EFFECT OF GLUCOSE CONCENTRATION ON PERIPHERAL NERVE AND ITS RESPONSE TO ANOXIA

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ABSTRACT: Introduction: Glucose has a significant effect on nerve function. Methods: The effects of glucose on the nerve action potential (NAP) were investigated for concentrations between 0 and 55.5 mmol/L in an in vitro system using rat sciatic nerve. The effects of glucose were investigated in nerves exposed to oxygenated perfusate and those subjected to anoxia. Multiple aspects of the NAP were analyzed. Results: Hypoglycemia produces immediate reductions in NAP amplitude and velocity, whereas hyperglycemia has the opposite effect in the short term. Over a 12-hour experiment, the amplitude of the NAP remained stable for glucose concentrations in the range 2.8–5.6 mmol/L, but, when the glucose concentration was <2.8 mmol/L or >27.8 mmol/L, the amplitude of the NAP declined. The deleterious effects of hyperglycemia (>27.8 mmol/L), or hypoglycemia (<4.2 mmol/L) were more pronounced in nerves exposed to intermittent anoxia. Conclusion: The present findings confirm the importance of glucose concentration for nerve function especially during anoxia.

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Neuropathy is a common neurological problem that is difficult to treat. Neuropathy affects up to 43% of patients with diabetes,1,2 and it significantly impacts their quality of life. Improving glycemic control3 is the only therapy generally recognized to improve the underlying pathologic processes. However, this has only limited effects in type 2 diabetes and may predispose patients to the serious risk of hypoglycemia. There have been a number of clinical trials5–12 of other treatments with the potential to improve neuropathy, including aldose reductase inhibitors, statins,6 alpha-lipoic acid,13 and neurolysis,14 but none have been sufficiently promising to enter into routine clinical practice. Other types of nerve injury are affected by the concentration of glucose, particularly critical illness polyneuropathy,15 which is more severe in the setting of hyperglycemia.

The purposes of this study are first to elucidate the acute effects of glucose concentration on rat peripheral nerve and then to describe the modulatory effects of glucose concentration on the response of the nerves to anoxia. Although a number of studies suggested that hyperglycemia worsens stroke,16–18 it may have different effects on other organs undergoing anoxia.19,20 There have been fewer studies of the effects of glucose concentration on the response of peripheral nerve to anoxia.21–24 Schneider et al.25 suggested that the response of peripheral nerves to hyperglycemic anoxia was worse than normoglycemic anoxia, possibly as the result of acidosis produced by anaerobic glycolysis. Grafe et al.26 supported this hypothesis and further suggested that acidosis contributes to block of potassium channels. The level of glucose concentration at which these effects of hyperglycemia are seen has not been determined to provide a more detailed multidimensional picture. Based on those findings, it is expected that glucose concentration will have significant effects on peripheral nerve function and that the effect of glucose concentration is different during periods of oxygenation and anoxia.

METHODS

A full description of the methods has been given in previous studies.25–28 Under a protocol approved by the animal care and use committee of our institution (Winthrop University Hospital Protocol WUH-MS#1), a total of 85 nerves from 45 Sprague-Dawley rats (Hilltop, Scottsdale, Pennsylvania) were studied. Each sciatic nerve was dissected and placed into a perfusion chamber and stimulated using stainless-steel subdermal electrodes. The stimulus consisted of paired unipolar pulses separated by 4 ms, each with a 15-mA peak current, a duration of 0.01 ms, and an overall pair repetition rate of 5 Hz. Bipolar recordings of the nerve action potential (NAP) were made, digitized at 99 kHz, averaged, and stored every 4 s. Three types of experiment were performed. In the first, termed “stability” experiments, the nerves were exposed continuously to oxygenated perfusate. In the others, termed “anoxia” experiments,
the nerves were subjected to 5 90-min periods of anoxia, each followed by a 90-min period of recovery in fully oxygenated perfusate. The 90-min periods of anoxia and recovery were chosen so as to be consistent with previous experiments. This allowed direct comparison between new experiments and those performed previously. Periods of anoxia significantly shorter than 90 min did not allow time for the NAP to disappear during anoxia and made it difficult to quantitate the time course of changes during different conditions. Longer periods of anoxia made the experiments so long that the effects of Wallerian degeneration became significant. Anoxic durations of the order of 90 min can be seen during surgical procedures using tourniquets and during aortic surgery.

The anoxia experiments were of the same total length as the “stability” experiments but divided into phases representing the responses to 5 cycles of anoxia (phases 2, 4, 6, 8, 10) and recovery (phases 3, 5, 7, 9, 11), with phase 1 being the baseline oxygenated state prior to the first period of anoxia. Thus, phase 5 refers to the recovery period after the first cycle of anoxia, and phase 11 is the recovery period after the fifth cycle of anoxia. To compare the NAP during the stability and anoxia experiments the data from the stability experiments were classified into the same time intervals (phases) as the anoxia experiments (Fig. 1). These comparisons are meaningful only for phases 3, 5, 7, 9, and 11, because the NAP disappears during phases 2, 4, 6, 8, and 10 in the anoxic phases.

The third type of experiment was designed to elucidate the acute effects of changing glucose concentrations. These experiments used the same timing of events as in the anoxia experiments but switched the perfusate between either 5.5-mmol/L and 55.5-mmol/L or 5.5-mmol/L and 0-mmol/L glucose concentrations and remained fully oxygenated at all times.

The entire experiment was under computer control. All experiments were performed with the nerve at 36°C. The base perfusate was composed of 10 mM HEPES, 110.2 mM NaCl, 17.8 mM NaHCO3, 4.0 mM MgSO4, 3.9 mM KCl, 3.0 mM KH2PO4, and 1.2 mM CaCl2, as in previous studies. The dextrose concentration was varied from 0 to 55.5 mmol/L. pH was measured before and after each experiment and did not vary by more than 0.15.

The NAP peak-to-peak amplitude, conduction velocity, duration, and area under the curve (AUC) for the response to the first stimulus were the primary parameters measured, which were abstracted automatically with manual supervision (Fig. 1). The ratio of the peak-to-peak amplitude of the conditioned (second) response to the unconditioned (first) response for each stimulus was recorded as the conditioned stimulus response (CSR). To compare the results from different nerves, the values of each of the abstracted parameters were normalized so that their mean values in the baseline oxygenated state (phase 1) equaled 1. We then obtained the time (“T50”) required for the peak-to-peak amplitude to change from its...
starting value in each phase to first reach a value halfway between the beginning and ending values in that phase.

Statistical analyses were based on repeated-measures analysis of variance (ANOVA). In these analyses, the variable ANOXIC described the experiment type (stability vs. anoxia). The variable CYCLE referred to the data from phases 3, 5, 7, 9, and 11, in which the nerves exposed to anoxia were recovering from the first through fifth cycles of anoxia. The normalized values of amplitude, velocity, duration, CSR, and AUC were described by the variable TYPE. Statistical analyses using variables other than amplitude and AUC were necessarily limited to a small range of conditions, because, at the conclusion of an experiment and at the extremes of glucose concentration, there may be no recordable NAP. Thus, the first analysis was a repeated-measures ANOVA to determine whether there was a statistically significant GLUCOSE × CYCLE × ANOXIC interaction for the NAP amplitudes that would suggest the effects of glucose were different in different phases in the stability and anoxic experiments. Analyses to find a GLUCOSE × TYPE × CYCLE interaction were limited to the stability experiments because of the loss of the NAP by the end of many of the anoxia experiments. In addition, the T50 values during both anoxia and recovery were computed for the anoxia experiments, and the repeated-measures ANOVA was used to determine whether there was a significant GLUCOSE × CYCLE × OXYGEN interaction. OXYGEN is the variable indicating whether in a given cycle the nerve was currently anoxic or fully oxygenated.

RESULTS

Stability Experiments. Figure 1 shows the average changes in normalized amplitude, velocity, and duration during experiments when the nerve was subjected to a constantly oxygenated perfusate at a glucose level of 5.5 mmol/L. There was a slow and gradual decline in amplitude and velocity over time. Repeated-measures ANOVA (Table 1) demonstrated a statistically significant interaction of TYPE × CYCLE × GLUCOSE \[ F(96, 288) = 6.29, P < 0.001 \] for glucose concentrations of 1.4 mmol/L through 55.5 mmol/L, as no NAPs could be obtained in the glucose-free solutions by the end of any experiment. This demonstrates that the measured variables reported different aspects of the NAP that changed differently with glucose and the number of previous episodes of anoxia. Figure 2a shows the normalized amplitude of the NAP as a function of glucose concentration for various cycles during the experiment. For glucose concentrations in the 2.8–27.8-mmol/L range, the NAP peak-to-peak amplitude changed only slightly during the experiment. There was only a slight reduction in the amplitude of the NAP by the end of the experiments with the 55.5-mmol/L glucose concentrations. The solutions with low glucose concentrations maintained high-amplitude NAPs for 30–60 min, but thereafter that the NAP amplitude dropped quickly to 0. Figure 3a shows that the mean AUC during each cycle depended on glucose concentration, similar to that for peak-to-peak amplitude. The velocity and CSR showed similar patterns of decline with time that were much more prominent at the lowest glucose concentrations, whereas the NAP duration increased over time.

<table>
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<tr>
<th>Comparison</th>
<th>Statistical effect</th>
<th>Comment</th>
<th>Number of nerves</th>
<th>Degrees of freedom</th>
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<th>( P )</th>
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<td>16, 288</td>
<td>84.81</td>
<td>&lt;0.001</td>
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<td>Only amplitude analyzed</td>
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Numbers in parentheses next to a factor represent the number of levels for that factor. The first test compares all measurements as a function of GLUCOSE and CYCLE, with glucose of 0 excluded. The second test compares results obtained during anoxia and stability experiments as a function of CYCLE and GLUCOSE using only NAP amplitude. The third test compares time-course measurement of changes in NAP amplitude as measured by the T50 during the first period of anoxia and recovery.
and predominantly at the low glucose concentrations. Overall, NAP was closest to its baseline state when glucose concentrations were in the 2.8–27.8 mmol/L range.

To further understand the acute effects of changing glucose concentrations on the NAP, a number of experiments were performed in which the nerve remained fully oxygenated at all times. In the first, the nerve was bathed by perfusate with either a 5.6 mmol/L or 55.5 mmol/L glucose concentration, switching between them every 90 min. On switching from the lower to the higher glucose concentration, there was a sudden increase in the NAP peak-to-peak amplitude that averaged roughly 2–3%. There remained a gradual reduction in NAP amplitude over hours as in the constant glucose experiments described earlier. However, the normalized NAP amplitude by the end of these experiments was 77% of baseline compared with 40% when the highest glucose concentration was maintained continuously. In the second set of experiments, the nerve was bathed first by 5.6 mmol/L and then by 0 mmol/L glucose concentrations, switching between them every 90 min. With each exposure to hypoglycemia there was a larger reduction in NAP amplitude over hours as in the constant glucose experiments described earlier. However, the normalized NAP amplitude by the end of these experiments was 77% of baseline compared with 40% when the highest glucose concentration was maintained continuously. In the second set of experiments, the nerve was bathed first by 5.6 mmol/L and then by 0 mmol/L glucose concentrations, switching between them every 90 min. With each exposure to hypoglycemia there was a larger reduction in NAP amplitude. In the first cycle of hypoglycemia, there was only a 10% decline, but by the fifth cycle there was a 50% decline. These changes in amplitude took place over roughly 2000 s. At the end of the alternating normoglycemic–hypoglycemic experiment, the amplitude of the NAP was roughly 50% of baseline compared with the case in which glucose was constant at 0 mmol/L, where there were no recordable responses by the end of the experiment.

Anoxia Experiments. There is a significant (Table 1) ANOXIA × CYCLE × GLUCOSE interaction \(F(28, 268) = 4.26, P < 0.001\), indicating that the effects of glucose concentration and experiment duration were different in the nerves exposed to anoxia, and this difference depended on the glucose concentration and cycle. Figure 2b shows that there were 2 primary differences in the pattern of changes in NAP amplitude for different glucose concentrations. First, the amplitude of the NAP fell more rapidly over time during anoxia than during the stability experiments at all glucose concentrations. Second, the amplitude of the NAP decreased markedly for glucose concentrations \(\leq 27.8\) mmol/L as well as for glucose concentrations \(< 4.2\) mmol/L. Figure 2b, d, f, and h shows the adverse changes in the other NAP-based measures. The duration increased rapidly over time although this was minimized at a glucose concentration of 4.2–5.6 mmol/L. The CSR was best preserved at 1.3–4.2 mmol/L. The velocity dropped rapidly with each cycle, most prominently after the first cycle of anoxia; it was lowest at the highest glucose concentrations and highest at the lowest glucose concentrations. Overall, all NAP parameters except...
velocity were best preserved at glucose concentrations of between 4.3 and 5.6 mmol/L.

Other important descriptors of the response to anoxia included the time required for the NAP to drop to half its baseline value during anoxia (anoxia T50) and the time required for the NAP to return to half its maximal value during reoxygenation (recovery T50). Because the NAP disappeared during the later cycles of anoxia at the highest and lowest glucose concentrations, there are data for all glucose concentrations only for the first cycle.

Using the data from this cycle (Table 1), there were significant main effects of both GLUCOSE and OXYGEN and a statistically significant GLUCOSE × OXYGEN interaction of the T50s for recovery and anoxia \( F(7, 44) = 10.1, P < 0.001 \). This demonstrates that T50s were: different during anoxia and recovery; dependent on glucose levels; and that the effect of glucose level was different during recovery and anoxia. Figure 4 shows that there was a significant and gradual increase in the anoxic T50 from about 600 s at the lower glucose concentrations to about 2000 s at the higher glucose concentrations.
concentrations to 1600 s at glucose concentrations of 55.5 mmol/L. As in previous studies, there was a gradual increase in the anoxic $T_{50}$s in the later cycles of anoxia. The recovery $T_{50}$s showed only minor changes with glucose and cycle.

**DISCUSSION**

These acute *in vitro* studies demonstrate that extreme glucose concentrations have immediate effects on nerve conduction that are not related to any effects on the vascular or immune systems. A number of *in vivo* studies have demonstrated changes in nerve conduction during acute changes in glucose,31–35 but were necessarily limited in time resolution and glucose concentrations. This study has shown that the initial effect of hyperglycemia is actually an improvement in nerve conduction, which confirms the *in vivo* results of Sindrup et al.36 Longer exposure to glucose concentrations of 55.5 mmol/L over hours had mild negative effects. There was a more dramatic immediate negative effect of severe hypoglycemia over roughly 1 hour that worsened with chronic exposure to hypoglycemia. In stability experiments, NAP amplitude was preserved optimally for glucose concentrations in the range of 2.8–11.1 mmol/L.

Although extremely high glucose values can affect acutely the function of peripheral nerve, this effect is enhanced significantly in the presence of anoxia. There are 2 competing effects. Increasing glucose concentration increases the time required for the NAP to disappear during anoxia (anoxia $T_{50}$), as suggested by Parry and Kohzu in a study of human nerves.37,38 This indicates that increased glucose concentrations may facilitate anaerobic metabolism. Prolongation in the anoxia $T_{50}$s of roughly the same magnitude were seen with hypothermia to 17°C in our previous studies,27,28 as a possible reflection of reduced metabolic activity at lower temperatures.

Despite prolongation of the anoxia $T_{50}$ by hyperglycemia, the amplitude of the NAP declined more quickly with repeated episodes of anoxia than at normoglycemia. This is very different from what was observed during hypothermia, where the NAP amplitude was better preserved as the anoxia $T_{50}$ was extended. There are a number of possible explanations for this phenomenon. One is that, during anoxia, anaerobic metabolism produces lactate and localized tissue acidosis. Although the pH external to the nerve was well controlled and did not change during the experiments, it is still possible that localized acidosis may exist due to diffusion limitations. This would be worse in the setting of higher glucose concentrations, especially if anaerobic metabolism is enhanced, and is consistent with findings by Grafe et al.23 Another possibility is that damaging metabolic byproducts, such as advanced glycation products or free radicals, may also be created during anoxia. Additional experiments would be required to answer this question.

A more complete picture of the changes in nerve physiology during anoxia is revealed by

**FIGURE 4.** The effects of glucose concentration on the $T_{50}$ values during anoxia (time to 50% reduction in amplitude) and recovery (time for the NAP to first reach 50% of the highest amplitude in the following recovery phase). For some of the extreme glucose values in the later cycles there are very few measurements because of the disappearance of the NAP.
reviewing the other NAP parameters. NAP duration, which is dependent on the kinetics of sodium channels, is shortest during anoxia at glucose concentrations near 4.2 mmol/L and is prolonged at either higher or lower concentrations. Similarly, the paired pulse response (CSR), which is also related to sodium channel kinetics, is preserved optimally during anoxia at glucose concentrations of 1.4-4.2 mmol/L. These patterns are different from those seen in the amplitude and AUC, suggesting that there may be more than 1 independent type of injury occurring during anoxia. Velocity shows a third pattern of gradually decreasing recovery with increasing glucose concentrations. Changes in velocity can be produced both by changes in myelin and in the kinetics of active channels. However, the pattern of changes in velocity is different than duration and CSR, which are most closely connected to channel kinetics. This may indicate involvement of myelin.

Under hypoglycemic conditions, the NAP diminishes quickly during anoxia but is slower than under stable oxygen conditions. This may be the result of the fact that, during anoxia, there is less utilization of any local metabolic sources of energy, and the NAP may persist for longer periods.

Overall, there is no glucose concentration that optimizes all aspects of recovery of peripheral nerve from anoxia, although concentrations in the 4.2-8.3 mmol/L range are probably the best compromise. This is a fairly narrow range for optimal glucose concentration during anoxia when compared with the much wider range of “optimal” glucose concentrations for nerves not exposed to anoxia. This confirms in peripheral nerve the importance of keeping the glucose concentration tightly regulated during periods of anoxia or hypoxia. It also suggests that periods of hypoglycemia on the order of 1 hour or periods of severe hyperglycemia on the order of 10 hours may cause significant changes in peripheral nerve, especially during periods of metabolic stress.

There are always questions regarding the applicability of an animal model, such as the one used in these experiments, to diseases in humans. This model and the effects described herein likely have more in common with such disorders as vasculitic neuropathy, critical illness neuropathy, and focal ischemic mononeuropathies, which are clearly related to periods of anoxia/ischemia. This model may also have relevance to the study of diabetic polyneuropathy, because there is evidence that ischemia is a contributing factor. One limitation in such a comparison is that the duration of anoxia/ischemia in the human diseases is likely not the same as the period of anoxia chosen for this study. Another limitation is that human nerve is different from rat nerve, especially in terms of size. Nonetheless, this study may provide guidance regarding the range of glucose concentrations that influence nerve function, both during the oxygenated state and during anoxia.

Additional studies would be required to determine the mechanism of the changes described above and are currently in progress, but one advantage of in vitro studies is that they remove the complexity of effects of glucose concentration on blood vessels and the inflammatory response. Although human nerves are different from rat nerves, many of the phenomena observed in this model may also be seen in human experiments.

REFERENCES