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# PLASMA MEMBRANE STUDIES ON DRUG-SENSITIVE AND -RESISTANT CELL LINES

## II. OUABAIN SENSITIVITY OF $(Na^+ + K^+)$-STIMULATED $Mg^{2+}$-ATPase

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SUMMARY

Mutant cell lines have been selected from the murine plasmocytoma MOPC 173 for their resistance to ouabain, dibutyryl cyclic AMP, theophyllin and concanavalin A. We have compared three wild-type cell lines with their seven resistant counterparts. All resistant mutants exhibited a $(Na^+ + K^+)$-stimulated $Mg^{2+}$-ATPase resistance to ouabain inhibition when measured in microsomes. The homogeneity of ouabain binding sites has been found in most of the cell lines; however, two different populations of sites have been detected in one wild-type and in one resistant cell lines.

These results led us to hypothetise the $(Na^+ + K^+)$-ATPase-ouabain interaction being modulated by a non-specific membrane structure.

## INTRODUCTION

Comparisons of drug resistant mutants [1–6] with their wild-type counter-parts are useful in biochemical investigation of cell functions. Most of the studies in this field have concentrated on the genetic implications of such traits as changes in drug binding [2, 7] and variations in metabolic pathways [3].

The isolation and characterisation of particular mutants altered in their plasma membranes should contribute substantially to our understanding of the functions and organization of that organelle. We have used this approach in selecting mutants (or variants) by their resistance to drugs which are supposed to be active at the cell surface level and are known to inhibit cell growth. Ouabain [8, 9] specific inhibitor of $(Na^+ + K^+)$-ATPase [10], dibutyryl cyclic AMP [11], theophyllin [12] and concanavalin A [13] were used.

We have shown that clones selected for their growth resistance to one of these agents show resistance to the three other drugs [14, 15]. Moreover this cross-resistance has been shown to be correlated with a lack in these mutants of the coupling between $(Na^+ + K^+)$-ATPase and adenylate cyclase found in different wild phenotypes derived from the murine plasmocytoma MOPC 173 [16].

TABLE 1

PROPERTIES OF WILD-TYPE CELLS AND CELLS SELECTED FOR OUABAIN, CONCANAVALIN A, DIBUTYRYL CYCLIC AMP AND THEOPHYLLIN RESISTANCE

| Cell lines | Selecting agents | Morphology | Density-dependent inhibition of growth | Number of cells $\times 10^{-4}/cm^2$ at confluency | Doubling time (h) |
|---|---|---|---|---|---|
| $ME_2$ | wild type | epitheloid | + | 5.6 ± 1.2 | 24 |
| $ME_2OR_1$ | ouabain | epitheloid | + | 10 ± 2 | 18 |
| $ME_2CR_1$ | concanavalin A | epitheloid | + | 20 ± 2 | 20 |
| $ME_2CAR_1$ | dibutyryl cyclic AMP | fibroblastic | − | 26 ± 2 | 15 |
| $ME_2TR_1$ | theophyllin | fibroblastic | − | 24 ± 4 | 24 |
| $MF_2$ | wild type | fibroblastic | − | 32 ± 4 | 12 |
| $MF_2OR_1$ | ouabain | epitheloid | + | 8 ± 1.5 | 18 |
| $MF_2OR_2$ | ouabain | fibroblastic | − | 40 ± 6 | 12 |
| $MF_2CAR_1$ | dibutyryl cyclic AMP | fibroblastic | − | 25 ± 4 | 12 |
| $MF_2S$ | wild type | $MF_2$ growing ascites cells in Balb/c mice | | | |

This paper deals with specific inhibition of the $(Na^+ + K^+)$-ATPase in a microsomal cell fraction isolated from seven different mutant and three wild-type clones in order to substantiate the evidence for cross-resistance at the molecular or supramolecular level. We shall see that each drug-resistant cell line, whatever the selecting agent, differs from the wild type by its ouabain sensitivity of the plasma membrane $(Na^+ + K^+)$-stimulated $Mg^{2+}$-ATPase (EC 3.6.1.3) ($(Na^+ + K^+)$-ATPase).

## MATERIALS AND METHODS

*Cell lines.* All cell lines were derived from the murine plasmocytoma MOPC 173. Three wild types were considered: $ME_2$, epitheloid, growing at low density and contact inhibited; $MF_2$, fibroblastic, growing at high density and not contact inhibited; $MF_2S$, derived from $MF_2$ by passaging in mice where the cells grow as ascites.

From the $ME_2$ cell line we selected mutants resistant to ouabain ($ME_2OR_1$), concanavalin A ($ME_2CR_1$), dibutyryl cyclic AMP ($ME_2CAR_1$) and theophyllin ($ME_2TR_1$). Similarly, we have obtained from the wild type $MF_2$, mutants resistant to ouabain ($MF_2OR_1$ and $MF_2OR_2$) and to dibutyryl cyclic AMP ($MF_2CAR_1$). As $MF_2$ is resistant to theophyllin and concanavalin A, we did not try to isolate mutants resistant to these drugs. Culture and cloning conditions have already been described [14, 15, 17]. In brief, all $ME_2$-derived cell lines are grown in an Eagle-actalbumin hydrolysate-yeast extract medium supplemented with vitamins, glutamin and 2 % calf serum. $MF_2$ cell lines were grown in the same medium with 10 % calf serum. All cell lines were grown on glass (Roux flasks or roller-bottles) or plastic flasks.

$ME_2$ and $MF_2$ cell lines were gradually adapted to grow in the presence of increasing amounts of each drug. After 20–50 passages, the cells were diluted and seeded. Single colonies were removed with trypsin, replated, grown, and again cloned two more times.

The general properties of the mutants under study are shown in Table I.

TABLE II

THE RELATIVE SUSCEPTIBILITY OF THE DIFFERENT CELL LINES TO THE DIFFERENT DRUGS EXPRESSED BY THEIR $G_{\frac{1}{2}}$ (see text)

| Cell lines | Membrane-active agents | | | |
|---|---|---|---|---|
| | Ouabain (mM) | Concanavalin A ($\mu$g/ml) | Dibutyryl cyclic AMP (mM) | Theophyllin (mM) |
| $ME_2$ | 0.02 | 25 | 0.02 | 0.5 |
| $ME_2OR_1$ | 0.2 | 50 | 0.5 | 1 |
| $ME_2CR_1$ | 0.15 | 100 | N.T. | N.T. |
| $ME_2CAR_1$ | 0.1 | 50 | 2 | 2 |
| $ME_2TR_1$ | 0.2 | 50 | 0.02 | 2 |
| $MF_2$ | 0.2 | 100 | 0.5 | 1 |
| $MF_2OR_1$ | 2 | 100 | 1 | N.T. |
| $MF_2OR_2$ | 2 | 100 | 2 | N.T. |
| $MF_2CAR_1$ | 0.5 | N.T. | 2 | N.T. |

N.T., not tested.

*Growth and cross-resistance studies.* $G_{\frac{1}{2}}$ was defined as the molarity (or weight) of the drug which gave 50 % fewer cells than the control after 5 days (for fibroblastic) or 7 days (for epitheloid cell lines) in culture. It can be seen on Table II that resistant cell lines, whatever the selective agent was, were more resistant to ouabain than their wild-type counterparts.

*Plasma membrane isolation.* The cells were collected by scraping the surface of the flask. After two washes in saline, the cells were resuspended in the lytic medium ($10^7$ cells/ml) [18]. After 20–30 min magnetic stirring, the lysed cell suspension was centrifuged at 27 000 $\times g$ for 20 min. No $(Na^+ + K^+)$-ATPase activity could be detected in the supernatant [18]. The pellet $P_0$ (47 $\pm$ 5 % of the protein) was suspended in 30 mM imidazol · HCl, 0.25 M sucrose and 1 mM EDTA, pH 6.8. The centrifugation technique of Jørgensen and Skou [19] was then followed.

*Protein determination and enzymatic assay.* The protein content of the microsomal preparations was determined according to the procedure described by Lowry et al. [20]. The procedure for $(Na^+ + K^+)$-ATPase assay was described by Jørgensen and Skou [19]. Kinetic studies were performed by transferring aliquots at varying times to test tubes where the reaction was stopped and inorganic phosphate measured [21].

$(Na^+ + K^+)$-ATPase activity was measured by the difference between the hydrolytic activity without and with 1 mM ouabain (prepared daily), which was shown identical to the stimulation by $Na^+ + K^+$ when added to the $Mg^{2+}$ ATPase. The ouabain susceptibility of $(Na^+ + K^+)$-ATPase was checked with increasing amounts of ouabain ($10^{-8}$–$10^{-3}$ M). The mixture was preincubated for 10 min, whatever the ouabain concentration, at 37 °C prior to addition of substrate. The reaction was linear with respect to both time from 3 to 15 min, and protein concentration from 10 to 100 $\mu$g/ml. Routinely, the assays were performed with 60 $\mu$g of protein per ml.

## RESULTS

*Protein yield and distribution.* The distribution of protein between the different pellets and supernatant for all cell types was as follows. 27 000 $\times g$ for 30 min ($P_0$): 47 $\pm$ 5 %; 7500 $\times g$ for 15 min ($P_1$): 26 $\pm$ 6 %; 31000 $\times g$ supernatant for 30 min: 14 $\pm$ 2 %.

The amount of protein in the 31 000 $\times g$ pellets (so called $P_2$) is given in Table III.

*$(Na^+ + K^+)$-ATPase ouabain-sensitive activity.* Whatever the cell type, the specific activity in the lysate was 1.2 $\pm$ 0.2 $\mu$mol $P_i$/h per mg of protein. In all studied cell lines, this activity was only recovered in $P_2$ fraction (31 000 $\times g$ pellet) with most cell lines the yield in the $P_2$ fraction was from 25 to 55 %. However, $ME_2CR_1$, both contact-inhibited and drug-resistant cell lines, gave final yields of enzymatic activity which were very low (approx. 10 %). In all cell lines tested, except for $ME_2OR_1$, the ouabain-sensitive activity was similar to $Na^+ + K^+$ stimulation of ATP hydrolysis activity. 20 % of the total ATPase activity in $ME_2OR_1$ plasma membranes was not inhibited by 2 mM ouabain.

A pattern is evident in Table III: with the exception of $ME_2CR_1$, the enzymatic activity in $P_2$ from wild-type and resistant $ME_2$ cell lines was higher than 10 $\mu$mol

TABLE III

PROTEIN AND ENZYME ACTIVITY RECOVERIES IN CRUDE PLASMA MEMBRANES FROM SENSITIVE AND RESISTANT CELL LINES

| Cell lines | Amount of protein in $P_2$[a] in respect to lysate (%) | $Na^+ + K^+$ stimulation of ATP hydrolysis (%)★ | Specific activity of $(Na^+ + K^+)$-ATPase ($\mu$mol/h per mg protein) | Final recovery[b] (%) | Purification[c] |
|---|---|---|---|---|---|
| $ME_2$[d] | 4.7 ± 0.2 | 60 ± 7 | 21 ± 1 | 55 | 12 |
| $ME_2OR_1$[d] | 4.7 ± 0.2 | 33 ± 4 | 10 ± 1 | 32 | 7 |
| $ME_2CAR_1$ | 4.3 ± 0.1 | 67 ± 10 | 9 ± 1 | 38 | 9 |
| $ME_2CR_1$ | 2 ± 0.1 | 60 ± 10 | 4.5 ± 0.1 | 8 | 4 |
| $ME_2TR_1$ | 5.7 ± 0.5 | 63 ± 5 | 10 ± 1.1 | 34 | 6 |
| $MF_2$ | 6 ± 0.8 | 70 ± 10 | 5.2 ± 0.3 | 30 | 5 |
| $MF_2OR_1$[d] | 3.7 ± 0.1 | 64 ± 2 | 4 ± 0.4 | 11 | 3 |
| $MF_2OR_2$ | 4.3 ± 0.3 | 70 ± 5 | 11 ± 0.4 | 34 | 8 |
| $MF_2CAR_1$ | 4.1 ± 0.3 | 71 ± 5 | 5.2 ± 0.2 | 17 | 4 |
| $MF_2S$ | 5.5 ± 0.2 | 90 ± 5 | 10 ± 0.4 | 40 | 7 |

[a] $P_2$, 31 000 × $g$ pellet

[b] Total activity of $(Na^+ + K^+)$-ATPase in $P_2$ with respect to the original activity in the cell lysate.

[c] Specific activity of $(Na^+ + K^+)$-ATPase in $P_2$/specific activity in the cell lysate.

[d] These cell lines have a decreased $(Na^+ + K^+)$-ATPase activity at confluency and, thus, were harvested at the growing phase. All other cell lines were harvested at confluency.

★ Total ATPase activity, $Mg^{2+}$ ATPase activity/$Mg^{2+}$ activity × 100

TABLE IV

THE RELATIVE SUSCEPTIBILITY TO OUABAIN OF $(Na^+ + K^+)$-ATPase FROM SENSITIVE AND RESISTANT CELL LINES

| | Cell line | Ouabain ($\mu$M) needed to inhibit enzyme activity for 50 % ($E_{\frac{1}{2}}$) |
|---|---|---|
| Wild types | $ME_2$ | 3.8 ± 0.6 |
| | $MF_2$ | 0.8 ± 0.1 and 90 ± 7 |
| | $MF_2S$ | 0.47 ± 0.03 |
| Ouabain selected | $ME_2OR_1$ | 170 ± 20 |
| | $MF_2OR_1$ | 14 ± 4 |
| | $MF_2OR_2$ | 60 ± 5 |
| Other drug selected | $ME_2CAR_1$ | 24 ± 4 |
| | $ME_2CR_1$ | 20 ± 5 |
| | $ME_2TR_1$ | 4 ± 0.4 and 97 ± 7 |
| | $MF_2CAR_1$ | 12 ± 1 |

$P_i$/h per mg of protein. In $MF_2$ and derived cell lines, specific activities did not exceed 5 excepted for $MF_2OR_2$. All resistant cell lines derived from $ME_2$ appear to have a lower specific activity as compared to the wild types (Table III). In contrast, the $MF_2$-resistant cell lines exhibited the same or higher specific activity than the wild type.

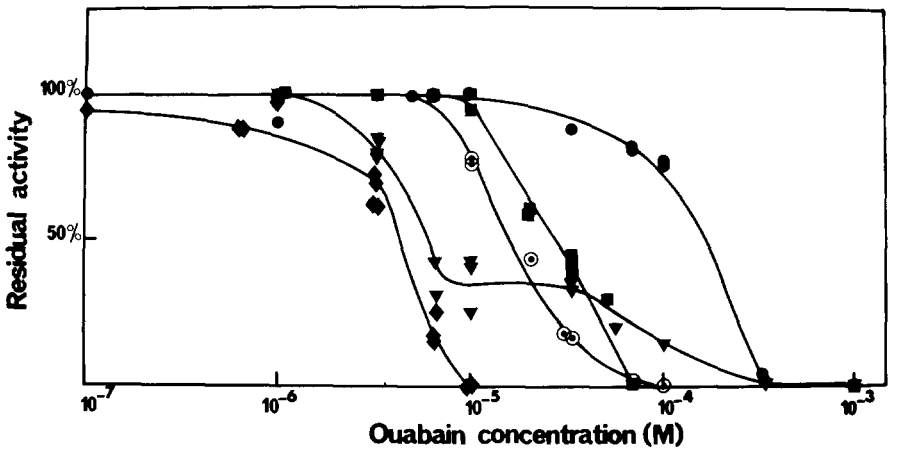


Fig. 1. Dose vs. response curves: $(Na^+ + K^+)$-stimulated $Mg^{2+}$-ATPase activity vs. logarithm of ouabain concentrations: $ME_2$ wild type and its resistant derivatives. ◆, $ME_2$; ●, $ME_2OR_1$; ■ $ME_2CAR_1$; ⊙, $ME_2CR_1$; ▼, $ME_2TR_1$.

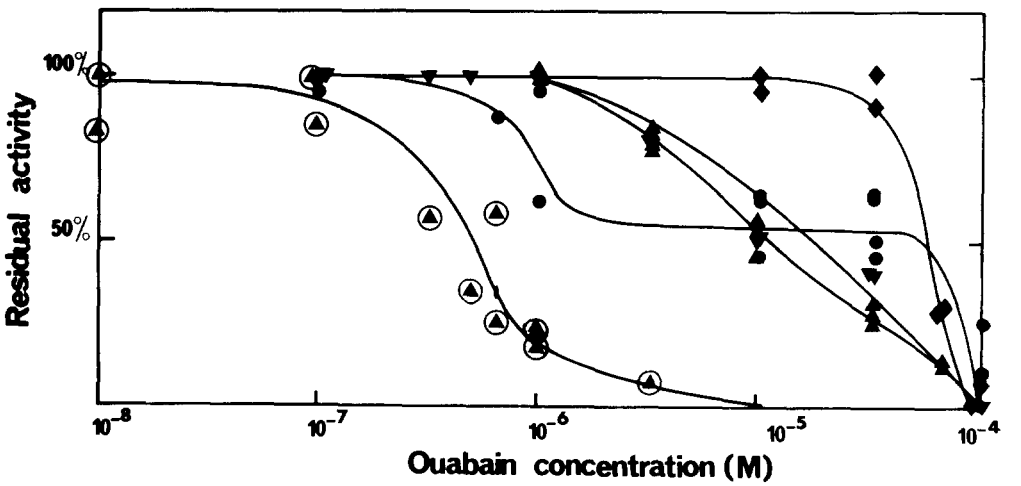


Fig. 2. Dose vs. response curves: $(Na^+ + K^+)$ stimulated $Mg^{2+}$-ATPase activity vs. logarithm of ouabain concentrations: $MF_2$ and $MF_2S$ wild types and $MF_2$-derived cell lines. ●, $MF_2$; ▼, $MF_2OR_1$; ◆, $MF_2OR_2$; ▲, $MF_2CAR_1$; ▲, in circle, $MF_2S$.

*The susceptibility of $(Na^+ + K^+)$-ATPase to ouabain inhibition.* We measured the $(Na^+ + K^+)$-ATPase activity for each cell line with ouabain concentrations varying between $10^{-8}$ and $10^{-3}$ M. Table IV shows the ouabain concentrations required for a 50 % inhibition of activity ($E_{\frac{1}{2}}$) for each 31 000 × *g* sediment. Note that the wild type lines give extracts which vary considerably in their sensitivities ($E_{\frac{1}{2}}$ = 0.47–3.8 μM). However, all the drug-resistant derivatives have increased ouabain resistance of their activities with respect to their respective wild types. The resistance of the activities from lines selected for ouabain resistance was consistantly higher

(20–100-fold over wild type) than that of the cross-resistant lines (selected for dibutyryl cyclic AMP, theophyllin or concanavalin A resistance).

No correlation could be found between the specific activity of $(Na^+ + K^+)$-ATPase (Table III) and the $E_{\frac{1}{2}}$ value (Table IV) in the same cell line.

The shapes of the ouabain dose response curves are shown in Figs. 1 and 2. In general the activities are fully resistant up to a critical concentration and then fall sharply to zero. Exceptions to this are the response of $MF_2OR_1$ and $MF_2CAR_1$ which show a shallow decline in resistance after the plateau. The wild-type line $MF_2$ gave a marked two-step dose vs. response curve suggesting the presence of two binding activities giving $E_{\frac{1}{2}}$ values of 0.8 and 90 $\mu$M. The low affinity binding sites were associated with $55 \pm 8$ % of the initial activity. It is interesting to note that all lines derived from $MF_2$ show single response curves as if one of the binding site classes is lost. $MF_2OR_2$ for example seems to have retained only the weak binding reaction ($E_{\frac{1}{2}} = 60$ $\mu$M) whereas the response of $MF_2S$ (N.B. not selected for drug resistance) resembles the strong binding reaction ($E_{\frac{1}{2}} = 0.47$ $\mu$M). $ME_2$ wild type and its resistant derivatives showed a single affinity dose vs. response curve with the exception of $ME_2TR_1$ which had acquired a two-step response with $E_{\frac{1}{2}}$ values of 4 and 97 $\mu$M, respectively. The low affinity binding sites were associated with $35 \pm 10$ % of the total activity.

Furthermore, the $E_{\frac{1}{2}}$ value could not be taken as the only criterion for resistance: the $E_{\frac{1}{2}}$ of $ME_2CAR_1$, $ME_2CR_1$, $MF_2CAR_1$ and $MF_2OR_1$ are approximately the same but the shapes of the dose vs. response curves differ clearly (Figs. 1 and 2).

## DISCUSSION

*Cross-resistance.* Microsomes prepared from ouabain, dibutyryl cyclic AMP, theophyllin and concanavalin A resistant cell lines exhibited a resistance of the $(Na^+ + K^+)$-stimulated $Mg^{2+}$-ATPase to ouabain inhibition. This resistance was found to be 5–100-fold as compared to that of the original wild-type cell lines. Thus, the original observation at the cell growth level [3] of cross-resistance to different drugs is supported by the fact that the $(Na^+ + K^+)$-stimulated $Mg^{2+}$-ATPases from the resistant cell lines are resistant to ouabain whatever the selective agent was. However, a comparison between the $G_{\frac{1}{2}}$ (amount of ouabain decreasing cell growth by 50 %) and the $E_{\frac{1}{2}}$ (amount of ouabain decreasing $(Na^+ + K^+)$-ATPase activity by 50 %) shows discrepancies in the relative susceptibility of the drug-resistant cell lines (Table V). For instance, $ME_2OR_1$ has a $G_{\frac{1}{2}}$ lower than that of $MF_2OR_1$ but the reverse is true for their respective $E_{\frac{1}{2}}$. $^{42}K$ flux has been shown to be inhibited by ouabain concentrations which did not affect the cell growth [7, 22]. Thus, the $E_{\frac{1}{2}}$ which measures a specific membrane-bound enzyme-inhibitor interaction may not correlate with the $G_{\frac{1}{2}}$ which corresponds to an integrated cellular response to a cytotoxic agent. Moreover, we cannot rule out the possibility that during the isolation of $P_2$ fractions some changes take place in the overall membrane structure [23] which affect the $(Na^+ + K^+)$-ATPase-ouabain interaction. The membrane-bound enzyme activity has been shown to be modified by membranes proteins [23] and/or phospholipids alterations [24], by increased membrane fragmentation [25] or by a relative permeability of the membrane to ATP [26]. Furthermore, the relative proportion of inside out (IO) and

TABLE V

CLASSIFICATION OF THE CELL LINES ACCORDING TO THEIR RESPECTIVE $G_{\frac{1}{2}}$ AND $E_{\frac{1}{2}}$

| In culture $G_{\frac{1}{2}}$ | The highest resistance | In plasma membranes $E_{\frac{1}{2}}$ |
|---|---|---|
| $MF_2OR_1$<br>$MF_2OR_2$<br>$MF_2CAR_1$ | ↑ | $ME_2OR_1$<br>$ME_2TR_1$ (low affinity)<br>$MF_2$ (low affinity) |
| $ME_2OR_1 = MF_2 = ME_2TR_1$ | | $MF_2OR_2$ |
| $ME_2CR_1$<br>$ME_2CAR_1$ | | $ME_2CR_1 = ME_2CAR_1$<br>$MF_2OR_1 = MF_2CAR_1$ |
| | The lowest resistance | $ME_2 = ME_2TR_1$ (high affinity) |
| $ME_2$ | | $MF_2$ (high affinity) |

right side out vesicles (RSO) has been shown to be correlated with the sensitivity of ATPase to ouabain [9, 27]. It was shown that the $(Na^+ + K^+)$-ATPase in IO vesicles had a specific activity three-fold higher than the RSO vesicle-bound ATPase [28]. A 30 % variation in specific activity in the cell lysate ($1{,}2 \pm 0.2$) might be at most associated with a 10 % variation in the percentage of IO to RSO. Thus we can assume a close distribution of IO and RSO in the $P_2$ fraction whatever the cell type. Moreover, in our case, the ouabain sensitivities of $(Na^+ + K^+)$-ATPase in inverted and right side out vesicles isolated from $MF_2S$ cell plasma membranes were found to be identical [29]. From these two evidences, we can assume that in our case the difference in ouabain sensitivity should not depend on the IO/RSO vesicle distribution. Moreover in preliminary experiments, the $P_2$ fractions isolated from the wild type and the ouabain resistant $MF_2$ cells were found to show the same sensitivity to ouabain before and after deoxycholate treatment. Thus the large differences found in ouabain susceptibility in the different cell lines could be due to a structural difference between each mutant

*Heterogeneity of ouabain binding sites.* ATP hydrolysis by $P_2$ fraction in the presence of ouabain was linear with time from 3 to 15 min, which strongly suggests an immediate ouabain binding to the $(Na^+ + K^+)$-ATPase even at low doses of inhibitor. On living cells, ouabain binding takes place within 1 min (Charlemagne, D. and Geny, B., personal communication) and which is consistant with the results described here. This rapid binding has also been reported for canine kidney cell $(Na^+ + K^+)$-ATPase preparations [30]. If we assume that ouabain binding paralled the $(Na^+ + K^+)$-ATPase inhibition [31] the comparison of the dose vs. response curves of $ME_2$, $MF_2S$ and $MF_2$ showed an homogeneous population of ouabain binding sites ($ME_2$, $MF_2S$) or an heterogeneous population ($MF_2$). In the latter case, two different sites expressing a 100-fold difference in ouabain binding are implied. The same observation was reported for brain microsomes [32, 33] and Hela cells [7]. All $MF_2$-resistant cell lines displayed an homogeneous population of ouabain binding sites implying a loss of the high affinity binding site found in the wild type. The $ME_2$ wild type and most of the $ME_2$-resistant cell lines have shown an homogeneous population of ouabain binding sites. However, $ME_2$ $TR_1$ while keeping the high affinity binding sites found in the wild type exhibited the appearance of a low affinity binding site

population as found in $MF_2$. Thus, by selecting cell populations resistant to different drugs to be active at the cell membrane level, we have obtained cells which have lost or acquired populations of binding sites for ouabain. Values for the range of increasing ouabain concentrations over which the $(Na^+ + K^+)$-ATPase activity decreases from 99 to 1 % give information about possible cooperativity of the ouabain binding. For the cell lines with an homogeneous population of sites, the total inhibition took place by a 3–16-fold increase ouabain concentration for $MF_2S$, $MF_2OR_2$, $ME_2$, $ME_2CAR_1$ and a 100-fold increase for $MF_2OR_1$ and $MF_2CAR_1$.

The Hill's coefficient is close to 2 for $ME_2$ cells and all $ME_2$-derived resistant mutants while varying from 2 to 8 for $MF_2$, $MF_2S$ and $MF_2OR_2$: we can assume for all these cells that ouabain binding occurs in a cooperative manner. In contrast to this the Hill's coefficient is 1 for $MF_2OR_1$ and $MF_2CAR_1$.

*A non-specific structure modulating the enzyme activity.* The cross resistance to ouabain inhibition found in the mutants selected for their resistance to dibutyryl cyclic AMP, theophyllin or concanavalin A lead us to assume that a membrane structure, distinct from the enzyme molecule, may be involved in the modulation of enzymatic activity. This hypothesis is suggested by different data: (1) membranes prepared from growing or contact-inhibited $ME_2$ cells did not exhibited the same $(Na^+ + K^+)$-stimulated $Mg^{2+}$-ATPase-specific activity [34]; (2) the modulation of the ATPase activity can be induced by specific ligands such as $K^+$, ATP but also by non-specific ligands such as concanavalin A [28, 35], antibodies against membrane antigens [36]; (3) the appearance of low affinity ouabain binding sites in $ME_2TR_1$ selected by theophyllin which is known to bind specifically the phosphodiesterase; (4) the ATPase dose vs. response curve to ouabain inhibition found in a wild type can be modified by a previous treatment with low concentrations of EDTA [37]. We are currently investigating the possibility that the low and high affinity binding sites differ only in their structural relationship to the membrane, and that the changes of binding site populations observed in drug-resistant cell lines are due to altered membrane organisation.

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