Biochimica et Biophysica Acta 852 (1986) 244–253
Elsevier

BBA 42071

Effects of various inhibitors of oxidative phosphorylation on energy metabolism, macromolecular synthesis and cyclic AMP production in isolated rat thymocytes. A regulating role for the cellular energy state in macromolecular synthesis and cyclic AMP production

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(Received 1 September 1986)

Key words: Trialkyltin compound; Oligomycin; Uncoupler; Macromolecular synthesis; cyclic AMP production; (Thymocyte)

Inhibitors of oxidative phosphorylation such as several triorganotin compounds, oligomycin, 2,4-dinitrophenol and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone suppress energy metabolism of isolated rat thymocytes as indicated by a reduction of ATP levels, an increase in glucose consumption and by a marked accumulation of lactate. Also these compounds effectively inhibit the incorporation of DNA, RNA and protein precursors into acid-precipitable material of thymocytes. Moreover, the prostaglandin E_1-induced elevation of cAMP is markedly reduced by these inhibitors. A correlation is observed between the effects on energy metabolism, macromolecular synthesis and cAMP production, since (i) from a series of trialkyltin chlorides, tri-*n*-propyltin, tri-*n*-butyltin and tri-*n*-hexyltin are very effective inhibitors of these functions, while trimethyltin and tri-*n*-octyltin affect neither of them; (ii) other inhibitors of oxidative phosphorylation, each of them with quite different mechanisms of action, also inhibit macromolecular synthesis and cAMP production. The finding that a rise in intracellular ATP concentrations leads to a reversion of the tri-*n*-butyltin-induced inhibition of cAMP production and uridine incorporation, indicates a regulating role for the cellular energy state in these aspects of cellular function.

Introduction

Of the triorganotin compounds, especially TBT, triphenyltin and tricyclohexyltin compounds are economically important. They are used as general biocides, agricultural fungicides and miticides in fruit-culture, respectively [1,2]. When given orally to rats, TPT, TBT or triphenyltin cause atrophy of the thymus [3] and subsequently immunosuppression [4,5]. The lower trialkyltin homologs, TMT or TET, are neurotoxic, whereas the long-chain trialkyltin compounds have only minor (THT) or no adverse (TOT) effects upon oral administration to rats [3].

The cytotoxicity of the triorganotin chlorides to isolated rat thymocytes was studied recently, using long-term incubations up to 30 h [6]. As indicated

Abbreviations: 2,4-DNP, 2,4-dinitrophenol; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; TMT(C), trimethyltin(chloride); TET(C), triethyltin(chloride); TPT(C), tri-*n*-propyltin(chloride); TBT(C), tri-*n*-butyltin(chloride); THT(C), tri-*n*-hexyltin(chloride); TOT(C), tri-*n*-octyltin(chloride).

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by several cytotoxicity assays, the lipophilic compounds TPTC, TBTC or THTC inhibited thymidine incorporation at concentrations ranging from 0.05 to 1 μM and caused membrane damage at higher levels (1 μM or more). TETC was less effective, whereas TMTC appeared the least cytotoxic homolog.

The mechanisms via which the trialkyltin compounds interfere with mitochondrial energy production have been intensively studied [7–9]. These compounds inhibit ATP synthesis of isolated rat liver mitochondria by three different mechanisms [9]. The respective concentrations for TMT, TET, TPT, TBT or THT that cause 50% inhibition of ATP synthesis of isolated liver mitochondria were found to be 4.0, 0.1, 0.32, 0.64 or 2.5 μM [9]. In a previous study [10], the effects of TBTC on several aspects of cell function were evaluated. Membrane integrity, energy metabolism, macromolecular synthesis and cAMP production of isolated rat thymocytes were all affected by this compound. The effects in the latter two processes were suggested to be caused by a disturbance of the cellular energy state. To verify further the role of the cellular energy state in these processes, the effects of a series of trialkyltin chlorides and other inhibitors of oxidative phosphorylation were compared. Especially by varying the intracellular ATP concentration of rat thymocytes, the regulating role of the cellular energy state in processes such as macromolecular synthesis and cAMP production became apparent.

Materials and Methods

Animals and chemicals. Male Wistar-derived rats (Central Laboratory for Animal Breeding, TNO, Zeist, The Netherlands) weighing 100–150 g were used. Trialkyltin chlorides were kindly provided by Dr. E.J. Bulten and Dr. H.A. Meinema, Institute for Applied Chemistry, TNO, Utrecht, The Netherlands. Purity of these compounds was more than 98% as established by thin-layer chromatography. [8-^{3}H]Adenosine 3′,5′-cyclic phosphate (26.5 Ci/mmol), L-[U-^{14}C]leucine (348 Ci/mmol), [*Methyl*-^{3}H]thymidine (40 Ci/mmol) and [5,6-^{3}H]uridine (48.7 Ci/mmol) were obtained from the Radiochemical Centre Amersham, England. Oligomycin (composition 65% A, 20% B, 15% C), FCCP, theophylline, 3-isobutyl-1-methylxanthine and prostaglandin E_1 (PGE_1) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. and 2,4-DNP from Serva Feinbiochemica, Heidelberg, F.R.G.

Isolation and incubation of thymocytes. Thymocytes were isolated and incubated in Dulbecco's phosphate-buffered saline supplemented with 2 mM D-glucose (phosphate-buffered saline/glucose; pH 7.4) as described previously [10]. The triorganotin compounds, oligomycin, 2,4-DNP and FCCP were dissolved in absolute ethanol just before each experiment. The final ethanol concentration of 0.1% did not affect any of the test systems.

Energy metabolism. The effects of a series of trialkyltin compounds on the energy metabolism of thymocytes ($4 \cdot 10^7$/ml) were studied as described before [10]. Upon 4 h of incubation with the organotin compounds, the glucose consumption and the production of lactate and pyruvate were determined. In 1-h incubation studies the effects of the trialkyltin compounds, oligomycin, 2,4-DNP, FCCP or anoxia on ATP levels and lactate production were investigated. The methods used to determine the concentrations of glucose, lactate, pyruvate and ATP are given in Ref. 10.

Incorporation of nucleosides and amino acids. To study the effects of the inhibitors of oxidative phosphorylation on macromolecular synthesis, the incorporation of thymidine (TdR), uridine (Urd) and L-leucine (Leu) into acid-precipitable material was determined. After a 30-min pre-incubation period with graded concentrations of inhibitor, 1 μCi/ml ^{3}H-TdR (final concentration, 20 nM), 1 μCi/ml ^{3}H-Urd (final concentration, 20 nM) or 50 nCi/ml ^{14}C-Leu (final concentration, 145 μM) was added to thymocyte suspensions ($2 \cdot 10^7$/ml). Studies were carried out as described in Ref. 10.

cAMP production and adenylate cyclase activity. In order to investigate the effects of various compounds on cAMP production, thymocytes ($2 \cdot 10^7$/ml) were pre-incubated with the organotin compounds, oligomycin, 2,4-DNP or FCCP for 10 min. cAMP levels increased rapidly upon addition of PGE_1 (1 μM) in the presence of phosphodiesterase inhibitor isobutylmethylxanthine (1 mM). 10 min after the addition of PGE_1, cAMP levels were determined as described previously [10]. The determination of unstimulated and

PGE_1-stimulated adenylate cyclase activity in lysates of isolated thymocytes ($2 \cdot 10^7$/ml) was carried out as indicated earlier [10].

Statistical analysis. Mean values ± standard deviation (s.d.) are given, while the number of experiments is indicated by *n*. Student's *t*-test was used to calculate significant differences between values of treated and control suspensions.

Results

Energy metabolism

Trialkyltin compounds. In order to evaluate the

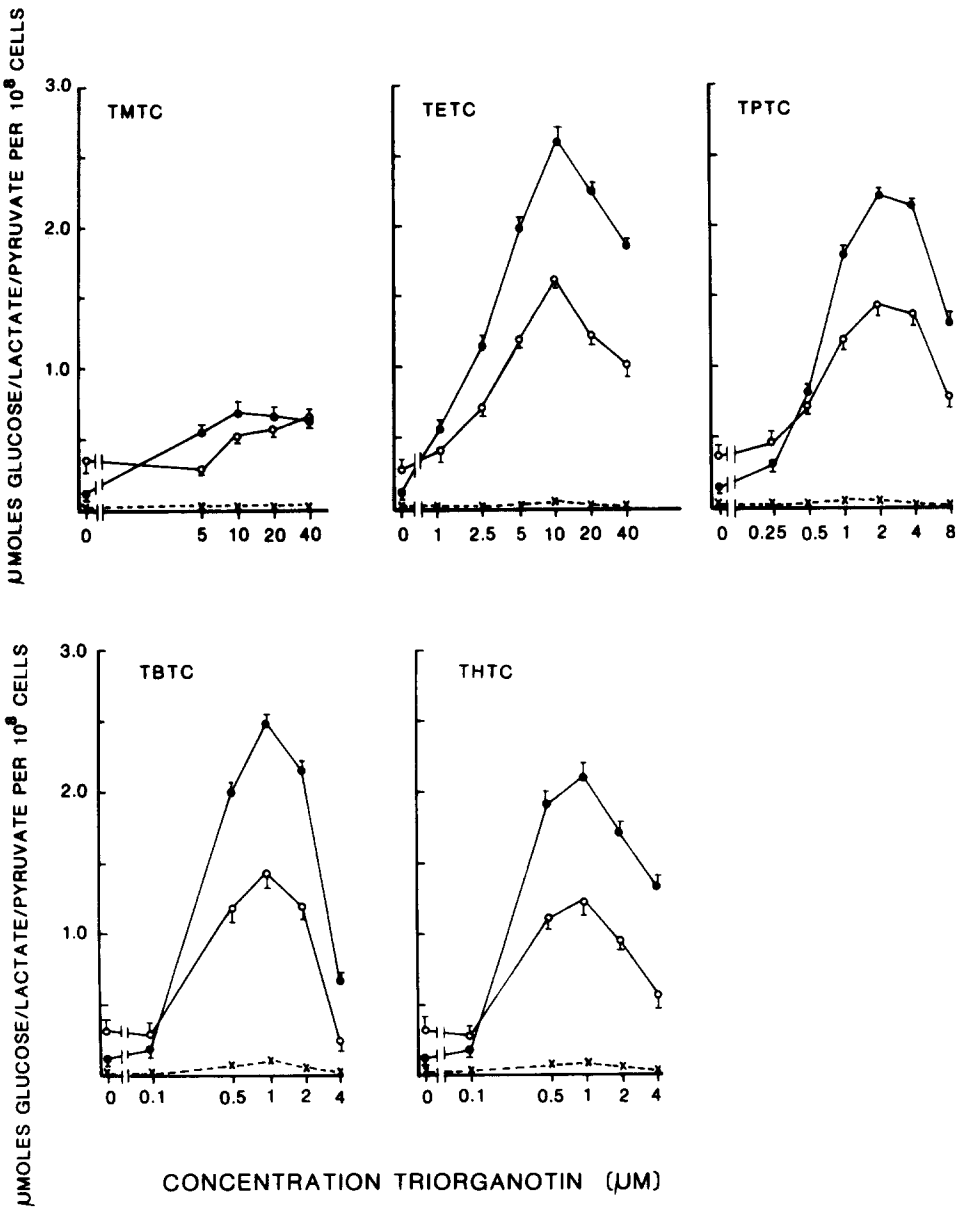

Fig. 1. Effects of a series of trialkyltin chlorides on the consumption of glucose (open circles), the production of lactate (closed circles) and pyruvate (crosses) of thymocytes after incubation in phosphate-buffered saline/glucose for 4 h. The mean values ± s.d. of three incubations are given.

effects of a series of trialkyltin chlorides on glucose metabolism, rat thymocytes were incubated with D-glucose (2 mM) as substrate for 4 h. As illustrated in Fig. 1, the water-soluble homolog TMTC had only a slight effect on the consumption of glucose and the production of lactate at concentrations up to 40 μM. The higher trialkyltin homologs caused a marked concentration-related increase in glucose consumption and lactate production. Only a small rise in pyruvate levels was observed. Maximal stimulation of all three parameters coincided at concentrations of 10, 2, 1 and 1 μM for TETC, TPTC, TBTC or THTC, respectively. At higher levels, glucose consumption and the accumulation of lactate and pyruvate progressively decreased in association with a reduction of cell viability. The most lipophilic homolog studied TOTC, did not affect any of these parameters at concentrations up to 10 μM (data not shown). The amount of glucose, taken up due to the action of trialkyltin compounds, was largely converted into lactate. Thereby shifting the fixed ratio of lactate to pyruvate from approx. 4 in control incubations [10] to very high values of 15–30 in treated suspensions.

Upon incubation of thymocytes for 1 h, the same trialkyltin compounds that affected glucose metabolism, reduced ATP levels (Table I). At concentrations up to 5 μM, the most water- and lipid-soluble homologs, TMTC and TOTC, respectively, had no effect on concentrations of ATP or lactate. The other compounds demonstrated a concentration-dependent reduction of ATP levels concurrently with an accumulation of lactate. With the exception of THTC, these compounds caused a maximal reduction of ATP levels within 10 min. THTC acted relatively slow, since ATP concentrations reached their lowest level only after 30 min of incubation (data not shown). Within the series of trialkyltin chlorides, homologs with 3–6 carbon atoms per side chain most effectively disturbed the energy metabolism of thymocytes.

Inhibitors of oxidative phosphorylation. Compounds known for their action on oxidative phosphorylation such as oligomycin, 2,4-DNP or FCCP markedly reduced ATP levels in thymocytes. Concomitantly with the reduction in ATP concentrations, the production of lactate increased drastically (Table II). Incubation of thymocytes under anoxic circumstances caused a similar disturbance of energy metabolism as was found for the energy poisons. None of the treatments affected cell survival as determined by cell count and trypan blue exclusion (data not shown).

TABLE I

CONCENTRATIONS OF ATP AND LACTATE (nmol/10^7 CELLS) IN THYMOCYTES INCUBATED FOR 1 h IN PHOSPHATE-BUFFERED SALINE/GLUCOSE WITH GRADED CONCENTRATIONS (μM) OF A SERIES OF TRIALKYLTIN CHLORIDES

Data are given as mean values ± s.d. of three experiments performed in duplo.

Compound	Concentration	ATP	Lactate
–	0	6.3 ± 0.8	13 ± 3
TMTC	1	6.2 ± 0.4	14 ± 3
	5	6.3 ± 0.5	12 ± 3
TETC	1	5.9 ± 0.3	23 ± 4
	5	4.7 ± 0.3	46 ± 1
TPTC	1	4.6 ± 0.2	63 ± 2
	2	3.9 ± 0.2	68 ± 2
TBTC	1	4.8 ± 0.4	76 ± 7
	2	4.0 ± 0.1	69 ± 2
THTC	1	4.7 ± 0.4	36 ± 6
	2	3.6 ± 0.3	58 ± 2
TOTC	1	6.3 ± 0.6	11 ± 3
	5	6.1 ± 0.3	10 ± 4

Macromolecular synthesis

Trialkyltin compounds. After a pre-incubation period of 30 min with various triorganotin compounds, the incorporation of ^{3}H-TdR, ^{3}H-Urd and ^{14}C-Leu was measured in order to evaluate effects on macromolecular synthesis. As demonstrated in Fig. 2, the incorporation of DNA, RNA and protein precursors was concentration-dependently reduced by all trialkyltin compounds, except for TMTC and TOTC. The latter homologs had virtually no influence on macromolecular synthesis at concentrations up to 4 μM. The effectivity to inhibit precursor incorporation increased in the following order, TMTC and TOTC < TETC < TPTC < TBTC and THTC. The incorporation of TdR was reduced by TPTC, TBTC or THTC at a concentration as low as 0.1 μM. The incorporation of Urd appeared relatively resistant to the inhibitory effects of the trialkyltin compounds.

Inhibitors of oxidative phosphorylation. A con-

TABLE II

CONCENTRATIONS OF ATP AND LACTATE (nmol/10^7 CELLS) IN THYMOCYTES INCUBATED FOR 1 h IN PHOSPHATE-BUFFERED SALINE/GLUCOSE WITH GRADED CONCENTRATIONS OF VARIOUS INHIBITORS OF OXIDATIVE PHOSPHORYLATION OR UNDER ANOXIC CIRCUMSTANCES

Data are given as mean values ± s.d. of a specific experiment carried out in 4-fold.

Compound and concentration	ATP	Lactate
Oligomycin		
0 ng/ml	5.8 ± 0.2	18 ± 2
1 ng/ml	5.8 ± 0.3	16 ± 3
10 ng/ml	3.3 ± 0.1	105 ± 1
100 ng/ml	1.7 ± 0.1	110 ± 4
2,4-DNP		
0 μM	5.8 ± 0.2	18 ± 2
10 μM	5.6 ± 0.2	19 ± 1
50 μM	3.9 ± 0.3	99 ± 2
100 μM	1.1 ± 0.3	115 ± 2
FCCP		
0 nM	5.9 ± 0.3	18 ± 1
10 nM	5.8 ± 0.2	17 ± 2
100 nM	5.1 ± 0.2	30 ± 1
1000 nM	0.9 ± 0.2	100 ± 2
Air		
–	5.7 ± 0.3	14 ± 1
Anoxia		
–	1.7 ± 0.2	103 ± 8

centration of 1 ng/ml oligomycin did not influence macromolecular synthesis of rat thymocytes (Table III). At a 5-times higher level, the incorporation of TdR, Urd and Leu was markedly inhibited. The uncoupler 2,4-DNP reduced precursor incorporation from a concentration of 10 μM. FCCP at a level of 100 nM also affected DNA, RNA and protein synthesis. All three inhibitors of oxidative phosphorylation reduced the incorporation of Leu most severely, while Urd incorporation was least affected.

cAMP production and adenylate cyclase activity

Trialkyltin compounds. The PGE_1-induced cAMP production of thymocytes was determined after a 10 min pre-incubation period with the more lipophilic trialkyltin compounds. A concentration of 0.1 μM TPTC, TBTC or THTC

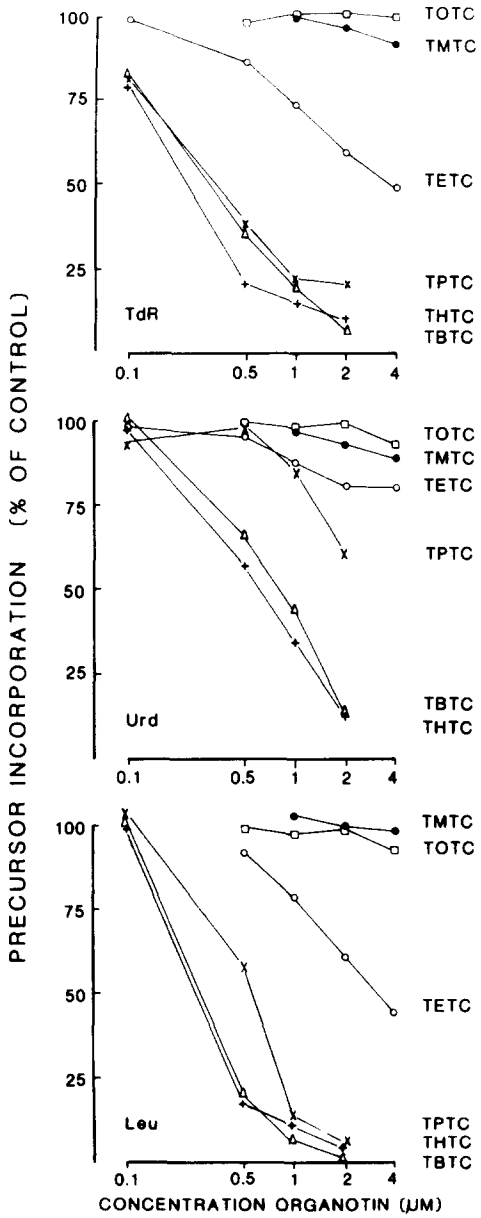

Fig. 2. Effects of a series of trialkyltin chlorides on the incorporation of [^{3}H]thymidine, [^{3}H]uridine and [^{14}C]leucine into acid-precipitable material of rat thymocytes. Incorporation was determined 1 h after the addition of labelled precursors, at which time the cells were incubated with the organotin compounds for 90 min. Each value is the mean ± s.d. of 4-fold determinations of a specific experiment.

caused already a reduction of cAMP production of 20% (Fig. 3). TBTC appeared the most powerful inhibitor of PGE_1-induced cAMP accumulation, whereas TOTC was ineffective over the con-

TABLE III

EFFECTS OF VARIOUS INHIBITORS OF OXIDATIVE PHOSPHORYLATION ON THE INCORPORATION OF THYMIDINE, URIDINE AND LEUCINE INTO THYMOCYTES ($2 \cdot 10^7$/ml)

After a 30-min pre-incubation period, cells were incubated for 1 h with radio-labelled TdR, Urd or Leu. The incorporation values at 1 h, determined in 4-fold, were expressed as percentage of the control incubation. Data presented are mean values ± s.d. of n experiments.

Compound and concentration	^{3}H-TdR	^{3}H-Urd	^{14}C-Leu
Oligomycin ($n = 4$)			
1 ng/ml	102± 6	97± 3	98± 5
5 ng/ml	28±11	50± 8	13± 2
10 ng/ml	25±10	42± 9	8± 1
100 ng/ml	19± 6	47± 4	7± 2
2,4-DNP ($n = 3$)			
10 μM	92± 3	92± 6	86± 2
50 μM	49±13	70±12	45±12
100 μM	13± 5	27± 6	7± 4
FCCP ($n = 3$)			
10 nM	95± 6	94± 5	93± 6
100 nM	70±13	78±10	66± 8
1000 nM	13± 5	12± 4	2± 1

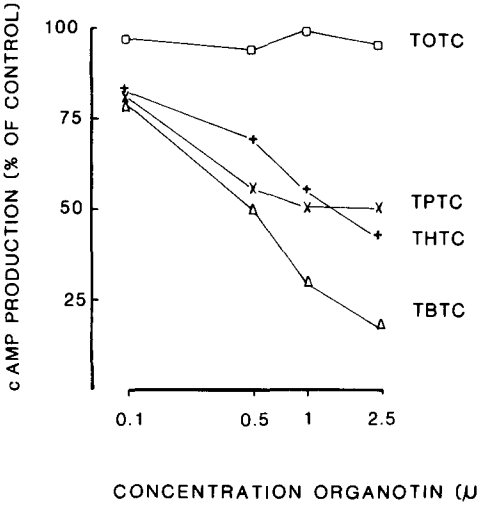

Fig. 3. Effects of a series of trialkyltin compounds on PGE_1-induced cAMP production of thymocytes pre-incubated with these compounds for 10 min in phosphate-buffered saline/glucose. 10 min after the addition of PGE_1 the concentration of cAMP was determined. Results are expressed as percentages of control incubations of at least two experiments performed in 4-fold. S.d. was smaller than 8% in each experiment.

centration range applied. The influence of the trialkyltin compounds on the adenylate cyclase activity in homogenates of thymocytes was determined in the presence or absence of the stimulator PGE_1. TPTC did not affect either the unstimulated or the stimulated adenylate cyclase activity at concentrations up to 1 μM. At a concentration of 2.5 μM, TPTC reduced both types of adenylate cyclase activity to 83 and 86%, respectively, of the control values. As was described before [10], TBTC inhibited both unstimulated and PGE_1-stimulated adenylate cyclase activity at concentrations of 1 μM or more. Reductions to 81% and 70% of control activity were found upon incubation with 1 and 2.5 μM TBTC, respectively, both in the absence and presence of PGE_1. THTC and TOTC had no effect on both types of adenylate cyclase activity at concentrations up to 2.5 μM (data are means of 2–6 experiments, each performed in triplo).

Inhibitors of oxidative phosphorylation

Oligomycin and the uncouplers 2,4-DNP and FCCP all reduced PGE_1-induced cAMP production at concentrations, that also affected energy metabolism of rat thymocytes (Table IV).

TABLE IV

EFFECTS OF OLIGOMYCIN, 2,4-DNP OR FCCP ON PGE_1-INDUCED cAMP PRODUCTION OF THYMOCYTES (AS IN FIG. 3)

Results are expressed as percentages of control incubations. Each value is the mean ± s.d. of 4-fold determinations of three experiments.

Compound and concentration	PGE_1-cAMP
Oligomycin	
1 ng/ml	100± 8
5 ng/ml	78± 2
10 ng/ml	53± 9
100 ng/ml	51± 7
2,4-DNP	
10 μM	84± 2
50 μM	59±10
100 μM	49± 7
FCCP	
10 nM	90± 9
100 nM	71± 4
1000 nM	42±12

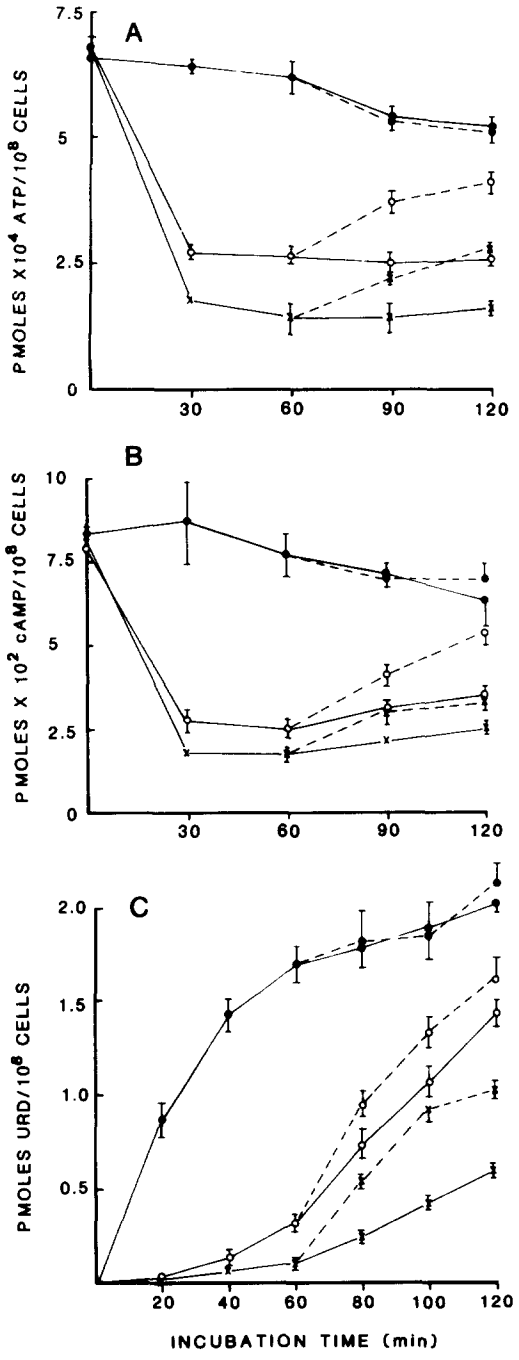

Fig. 4. The effects of TBTC on ATP concentrations (A), cAMP production (B), and Urd incorporation (C). TBTC was added on $t = 0$ at concentrations of 0 (closed circles), 1 (open circles) or 2 (crosses) μM to isolated rat thymocytes ($4 \cdot 10^7$/ml) suspended in phosphate-buffered saline/β-hydroxybutyrate (solid lines). After 60 min, glucose was added to a final concentration of 2 mM (broken lines). Data are mean values ± s.d. of one combined experiment performed in 4-fold.

Combined study

As described previously [10], the effects of TBTC on cellular ATP levels appeared substrate-dependent. Incubation of thymocytes ($4 \cdot 10^7$/ml) in phosphate-buffered saline supplemented with 2 mM β-hydroxybutyrate (phosphate-buffered saline/β-hydroxybutyrate) resulted in a concentration-related decrease of ATP, which was more severe than in phosphate-buffered saline/glucose-incubated thymocytes (Table I and Fig. 4A). In addition, the inhibition or Urd incorporation by TBTC was more pronounced, when cells were incubated in phosphate-buffered saline/β-hydroxybutyrate rather than in phosphate-buffered saline/glucose (Figs. 2B and 4C). When after 1 h of incubation in phosphate-buffered saline/β-hydroxybutyrate, D-glucose was added to a final concentration of 2 mM, the ATP levels of the TBTC-treated cells increased relative to the cells maintained in phosphate-buffered saline/β-hydroxybutyrate (Fig. 4A). Within the same incubations, PGE_1-induced cAMP production and Urd incorporation were determined. As demonstrated in Fig. 4B and C, the TBTC-induced reduction of both cAMP production and Urd incorporation paralleled the recovery of ATP levels upon addition of glucose.

Discussion

The energy metabolism of isolated rat thymocytes is characterized by a dominative oxidative metabolism, since glucose is readily taken up as energy substrate without any appreciable aerobic lactate accumulation. Another characteristic is a high Pasteur effect. When thymocytes are incubated under anoxic circumstances or when treated with energy poisons, glycolysis is drastically increased (Table II) [11,12].

In the present experiments a series of trialkyltin chlorides were used as tools to block oxidative phosphorylation in isolated rat thymocytes. Incubation of cells in phosphate-buffered saline/glucose with TETC, TPTC, TBTC or THTC resulted in a pronounced disturbance of energy metabolism. Concomitantly with a decrease in ATP levels, lactate production was markedly elevated (Table I). Glucose consumption and

lactate accumulation were increased by these trialkyltin compounds, while pyruvate production was only slightly raised (Fig. 1). The latter parameters reached a maximum at the same organotin concentration. At higher levels, when membrane integrity was affected [6,10], stimulation decreased. The most water- and lipid-soluble homologs, TMTC and TOTC, demonstrated virtually no effects on parameters of energy metabolism at concentrations up to 10 μM.

In contrast to TETC, TPTC and TBTC, the hexyl homolog reduced ATP levels rather slowly. In the 1-h study, the lactate concentrations were therefore less increased when compared to TBTC-exposed cells (Table I).

The order of effectiveness to inhibit ATP synthesis in isolated mitochondria (see Introduction) differs considerably from the order of trialkyltin compounds disturbing thymocyte energy metabolism. Not TET is most effective, but the more lipophilic homologs TPT, TBT or THT. Apparently to penetrate the cell and interfere with mitochondrial respiration, a trialkyltin compound should not be hydrophilic or too lipophilic. Both TMTC and TOTC were virtually inactive and also the delayed effects of THTC suggest that this compound reaches the limit of lipid solubility beyond which homologs are ineffective.

In addition to the trialkyltin compounds, other inhibitors of oxidative phosphorylation such as oligomycin, 2,4-DNP and FCCP were used. Oligomycin interacts with the energy-conserving system, while the other two compounds uncouple oxidative phosphorylation from the respiratory chain [13]. In contrast to 2,4-DNP, the uncoupling effect of FCCP can be reversed by dithiol compounds [14]. Despite these differences in mode of action, all three inhibitors effectively reduced intracellular ATP levels concurrently with an increase in lactate production in rat thymocytes (Table II). The concentrations of these three inhibitors did not affect cell survival as determined by cell count and trypan blue exclusion. In contrast to this, TPTC, TBTC or THTC disturb membrane integrity at concentrations higher than 1 μM [6,10]. Probably for this reason, treatment with oligomycin, the uncouplers and anoxia resulted in a more severe decrease of ATP levels, while lactate concentrations rose over 100 nmoles/10^7 cells per h (cf. TBTC: 70 nmol/10^7 cells per h [10]).

Those trialkyltin compounds, which affected energy metabolism also demonstrated distinct effects on the incorporation of TdR, Urd and Leu into acid-precipitable material of thymocytes (Fig. 2). The structure-activity relationship for the effects on energy metabolism and macromolecular synthesis are identical. TMTC and TOTC hardly influenced DNA, RNA or protein synthesis, whereas TPTC, TBTC or THTC were strong inhibitors of macromolecular synthesis. TETC was again of intermediate toxicity. The other inhibitors of oxidative phosphorylation (Table III) also revealed that concentrations capable of disturbing energy metabolism, markedly affected the incorporation of TdR, Urd and Leu. Most severe effects, caused by all compounds affecting energy metabolism, were noticed for the incorporation of amino acids, leucine (this study), but also proline [10]. Urd incorporation into thymocytes appeared somewhat more resistant to the energy poisons studied.

As illustrated in Fig. 3, TPTC, TBTC or THTC inhibited the PGE_1-induced cAMP accumulation in a concentration-dependent fashion. TOTC was ineffective. The inhibition of cAMP production does not seem to be due to an increase in phosphodiesterase (PDE) activity, since in the assay used the PDE-inhibitor isobutylmethylxanthine was present. The observed effects are not likely caused by inhibition of adenylate cyclase activity, since this enzyme was not affected by TPTC up to 1 μM and not by THTC or TOTC up to 2.5 μM. TBTC decreased adenylate cyclase activity from a concentration of 1 μM, which may explain the severe effects of TBTC on cAMP production at these levels. Also oligomycin and the uncouplers were able to reduce PGE_1-induced cAMP production (Table IV). Concentrations which affected energy metabolism, markedly inhibited cAMP accumulation.

The results demonstrate a correlation between effects on the cellular energy state, as judged by ATP levels, and effects on macromolecular synthesis or cAMP production of rat thymocytes. As described by Plagemann and co-workers [15,16], macromolecular synthesis is critically dependent on intracellular ATP levels in the process of

nucleoside phosphorylation. Guanine nucleotides, which may be regulated by adenine nucleotides, are thought to be essential in the initiation of protein synthesis [17]. Therefore it can be understood that disturbances of the energy metabolism may very well influence macromolecular synthesis. Rather unexpected is the correlation between the effects on the energy state and cAMP production, since the basal levels of ATP and cAMP differ 1000–3000 times. Upon stimulation with PGE_1, cAMP levels are approx. 100 times less than the ATP concentrations (Fig. 4).

To further study the correlation between ATP levels and Urd incorporation or cAMP production a combined experiment was carried out (Fig. 4). As described previously [10], the effects of TBTC on ATP are substrate-dependent. Using phosphate-buffered saline/β-hydroxybutyrate, ATP levels were more decreased than in phosphate-buffered saline/glucose (cf. Table I and Fig. 4). Addition of glucose to thymocytes incubated in phosphate-buffered saline/β-hydroxybutyrate resulted in a considerable increase in ATP levels in TBTC-exposed cells. The extra ATP formed is most likely from glycolytic origin, since lactate levels were found to increase concomitantly (data not shown). As Fig. 4 points out the correlation between ATP and cAMP is very close. Not only a decrease but also a rise in ATP levels is paralleled by cAMP, albeit at a 100-times lower scale. Therefore a precise regulatory mechanism must be operative. The incorporation of Urd in phosphate-buffered saline/β-hydroxybutyrate is severely affected, much more than in phosphate-buffered saline/glucose (cf. Figs. 2 and 4). The addition of glucose induced an immediate increase of Urd incorporation as compared to the phosphate-buffered saline/β-hydroxybutyrate controls. Therefore, in addition to the results obtained in the previous paper [10], the effects of inhibitors of oxidative phosphorylation on macromolecular synthesis and cAMP production are most likely regulated by the cellular energy state.

Studying the effects of cortisol on rat thymocytes, Young and co-workers [18–21] framed the hypothesis that the cortisol-induced inhibition of growth and development of lymphoid cells is a consequence of its ability to suppress the cellular energy state. Slight changes in adenine nucleotide ratios were found to influence the incorporation of Urd and amino acids markedly. They suggested that slight disturbances in the energy supply induce a reordering of metabolic priorities in favor of those essential for immediate cell survival. The results presented here, using various inhibitors of oxidative phosphorylation, are consistent with these suppositions and they extent them. Suppression of the energy supply not only correlates with a decreased incorporation of RNA and protein precursors, but also with TdR incorporation. The concentration-effect curve for the incorporation of each precursor is found to be different. The incorporation of amino acids is most severely inhibited, whereas Urd incorporation appears relatively resistant (Fig. 2 and Table III). Moreover, very low concentrations of TPTC, TBTC or THTC (0.1 μM) significantly reduce TdR incorporation (Fig. 2) and cAMP production (Fig. 3) without any effect on the energy metabolism (Fig. 1 and Table I). Although a specific effect cannot be excluded, the latter observation may be explained by assuming that a small shift in metabolic priorities may even prevent parameters reflecting the cellular energy state from changing.

The observed regulatory properties of the cellular energy state could be a special attribute of thymocytes. As described by Donofrio et al. for mouse thymocytes [22], pool sizes of ATP, GTP, dATP and dTTP are less than 10% of pool sizes commonly observed in mammalian cells. This, in combination with a high proliferative capacity, may render thymocytes very sensitive to minor changes in the cellular energy state.

Acknowledgements

The authors wish to express their thanks to Marianne Bol-Schoenmakers, Petra Punt and Ton Ermens for excellent technical assistance, and to Dr. W.J. Vaartjes, Department of Veterinary Biochemistry, Utrecht, for helpful discussions. This work was supported by the Dutch Technology Foundation (STW).

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