central nicotinic effect of soman, although it is unlikely since neither nicotinic agonists nor antagonists have any demonstrated effect on cGMP concentrations in brain (Lee & others, 1972; Mao & others, 1974b).

These studies show clearly that anticonvulsants which are believed to act primarily via GABA-ergic mechanisms, block soman convulsions as well as certain neurochemical changes induced by this compound.

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