Biochemical Pharmacology, Vol. 28, pp. 2441–2444

0006-2952/79/0815-2441 $02.00/0

# EFFECTS OF LIDOCAIN ON THE MEMBRANE OF HUMAN LYMPHOCYTES

JULIA BOJTA and FERENC ANTONI

First Institute of Biochemistry, Semmelweis University Medical School, Budapest, Hungary

(*Received* 19 *July* 1978; *accepted* 12 *February* 1979)

**Abstract**—(1) Lidocain inhibited [$^3$H]thymidine and [$^3$H]uridine incorporation in human tonsillar lymphocytes known to synthetize DNA in the absence of mitogens. This effect of lidocain was shown to be due to an inhibition by the anaesthetic of the uptake of the labeled precursors. (2) Lidocain was found to sensitize lymphocytes to the cytolytic effect of antilymphocyte serum. Lidocain is assumed to inhibit a hypothetic repair mechanism responsible for the integrity of the plasma membrane.

Local anaesthetics are known to alter the physicochemical state of the plasma membrane of cells by increasing membrane fluidity [1–3]. These compounds decrease the binding of calcium to the membrane [4, 5], and inhibit the function of microtubules and microfilaments [6]. Their anaesthetic effect, i.e. the inhibition of neural conductivity, is due to the inhibition of the influx of sodium ions. Local anaesthetics also affect other kinds of cells: they cause membrane expansion in erythrocytes [7, 8], destroy the "cap" formed on lymphocytes and inhibit "cap" formation [6, 10–12].

Prevention of the mitogen-induced stimulation of lymphocytes by local anaesthetic has been subject of several reports [9, 13]; however, the underlying mechanism has remained obscure. Ferguson has demonstrated that the binding of mitogen to cells is not influenced by the drugs [9, 13]; therefore it has been assumed that either the processing of the stimulation signal or the synthesis of DNA is inhibited [9, 13]. We attempted to gain information on the mechanism of the inhibition of mitogen-induced stimulation of lymphocytes by studying the uptake and incorporation of nucleic acid precursors into human tonsillar lymphocytes which are known to synthetize DNA in the absence of mitogen [14].

Another line of investigation of the membrane alterations caused by Lidocain was concerned with the resistance of lymphocytes to the cytolytic action of antilymphocyte serum (ALS).

## MATERIALS AND METHODS

Lymphocytes were prepared from tonsils of 5 to 10 year-old children as described earlier [15, 16], and suspended for incubation in Eagle's minimal essential medium completed with penicillin and streptomycin (100 $\mu$g per ml, each).

Uptake and incorporation of precursors were studied as follows. Cells ($10^7$ in 1 ml) were incubated at 37° for 60 min with 1.5 $\mu$Ci [$^3$H]thymidine (26 Ci per m-mole) or with 3 $\mu$Ci [$^3$H]uridine (25.5 Ci per m-mole). Labeled precursors were products of the Institute of Isotopes, Prague. After incubation the cells were washed twice with ice-cold Hanks' solution, then homogenized in cold 0.5 N perchloric acid (PCA), and centrifuged, at 2500 r.p.m., at 0° for 10 min. Radioactivity of the acid supernatant was determined. The acid precipitate was washed three times with cold 0.5 N PCA, then treated with 0.5 N PCA at 90° for 60 min, and centrifuged. The radioactivity of the supernatant was determined in a Packard Tri-Carb liquid scintillation spectrometer using toluene based cocktail to which absolute ethanol was added. Radioactivity extracted by hot PCA from the fraction insoluble in cold acid was regarded as a measure of incorporation of precursors into nucleic acids ("incorporation"). The total amount of precursors taken up by the cells ("uptake") was the sum of cold acid soluble radioactivity and the amount incorporated into nucleic acids. Results are expressed as c.p.m. per $10^7$ cells. In each experiment three parallel samples were studied. The coefficients of variation were between 11.7 and 15 per cent.

Antilymphocyte serum was produced by the inoculation of human tonsillar lymphocytes ($10^7$ cells in 0.1 ml) into the paw of 6 to 8 week-old male rats. After 8 days the animals were bled and the serum obtained was stored at −20° for up to two weeks. Serum of untreated animals of similar sex and age was used as control.

Labelling of lymphocytes by $^{51}$Cr was performed in Eagle's medium. Cells ($10^7$ per ml) were incubated at 37° for 30 min with 10 $\mu$Ci of $Na_2{}^{51}CrO_4$ (30 to 10 mCi per mg Cr purchased from the Isotope Institute of the Hungarian Academy of Sciences, Budapest). Subsequently the cells were washed three times with cold Hanks' solution in order to remove free radiochromate. Cells were centrifuged at 0°, at 1000 r.p.m. for 5 min.

Immune cytolysis was investigated by the use of $^{51}$Cr-labeled lymphocytes ($10^6$ cells) suspended in 0.5 ml Eagle's medium containing ALS and additions. Serum concentration was 10% (v/v) and the mixture was incubated at 37° for 40 min, if not stated otherwise. The incubation was terminated by centrifugation. Radiochromium released by the cells into the medium was determined using a well-type crystal scintillation detector (Gamma Works, Budapest). Released $^{51}$Cr was expressed as a percentage of the original total label. In each experiment three parallel samples were studied.

Abbreviations: PCA, perchloric acid; ALS, antilymphocyte serum; c.p.m., counts per minute.

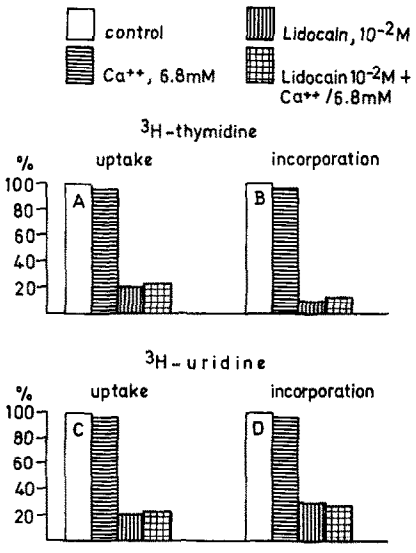


Fig. 1. Effect of Lidocain on the uptake and incorporation of nucleic acid precursors. (Control values, expressed as c.p.m. per $10^7$ cells, were 16,000 for A, 8100 for B, 365,000 for C and 144,900 for D.) Lidocain inhibited the uptake and incorporation of nucleic acid precursors. Calcium ions did not counteract the effect of Lidocain.

The coefficients of variation were between 3.37 and 15.17 per cent.

## RESULTS

1. *Effect of local anaesthetics on the uptake and incorporation of nucleic acid precursors.* Incorporation of [$^3$H]thymidine into the DNA of tonsillar lymphocytes was found to be inhibited by all local anaesthetics tested. The inhibitory effect of the compounds (at $10^{-3}$ M concentration) correlated with their anaesthetic efficiency, Nupercain being the most inhibitory followed by Tetracain, Lidocain and Procain (Table 1).

The effect of Lidocain is shown in Fig. 1. It can be seen that the cellular uptake of both [$^3$H]thymidine and [$^3$H]uridine were strongly inhibited by Lidocain, to about the same extent. Inhibition of incorporation was more marked in the case of thymidine where the precursor pool was smaller. Addition of calcium ions did not counteract any of these inhibitory effects of Lidocain. Uptake and incorporation of precursors were restored (85.1–92.5 per cent of the control value) after the removal of the local anaesthetic by changing the suspending medium. The uptake and incorporation of lymphocytes, which have been submitted to the same experimental procedure but without Lidocain, were 96.2–98.2 per cent of the control value.

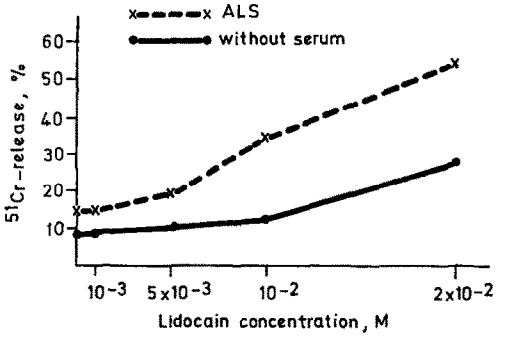


Fig. 2. Effect of Lidocain on the spontaneous and ALS-induced $^{51}$Cr release. The spontaneous $^{51}$Cr release was increased by Lidocain only at a concentration higher than $10^{-2}$ M. The ALS-induced $^{51}$Cr release was potentiated by Lidocain at $5 \times 10^{-3}$ M.

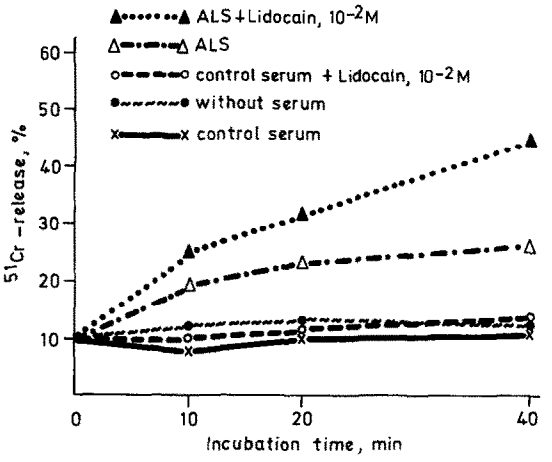


Fig. 3. Time course of $^{51}$Cr release in the presence of Lidocain.

2. *Sensitization of lymphocytes to the cytolytic effect of ALS by Lidocain.* Immune cytolysis induced by ALS was quantitated by measuring the amount of $^{51}$Cr released from lymphocytes prelabeled with radiochromium. Lidocain increased markedly the ALS-induced $^{51}$Cr-release of lymphocytes, although it did not influence significantly the spontaneous $^{51}$Cr-release of cells up to a concentration of $10^{-2}$ M (Fig. 2), therefore the drug was applied at this concentration in the following experiments.

The time course of cytolysis (Fig. 3) as well as its dependence on serum concentration (Fig. 4) show that Lidocain had a characteristic potentiating effect on the cytotoxicity of ALS. The potentiating effect of Lido-

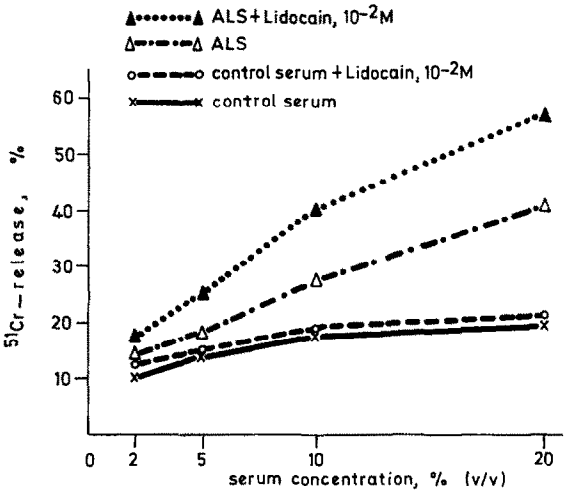


Fig. 4. Effect of serum concentration on $^{51}$Cr release in the presence of Lidocain.

Table 1. The relative anaesthetic efficiency and the per cent inhibition of [$^3$H]thymidine incorporation

| Anaesthetics | Relative anaesthetic efficiency | Per cent inhibition of [$^3$H]thymidine incorporation at $10^{-3}$ M | $10^{-2}$ M |
|---|---|---|---|
| Procain | 1 | 20.0 | 73.0 |
| Lidocain | 2 | 44.3 | 91.3 |
| Tetracain | 25 | 98.8 | 99.7 |
| Cinchocain | 25 | 99.6 | 99.8 |

cain on immune cytolysis was counteracted by calcium ions which had no effect on either the spontaneous or the ALS-induced $^{51}$Cr-release (Fig. 5).

The experiment presented in Fig. 6 was devised for the elucidation of the peculiar effect of Lidocain described above. Labeled lymphocytes were preincubated for 10 min in control medium, or with 5% ALS, in the presence and absence of Lidocain. Then the cells were centrifuged and resuspended in fresh media containing various additions as indicated (when present, ALS was 10%), and incubated for further 30 min.

Preincubation of the cells in control medium had no effect. The spontaneous $^{51}$Cr release was about the same during the (second) incubation as in the experiments without preincubation. ALS increased the $^{51}$Cr release, Lidocain did not affect the $^{51}$Cr release in the absence of ALS.

The same results were obtained when cells were preincubated for a short time (10 min) in the presence of ALS. Thus the effect of ALS is reversible; after its removal it does not affect the $^{51}$Cr release during the (second) incubation. In contrast, after preincubation of cells with ALS and Lidocain, $^{51}$Cr release continued even in the control medium. Further, Lidocain in itself was as effective in maintaining the leakage of $^{51}$Cr from cells as ALS. Cells, simultaneously exposed to ALS and Lidocain, are more susceptible to $^{51}$Cr release.

Accordingly, the reversible injury of cells caused by ALS (and complement) changed to an irreversible damage in the presence of Lidocain. Calcium ions were shown to counteract the ALS-potentiating effect of Lidocain, and in the latter system calcium restored the reversible character of the cellular injury.

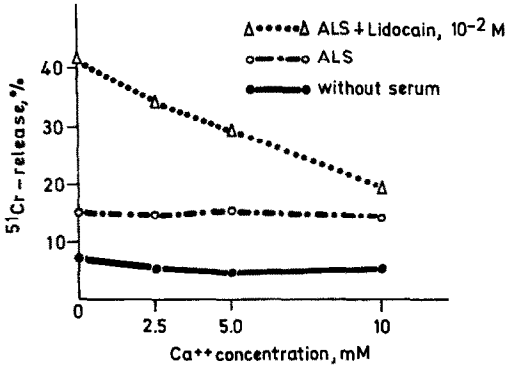


Fig. 5. Effect of calcium ions on $^{51}$Cr release in the presence of Lidocain. Calcium ions had no effect on the $^{51}$Cr release in the absence of Lidocain, but inhibited the potentiating effect of Lidocain on the ALS-induced $^{51}$Cr release.

These experiments were carried out 7 times. The coefficients of variation were between 4.26 and 14.8 per cent.

## DISCUSSION

Local anaesthetics are known to affect the plasma membrane of cells in general. This is why the observations that these compounds inhibit mitogen stimulation without interfering with the membrane-binding of mitogens have been difficult to interpret. Human tonsillar lymphocytes, which contain many stimulated cells syn-

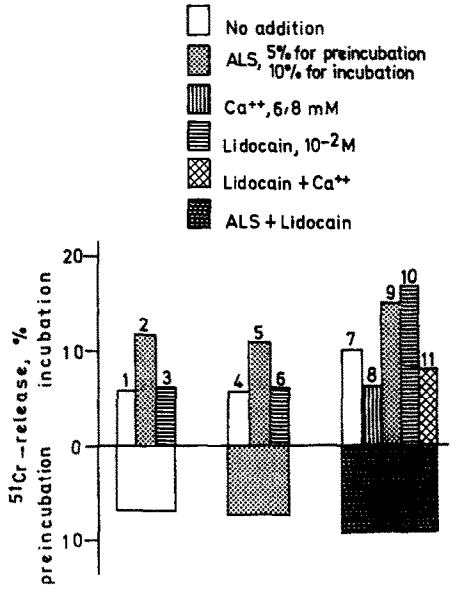


Fig. 6. Reversibility of the $^{51}$Cr release of ALS-treated lymphocytes. The lower columns show the $^{51}$Cr release during preincubation in control medium, ALS (5%) containing medium, and in the presence of ALS (5%) and Lidocain ($10^{-2}$ M), respectively. After preincubation, taking 10 min, the media were changed and cells were incubated for a further 30 min.

Control media were exchanged for: column 1—control medium; column 2—ALS (10%)-containing medium; column 3—Lidocain ($10^{-2}$ M)-containing medium.

ALS containing media were exchanged for: column 4—control medium; column 5—ALS (10%)-containing medium; column 6—Lidocain ($10^{-2}$ M)-containing medium.

ALS and Lidocain containing media were exchanged for: column 7—control medium; column 8—calcium (6.8 mM)-containing medium; column 9—ALS (10%)-containing medium; column 10—Lidocain ($10^{-2}$ M)-containing medium; column 11—calcium (6.8 mM)- and Lidocain ($10^{-2}$ M)-containing medium.

thetizing DNA in the absence of mitogen [14] are particularly suited for the examination of this problem, since in these cells mitogenic stimulation has occurred *in vivo*. There remained to be investigated whether the incorporation of externally supplied precursors is blocked at some stage. As shown by our results, at least one of the impaired steps is the membrane transport of nucleosides. This may account for all findings according to which mitogen-induced incorporation of radioactive thymidine has been inhibited by local anaesthetics [9, 13]. Neither the inhibition of mitogen stimulation [9] nor the inhibition of transport have been prevented by calcium ions. The inhibitory effect of local anaesthetic was found to be reversible.

The enhancement by Lidocain of the ALS-induced, complement-mediated cytolysis exhibited the characteristics of a potentiating effect. Lidocain may sensitize the plasma membrane to the action of the complement and/or it may inhibit some mechanism responsible for the reparation of complement induced lesions. According to the doughnut hypothesis [17] these lesions are transmembrane channels produced by the annular insertion of terminal complement components into the phospholipid bilayer. However, according to recent concepts complement lysis is accompanied by a major rearrangement of the membrane structure [18], involving lateral seggregation of membrane lipids and proteins [19], probably due to the detergent like effect of C8 and C9 [20]. Local anaesthetics are known to increase the fluidity of membranes [1–3], thus changes in the physico-chemical state of the membrane caused by Lidocain may predispose the cells to the disorganizing effect of the complement.

We observed that ALS-treated lymphocytes recovered from the state of increased permeability after the removal of ALS (Fig. 6). Similarly, $^{86}Rb$ release has been found to be a reversible, nonlethal event in hepatoma cells treated with antibody and complement [21]. These findings suggest the existence of a repair mechanism which can restore membrane integrity following complement-induced damage. Decreased resistance of tumour cells to immune cytolysis after pretreatment with the chemotherapeutics 6-fluorouracil and 6-mercaptopurin [16] may also be explained by an inhibition of such a repair mechanism. Our finding that Lidocain, when present during the exposition of lymphocytes to ALS, sustained the increased permeability of cells even after removal of the antiserum (Fig. 6), suggests that Lidocain interferes with the alleged repair mechanism. Probably the permeability of the plasma membrane is determined by the extent of the complement-induced damage and by the efficiency of the repair mechanism.

## REFERENCES


1. A. G. Lee, *Biochim. biophys. Acta.* **448**, 34 (1976).
2. A. G. Lee, *Nature, Lond.* **262**, 545 (1976).
3. M. K. Jain, N. Yen-Min Wu and L. V. Wrong, *Nature, Lond.* **255**, 494 (1975).
4. W. O. Kwant and Ph. Seeman, *Biochim. biophys. Acta.* **193**, 338 (1969).
5. W. Kazimierczak, M. Perct and Cz. Maslinski, *Biochem. Pharmac.* **25**, 1747 (1976).
6. G. L. Nicolson, *Biochim. biophys. Acta.* **457**, 57 (1976).
7. P. Seeman, W. O. Kwant and T. Sauks, *Biochim biophys. Acta.* **183**, 499 (1969).
8. S. Roth and P. Seeman, *Nature, New Biol.* **231**, 284 (1971).
9. R. M. Ferguson, J. R. Schmidtke and R. L. Simmons, *J. Immun.* **116**, 627 (1976).
10. G. Poste, D. Papahadjopoulos and G. L. Nicolson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 4430 (1975).
11. G. F. Schreiner and E. R. Unanue, *Clin. Immun. Immunopathol.* **6**, 264 (1976).
12. G. B. Ryan, E. R. Unanue and M. J. Karnovsky, *Nature, Lond.* **250**, 56 (1974).
13. R. M. Ferguson, J. R. Schmidtke and R. L. Simmons, *Nature, Lond.* **256**, 744 (1975).
14. M. Staub, F. Antoni and M. Sellyei, *Biochem. Med.* **15**, 246 (1976).
15. F. Antoni in *Tonsils: Structure, Biochemistry and Immunology* (eds. F. Antoni and M. Staub) p. 81. Akadémiai Kiadó, Budapest (1978).
16. P. Piffkó, H. G. Köteles and F. Antoni, *Prac. Otorhinolaryng* **32**, 350 (1970).
17. S. Bhakdi, V. Speth, H. Knüfermann, D. F. Hoelzl Wallach and H. Fischer, *Biochim. biophys. Acta.* **356**, 300 (1974).
18. S. L. Kinsky, *Biochim. biophys. Acta.* **265**, 1 (1972).
19. M. D. P. Boyle, S. H. Ohanian and T. Borsos, *J. Immun.* **117**, 1346 (1976).
20. M. Segerling, S. H. Ohanian and T. Borsos, *Science*, **188**, 55 (1975).
21. G. Poste, D. Papahadjopoulos, K. Jacobson and W. J. Vail, *Biochim. biophys. Acta.* **394**, 520 (1975).
22. P. G. W. Plagemann and D. P. Richey, *Biochim. biophys. Acta.* **344**, 263 (1974).