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Interactions between γ-Aminobutyric Acid and Guanosine Cyclic 3′,5′-Monophosphate in Rat Cerebellum

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SUMMARY

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The concentrations of adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) were measured in various brain areas of rats either kept at 22° or exposed to 4° for various periods. Brain metabolic activity was terminated by focusing a microwave beam (70 mW/cm²) on the skull for 2 sec. Pituitary was the only tissue in which cAMP concentration was increased by exposure to cold. The concentrations of cGMP in cerebellum, hypothalamus, and brain stem were significantly increased between 5 and 15 min of exposure to 4°. The increase in cerebellar cGMP elicited by cold was prompt but transient. When rats were exposed to cold, cerebellar cGMP content increased 3-fold over the control value in 5 min and returned to basal level after 30 min of cold exposure. This increase of cerebellar cGMP was unimpaired when cholinergic, catecholaminergic, and serotonergic neuronal mechanisms were blocked pharmacologically. Of the three drugs —aminooxyacetic acid, hydrazine sulfate, and hydroxylamine—which increase cerebellar concentrations of γ-aminobutyric acid (GABA), the first two inhibited the increase in cGMP concentration elicited by cold exposure. The inhibition of the increment in cerebellar cGMP elicited by cold correlated with the extent of the increase in cerebellar GABA concentrations. Isonicotinic acid hydrazide decreased the concentration of cerebellar GABA and caused an increase of cGMP concentration in the cerebellum of rats either kept at 22° or exposed to cold for 5 min. This drug, up to 10 mM, failed to inhibit the cyclic nucleotide phosphodiesterase activity of brain homogenates. The increase or decrease of cerebellar concentrations of GABA elicited pharmacologically failed to change the concentrations of cAMP in cerebellum.

INTRODUCTION

Several lines of evidence suggest that adenosine 3′,5′-cyclic monophosphate and guanosine 3′,5′-cyclic monophosphate function as second messengers in central and peripheral synapses (1-5). At one time it appeared that the type of transmitter involved at a given synapse would determine whether adenylate or guanylate cyclase was activated (3, 6-8); however, other lines of evidence suggest that the molecular nature of the transmitter released at synapses may not be the only significant element predicting whether the concentrations of cAMP or cGMP in the target cells will increase as a result of synaptic activity (5). The addition of catecholamines, histamine, serotonin, acetylcholine, membrane-depolarizing

agents, or electrical stimulation can promote the accumulation of cAMP[1] in slices of brain and adrenal medulla (9–13). A role of cGMP in the mediation of synaptic events may also be considered, because in brain slices an increase of cGMP concentration is elicited not only by the activation of cholinergic synapses but also by the action of depolarizing agents (8, 14, 15).

The presence in mammalian central nervous system of specific protein kinases activated by either cAMP (16) or cGMP (17) and the observation that a single transmitter can change the cAMP and cGMP concentrations of a uniform population of postsynaptic cells in opposite directions (13) suggested that the concept of the "biological dualism" (18) of cAMP and cGMP can be extended to their role in synaptic mechanisms. Synaptic activity, by changing the cAMP:cGMP ratios in neuronal membranes, can activate the protein kinases of these membranes, thus promoting the phosphorylation of specific membrane constituents (19). Phosphorylation and dephosphorylation of these proteins may regulate the impedance of the neuron membranes and change their excitability (20).

The present report describes the increase of cAMP and cGMP concentrations in various brain regions of rats exposed to 4°. In cerebellum, where the increase of cGMP concentration reaches its maximum within 5 min of cold exposure, this change does not appear to require the participation of adrenergic, cholinergic, or serotonergic synaptic mechanisms. However, the extent of cGMP increase elicited by cold is modulated when the cerebellar concentrations of γ-aminobutyric acid are increased or decreased by drugs. Our experiments indicate that neither the increase of GABA concentrations nor the exposure to 4° influence the cerebellar concentrations of cAMP.

METHODS

Adult male Sprague-Dawley rats were placed in single cages at 4° for various time intervals after having their fur wetted with water at 22°. Control animals were housed in the same manner, but at 22° and without the wetting procedure. Rats were killed by exposing their heads for 2 sec to a high-intensity microwave beam (70 mW/cm²) (21). A group of rats was killed with the freeze-blowing technique described by Veech *et al.* (22); the concentrations of cAMP, cGMP, and the activities of cyclic nucleotide phosphodiesterase and adenylate cyclase of their brains were compared with those of rats killed by a microwave beam. Adenylate cyclase was assayed as previously described (23). Cyclic AMP phosphodiesterase activity was determined according to Weiss *et al.* (24), and cGMP phosphodiesterase, according to Thompson and Appleman (25).

Cyclic nucleotide purification. After microwave irradiation, various brain parts were homogenized in 1 ml of 0.4 N $HClO_4$ and centrifuged at 4° for 20 min at 30,000 × *g*. The supernatant fluid was neutralized with 0.15 ml of 3 M Tris containing 0.02 pmole of [³H]cAMP (about 10,000 dpm) and 0.05 pmole of [³H]cGMP (about 7500 dpm). The radioactive nucleotides were added to monitor the recovery of cyclic nucleotides in each sample throughout the procedure.

The cAMP and cGMP present in the supernatant extracts were isolated and purified according to Mao and Guidotti (26). In brief, the neutralized supernatant was placed on an aluminum oxide column (0.4 × 4.3 cm) equilibrated in 0.0 M6 Tris-chloride buffer, pH 7.5. The cyclic nucleotides were eluted from the column with 4 ml of 0.6 M Tris-chloride buffer, pH 7.5, onto a Dowex 1-X2 column (0.4 × 8.0 cm, 200–400 mesh, chloride form, in H_2O). This column was rinsed with 4 ml of H_2O, and cAMP was eluted with 6 ml of 0.05 N HCl. The second and third milliliters of the eluate contained about 80% of the cyclic nucleotide present in the original sample. cGMP was eluted with another 4 ml of 0.5 N HCl, with the second and third milliliters likewise containing about 80% of this nucleotide. The cAMP eluate was neutralized with 0.2 ml of 0.6 M Tris, and the cAMP was adsorbed onto a Dowex 50-X8 column (0.4 × 3 cm, 200–400 mesh, H^+ form, in H_2O). The column was

[1] The abbreviations used are: cAMP, adenosine cyclic 3′,5′-monophosphate; cGMP, guanosine cyclic 3′,5′-monophosphate; GABA, γ-aminobutyric acid.

then rinsed with 2.5 ml of H_2O, and the cAMP was eluted by an additional 2 ml of H_2O; the eluate was lyophilized. The cGMP eluate from the Dowex 1-X2 column was lyophilized and taken up in 1 ml of H_2O. This solution was adsorbed onto a Sephadex G-10 column (0.4 × 7.5 cm, in H_2O), and cGMP was eluted with 2 ml of H_2O. This eluate was lyophilized.

cAMP was assayed with the luciferin-luciferase method (27), and cGMP, with the cGMP-dependent protein kinase assay (8). The assays were always performed in duplicate. The purity of the cyclic nucleotides isolated from various tissues with our procedure was verified by silica gel thin-layer chromatography (26), hydrolysis with phosphodiesterase (26), and lack of ATP production from purified cGMP by an incubation mixture containing phosphodiesterase myokinase and pyruvate kinase (26). Moreover, cAMP or cGMP, passed through the columns in amounts ranging from 0.5 to 30 pmoles, showed linear calibration curves by the respective detection systems. The slopes of these lines, when the values are corrected for recovery, were equal to those obtained with equal amounts of authentic cAMP or cGMP which had not been adsorbed on the various columns used for the purification of tissue samples.

When GABA and cyclic nucleotides were determined in the same tissue sample, the tissue was homogenized in 1 ml of 0.4 N $HClO_4$ containing 0.1 M EDTA and centrifuged at 4° (30,000 × *g*). The supernatant fluid was neutralized with 0.15 ml of 4 N KOH and 0.15 ml of 3 M Tris-chloride buffer, pH 7.0, containing tritiated cyclic nucleotides. The precipitate ($KClO_4$) was separated by a second centrifugation. An aliquot of this supernatant fraction was used to determine GABA concentrations (28), and the rest was used to assay cAMP and cGMP.

Drugs and materials. Atropine sulfate (Sigma Chemical Company), chlorisondamine (Ciba), mecamylamine (Merck Sharp & Dohme), *p*-chlorophenylalanine methyl ester HCl (Regis Chemical Company), and isonicotinic acid hydrazide (Calbiochem) were dissolved in saline. Aminooxyacetic acid HCl (Sigma), hydroxylamine HCl (Eastman Organic Chemicals), pargyline (Abbott), and hydrazine sulfate (Eastman) were dissolved in saline and then titrated to approximately neutral pH. Picrotoxin (K & K Laboratories) was dissolved in hot water. Indomethacin (Merck Sharp & Dohme) was suspended in 0.1 M phosphate buffer, pH 7.0. Reserpine (10 mg) (Sigma) was dissolved in 50 µl of concentrated acetic acid, then diluted with distilled water to a suitable concentration. 6-Hydroxydopamine (Regis) was dissolved in 0.85% NaCl containing 0.001% $Na_2S_2O_5$ (29).

6-Hydroxydopamine (200 µg) in 50 µl was injected intracisternally into rats lightly anesthetized with ether. Two other injections, of 50 µg each, were given 72 and 96 hr after the first dose. These rats were exposed to cold 7 days after the last dose of 6-hydroxydopamine. GABA transaminase–succinate semialdehyde dehydrogenase mixture (Gabase) in 50% glycerol was obtained from Sigma Chemical Company.

RESULTS

Steady-state concentrations of cyclic nucleotides, cyclic nucleotide phosphodiesterase, and adenylate cyclase activities after microwave irradiation. Previous reports (30) showed that after microwave irradiation of the whole rat the brain concentration of cAMP is lower than after the freeze-blowing procedure developed by Veech *et al.* (22). Since the microwave irradiation used in the present experiments (see METHODS) differs from that used by Lust *et al.* (30), we determined whether this new procedure would affect the steady state of brain cyclic nucleotides. The data reported in Table 1 show that the cAMP and cGMP concentrations in brains of rats killed with freeze-blowing or with microwaves are identical. In addition, we measured the brain adenylate cyclase and phosphodiesterase activities of rats killed with microwave irradiation and found that these enzymes were completely inactivated after 2 sec of treatment.

Location of cyclic nucleotides in rat brain and effect of cold exposure on cyclic nucleotide concentrations. The concentrations of cAMP and cGMP in various brain areas of rats kept at 22° or exposed for 15 min to 4° are re-

TABLE 1

cAMP and cGMP concentrations in brains of rats killed with microwave irradiation or freeze-blowing

The heads of rats weighing 175 g were exposed for 2 sec to a focused microwave radiator (75 mW/cm²), or the brains were frozen for 0.5 sec with the technique described by Veech *et al.* (22). The values represent means ± standard errors for eight animals.

Method of killing	Time required to inactivate brain enzymes	cAMP	cGMP	cAMP:cGMP
	sec	*pmoles/mg protein*		
Microwave	2	7.6 ± 0.52	0.31 ± 0.007	24
Freeze-blowing	0.5	8.3 ± 0.60	0.28 ± 0.018	29

TABLE 2

Concentrations of cAMP and cGMP in various brain regions of rats kept at 22° or 4° for 15 min

The values are the means ± standard errors of at least four determinations. The assays for cGMP and cAMP in hypothalamus and pituitary were performed in four pooled tissue samples.

Brain region	cAMP		cGMP	
	22°	4°	22°	4°
	pmoles/mg protein		*pmoles/mg protein*	
Cerebellum	9.4 ± 1.2	10 ± 1.1	6.8 ± 0.79	14 ± 2.1[a]
Caudate	10 ± 0.73	13 ± 0.97	0.50 ± 0.13	0.61 ± 0.09
Cerebral cortex	11 ± 0.66	12 ± 0.60	0.58 ± 0.09	0.48 ± 0.10
Brain stem	6.6 ± 0.21	8.9 ± 1.4	1.0 ± 0.12	1.8 ± 0.24[b]
Hypothalamus	12 ± 1.2	13 ± 1.9	0.48 ± 0.04	0.68 ± 0.02[b]
Pituitary	10 ± 0.78	84 ± 4.1[a]	0.53 ± 0.06	0.60 ± 0.05

[a] $p < 0.005$ compared with results at 22°.
[b] $p < 0.05$ compared with results at 22°.

ported in Table 2. The cAMP concentrations were higher than those of cGMP in all brain regions studied except the cerebellum, where the concentrations of the two cyclic nucleotides were similar.

When rats were exposed to 4° for 15 min the pituitary was the only tissue in which the cAMP concentration was increased ($p < 0.001$). On the other hand, this environmental stress increased the cGMP concentrations in cerebellum, hypothalamus, and brain stem. Wetting the fur of the rats with 22° water also increased the cGMP concentrations of cerebellum, but by only 50%. cAMP concentrations remained unchanged after fur wetting.

Time course of cGMP increase in cerebellum of rats exposed to cold. When rats were exposed to 4° for periods of 5 min to 2 hr after wetting their fur with 22° water, the cGMP concentrations rose promptly (Fig. 1) from 6.8 ± 0.79 to 20 ± 1.8 pmoles/mg of protein. When the cold exposure lasted longer than 15 min the cerebellar concentration of cGMP slowly declined and returned toward the basal level in 30 min. The cerebellar concentrations of cAMP remained unchanged throughout (Fig. 1). The body temperature of the rats was unchanged during the first 30 min and decreased 2–3° after 2 hr of continued exposure at 4°.

Drugs affecting adrenergic, cholinergic, and serotonergic synapses and cerebellar cAMP and cGMP concentrations of rats kept at 22° or 4°. In order to elucidate which synaptic mechanism is involved in the increased cerebellar cGMP concentrations of rats exposed to cold, we injected several drugs which act upon specific processes of various neural pathways. Atropine (100 mg/kg

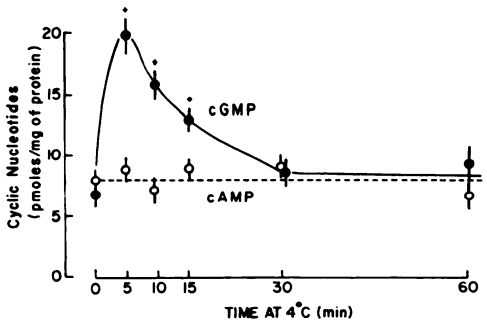

FIG. 1. *Cyclic AMP and cGMP concentrations in cerebella of rats exposed to 4° for different time periods*

The cGMP concentration returned to basal level after 30 min of cold exposure. The concentration of cAMP remained unchanged. Each point is the mean ± standard error (vertical bars) of at least six rats.
$p < 0.025$.

intraperitoneally), chlorisondamine (10 mg/kg intraperitoneally), and mecamylamine (2.5 mg/kg subcutaneously) injected 15 min before the experiments neither modified the concentrations of cAMP and cGMP nor changed the increase of cerebellar cGMP elicited by cold exposure (Fig. 2). Atropine caused moderate hyperthermia (1–2°), while nicotinic receptor blockers caused slight hypothermia and ptosis.

An intracisternal dose schedule of 6-hydroxydopamine that lowers the cerebellar concentration of norepinephrine by about 70% (29) or the intravenous injection of *p*-chlorophenylalanine methyl ester at 100 mg/kg, which decreases the brain serotonin concentration and tryptophan hydroxylase activity by 70–80%,[2] failed to change the cGMP and cAMP concentrations in cerebellum (Fig. 2). Moreover, the increase of cerebellar cGMP elicited by cold exposure remained unaltered in rats receiving these drugs (Fig. 2).

The possibility that either serotonin or catecholamines are involved in the increase of cerebellar cGMP concentrations was further tested by intraperitoneal injection of reserpine (5 mg/kg) or pargyline (75 mg/kg). In rats kept at 22° neither drug changed the cerebellar concentrations of cAMP or cGMP by 2 hr; moreover, they did not affect the increase in cerebellar cGMP elicited by cold (Fig. 2). Indomethacin (10 mg/kg intraperitoneally) neither changed cAMP or cGMP concentrations in cerebella of rats kept at 22° nor reduced the increase in cGMP content in rats exposed to cold for 5 min (Fig. 2). Reserpine and indomethacin slightly reduced the body temperature of the rats.

Cerebellar cyclic nucleotide concentrations in rats treated with drugs increasing GABA content in brain. Aminooxyacetic acid, hydroxylamine, and hydrazine increase the GABA concentrations in brain, presumably by inhibiting GABA transminase activity (31, 32). When the rats received any one of the three drugs at the doses indicated in Table 3, the cerebellar cAMP concentrations in animals kept at either 22° or 4° remained unchanged. In contrast, the cGMP concentrations in cerebella of rats kept at 22° and receiving hydrazine was reduced by 66% (Table 3). Aminooxyacetic acid and hydroxylamine failed to change cGMP in the cerebella of rats kept at 22° (Table 3). Of the three drugs only aminooxyacetic acid and hydrazine reduced the amount of cerebellar cGMP accumulated during 5 min of cold exposure, but none affected the cyclic nucleotide phosphodiesterase activity of cerebellar homogenates *in vitro*. The increment in cerebellar cGMP elicited by cold exposure was plotted against the logarithm of GABA concentrations found in the cerebella of rats receiving either NaCl or these drugs. The regression line indicates a highly significant correlation between the extent of increase in GABA concentration and inhibition of the increment in cerebellar cGMP elicited by cold (Fig. 3). Cold exposure per se changed neither the basal GABA concentration nor the drug-induced increase in cerebellar GABA. At various times after the injection of each drug the body temperatures of rats kept at 22° or exposed to 4° for 5 min remained unchanged.

Cerebellar cyclic GMP concentrations after administration of drug decreasing cerebellar GABA concentration. In rats receiving 300 or 600 mg/kg of isonicotinic acid hydrazide subcutaneously, the cerebellar GABA concentration decreased while the cerebellar

[2] B. Zivkovic, personal communication.

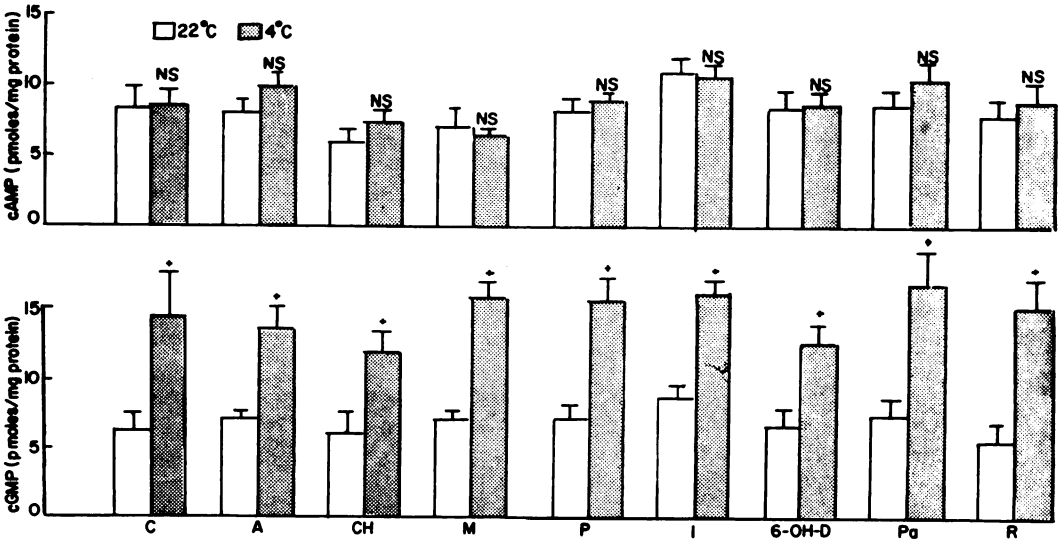

FIG. 2. *Effects of various drugs that affect cholinergic, adrenergic, and serotonergic transmissions on cerebellar cAMP and cGMP concentrations in rats kept at 22° or 4° for 5 min*

The dose and time elapsed between drug treatment and experiment are: C, control; A (atropine sulfate), 100 mg/kg intraperitoneally, 15 min; CH (chlorisondamine), 10 mg/kg intraperitoneally, 20 min; M (mecamylamine methyl ester), 2.5 mg/kg subcutaneously, 20 min; P (*p*-chlorophenylalanine methyl ester), 100 mg/kg intravenously, 2 days; I (indomethacin), 10 mg/kg intraperitoneally, 30 min; 6-OH-D (6-hydroxydopamine), 200, 50, and 50 μg injected intracisternally 11, 8, and 7 days before the experiment; Pa (pargyline), 75 mg/kg intraperitoneally, 2 hr; R (reserpine), 5 mg/kg intraperitoneally, 2 hr. Each value is the mean ± standard error (vertical bars) of at least six animals.

$p < 0.025$ when compared with the cGMP concentrations in rats receiving a similar injection but kept at 22°.

TABLE 3

Concentrations of cerebellar cAMP and cGMP in rats kept at 22° or 4° after injection of drugs that increase brain GABA concentrations

Aminooxyacetic acid, hydroxylamine, and hydrazine sulfate were injected 5 hr, 90 min, and 3 hr, respectively, prior to the experiments. Rats were kept at 4° for 5 min. Numbers of animals used are shown in parentheses.

Treatment	cGMP		cAMP	
	22°	4°	22°	4°
	pmoles/mg protein		*pmoles/mg protein*	
Saline	6.8 ± 1.4 (13)	24 ± 2.1 (11)	6.9 ± 0.60 (16)	7.1 ± 1.11 (10)
Aminooxyacetic acid				
25 mg/kg ip	7.6 ± 1.3 (13)	12 ± 2.7 (11)[a]	8.4 ± 0.59 (13)	8.4 ± 1.03 (11)
50 mg/kg ip	5.1 ± 1.90 (3)	6.1 ± 0.33 (9)[a]	8.1 ± 0.90 (5)	8.4 ± 1.16 (6)
Hydroxylamine, 75 mg/kg ip	6.3 ± 1.04 (4)	20 ± 1.25 (5)		
Hydrazine, 150 mg/kg sc	2.3 ± 0.54 (4)[a]	6.6 ± 0.50 (6)[a]	5.3 ± 0.71 (3)	6.7 ± 0.92 (5)

[a] $p < 0.025$ compared with saline-treated controls.

concentration of cGMP rose (Table 4). Both changes appeared to be dose-related. Furthermore, the cerebellar cGMP concentrations of rats receiving isonicotinic acid hydrazide were elevated to the same levels in the rats kept both at 4° and at 22° (Table 4). The rats treated with 600 mg/kg of isonicotinic acid hydrazide exhibited convulsions, which were not observed in rats receiving 300 mg/kg. When tested *in vitro* this drug failed to inhibit cyclic nucleotide phosphodiesterase activity of cerebellar homogenates.

DISCUSSION

When rats are killed by focusing a microwave beam on their skulls for 2 sec the brain enzymes involved in the metabolism of cAMP and cGMP are promptly inactivated. Hence microwave irradiation allows one to study how drugs or environmental conditions affect the steady-state concentrations of the two "second messengers" in various brain parts. By showing that the concentrations of these cyclic nucleotides change rapidly in the brain post mortem, previous studies (33–35) suggested that the participation of cAMP and cGMP in the response to environmental stimulus or in the mechanism of drug action could be evaluated only after instantaneous inactivation of tissue enzymes. Application of microwaves to the whole rat, as previously performed (36), was inappropriate because the enzyme was inactivated in about 30 sec. We compared our focused high-power microwave irradiation with the freeze-blowing technique (22) and found that the concentrations of cAMP and cGMP in the brains of rats killed with the two procedures are identical. However, the microwave application has the great advantage of allowing dissection of discrete brain parts while freeze-blowing does not.

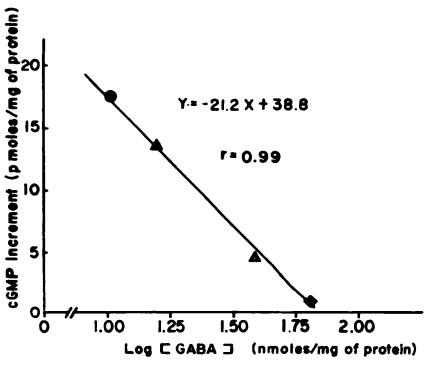

FIG. 3. *Correlation between cerebellar GABA concentrations and increments of cerebellar cGMP elicited by exposure to 4°*

●, control; ▲, hydroxylamine, 75 mg/kg intraperitoneally; △, hydrazine sulfate, 150 mg/kg subcutaneously; ◆, aminooxyacetic acid, 50 mg/kg intraperitoneally. $r = 0.99$; $p < 0.001$.

In the various brain areas we studied the cAMP concentrations were 6–10 times those of cGMP, with the exception of the cerebellum, which contained similar concentrations of cAMP and cGMP. Cerebellar cGMP concentrations increased 3-fold when the rats were exposed to 4° for 5 min, but declined and approached the basal value within 30 min thereafter. The cGMP concentrations also increased in hypothalamus

TABLE 4

Concentrations of cerebellar cGMP and GABA in rats kept at 22° or 4° after treatment with isonicotinic acid hydrazide

The rats received isonicotinic acid hydrazide 45 min prior to the experiment, and were exposed to cold for 5 min. The numbers of rats used are shown in parentheses.

Treatment	cGMP		GABA	
	22°	4°	22°	4°
	pmoles/mg protein		*nmoles/mg protein*	
Saline	6.9 ± 1.6 (11)	17 ± 1.8 (18)	9.2 ± 0.30 (5)	11 ± 0.7 (9)
Isonicotinic acid hydrazide				
300 mg/kg sc	25 ± 6.8 (5)[a]	27 ± 1.8 (6)[a]	7.8 ± 0.3 (5)[a]	7.2 ± 0.5 (6)[a]
600 mg/kg sc	55 ± 14 (4)[a]	51 ± 6.9 (5)[a]	6.7 ± 1.2 (4)[a]	4.9 ± 0.4 (5)[a]

[a] $p < 0.025$ compared to rats receiving saline.

and brain stem. In every brain part we studied except the pituitary, the cAMP concentration remained unaltered by 4° exposure. An 8-fold increase in cAMP concentration was observed in the pituitaries of rats exposed for 15 min to 4°. The observation that during cold exposure the cerebellar cAMP remained unchanged while cGMP increased, suggests that the increase in cGMP was unrelated to over-all inhibition of cyclic nucleotide phosphodiesterase. However, there are several forms of phosphodiesterase in brain (37–39), some of which appear to be cell-specific (neurons vs. glia) (40, 41). Cold exposure may inhibit the cyclic nucleotide phosphodiesterase in a specific neuronal population (e.g., Purkinje cells) which contains the cGMP pool.

Exposure to low temperatures has long been known to increase the firing rates of certain neurons in mammalian central nervous system. For example, cold exposure accelerates the brain turnover rate of norepinephrine (42, 43) but not that of dopamine (44). Since cAMP and cGMP concentrations in brain are regulated by putative brain neurotransmitters (9, 10, 14, 15, 45–47), we investigated whether the increase in cerebellar cGMP concentrations by cold is related to the activation of a specific synaptic mechanism.

In order to identify the molecular nature of such a mechanism we utilized the pharmacological blockade of postsynaptic muscarinic and nicotinic cholinergic receptors, the central chemical sympathectomy elicited by 6-hydroxydopamine injected intracisternally, the reduction of serotonergic activity by inhibiting tryptophan hydroxylase, the reduction of serotonin and catecholamines neurotransmitter stores by depletion with reserpine, and the increase of the availability of these amines by blocking their metabolism with pargyline. None of these procedures curtailed or facilitated the increase in cerebellar cGMP concentrations elicited by cold. Negative results were also obtained when we tried to influence the increase in cGMP by injecting indomethacin, a prostaglandin synthetase inhibitor (48).

Several cerebellar neurons release inhibitory neurotransmitters which hyperpolarize the membranes of the neurons they innervate (49). We are not aware of any study concerning the involvement of cyclic nucleotides in these inhibitory synaptic mechanisms. GABA has been repeatedly proposed as the neurotransmitter released by Purkinje and other cerebellar neurons (50–52). In accord with other reports (31, 32), we found that aminooxyacetic acid, hydroxylamine, and hydrazine elevate the cerebellar concentrations of GABA. Our experiments suggest that the increase of cerebellar concentrations of GABA reduce or abolish the increase in cerebellar cGMP elicited by cold exposure. The present report also excludes the possibility that steady-state concentrations of cAMP in cerebellum change when the concentrations of GABA are increased by drug injections. The degree of inhibition of cGMP increase correlates ($p < 0.001$) with the extent of the increase in cerebellar GABA concentration. However, our experiments do not exclude action of GABA in other brain areas to inhibit the increase of cerebellar cGMP elicited by cold, because in rats receiving the three drugs mentioned above GABA concentrations were increased throughout the brain.

We found that both convulsive and subconvulsive doses of isonicotinic acid hydrazide decreased the cerebellar content of GABA and simultaneously increased the concentration of cGMP in rat cerebellum. In rats receiving isonicotinic acid hydrazide the cerebellar cGMP concentration could not be augmented by exposure to 4° for 5 min. We also found that this drug does not inhibit cyclic nucleotide phosphodiesterase *in vitro*. Our preliminary experiments showed that a nonconvulsant dose (3 mg/kg intraperitoneally) of picrotoxin, a blocker of GABA receptors (51, 53, 54), failed to change the steady state of cerebellar GABA but increased the cerebellar concentrations of cGMP 9–10-fold. Thus, when GABA levels were depressed below normal, or when GABA receptors were blocked, the basal cGMP concentrations in cerebellum were elevated. Moreover, the mechanism that promotes the increase of cerebellar cGMP concentration during cold exposure seems inoperative when GABA availability is

reduced. Conversely when GABA levels are elevated, the mechanism that elevates cGMP in cerebella of rats exposed to cold is inhibited.

In conclusion, our experiments show that the increase of cGMP elicited by cold in rat cerebellum fails to involve axon terminals that release catecholamines, serotonin, and acetylcholine. We have provided evidence suggesting that GABA inhibits the increase of cerebellar cGMP elicited by cold exposure, but we have not yet determined the molecular nature of the transmitter that promotes the increase of cGMP concentration in rats exposed to 4°. This component of our model requires clarification if we are to understand the correlation between cGMP and GABA in the mode of action of GABA as a neurotransmitter in cerebellum.

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