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EFFECT OF MONOVALENT CATION IONOPHORES ON LYMPHOCYTE CELLULAR METABOLISM

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Summary

The effect of valinomycin, nigericin and gramicidin on the cellular O_2 consumption and on ATP content has been investigated. It has been found that while valinomycin and nigericin interfere with mitochondrial functions, gramicidin D does not show any appreciable effect.

These results are explained in terms of the differing abilities of ionophores to redistribute among intracellular membranes.

Introduction

Monovalent cation ionophores have been used to study the influence of ion redistribution across membranes on a variety of biological functions [1]. The most widely used monovalent cation ionophores are valinomycin, which translocates selectively potassium across membranes, nigericin, which exchanges potassium for protons, and gramicidin, which rapidly and unspecifically permeabilizes membranes to monovalent cations.

Valinomycin and nigericin have been shown to inhibit lectin-induced lymphocyte mitogenesis [2,3]. Valinomycin inhibits also the 'capping' of surface immunoglobulins in human peripheral blood lymphocytes [4] and in mouse spleen lymphocytes [5]. Serotonin transport in human blood platelets has been shown by using nigericin and monesin to be an Na^+-dependent carrier-mediated process [6]. Furthermore, the involvement of potassium in meiosis of *Xenopus laevis* oocytes has been inferred by using valinomycin [7]. The role of monovalent cations on the antigen-induced histamine release from mast cells has

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DMSO, dimethyl sulfoxide.

been studied by using gramicidin and valinomycin [8]. Moreover, membrane potential has been assessed in many instances by using cation ionophores [9—13].

In most cases little attention has been paid to ionophore interference with cellular metabolism, which in turn can affect the value of membrane potential and the biological phenomena under study. We have, therefore, attempted to investigate in rat spleen lymphocytes the effect of valinomycin, nigericin and gramicidin on both respiratory rate and cellular ATP level, which are important parameters of cellular metabolism.

Methods and Materials

Rat spleen lymphocytes were prepared from spleens of male albino Wistar rats as previously reported [14]. Viability, as measured by eosin red exclusion, was always over 95%. Cells were maintained at a concentration of $(2—3) \cdot 10^{-7}$ cells/ml in Hank's minimal essential medium (Gibco, NY, U.S.A.) buffered with 10 mM Hepes, pH 7.3.

ATP was measured with the luciferase assay as previously reported [14] with a Dupont luminescence biometer.

O_2 consumption was measured either with a vibrating platinum electrode (American Instrument Co., U.S.A.) or with a Clark-type oxygen electrode in a thermostatically controlled cell. The signal was amplified with a linear amplifier (Johnson Foundation Instrument, Philadelphia, U.S.A.) [15].

Oligomycin, FCCP, rotenone, valinomycin, gramicidin S and nigericin were purchased from Sigma (St. Louis, MO, U.S.A.) and gramicidin D from Koch-Light Laboratories (Colnbrook, U.K.) and they were added from freshly distilled DMSO stock solutions. Final DMSO concentrations were always lower than 0.5% (v/v).

All chemicals used were of the highest purity commercially available. DMSO was purified by distillation under vacuum.

Results and Discussion

Fig. 1 shows the oxygen consumption rate of rat spleen lymphocytes and the effect of oligomycin, FCCP and rotenone. The addition of oligomycin, a specific inhibitor of mitochondrial ATP synthesis, lowers the rate of oxygen consumption to approx. 50% of the initial value. The addition of FCCP after oligomycin, restores and slightly enhances the initial O_2 consumption. FCCP, in the absence of oligomycin, causes an increase in the rate of respiration, varying in different preparations from 30 to 50%. 90% of total O_2 consumption is inhibited by rotenone, an inhibitor of NADH oxidase.

The effect of rotenone indicates that most of the O_2 consumption is due to mitochondrial oxidation of NAD-linked substrates. The effect of oligomycin and the small increase in the basal O_2 consumption rate after addition of the uncoupler FCCP indicate that O_2 consumption of resting lymphocyte mitochondria is nearly maximum and does not show a controlled or resting rate. The measurement of the respiration rate cannot, therefore, distinguish between normal high O_2 consumption leading to ATP synthesis (basal rate) and an

increased O_2 consumption due to an uncoupling or ion cycling from the added drugs.

However, once ATP synthesis has been inhibited by oligomycin the effect of uncoupling or ion cycling can be clearly observed as an increase in O_2 consumption.

Fig. 2 shows the effect of valinomycin (panel A), nigericin (panel B) and gramicidin D (panel C) on the O_2 consumption of rat spleen lymphocytes. Valinomycin (panel A), which selectively increases membrane permeability to K^+, causes an increase in O_2 consumption. This is not unexpected, since the potassium uptake induced by valinomycin results in an increased O_2 consumption in isolated mitochondria. Hence, valinomycin acts as an uncoupler by dissipating membrane potential.

Nigericin (10^{-7} M) (panel B), which exchanges K^+ for H^+ or K^+ for Na^+ according to their concentration gradients, decreases cellular O_2 consumption. Higher nigericin concentrations (10^{-5} M) cause an increase in O_2 consumption (not shown). The nigericin effect is probably a complex one: it is known that it inhibits the oxidation of NAD-linked substrates in isolated mitochondria in low

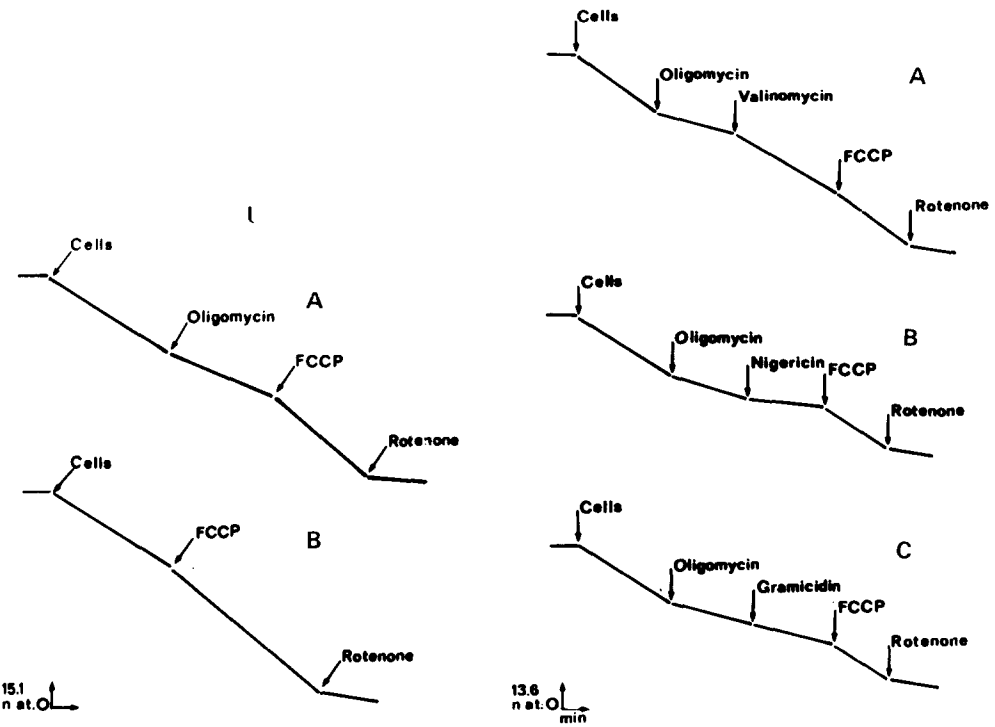

Fig. 1. Effect of oligomycin, FCCP and rotenone on the O_2 consumption rate. Medium, Hank's minimal essential/Hepes, pH 7.4; temperature, 37° C; final vol., 3 ml. Where indicated: $2 \cdot 10^{-7}$ cells/ml, oligomycin, 1 mg/ml, FCCP, 1 μM and rotenone, 5 μM.

Fig. 2. Effect of valinomycin (A), nigericin (B) and gramicidin D (C) on O_2 consumption rate. Experimental conditions as in Fig. 1. Cell concentration, oligomycin, FCCP and rotenone concentrations as in Fig. 1. Valinomycin, nigericin and gramicidin D, when added, were respectively: 1, 0.1 and 0.1 μM.

K^+ medium [16]. This inhibition is relieved by increasing the K^+ concentration of the medium. As the cytoplasm is a high K^+ medium, the nigericin effect was unexpected [5]. A possible interpretation is that nigericin depletes cytoplasmic K^+ by exchanging it with external Na^+. Once cytoplasmic K^+ concentration is decreased, the inhibitory effect of nigericin on mitochondria becomes apparent. This explanation is supported by the observation that in intact lymphocytes also an increased concentration of K^+ in the external medium relieves the nigericin inhibitory effect (not shown).

Gramicidin D (panel C), which increases membrane permeability to monovalent cations, does not affect cellular respiration. Gramicidin D has been shown to be a potent uncoupler in isolated mitochondria [17] and its lack of effect on rat lymphocytes is not due to absence of interaction with the cell membrane since, at the concentration used, it effectively exchanges internal K^+ for external Na^+ and collapses membrane potential [11]. At variance, gramicidin S shows an uncoupling effect on cellular O_2 consumption and lowers the cellular ATP level (not shown). Our interpretation of this result is that gramicidin D is sequestered by plasma membrane and does not redistribute among intracellular membranes within the time of the experiment. It is possible that gramicidin D requires a longer incubation in order to reach intracellular membranes. In Table I the effects of ionophores and of some mitochondrial inhibitors on the cellular ATP content of rat spleen lymphocytes, measured under conditions similar to those used for measuring O_2 consumption, are reported. Cellular ATP level is lowered to less than 30% of the controls by the addition of valinomycin and nigericin, whereas gramicidin D, as expected from O_2 consumption data, shows only a negligible effect. The ATP-depletion induced by valinomycin and nigericin is similar to that caused by the specific mitochondrial poisons oligomycin and FCCP. Experiments have been performed also with lower cell concentrations and ionophores at the same concentrations as reported in Fig. 1. As expected, the ionophore effects on cells were enhanced. Conversely, when cell and ionophore concentrations were scaled down proportionally, the ionophore effects on cellular O_2 consumption and

TABLE I

EFFECT OF MONOVALENT CATION IONOPHORES AND MITOCHONDRIAL POISONS ON CELLULAR ATP CONTENT

Experimental conditions as described in Fig. 1 except that the final volume was 100 µl. Lymphocytes were incubated 5 min with the drugs at 37°C before blocking the reaction with perchloric acid. ATP was assayed as in Ref. 5. The results are the mean of six independent experiments ± S.D.

	Concentration (M)	% ATP
Valinomycin	10^{-7}	38.2 ± 10.3
Nigericin	10^{-7}	29.8 ± 5.5
	10^{-5}	20.9 ± 5.8
Gramicidin D	10^{-7}	93.3 ± 10.3
Gramicidin S	10^{-7}	30.1 ± 7.9
FCCP	10^{-6}	24.3 ± 5.6
Oligomycin	$5 \cdot 10^{-6}$	31.5 ± 3.7
Rotenone	$5 \cdot 10^{-6}$	31.2 ± 3.1

ATP content were present and were comparable to ionophore effects obtained with higher cell concentrations. Similar results have been obtained in mouse spleen lymphocytes and in human spermatozoa.

Conclusions

In rat spleen lymphocytes valinomycin and nigericin redistributes rapidly among intracellular membranes affecting mitochondrial functions. Gramicidin D, on the other hand, appears to confine itself to the plasma membrane in short-term (30-min) incubation. Due to this potential side effect, caution should be taken in interpreting some of the results obtained using monovalent cation ionophores in living cells.

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