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Cerebellar cGMP levels reduced by morphine and pentobarbital on a dose- and time-dependent basis

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The role of 3′,5′-cyclic guanosine monophosphate (cGMP) in cerebellar function is unknown, although the presence of a specific, high affinity cGMP binding protein in rat cerebellum [1], a distinct cGMP-dependent protein kinase in bovine cerebellum [2], and the relatively high cGMP concentration in rat cerebellum [3] emphasize the possibility that cGMP may have an important function in the regulation of cerebellar activity. 3′,5′-Cyclic adenosine monophosphate (cAMP) is present in cerebellum and its levels can be altered by a variety of drugs *in vivo* [4] and by putative neurotransmitters in cerebellar slices [5]. However, selective and independent changes in cerebellar cGMP levels can be achieved by administration of harmaline or diazepam [6], glutamic acid, picrotoxin [7] and genetic mutation [8]. Our study has dealt with and compared the effects of morphine and pentobarbital on cGMP levels in rat cerebellum, demonstrating a dramatic depression of cGMP by morphine and confirming and extending related work on pentobarbital effects on cerebellar cGMP levels [4].

Naive animals (male Sprague–Dawley rats weighing 150 g) were injected (i.p.) with either morphine or pentobarbital, in varying dosages, and sacrificed 15 min post-injection by exposing the head to focused microwave irradiation (1.3 kW, 4.0-sec exposure) which inactivated brain enzymes and prevented post-mortem changes in cyclic nucleotide levels (microwave source was a Litton Menumaster oven modified by Medical Engineering Consultants, Lexington, Mass., model 70/50). cGMP was extracted from the cerebellum [9] and the quantity measured with a competitive protein binding assay [10]. Figures 1 and 2 display the dose/response curves relating the morphine and pentobarbital dose to cGMP level in the cerebellum. Ataxia and catatonia were pronounced 15 min after injection in rats treated with 45 mg/kg of morphine or 12.5 mg/kg of pentobarbital, dosages which depressed cerebellar cGMP content to 29 and 38 per cent of control levels respectively. Figure 3 presents time course studies on the cGMP-depressant effects of morphine and of pentobarbital.

A second group of rats was made tolerant to morphine through twice-daily injections of 60 mg/kg of morphine for 10 days. Using a noxious heat stimulus (hot plate technique) [12, 13], rats chronically treated with morphine were defined as tolerant when they responded as quickly as controls to the stimulus [12, 13]. Rats acutely treated with morphine responded much more slowly to the stimulus, and as tolerance to morphine was acquired over the 10-day schedule, response time decreased until it equaled that of the controls. As shown in Fig. 4, the cGMP content of cerebella from tolerant rats challenged with 45 mg/kg

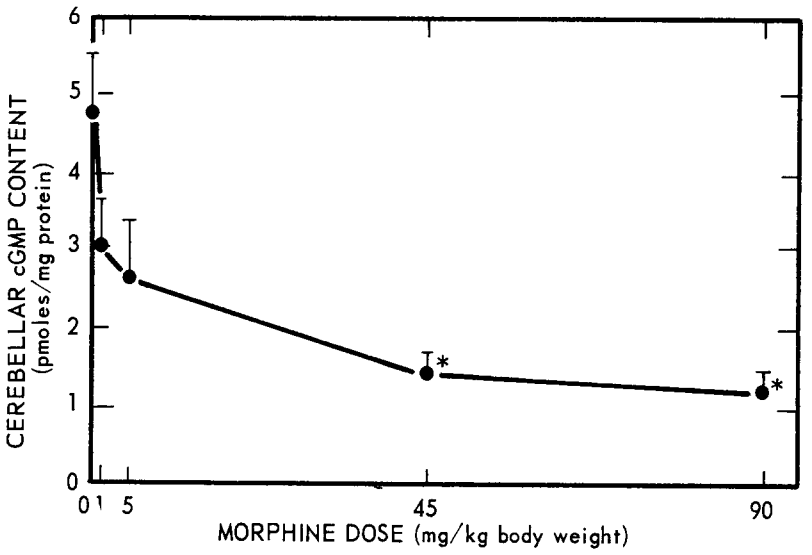

Fig. 1. Morphine dose/response curve: dose-dependent reduction in cerebellar cGMP content. Groups of 150-g Sprague–Dawley rats (n = 7, means ± S. E.) were injected with varying amounts of morphine sulfate (i.p.) and euthanatized 15 min later by focused microwave irradiation of the head (1.3 kW power density, 4.0-sec exposure). Cerebella were removed and their cGMP content assayed as previously described [9, 10]. Cerebellar protein content was measured with the method of Lowry *et al.* [11] and the level of cGMP in each cerebellum was expressed in pmoles/mg of protein. cGMP values were not corrected for recovery during extraction and purification. Asterisks indicate values significantly different from control, $P < 0.001$.

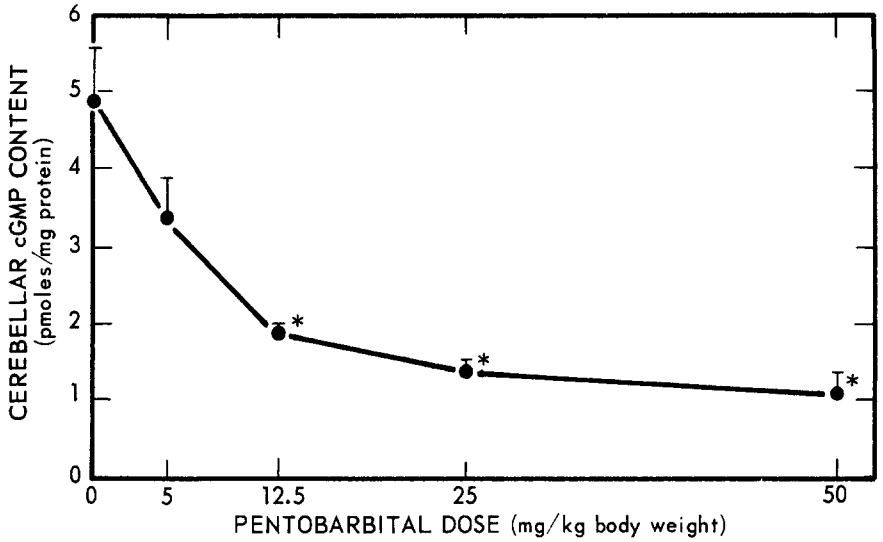

Fig. 2. Pentobarbital dose/response curve: dose-dependent reduction in cerebellar cGMP content. Groups of 150-g Sprague–Dawley rats (n = 6, means ± S. E.) were injected with varying amounts of sodium pentobarbital (i.p.) and euthanatized 15 min later. Techniques were identical to those referenced in Fig. 1. Asterisks indicate values significantly different from control, $P < 0.005$.

of morphine was not significantly different from that of naive controls. Furthermore, tolerant rats undergoing precipitated withdrawal induced by the narcotic antagonist naloxone (2.5 mg/kg, i.p.) had cerebellar cGMP levels equal to those of morphine-challenged tolerant rats and naive controls. This observation is surprising in light of a compensatory overshoot in cGMP content which might have been expected at this time.

The significance of the changes in cerebellar cGMP levels after narcotic or hypnotic drug administration is unknown. The location of cGMP within the cytoarchitecture of the cerebellum is no definitely known. Although all cerebellar cGMP may not be physically associated with the Purkinje cells—the efferent cerebellar cortical cells—and could conceivably be associated with cerebellar cortical interneurons, glia or deep cerebellar nuclei, there is suggestive evidence for the presence of a large cGMP pool in, or functionally associated with, Purkinje cells. The level of cGMP is low in infant rat cerebellum, but upon achievement of synaptic contact between climbing fibers of the inferior olivary nucleus and the Purkinje cells, cGMP levels rise to those found in the adult [14]. Mutant mice

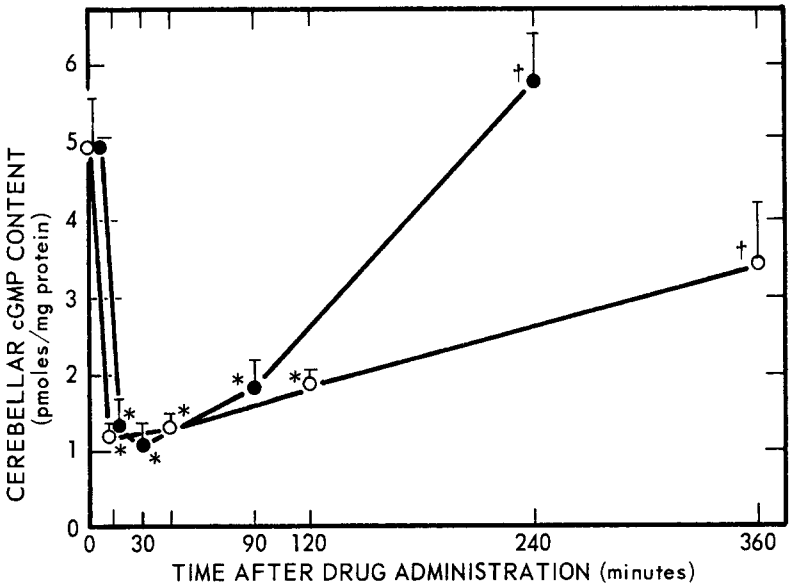

Fig. 3. Time course studies: time-dependent effects of acute doses of morphine (45 mg/kg) or pentobarbital (25 mg/kg) on cerebellar cGMP content. All injections were given intraperitoneally. For morphine study, n = 7, means ± S. E. M. For pentobarbital study, n = 5, means ± S. E. M. Measurement techniques were as referenced in Fig. 1. Asterisks indicate values significantly different from control, $P < 0.005$. Daggers indicate values not significantly different from control, $P > 0.20$. Key: (●) morphine time course; and (○) pentobarbital time course.

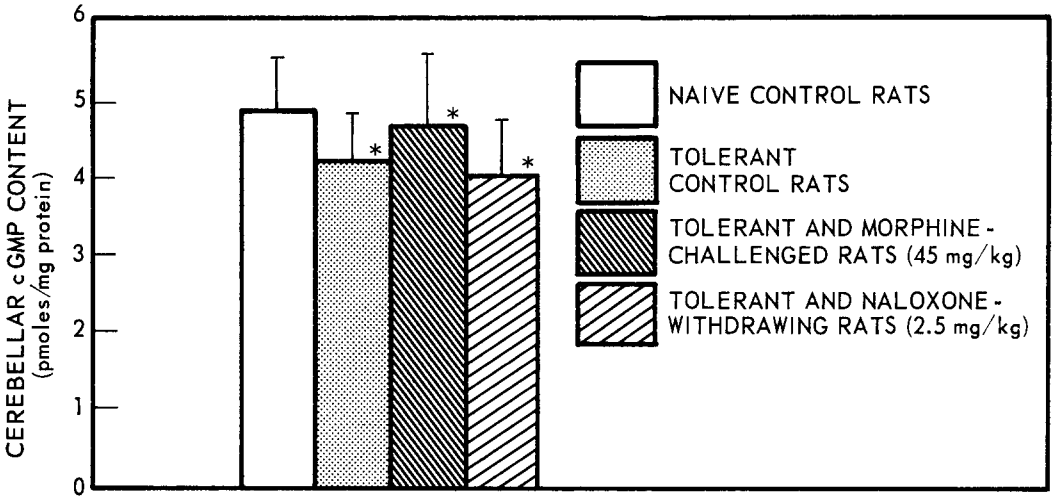

Fig. 4. Effect of morphine tolerance, and morphine or naloxone challenge in morphine-tolerant rats on cerebellar cGMP levels. Rats were made tolerant to morphine as defined and determined by their response to minimize a standardized noxious heat stimulus [12, 13]. Tolerant animals were defined as responding as quickly as animals never treated with morphine. Animals were made tolerant by twice-daily injections of morphine sulfate (60 mg/kg, i.p.) for 10 days. A tolerant control group was euthanatized by focused microwave irradiation just prior to the final scheduled morphine dose. Tolerant, morphine-challenged rats were injected with 45 mg/kg of morphine, i.p., at this time, and tolerant, naloxone-withdrawing rats were injected with 2.5 mg/kg of naloxone, i.p., at the same time. Both groups were euthanatized 15 min later, at which time naloxons-withdrawing rats exhibited the typical abstinence syndrome of diarrhea, shaking and hypersensitivity to handling. Techniques identical to those referenced in Fig. 1 were used; n = 7, means ± S. E. M. for each group. Asterisks indicate values not significantly different from naive controls, $P > 0.50$.

which have a selective loss of 90 per cent of cerebellar Purkinje cells, with an otherwise intact cerebellar cortex, similarly have very low levels of cerebellar cGMP but normal levels of cAMP [8]. Such mice move with an ataxic gait [8]. Current thought points to the possible role of cGMP and cAMP as mediators of opposing cellular responses induced by hormones and putative neurotransmitters [15]. cGMP has been suggested as a specific cholinergic mediator in superior cervical ganglion [16] and rat cerebellar cortex [17]. cGMP levels were similarly elevated by acetylcholine in rabbit cerebellar slices [5]. Consistent with the general hypothesis of cAMP and cGMP as opposing cellular regulatory mediators, cAMP and cGMP were found to respectively inhibit and excite pyramidal tract neurons in rat cerebral cortex when applied microiontophoretically, mimicking, respectively, the actions of norepinephrine and acetylcholine [18].

However, other work indicates that cAMP and cGMP may mediate neuronal responses to putative transmitters other than norepinephrine and acetylcholine. For example, pharmacologic manipulation of cerebellar levels of gamma-amino butyric acid (GABA), and intraventricular injection of either GABA or glutamate have been found to respectively depress and elevate cerebellar cGMP levels [7]. Iontophoresis of GABA [19] and glutamate [19, 20] onto Purkinje cells is known to respectively depress and elevate simple spike firing rates; iontophoretic experiments with cAMP and cGMP, respectively, suggest that these nucleotides may mediate these putative neurotransmitter effects also [19].

We have shown that morphine and pentobarbital reduce cerebellar cGMP upon acute administration, as well as induce ataxia/catatonia. Although the correlation between the motor effects of morphine and pentobarbital and reduced cerebellar cGMP levels does not prove that reduced nucleotide levels are directly responsible for altered motor activity, it nonetheless offers a possible biochemical and pharmacological locus of an altered cerebellar state of excitability, which may be partly responsible for the motor signs incident to narcotic or hypnotic administration.

We are currently studying the enzymatic regulatory basis underlying the morphine and pentobarbital-induced depression of cerebellar cGMP levels; however, the mode of regulation of cGMP synthesis and degradation is unknown. The depression of both evoked [21] and spontaneous [22, 23] Purkinje cell activity in barbiturate-treated cats has been observed, and it has been suggested that these effects are caused in part by the depressant action of barbiturates upon the excitatory drive to the Purkinje cells through hypothesized direct action on the mossy fiber-granule cell system of the cerebellum [21, 22]. By analogy, a similar mechanism of direct depression of cerebellar cortical activity, including spontaneous Purkinje cell activity, may occur with morphine administration. Also, it has been suggested that modification of climbing fiber input from the inferior olivary nucleus to the cerebellum is responsible for the elevation of cerebellar cGMP upon administration of harmaline [6]. Thus, morphine and barbiturates may possibly influence cerebellar cGMP levels through this pathway as well. The lack of measurable and specific opiate receptor binding in cerebellar homogenates, in singular contrast to the presence of such stereospecific opiate receptor binding in homogenates of other brain areas [24], is evidence against a direct effect by morphine. However, a lack of measurable opiate receptors does not conclusively rule out a direct effect. A recent study has shown that ethanol administration also drastically reduced cerebellar cGMP levels [25]. The similarity of morphine, barbiturate and alcohol effects on cerebellar cGMP levels suggests that these agents act through common mechanisms. The absence of morphine receptors in the cerebellum [24] and the lack of documentation of specific ethanol receptors in any part of the brain suggest that

these biochemical changes in cerebellum may be secondary to changes in input activity from other brain areas.

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