CONTINUOUS Sr^{2+}-INDUCED OSCILLATIONS OF THE IONIC FLUXES IN MITOCHONDRIA

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Received 30 March 1976

1. Introduction

Investigation of oscillation reactions in mitochondria [1–5] may serve as a promising way to study the control of oxidative phosphorylation as was so with glycolysis. However the stability and the ability to experimentally regulate mitochondrial oscillations still does not reach the levels available for glycolytic oscillations. In this paper the oscillations of K^+ and H^+ fluxes in rat liver mitochondria which proceed in the form of well-correlated quasi-harmonic and saw-tooth curves are described. Oscillations are induced by addition of Sr^{2+} in the presence of small amounts of valinomycin and rotenone and may be sustained for an hour at physiological pH. The determination of certain narrow ranges conditions such as combinations of specific Sr^{2+}, valinomycin and rotenone concentrations with the level of $[K^+]$ and pH, variations in the substrate and energy supply allows one to obtain well-pronounced oscillations with the period of 2–15 min under different conditions.

2. Materials and methods

Rat liver mitochondria (RLM) (Wistar, males, 200–250 g) were isolated in 0.3 M sucrose and 0.01 M Tris-HCl, pH 7.5. RLM were washed resuspended in the same medium (40 mg mitochondrial protein (MP) per ml) and kept at + 4°C. The measurements were done using a NaAl-2704 glass K^+-selective electrode and a glass pH-electrode in an open cell (total volume 2 ml, surface about 1 cm^2) with constant mixing.

3. Results

Examples characteristic of the prolonged oscillations are given in fig.1 and in fig.2(3), fig.3(2), and fig.5. They represent the simultaneous recording of $\log[K^+]$ and pH changes in RLM suspension induced by Sr^{2+} addition. The oscillations have a period (T) of 4–7 min and continue for 40–60 min. A more or less prolonged increase of $[K^+]$ oscillation amplitude (A) is observed which indicates the self-oscillating character of the processes. The oscillations begin with intensive output of K^+ and H^+ from RLM in response to Sr^{2+} addition. At a certain moment K^+ efflux is changed to spontaneous K^+ influx and the variations in $[K^+]$ and $[H^+]$ become oscillatory. The oscillations of the K^+ fluxes are quasi-harmonic whereas the H^+ fluxes have a saw-tooth shape. H^+ pumping from mitochondria occupies nearly the whole T, starting

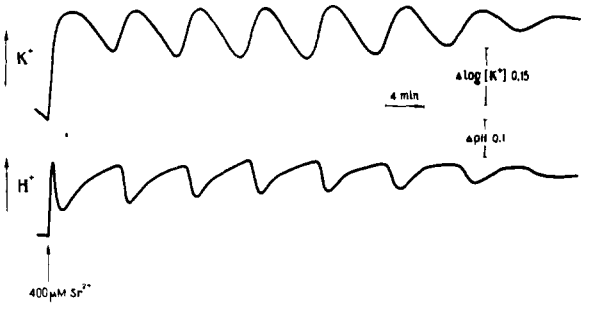

Fig.1. The Sr^{2+}-induced oscillations of K^+ and H^+ ion fluxes in rat liver mitochondria. The basal medium: sucrose (20 mM), KCl (1 mM), Tris (12 mM). Additions: succinate (5 mM), valinomycin (4 ng/mg MP), rotenone (4.8 ng/mg MP), MP (2 mg/ml), t = 22°C, pH = 7.5.

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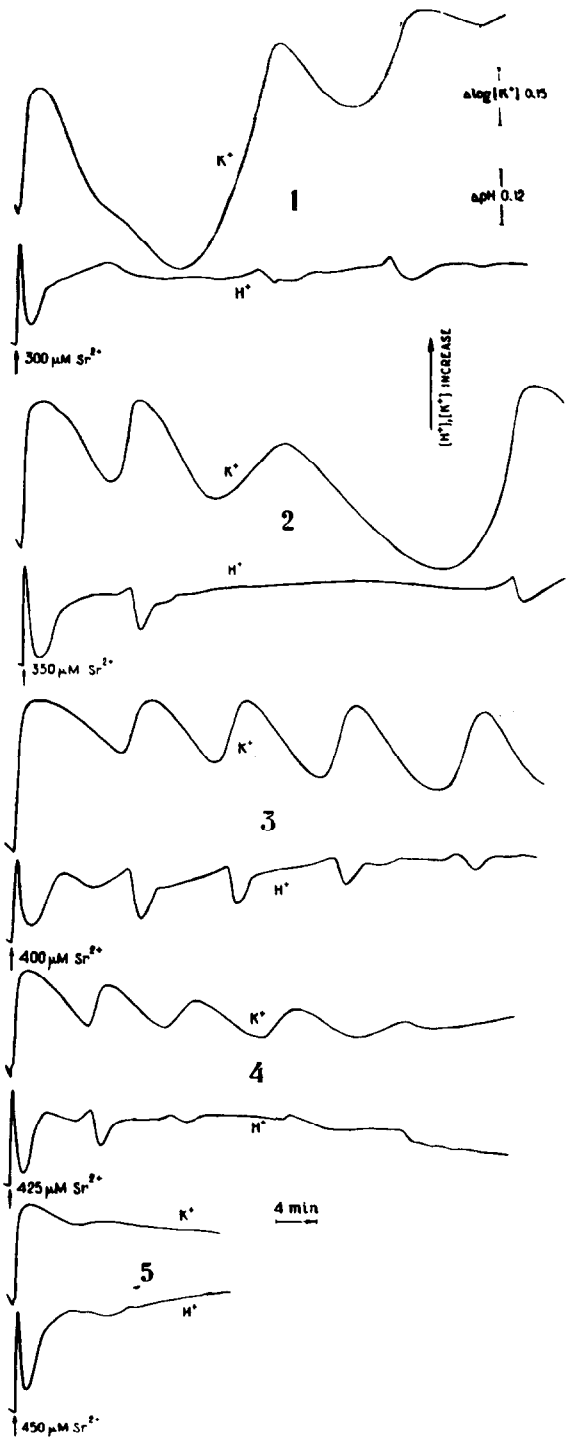

Fig.2. The dependence of oscillations on $[Sr^{2+}]$. Basal medium, MP, t, pH and succinate as in fig.1, Valinomycin (8.3 ng/mg MP).

rapidly and then slowing down. The sharp and short influx of H^+ lasting less than 0.2 T completes the period. The comparison of K^+ and H^+ fluxes during the cycle shows that H^+ influx coincides with the maximum rate of K^+ efflux, whereas the main bulk of K^+ input corresponds to the slow-rate H^+ pumping from mitochondria.

3.1. *Sr^{2+} dimensionless concentration*

The oscillation parameters depend greatly on the ratio between the quantities of Sr^{2+} and RLM (fig.2). A decrease of $[Sr^{2+}]$ results in an increase of T. The amount of Sr^{2+} necessary to obtain well-pronounced oscillations with minimal damping can be determined by α, the dimensionless ratio of Sr^{2+} to mitochondrial protein. For pH 7.5, KCl 1 mM, T = 22°C $\alpha = 0.018 \pm 0.0013$ for RLM preparations with MP 1–4 mg/ml. The value of α depends on both $[K^+]$ and $[H^+]$. For a particular RLM preparation for $[K^+]$ of 0.3–1.0 mM, $\alpha = 0.019$–0.017.

Increasing pH influences α like a decreasing $[Sr^{2+}]$. The corresponding values of pH and α are: 7.0–7.7 and 0.023–0.015.

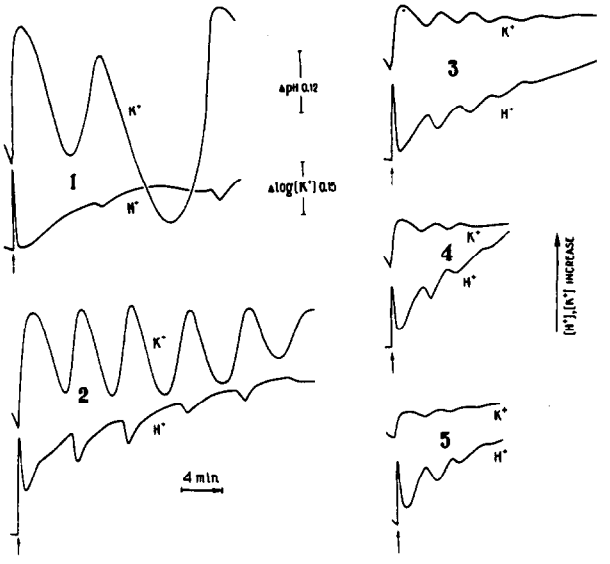

Fig.3. The dependence of oscillations on $[K^+]$. pH, MP, succinate and basal medium as in fig.1 except for K^+ and sucrose. KCl and sucrose (both in mM) for curves 1,2,3,4,5 was 0.7, 1.0, 2.0, 3.0, 5.0, and 20, 20, 18, 16, 12 respectively. Valinomycin (10 ng/mg MP), t = 30°C. Additions of 370 μM $SrCl_2$ are indicated by arrows.

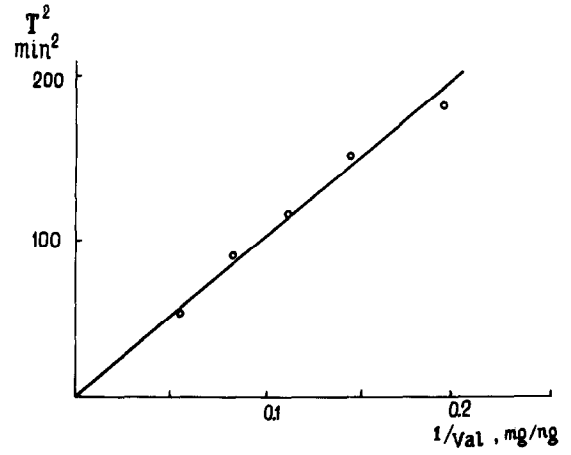

Fig.4. Plot of T^2 on the inverse specific valinomycin concentration. The conditions as in fig.1 except for absence of rotenone.

3.2. *Valinomycin specific concentration*

The plot of T^2 against the inverse specific concentration of valinomycin is practically linear within the range of 6–18 ng/mg mitochondrial protein (fig.4). The data obtained are similar to those of Packer's laboratory [5].

3.3. *K^+ and pH*

The dependence of oscillations on $[K^+]$ is given in fig.3. At $\alpha = 0.018$, pronounced oscillations are observed in the narrow region around 1 mM KCl. An increase in $[K^+]$ reduces T and increases the damping factor. Similarly at $\alpha = 0.018$, oscillations occur in the narrow region of pH 7.4–7.6.

Strong dependence of oscillation on external $[K^+]$ and $[H^+]$ might suggest that concentrations of these cations in media are essential variables of the oscillatory mechanism. An instant change in any essential variable in the course of oscillation results in a shift of phase without any other changes [6,7]. The amount of such an addition should not exceed that of an oscillation amplitude. Fig.5. demonstrates that corresponding additions of KCl and HCl, during the most sensitive parts of the cycle, cause no shift of phase. Therefore the extramitochondrial $[K^+]$ and $[H^+]$ should be treated as parameters rather than variables of the oscillation mechanism.

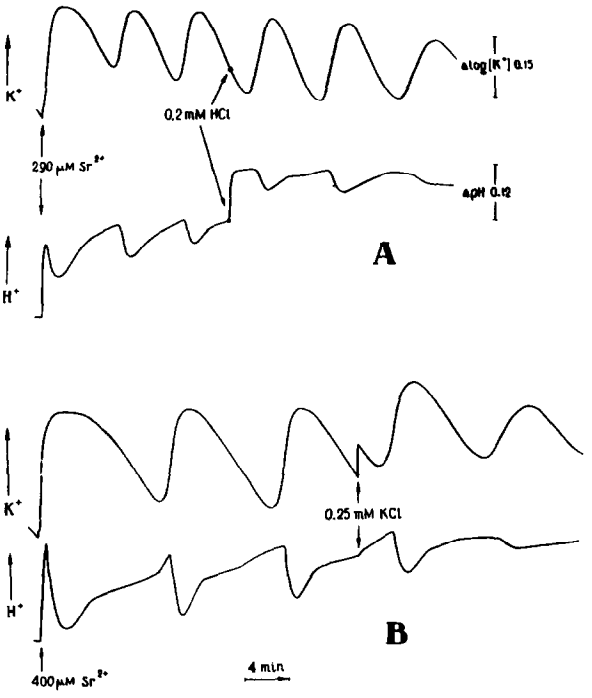

Fig.5. The absence of phase shift on an instantaneous change of extramitochondrial $[K^+]$ and $[H^+]$. Basal medium, pH, succinate as in fig.1. MP (1.6 mg/ml), t = 30°C (A) and MP (2.0 mg/ml), t = 20°C (B).

3.4. *Substrate, rotenone and energy supply*

Oscillations demand substrate oxidation because they are not observed in the presence of antimycin A and CN^-.

Succinate, preferably with low concentrations of rotenone (4–5 ng/mg MP) which reduced T, and α-ketoglutarate may be used as substrates to support oscillations. In the presence of β-hydroxybutyrate or succinate with high concentrations of rotenone (250 ng/mg MP) oscillations do not occur, without the output of K^+ being followed by its uptake.

This suggests the necessity for substrate-level phosphorylation. GTP or phosphoenolpyruvate (fig.6) actually restore the K^+ input and oscillations when succinate with large concentrations of rotenone was used. Energy is not provided via ATPase, since oligomycin does not abolish oscillations during substrate oxidation (as in L. Packer's results [5]) and does not prevent oscillations being restored by ATP in the presence of succinate with high rotenone.

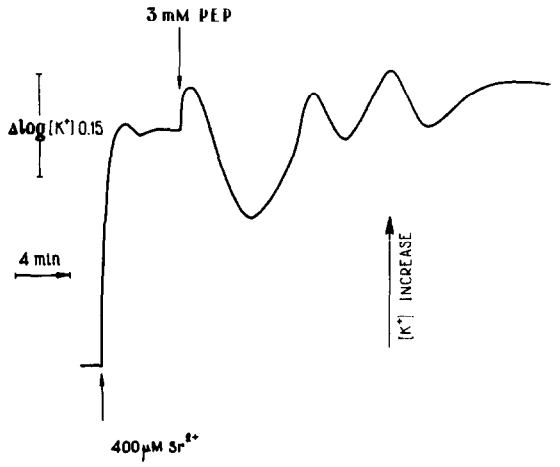

Fig.6. The effect of phosphoenolpyruvate on oscillations. Basal medium, MP, t, pH, succinate and valinomycin as in fig.1. Rotenone (250 ng/mg MP).

4. Discussion

The sequence of events during the oscillatory cycle may be outlined as follows. The substrate supported membrane potential (φ) being present, Sr^{2+} and K^+ (due to valinomycin) enter mitochondria in exchange for H^+. The latter process results in alkalinization of the intramitochondrial space. The increase of pH and the presence of Sr^{2+} (directly or through endogeneous Ca^{2+}) stimulate phospholipase resulting in an increase in the permeability of the mitochondrial membrane and in a fall of φ. Cations leak out of mitochondria inducing a portion of H^+ to move rapidly into mitochondria. The loss of cations and acidification of the matrix inhibits phospholipase. The original state of the membrane is restored by the GTP-dependent phospholipid resynthesis from lysophospholipids and fatty acids [8]. Shortening of T by low concentrations of rotenone is compatible with this explanation, because it stimulates fatty acid synthesis in mitochondria [9]. The original φ value being restored, cations move into mitochondria and a new cycle begins.

Acknowledgements

We wish to thank Dr Yu. G. Kaminsky for his kind help in providing the equipment used and Dr A. D. Vinogradov for his helpful criticism and discussion.

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