Biochemical Pharmacology, Vol. 30, No. 21, pp. 2989–2992, 1981.
Printed in Great Britain.

0006-2952/81/212989-04 $02.00/0

EFFECT OF LOCAL ANAESTHETICS ON LYMPHOCYTE CAPPING AND ENERGY METABOLISM

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(*Received* 13 *January* 1981; *accepted* 25 *May* 1981)

Abstract—Several local anaesthetic and antipsychotic drugs have been tested for their ability to inhibit the capping of s-Ig in mouse spleen lymphocytes and for their effect on the cellular ATP level. Drug concentrations which inhibit capping also lower cellular ATP content below the minimum amount required for a lymphocyte to cap. The two effects show similar kinetics. The ATP depletion caused by these drugs may help to explain their inhibition of lymphocyte capping as well as other effects they cause on living cells.

Capping is an energy-requiring redistribution of membrane proteins triggered by multi-valent ligands [1, 2]. Capping is a general phenomenon of nucleated cells, which is inhibited by metabolic poisons such as sodium azide or dinitrophenol [1, 2]. Several hypotheses have been advanced in order to explain the mechanism of capping [3–8]. Most of them imply an active role of microfilaments in driving the receptors, scattered on the cell surface, toward one pole of the cell. It has been proposed that the connection between cross-linked receptors and microfilaments is mediated by membrane bound calcium [3, 9]. This proposal is based on the inhibitory effect of local anaesthetics on capping [10–12] and on the well established release of membrane bound calcium induced by local anaesthetics both from liposomes and from red cell membranes [13–15]. However, these drugs appear to exert a variety of effects on cells [16] and in particular they affect some enzymatic systems involved in the production of ATP [17, 23]. Hence we have analysed the effects on capping and ATP levels of several amine local anaesthetics and antipsychotic agents.

In this report we show that in most cases the inhibition of capping is quantitatively correlated with a decrease of cellular ATP level. This metabolic effect should be taken into account in order to explain the inhibition by local anaesthetics of lymphocyte capping and in general when considering other effects of these drugs on intact cells.

MATERIALS AND METHODS

Spleen cells were isolated from 2–3 month old Balb/c mice and purified by Ficoll-Hypaque Pharmacia (Uppsala, Sweden) centrifugation. Lymphocytes were suspended at 2×10^7 cells/ml in Hank's minimum essential medium (H-MEM) (GIBCO, Grand Island, NY) buffered with 10 mM Hepes (*N* - 2 - Hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid), (Sigma, St. Louis, MO) at pH 7.3. Cells were preincubated for 10 min in the dark with or without drugs (all from freshly prepared stock solutions in purified DMSO). Capping was carried out at 37° with fluorescein-conjugated rabbit anti-mouse Ig (Miles-Yeda, Rehovot, Israel) extensively dialysed against phosphate buffered saline (PBS) as reported previously [24]. Cells were fixed with paraformaldehyde (2.5% in PBS), washed, resuspended in 30% glycerol/PBS (phosphate buffered saline) and examined by u.v. fluorescence microscopy in a Leitz fluorescence microscope (Leitz GMBH, Wetzlar, West Germany). Capping was scored as the percentage of fluorescent cells with caps covering less than 50% of the cell surface. Over two hundred stained cells were counted per sample. ATP was measured with the luciferase method as previously reported [25] in a DuPont 760 luminescence Biometer, calibrated with standard ATP solutions. ATP results are expressed as a percentage of control samples incubated without drugs, but with DMSO. No drugs interfered at the concentrations used with the luciferase ATP assay. The average mouse spleen lymphocyte ATP content was 625 ± 24 S.D. pmoles × 10^6 cells. Butacaine, chlorpromazine and propranolol were products of Sigma (St. Louis, MO). Lidocaine, benzocaine, tetracaine, procaine, quinidine, dibucaine and phenacaine were from Pfaltz & Bauer (New York, NY). Fluphenazine was kindly provided by Dr. P. Palatini

RESULTS

Some local anaesthetics have been shown to block the capping of s-Ig on mouse B lymphocytes and this effect has been related to their ability to displace membrane-bound calcium [3, 9–12]. Blaustein and Goldman [13] have ordered some amine anaesthetics on the base of their ability to release calcium from model membranes: dibucaine > tetracaine > lidocaine > procaine. We have tried to see if the same order of potency is exhibited in blocking lymphocyte capping by determining the dose–inhibition curves for many drugs showing local anaesthetic effect, most of them previously untested for their effect on capping. Figure 1 shows the curve obtained with pro-

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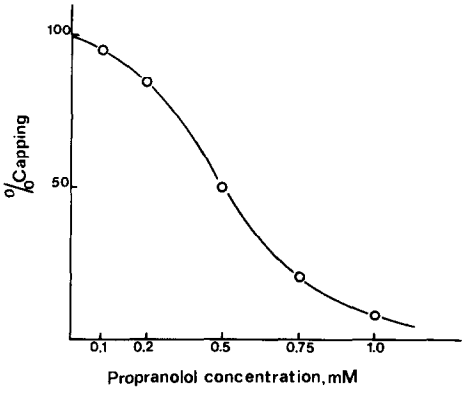

Fig. 1. Inhibition of s-Ig capping on mouse spleen lymphocyte by propranol.

pranolol, a β-blocking agent, which has been shown to displace calcium from rat-heart plasma membranes [26]. Similar curves were obtained with the local amine anaesthetics benzocaine, butacaine, dibucaine, lidocaine, phenocaine, procaine, quinidine, tetracaine and the antipsychotic agents chloropromazine and fluphenazine. Their half-inhibitory and fully inhibitory concentrations are reported in Table 1. We have observed some variability in the fully inhibitory concentrations and Table 1 reports drug concentrations (and corresponding ATP levels) which always caused at least 90% inhibition of capping. It may be noted that the potency of these drugs in inhibiting the capping of s-Ig on mouse spleen lymphocytes correlates with their calcium displacing ability. These findings support the calcium-displacement hypothesis of Schreiner and Unanue, and of Nicholson and Poste [3, 9, 11].

However it has been reported that these drugs inhibit several mitochondrial functions and some enzymes involved in glucose metabolism [17–23]. We have recently shown that, when cellular ATP level is lower than 60–70% of the control, mouse lymphocytes are unable to cap s-Ig, Con-A and the Thy-l antigen [24]. Table 1 shows that drug concentrations that completely inhibit capping also lower cellular ATP below 60–70% of the control value, with the exception of benzocaine. Both the effects on capping and on cellular ATP were fully reversible for all the drugs tested after cell washing and resuspension in medium without drug. Figure 2 shows the correlation between per cent ATP and per cent of capping for six of these drugs with is closely similar to that found for several specific mitochondrial inhibitors [24].

It has been claimed that chlorpromazine does not block Thy-I antigen capping on mouse thymocytes [27]. This result is not in agreement with the present data since we have shown previously that Thy-I capping has the same quantitative dependence on ATP as s-Ig capping. We have been unable to confirm this observation and found that chlorpromazine causes inhibition of Thy-I capping and ATP depletion of mouse thymocytes (Pozzan *et al.*, unpublished results).

Schreiner and Unanue have also shown that increasing the calcium concentration of the medium counteracts the inhibitory effect of chlorpromazine on s-Ig capping [11]. We have confirmed this effect, but also observed that calcium prevents the cellular ATP depletion induced by this drug (not shown).

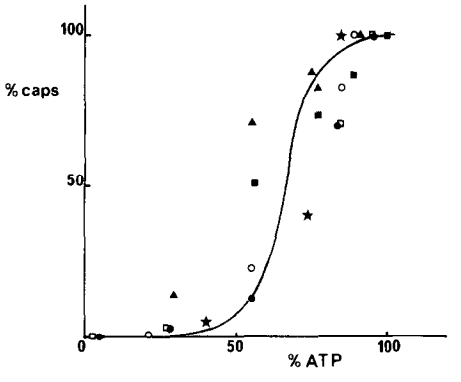

Fig. 2. Relation between capping and ATP content in mouse spleen lymphocyte treated with quinidine (★), propranolol (●), chlorpromazine (■), fluphenazine (▲), tetracaine (○) and dibucaine (□). The solid line, taken from Ref. [24] represents the ATP/capping relationship found for several specific mitochondrial inhibitors.

Table 1. Effects of various concentrations of local anaesthetics on lymphocyte capping and ATP level

Substance	Drug concentration which inhibits capping at 50%	Drug concentration which inhibits capping ≥90%	Per cent of ATP with respect to the control when capping is inhibited ≥90%*
Control	—	—	100%
Chlorpromazine	0.080 mM	0.20 mM	25 ± 8
Fluphenazine	0.025 mM	0.10 mM	17 ± 7
Lidocaine	3.0 mM	8.0 mM	44 ± 10
Phenacaine	0.2 mM	0.6 mM	34 ± 10
Propanolol	0.5 mM	1.0 mM	43 ± 15
Butacaine	0.3 mM	2.0 mM	18 ± 6
Procaine	8.0 mM	15.0 mM	17 ± 4
Tetracaine	0.5 mM	1.0 mM	15 ± 2
Quinidine	0.25 mM	1.0 mM	17 ± 4
Dibucaine	0.5 mM	1.5 mM	15 ± 5
Benzocaine	0.5 mM	2.0 mM	84 ± 7

* Values are the mean ± S.E. of at least five duplicate experiments.

Taken together these results suggest a correlation between the effects of these drugs on capping and on cellular ATP. However the two effects could be kinetically unrelated. We therefore compared the time-course of ATP depletion caused by chlorpromazine on mouse spleen lymphocytes with that of oligomycin, a capping inhibitor, which specifically blocks mitochondrial ATP synthesis. Figure 3 shows that these two drugs lower cellular ATP with similar kinetics, which are comparable to that of capping at 37° (Fig. 3 inset). Similar rates of ATP depletion have been observed with the other drugs tested in this study (not shown).

DISCUSSION

Apart from blocking nerve conduction amine local anaesthetics have been shown to exert a large variety of effects on cells [16]. Among others they inhibit lymphocyte mitogenesis [28], induce changes in cell shape [9] inhibit cell adhesion to substrate [29] promote the lectin-induced agglutination of fibroblast [12]. Moreover they inhibit lymphocyte capping [10, 12] and reverse preformed s-Ig caps [11, 12]. These latter effects have been explained with the ability of local anaesthetics to displace membrane-bound calcium and they have been taken as indication that calcium is involved in the binding of the cellular contractile apparatus to the patches of s-Ig on the plasma membrane [3, 9].

We have previously shown that high cellular ATP levels are required for a lymphocyte to cap [24]. Since local anaesthetics have been shown to interfere with various components of the cell energy producing system [17, 23] we have tested a large series of these compounds for their effects on capping and on cellular ATP content. The results reported here show that all compounds tested, with the exception of benzocaine, lower cellular ATP below the level required for capping to occur. The lack of effect of benzocaine on ATP may be related to its lack of charge at physiological pH. Their action is fast enough to explain their effect on the rapid capping process, which takes place at 37°, and it is very similar to that of specific mitochondrial inhibitors such as oligomycin. These findings demonstrate that local amine anaesthetics act on cellular energy metabolism on the intact cell as well as on isolated components. This effect on cellular ATP may help to explain their inhibition of lymphocyte capping as well as other effects they perform on cells [9, 12, 16, 28, 29]. However local amine anaesthetics also reverse caps once they are formed, while mitochondrial inhibitors are largely uneffective [11, 12]. We have extended this finding to the drugs used in this study and found that they effectively reverse s-Ig caps. This observation and the benzocaine effect suggest that, in addition to inhibit capping by lowering cellular ATP, local anaesthetics may affect directly the capping machinery. This is also supported by the fact that chlorpromazine alters the polymerisation and gelation of isolated actin, a major component of microfilaments [30]. On the basis of spin label examination of membrane fluidity De Foor, Davis and Sondberg [31] have suggested that a membrane expansion caused by the anaesthetic should also be taken into account in order to explain their inhibition of capping.

Phenothiazine drugs have been also shown to inhibit calmodulin regulated processes by binding to calmodulin [32–34]. Then the inhibition effect of these drugs on lymphocyte capping could be related to their effect on calmodulin.

However it appears that these, as well as others drugs, exert such a variety of effects on living cells that any conclusion based on their effects on model system should be considered after an analysis of other possible cellular side effects. The measurement of metabolic parameters such as cellular ATP content and oxygen consumption can exclude an impairment of cellular metabolism.

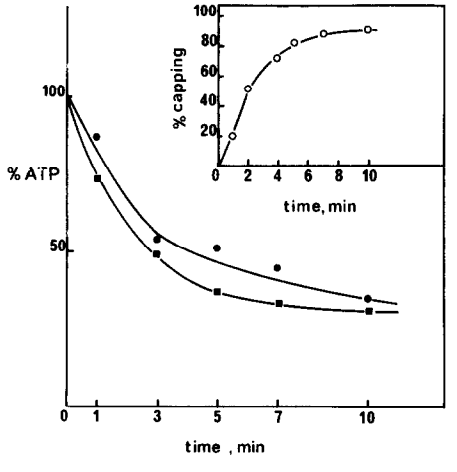

Fig. 3. Time-courses of mouse spleen lymphocyte ATP depletion induced by oligomicin, 2 μg/ml (■) and chlorpromazine, 0.2 mM (●). In the inset the time course of s-Ig capping at 37° is shown.

Acknowledgements—We gratefully acknowledge the support and encouragement of Prof. G. F. Azzone. We are indebted to Prof. G. Fassina, Institute of Pharmacology, for the use of a luminescence Biometer. This work was supported by a grant from C.N.R. for the study of the physiology of mitochondria. During part of this work T. Pozzan was a recipient of an E.M.B.O. long term fellowship.

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