The in-vitro sciatic nerve model: new Insights into neuropathy

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Polyneuropathy is a prevalent condition that can severely impair a person’s quality of life. In-vitro experiments were conducted in order to study external factors associated with polyneuropathy in isolation from systemic effects in live models. Sciatic nerves were isolated from Sprague-Dawley rats and placed in perfusion chambers that contained artificial cerebral spinal fluid. The nerves were stimulated, the averaged nerve action potential (NAP) waveforms were digitized, and the NAP amplitude, velocity, duration, and amplitude of the paired pulse response were analyzed. The effects of anoxia, hypothermia, and external metabolic substrates on the NAP were analyzed. Results showed a complex interaction between the three categories and their effects on the NAP parameters. Cyclic episodes of anoxia had different effects on NAP amplitude and velocity and also resulted in ischemic preconditioning of the nerve, which is perhaps due to the build-up of glycogen during the re-oxygenated (recovery) phases. Hypothermia appeared to best preserve nerve function when present during the anoxic phases and absent during the recovery phases, perhaps allowing for glycogenolysis during the recovery phases. The metabolic function of the nerve during intermittent anoxia was then analyzed, and it was determined that hyperglycemia improved ischemic preconditioning while decreasing NAP amplitude overtime, suggesting that there are other contributing processes such as the production of free radicals and advanced glycation end-products. Lactate uniquely supported the nerve in high and low concentrations during both continuous oxygenation and intermittent anoxia. The existence of a lactate shuttle between Schwann cells and peripheral nerves may explain these results. The findings from these in-vitro experiments may be important in future hypothesis testing in search for effective treatment of polyneuropathy.

Keywords: polyneuropathy; in-vitro studies; anoxia, hypothermia, nerve conduction, glucose, lactate, sorbitol, diabetes


Introduction

There are many types of peripheral nerve injury but distal symmetric polyneuropathy, particularly affecting nerves of the distal extremities, is the most common. Studies of large populations in India [1] and Sicily [2] suggest that the prevalence of neuropathy is 2-7%. However, in patients with diabetes, the risk of neuropathy increases to 50% [3]. Patients with neuropathy often describe symptoms of symmetric sensory loss in the lower legs and arms, accompanied by burning or weakness. Sometimes the sensory deficit coexists with motor impairment, which can affect ambulation and dexterity. The symptoms can range from mild to debilitating, and can severely impair quality of life [4]. Usually, the symptoms progress gradually and effective treatments are generally lacking [5], except for the neuropathies caused by vitamin deficiencies or autoimmune processes. Additional investigations into the processes responsible for neuropathy may lead to future effective treatments.

Many factors are associated with neuropathy. A study of the direct effects of these factors on the peripheral nerve is
difficult in-vivo because of the associated secondary systemic effects. An in-vitro system allows for the systematic study of these effects on only the peripheral nerve. The function of the peripheral nerve is readily probed by studying the nerve action potential (NAP), which has similar physiology in both humans and other mammals. In-vitro studies can provide information about the number of conducting axons, the excitability of the nerve, as well as the ability of myelin to support conduction in the largest fibers. One in-vitro approach [6] begins with extracting sciatic nerves from Sprague-Dawley rats and placing them in perfusion chambers containing artificial cerebral spinal fluid (ACSF). The environment of the peripheral nerve can then be manipulated in order to study the affects of the different factors on NAP in isolation. The standard protocol involves stimulating the nerve at 5Hz with the averaged waveforms saved every 4 seconds and digitized. Automated algorithms are used with manual supervision to abstract the amplitude, velocity, duration and the amplitude of the paired pulse response from each NAP tracing. Changes of these parameters are then used to study the effects of temperature, oxygenation, and external substrates on nerve function.

Hyoxia is one factor that has repeatedly been shown to influence peripheral nerve function [7-9]. In long-standing diabetes, advanced glycation products (AGEs) accumulate in vessel walls, resulting in increased vascular permeability and hypoxia [10-11]. Many diabetics also develop atherosclerosis, which impairs oxygen delivery to tissue [12]. By creating a hypoxic environment in an in-vitro model, an aspect of the peripheral nerve environment in a diabetic patient was mimicked and the effect on nerve function was analyzed [6] without the secondary effects that hypoxia might have on other tissues. The paradigm for studying the effect of hypoxia in these studies of peripheral nerve involved repeated 90 minute periods of anoxia followed by 90 minutes of reperfusion. There were substantial changes in the response of the nerve to each successive episode of anoxia, which provided additional information about the state of the nerve. Repeated episodes of anoxia resulted in progressively decreasing NAP amplitude and increasing NAP duration. The velocity drops with the first period of anoxia but then changes over time in just the same way as nerves not exposed to anoxia. This suggests that different aspects of the NAP are affected differently by anoxia. Additionally, the NAP disappears slowly when the nerve is made hypoxic, but reappears more quickly when re-perfused with oxygen. The rate of disappearance of the NAP during anoxia is a function of both the ability of the nerve to use metabolites in the perfusate in the absence of oxidative phosphorylation as well as its ability to use endogenous glycogen produced during periods of oxygenation. The rate of reappearance of the NAP relates to the ability of the nerve to generate energy by oxidative phosphorylation. Because more energy is generated per molecule of glucose during oxidative phosphorylation than glycolysis, it is understandable that the recovery time during re-oxygenation would be shorter than the time required for the NAP to disappear during anoxia. Over repeated cycles of anoxia the time for the NAP to disappear during the anoxic phase increases, demonstrating the development of anoxic tolerance, but the time for the NAP to reappear during re-oxygenation remains constant. This pre-conditioning effect suggests that there could be a build-up of glycogen over repeated episodes of anoxia [6] among other possibilities.

Temperature has many effects on the nervous system. The anoxic tolerance of the brain can be increased by 7-8 fold by lowering the temperature from 37°C to 18°C [15-14]. Are the same processes occurring in the peripheral nerve? Nerves were exposed to hypothermia in two types of experiments: one in which the nerve remained continuously hypothermic throughout the entire experiment and one in which the nerve was hypothermic during the anoxic state and normothermic during the oxygenated state. In both experiments, hypothermia improved the survival of the nerve after anoxia. However, optimal preservation occurred when the nerves were hypothermic only during the anoxic phases. This indicates that hypothermia helps to preserve nerve function during anoxia, but suppresses active restorative processes during the oxygenated state. The time for the NAP to change during anoxia prolonged by roughly a factor of 3 on lowering the temperature from 37-17°C which is relatively consistent with the rate of change seen in evoked potentials with cardiac arrest at different temperatures [15] and the changes in brain metabolic rate over a similar temperature range [16-17]. If glycogen is the major source of energy during anoxia [18], and the rate of glycogenolysis is less suppressed by hypothermia than the overall metabolic rate, then the time that the NAP persists into anoxia would increase. This hypothesis also explains the improvement when the nerve is normothermic during the oxygenated state because this allows for the synthesis of additional glycogen.

One implicit consideration is that the outcome of anoxia is related to the ability of the nerve to maintain activity during anoxia. In order to address this, experiments were designed to manipulate the metabolic function of the nerve in a different way than hypothermia by studying how different concentrations of glucose affect the anoxic tolerance of the peripheral nerve [19]. In these experiments, nerves were perfused with different concentrations of glucose ranging from 0 mmol/L to 55.5 mmol/L glucose. It was determined that increasing glucose concentrations above 5.5mmol/L resulted in prolonged times for the NAP to disappear during anoxia, demonstrating increased immediate resistance to
anoxia. Although increasing glucose concentrations produced the same effects as lowering temperature on the time for the NAP to disappear during anoxia, the hyperglycemic nerves had markedly smaller NAP amplitudes than the normoglycemic nerves after anoxia. This result conflicts with the idea that prolonged preservation of NAP into anoxia is responsible for improving the outcome of anoxia, suggesting that there are other contributing processes. One might speculate that hyperglycemia is associated with the production of metabolic byproducts from glycolysis, such as free radicals and advanced glycation products, which are increased during anoxia and are found in higher concentrations when the nerve is in a hyperglycemic environment.

Exploring the metabolic substrates that might support nerve function both during the continuously oxygenated state and during intermittent anoxia can help clarify these issues [20]. Specifically the effects of glucose, fructose, galactose, sorbitol, beta-hydroxybutyrate, acetate and lactate were studied at 5.5mmol/L and 55mmol/L concentrations. Acetate and beta-hydroxybutyrate, which bypass glycolysis and feed into the tricarboxylic acid (TCA) cycle, do not support continuously oxygenated or intermittently anoxic nerves. This suggests that glycolysis is important to the function of the nerve, not just energy generation. Galactose was ineffective at preserving nerve function at either high or low concentrations. Only glucose showed the pattern in which low concentrations supported both oxygenated and intermittently anoxic nerves well but high concentrations produced marked damage to nerves that were intermittently anoxic. On the other hand, fructose supported nerve function well except that the lower concentration was able to support only intermittently anoxic nerves. Sorbitol supported both oxygenated and intermittently anoxic nerves at high concentrations. Especially since only Schwann cells contain sorbitol dehydrogenase, it is likely that sorbitol is metabolized in the Schwann cells that then produce another substrate, such as lactate, that the axon can use. The effects of lactate were unique in that it supported the peripheral nerve in both high and low concentrations during continuous oxygenation and intermittent anoxia. The time from anoxia to the disappearance of the NAP declined over successive periods of anoxia when lactate was the substrate while it increased with successive periods of anoxia with glucose or fructose as substrate. This implies that either the process responsible for the preconditioning acts proximally to the entry of lactate into metabolic pathways or that anoxia inhibits gluconeogenesis from lactate, decreasing its effectiveness in energy production. In general, these results indicate that an anoxic environment can alter the effectiveness of certain substrates in preserving nerve function when compared with a stable, oxygenated environment, and that the concentration of certain substrates plays an important role on how well the nerve is preserved. These results also demonstrate that anoxia can modify the metabolic pathways utilized by the peripheral nerve.

Multiple studies have shown that in the central nervous system a lactate shuttle exists between astrocytes and neurons and that astrocyte-derived lactate could be used as a substrate to meet increased metabolic demands of neurons [21-23]. It has been argued that glycogen and glycolytic pathways are predominant in astrocytes and that lactate is utilized by neurons at least in part because of the increased expression of lactate dehydrogenase and monocarboxylate transporters (MCT) [24] in neurons of the central nervous system. This metabolic compartmentalization, in which astrocytes display predominately anaerobic activity and central neurons rely mostly on oxidative phosphorylation, might also occur in the peripheral nervous system. Schwann cells could produce lactate, which could then be transported to neurons via MCT and, once within the intracellular space, can be converted to pyruvate and acetyl-CoA (producing NADH) or take part in gluconeogenesis.

Can any of this information lead to clinically useful results? First, they demonstrate the complexity of the interactions between temperature, anoxia and metabolic substrate and hence suggest that testing of agents that might improve nerve function should assess their function in some of the different conditions described above. Second, certain models of diabetic neuropathy show changes similar to those seen in the above acute studies. Hyperglycemia has been shown to induce neuronal dysfunction by affecting subpopulations of neurons and nerve terminals located within parasympathetic and sympathetic ganglia [25]. Insulin resistance has been associated with nerve conduction velocity deficits and thermal hyperalgesia around the time of diabetes onset [26]. In addition, similar changes are seen in the NAP in various animal models of diabetes [27-28] and in humans with diabetes neuropathy [29]. However, a more direct comparison with the above animal studies and with the human entities of critical illness neuropathy [30] and vasculitic neuropathy [31] is in need. Despite this, acute in-vitro experiments can be carried out more quickly than in-vivo testing in animals or clinical trials in humans and hence may be important in the process of hypothesis testing and testing of agents that could have value in various types of neuropathy.

Conflict of Interest

There are no conflicts of interest.

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References


