

THE INFLUENCE OF HIGH HYDROSTATIC PRESSURE ON COCAINE AND VERATRINE ACTION IN A VERTEBRATE NERVE

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ABSTRACT

The application of high hydrostatic pressure to toad sciatic nerve causes a gain in sodium and a loss of potassium which are not affected by cocaine. However, cocaine action is enhanced by high pressure when counteracting veratrine depolarization and when blocking the action potential. Various effects of elevated pressure on the afterpotentials are presented and the role of ions in these processes is discussed.

INTRODUCTION

The antagonistic effect of cocaine upon the action of the veratrine alkaloids on cellular electrochemical activity has been well described. Shanes (5-8) has shown that the addition of 2 mg. per cent (1:50,000) veratrine to frog-Ringer's solution depolarizes frog sciatic nerve with an accompanying exchange of external Na^+ for internal K^+ at a rate of approximately $1.5 \mu\text{M}/\text{gm. hr.}$ The depolarization and ionic exchange are prevented by the addition of 0.1 per cent cocaine to the medium. More recent observations and the theoretical implications are summarized in a recent review (9).

This report describes observations on the effects of elevated hydrostatic pressures on the action of veratrine and cocaine on the electrical and ionic properties of the sciatic nerve of the toad (*Bufo marinus*).

Methods

Ion Distribution.—In the experiments on the ionic alterations with high hydrostatic pressure, upwards of six nerves in their experimental solutions (not oxygenated to minimize oxygen "poisoning" (2, 3)), were placed in the innermost of three coaxial glass tubes which had a total volume of 200 ml. Each tube was capped by a rubber dam to permit the transmission of the applied pressure. The outer two annular spaces were filled with Ringer's solution. This arrangement limits the temperature change, which Spyropoulos (12) has noted occurs with compression of the mineral oil used as

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the hydraulic fluid, to about 1°C. This arrangement also prevents contamination of the pressure bomb apparatus by partitioning of the drugs between the Ringer's solution and the oil. A sheet of saran wrap covered the outermost tube to prevent deterioration of the outermost rubber dam by the oil. Paired, freshly dissected, desheathed nerves were used. One nerve from each animal was placed in the experimental group and its mate in the control group. Pressure was applied for a period of 3 hours. Following the release of pressure, middle segments of the nerves were dried by blotting, weighed, and leached overnight in distilled water (8); the resulting solutions were analyzed for Na^+ and K^+ with the Beckmann D.U. flame spectrophotometer. The Ringer's solution contained 107 mM/liter NaCl, 1.8 mM/liter KCl, 1.1 mM/liter CaCl_2 , and an all sodium Sørensen buffer which added only 1.7 mM/liter sodium.

Action Potential.—The cell employed for measurement of the action potential consisted of two lucite tubes, the smaller of which (3 mm. inside diameter) was supported within the larger one (25 mm. inside diameter) by a special silicone resin. This resin also acted as an insulator for ten silver electrodes, 5 mm. apart, which extended from the inside wall of the inner tube to the outside wall of the outer tube; such an arrangement permitted flexibility in the choice of the stimulating, pickup, and ground electrodes. The open ends of the tubes were closed with removable latex caps. With the nerve in place, 0.5 ml. of solution was required in the inner tube; the annular space was filled with water. Prior to exposure to the experimental conditions, the distal end of the nerve trunk was injured by crushing it with a glass rod to improve monophasicity of the action potential.

Action potentials were measured with a DuMont type 304 cathode ray oscillograph used in conjunction with a Tektronix square pulse generator and a Tektronix pre-amplifier (type 122). The response was flat from 0.2 cycle per second to 40 kc per second. Repetition rates were limited to one shock every 2 seconds; preliminary observations showed that five shocks per second gave essentially the same results. Each of the results reported was observed in at least two preparations.

Pressure Apparatus.—Pressures up to 10,000 lb./in.² were obtained with a hand-operated hydraulic pump. The pressure bomb was of the "outside-cap compression closure" type made by the American Instrument Co. with an inside diameter of 2½ inches and a depth of 10 inches. The bomb was fitted with a special head containing ten electrical leads to permit measurement of action potentials of the nerves while under compression. Mineral oil was used as the compression fluid.

All experiments were carried out at a room temperature of about 25°C.

RESULTS

Ion Distribution.—Table I summarizes the results obtained with different hydrostatic pressures under a variety of experimental conditions. It is apparent in series I A that high pressure causes a loss of potassium and a gain of sodium. Series I B shows that cocaine has no significant effect on this loss of potassium induced by high pressure. In series I C it can be seen that the exchange of Na^+ for K^+ brought on by veratrine at atmospheric pressure also appears to be unaffected by pressure. Cocaine is able to reduce the ionic leak of the veratrinized nerve at pressures as high as 10,000 lb./in.²; indeed, the potas-

sium data indicate that the anesthetic counteracts veratrine more effectively at the elevated pressures. The greater variability of the sodium data does not permit a verification of this effect from the behavior of sodium.

Table II gives the results of more detailed experiments that compare the loss of potassium due to pressure alone (series II A) and that due to the combined effects of veratrine and pressure (series II B). To improve the significance of the

TABLE I*
Comparison of the Na⁺ and K⁺ Contents of Paired Toad Sciatic Nerves during 3 Hours' Exposure to Various Experimental (X) and Control (C) Conditions

	Conditions	Pressure, lb./in. ²	No. of pairs	Na, $\mu\text{M}/\text{gm. wet weight}$			K, $\mu\text{M}/\text{gm. wet weight}$		
				X	C	X - C	X	C	X - C
A	X = Ringer's at indicated pressure	2,500	6	71.2 \pm 2.2	67.7 \pm 2.1	+3.5 \pm 1.0	38.1 \pm 1.3	41.5 \pm 1.7	-3.4 \pm 0.6
	C = Ringer's, 14.7 lb./in. ²	10,000	6	71.7 \pm 2.3	67.9 \pm 1.7	+3.8 \pm 1.2	29.4 \pm 0.8	36.6 \pm 0.4	-7.2 \pm 0.7
B	X = 0.1 per cent cocaine in Ringer's + pressure	2,500	12	66.2 \pm 1.5	68.3 \pm 1.3	-2.1 \pm 0.6	31.5 \pm 1.3	30.9 \pm 2.0	+0.6 \pm 0.3
		5,000	6	71.4 \pm 0.8	72.1 \pm 2.0	-0.7 \pm 1.7	29.8 \pm 1.4	29.2 \pm 1.0	+0.6 \pm 0.6
	C = Ringer's + pressure	7,500	6	69.4 \pm 1.6	68.9 \pm 1.4	+0.5 \pm 0.2	30.2 \pm 2.4	30.3 \pm 2.1	-0.1 \pm 0.6
C	X = 1:75,000 veratrine + 0.1 per cent cocaine in Ringer's + pressure	14.7	5	70.1 \pm 1.2	77.3 \pm 0.5	-7.2 \pm 0.9	27.9 \pm 1.0	24.5 \pm 1.0	+3.4 \pm 1.2
		2,500	6	76.8 \pm 1.9	81.3 \pm 3.6	-4.5 \pm 3.0	28.1 \pm 1.7	21.0 \pm 1.7	+7.1 \pm 0.5
		7,500	3	75.8 \pm 0.5	89.2 \pm 3.6	-13.4 \pm 3.9	33.2 \pm 1.5	23.2 \pm 1.6	+10.0 \pm 1.0
	C = 1:75,000 veratrine in Ringer's + pressure	10,000	6	75.4 \pm 1.7	79.8 \pm 1.4	-4.4 \pm 2.3	28.8 \pm 1.8	21.3 \pm 2.1	+7.5 \pm 1.6

* All values are given with the appropriate standard error, those in the X - C columns being based on paired nerves.

data, these observations were made in K⁺-free Ringer's solution, with each nerve in its own vial, so that it was possible to measure the increments of potassium in the media as well as the losses from the nerves. It can be seen that potassium leak due only to the application of pressure is superimposed upon that due to veratrine.

Action Potential.—In Ringer's solution, with no drug present, elevation of the pressure to 5000 lb./in.² causes in some cases a slight increase in the spike amplitude with no change in duration, and at other times no change in the spike amplitude but an increase of about 20 per cent in the duration.

When 0.1 per cent cocaine is present in the Ringer's solution the spike ampli-

tude, in addition to undergoing a slow decline at atmospheric pressure, decreases reversibly with a rapidly applied increase in pressure. Complete block occurs at 7500 lb./in.². In cocaine the spike duration and threshold are also increased reversibly with pressure. The threshold increases particularly rapidly

TABLE II*
Comparison of the Effects of Veratrine and Pressure on the 3 Hour Loss of Potassium from Toad Sciatic Nerve in K⁺-Free Ringer's Solution

	Conditions	No. of pairs	Nerve	Medium
			K, $\mu\text{M/gm. wet weight}$	K, $\mu\text{M/gm. wet weight}$
A	X = Ringer's solution at 2500 lb./in. ²	3	22.3 \pm 1.33	4.09 \pm 0.97
	C = Ringer's solution at 14.7 lb./in. ²	3	24.6 \pm 1.3	1.83 \pm 0.22
	X - C		-2.3 \pm 0.7	+2.26 \pm 1.10
B	X = 1:75,000 veratrine + Ringer's at 2500 lb./in. ²	3	18.1 \pm 1.3	7.84 \pm 0.85
	C = 1:75,000 veratrine + Ringer's at 14.7 lb./in. ²	3	21.8 \pm 1.6	4.33 \pm 0.55
	X - C		-3.7 \pm 0.4	+3.51 \pm 0.58

* All values are given with the appropriate standard errors. The X - C values are based on paired nerves.

with pressure, as may be seen in Table III. The data approximately follow the relation:

$$\frac{t_p}{t_0} = 2 \left(\frac{P}{1000} \right)$$

in which t_p and t_0 are the thresholds obtained at an applied pressure P in pounds per square inch and at atmospheric pressure, respectively. It was observed that when cocaine was removed by soaking the nerve in Ringer's overnight, its effects at atmospheric and high pressure were eliminated.

The alkaloids of veratrine increase the negative after-potential of nerve (9). Though the relation of pressure to the after-potential has been studied (1), it seemed worth while to repeat these experiments in view of experimental evidence that the earlier results may have been obscured by temperature changes (12). The nerve was soaked for 30 minutes in 1.3 mg. per cent veratrine-Ringer's, then washed 30 minutes in Ringer's prior to insertion into the pressure bomb.

This procedure gives a stable preparation with well maintained after-potentials (7). The following events were observed for the negative after-potential as pressure was increased to 7500 lb./in.²: (a) Both the duration and amplitude decreased only slightly with increase in pressure; (b) spontaneous firing at the peak of the after-potential appeared at 3000 lb./in.² and disappeared when the pressure was lowered or raised further to 7500 lb./in.²; (c) when pressure was gradually released from 7500 lb./in.², an apparent rise phase to the negative after-potential developed at 4000 lb./in.²; this remained upon return to 14.7 lb./in.². During observations (a) to (c) no change in the spike amplitude was observed during elevated pressures.

TABLE III

The Effect of Elevated Pressure on the Threshold of Excitability, t , in Arbitrary Units, of Toad Sciatic Nerve in Ringer's Solution with 0.1 Per Cent Cocaine Added

Pressure	t	t_p/t_0^*	$\left(\frac{P}{1000}\right)_2$
lb./in. ²	units		
14.7	1.3	1	1
2000	4.1	3.2	4
3000	10	8	8
4000	25	19	16
5000	45	34	32

* t_p and t_0 are the thresholds obtained at an applied pressure, P , and atmospheric pressure, respectively.

It was also noted that when 5000 lb./in.² was applied to a freshly prepared, veratrinized nerve for 15 minutes, a positive potential (*i.e.* a transitory hyperpolarization following the spike) appeared. With subsequent reduction of pressure, this hyperpolarization became larger. Upon return to 7500 lb./in.², the positive potential disappeared, but reappeared slowly with time. These effects could be observed repeatedly in the same preparation.

DISCUSSION

High pressure resembles either anoxia or veratrine in causing an increased sodium and potassium exchange (6). While cocaine will reduce ionic interchange brought on by anoxia and veratrine (6), it appeared to have no significant effect on the ionic losses induced by high pressure.

For the resting nerve trunk, elevated pressure up to 10,000 lb./in.² exerts little or no effect when veratrine and cocaine are present by themselves. This is in keeping with observations on nerve (10) and skeletal muscle (4) that the injury potential is unaffected by high pressure. However, the effectiveness of

cocaine in reducing the ionic exchange brought on by veratrine is augmented by high pressure.

When no drug is present, the action potential remains relatively unaffected by high pressures. An increase in duration of the spikes of individual fibers, such as described by Tasaki and Spyropoulos (13), can bring about a better summation and hence a greater amplitude as well as a prolongation of the multifibered spike. These effects were actually found.

The most striking aspect of high pressure was its ability to increase the effectiveness of cocaine in reducing the height of the action potential and in raising the threshold of excitability. This differs from Spyropoulos' finding with ethanol; "narcosis" of squid axons by this alcohol is reported to be removed at 7500 lb./in.² (11).

The development of a rise phase to the negative after-potential under elevated pressure reported here probably reflects a small positive potential (5) that anticipates the large positive potential which subsequently appears under prolonged high pressure. An increase in positive potential also occurs with elevation of the Ca⁺⁺ level in the medium (5).

From these results it is clear that high pressure will influence cocaine activity under those conditions at which the anesthetic is normally effective at atmospheric pressure. For example, cocaine will reduce ionic exchange in veratrinized nerves, and block the action potential (9) at atmospheric pressure; under elevated pressure the ability of cocaine to antagonize veratrine and to block the action potential is enhanced. There is overwhelming evidence that production of the action potential depends on an increase in ion permeability and considerable evidence is available that "stabilizers" such as cocaine interfere with this increase in permeability (9). Inasmuch as veratrine also increases cellular permeability and this is reduced by cocaine (8), it follows pressure influences only that aspect of cocaine action that interferes with increases in ion permeability. Thus, it appears that the permeability changes themselves, which are relatively insensitive to hydrostatic pressure, are not the result of large volume changes, whereas the action of cocaine may involve appreciable volume changes.

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