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High pressure neurological syndrome: antagonistic effects of helium pressure and inhalation anesthetics on the dopamine-sensitive cyclic AMP response

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Fish, E., R. Shankaran, and J. C. Hsia. 1979. High pressure neurological syndrome: antagonistic effects of helium pressure and inhalation anesthetics on the dopamine-sensitive cyclic AMP response. Undersea Biomed. Res. 6(2): 189–196.—The antagonistic effects of helium pressure and certain inhalation anesthetics on the dopamine-sensitive cAMP response in the caudate nucleus of rat brain were examined. Helium pressure suppressed basal, dopamine-stimulated, and anesthetic-stimulated cAMP levels. These data indicate that by reversing the effects of pressure on dopaminergic transmission in the brain, inhalation anesthetics may account for the improvement of some clinical symptoms of high pressure neurological syndrome (HPNS).

high pressure neurological syndrome
anesthetic
dopamine-sensitive cAMP response

The incidence of high pressure neurological syndrome (HPNS) among divers breathing He-O_2 mixtures at 20–60 atmospheres' pressure is well documented (Hunter and Bennett 1974). This HPNS is typically characterized by tremor, lowered vigilance, and electroencephalographic anomalies. The onset and intensity of the symptoms depend on the rate of compression and ultimate depth reached (Fructus and Vigreux 1970; Hsia and Shankaran 1976). During the past decade there has been an increasing realization of the importance of dopamine as a neurotransmitter in the brain. In addition, there is evidence that abnormalities of dopaminergic transmission in the central nervous system may be of clinical importance. For example, degeneration of the dopaminergic pathway that normally connects the substantia nigra to the caudate nucleus and putamen is thought to be the primary feature in the etiology of Parkinson's disease (Hornykiewicz 1973; Vogt 1973). Though tremor associated with Parkinsonism may be associated with lesions of the dopaminergic pathway, tremor in HPNS is more complex, since pressure can affect more than one of the synaptic processes in the brain that control involuntary movements. Moreover, the tremor in Parkinsonism has a frequency of 3–6

Hz, is proximal, and is seen in relaxation. Tremor in HPNS has a frequency of 8–13 Hz, and it is seen at the extremities during intentional or postural movements.

At any given time within the caudate nucleus and putamen, there exists a delicate balance between excitory and inhibitory pathways, controlled in part by the interrelation between the acetylcholine and dopamine neurons, respectively (Stone, Taylor, and Bloom 1975). When, as a consequence of extrinsic factors, this balance is upset such that dopaminergic transmission is blocked, excitory impulses occur that may be expressed as an involuntary motor reflex or tremor. The modulation of dopamine receptors in the brain appears to be coupled to adenosine 3′5′-cyclic phosphate (cAMP) formation. Recently, attention has focused on the possible role of neurotransmitter-sensitive adenylate cyclases in mediating synaptic transmission between neurons (Iverson 1975). Thus, by closely monitoring dopamine-sensitive cAMP levels in the brain, the extent of dopaminergic transmission may be evaluated. The phenomena of antagonism of anesthesia by pressure (originally reported by Johnson, Brown, and Marsland 1942a,b) and the action of cAMP as a mediator in anesthetic processes (Seager 1975) have been well documented. Johnson and Miller (1970) suggest that if pressure can reverse anesthesia, the addition of anesthetics might reverse the effects of hydrostatic pressure and, hence, abolish HPNS.

This article reports the modulation of a dopamine-sensitive adenylate cyclase located in the caudate nucleus of a rat brain. In addition, we have attempted to investigate the effects of helium pressure on the dopamine-sensitive cAMP response elicited by anesthetics, in order to correlate anesthetic stimulation with pressure depression of cAMP levels. Preliminary results have been reported previously (Hsia and Shankaran 1976).

MATERIALS AND METHODS

Charcoal and 3′5′-cyclic AMP binding protein were purchased from BDH Chemicals Ltd., Poole, U.K. 3-Hydroxy tyramine HCl (3,4-dihydroxy phenylethylamine HCl or dopamine), adenosine 5′-triphosphate, adenosine 3′5′-cyclic phosphate (cAMP), bovine serum albumin (Fraction 5) and theophylline were supplied by Sigma Chemical Co., Missouri. Adenosine 3′5′-cyclic phosphate (8^3H) at a specific activity of 2 Ci/mmole was purchased from Schwarz/Mann. Fluothane (halothane) was obtained from Ayerst Laboratories, Montreal, Quebec; chloroform was supplied through Fisher Scientific Co., New Jersey; ethyl alcohol through Consolidated Alcohols Ltd., Toronto, Ontario; helium gas (99.9%) through Matheson of Canada, Ltd., Whitby, Ontario, and levophed bitartrate monohydrate (0.2% solution) (Noradrenalin) through Winthrop, Aurora, Ontario. 2-Mercaptoethanol was purchased from J. T. Baker Chemical Co., New Jersey. Aquasol was obtained from Massachusetts. Young adult male Wistar rats were purchased from High Oak Ranch Ltd., St. Agatha, Ontario.

Rat caudate nuclei homogenates were routinely prepared by killing the animal, exposing the brain, and dissecting out the circular caudates from the basal ganglia. Since the levels of cAMP alter with time after decapitation, all ensuing operations were performed rapidly and at 4°C. The caudates were initially washed with Ringer-bicarbonate solution, pH 7.4 (Biochemists Handbook 1961), then homogenized in 25 volumes of 2 mM trismaleate buffer, pH 7.4, containing 2 mM EGTA, by means of a Dounce Homogenizer. The supernatant, S, was obtained by centrifugation of the homogenate at 2000 rpm for 5 min in a Sorval GLC-1 centrifuge.

General principle of the method

Cold cAMP in the caudate nucleus is estimated by its ability to compete with externally supplied ^{3}H-cAMP for the binding sites on the cAMP binding protein. At the start of the experiment, ^{3}H-cAMP is bound to the cAMP-binding protein. The endogenous cAMP in the caudate nucleus then displaces the ^{3}H-cAMP from its binding sites. An estimate of the bound cAMP can then be made after the free cAMP has been removed by charcoal.

To examine the effects of pressure on basal and dopamine stimulated cAMP levels, the following procedure was carried out: 100 μliter aliquots of the supernatant, S, were added to a reaction mixture consisting of 80 mM Tris-maleate, 2 mM $MgSO_4$, 10 mM theophylline, 0.2 mM EGTA, and 0.5 mM ATP in a final volume of 500 μliter at pH 7.4; 0.3 mM dopamine was used to stimulate adenylate cyclase activity, where indicated. Simultaneously, a control experiment was set up for which varying amounts of noradrenaline were added to the basic reaction mixture, together with the supernatant, S. Essentially, these controls were set up to substantiate the claim that all the cAMP responses studied in this system are a consequence of modulation of a dopamine-sensitive adenylate cyclase. The ice-cold sample tubes were preincubated for 2 min at 30°C in a pressure chamber and flushed with helium. Pressure was applied at different levels for 3 min. To ensure that bubble formation did not occur on decompression, narrow tubes that were completely filled were used for the reaction. Moreover, due to the low solubility of helium gas in the reaction buffer, the resultant effects of pressure can be attributed more accurately to hydrostatic than hyperbaric pressure. Two minutes after decompression, the tubes were immersed in a boiling water bath to inactivate the adenylate cyclase. The tubes were then centrifuged at 3000 rpm for 10 min at 4°C in a Sorval GLC-1 centrifuge, and cAMP was estimated in 50-μliter aliquots of the supernatant, after Brown's Kinase Method (Brown, Albano, Ekins, Sgherzi, and Tampion 1971). Briefly, 50 μliter (^{3}H)-cAMP, together with 50 μliter standard/sample cAMP, 100 μliter diluted binding reagent, and 150 μliter 50 mM Tris-HCl buffer, pH 7.4, containing 8 mM theophylline and 6 mM 2-mercaptoethanol were mixed together, and the incubation tubes were subsequently held at 4°C for 90 min. After this incubation period, separation of the free from bound moieties was effected by the addition to the tubes of 100 μliter of a stirred suspension of 10% (w/v) charcoal and 2% (w/v) bovine serum albumin in the assay buffer. The tubes were then briefly agitated on a Thermolyne "Maxi-Mix" and centrifuged at 3000 rpm for 10 min in a Sorval GLC-1 centrifuge, at 4°C. Aliquots (100-μ liter) of the supernatants were subsequently transferred into liquid scintillation vials containing 10 ml of Aquasol, for the estimation of radioactivity. All samples were counted in a Nuclear Chicago Liquid Scintillation Spectrometer. A calibration curve was plotted for known cAMP standards, and the amount of cAMP in unknown samples was determined by reference to this plot.

To study the combined effects of selected anesthetics and pressure on cAMP levels in the caudate nucleus, an additional series of experiments was set up similar to those previously outlined: the anesthetics ethanol, ether, chloroform, and halothane at concentrations similar to those that produce general anesthesia (Seeman and Roth 1972) were added to separate basic reaction mixtures, together with the supernatant fraction, S, and the cAMP levels elicited in response to an increase in applied pressure were then examined as previously outlined.

RESULTS

Each point in the accompanying figures represents the mean value of duplicate measurements.

Pressure depression of dopamine-sensitive cAMP levels

The results shown in Fig. 1 indicate that up to a concentration of 1.0 mM, noradrenaline does not modulate the adenylate cyclase activity of the caudate nucleus homogenate. Taken in conjunction with the results demonstrated in Fig. 2, namely the stimulation of adenylate cyclase activity by 0.3 mM dopamine, it appears that these variations in cAMP levels are a consequence of modulation of a dopamine-sensitive adenylate cyclase. Furthermore, the results in Fig. 2 demonstrate that helium pressure from 1–170 ATA depressed both the basal and dopamine-stimulated cAMP response in the caudate nucleus in a linear fashion. At 100 ATA helium pressure, the basal cAMP level is depressed to approximately 65% and the dopamine-stimulated cAMP level to approximately 72% of that of control at 1 atmosphere.

Anesthetic stimulation and pressure depression of cAMP levels

Figure 3 summarizes the effects of selected anesthetics and helium pressure on the dopamine-sensitive cAMP levels in the caudate nucleus. At concentrations similar to those that produce general anesthesia, ethanol (A), ether (B), chloroform (C), and halothane (D) stimulate the dopamine-sensitive cAMP response. Our preliminary results suggest that the dose response curves for anesthetic stimulation of cAMP levels may be biphasic. Additional results in Fig. 3 demonstrate that a helium pressure of 58.5 ATA depresses anesthetic-stimulated cAMP responses.

DISCUSSION

Anesthesia is the result of some physical interaction of the anesthetic with certain membrane constituents (Schoenborn 1968; Paterson, Butler, Huang, Labelle, Smith, and Schneider 1972; Seeman and Roth 1972; Trudell, Hubell, and Cohen 1973a; Trudell, Hubell, and Cohen 1973b; Johnson, Miller, and Bangham 1973; Trudell, Payan, Chin, and Cohen 1975; Hsia and Boggs 1975) that causes expansion of the membrane. Several studies have demonstrated that pressure can reverse the anesthetic state by counteracting the expansion produced (Johnson and Flager 1950; Lever, Miller, Paton, and Smith 1971; Miller, Paton, Smith, and Smith 1973; Spyropoulos 1975; Roth 1975; Roth, Smith, and Paton 1976) and the lipid bilayer was proposed as the primary site of this action, via fluidization of the lipid fatty acid chains. Earlier work from this laboratory failed to support this fluidization hypothesis (Boggs, Yoong, and Hsia

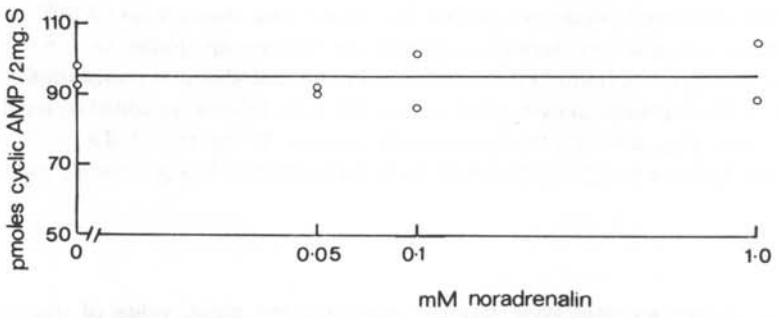

Fig. 1. Effect of noradrenalin on adenylate cyclase activity of the caudate nucleus homogenate, S.

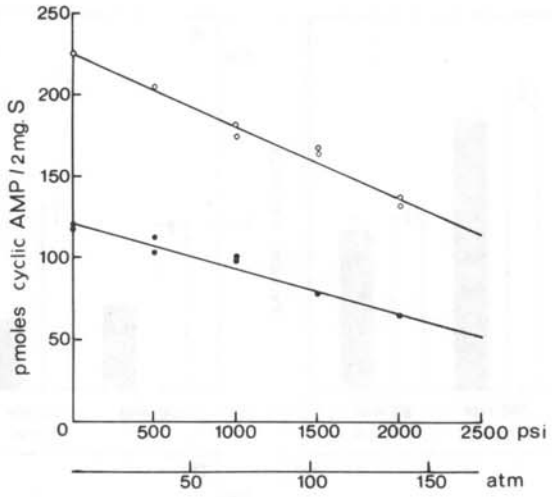

Fig. 2. Effect of helium pressure on the basal- (—●—●) and 0.3 mM dopamine-stimulated (—○—○—) cyclic AMP response in the caudate nucleus homogenate, S.

1976a; Boggs, Roth, Yoong, Wong, and Hsia 1976b) and a protein perturbation hypothesis was developed (Hsia and Boggs 1975) that suggests that the binding of anesthetic molecules to definite protein sites involved in synaptic transmission may modulate the function of associated sites. Differences in the affinities of anesthetics for the various binding sites associated with the nerve membranes, as well as differences in their lipid solubilities, may account for the variations in anesthetic potency. In connection with a dopamine receptor site, if the mode of action of an anesthetic is to bind to the postsynaptic receptor in such a way as to promote a conformation change, such a change may activate the adenyl cyclase and promote cAMP production. For the anesthetics used in this study this appeared to be the case. By stimulating the inhibitory dopaminergic pathway, the balance between inhibitory and excitory transmission alters, and motor function subsequently declines as anesthesia sets in. It must be expected that there are minor variations in the sequence in which some anesthetics modulate synapses. For example, if a particular anesthetic modulates excitory synapses at somewhat lower concentrations than inhibitory synapses, then there will be a range of anesthetic concentrations when the subject is hyperexcitable. To characterize anesthetic action better, dose response curves for different synapses should be determined. Thus, pressure may cause reversal or enhancement of anesthesia simply by counteracting an anesthetic-induced protein conformation change that results in membrane expansion or contraction.

The results of this study clearly indicate that helium pressure depresses the dopamine-sensitive adenylate cyclase activity such that cAMP production falls off in a linear fashion. Both basal and dopamine-stimulated cAMP levels are affected. Without knowing whether pressure has an effect on cholinergic neuron activity, it can be assumed that pressure has an effect on cholinergic neuron activity, it can be assumed that pressure suppression of dopaminergic activity alone could lead to involuntary tremor since it is known that inhibitory dopaminergic neurons in the central nervous system are balanced by the excitory cholinergic neurons. The transient, short-lived nature of the symptoms of HPNS suggests that a compensatory mechanism may exist which allows an individual to adjust to variations in pressure levels. Although this compensatory mechanism may operate to restore a balance between the

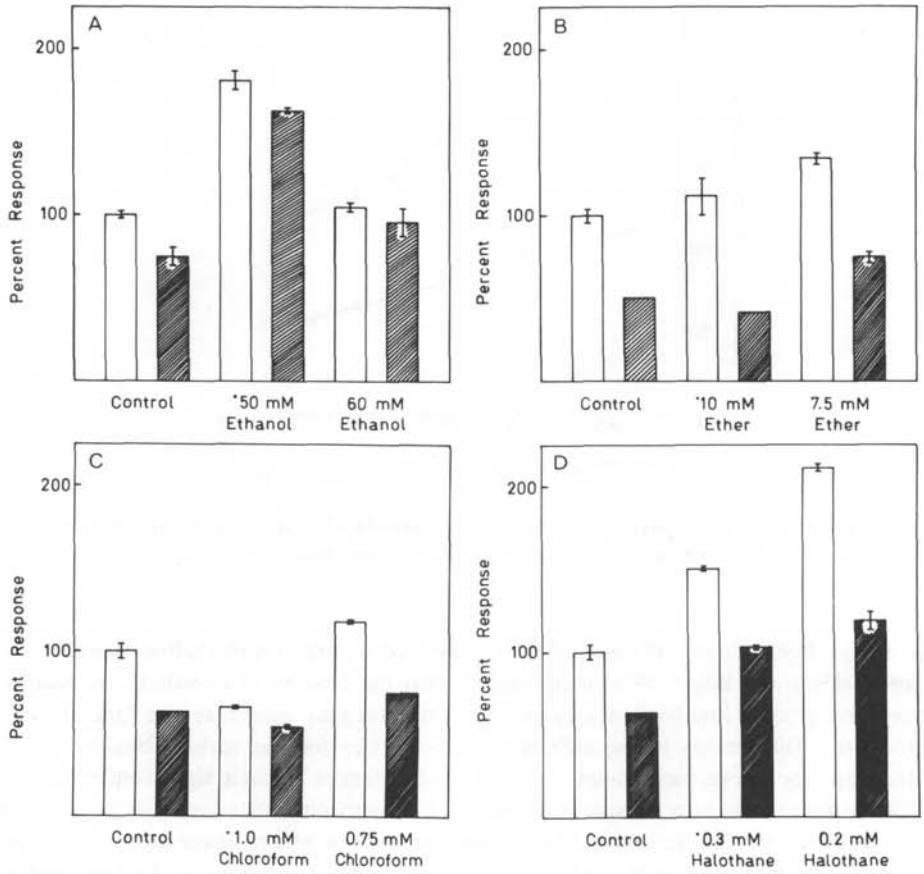

Fig. 3. Anesthetic stimulation and pressure depression of the dopamine-sensitive cAMP response in caudate nucleus. Caudate nuclei were removed and homogenates, S, prepared as described in MATERIALS AND METHODS. Inhalation anesthetics were included in the reaction mixture, where indicated. Each histogram represents mean of 4 experiments and each bar range of values for these experiments.*-Anesthetic concentration that produces general anesthetic (Seeman and Roth 1972). A clear bar corresponds to atmospheric pressure whereas a shaded bar corresponds to 58.5 atmospheres' pressure.

respective inhibitory and excitory pathways of the dopaminergic and cholinergic neurons, it would nevertheless be desirable to alleviate the symptoms of HPNS altogether. The preliminary results reported here imply that although pressure suppresses dopaminergic transmission in the caudate nucleus, certain anesthetics may reverse this suppression by stimulating dopaminergic activity. Anesthetic reversal of pressure effects of dopaminergic transmission may provide a mechanistic approach for the prevention of certain symptoms of clinical HPNS.

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Fish, E., R. Shankaran, and J. C. Hsia. 1979. Syndrome nerveux des hautes pressions: effets antagonistes de la pression d'helium et des anesthésiques d'inhalation sur la preponse sensible à la dopamine de cAMP. Undersea Biomed. Res. 6(2): 189–196.—Les effets opposés de la pression d'hélium et de certains anesthésiques d'inhalation sur la réponse sensible à la dopamine de cAMP été étudiées au niveau du noyau caudé chez le rat. La pression d'hélium a diminué les valeurs basales de cAMP, ainsi que celles observées après stimulation par dopamine et par anesthésiques. Ces résultats suggèrent que c'est en annulant les effets de la pression sur la transmission dopaminergique cérébrale que les anesthésiques d'inhalation pourraient être responsables de l'amélioration de certains symptômes cliniques du syndrome nerveux des hautes pressions.

anesthésiques
syndrome nerveux des hautes pressions
réponse sensible à la dopamine de cAMP

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