1 Paper No. 70 of the series: Defense Mechanisms of Arthropods.
2 Acknowledgments. Study supported in part by the NIH (grants AI-02908 and AI-12020). We are indebted to John Doyen and the late Robert E. Silberglied for providing respectively the *Metrius* and *Goniotropis*.
3 Current address: Department of Chemistry, U.S. Naval Academy, Annapolis (MD 21402, USA).
4 Current address: Department of Biology, Queens College, Flushing (NY 11367, USA).
5 To whom reprint requests should be addressed.
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0014-4754/83/040366-03$1.50 + 0.20/0

Sucrose gap longevity is markedly improved by addition of lanthanum to the sucrose

J.P. Pooler

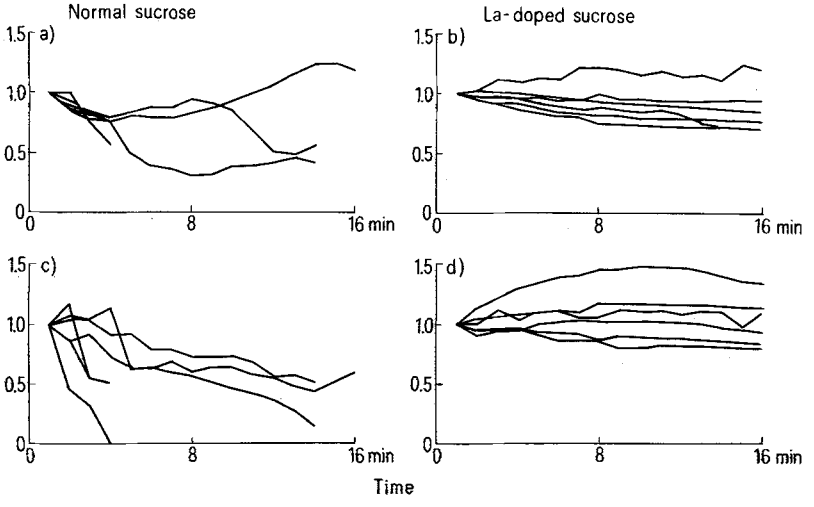
Department of Physiology, Emory University School of Medicine, Atlanta (Georgia 30322, USA), October 27, 1982

Summary. The addition of low concentrations of lanthanum to the sucrose in the double sucrose gap technique for the study of giant axons significantly prolongs preparation longevity and decreases drift.

The sucrose gap technique for the assessment of membrane potentials on small nerve cells without intracellular electrodes was introduced by Stämpfli[1] and developed for voltage clamping of lobster giant axons by Julian et al.[2,3]. Despite the usefulness of the technique it suffers from the drawback of short preparation viability and high drift. This report describes a dramatic improvement in both longevity and reduction of drift by the simple expedient of doping the sucrose with low concentrations of lanthanum.

In the double sucrose gap technique as applied to giant nerve axons an artificial node is formed by the patch of membrane between the sucrose streams. Many such nodes may be studied on just one axon by translating the whole axon through the experiment chamber in short steps, thereby bringing successive fresh areas of membrane into the region between the sucrose streams. The longevity of any one node is often only a few minutes and rarely exceeds 30 min. During this time the resting potential tends to meander and often falls by tens of mV in abrupt steps. There is usually a corresponding rise in leakage current. Furthermore, the kinetics and magnitude of active sodium and potassium currents studied in voltage clamp usually drift during the life of any one node. If a node deteriorates too much during an experiment one simply translates the axon to form a new node and begins the experiment all over, but it is difficult to do experiments which require comparative measurements spaced more than a few minutes apart.

In 1972 New and Trautwein[4] suggested that low concentrations of calcium in the sucrose improved the stability of muscle preparations in sucrose gap and this was soon found



Normalized peak sodium current for clamp steps to −15 mV (a and b) and resting potential (c and d), assessed at 1-min intervals in normal or La-doped sucrose. Each determination is normalized to the value at 1 min. Three nodes in normal sucrose and 3 nodes in La-doped sucrose from each of 2 axons are shown. In normal sucrose all but 1 experiment had to be terminated before 16 min because the current required to hold the potential at −100 mV went up precipitously. In La-doped sucrose all nodes easily survived 16 min.

to improve giant axon stability also[5]. In the present work the longevity of lobster axon nodes has been studied as a function of sucrose solution composition. Normal isosmotic sucrose or isosmotic sucrose doped with the chloride salts of sodium, calcium or lanthanum, all matched for equal solution resistivity, were employed. Normal de-ionized sucrose had a resistivity of 1.5×10^6 Ω cm and gave a gap resistance in the absence of an axon of 2×10^8 Ω. Doped sucrose had a resistivity of 6.5×10^4 Ω cm and gave a gap resistance of 10^7 Ω. The doping concentrations were NaCl 2.1×10^{-4} M; $CaCl_2$ 1×10^{-4} M; $LaCl_3$ 6.7×10^{-5} M. Axons were the medial giants from the circumesophageal connective nerve in lobster *(Homarus americanus)*.

The results with sodium-doped sucrose show no improvement over unmodified sucrose and perhaps an exacerbation in the rate of rundown. With calcium there is a significant increase in longevity and a decrease in variation of resting potential. With lanthanum the improvement is even more dramatic. In 15 experiments employing lanthanum as the doping ion no node which was originally viable had to be terminated because of deterioration. This includes several experiments which ran for 60 min and one for 80 min. All were in good condition at the end. In addition, the stability during the experiments was significantly better than in normal sucrose. The figure shows 16 min records of peak sodium current at -15 mV and resting potential for 6 nodes from 2 axons in normal sucrose (panels a and c) and another 6 nodes from the same axons in lanthanum-doped sucrose (panels b and d). The smallest decline in resting potential with normal sucrose exceeded the largest decline with lanthanum-doped sucrose and only 1 of the nodes in normal sucrose survived the 16 min.

Lanthanum exerts strong pharmacological effects on active sodium and potassium conductances[6,7] but at the low concentrations used here there is no observable influence. When the artificial sea water bathing the node was switched to one containing 6.7×10^{-5} M lanthanum, using normal sucrose in the gaps, no visual change in the pattern of clamp currents at 0 mV could be detected. Lanthanum has been described as a 'super calcium'[6], being about 20 times more effective in shifting the voltage dependence of active conductances than calcium. The artificial sea water contained 25×10^{-3} M calcium and the addition of 6.7×10^{-5} M lanthanum would be equivalent to raising calcium to 26.3×10^{-3} M, a change which would be undetectable in terms of conductance vs voltage plots. On the other hand the addition of 6.7×10^{-5} M lanthanum to the low ionic strength sucrose could bring about a major reduction in surface potential at the membrane under sucrose. To account for the influence of lanthanum I postulate that the membrane resistance under normal sucrose is very low, because the low ionic strength bathing medium and the absence of multivalent cations allow the existence of a large negative surface potential. According to this hypothesis the influence of lanthanum is to lower the surface potential and thereby raise the resistance back toward the nodal membrane resistance. This, in turn, decreases the perturbations in measured potential associated with changing properties of membrane under sucrose. The findings reported here make the double sucrose gap technique much more attractive as a means to study pharmacological modifications of channel properties because of the improvement in longevity and decrease in drift.

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0014-4754/83/040368-02$1.50 + 0.20/0

Demonstration of vascular redistribution after carotid clamping in rats

P. Lespinasse, M. Le Poncin-Lafitte, P.C. Pesquies and J.R. Rapin[1]

Cerebro-vascular Neurophysiology, C.H.U. St-Antoine, F-75012 Paris (France), and CERMAS, Porte de Sèvres, F-75731 Paris Air Cedex 15 (France), January 20, 1982

Summary. In Long Evans rats, the vertebral arteries supply only the brain stem. Simultaneous application of clamps to both carotid arteries results in a considerable fall of cerebral blood flow and eventually death. If an interval of about 4 days is allowed between the clamping of the 2 carotids, redistribution of blood flow takes place. Owing to this mechanism, 50% of the rats survive with a satisfactory restoration of cerebral blood flow.

In many species the ponto-medullary areas are supplied with blood by the vertebral arteries, whereas the rest of the brain is supplied by the carotid arteries. However, in the rat there is evidence that the vertebral arteries do not supply the brain[2] or that they only supply the spinal bulb-pons area[3]. The latter was demonstrated indirectly by the injection of radioactive microspheres by heart puncture into the left ventricle or into the carotids and by comparing their distribution in the brain: these studies were carried out in healthy animals and it was therefore possible to show any redistribution of cerebral blood flow, which occurs in certain disease states. In our study a direct method was used to investigate the changes of cerebral blood flow after simultaneous clamping of both internal carotids. The redistribution of cerebral blood flow was analyzed allowing a time interval between the ligation of the 2 vessels.

Material and methods. 1. Ligation of the carotids. Male Long Evans rats weighing 200 ± 20 g were anesthetized with chloral hydrate (300 mg/kg i.p.). After careful dissection the internal carotid arteries were ligated just above the bifurcation. The ligation of the 2 arteries were performed simultaneously or at an interval of 15 min, 24 h, 48 h, 72 h,