NERVE CONDUCTION WITHOUT INCREASED OXYGEN CONSUMPTION; THE ACTION OF AZIDE AND FLUOROACETATE*

by

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The precise correlation of an extra oxygen consumption of active nerve with an extra heat production was established nearly a quarter of a century ago by one of us in PROF. MEYERHOF's laboratory. It is an especial pleasure to report the present extension of such studies, in his honour. Nor can we refrain from an expression of admiration for his continued vigour of thought and research despite a weight of personal disaster that would have crushed most men.

That the extra energy release of nerve activity is essential to conduction and recovery was taken for granted since its discovery. With energy sources blocked by oxygen lack or IAA poisoning, conduction failed. With tetanization at a rate to limit full development of the delayed heat and oxygen consumption, conduction was depressed. Restoration of full metabolism restored full conduction in all cases. The actual fuel burned proved not identical for rest and activity. True, both resting and active metabolism seemed to focus on the production of energy-rich phosphate bonds, especially as creatine phosphate. And true, also, that the procedures that blocked conduction affected resting as well as active respiration. Nonetheless, there seemed no reason to question the essential contribution of the active respiration to actual conduction. A tentative report by SCHMITT, of a fall in oxygen consumption on stimulation of yohimbinized nerve, was given little weight; and LORENTE DE NO's finding, that excitation could be restored in a nerve blocked by anoxia, with the aid of a repolarizing current, did not really question the necessity of the metabolism as a normal source of membrane polarization.

Yet it was early shown by FENG and in this laboratory that lactate, indifferent to nerve conduction and metabolism under normal conditions, could restore resting oxygen consumption and active conduction after IAA poisoning — suggesting some interchangeability of resting and active metabolic energy. Further, 90 to 97% of the energy of activity is liberated after an impulse has traveled and the nerve again reset for action. Moreover, a factor of safety of five for the resting metabolism could be estimated. Activity might, then, be supported under emergency conditions by a portion of the resting metabolism. Sodium azide, found by STANNARD to eliminate the contraction respiration of muscle, was tested on nerve in BRONK's laboratory and here and found indeed able to abolish the extra oxygen consumption of active nerve while leaving conduction intact and resting respiration largely so. We found, further, that methyl

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fluoroacetate can reduce the resting oxygen consumption below half normal while leaving conduction and the attendant respiration increase intact. Resting and active respiration are thus sharply separable, yet they are effectively interchangeable in support of function.

For these studies, a modified GERARD-HARTLINE capillary respirometer was developed. Ten slots in a plexiglass block served as nerve chambers, each fitted with stimulating and lead-off electrodes. Capillaries led from each into a large chamber machined in the same block, the whole being covered with a plexiglass sheet and mounted in a glass-walled water bath. The movement of dodecane indicator drops in the capillaries was followed with a horizontal microscope mounted on the compound rest of an 11 inch lathe. Stimuli at 120/sec gave an action spike of about 25 mm measured on the cathode ray tube face.

The resting Q_{O_2} of twenty four pairs of frog sciatics at 24° C (22 to 26) centered around 65 and the two nerves of a pair agreed within 12% (aver. 4%) in all but three cases. The increased Q_{O_2} on maximal stimulation averaged 21, but with an average difference between members of a pair of nearly 30%. The coefficient of correlation between spike height and activity Q_{O_2} was only 0.4 for 67 normal nerves, and that between resting Q_{O_2} and the active increase, —0.1. Even allowing for methodological errors, these data suggest some real independence of the three variables.

In ten experiments with Na azide (0.1 or 0.3 mM, p_H 7.5, 1 hour soak), spike height of the exposed nerves averaged 88% of their undrugged partners, while the Q_{O_2} increase on tetanization was only 12% of the normals. In four experiments with spike height in both nerves of a pair alike, the Q_{O_2} increase in the azide member was 0 or 1. Even these azide concentrations do not fully spare the resting metabolism, which was depressed by 0 in 4 experiments to some 50% in 2. When resting oxygen was cut in two and the active increase abolished, spike height was greatly reduced. Stronger azide (5 or 10 mM) cut resting Q_{O_2} to 20–35% of normal and stopped conduction. Full conduction without increased Q_{O_2} is possible for at least 4 hours.

In 11 experiments with MFA (1 to 2.5 mM), the spike height and the extra Q_{O_2} of activity remained entirely normal in the exposed nerves, while the resting Q_{O_2} was depressed 25% on the average, one third maximum. This depression cannot be solely of non-axonal tissue (*e.g.*, SCHWANN cells), for fiber thresholds rise acutely. With stronger MFA (13 experiments at 5 or 7.5 mM), resting and active Q_{O_2} were both cut to about half and spike height to under two-thirds normal. In individual cases, the active spike and Q_{O_2} were essentially normal with resting Q_{O_2} depressed to one-third; in one case activity responses remained normal for 7 hours with resting Q_{O_2} at 50%. More usually with resting Q_{O_2} cut in half the active increase was also abolished while spike height remained close to normal.

A nerve can thus continue to conduct for hours with no increase in oxygen consumption and even with some half its resting respiration lost. Whether other energy sources are being tapped or even whether the small initial heat persists without delayed heat under such drug action, could be determined by heat measurements; but it seems most likely that the extra energy for activity is somehow derived from the resting metabolism by virtue of the considerable safety factor normally present.

SUMMARY

Using a modified GERARD-HARTLINE capillary respirometer the resting respiration of frog nerve at 24° C was measured, Q_{O_2} 65, as well as the increase on tetanization at 120/sec, Q_{O_2} 21, and the

action spike. Azide (0.1–0.3 mM) can abolish the activity increase of oxygen consumption while leaving intact (sometimes) the resting level and conduction. Methylfluoroacetate (2 mM), conversely, can reduce the resting oxygen consumption below half while leaving intact the activity increase and conduction. Resting and active metabolism are thus separable and conduction can continue at least seven hours with no extra respiration and even with half depression of the resting level.

RÉSUMÉ

Au moyen d'un respiromètre capillaire GERARD-HARTLINE modifié, on a mesuré à 24° la respiration de nerfs de grenouille au repos (Qo_2 65), son augmentation par tétanisation à 120/sec (Qo_2 21), et la "pointe" d'action. L'ion N_3 (0.1–0.3 mM) peut abolir l'accroissement de consommation d'oxygène dû à l'activité, tout en laissant intacts (parfois) le niveau du repos et la conduction. Le fluoracétate de méthyle (2 mM) par contre peut réduire la consommation d'oxygène au repos de plus de la moitié tout en laissant intacts l'accroissement dû à l'activité et la conduction. Le métabolisme au repos et pendant l'activité sont ainsi séparables, et la conduction peut continuer pendant au moins 7 heures sans respiration supplémentaire et même avec un abaissement de moitié du niveau du repos.

ZUSAMMENFASSUNG

Mittels eines abgeänderten GERARD-HARTLINE Kapillar-Respirometers wurde die Atmung des ruhenden Frischnervs bei 24° gemessen (Qo_2 65), desgleichen die Steigerung durch Tetanisierung bei 120/sek. (Qo_2 21) und die "Wirkungspitze". Azid (0.1–0.3 mM) kann die Steigerung des Sauerstoffverbrauchs bei der Arbeit unterdrücken, während der Verbrauchsspiegel bei Ruhe (manchmal) und die Übertragung unverändert bleiben. Methyl-fluoracetat (2 mM) dagegen kann den Sauerstoffverbrauch bei Ruhe unter die Hälfte herabdrücken, während die Steigerung bei Arbeit und die Übertragung unberührt bleiben. Ruheumsatz und Arbeitsumsatz sind also trennbar, und die Übertragung kann mindestens 7 Stunden lang fortbestehen ohne zusätzliche Atmung, und sogar mit einem auf die Hälfte herabgeminderten Ruhespiegel.

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