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Effects of halothane and hydrostatic pressure on sodium channels in squid axon

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Parmentier J, Shrivastav B, Bennett PB. Effects of halothane and hydrostatic pressure on sodium channels in squid axon. Undersea Biomed Res 1985; 12(3):259–268.—The effects of halothane and hydrostatic compression have been studied on the voltage-dependent sodium conductances in the squid giant axon. Compression to 150 atmospheres reduced the time course of both the activation and inactivation phases of the sodium current, reduced the amplitude of the peak inward current, and shifted the sodium reversal potential in a slightly hyperpolarizing direction. Halothane also depressed the sodium current amplitude and shifted the reversal potential but did not alter the membrane kinetics. Subsequent compression to 100 ATA did not restore the inward current flux to its control value, nor reverse the shift in the sodium reversal potential. We conclude that pressure and halothane do not act antagonistically at common sites in this preparation.

pressure reversal
anesthesia
voltage clamp
ionic conductances

Raised hydrostatic pressure causes hyperexcitability in animals and man, which has come to be known as the high pressure nervous syndrome (HPNS). In animals the syndrome is characterized by an increasingly severe pattern of motor outputs, ranging from tremors through myoclonic jerking to seizures and eventual death if pressure is not relieved (1). In man, HPNS produces complex neurological and performance decrements, hyperreflexia, and motor incoordination (2). By adding a narcotic gas such as nitrogen to deep diving breathing mixtures it is possible to alleviate some of the effects of pressure-induced hyperexcitability (3). This procedure may be of practical value in the development of deep human diving programs (4). Conversely, pressure can counteract the narcotizing effects of barbiturates, steroid anesthetics, inert gases, and volatile general anesthetic agents in intact animals (5–8).

Pressure reversal of anesthesia has attracted considerable attention because of the constraints it may place on various hypotheses of anesthetic action. The "critical volume hypothesis" proposed that general anesthesia or pressure-induced convulsions occur when a hydrophobic region is expanded or compressed, respectively (3).

This hypothesis was attractive because it did not require that specific sites be identified. The simplest conditions under which the critical volume hypothesis could be met would be if different anesthetics operated by a fundamentally similar mechanism at a specific cellular location or by disturbing a specific function. Pressure reversal might then involve the opposition of this disturbance by pressure, regardless of how many sites or what specific membrane functions are affected.

Since HPNS can be alleviated by compounds that do not produce anesthesia, and since pressure reversal or anesthesia only occurs within a narrow pressure range, the site (or sites) responsible for anesthesia and HPNS are thought to be physically different (9). However, the site (or sites) at which pressure reverses the effect of narcosis, or where narcotics oppose pressure excitability, have not yet been identified (10). The simplest hypothesis is that the antagonism is by direct interaction at a common site. This "common site hypothesis" provides a rationale for the safe use of a narcotic gas to alleviate HPNS symptoms in deep diving because it predicts that all pressure-induced effects will be antagonized by the narcotic additive. A voltage-dependent influx of sodium constitutes the primary biophysical event responsible for excitability in nerve. To test the common site hypothesis we have measured the amplitude and time course of sodium currents in isolated squid giant axons following halothane exposure and hydraulic compression. We report here that compression to 100 atmospheres (ATA) does not reverse the effects of halothane on ionic conductances in this preparation.

MATERIALS AND METHODS

Single squid axons from *Loligo peali,* collected by the Marine Biological Laboratory in Woods Hole, MA, were isolated, cleaned, cannulated, and suspended vertically in a pool of sea water. A "piggy back" axial wire electrode, consisting of an external platinized platinum wire insulated except at the tip and a quartz capillary filled with 0.6 *M* KCl and shunted by an internal platinum wire, was mounted on a micromanipulator above the cannulated axon. The electrode was lowered through the cannula and into the axon until its holder made contact with the holder of the cannula. The two were then screwed together to make one unit. Appropriate connections were made to pass a stimulating current into the axon and to record an action potential. Only those axons giving an action potential larger than 100 mV were included in the experiment.

The axon, with the electrode system inside, was transferred to a cuvette 15 mm diameter by 50 mm high which was filled with artificial sea water (400 m*M* Na^{+}, 10 m*M* K^{+}, 10 m*M* Ca^{++}, 50 m*M* Mg^{++}, 530 m*M* Cl^{-}, 5 m*M* HEPES buffer, adjusted to pH 8.0). The inside of the cuvette was fitted with 3 rings of 4 mm-wide platinized platinum foil. The center ring served as a current measuring electrode while the two outer rings serve as grounded guard electrodes. When in position, the tip of the potential-measuring electrode reached the midhorizontal plane of the current measuring electrode ring. A reference electrode of Ag-AgCl was hung vertically parallel to the nerve fiber. The cuvette was than placed in the unassembled Wilson High Pressure Chamber (11) and the appropriate electrical connections made to external equipment. Sodium currents were studied in isolation after blocking potassium currents by the addition of 3,4 diamino pyridine directly to the cuvette by

syringe to a final concentration of 50 μM. No drugs were added from outside the Wilson chamber once it was sealed.

The voltage clamp technique for these experiments was conventional and has been described previously (12). The standard holding potential was -80 mV. Data were collected at 1, 100, and 150 ATA with current families taken over a range of -60 to $+80$ mV in steps of 5 or 10 mV. The currents were monitored on a Nicolet digital oscilloscope and analyzed by a Wang 2200 calculator. Membrane deterioration was monitored by frequent leakage current measurements at -100, -120, and -140 mV. Axons in poor condition were discarded. Appropriate values for leakage currents were subtracted from the data.

Once control clamp currents were recorded, a mixture of halothane in sea water was added to the cuvette such that the final concentration in the cuvette was 10% of a halothane-saturated sea water solution. The Wilson chamber was filled with mineral oil and smoothly compressed by commercially available helium from a 3000 psi gas cylinder. The compression rate of 6 to 8 atmospheres/min was monitored by a transducer in the pressure chamber wall. For pressure reversal experiments the Wilson Chamber was filled with mineral oil that had been preequilibrated with a specified amount of halothane according to the oil-water partition coefficient for halothane in saline. The halothane-ladened oil supplied 10% of a saturated solution of halothane to the oil/seawater interface and thus served as an infinite reservoir of anesthetic to the sea water in the cuvette.

A cooling system consisting of ⅛″ stainless steel tubing was coiled tightly around the outside of the aluminum vial and projected through the base of the pressure vessel to connect with a Lauda circulating bath which operated at ambient pressure. During the course of a 3-mo. period the various experiments described here were conducted at temperatures between 6.5 and 10.5°C. However, for any given experiment, the cooling arrangement maintained temperature in the cuvette to within 0.1°C. Once test pressure was reached the system was allowed to stabilize for 15 min before collecting data.

Data from a total of 14 axons are reported here. Six control axons were successively compressed to and recovered from 150 ATA. Eight axons were exposed to halothane and then compressed, of which six were compressed to 100 ATA and two compressed to 150 ATA. Data are given as mean values (± SEM).

The peak transient (sodium) current in the squid giant axon is described by the Hodgkin-Huxley equation (13):

$$I_{Na} = g_{Na}(V - V_{Na})$$
$$g_{Na} = \bar{g}_{Na}\,\mathrm{m}^3\mathrm{h}$$

I_{Na} and g_{Na} are the peak transient current and conductance, respectively, for the membrane potential V. The maximum value of g_{Na} is $\bar{g}_{Na}$. The g_{Na} ($t = 0$) value is the zero time intercept of the slope of the falling phase of the logarithmic plot of each current curve against time. The mean of the steady state values of g_{Na} ($t = 0$) for each axon was taken as $\bar{g}_{Na}$. Although the small number of experiments precludes proper statistical analysis, the data are presented as means with a standard deviation.

RESULTS

Pressure experiments

Hydrostatic pressure up to 150 ATA had no effect on the resting potential of the membrane. Figure 1A shows superimposed sodium currents in a representative squid

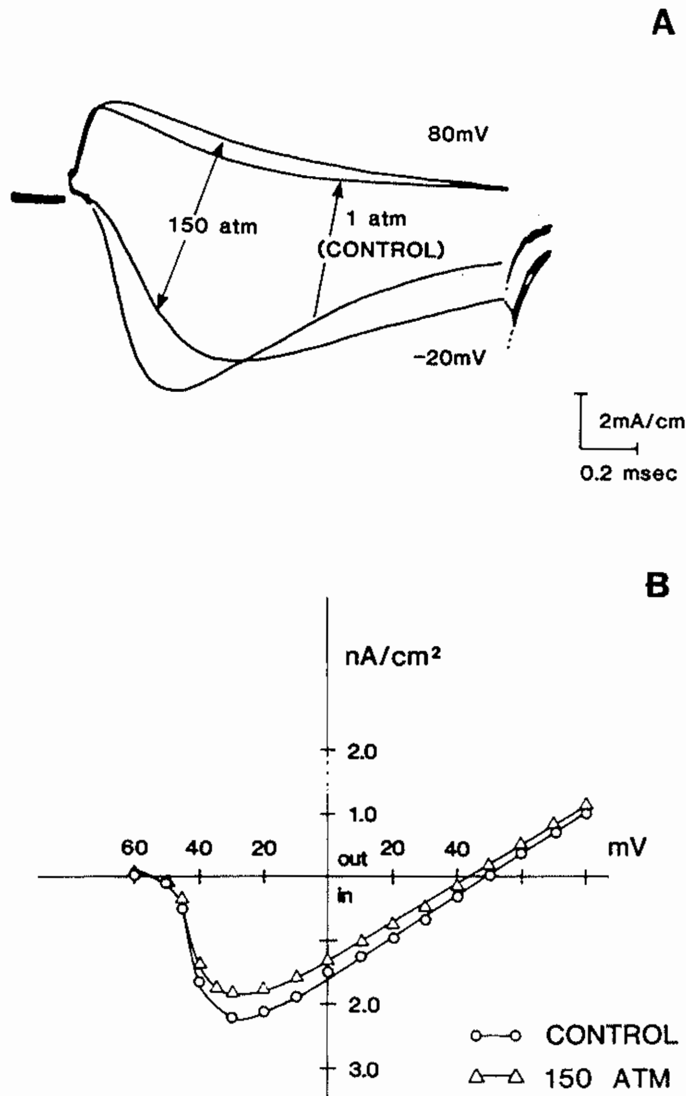

Fig. 1. *A:* Effect of pressure on sodium currents which result from voltage steps from −80 mV to −20 and +80 mV. Increased pressure slows down both the rising and falling phases of the currents. *B:* Current-voltage plot of peak inward transient currents of a representative axon compressed to 150 ATA. Primary effects of pressure are to decrease peak inward current and to shift the reversal potential toward more negative values. This axon was not exposed to DAP.

axon at 1 ATA and 150 ATA. The traces resulted from voltage steps to −20 and +80 mV from a holding potential of −80 mV. Pressure decreases the peak inward current and slows both the activation and inactivation processes of the inward current. Both processes recover with decompression. The amplitudes of the peak transient inward currents at each test membrane potential are plotted in Fig. 1*B* for an axon compressed to 150 ATA. The currents are decreased at all potentials higher than threshold and the sodium reversal potential V_{Na}) is shifted slightly toward more negative membrane

potentials. The average values of V_{Na} for six axons at 1 (control), 100, and 150 ATA was 50.2 (± 4.3), 42.6 (± 6.1), and 41.3 (± 5.1) mV, respectively.

The mean value of $\bar{g}_{Na}$, calculated as the mean of steady state values of conductance of each axon at $t = 0$, was reduced at pressure for all six axons. The value of $\bar{g}_{Na}$ decreased from 141.6 mmho/cm 2 (± 15.6) in control to 117.1 mmho/cm 2 (± 8.1) at 150 ATA. These values amount to a 17.3% reduction in mean conductance relative to control values. Upon decompression the maximum $\bar{g}_{Na}$ recovered to 144.0 (± 12.6) mmho/cm 2, which is very near to the control value. Figure 2 presents the sodium conductance, $\bar{g}_{Na}$ ($t = 0$), normalized to $\bar{g}_{Na}$, as a function of membrane potential. Increased pressure shifted the conductance curve toward more positive membrane potentials. When compared to control values, the estimated mean shift along the membrane axis at the point where $\bar{g}_{Na}$ $(t = 0)/\bar{g}_{Na} = 0.125$ is 7 mV at 150 ATA. During these experiments the mean leakage conductance (g_L) was 0.29 (± 0.10), and 0.48 (± 0.16) mmho/cm 2, respectively, for ambient pressures of 1 and 150 ATA. The small size of these changes (less than 2% of the shift of the active conductances) indicates that the axons were undamaged by the compression.

Halothane and pressure reversal experiments

Figure 3*A* shows sodium current traces generated by step depolarizations to −20 and +80 mV in an axon exposed to a 10% saturated solution of halothane in sea

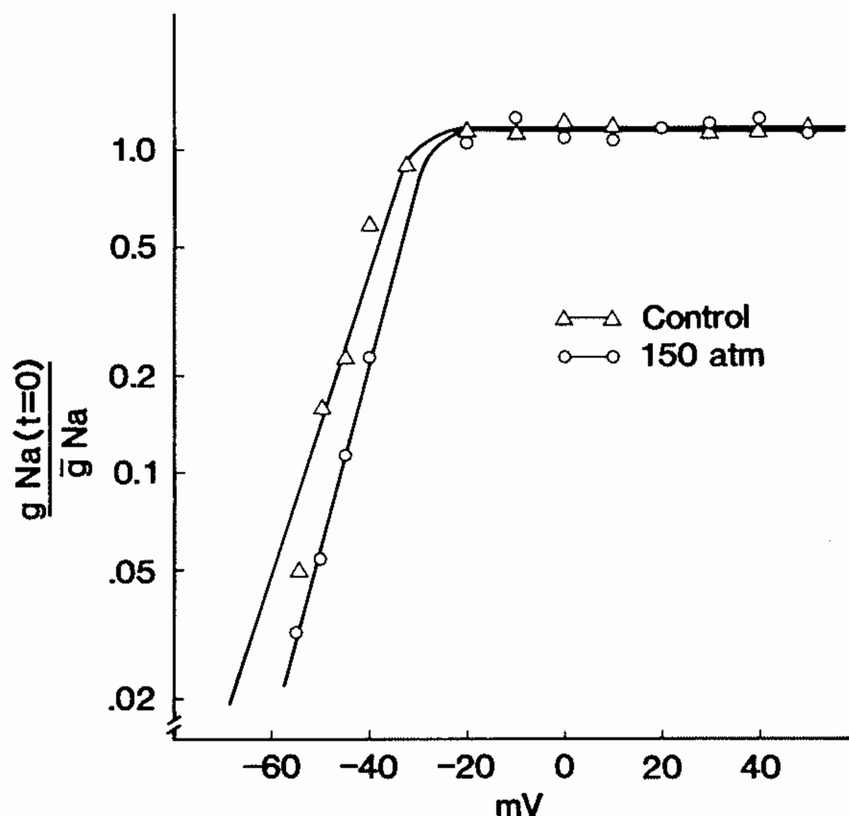

Fig. 2. Effect of pressure on the steady-state conductance, g_{Na} ($t = 0$) (normalized to $\bar{g}_{Na}$). Increased pressure shifted these curves toward more positive membrane values. Each point is a mean of six axons.

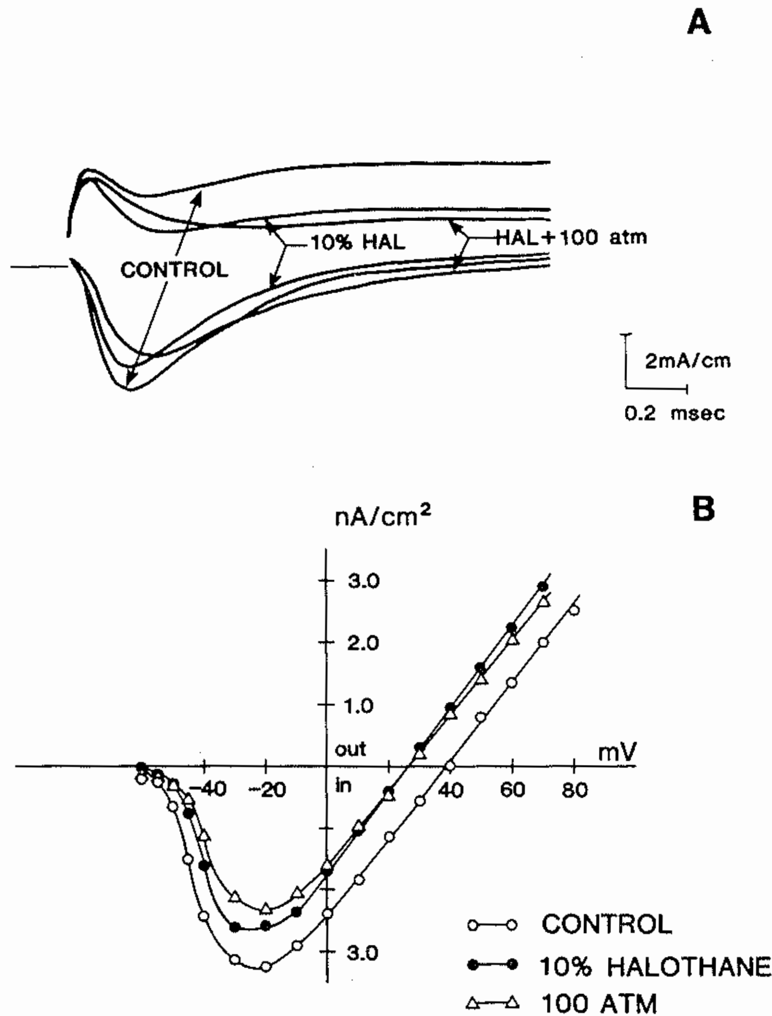

Fig. 3. *A:* Effects of both halothane and hydrostatic pressure on representative inward sodium currents following step depolarizations to −20 mV and +80 mV from a holding potential of −80 mV. The pressure was applied after the axon had been exposed for 20 min to 10% of a saturated solution of halothane. *B:* Representative plot of the current-voltage relationship of an axon exposed to halothane and subsequently compressed hydraulically to 100 ATA.

water for 20 min and then compressed to 100 ATA. The currents are representative of the seven axons that were studied in this manner. Halothane reduced the sodium current amplitude but had little effect on the time course of current flow. The time-to-peak of the currents remain substantially unchanged. Subsequent hydraulic compression clearly delayed the time course of the membrane currents in a manner similar to that seen in Fig. 1*A*, where no anesthetic was present. Instead of reversing the effect of the halothane, pressure superimposed its own slowing effect on the kinetics of the narcotized axon.

Figure 3*B* shows the current-voltage relationship of the same axon presented in Fig. 3*A*. Halothane suppressed peak transient currents at all membrane potentials more positive than −45 mV, did not alter the slope of the I-V curve. In seven axons

the reversal potential was shifted by an average of 11.8 mV in the hyperpolarizing direction and the inward current reduced by 28.6 (± 4.9) mA/cm 2. Compression to 100 ATA failed to restore either the current amplitude or the reversal potential of these axons to control levels. Rather, it caused a further decrease of inward current in five of the axons and, moreover, lowered V_{Na} in three others still further. Both of the responses were further enhanced in the two narcotized axons that were compressed to 150 ATA. In none of the eight axons studied for pressure reversal was there a significant change in leakage conductance following either the halothane exposure or the subsequent compression. The mean control value of g_L, for voltage steps to 100, 120, and 140 mV, was 0.29 mmho/cm 2. After 30 min in halothane-sea water at 1 ATA, and again at 100 ATA, the mean values were 0.25 and 0.32 mmho/cm 2, respectively. This low value of the leakage, or nonactive conductance, indicates that the charge separation characteristics of the axon remained undamaged during the anesthetic exposures.

DISCUSSION

Cellular excitability results from the activation of voltage-dependent conductances in nerve and muscle membranes, and it is these conductances that are ultimately affected by anesthetics and by pressure. During the past decade several laboratories, including our own, have studied the interactions of anesthetics and high hydrostatic pressure on excised neural preparations. Measurements of ionic currents under high pressure have been made in neurons from squid (14–18), snail (19–22), node of Ranvier (23–25), and arthropod preparations (26,27). These studies have consistently shown that pressures above 50 ATA slow the rate of rise and fall of voltage-dependent currents and reduce the amount of peak inward current that flows following its activation. Kendig has reported a depolarizing inward current in certain nodes of Ranvier (23), and a tendency for crustacean axons to fire spontaneously under pressure (27). Under appropriate conditions these characteristics could lead to spontaneous neural excitation. However, the predominant single finding in excised tissue studies to date has been that pressure causes decrements in membrane excitability (10). Our experiments agree with this finding and confirm that the primary effect of pressure is to decrease the time course of membrane current kinetics.

The reduction in peak inward current amplitude, and the 17.3% decrease in maximum conductance which was noted at both 100 and 150 ATA, may result from either of two causes. The first is that pressure decreased the ion-carrying capacity of each of the sodium channels. The second is that the total number of sodium channels available to the membrane was decreased, or were not in a position to conduct current. Our present data cannot distinguish between these two mechanisms. Conti et al. (14) noted a small shift in the I-V relationship at threshold voltages and a slight shift in the voltage at which the peak I_{Na} appeared. These did not appear in our data. Kendig has shown that compression shifts steady-state inactivation in the node of Ranvier in a hyperpolarizing direction, indicating a differential pressure sensitivity of the mechanisms controlling development of the active and inactive states. In earlier studies neither we (17) nor Conti et al. (14) saw a shift in the h^{inf} curve for squid axon.

The primary effect of the halothane was to decrease the peak inward current. The anesthetic had little or no effect on the kinetics of current flow. The effect of halothane

is similar to that reported for 6 ATA nitrogen at the node of Ranvier (25). In our experiments halothane also caused a shift of the sodium reversal potential in the hyperpolarizing direction, as had been reported earlier for other anesthetics (12). Both of these effects would lead to a reduction in or loss of action potential generation in the unclamped axon. Our data indicate that pressure and halothane alter different properties of the ion channels and that compression of a narcotized axon does not directly reverse the effects of the anesthetic.

Kendig has shown that pressure can restore the amplitude of halothane-depressed compound action potentials in the rat superior cervical ganglion if the amplitude has not been reduced by more than 10% (28). She has postulated that pressure reversal in the node is due to an activation or an increase in the number of resting sodium channels which can then function in place of those channels that were blocked by the anesthetic. This interpretation of pressure reversal does not employ the common site hypothesis, but instead supports the concept that pressure and anesthetics produce their observed antagonism by compensatory effects of different aspects of the membrane excitability processes. This hypothesis is amenable to testing through appropriate pharmacological manipulation of ion channel blockers. Our data are consistent with such a concept.

The observation that pressure and halothane are not mutually antagonistic at the sodium channel in nerve does not disprove the common site hypothesis of pressure reversal. It is possible that some as yet unexamined membrane function may be responsible for narcosis and may be directly antagonized by hydrostatic pressure. However, the mounting evidence that pressure affects membrane kinetics while anesthetics primarily alter peak conductance strongly suggests that a hypothesis of direct antagonism is not tenable. Most of the arguments that support the experimental use of a narcotic gas to alleviate symptoms of high pressure tremor and convulsions in mammals are predicated on the concept that any potential consequences of HPNS are controllable by appropriate titration with the anesthetic. However, without the common site hypothesis researchers must seriously consider the possibility that the narcotic gas may be missing or masking certain potentially deleterious manifestations of HPNS.

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Parmentier J, Shrivastav B, Bennett PB. Effets de l'halothane et de la pression hydrostatique sur les canaux de sodium dans l'axone du calmar. Undersea Biomed Res 1985; 12(3): 259–268.—Les effets de l'halothane et de la compression hydrostatique ont été étudiés sur la tension des conductances dépendantes du sodium dans l'axone géant du calmar. La compression à 150 atmosphères reduisit le décours de toutes les deux phases d'activation et d'inactivation du courant sodique, diminua l'amplitude et le pic du courant entrant, et déplaca le potentiel d'inversion du sodium dans une direction légèrement hyperpolarisante. L'halothane déprima également l'amplitude du courant sodique et déplaça le potentiel d'inversion mais ne modifia pas les propriétés cinétiques membranaires. La décompression subséquente à 100 ATA ne rétablit pas le flux de courant entrant à sa valeur témoin, et ne renversa pas le déplacement du potentiel d'inversion du sodium. Il est conclu que la pression et l'halothane n'agissent pas de façon antagonistes à des sites communs dans cette préparation.

pression d'inversion — voltage en clampe
anesthésie — conductances ioniques

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