

AD-A214 336

DTIC FILE COPY

Undersea Biomedical Research, Vol. 16, No. 3, 1989

DTIC
ELECT
NOV 09 1989
S B U

Pressure-dependent changes in the release of GABA by cerebrocortical synaptosomes

S. C. GILMAN, J. S. COLTON, and A. J. DUTKA

Diving Medicine Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20814-5055

Gilman SC, Colton JS, Dutka AJ. Pressure-dependent changes in the release of GABA by cerebrocortical synaptosomes. Undersea Biomed Res 1989; 16(3):253-258. → Previous studies describe a depression in potassium-evoked, calcium-dependent transmitter release from guinea pig cerebrocortical synaptosomes in response to compression to 68 ATA with heliox. The study described in this paper was designed to evaluate whether a similar effect could be detected at lower pressures and to determine whether the effect had an abrupt onset with a fixed response or a progressive response varying over a range of pressures. A typical response to potassium-evoked depolarization was observed at all pressures studied. This consisted of an initial rising phase lasting 2 to 3 min followed by a falling phase. There was a pressure-dependent depression in the absolute amount of transmitter released as well as a depression in the rate of release in the first minute following stimulation. The mean depression in γ -aminobutyric acid release during the first minute was an average of 15% at 19 ATA, 28% at 37 ATA, 38% at 50 ATA, and 54% at 62 ATA when compared to a 1 ATA control. *synt. acc. dec.*

high pressure nervous syndrome;
 γ -aminobutyric acid;
 synaptosome;
 neurotransmitter;
 pressure; *in print* (K.T.)

→ Exposure of animals to ambient pressures of 19 ATA and greater results in a disturbance of neurologic function referred to as the high pressure nervous syndrome (HPNS). This disorder progresses through a number of symptomatic stages beginning with myoclonic jerks followed by alterations in electroencephalographic (EEG) activity, tremor, and finally convulsions (1-3). Earlier studies have indicated that these clinical manifestations can vary with the particular gas mixture breathed throughout compression, the rate of compression, and the species of animal being compressed. For example, mice subjected to pressurization with He-O₂ demonstrated tremor activity at 75-80 ATA (4), whereas rhesus monkeys convulsed at only 50 ATA (5). Spectral analysis of data from hindlimb movement in guinea pigs revealed that the onset of pressure-induced tremor developed around 19 ATA and increased in severity (i.e., the number of events and amplitude) with compression to 68 ATA (6). Overall, the most predominant clinical signs appear to be neurological.

DISTRIBUTION STATEMENT A

Approved for public release;
 Distribution Unlimited

253

89 11 09 043

At the cellular level, neurotransmitter release has been shown to be depressed in various synaptic preparations during exposure to elevated pressures (7–12). For example, intracellular electrophysiologic studies have indicated that He pressure as low as 10 ATA can produce a reduction in the amplitude of the glutamate-mediated excitatory end plate potential in lobster muscle (13). In this same pressure range, miniature end plate potential frequency in the frog neuromuscular junction was reported to be significantly depressed (7). These findings coupled with the data reported by other investigators from studies performed at pressures greater than 10 ATA suggest that the predominant effect of pressure may be on the presynaptic terminal (8, 10–12, 14). It is not surprising then that elevated pressure (68 ATA) could significantly alter calcium-dependent, potassium-evoked [^3H]- γ -aminobutyric acid (GABA) release from isolated presynaptic terminals (synaptosomes) (15, 16).

The current study was designed to evaluate whether release could be altered (i.e., depressed) at pressures less than 68 ATA. Therefore, in this study, release of [^3H]GABA by cerebrocortical synaptosomes was monitored during exposure to a preselected range of helium pressures.

MATERIALS AND METHODS

Animals

Adult male Hartley guinea pigs (300–400 g) were housed at the Laboratory Animal Facility, Naval Medical Research Institute, under a 12-h dark cycle with food and water provided ad libitum.

Materials

Radioactive γ -[2,3- ^3H]aminobutyric acid (specific activity 25–49 Ci/nmol) was purchased from New England Nuclear Corporation (Boston, MA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Method

Each guinea pig was killed by rapid cervical dislocation. The cortex was dissected and synaptosomes prepared by density gradient centrifugation as previously described (15). The method used for loading radioactive GABA into synaptosomes was the same as the procedure described in an earlier publication (17). The final synaptosome pellet was suspended in 10 vol of a buffer medium consisting of a high sodium, calcium-free solution. The composition was (in mM): NaCl, 145; KCl, 5; MgCl_2 , 3.7; KH_2PO_4 , 1.2; HEPES, 20; glucose, 10; amino-oxyacetic acid (AOA) 0.1; GABA, 0.01; pH 7.4. Radiolabeled GABA was added and the mixture incubated at 37°C for 5 min to allow uptake by the tissue.

Following uptake, compression studies were conducted using a previously described procedure (16). A brief description follows. A 1.0-ml aliquot of the radioisotope-loaded synaptosome suspension containing 1–1.5 mg protein was placed on a filter unit positioned on a multiperforated support of a perfusion chamber. The perfusion chamber was then connected to a peristaltic pump. After the filter was washed with

50 ml of buffer medium containing 1.2 mM Ca^{2+} to remove unbound radioactivity, the filter unit and superfusion apparatus were placed in a hyperbaric chamber. A line from the filter unit was directed over a scintillation vial inserted in a compartment of a turning carousel. This carousel was positioned on a Plexiglas stand, to which was mounted two stepping pump systems. Pump 1 was programmed to deliver 1 ml of buffer medium containing 1.2 mM Ca^{2+} ; pump 2 to deliver 5 ml of 55 mM K^{+} containing "efflux" medium. The composition was (in mM): NaCl 95; KCl, 55; CaCl_2 , 1.2; MgCl_2 , 2.5; KH_2PO_4 , 1.2; HEPES, 20; glucose, 10; AOA, 0.1; pH 7.4. Buffer medium was delivered as a bolus over the filter. The hyperbaric chamber was sealed, flushed with pure oxygen for 1 min, pressurized to 1.3 ATA with pure oxygen, and then compressed to a final pressure of 19, 37, 50, or 62 ATA with helium at a compression rate of 3 atm/min. During compression, the filter was washed continuously with buffer medium containing 1.2 mM Ca^{2+} . Upon reaching the predetermined pressure, release studies were initiated by delivering 1 ml of buffer medium from pump 1 over the filter unit and two "wash" samples collected directly into scintillation vials. After 2 min at pressure, 5 ml of a depolarizing, 55 mM K^{+} "efflux" buffer from pump 2 was delivered over the filter unit. Fractions containing 500 μl each of perfusate were collected directly into scintillation vials every minute.

During the release studies the temperature of the buffer media and perfusion chamber was constantly monitored by microthermistors and maintained at $37^\circ \pm 1^\circ\text{C}$. In all experiments aliquots from the same synaptosome preparation were used for obtaining the 1 ATA and increased pressure GABA-release values. Order of experimental exposures was randomized to eliminate any time effect. The control preparations were treated identically to the experimental groups, except that pressure exposures were sham (1 ATA). Fractional [^3H]GABA efflux was expressed as percentage of total radioactivity, where total radioactivity was the sum of all fractional filtrate radioactivity and radioactivity remaining on the filter. Statistical significance was determined by analysis of variance (ANOVA). A $P < 0.05$ was considered significant.

RESULTS

Application of incubation media containing 1.2 mM calcium and sufficient KCl (55 mM) to produce depolarization of the synaptosomes, induced an increase in release of [^3H]GABA from the 1 ATA control group and the 4 groups exposed to elevated pressure. All 5 groups maintained a similar release profile, that is, an initial rising phase that lasted 2 to 3 min, followed by a falling phase during the remainder of the observation period (Fig. 1). However, there was a pressure-dependent depression in the amount and rate of the potassium-evoked GABA release from the synaptosomes during minute 1 of transmitter efflux. Mean GABA release during this first minute was decreased by an average of 15% in the 19 ATA group, 28% in the 37 ATA group, 38% in the 50 ATA group, and 54% in the 62 ATA group when compared to the 1 ATA value. Although there was an increase in the rate of release observed in minute 2, the amount of stimulated GABA efflux at increased pressure remained depressed from the 1 ATA control level (Fig. 1).

The effect of pressure on [^3H]GABA release was assessed using a 5×7 [pressure and/or dosage (1, 19, 37, 50, or 62 ATA) by time (1, 2, 3, 4, 5, 6, or 7 min)] ANOVA based:

☒
☐
☐

ty Codes

A-1 | 20

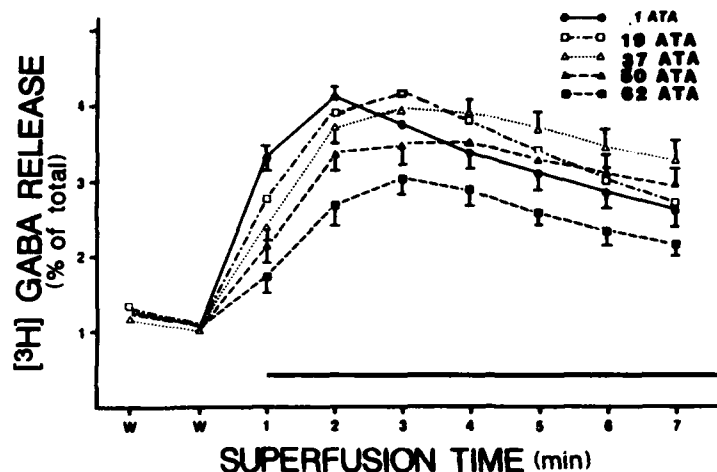


Fig 1. Effect of increased He-O₂ pressure on the depolarization-induced release of [³H]GABA from cerebrocortical synaptosomes. Each point represents the mean $\pm$ SEM of 12 experiments for each pressure condition.

on repeated measurements. Results revealed a significant pressure effect and time-pressure interaction. As seen in Fig. 1, mean level of GABA release at 1 min was reduced from the 1 ATA control for 37, 50, and 62 ATA. At 2 min the reduction remained only in the 50 and 62 ATA groups.

In addition to evaluating the effect of pressure on the amount of [³H]GABA released during a given time period, the rate of transmitter efflux was also analyzed. The rate of [³H]GABA release is presented for the first 4 min after stimulation with perfusion media containing 55 mM K⁺ (Table 1). In the first 1 min of observation there was a pressure-dependent decrease in the rate of transmitter efflux, with GABA release slowing from 2.18% per min at 1 ATA to 0.70% per min at 62 ATA (Table 1). In minute 2 of observation, the rates of [³H]GABA release at all elevated pressures were greater than control (1 ATA) values. In addition, there was a slight increase in rate at 50 and 62 ATA from preceding values. In minute 3 the rate of transmitter release at all pressures above 1 ATA dropped below their rates in the first 2 min, and the amount released also peaked at this time (Table 1). In minute 4, rate of release declined for all pressures observed with the exception of 50 ATA (the slight positive release rate at 50 ATA is small and not considered significant) (Table 1).

DISCUSSION

Elevated hydrostatic or helium pressure has been shown to depress the amplitude of intracellularly recorded excitatory and inhibitory junctional potentials from lobster (8–10, 13), reduce peak inward currents recorded in ganglion cells from *Aplysia* or *Helix* (11, 12), and slow the rate of rise of excitatory postsynaptic potentials in voltage-clamped neurons of the squid giant synapse (18). In this study an alteration in the rate and magnitude of transmitter release was observed in guinea pig cerebrocortical synaptosomes in response to helium pressure in the range of 19–62 ATA.

It was within minute 1 of synaptosomal transmitter release that the most profound effects of elevated helium pressure were observed. In this time frame, the depression in the rate and amount of [³H]GABA efflux was progressive and was most predominant at 62 ATA.

TABLE 1
EFFECT OF PRESSURE ON RATE OF STIMULATED [³H]GABA RELEASE
BY SYNAPTOSOMES^a

Pressure ATA	Rate of [³ H]GABA Release, %/min			
	Minute 1	Minute 2	Minute 3	Minute 4
1	2.18	0.80	-0.32	-0.40
19	1.57	1.21	0.16	-0.16
37	1.32	1.32	0.20	-0.03
50	1.09	1.21	0.10	0.13
62	0.70	0.93	0.33	-0.13

^aValues given represent the percent of [³H]GABA released per min and were obtained by determining the difference in percent release at 1-min intervals. For minute 1 values, percent released was subtracted from the percent released in the previous unstimulated wash. Data at each pressure represent the mean of 12 observations at each time period.

The phenomenon of a "progressive" decrease in response has been observed in other systems. For example, Parmentier et al. (12) reported an exponential fall in synaptic currents recorded from *Aplysia*, in the pressure range of 1–100 ATA. Campenot (8) reported a progressive decrease in the excitatory junctional potential amplitude in lobster, Ashford et al. (7) demonstrated an exponential decrease in the frequency of the miniature end plate current at the frog neuromuscular junction, and Grossman and Kendig (10) have recently shown that excitatory junctional potential amplitude in two different species of lobster (*Homarus* and *Panulirus*) are progressively depressed with increasing pressure. The mechanism of the pressure-induced depression is not completely understood at this time, but possible causes may include the following: decreased calcium ion influx through channels in the terminal membrane, interruption of the intraterminal calcium-dependent cascade necessary for transmitter release (e.g., calcium-calmodulin interactions or calcium-dependent protein phosphorylation), vesicle-terminal membrane fusion, level of terminal transmembrane potential, or the rate of transmitter synthesis or replenishment (i.e., re-uptake). Pressure seems to have multiple effects on the terminal, inasmuch as high pressure has been shown to depress calcium ion flux into the terminal (17) and to interfere with membrane fusion (19).

If this depression in transmitter release from potassium-stimulated synaptosomes is representative of release from nerve terminals in intact brain, then depression in the initial rate and amount of transmitter release could significantly alter the timing of the inhibitory and excitatory transmitter interactions. This discrepancy in neuronal synchronization could in turn result in dramatic psychologic and sensory-motor dysfunction such as those that comprise the HPNS.

The authors give special thanks to Elizabeth Baker and Remy Cunamay for valuable technical assistance. The authors also thank Susan Cecire and Janet Gaines for their assistance in the preparation of this manuscript.

This study was supported by the Naval Medical Research and Development Command Research Task MR041.01.01.1124. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department of the naval service at large.

The experiments reported herein were conducted according to the principles set forth in the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

This manuscript was prepared by United States Government employees as part of their official duties and, therefore, cannot be copyrighted and may be copied without restriction.—*Manuscript received December 1988; accepted March 1989.*

REFERENCES

1. Bennett PB, McLeod M. Probing the limits of human deep diving. *Philos Trans R Soc Lond B Biol Sci* 1984; 304:105–117.
2. Lever MJ, Miller KW, Paton WDM, Street WB, Smith EB. Effects of hydrostatic pressure on mammals. In: Lambertsen CJ, ed. *Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology*. New York: Academic Press, 1971:101–108.
3. Naquet R, Lemaire C, Rostain J-C. High pressure nervous syndrome: psychometric and clinico-electrophysiological correlations. *Philos Trans R Soc Lond B Biol Sci* 1984; 304:95–102.
4. MacInnis JB, Dickson JG, Lambertsen CJ. Exposure of mice to a helium-oxygen mixture at pressures of 122 atmospheres. *J Appl Physiol* 1967; 22:694–698.
5. Brauer RW, Johnsen DO, Pessotti RL, Redding RW. Effects of hydrogen and helium at pressures to 67 atmospheres on mice and monkeys. *Fed Proc* 1966; 25:202.
6. Gilman SC, Hallenbeck JM, Ackerman MJ, Bradley ME. Antagonism by taurine of pressure-induced tremor in the guinea pig. *J Appl Physiol* 1984; 57:1409–1411.
7. Ashford MJL, MacDonald AG, Wann KT. The effects of hydrostatic pressure on the spontaneous release of transmitter at the frog neuromuscular junction. *J Physiol (Lond)* 1982; 333:531–543.
8. Campenot RB. The effects of high hydrostatic pressure on transmission at the crustacean neuromuscular junction. *Comp Biochem Physiol* 1975; 52:133–140.
9. Colton CA, Colton JS. The action of oxygen and oxygen at high pressure on inhibitory transmission. *Brain Res* 1986; 364:151–158.
10. Grossman Y, Kendig JJ. Synaptic integrative properties at hyperbaric pressure. *J Neurophysiol* 1988; 60:1497–1512.
11. Harper AA, MacDonald AG, Wann KT. The action of high hydrostatic pressure on the membrane currents of helix neurons. *J Physiol* 1981; 311:325–339.
12. Parmentier JL, Shrivastav BB, Bennett PB. Hydrostatic pressure reduces synaptic efficiency by inhibiting transmitter release. *Undersea Biomed Res* 1981; 8:175–183.
13. Colton JS, Colton CA. An electrophysiological analysis of oxygen and pressure on synaptic transmission. *Brain Res* 1982; 251:221–227.
14. Wann KT, MacDonald AG. Actions and interactions of high pressure and general anaesthetics. *Prog Neurobiol* 1988; 30:271–307.
15. Gilman SC, Colton JS, Hallenbeck JM. Effect of pressure on [³H]GABA release by synaptosomes isolated from the cerebral cortex. *J Appl Physiol* 1986; 61:2067–2073.
16. Gilman SC, Colton JS, Dutka AJ. Effect of pressure on the release of radioactive glycine and GABA from spinal cord synaptosomes. *J Neurochem* 1987; 49:1571–1578.
17. Gilman SC, Kumaroo KK, Hallenbeck JM. Effect of pressure on the uptake and release of calcium by guinea pig brain synaptosomes. *J Appl Physiol* 1986; 60:1446–1450.
18. Henderson JV, Lowenhaupt MT, Gilbert DL. Helium pressure alteration of function in squid giant synapse. *Undersea Biomed Res* 1977; 4:19–26.
19. Heinemann SH, Conti F, Stuhmer W, Neher E. Effect of hydrostatic pressure on membrane processes. *J Gen Physiol* 1987; 90:765–778.

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited	
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 89-66		7a. NAME OF MONITORING ORGANIZATION Naval Medical Command	
6a. NAME OF PERFORMING ORGANIZATION Naval Medical Research		6b. OFFICE SYMBOL (If applicable)	
6c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055		7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, D.C. 20372-5120	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION Naval Medical Research and Development Command		8b. OFFICE SYMBOL (If applicable)	
8c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
10. SOURCE OF FUNDING NUMBERS		11. TITLE (Include Security Classification) Pressure-dependent changes in the release of GABA by cerebrocortical synaptosomes	
PROGRAM ELEMENT NO. 61153N		PROJECT NO. MR04101.01	
TASK NO. 1005		WORK UNIT ACCESSION NO. DN277001	
12. PERSONAL AUTHOR(S) Gilman SC, Colton JS, Dutka AJ			
13a. TYPE OF REPORT journal article		13b. TIME COVERED FROM TO	
14. DATE OF REPORT (Year, Month, Day) 1989		15. PAGE COUNT 6	
16. SUPPLEMENTARY NOTATION Reprinted from: Undersea Biomedical Research 1989 Vol.16(3) pp. 253-258			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS			
21. ABSTRACT SECURITY CLASSIFICATION Unclassified			
22a. NAME OF RESPONSIBLE INDIVIDUAL Phyllis Blum, Information Services Division		22b. TELEPHONE (Include Area Code) 202-295-2188	
		22c. OFFICE SYMBOL ISD/ADMIN/NMRI	