AJNR

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R N Bryan and D Johnston

AJNR Am J Neuroradiol 1982, 3 (2) 117-120
http://www.ajnr.org/content/3/2/117

Epileptogenic Effects of Radiographic Contrast Agents: Experimental Study

R. Nick Bryan[1]
Daniel Johnston[2]

Electrical activity in cells directly exposed to water-soluble radiographic contrast agents was investigated by intracellular recordings from neurons of the abdominal ganglia of *Aplysia*. Measurements of membrane resting potential, membrane conductance, synaptic and action potential configuration, and spontaneous electrical activity were performed. Test solutions included sodium diatrizoate, meglumine iothalamate, metrizamide, and control solutions. Solutions (100 and 200 mOsm) of these agents did not significantly alter resting membrane potential, membrane conductance, action potential frequency or configuration, or excitatory postsynaptic potentials. Inhibitory postsynaptic potentials were suppressed by contrast agents. These results suggest that contrast agents affect at least some neurons by disinhibition.

Imaging of the cerebrospinal fluid spaces and adjacent brain surfaces requires contrast enhancement, particularly in the posterior fossa and spine. Recent developments in water-soluble radiographic contrast media have permitted their substitution for oily contrast material and air in many cases. Unfortunately, even the newest water-soluble contrast materials have significant side effects, some apparently related to direct neurotoxicity. Older water-soluble contrast agents have long been known to be extremely toxic when they are directly applied to the central nervous system (CNS). In fact, with older agents, the CNS was the critical organ in pharmacologic terms [1, 2].

This direct neurotoxicity is primarily reflected by abnormal electrical activity such as electroencephalographic (EEG) spikes and grossly evidenced as motor seizures. The newer, nonionic, water-soluble contrast agents have markedly diminished epileptogenic effects, but motor seizures remain an occasional clinical problem [3]. Mental dysfunction reflected by short-term memory loss, confusion, and disorientation is a more frequent problem with nonionic agents [4].

The cellular basis for the clinical symptoms and the altered electrical activity of the brain is essentially unknown. Numerous studies show alteration of EEG patterns with spike activity [5–7], but more specific neurophysiologic changes have seldom been studied.

Spinal root electrical activity is depressed by hypertonic solutions of ionic contrast agents [8], but not by isotonic concentrations [9]. Spinal long-tract electrical activity is generally depressed or unaltered [10–12]. The spinal monosynaptic reflexes are little affected by contrast agents, but some polysynaptic activity is markedly increased [11]. Certain spinal inhibitory pathways have been shown to be depressed [9]. The latter study [9] suggests a more specific central synaptic effect of contrast agents which produce epileptogenic activity by disinhibition. While these studies are more specific than EEG records, no single unit or intracellular studies on neurons exposed to radiographic contrast agents have previously been performed. To further elucidate the specific neurotoxic effects of contrast agents, this study involves intracellular electrical recordings during exposure of neurons to various contrast agents.

Received July 10, 1981; accepted after revision September 24, 1981.

This work was supported by U.S. Public Health Service grant NS14907.

Presented at the annual meeting of the American Society of Neuroradiology, Los Angeles, March 1980.

[1] Department of Radiology, Baylor College of Medicine, Houston, TX 77030. Address reprint requests to R. N. Bryan, M.S. NB703, Methodist Hospital, 6565 Bertner, Houston, TX 77030.

[2] Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

AJNR 3:117–120, March/April 1982
0195-6108/82/0302-0117 $00.00

Materials and Methods

The abdominal ganglia of *Aplysia californica*, a marine mollusc, were removed and placed in a circulating seawater bath to which various solutions could be added. Selected neurons [13] were then impaled with potassium-acetate-filled glass microelectrodes with tips of less than 1 μm. In the giant cell R2, two electrodes were placed intracellularly. In other cells, only one electrode was used. Intracellular electrical activity was monitored by a high impedence preamplifier and fed to a differential amplifier for oscilloscope or strip-chart display (fig. 1). Intracellular recordings allowed measurements of membrane resting potential and membrane resistance, spontaneous spike frequency and configuration, and electrical characteristics of spontaneous postsynaptic potentials.

In some preparations, connectives (nerves) to the ganglia were stimulated with a constant current suction electrode to produce orthodromic postsynaptic potentials and action potentials. This allowed measurement of thresholds and waveforms of the synaptic and action potentials.

The test solutions used to bathe the ganglia included seawater (1,000 mOsm), and solutions of seawater plus 10% by volume 100 mOsm NaCl; 1,500 mOsm NaCl; 1,500 mOsm sucrose; 100, 200, and 300 mOsm sodium diatrizoate; meglumine iothalamate; and metrizamide. All solutions had a final total osmolarity of 950–1,150 mOsm. The contrast solutions then had iodine levels of 6–18 mg/ml. The ganglia were exposed to the test solutions for 20–60 min and then rinsed with fresh seawater for a comparable period of time.

Results

Membrane resting potential was evaluated in 20 cells, including 11 silent and nine spontaneously active cells. No change in membrane resting potential was produced by any solution (fig. 2). This was further substantiated by the double electrode–current injection method of evaluating membrane resistance, performed on five cells, all R2. No change in membrane resistance was obtained by comparison of the current-voltage curves of membrane resistance, and no displacement of the curves indicative of membrane resting potential change was noted (fig. 3).

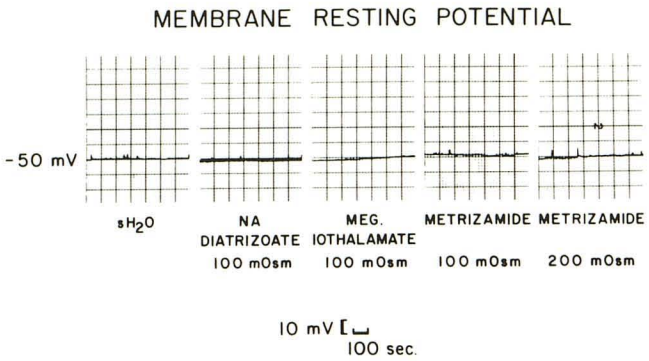


Fig. 2.—Representative recordings from cell R2 of abdominal ganglion of *Aplysia*. Each recording was made 30 min after application of test solution at stated concentrations. No change in membrane resting potential.

Spike configuration, both spontaneous and induced by orthodromic stimulation, was evaluated in 12 cells. No alterations in the spikes were seen as determined by measurements of spike amplitude, duration, and overall waveform (fig. 4).

Spontaneous spike activity was evaluated in 11 silent cells, mainly R2. No alterations (i.e., increase in spontaneous firing rate) were noted. Spontaneous firing pattern was also evaluated in nine intrinsically spiking cells. These cells included L11, R15, and those from the L2–6 complex. In these cells, no consistent change was seen. In seven experiments, the spike activity was unchanged; in five experiments, there was a slight increase in spike rate (4/sec to 6/sec); and in five experiments, a mild decrease in spike rate was noted (3.5/sec to 1.5/sec). There was no consistent relation between the minor alterations in spike activity and the various test solutions, including contrast agents.

Excitatory postsynaptic potentials (EPSP) were evaluated in six cells, all R2 by stimulation of the left connective. In three experiments with metrizamide, no change was seen, while in one there was a slight (2–3 mV) depression in amplitude. In six experiments with meglumine iothalamate, no change was seen; in one experiment, there was slight diminution in amplitude of the EPSP (2 mV); in two other experiments, there was a slight increase in EPSP amplitude (3 mV). In no experiment was there any alteration in duration of the postsynaptic potential (fig. 5). In no experiment was the EPSP increased to spike threshold.

In three cells, all L11, spontaneous and orthodromic inhibitory postsynaptic potentials (IPSP) were evaluated. In all cases, there was a diminution in amplitude and frequency of spontaneous IPSP by both ionic and nonionic agents, but more so by the nonionic agent metrizamide (fig. 6). Likewise, the orthodromic IPSP elicited by stimulation of the left connective was blocked by the contrast agents; however, not by the control solutions of NaCl or sucrose (fig. 7). This alteration of inhibitory postsynaptic potentials was unassociated with alteration in membrane resting potential.

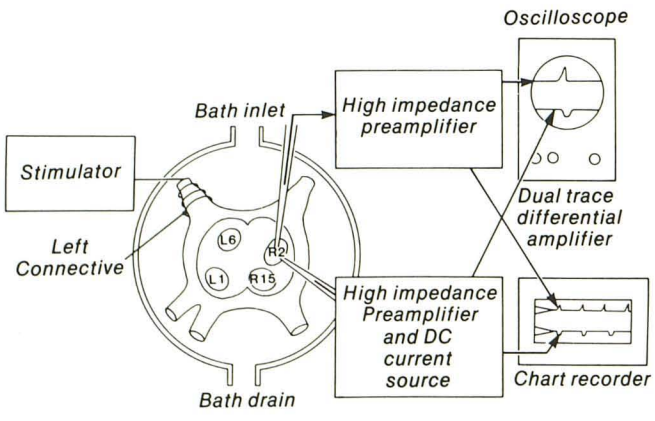
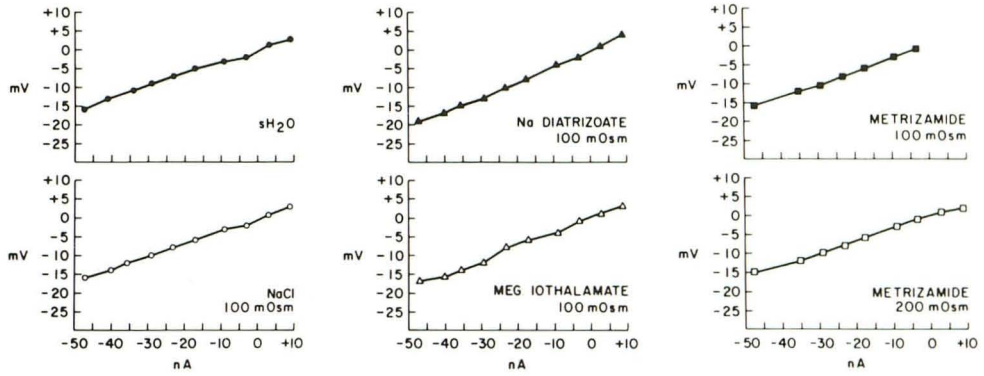


Fig. 1.—Preparation of experiment. Resected abdominal ganglia of *Aplysia* are placed in small chamber in which they are continuously bathed in various circulating test solutions. Specific neurons (such as R2) are then impaled with one or two glass microelectrodes. One electrode is coupled by high impedence preamplifier to oscilloscope differential amplifier for display of intracellular potentials. If second electrode is used, it is coupled to DC current source by high impedence preamplifier. Second electrode is then used to alter transmembrane potential by current passage, which is displayed on oscilloscope or strip chart recorder. In addition, various connectives (nerves) are placed in suction electrodes for orthodromic stimulation.

Fig. 3.—Passive membrane resistance. Intracellular transmembrane potential (mV) is plotted on ordinate as function of intracellular current (nA) passed through separate electrode, displayed on abscissa. Slopes of curve indicate membrane resistance and reveal no change after 30 min exposure to various contrast agents. Dual electrode recordings from R2. OmV = −57 mV RP.



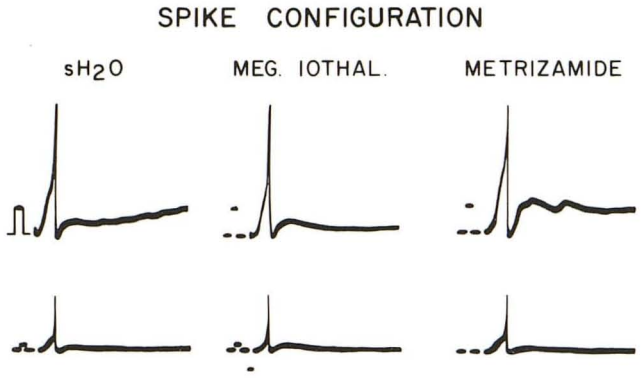


Fig. 4.—Spike configuration, recording from R2. Orthodromic spikes elicited by stimulation of left connective showed no alteration in configuration after exposure to meglumine iothalamate and metrizamide. Variations in afterpotentials not consistent, related to spontaneous baseline alterations. Calibration 10 mV/10 msec.

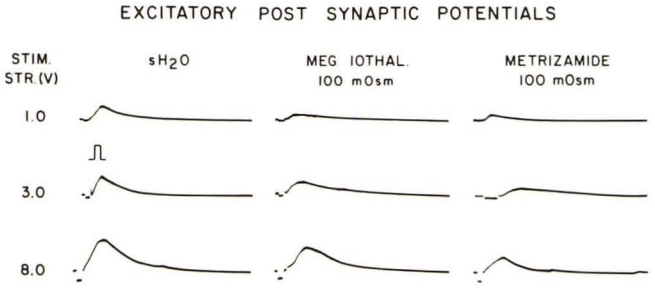
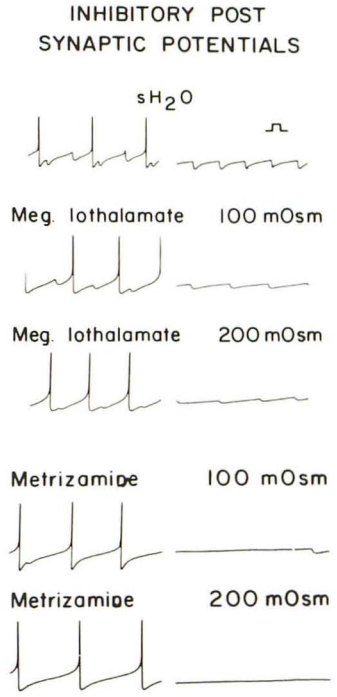


Fig. 5.—Intracellular recordings of excitatory postsynaptic potentials (EPSP) from R2 after stimulation of left connective. Stimulus strengths are at 2, 6, and 16 times threshold (last is supramaximal). Mild diminution in amplitude of EPSP after exposure to meglumine iothalamate and metrizamide. Calibration 10 mV/10 msec.

Fig. 6.—Intracellular recordings of spontaneous inhibitory postsynaptic potentials (IPSP) from L11. Recordings on left are at resting potential; those on right are hyperpolarized to block spontaneous spike. Meglumine iothalamate slightly diminishes both frequency and amplitude of IPSP, while metrizamide completely blocks IPSP.



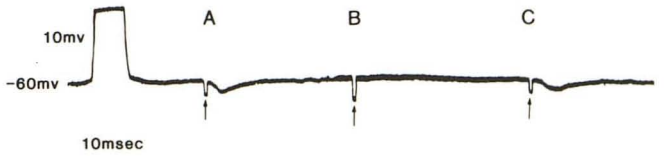


Fig. 7.—Orthodromic IPSP in cell L11 from stimulation of left connective. **A**, Control recording. **B**, Blockage of IPSP after 15 min exposure to 100 mOsm metrizamide. **C**, Reversal of blockage after 15 min of seawater rinse. Stimulus artifact (*arrows*).

Discussion

Our results indicate that water-soluble radiographic contrast agents do not greatly alter a variety of electrical phenomena in neurons of the abdominal ganglia of *Aplysia*, except for inhibitory postsynaptic potentials, which they consistently depress. The most sensitive neuronal activity to the effects of radiographic contrast agents seems to be inhibitory postsynaptic potentials. This would imply that at least part of the epileptogenic properties of these drugs is due to disinhibition rather than a direct excitatory effect. This is compatible with previous experimental results in the cat spinal cord [9]. Although these are limited data, they

suggest that these contrast agents may affect neurons similarly to other known convulsant agents, such as picrotoxin which also seems to produce epileptogenic effects by diminution of inhibitory synaptic activity [14]. In our study relatively low concentrations of the contrast agents altered inhibitory postsynaptic activity and this effect is independent of osmolarity. The normal fluid osmolarity of these animals is about 1,000 mOsm and the total osmolarity of the test solutions in these experiments was 950–1,150 mOsm. Control solutions of NaCl and sucrose have no significant effect on IPSP (or any of the other parameters measured).

These observations imply that contrast agents directly affect specific synaptic activity and that their neuronal effects are not due to their hypertonicity or generalized alteration of membrane properties, such as resting potential. The exact mechanism of the decreased inhibitory synaptic potentials cannot be determined from these experiments, but alterations in synaptic C1 mechanisms is a possibility. Why the nonionic agents should have a greater effect than the clinically more toxic ionic drugs is unclear, but may relate to an overall depressant activity of nonionic compounds recently demonstrated in mammalian hippocampus (N. Bryan and N. Hershkowitz, unpublished data) and seen in clinical EEGs.

Since the effects of these drugs on neurons involves a rather specific chemical activity, it seems that future modifications of these agents should be directed toward chemical structure relating to synaptic activity rather than to decreasing osmolarity or even lipid solubility. It may, in fact, be fortuitous that the newer nonionic drugs have less neurotoxicity than their ionic predecessors. The chemical changes resulting in diminution of osmolarity and lipid solubility may have limited their access to neuronal membrane surfaces, but more importantly, may have altered their effect on inhibitory synapses.

One must admit that while these drugs do have what may be an appropriate effect (i.e., disinhibition) on the neurons of this invertebrate animal preparation, there is an important difference between the degree of these effects of *Aplysia* neurons and neurons of mammalian systems. These drugs produce frank "convulsive" activity in various mammalian preparations including cat cortex and spinal cord, rabbit cortex, and rat cortex and hippocampus (N. Bryan and N. Hershkowitz, unpublished data). In none of the *Aplysia* neurons that we investigated was there ever rapid repetitive neuronal spiking or "burst" activity, which is generally taken to be "epileptogenic." Certainly, other convulsants such as picrotoxin, strychnine, and Metrazol produce more obvious neuronal spike activity in *Aplysia* neurons. It is unclear why contrast agents do not produce such dramatic effects. Two possibilities are significant species difference in neuronal sensitivity to the drugs and/or a significant binding of these drugs to the membranous capsule that surrounds the neurons in the abdominal ganglia of *Aplysia*. Further experiments, both in this preparation and other epilepsy models, will be necessary to clarify this discrepancy.

While these experiments are relatively limited, they do offer the first information of what may be some of the specific cellular mechanisms of neurotoxicity of water-soluble contrast agents. This type of approach seems important, not only in determining the factors of neurotoxicity of these drugs, but also in evaluating future compounds for improved clinical usefulness.

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