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INTERACTIONS OF CARDIAC GLYCOSIDES WITH CARDIAC CELLS

III. ALTERATIONS IN THE SENSITIVITY OF $(Na^+ + K^+)$-ATPase TO INHIBITION BY OUABAIN IN RAT HEARTS

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The causes of the resistance of adult rats to cardiac glycosides were examined using two approaches: (a) alterations in the binding parameters of ouabain to post-natal heart cells during their maturation in culture, a process which could be indicative of aging; and (b) changes in the I_{50} values of cardiac $(Na^+ + K^+)$-ATPase and K^+-phosphatase activities by ouabain during the process of enzyme's purification, from post-natal to adult rats, which could be indicative of structural changes. The amounts of the cell-bound ouabain decreased from 120 to 12 fmol/mg protein or from 8 to 2 fmol/μg DNA between the 2nd and the 10th day or between the 2nd and the 8th day of growth, respectively. Furthermore, partially purified preparations of $(Na^+ + K^+)$-ATPase from post-natal rat hearts, obtained following treatment with deoxycholate and high concentrations of NaI, were most sensitive to inhibition by ouabain ($I_{50} = (4.6 \pm 0.6) \cdot 10^{-5}$ M) than similar preparations from adult rats ($I_{50} = (22.4 \pm 5.3) \cdot 10^{-5}$ M, $P < 0.005$). The I_{50} values for the detergent-treated enzyme and the salt-treated enzyme in the adult rat or adult cat hearts were: $(22.4 \pm 5.3) \cdot 10^{-5}$ M and $(4.89 \pm 0.83) \cdot 10^{-5}$ M ($P < 0.001$), respectively, for the rat and $(178 \pm 52) \cdot 10^{-8}$ M and $(2.0 \pm 0.48) \cdot 10^{-8}$ M ($P < 0.005$), respectively, for the cat. Frozen and thawed homogenates of either species responded neither to the cations nor to inhibition by ouabain. The K^+-activated, ouabain-inhibited changes in *p*-nitrophenylphosphatase sensitivity to inhibition by the cardiac glycoside were minimal during the purification of the enzyme from both the cat or rat hearts, although the purification factors were 6.4 and 9.0, respectively. The decrease in ouabain binding to new-born rat cardiac muscle cells, but not to non-muscle cells, during maturation in culture; the decrease in sensitivity of $(Na^+ + K^+)$-ATPase from new-born rats, compared to adult rats, to inhibition by ouabain; and the increase in the sensitivity of the enzyme to inhibition by the drug during the process of the enzyme's purification from adult rat or from cat suggested that certain structural alterations could occur in the ouabain receptor during aging in culture or maturation in vivo. These may be reflected by the removal of certain components during the process of the enzyme's purification and may provide a partial explanation for the resistance of the rat to ouabain.

Introduction

$(Na^+ + K^+)$-ATPase (EC 3.6.1.3), the enzyme responsible for the translocation of sodium and potassium across the sarcolemma, is believed to be the putative receptor for cardiac glycosides, and hence the mediator for their positive inotropic action [1,2].

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Adult rats have been shown to be digitalis-insensitive with respect to both pharmacological as well as toxic doses [3–5]. Complete inhibition of the $(Na^+ + K^+)$-ATPase activity from rat tissues requires ouabain concentrations in the range of 10^{-4}–10^{-3} M, as compared to other species requiring 10^{-8}–10^{-6} M [4].

It could be reasoned that ouabain resistance is due to genetic inheritance and, indeed, ouabain-resistant mutants have been isolated from muscle myoblast lines [6], mouse L cells and Chinese hamster ovary cells [7]. Alternatively, such resistance could be related to the primary structure of the enzyme [8] which leads to a low stability of the digitalis-enzyme complex [4,9] due to its short half-lives in moderately sensitive species, i.e., guinea-pig or rabbit with $t_{1/2}$ 10–16 min, compared to highly sensitive species such as dog or cat with $t_{1/2}$ 65–85 min [10].

In previous communications, we have described several properties of the receptors for digitalis in human and rat erythrocytes as well as in cultured cardiac muscle cells from post-natal rats [11–13].

In this communication, the problem of digitalis resistance has been examined by determining two parameters centering around: (a) the interaction of ouabain with cultured cells of different ages; and (b) the inhibition of the catalytic activity by ouabain, expressed as I_{50} values, as a function of both the age of the rat and the complexity of the enzyme structure. Alterations in enzyme structure may be deduced from the measured changes in I_{50} values due to applications of deoxycholate.

Our data support the concept of 'aging' of a digitalis receptor in a sense that changes in the pharmacological response (i.e., resistance) towards digitalis in the adult rat are accompanied by a decrease in the inhibition of the enzymatic activity of $(Na^+ + K^+)$-ATPase by ouabain. It also suggest an influence of structural elements on the binding of ouabain to its receptor following partial removal of membrane components during purification of the receptor. The possible relationship between these data and the resistance to digitalis is discussed.

Materials and Methods

Ouabain (octahydrate), ATP (disodium), tris-(*p*-nitrophenyl)phosphate, deoxycholate (sodium) and *p*-nitrophenol were purchased from Sigma Israel (Tel Aviv).

The procedures for preparation of cultures enriched with respect to muscle (myoblast or myocyte) or non-muscle (endothelial or fibroblast) cells and for the binding of ouabain to cells have been published elsewhere [13].

Partially purified preparations of $(Na^+ + K^+)$-ATPase were obtained from rat hearts using the procedure developed by Pitts and Schwartz for bovine heart [14]. The principal steps included homogenization, a first extraction with deoxycholate followed by treatment with high concentrations of NaI and a second extraction with deoxycholate/citrate. The same procedure was applied to other species (e.g., cat).

The activity of $(Na^+ + K^+)$-ATPase was assayed according to Kidwai et al., with some slight modifications [15]. The enzyme (10–50 μg protein) was pre-incubated at room temperature for 15 min with 50 mM Tris-HCl buffer (pH 7.4) 6 mM $MgCl_2$ 100 mM NaCl/1 mM EDTA either with varying concentrations of ouabain or in the absence of ouabain. Afterwards, 10 mM K^+ was added followed by 6 mM Na_2ATP, and the incubation, in a final volume of 0.5 ml, was continued at 37°C for an additional 15–60 min with constant shaking. The amount of P_i liberated at the end of the incubation was measured by the method of Yoda and Hokin [16].

The K^+-activated phosphatase was assayed according to Heller and Hanahan [17], using tris(*p*-nitrophenyl)phosphate instead of ATP as substrate and the same reagents of the ATPase assay (omitting Na^+).

Enzymatic activities are expressed in munits/mg (nmol product formed/min per mg protein).

Inhibition by cardiac glycosides is expressed in terms of I_{50} which represents the concentration of the drug causing 50% inhibition of either $(Na^+ + K^+)$-ATPase or K^+-activated phosphatase. The data were processed statistically according to Wilkinson [18].

The DNA content was determined by the method of Kissane and Robins [19] with slight modifications. The second extraction with methanol was replaced by an extraction with chloroform/methanol (1 : 1, v/v) to improve sensitivity and reproducibility by removing interfering lipids.

Fluorescence was determined using a Perkin-Elmer spectrofluorimeter model MPF 77A. Protein content was determined by the method of Lowry et al. [20], including sodium dodecyl sulphate at a final concentration of 0.0075% (w/v) to dissolve membranes.

Results

Binding of ouabain to cells as a function of culture age

Cultures enriched with respect to myoblasts or fibroblasts were grown for periods up to 14 days. Ouabain binding to each type of cell was measured during this period. Fig. 1 demonstrates a 19-fold decrease in the amount of ouabain bound per myoblast during the first 10 days in culture. In contrast, under similar conditions, there was little change in the binding of ouabain to fibroblasts; on the 4th day of culture there was a slight increase in binding followed by a slow decrease to the initial values.

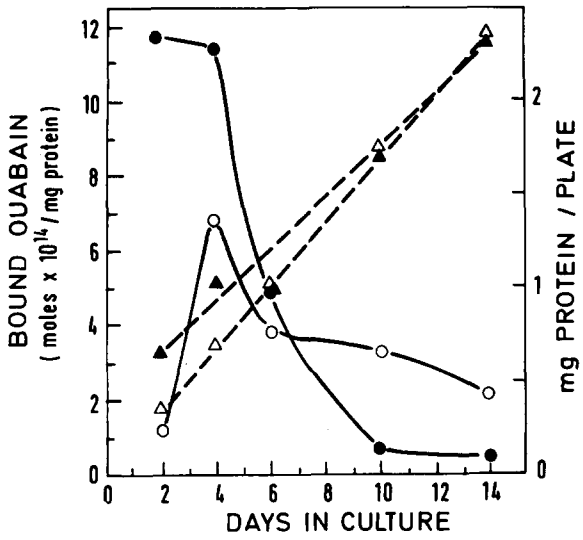

Fig. 1. Binding of ouabain as a function of culture age and protein content. Binding of [^{3}H]ouabain to cultures, 2–14 days old, enriched with respect to muscle cells (●———●) or non-muscle cells (○———○) was estimated according to Friedman et al. [13]. Duplicate plates were incubated either with $7.5 \cdot 10^{-9}$ M [^{3}H]ouabain (approx. $7 \cdot 10^5$ dpm/plate) or the same plate with added $1 \cdot 10^{-3}$ M unlabelled ouabain to determine nonspecific binding. Incubation was carried out at 37°C for 30 min. Following rinsing, the cells were scraped and counted. The values represent only specifically bound ouabain, at or close to equilibrium. Changes in protein content (mg/plate) are given for muscle (▲———▲) and non-muscle cells (△———△). The points shown represent values obtained from at least three experiments.

The protein content of both types of plate, containing myoblasts and fibroblasts, respectively, continued to increase during the same period. This observation corroborates an earlier one made by Lewis and Harary [21] who found a good correlation between the amount of protein and the cell count in plates during the first 12 days of a heart cell culture.

The decrease in the amount of ouabain bound to myoblast cells represents a reduction in the number of ouabain molecules bound per cell and this decrease could be a consequence of: (a) a decrease in the number of receptor sites for ouabain in the sarcolemma; or (b) an alteration in the affinity of ouabain to its receptor, causing dissociation of the receptor-ouabain complex as the cell ages, a phenomenon which could be faster in older than in younger cells.

In order to examine these possibilities, taking into account the fact that both the dimensions of the cells and their content of protein may vary in culture, we have sought another parameter. It is well established that the amount of DNA remains at a constant level in heart cells [22], hence determination of DNA content might serve as a

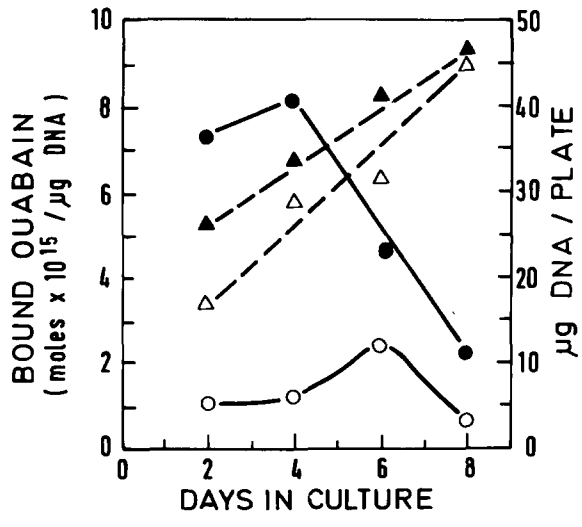

Fig. 2. Binding of ouabain as a function of culture age and DNA content. The details and designation are the same as in Fig. 1, except that µg DNA/plate was measured.

more reliable indicator of cell number in culture than the protein content.

Fig. 2 illustrates a typical decrease in the amount of ouabain bound to cultured myoblasts in enriched plates during the first 8 days in culture expressed as a function of DNA content. Under the same conditions the binding per cell did not vary to any significant extent in plates enriched with fibroblasts. The observed shift of the small peak on day 4 in Fig. 1 to day 6 in Fig. 2 is probably due to small differences between the groups of plates in both experiments. Such shifts were ocassionally detected and should not be considered significant.

We may conclude at this stage that the receptors for ouabain display related alterations in cardiac muscle cells in culture.

An alternative analysis of the data may be carried out using the following premises for evaluation. (1) On the 4th day the number of cells was approx. $5 \cdot 10^6$/plate. From Fig. 2, we calculated $5.8 \cdot 10^{-6}$ μg DNA/cell. (2) The vital counting of the myoblast-containing plates has revealed the presence of about 15% fibroblasts on the 4th day which increased on the 8th day to 40%. (3) On the 4th day each plate contained 29 μg DNA and on the 8th day 41 μg DNA (Fig. 2). Consequently, the cell count on the 8th day is $(41/29) \cdot 5 \cdot 10^6 = 7 \cdot 10^6$ cells/plate. Knowledge of cell count, number of sites per cell (i.e., 147000/myoblast, 33000/fibroblast (from Ref. 13)) and the relative abundance of each cell type in the plate allows the calculation of the total number of binding sites: (a) on the 4th day the calculated total number of sites in the myoblast enriched plates was $6.5 \cdot 10^{11}$ and additional data, based on the amount of bound ouabain yielded a total number of $3 \cdot 10^{11}$ sites on the 8th day; (b) if the total number of sites on the myoblasts only is X, on the 8th day the total number of sites on the fibroblasts alone will be $(3 \cdot 10^{11} - X)$, in the same plate; (c) having a total of Y myoblasts in the plate, the total number of sites on all the myocytes is $X = 147000\ Y$, hence $Y = X/147000$ myocytes; the total number of fibroblasts is $(7 \cdot 10^6 - X)/147000$. From the data shown in Fig. 2 and the calculated unchanged number of sites/cell in the fibroblast (the number is 33000 sites/cell), we may estimate $6 \cdot 10^5$ myoblasts on day 4 or 8.4% of the total number of cells present in the plate. This figure does not agree with the results obtained by vital staining; consequently, the binding parameters on the 8th day have to be different from those on the 3rd or 4th day and could therefore represent aging of the receptors in the myocytes.

In a similar manner, we could assume that the levels of DNA do not change during the period between day 4 and day 8; consequently, the total number of cells does not change, but only the proportion between myocytes and fibroblasts alters. Based on DNA content, we have calculated that only 23% of the total number of cells are myoblasts, which disagrees with cell counting based on vital staining; hence again we have to assume a process of receptor aging.

Purification of $(Na^+ + K^+)$-ATPase and K^+-Phosphatase from adult rat hearts

The complex formed between the intact cell's receptor and ouabain provides only limited information on the nature of the binding. We have therefore attempted to purify the 'receptor', believed to be the putative sarcolemmal entity and probably identical with $(Na^+ + K^+)$-ATPase [1,2]. The procedure of purification was adopted from Pitts and Schwartz [14], and during the various stages of purification, the sensitivity of the enzymatic activities (i.e., the hydrolysis of ATP, activated by Na^+ and K^+, and the hydrolysis of *p*-nitrophenylphosphate, activated by K^+) to inhibition by ouabain was also determined. We have employed the parameter I_{50} as a measure of sensitivity and have used the accepted definition, i.e., the concentration of the drug (ouabain) causing 50% inhibition of the enzyme's activity. This takes into consideration that the I_{50} value is a function of the Na^+/K^+ ratio [23,24] but not of the time or temperature of incubation [25].

Almost no activity of the $(Na^+ + K^+)$-ATPase was detected in crude homogenates of rat hearts, and even repeated cycles of freezing and thawing (cf. Michael et al. [26]), exposed the activity to only a very limited extent. On the other hand, detergents such as deoxycholate 'activated' the enzyme. These results are in agreement with those of Jörgensen [27] and point to the latency of the enzyme. Such a latency could result from the presence of impermeable, right-side-out vesicles in

the homogenates [28]. However, in spite of the latency of the activity, and the cycles of freezing and thawing or application of detergents, we have failed to detect inhibition by ouabain of enzymatic activities in homogenates. In contrast to the ATPase, the activity of the K^+-activated phosphatase, with *p*-nitrophenylphosphate as substrate, was easily detected either in the fresh homogenates or following freezing and thawing. Their specific activities in the newborn and adult rats were 8.5 and 11.1 mU/mg protein, respectively. Under these circumstances, we have determined the value of inhibition by ouabain which was $I_{50} = (2.24 \pm 0.65) \cdot 10^{-3}$ M (Table I). Further purification of the enzyme from rat heart included using controlled concentrations of deoxycholate (i.e., 1 mg deoxycholate/6.5 mg protein [14]) followed by high concentrations of NaI and a second step of deoxycholate. The detergent was removed prior to assay of enzymatic activities. The purified preparations exhibited increased specific activity of both the $(Na^+ + K^+)$-ATPase and the K^+-activated phosphatase as well as an increased sensitivity of both activities to inhibition by ouabain. The differences in the values were statistically significant. The last step of purification which employed deoxycholate in the presence of citrate [14] sometimes inactivated the enzymatic activity, probably as a consequence of the enhanced sensitivity of the enzyme to deoxycholate; however, this procedure did not affect its response to ouabain. The phosphatase's measured specific activity was about 50% that of the ATPase and its response to inhibition by ouabain was not altered appreciably during the process of purification.

A 28-fold purification of the ATPase was accompanied by a 5-fold increase in its sensitivity to inhibition by ouabain; the phosphatase, which was less sensitive to the inhibition (range of I_{50} at $(1-2) \cdot 10^{-3}$ M), was also less affected by the process of purification, being only 6- to 7-fold purified. By comparison, the same procedure of purification was applied to cat heart $(Na^+ + K^+)$-ATPase, resulting also in a 28-fold purification to

TABLE I

PURIFICATION OF RAT CARDIAC $(Na^+ + K^+)$-ATPase AND ITS SENSITIVITY TO OUABAIN IN ADULT AND NEWBORN RATS

The enzyme was purified according to Pitts and Schwartz [14]. I_{50} denotes the concentration of ouabain causing 50% inhibition of catalytic activity, the values of which are expressed as I_{50}, mean±S.E. (S.E.). The data were statistically processed according to Wilkinson [18]. The numbers in parentheses indicate the degrees of freedom. (*a*) At the apparent, maximal solubility of ouabain (i.e., 10 mM) the activity was not inhibited. *, $P < 0.001$ v.s. the deoxycholate (I)-treated enzyme; **, $P < 0.005$ and *** $P < 0.02$ v.s. the value of adult rats enzyme at same stage of purification. *n*, number of experiments.

	$(Na^+ + K^+)$-ATPase			K^+-phosphatase		
	n	Activity (mU/mg)	I_{50} (M) ($\times 10^5$)	*n*	Activity (mU/mg)	I_{50} (M) ($\times 10^3$)
A. Adult rats:						
Frozen and thawed homogenate	10	5	(*a*)	4	11.1	2.24±0.65 (28)
Deoxycholate (I)-treated enzyme	3	60	22.4 ±5.3 (21)	8	30	0.94±0.15 (47)
NaI-treated enzyme	4	140	4.89±0.83 * (22)	4	71	0.96±0.12 (25)
B. Newborn rats:						
Deoxycholate (I)-treated enzyme	4	81	4.6 ±0.6 ** (29)	5	29	0.54±0.07 *** (36)

the same stage of the catalytic activity, which agrees well with published data (cf. Table I in Ref. 14 for bovine heart), and a 10- to 90-fold increase in sensitivity to inhibition by ouabain (data are not included in tables).

In spite of the alteration in sensitivity to ouabain, the range of its inhibitory concentrations in the case of the rat showed I_{50} values from $(22.4 \pm 5.3) \cdot 10^{-5}$ to $(4.89 \pm 0.43) \cdot 10^{-5}$ M, whereas those for the cat enzyme ranged from $(178 \pm 52) \cdot 10^{-8}$ to $(2 \pm 48) \cdot 10^{-8}$ M. In both species, the range of I_{50} values for phosphatase inhibition by the drug was two orders of magnitude higher than for the ATPase (i.e., 10^{-3} to 10^{-6} M in the rat vs. 10^{-5} to 10^{-8} M in the cat).

Sensitivity of cardiac ($Na^+ + K^+$)-ATPase from new-born rats to ouabain

Hearts from adult rats demonstrate certain unusual physiological and pharmacological characteristics, particularly resistance to the action of cardiac glycosides. However, Langer et al. [29] have shown that neonatal rat hearts differ in this respect. We have attempted to corroborate these data biochemically by comparing the sensitivity of ($Na^+ + K^+$)-ATPase from neonatal and adult rats to ouabain inhibition. Table I shows such a comparison in I_{50} values of the enzyme purified to stage 1 (i.e., deoxycholate I, for adult and new-born rats; cf. also Ref. 14). At this stage of purification, the sensitivity of the enzymatic activity in the new-born rat heart is 5-times higher than in the adult; the very same value was also observed for the enzyme from adult heart after one further step of purification, i.e., from the deoxycholate I to the NaI-treated enzyme, the value of I_{50} decreased from $(22.4 \pm 5.3) \cdot 10^{-5}$ to $(4.89 \pm 0.83) \cdot 10^{-5}$ M, cf. Table I). These data of change in sensitivity to ouabain inhibition between neonatal and adult resembled the binding data shown in Figs. 1 and 2, in which the cells obtained from hearts of 2-day-old rats bound more ouabain than the 8- or 10-day-old cells.

Discussion

In the present study, we have attempted to clarify some problems related to the resistance of certain species such as rats towards digitalis. Remarkable changes have been observed in rat hearts during the first 3 weeks of postnatal life, as shown by electrophysiological studies as well as electron microscopic examination [29,30]. In addition to these and other data, it has also been observed that the younger the post-natal rat heart, the more were its responses characteristic of other adult species: for example, prominent action potential plateau, 'ascending staircase' response and significant increase in contractility following cardiac glycoside administration [29]. These data corroborate the data presented in Table I: namely, the sensitivity of enzymatic activity of new-born rat hearts to ouabain is 5-times higher than in the adult. Similar results have been described by Intrussisi et al. [31]. Akera et al. [32] had found a similar sensitivity to ouabain in ($Na^+ + K^+$)-ATPase prepared from hearts of 10- and 50-day-old rats. Their results do not compare with ours, since the enzyme in the present study was prepared from 1-day-old rats. It was found that the sensitivity of the enzyme prepared from 14-day-old rats was similar to that from adult rats (data not shown). The shift from sensitivity to insensitivity occurs, probably, during the 2nd week of the rat's life.

In heart cells obtained from 3- to 5-day-old rats, two types of receptor for ouabain were observed in fibroblasts and in myoblasts. The receptor with the higher affinity for ouabain had dissociation constants of $8.9 \cdot 10^{-8}$ and $2.3 \cdot 10^{-8}$ M for the muscle and non-muscle, respectively. Furthermore, the total number of binding sites in myoblasts and fibroblasts on the third day in culture, as calculated from a Scatchard plot [33], were 147000 and 33000 per cell, respectively [13].

We have demonstrated a decrease in the amount of ouabain bound at equilibrium to cultured myoblasts as the cell ages in culture starting from the 2nd day (Figs. 1 and 2). A good correlation between the protein content and cell count, both of which increase during the experimental period [21], could support the validity of these observations. However, cellular protein concentrations are not always constant; therefore, we have added another parameter – the cellular DNA content, which remains constant during cell's growth. Moreover, polyploidity in heart cells is rather low [22]. Hence, an increase in DNA is proportional to an increase in the number of cells in culture, and

this was accompanied by a decrease in the amount of ouabain as the cultures aged. An apparent process of 'receptor aging' could explain these data, either by a decrease in the number of binding sites on the sarcolemma or alternatively a decrease in affinity (dissociation constant, K_d) or both. A change in affinity could result from either alterations in the conformation of the receptor or from modifications in lipid or protein composition in its immediate vicinity.

Allen and Schwartz [4] have suggested an unstable complex between the enzyme and the drug as an explanation for the lack of sensitivity of adult rats to cardiac glycosides. The activity of cardiac $(Na^+ + K^+)$-ATPase in chicken embryos and following hatching has been determined. In the adult rooster the activity was 30% lower than in the younger chicken, and its decrease in enzymatic activity of skeletal muscle showed an impressive value up to 100% [34]. A similar observation was also made in the cardiac muscle of the rats [35]. Furthermore, the $(Na^+ + K^+)$-ATPase activities, active K^+ influx and ouabain binding levels were reported to decline as a function of the time in cells from ground squirrel kidney cortex. The rate of K^+ turnover/site and the K^+ exchange both increased in later cultures [36]. However, the increase in sensitivity towards inhibition by ouabain upon purification of $(Na^+ + K^+)$-ATPase from adult rat hearts should be seriously considered, although it was only 5-fold (with a statistical evaluation) and with I_{50} values around 10^{-5} M ouabain (Table I), as compared to a 12- to 90-fold increase for a similar preparation from cat heart, and I_{50} values in the region of 10^{-8} M ouabain. It is worth mentioning that the sensitivity of the K^+-activated phosphatase in both species was only slightly affected by the process of purification. A decrease in the I_{50} value upon purification may be due to an exposure of the ATP binding site, but also to a faster rate of ATP binding, which in turn favours ouabain binding. Differences in I_{50} for ATPase and phosphatase are due to the fact that during the ATPase assay, ATP is present which increases ouabain binding [37].

Kline et al. [38] have studied several kinetic parameters of $(Na^+ + K^+)$-ATPase purified from bovine serum brain starting from NaI-treated microsomes (2.5% pure) through to the final ammonium sulphate fractionation (50% pure). These steps of purification had no effect on the sensitivity towards ouabain, a finding which agrees well with ours, since the starting step in Kline's paper has already obtained maximal ouabain sensitivity in the present paper, for both rat and cat enzymes, being $(4.89 \pm 0.83) \cdot 10^{-5}$ M ($P < 0.005$) and $(2.0 \pm 0.48) \cdot 10^{-8}$ M ($P < 0.001$), respectively.

Choi and Akera [39] found changes of the kinetic parameters with purification of the enzyme from different tissues of the dog. These changes may explain the changes in ouabain sensitivity found in the present study. Periyasamy et al. [8] have not found changes in ATPase sensitivity during purification and have concluded that the determinant of the rat kidney $(Na^+ + K^+)$-ATPase insensitivity is contained within the primary structure of the protein, based on an experimental reasoning of a similar nature.

This difference in results could occur because of the different conditions and tissues employed in the two studies. Periyasamy and co-workers have used frozen, renal tissue, whereas fresh hearts were employed in the present work.

It therefore seems that structural components in the membrane involved in the binding stability of the cardiac glycosides could be removed or altered at an early stage by the appropriate treatment. However, their removal does not succeed in converting a 'resistant' rat into a 'sensitive' animal.

These data do not explain the alterations occurring in the binding properties of isolated myocytes from young and older rats. Therefore, based on (a) the data presented here and in unpublished observations related to modifications in lipid content of sarcolemma in cultured myocytes and (b) the fact that even the most highly purified preparations of the $(Na^+ + K^+)$-ATPase are membranous fragments containing up to 40% lipids (by weight) [35], we tend to support the idea that structural elements other than the primary structure of the enzyme play a crucial role in defining the characteristics of the interaction of cardiac glycosides with their receptors.

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