American Journal of Physiology

Glucose availability and sensitivity to anoxia of isolated rat peroneal nerve

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STRUPP, M., R. JUND, U. SCHNEIDER, AND P. GRAFE. *Glucose availability and sensitivity to anoxia of isolated rat peroneal nerve.* Am. J. Physiol. 261 (Endocrinol. Metab. 24): E389–E394, 1991.—The contrast between resistance to ischemia and ischemic lesions in peripheral nerves of diabetic patients was explored by in vitro experiments. Isolated and desheathed rat peroneal nerves were incubated in the following solutions with different glucose availability: *1*) 25 mM glucose, *2*) 2.5 mM glucose, and *3*) 2.5 mM glucose plus 10 mM 2-deoxy-D-glucose. Additionally, the buffering power of all of these solutions was modified. Compound nerve action potential (CNAP), extracellular pH, and extracellular potassium activity (a_e^K) were measured simultaneously before, during, and after a period of 30 min of anoxia. An increase in glucose availability led to a slower decline in CNAP and to a smaller rise in a_e^K during anoxia. This resistance to anoxia was accompanied by an enhanced extracellular acidosis. Postanoxic recovery of CNAP was always complete in 25 mM HCO_3^--buffered solutions. In 5 mM HCO_3^- and in HCO_3^--free solutions, however, nerves incubated in 25 mM glucose did not recover functionally after anoxia, whereas nerves bathed in *solutions 2* or *3* showed a complete restitution of CNAP. We conclude that high glucose availability and low Po_2 in the combination with decreased buffering power and/or inhibition of HCO_3^--dependent pH regulation mechanisms may damage peripheral mammalian nerves due to a pronounced intracellular acidosis.

diabetic neuropathy; pH homeostasis; extracellular ion activities; resistance to ischemia; ischemic damage

PATHOPHYSIOLOGY of diabetic neuropathy is not very well understood, and there is a great variety of hypotheses. In the present study, we have focused our experiments on the combined effects of two factors very likely involved in the development of this disorder. First, epidemiological and neurophysiological studies favor the hypothesis that high blood glucose levels promote diabetic neuropathy (7). Second, there is also good evidence that low endoneurial Po_2 due to microangiopathy and rheological abnormalities (4, 10) accompanies diabetic neuropathy (4, 16, 34). Early data in crab (23) and frog (15) nerve have shown that high glucose concentrations induce resistance to anoxia and depress postanoxic recovery. However, the precise link between hyperglycemia, low Po_2, and nerve damage remained unclear, although acidification was discussed as a possible factor. In the central nervous system, on the other hand, good evidence exists to assume that pronounced intracellular acidosis (3, 12, 21, 26) produces neuronal damage when hypoxia or ischemia occur during hyperglycemia (20, 25, 36).

The experiments of our study were designed to investigate the following questions. *1*) Can high glucose concentrations in vitro induce resistance to anoxia in a mammalian peripheral nerve? *2*) Does high glucose availability impair postanoxic recovery of nerve function? *3*) If nerves do not recover functionally after anoxia in high glucose concentrations, what causes the damage? To answer these questions, we have measured simultaneously changes in the amplitude of the compound nerve action potential (CNAP) and the extracellular K^+ activity (a_e^K) as well as extracellular pH (pH_e). To modify extra- and intracellular buffering power and HCO_3^--dependent pH regulation mechanisms, different buffer solutions were used. In one set of experiments the HCO_3^- concentration was reduced to 5 mM to imitate the in vivo situation in a "closed buffer system," when HCO_3^- stores are exhausted by complete ischemia during hyperglycemia. Kraig et al. (13) reported that, under these conditions, brain HCO_3^- is reduced to ~6.3 mmol/kg.

Our data show that high glucose availability in a mammalian peripheral nerve induces resistance to anoxia and impairs postanoxic functional recovery. We conclude from our observations that acidosis is most probably a pathogenetic factor for hypoxia-related lesions in diabetic nerves. Parts of the results have been published in an abstract form (30).

MATERIALS AND METHODS

Animals and preparation. Male Wistar rats (300–400 g) were obtained from Thomae, Biberach, FRG. Under urethan anesthesia (1.5 g/kg ip) the peroneal nerves were removed and transferred to a culture dish. Immediately after removal of the nerves the rats were killed by intracardial injection of urethane. Subsequently, the isolated nerves were desheathed and incubated for at least 3 h in different solutions. In experiments with 2-deoxy-D-glucose (2-DG) the nerves were additionally pretreated for 1 h with 10 mM 2-DG.

Solutions. The standard solution contained (in mM) 118.0 NaCl, 3.0 KCl, 1.5 $CaCl_2$, and 1.0 $MgCl_2$. The glucose concentration was either 2.5 or 25 mM. Normal HCO_3^--buffered solution consisted of the standard solution plus 25 mM $NaHCO_3$ and 1.2 mM NaH_2PO_4, bubbled with 95% O_2-5% CO_2 (normoxia) or 95% N_2-5% CO_2 (anoxia). Low HCO_3^--buffered solution consisted of the standard solution plus 5 mM $NaHCO_3$ and 1.2 mM

NaH_2PO_4, bubbled with 99% O_2-1% CO_2 (normoxia) or 99% N_2-1% CO_2 (anoxia). The osmolarity was kept constant by the addition of NaCl. N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; 6 mM) and 20.0 mM sodium gluconate were used in HCO_3^--free solutions. The normoxic HEPES-buffered solution was equilibrated with 100% O_2, and the anoxic solution was equilibrated with ultrapure argon (>99.9999%) for at least 20 min. The final pH of all solutions was 7.35 (adjusted with NaOH). At this pH the different buffer solutions have a (calculated) buffering power (22) of 57 mM (25 mM HCO_3^-), 11 mM (5 mM HCO_3^-) and 3.5 mM (6 mM HEPES). Additionally, HEPES is unable to cross the cell membrane and therefore does not contribute to intracellular buffering (32). Drugs were applied via the bathing solution. All chemicals in this study were purchased from Sigma, Munich, FRG. Ultrapure argon (argon 6.0) was obtained from Linde, Unterschleissheim, FRG.

Ion-sensitive microelectrodes. The methods used for construction and calibration of double-barreled ion-sensitive microelectrodes (tip size 1.0–1.5 μm) and their properties are described in detail elsewhere (6). For the present study, microelectrodes were drawn on a Brown Flaming micropipette puller (Sutter Instruments, San Rafael, CA). The following combinations of ion-exchanger, backfilling solution, and reference solution were used: K^+-sensitive microelectrodes (Corning 477317, 200 mM KCl, and 1 M magnesium acetate); pH-sensitive microelectrodes (Fluka Hydrogen Ion Ionophore I-Cocktail A 95291, 100 mM sodium citrate + 100 mM NaCl at pH 6.80, and 1 M magnesium acetate). Extracellular K^+ was measured as a_e^K. An activity coefficient of 0.74 was used to calculate a_e^K from defined K^+ concentrations in calibration solutions.

Experimental setup. As shown in Fig. 1, the isolated rat peroneal nerve was superfused in a closed experimental chamber (at 37°C), which allowed nearly complete exclusion of atmospheric oxygen. Gas was delivered through stainless steel tubing. The liquid flow was regulated by positive gas pressure in the buffer flask. The flow rate was 10 ml/min (volume of the experimental chamber 1.2 ml). Contact of the solution with pumps or with plastics was avoided. P_{O_2} was monitored continuously in the experimental chamber by a Clark-style electrode (Diamond Electro-Tech, Ann Arbor, MI). P_{O_2} in the solution was ~650 mmHg (95% O_2) during normoxia and fell to <0.5 mmHg within 5 min after the onset of anoxia. The nerve ends were drawn into a pair of suction electrodes that were used for stimulation (supramaximal single square voltage pulses, duration 0.05 ms, stimulation rate 1 Hz) and recording of the CNAP. The amplitude and latency of the CNAP were measured continuously by a peak detector. The ion-sensitive microelectrodes were inserted into the center of the nerves through small holes in the upper lid of the organ bath. These holes represented a potential source of oxygen during anoxia. Nevertheless, P_{O_2} levels <0.5 mmHg were reached during anoxia due to the high flow rate (10 ml/min) of the perfusion system.

Data and statistics. Data were recorded, stored, and analyzed (averaged) on a digital oscilloscope (Nicolet 4562). Because the absolute values of the amplitude of the CNAP varied between different nerves, the digitized data were normalized to the same preanoxic level. This enabled the data to be compared with respect to percentage changes in CNAP, and it also enabled us to obtain unbiased averages of data from different groups. Data are expressed as means ± SE. Statistical analysis was performed by an unpaired two-tailed t test to assess significance of differences. Differences were considered to be significant at $P < 0.05$.

RESULTS

General observations. Only one exposure to anoxia was performed on each nerve. Therefore, a standardized experimental protocol was necessary for comparison of data obtained from different nerves. Typical recordings sampled during such a standardized experiment are illustrated in Fig. 2. The data obtained were amplitude of CNAP, a_e^K, and pH_e. The nerves were stimulated once per second during the entire recording period. This stimulation frequency did not have an influence on CNAP, a_e^K, and pH_e. Under these conditions (without exposure to anoxia) the preparation was stable for >3 h (decline of CNAP amplitude <5%). The protocol for experiments with exposure to anoxia was as follows. Three minutes after the beginning of the recording the nerves were stimulated with a mean frequency of 120 Hz for 60 s (300 Hz for 400 ms/s). This stimulation led to an increase of a_e^K, a small decrease in CNAP, and no change in pH_e. Six minutes later, anoxia was started. This resulted in a slow decline of CNAP, a rise in a_e^K, and an acid-going shift in pH_e. The anoxia lasted for 30 min. Subsequently, the postanoxic recovery was observed for another 20 min. During reoxygenation under the conditions of the experiment illustrated in Fig. 2 (2.5 mM glucose, HEPES-buffered solution), a fast recovery of CNAP, a rapid fall of a_e^K, followed by an a_e^K undershoot, and a slow normalization of pH_e were observed.

The standardized experimental protocol illustrated in Fig. 2 was performed on 64 isolated rat peroneal nerves. The only modifications concerned the availability of glucose and/or the buffering power of the bathing solutions.
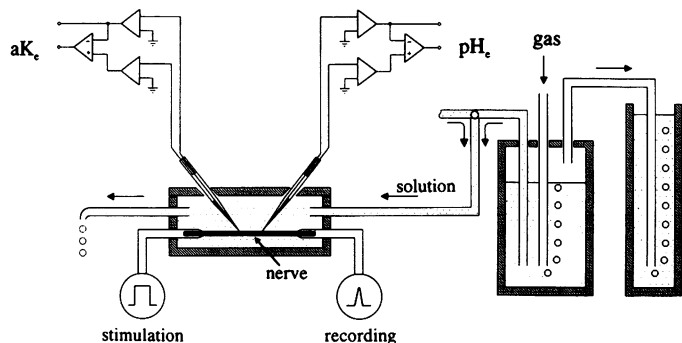

FIG. 1. Experimental setup. Ends of desheathed peroneal nerves were drawn into pair of suction electrodes, which allowed stimulation and recording of supramaximal compound nerve action potential (CNAP). Extracellular pH (pH_e) and potassium activity (a_e^K) were recorded with double-barreled ion-sensitive microelectrodes. Closed system was used to induce anoxia (see MATERIALS AND METHODS).
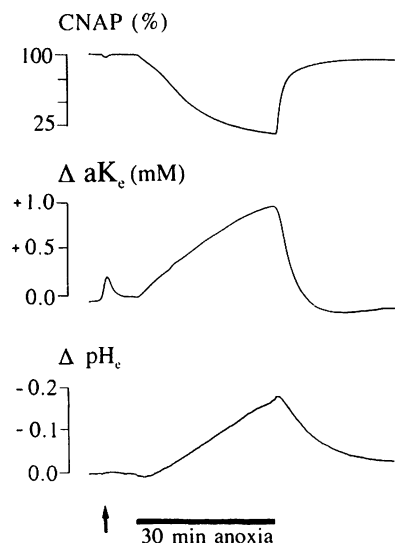
CNAP (%)

Δ aK_e (mM)

Δ pH_e

30 min anoxia

FIG. 2. Typical recordings sampled during standardized experimental protocol. Simultaneous measurements were made of amplitude, of compound nerve action potential (CNAP) normalized to 100% of changes in extracellular potassium activity (Δa_e^K), and of the extracellular pH (ΔpH_e) before, during, and after period of 30 min of anoxia. Arrow, period of intense electrical stimulation (mean frequency 120 Hz, 1 min in duration). Data stem from rat peroneal nerve incubated in HEPES-buffered solution containing 2.5 mM glucose.

CNAP during anoxia. The amplitude of the CNAP was used to quantify the sensitivity to anoxia and the postanoxic recovery. Figure 3 shows superimposed curves of the amplitude of the CNAP, normalized to 100% (the absolute values of the CNAP ranged between 3 and 10 mV and were mainly dependent on the resistance between the nerve and the recording electrode). As mentioned before, experiments were performed in solutions buffered with 25 or 5 mM HCO_3^- or 6 mM HEPES. In all cases, nerves bathed in 2.5 mM glucose displayed a marked sensitivity to anoxia. The sensitivity to anoxia was even more pronounced in the experiments with 2.5 mM glucose plus 10 mM 2-DG. In particular, a faster decline in CNAP after the onset of anoxia was found in nerves with

an inhibition of glycolysis by 2-DG (Fig. 3*D*). In contrast, the CNAP of nerves bathed for at least 3 h in 25 mM glucose showed significant resistance to anoxia compared with 2.5 mM glucose (Fig. 3, *A-C*). This long incubation period was necessary, since resistance to anoxia was not observed after acute elevation of bath glucose concentration (not illustrated). Resistance to anoxia was independent of the buffer used. In general, we found an inverse correlation between glucose availability and sensitivity to anoxia.

Postanoxic recovery of CNAP. In all cases, nerves bathed in 2.5 mM glucose showed a nearly complete or complete postanoxic recovery (Fig. 3): 15 min after reoxygenation the amplitude reached ~95% of the control value. In addition, we observed that CNAP amplitude was stable for at least 2 h after anoxia (5 experiments). Such a good recovery was also observed in nerves incubated in 2.5 mM glucose plus 10 mM 2-DG despite the faster decline in CNAP during anoxia (Fig. 3*D*).

In the set of experiments with 25 mM bath glucose the postanoxic recovery was dependent on the buffer solution used. In the solution buffered with 25 mM HCO_3^- (Fig. 3*A*) the amplitude reached 93.9 ± 2.4% (mean ± SE, n = 13) of control 15 min after the end of anoxia. However, anoxia induced in 5 mM HCO_3^-- or HEPES-buffered solutions containing 25 mM glucose caused electrophysiological damage of the nerves (Fig. 3, *B* and *C*): the amplitude of the CNAP reached only 62.0 ± 4.2% (n = 5) and 52.1 ± 5.4% (n = 9), respectively. These values are significantly smaller than the corresponding postanoxic amplitudes of the CNAP in 2.5 mM glucose. The postanoxic period of the standardized experimental protocol lasted 20 min (see Fig. 2). However, in the experiments with HEPES-buffered solutions and 25 mM bath glucose, no further improvement of nerve function was observed when the CNAP amplitude was monitored for 2 h after anoxia (5 experiments). Also, recovery was not improved when the HEPES concentration was increased

A 25 mM HCO₃⁻ / 5 % CO₂

CNAP (%)

B 5 mM HCO₃⁻ / 1 % CO₂

CNAP (%)

○ 2.5 mM glucose
● 25 mM glucose
＊ p < 0.05
＊＊＊ p < 0.001

C HEPES

CNAP (%)

30 min anoxia

D HEPES

CNAP (%)

30 min anoxia

○ 2.5 mM glucose
● 25 mM glucose
▲ 2.5 mM glucose
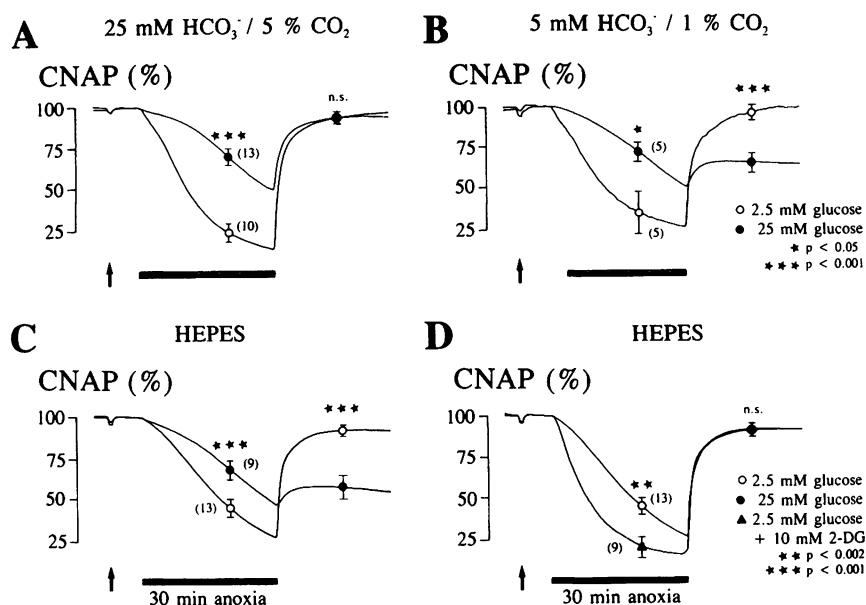   + 10 mM 2-DG
＊＊ p < 0.002
＊＊＊ p < 0.001

FIG. 3. Changes in amplitude of CNAP before, during, and after anoxia. Standard protocol described in Fig. 2 was used. Peroneal nerves were incubated in solutions containing 25 mM glucose, 2.5 mM glucose, or 2.5 mM glucose + 10 mM 2-deoxy-D-glucose (2-DG). Another variable was buffer used (25 mM HCO_3^--5% CO_2, 5 mM HCO_3^--1% CO_2, or 6 mM HEPES). Data are digitized and averaged (mean ± SE, no. of observations given in parentheses; NS, not significant).

fourfold ($n = 3$).

a_e^K. As expected, anoxia led to an increase of a_e^K, and the magnitude was influenced by the glucose availability (Fig. 4). Quantitatively, in 2.5 mM glucose, a_e^K reached a level of ~1.2 mM above resting nerve K^+ activity at the end of the 30-min period of anoxia (the resting nerve a_e^K was ~0.2 mM above bath K^+ activity). Resistance to anoxia of the nerves in 25 mM glucose was reflected by a significantly smaller change in a_e^K. This observation was made in 25 mM HCO_3^- (Fig. 4A) as well as in HEPES-buffered solutions (Fig. 4B). Inhibition of glycolysis by 2-DG, on the other hand, enhanced the initial release of K^+ during anoxia. However, at the end of the anoxic period, there was no significant difference between the solutions with 2.5 mM glucose and with 2.5 mM glucose plus 10 mM 2-DG (Fig. 4C). These data clearly show that glycolysis is important for K^+ homeostasis during anoxia. After anoxia, a_e^K in 2.5 mM glucose and in the presence of 2-DG recovered within 10 min to the baseline and showed an undershoot. Such undershoots in a_e^K have been previously reported in peripheral mammalian nerve and have been explained by the activation of electrogenic Na^+-K^+ transport (2, 5).

We also explored how glucose availability influences the stimulus-induced rise in a_e^K under normoxic conditions (see arrows in Fig. 4). Hereby, no differences were found between K^+ accumulation during stimulus trains in high or low glucose concentrations.

pH_e. Under resting conditions, pH_e in the center of the peroneal nerves was ~7.25. This value is 0.1 pH units below the bath pH. A stimulus-induced change in pH_e was never observed (Figs. 2 and 5). Measurements of changes in pH_e during and after anoxia were made in two different HCO_3^-- and in a HEPES-buffered solution. The anoxia-induced changes of pH_e ranged between 0.06 ± 0.01 (2.5 mM glucose, 25 mM HCO_3^--buffered solution;

mean ± SE, $n = 4$) and 0.31 ± 0.02 (25 mM glucose, HEPES-buffered solution, $n = 9$), depending on glucose concentration and buffer solution used. As shown in Fig. 5, the extracellular acidosis was more pronounced in HEPES-buffered solutions (Fig. 5, C and D) than in 5 or 25 mM HCO_3^--containing solutions (Fig. 5, A and B), i.e., that changes in pH_e were dependent on the buffering power of the solutions (see MATERIALS AND METHODS for calculated buffering power). It is important to note that, in all cases, 25 mM bath glucose led to a significantly stronger extracellular acidosis than 2.5 mM glucose. Nerves incubated in 2-DG, on the other hand, showed very little extracellular acidosis, even in the HEPES-buffered solution (Fig. 5D).

DISCUSSION

Effects of anoxia on peripheral nerve. The general effects of anoxia on isolated rat peroneal nerves seen in our study resemble previous descriptions given in the literature for other nerves (1, 15, 18). The rise in a_e^K has been explained as a result of a breakdown in ATP-dependent K^+ transport. The accompanying acidosis, on the other hand, seems to be the result of an enhanced anaerobic glycolysis. However, the reason(s) for the decrease in CNAP amplitude is not yet clear. There might be either a secondary effect due to membrane depolarization (15) or a direct effect of anoxia on voltage-dependent Na^+ conductance (18). Our data are based on extracellular recordings of action potentials and do not allow a precise analysis of underlying changes in electrophysiological membrane parameters.

Changes in the postanoxic period can be explained by the immediate reactivation of the Na^+-K^+ pump. This mechanism explains the rapid recovery in CNAP due to electrogenic membrane hyperpolarization (29) and also the undershoot in a_e^K (2).

Glucose availability and response during anoxia. Nerves preincubated in high glucose concentrations showed resistance to hypoxia, i.e., there was a slower decline in the amplitude of the CNAP and a diminution of the anoxia-induced rise in a_e^K (see Figs. 3 and 4). Resistance to ischemia is a well-known abnormality of peripheral nerves in diabetics (27). However, due to the complex in vivo situation, the underlying mechanisms have been a matter of debate (16, 33). Our in vitro data clearly show that tolerance to anoxia can be induced by high glucose concentrations in an isolated mammalian nerve. In frog and crab nerves, such an observation was made many years ago (15, 23). We assume that, also for human diabetic nerves, an increased glucose availability induces resistance to ischemia (16, 17, 19, 24, 29). This view is supported by our recent observation of a good correlation between ischemia-related changes in excitability of human peroneal nerve in situ and of the mean blood glucose concentration of the last 24 h before examination (29).

Resistance to anoxia could not be induced by acute elevations of the bath glucose concentration (present study, not illustrated). Similarly, in human subjects, resistance to ischemia was not observed 5–10 min after an intravenous injection of glucose (28). This indicates that intraneural glucose stores and not high extracellular
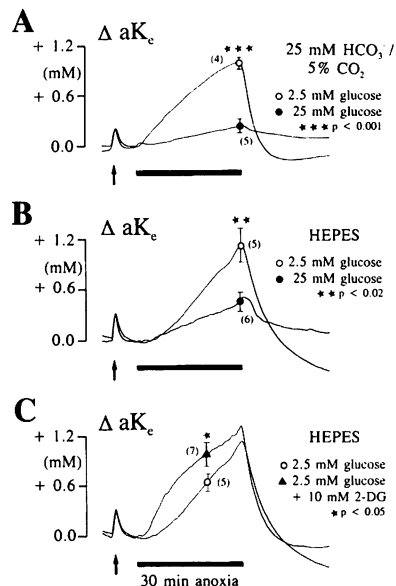
FIG. 4. Changes in extracellular K^+ activity. Standard protocol described in Fig. 2 was used. Peroneal nerves were incubated in solutions containing 25 mM glucose, 2.5 mM glucose, or 2.5 mM glucose + 10 mM 2-DG. Another variable was buffer used (25 mM HCO_3^--5% CO_2 or 6 mM HEPES). Data are digitized and averaged (mean ± SE, no. of observations are given in parentheses).
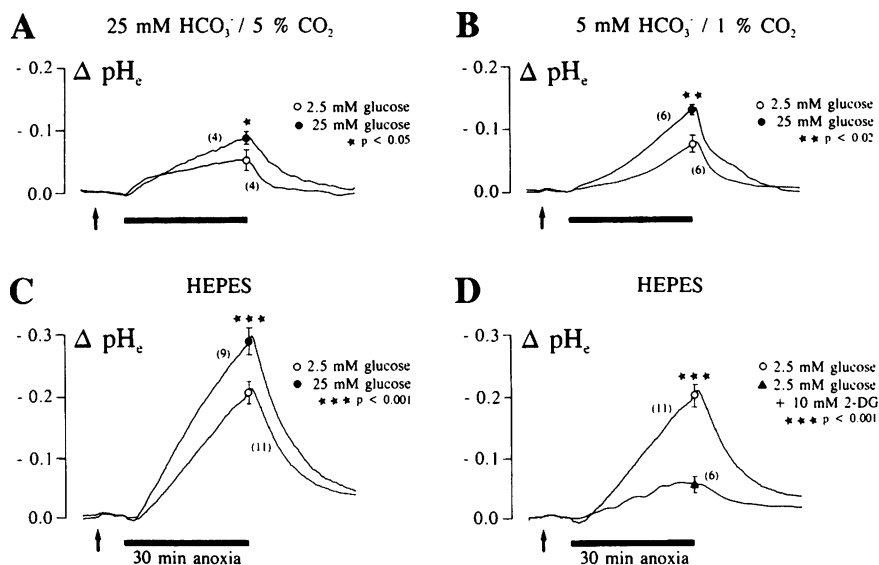
FIG. 5. Changes in pH_e before, during, and after anoxia. Standard protocol described in Fig. 2 was used. Peroneal nerves were incubated in solutions containing 25 mM glucose, 2.5 mM glucose, or 2.5 mM glucose + 10 mM 2-DG. Another variable was buffer used (25 mM HCO_3^--5% CO_2, 5 mM HCO_3^--1% CO_2, or 6mM HEPES). Data are digitized and averaged (mean ± SE, no. of observations are given in parentheses).

glucose concentrations alone are responsible for the change in sensitivity to ischemia. Energy derived from enhanced anaerobic glycolysis seems to be the main mechanism underlying resistance to anoxia under our experimental conditions. This view is supported by the following two observations: *1*) direct recordings of pH_e showed an enhanced extracellular acidosis in nerves with resistance to anoxia, and *2*) inhibition of glycolysis due to the application of 2-DG increased sensitivity to anoxia of nerves and decreased extracellular acidosis.

Resistance to anoxia was reflected by the diminution of anoxia-induced rise in a_e^K. Also in the central nervous system, a delayed rise in a_e^K has been reported when ischemia was induced during hyperglycemia (8). Quantitatively, the rise in a_e^K observed in the rat peroneal nerves during anoxia was not sufficient to explain the changes in the amplitude of the CNAP. The maximal rise in a_e^K did not exceed 1.5 mM. A passive increase in a_e^K by this amount does not markedly alter CNAP (5). Consequently, changes in nerve a_e^K during anoxia do not causally determine sensitivity to ischemia. Rather, this parameter is a consequence of sensitivity to anoxia, not the cause. Therefore, our data do not support the idea that low endoneural a_e^K, as a consequence of nerve edema (9), induces resistance to ischemia in peripheral nerve.

Glucose availability and recovery from anoxia. We found an inverse correlation between glucose availability and recovery from a period of anoxia (see Fig. 3): the higher the glucose availability the worse the postanoxic recovery. To explain this finding, we assume that enhanced anaerobic glycolysis leads to an anoxia-induced acidification capable of damaging the nerve (26). This conclusion is based on direct recordings of pH_e and on observations in low HCO_3^- or HCO_3^--free solutions. First, high glucose concentrations caused a more pronounced extracellular acidosis during anoxia than low glucose concentrations. Second, the observation that just a decrease of the HCO_3^- concentration worsened postanoxic recovery closely relates anoxic nerve damage and acidosis. Lack or reduction of HCO_3^- inhibits transmembranous pH regulation mechanisms and decreases buffering power (11, 22, 32). Both factors together should result in

stronger intracellular acid shifts during anoxia.

Our data provide evidence that intracellular and not extracellular acidosis underlies the deleterious effects of a combination of high glucose availability and low P_{O_2}. This conclusion is based on a quantitative comparison: there was a stronger anoxia-induced acid shift in nerves incubated in HEPES-buffered solutions with 2.5 mM glucose than in nerves incubated in 5 mM HCO_3^--buffered solutions with 25 mM glucose (Fig. 5). However, recovery from anoxia was worse in the second case (Fig. 3). Consequently, no direct correlation between changes in pH_e and outcome from anoxia can be made. This observation is in agreement with the general view that intracellular and not extracellular acidification causes neuronal and/or glial damage (35).

The assumption of a correlation between anoxia-induced acidosis and lack of recovery from ischemia indicates that prevention of acidosis should improve postanoxic outcome. This, in fact, is what we found. Nerves with inhibition of glycolysis due to application of 2-DG showed a full recovery in CNAP after anoxia, although they were more sensitive during ischemia. Also, in in vivo studies, an improvement of postischemic outcome by inhibition of glucose uptake and glycolysis has been described (14).

Experiments in rat optic nerve have recently indicated that the availability of extracellular Ca^{2+} determines the functional recovery from anoxia (31). Such a mechanism may be a general factor underlying effects of hypoxia. However, we do not think that this mechanism contributes to the differences between postanoxic outcome in hypoglycemic and hyperglycemic nerves, since high glucose availability reduces sensitivity to anoxia. It is very likely that such nerves also gain less Ca^{2+} during anoxia. Yet, there is bad postanoxic recovery.

Relevance to diabetic neuropathy. Peripheral nerves of diabetic patients show a paradoxical contrast between their resistance to ischemia and their liability to ischemic lesions (16). The underlying mechanism(s) is not very well understood. However, the results presented here closely relate glucose availability, acidosis, and postanoxic recovery. We suggest that acidification due to en-

hanced anaerobic glycolysis in high glucose concentrations may contribute to the damage. We have used low HCO_3^- or HCO_3^--free bathing solutions in our in vitro experiments to provoke postanoxic electrophysiological damage after a short period of anoxia. However, we do not think that in vivo low buffering power or lack of HCO_3^- "before" ischemia is a prerequisite for ischemia-induced nerve damage, since tissue HCO_3^- is exhausted "during" longer-lasting hyperglycemic complete ischemia (13). In diabetic patients, a hazardous situation exists. Resistance of their peripheral nerves to ischemia (lack of paresthesia) results in a decreased sensation of nerve hypoxia due to pressure and/or ischemia. Therefore, in contrast to normoglycemic subjects, nerves of diabetics may be exposed much longer to ischemia. Under such circumstances, acidosis may reach dangerous levels due to a long-lasting activation of anaerobic glycolysis and to a decrease in the nerve's capability to regulate pH due to a use-dependent HCO_3^- decrease. Very likely, such a mechanism contributes to abrupt onset ischemic lesions in diabetics. However, it may well contribute to the development of diabetic neuropathy in general.

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