Energy-Rich Metabolites in Stimulated Mammalian Non-Myelinated Nerve Fibres

A particularly high rate of metabolic turnover is expected to be found in mammalian non-myelinated nerve fibres, because of their small diameter and their consequently high surface to volume ratio (see [1]). Electrical activity in these fibres is thus followed by a long lasting period of increased heat production (recovery heat)[2], and oxygen consumption[3]. The purpose of this study was to measure the concentration of energy-rich metabolites during this period.

Vagus nerves of rabbits were used. These nerves are known to contain a large proportion of non-myelinated fibres (C-fibres)[1]. The nerves were desheathed, divided into 2 equal parts, mounted on platinum electrodes, stimulated with single shocks and shown to conduct impulses by monitoring the action potential. In the first series of experiments one of the preparations was stimulated for 15 sec, while the other served as a non-stimulated control. In the second series of experiments one of the preparations was stimulated for 2 periods of 15 sec and the other for 1 period of 15 sec. The stimulation frequency was 50 shocks/sec, the stimuli were supramaximal for C-fibres. For the analysis of metabolites, the nerves were plunged into boiling buffer solution, rapidly cooled, homogenized and deproteinized. After centrifugation, the supernatant was analysed for ATP and ADP by enzymatic fluorimetric methods, as described by GREENGARD[4,5].

Resting nerves were found to contain 1.86 $\pm$ 0.11 μM ATP/g wet weight (28 experiments) and 0.23 $\pm$ 0.02 μM ADP/g (8 experiments), (see also [6]). The ATP content at various times after a period of stimulation is shown in Figure 1, in which the amount of ATP in the stimulated nerve is expressed as a percentage of that found in the non-stimulated controls. Immediately at the end of the period of stimulation, the ATP level fell to 83.4%. When the ATP was measured some time after the tetanus, a further decrease was found: 40 sec after stimulation the ATP content was lowered to 30%. With longer resting periods ATP concentration increased again and returned, within 25 sec, to the resting level. The ADP concentration, on the other hand, increased during the post-tetanic period (Figure 1), and 30 sec after the end of the stimulation the content rose to 145% of the amount in non-stimulated controls. After a longer resting period the ADP decreased and tended to return to the resting level.

Figure 2 illustrates the results of the second series of experiments in which the effects of 2 periods of stimulation were studied. In these experiments the nerves were re-stimulated after a resting interval of variable duration, and the amount of ATP immediately after the second period of stimulation was compared to that after the first. The experiments showed that the second stimulation produced a further decrease in the ATP content, which was lowered to 15% when the 2 periods of stimulation were 15 sec apart. With longer resting intervals, progressively higher levels of ATP were found.

In Figure 3 the ATP after the second period of stimulation is expressed as a percentage of the non-stimulated controls (full circles). This way of calculation shows that the ATP was lowered to 5% of the resting level when the 2 periods were 25 sec apart. With increasing resting periods

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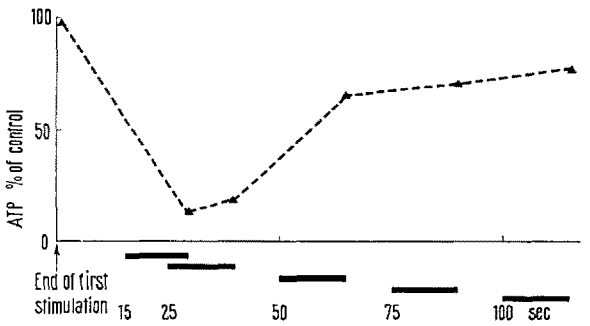

Fig. 2. ATP content in desheathed rabbit vagus immediately after two 15 sec periods of stimulation at 50 shocks/sec (———). The intervals between the 2 periods were varied as illustrated. Ordinate indicates ATP (means of 2 experiments) after the second stimulation in % of the ATP after a single period of stimulation. Temperature, 37 °C.

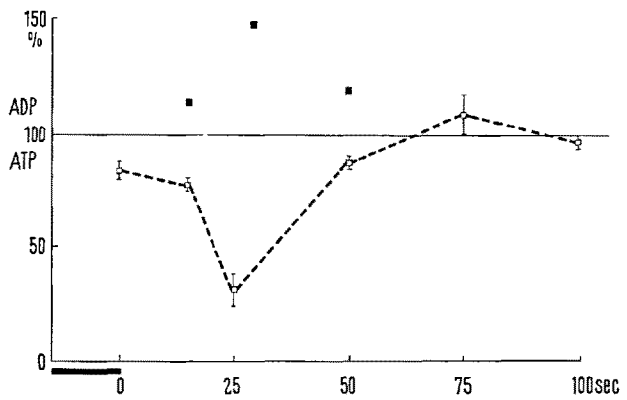

Fig. 1. ATP (□) and ADP (■) content in desheathed rabbit vagus at various times after a 15 sec period of stimulation at 50 shocks/sec (———). ATP means and S.E. of 5–9 experiments, ADP means of 2 experiments calculated in % of non-stimulated controls. Temperature, 37 °C.

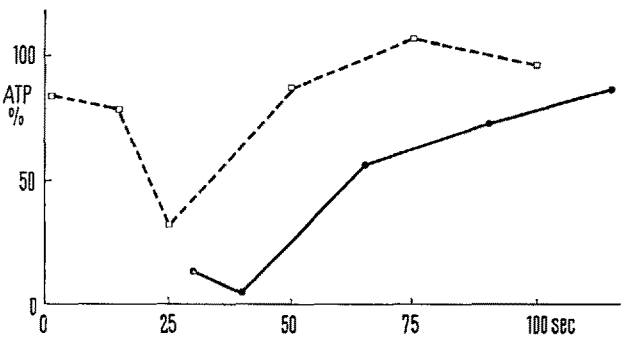

Fig. 3. ATP content (●) immediately after the second of two 15 sec periods of stimulation in % of resting controls, calculated from Figure 1 and 2. ATP (□) after a single period of stimulation in % of resting controls, as in Figure 1. Stimulation frequency 50/sec. Temperature, 37 °C.

the ATP concentrations after the second period of stimulation were larger, and the amount found with a resting interval of 100 sec was close to the value immediately after a single period of stimulation. For comparison, the ATP after one period of stimulation is also plotted in Figure 3.

The experiments demonstrate the utilization of ATP during electrical activity and during the post-tetanic period. The peaks of ATP degradation and ADP synthesis correspond approximately to the peaks of heat production and oxygen consumption. The experiments further show that ATP is quickly resynthetized. This rapid synthesis is suggested also by the experiments of GREENGARD and STRAUB[7], who measured the ATP concentration in vagus nerve trunks immediately after a 15 sec tetanus, and found no significant change in ATP with stimulation frequencies of 6 and 15 c/sec. Our experiments with 2 periods of stimulation demonstrate that the ATP concentration can be depressed to extremely low levels. This large depression suggests that, in addition to the non-myelinated axons, myelinated fibres as well as Schwann cells participate in the increased utilization of ATP after electrical activity[8].

Résumé. Le métabolisme des esters phosphorés riches en énergie a été étudié dans des fibres non-myélinisées pendant la période post-tétanique. Après une période de stimulation, le stock d'ATP est utilisé dans une proportion de 70%, puis, une rapide resynthèse a lieu en 90 sec. On constate également une augmentation concommittante de la teneur en ADP. Les pics de dégradation de l'ATP et de synthèse de l'ADP correspondent approximativement à ceux du dégagement de chaleur et de la consommation d'oxygène. Les expériences effectuées avec 2 périodes de stimulation montrent que le contenu en ATP peut s'abaisser à un niveau très bas. Cette importante diminution suggère une participation possible des cellules de Schwann au métabolisme post-tétanique.

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Effect of X-Rays on the Proteolytic Enzymes of Granulocytes and on Phagocytosis Index in Guinea-Pigs

In a previous communication[1] it was reported that the cytostatic agents decrease fibrinolytic and proteolytic enzymes of granulocytes but they do not affect phagocytosis index.

It is generally accepted that irradiated animals are more susceptible to the infection than non-irradiated ones. Numerous reports have been undertaken to elucidate the effects of irradiation on phagocytosis[2-4]. BERCOVICI[4] reported that irradiation depresses the phagocytic activity of leucocytes in both in vivo and in vitro experiments but it did not affect the proportion of cells exhibiting phagocytosis.

Migratory capacity of phagocytic cells was not affected by irradiation, but the proliferation of these cells was depressed[5]. No reports appeared which describe the effect of irradiation on the activities of fibrinolytic and proteolytic enzymes of the leucocytes. The purpose of this work was to investigate this problem.

Seventeen guinea-pigs of both sexes (weight 300–400 g) were used in these experiments. Peritoneal exudates were induced by injection of 0.1% solution of glycogen in amounts of about 70 ml. The exudates were collected 15 h after injection of glycogen. Phagocytosis index was calculated according to DAVIES' methods[6], using light microscopy. PMN leucocytes from exudates were mixed in tube with opsonized strain of staphylococcus 209 P. 100 cells were counted from each sample.

Whole-body irradiation of guinea-pigs was carried out in plastic cages at a target distance of 50 cm operated at 160 kVp, 10 mA, with a filter of 5 mm Cu and 10 mm Al at the rate of 23.2 rpm. Whole dose was 350 r.

The control group comprised 9 animals. PMN exudate obtained from these animals were considered as controls. The leucocyte count of all samples amounted to 10,000 per mm^3. Homogenate of the leucocytes were dialysed against phosphate buffer at 4 °C for 18 h. The activity of alkaline and acid leucocyte protease was determined by the caseinolytic method. The fibrinolytic enzymes: plasminogen, plasminogen activator and spontaneous fibrinolytic activity were studied, using ASTRUP and LASSEN plate method. Details concerning preparation of leucocyte homogenate and proteolytic and fibrinolytic methods are presented elsewhere[7].

The Table shows that irradiation induces a significant decrease of the activities of all investigated fibrinolytic and proteolytic enzymes. This decrease was statistically significant $p < 0.01$ when 9 samples of control leucocytes were compared with 8 samples of leucocytes collected from irradiated animals.

Phagocytosis index was slightly decreased but this change is not statistically significant, except in animals in which leucocytes were collected 20 days after irradiation.

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