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Some effects of P_{CO_2} and pH on nerve tissue

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1. Rat phrenic nerve preparations were used *in vitro* to study action potential amplitude and nerve conduction time under varying conditions of P_{CO_2} and sodium bicarbonate concentration in the surrounding Krebs solution.

2. A correlation was found between action potential amplitude and pH but not between action potential amplitude and P_{CO_2}.

3. A better correlation was found between conduction time and pH than between conduction time and P_{CO_2}.

Since 1896, when carbon dioxide was shown to modify nerve action potentials *in vitro* (Waller, 1896), there have been many papers describing experiments evaluating its physiological effects. There was general agreement among early workers that the addition of carbon dioxide to the " gassing mixture " increased the action potential amplitude ; in some cases concentrations in excess of 50% F_{CO_2} were used (Necheles & Gerard, 1930). It has been suggested (Lehmann, 1937) that the change in action potential was mediated by pH, but no distinction was made between pH and the direct action of carbon dioxide on the nerve. More recently, Carpenter (1963) found no significant difference in action potential amplitude when his preparation was gassed with pure oxygen as compared with a 92% oxygen and 8% carbon dioxide gas mixture, and de Jong & Wagman (1963) stated that the extracellular pH can carry from 5.5 to 8.0 pH units before an appreciable effect is seen on the action potential, conduction velocity and resting potential. It was decided to determine the action of carbon dioxide and of pH on the action potential amplitude and conduction time in a peripheral medullated mammalian nerve at its normal *in vivo* temperature.

Methods

Female hooded Lister rats weighing between 250 and 400 g were killed by a blow on the head and bled. Within 10 min the left phrenic nerve was removed and mounted on four platinum hook electrodes suspended in a tissue bath which contained 60 ml. of Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, (+)-glucose 10.1, $NaHCO_3$ (see text).

The tissue bath was maintained at a predetermined and thermostatically controlled temperature (37° ± 1° C) by the surrounding water bath, and the preparation was continually gassed with mixtures of carbon dioxide and oxygen from cylinders. The F_{CO_2} of each mixture was determined by the Haldane apparatus and the P_{CO_2}

calculated from the barometric pressure assuming 47 mm Hg saturated water vapour pressure in the tissue bath. The gas flow was measured by a rotameter calibrated for oxygen and it was maintained at 200 ml./min. The gas was piped through the water bath to allow temperature equilibration before entering the tissue bath.

The concentration of sodium bicarbonate in the Krebs solution was kept constant throughout each experiment, but four different concentrations were used in the sixteen experiments to be described, namely, 6.25 mM, 25 mM, 50 mM and 100 mM.

The preparation was stimulated via two platinum electrodes by a rectangular electrical impulse derived from a Palmer D 45 stimulator set to deliver a supramaximal stimulus of 60 V for 20 μsec repeated every 2 sec. The action potential was picked up by two further platinum electrodes and the signal was fed into a cathode follower and an oscilloscope, where the trace was photographed.

To minimize time-dependent effects, each experiment followed a Latin square format, the variables being the different concentrations of carbon dioxide in oxygen used to gas the preparation. Before a recording was made the gas was passed through the tissue bath for 15 min, after which time equilibration had occurred.

One minute before a recording was made the Krebs solution was drained from the tissue bath ; it was replaced by fresh Krebs solution at 37° C after the recording had been made.

The pH of the experimental Krebs solution was measured in an Astrup capillary glass electrode and compared with the theoretically derived pH using the Henderson–Hasselbalch equation.

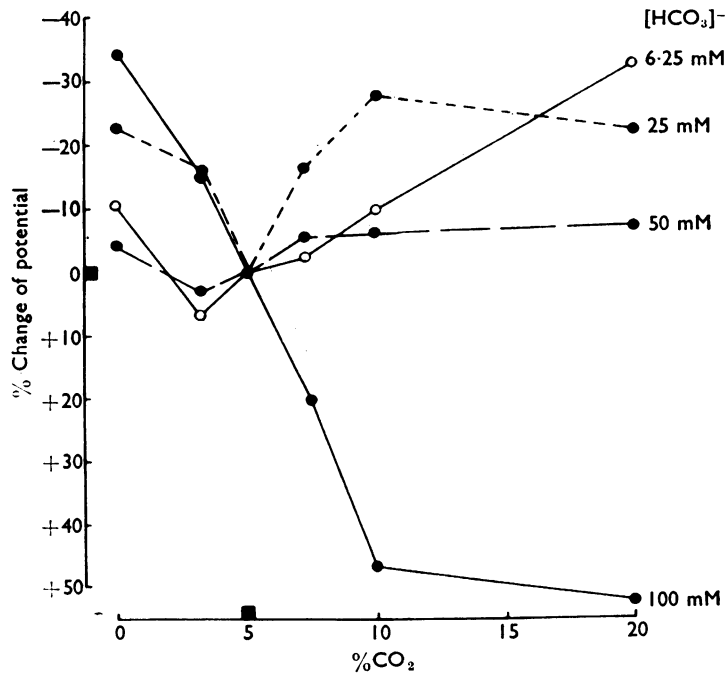

FIG. 1. Graph of percentage CO_2 in gassing mixture and percentage change in action potential amplitude; control value taken to be the 5% CO_2 potential. Isobicarbonate points connected.

Results

Action potential amplitude

The action potential amplitudes of one experiment were not directly comparable with those of a similar experiment due to certain non-reproducible factors, such as nerve diameter, and so the amplitudes are expressed as percentages of control values.

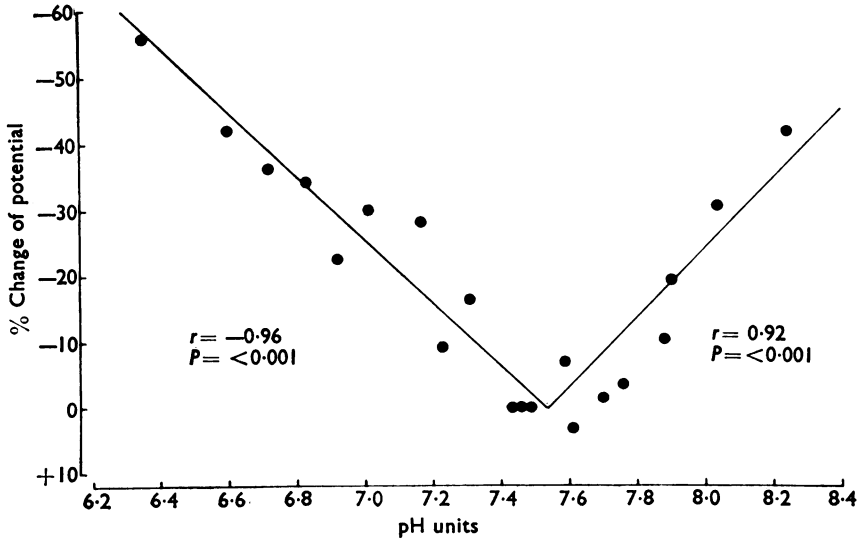

FIG. 2. Graph of pH of Krebs solution and percentage change in action potential amplitude; control value taken to be at pH 7.45. Two regression lines are drawn for the experimental points below and above the control value.

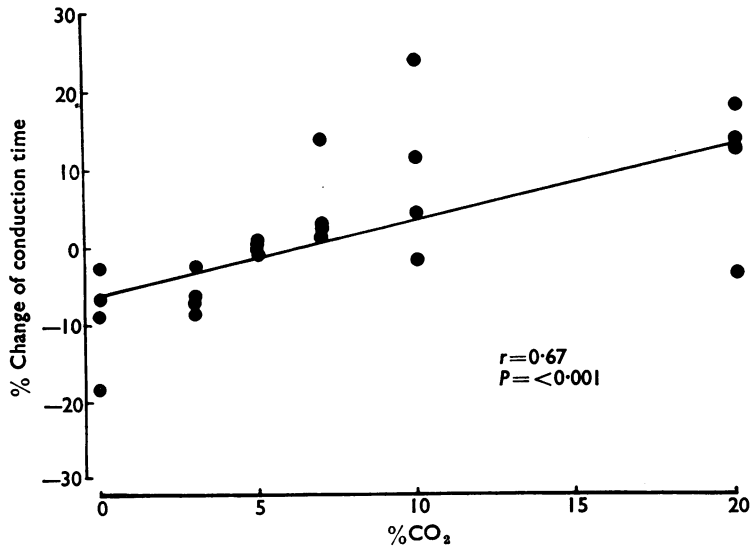

FIG. 3. Graph of percentage CO_2 in gassing mixture and percentage change of conduction time; control value taken to be the 5% CO_2 conduction time. Regression line is drawn.

In Fig. 1 the deviation of the action potential amplitudes from the control value (with 5% F_{CO_2}) is plotted against the percentage of CO_2 in the gassing mixture. Each point represents the mean of eight independent readings taken from two nerve preparations and connecting lines have been drawn between points representing identical bicarbonate concentrations in the Krebs solution. Because there was no correlation with F_{CO_2}, the control action potential amplitude was taken in each experiment to be that obtained when the pH of the Krebs solution was closest to the physiological norm of 7.45 pH units, and the other amplitudes expressed as a percentage of this control value.

A close correlation was found for pH and action potential amplitude providing the pH scale was divided, and regressions for values above and below pH 7.45 can be seen in Fig. 2. For the lower pH values the correlation coefficient $r = -0.96$ and $P<0.001$, and for the higher pH values $r=0.92$ and $P<0.001$.

Conduction time

A measure of the conduction time was taken as the distance between the stimulus artefact and the peak of the action potential.

A correlation between the percentage alteration of conduction time and F_{CO_2} was again sought, taking the 5% CO_2 conduction time as the control value. This gave a coefficient of correlation (r) of 0.67 with $P<0.001$. Figure 3 shows this relationship, and although the scatter is appreciable there is an obvious trend.

If now the control conduction time in each experiment is taken to be that obtained with a Krebs solution having a pH closest to 7.45 pH units, an improved correlation is found ($r = -0.86$ and $P<0.001$) as shown in Fig. 4.

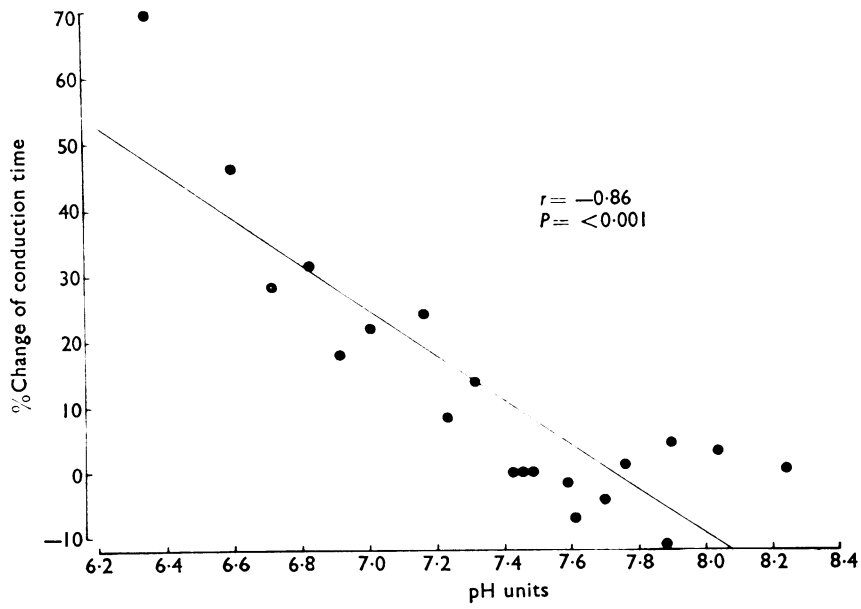

FIG. 4. Graph of pH of Krebs solution and percentage change of conduction time; control value taken to be at pH 7·45. The regression line is drawn.

Discussion

In order to demonstrate the relationship between pH and action potential amplitude it was convenient to construct the two rectilinear regressions shown in Fig. 2. It is not suggested that these regressions represent the true relationship, which might be better expressed using a continuous curvilinear regression. Rectilinear regressions were used for mathematical simplicity and because the correlation was good.

The regression line shown in Fig. 4, however, does not correlate so well. A better correlation can be obtained when a curve is fitted to the experimental points using the least squares technique. If the influence of the two extreme points is removed, the rectilinear correlation appears more satisfactory, especially in the low pH range. In the high pH range the conduction time appeared to be uninfluenced by pH change.

The importance of maintaining a constant extracellular pH during *in vitro* tissue experiments rather than a constant P_{CO_2} is apparent. Maintenance of constant pH during an experimental procedure, possibly involving the use of drugs, cannot be achieved by changing the sodium bicarbonate concentrations of the Krebs perfusate unless there is an equimolar decrease in the sodium chloride concentration to keep the sodium ion concentration unaltered. It is easier in practice to vary the F_{CO_2} of the gassing mixture.

Change in temperature of *in vitro* tissue preparations cause significant changes of pH if the P_{CO_2} and $[HCO_3]^-$ are kept constant, due to the temperature dependence of the dissociation constant of carbonic acid and the solubility of carbon dioxide.

The action potential amplitude is reduced when the pH either falls below or rises above the normal range whereas the conduction time is altered only by a fall in pH.

These phenomena point to an independence between the action potential amplitude and conduction time and it would be incorrect to assume a common mechanism just because both variables are dependent on one parameter (pH).

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