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Cultured heart cell reaggregates: a model for studying relationships between aging and lipid composition

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Cultured heart cells serve as a common model for studying the electronphysiology and pharmacology of intact cells of the myocardium from which they are derived (Sperelakis, N. (1982) in Cardiovascular Toxicology (Van Stel, E.W., ed.), pp. 57–108, Raven Press, New York). In this study, heart cell reaggregates were used for investigating the relationship between lipid composition and aging of the heart cells. Spherical reaggregates were prepared from newborn, 3- and 18-month-old rats, respectively. They were grown for 6 days in culture and then analyzed for their lipid composition and creatine phosphokinase levels. There was an age-related increase in total phospholipids and cholesterol level per unit of cell protein. Due to a relatively greater increase in the cholesterol, the mole ratio of cholesterol to phospholipids increased with animal age. The phospholipid composition was also affected. Thus, sphingomyelin levels increased, while those of phosphatidylcholine decreased; these alterations became much more pronounced with increasing animal age. All these changes could be affected by adding small unilamellar vesicles composed of egg phosphatidylcholine to the growth medium on the 5th day after seeding. Such treatment resulted in a lesser ratio of cholesterol to phospholipid as well as sphingomyelin to phosphatidylcholine, without reducing the total phospholipid per unit protein; the level of creatine phospholinase was also reduced. This study demonstrated that cultured heart reaggregates can serve as a model for studying aging of the whole animal. Its main advantage is the ability to employ cells from rats of any desired age. Currently this is not possible for cultured heart monolayers.

Introduction

Certain mammalian tissues undergo major changes in membrane lipid composition during aging This is expressed in increase in the mole ratio of cholesterol to phospholipid and of sphingomyelin to phosphatidylcholine as well as in the degree of phospholipid acyl chain saturation (for review, see Refs 1,2) Similar changes were observed recently in monolayers of cultured rat heart myocytes and rat heart fibroblasts [3–5] The relevancy of such alterations of membrane lipid composition to the complex aging process is supported by the role that lipid composition plays in the activity of various proteins in cellular features and functions in general (Refs 1, 2 and references therein) Our studies on monolayers of cultured heart cells [3–5] suggest that the effect of membrane lipid composition on cell properties is mediated through effects on lateral organization of membrane lipids and proteins Lateral mobility of membrane components seem to be a less important factor [3–5] One of the main issues of the above studies is their relevancy to the similar in

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Abbreviations PC, phosphatidylcholine, SUV, small unilamellar vesicles

vivo results on the relationship between lipid composition and aging [6] The drawback in the experiments on the aging of myocytes in culture is that, as yet, only myocytes of newborn rats but not of adult rats can grow in monolayers for a reasonable length of time Cultured heart cell reaggregates were used here as an alternative model for heart cell aging in vivo This model was used extensively before (Ref 7, and references therein) The main advantage of the reaggregates is the ability to study the effect of animal age as well as aging in culture on the properties of the heart cells The reaggregates are an easier system than cultured monolayers for electrophysiological work They are highly differentiated and possess pharmacological receptors which are almost identical to those of cells in the intact myocardium from which they originate [7]

The current study describes the lipid composition of free reaggregates prepared from hearts derived of rats of various ages, and how their treatment by small unilamellar vesicles made of egg phosphatidylcholine affects their sphingomyelin-to-phosphatidylcholine and cholesterol-to-phospholipid mole ratios It also relates these alterations to the level of creatine phosphokinase in the cells

Materials and Methods

Preparation of free rat heart reaggregates

Free reaggregates were prepared from hearts of Wistar rats of the desired age by the procedure of Jourdon et al [7,8] with minor modifications Free dispersed cells were obtained using collagenase (Type III, Sigma St , St Louis, MO) in Mg^{2+}-free F-10 medium (Gibco, Grand Island, NY) containing 0 1 mM $CaCl_2$ The free cells were suspended in medium A (normal F-10 HAM medium containing 1 2 mM $CaCl_2$, 10% fetal calf serum (v/v), 10% horse serum (v/v) both from Gibco, 2 10^5 IU/liter, sodium penicillin and 200 mg/liter streptomycin 3 ml of suspension of free cells in medium A (5 10^6 cells per ml) were introduced into a 25 ml Erlenmayer flasks Cells were grown in gyrotatory shakers at 37°C Reaggregates were formed 24–48 h after seeding Therefore, all treatments of the reaggregates started after the first 48 h

Treatment of reaggregates with phosphatidylcholine liposomes

Egg phosphatidylcholine (PC) was purified from egg yolk by established procedures [9] Egg PC liposomes (small unilamellar vesicles – SUV) were prepared and fractionated as described elsewhere [10] The SUV were sterilized by filtration through 0 22 μm Millipore filter and added to 4-day-old cells in a final concentration of 2 5 mM The liposomes were incubated with the reaggregates for 48 h It is worth noting that addition of egg pC SUV has to be performed after cell recovery and formation of reaggregates, which take about 48 h When added immediately after cell seeding the egg PC SUV prevented the formation of reaggregates, while, if added during the first 48 h of cell growth, liposomes penetrated into the cells, as was revealed by electron microscopy (data not shown)

Analytical methods

Determinations of the content of total phospholipids, sphingomyelin, PC, cholesterol, total protein, and creatine phosphokinase (EC 2 7 3 2) activity were performed according to established procedures as described in Ref 3, minisupplement, and references listed therein

Results

Free reaggregates were prepared from 3-day (newborn), 3-month and 18-month-old Wistar rats All the cells were grown for 6 days under conditions described in Materials and Methods Table I describes the lipid composition of the cells after 6 days culture Comparing reaggregates obtained from rats of the above three ages, it is clear that there is an age-dependent increase in total phospholipids and free cholesterol (both calculated per total cellular protein) The increase in this ratio is much more pronounced between the 18 and 3 months than from 3 months and newborn rats The increase in cell cholesterol exceeds that of the phospholipids, while phospholipids increased by about 60%, the cholesterol level more than doubled This resulted in a 1 3-fold increase in cholesterol-to-phospholipid mole ratio Regarding the phospholipid composition, only the three major phospholipids (phosphatidylcholine, sphingomyelin and phosphatidylethanolamine), which to-

TABLE I

LIPID COMPOSITION AND CREATINE PHOSPHOKINASE (CPK) ACTIVITY OF FREE RAT HEART REAGGREGATES EFFECT OF RAT AGE AND TREATMENT OF REAGGREGATES WITH EGG PHOSPHATIDYLCHOLINE SMALL UNILAMELLAR VESICLES

Free reaggregates were prepared from hearts of 3-day, 3-month- and 18-month-old Wistar rats (for more details, see Materials and Methods) All results are described per total cell protein, the creatine phosphokinase determination was done using a Boehringer diagnostic kit under conditions of saturation by the substrate S D on the results was always better than ±4 0% SM sphingomyelin

Reaggregates prepared from	PC SUV treatment	Total phospholipids (nmol/mg protein)	Cholesterol (nmol/mg protein)	Phospholipid composition (%)			Cholesterol phospholipids (mole ratio)	PC/SM (mole ratio)	CPK (U/mg protein)
				SM	PC	PE			
Newborn rates	–	100	48	30	51	19	0 48	1 70	138
	+	113	36	25	47	22	0 32	1 88	69
3-month old rats	–	115	58	30	50	20	0 50	1 67	278
	+	138	41	24	51	23	0 30	2 12	89
18-month-old rats	–	157	98	42	34	24	0 62	0 80	359
	+	190	50	27	53	20	0 26	1 96	88

gether comprise more than 85% of the cell phospholipids (Ref 11 and our data), were determined From Table I it is clear that major changes in phospholipid composition started only after 3 months and are well expressed for reaggregates prepared from 18-month-old rats Sphingomyelin and, to a smaller degree, phosphatidylethanolamine levels increase while phosphatidylcholine level decreases accordingly This resulted in a more than two-fold decrease in the PC to a sphingomyelin mole ratio comparing free reaggregates prepared from 3-month-old rats with those prepared from 18-month-old rats

Egg PC SUV when added to the growth medium of the reaggregates, after 4 days in culture, have a major effect on cell lipid composition (see Table I) (a) They increase total phospholipid per protein by 10–20%, (b) they deplete cell cholesterol in all cases, however, the degree of deletion increased with animal age, being most pronounced for reaggregates prepared from 18-month-old rats in which the cholesterol-to-phospholipid mole ratio was reduced to half of its original value, reaching a level even lower than reaggregates prepared from newborn rats, (c) similar effects were obtained for phospholipid composition, although increases in sphingomyelin to PC mole ratios obtained for all cases were small for reaggregates prepared from newborn and 3-month-old rats, but were very dramatic for 18-month-old rats It seems that in all cases this ratio approaches the same value of 1 9–2 1, which resembles the value of the young rats No significant changes were obtained in phosphatidylethanolamine levels

Cellular creatine phosphokinase specific activity increased dramatically with the age of the rats used as the source of reaggregates preparation (2 6-fold from newborn vs 18-month-old rats) The specific activity of the enzyme was reduced by treatment of the reaggregates with egg PC SUV to a level similar to this of reaggregates from newborn rats Thus, again, the older the animals, the larger was the effect

Discussion

The changes in lipid composition of many tissues with mammalian age is a well-established phenomenon (Refs 1, 2 and references therein) This change includes an increase in total phospholipid and cholesterol content per cell as well as an increase in cholesterol to phospholipid and sphingomyelin-to-PC mole ratio, and the degree of acyl chain saturation [1,2] Data obtained for various cell lines have given controversial results on changes of cell lipid composition and it seems that results differed for the various cell lines Recently we demonstrated that monolayers prepared from

newborn rat heart myocytes and from nonmuscle heart cells (fibroblast and endothelial cells) show similar patterns of changes with aging of cells in culture Namely, sphingomyelin-to-PC and cholesterol-to-phospholipid mole ratios increase with culture age [3–5] These changes in lipid composition were correlated with alterations in the dynamics and organization of membrane lipids and proteins and with some biological features of the cell However, the similarity of the culture monolayers of heart cells to the in vivo system is not complete, because preparation of cultured monolayers is at yet limited to newborn rats only The model of free reaggregates is therefore advantageous, since reaggregates can be prepared from rats of almost any age This model is very similar to tissues and organs in vivo, since the changes in lipid composition of the free reaggregates resemble very much changes in the whole animal, total phospholipid and cholesterol level increased with animal age (Table I) This was not the case for cultured monolayers, in which the level of total phospholipids was unaltered by culture age [1,3] The changes in sphingomyelin to PC mole ratio and cholesterol to phospholipid mole ratio also increased with age as in the whole rats and in the cultured monolayers [2] It should be stressed that the changes became much more pronounced in old age Reaggregates prepared from 18-month-old rats show a very dramatic change in lipid composition Also, they were the most affected by treatment with egg PC SUV, which reversed the changes in their lipid composition back to the levels which more resemble those of reaggregates prepared from newborn rats It seems that the effect of egg PC liposomes on the lipid composition is controlled by the cells by an as yet unknown mechanism This is suggested by the fact that there is a limit to the alteration in lipid composition of the reaggregates to that which is the composition of reaggregates prepared from newborn rats It should be stressed that the treatment with PC liposomes did not reduce the total phospholipid content, on the contrary, it even slightly increased it, which suggests that both exchange and net transfer of lipid molecules between the cells and the medium are going on

The effect of PC liposomes on the lipid composition of the reaggregates is overwhelming and therefore it has to include all cells in the reaggrage and not only the cells in the reaggregegage surface which are in direct contact with the growth medium Therefore, one must assume that the process of lipid transfer and lipid exchange among all cells of the reaggregates are fast enough to explain such major alterations in cellular lipid composition Probably in the same order of magnitude as in the cultured heart myocyte monolayers, in which a complete PC exchange with the PC SUV was obtained in about 24 h [3] Creatine phosphokinase was selected as a test case for the relationship between cell lipid composition and cell biology This enzyme was chosen because of its relevancy to myocyte function, it plays a major role in intracellular energy transport from mitochondria to myofibrils and in the regulation of energy transport coupled to energy utilization Recently we demonstrated that the level of creatine phosphokinase was responsive to the age of cultured myocyte monolayers and could be manipulated by alteration of cellular lipid composition [3] The activity of this enzyme is not restricted to a single subcellular organell and can be detected in various subcellular fractions including mitochondria, myofibrils and cytosol [3,12,13]

As in the case of the cultured myocyte monolayers the creatine phosphokinase level of the reaggregates increased with animal age and decreased upon treatment with PC liposomes The reduction in creatine phospholinase level reaches its plateau having a value which is similar for reaggregates prepared from the hearts of rats of the three different ages (newborn, 3 months and 18 months) The reasons for these age-dependent changes in cell lipid composition and creatine phosphokinase levels are not yet available However, one can speculate that the situation is similar to that found with cultured myocyte monolayers in which lipid composition seem to have major effects on membrane organization through changes in domain structure [3–5] Increase in sphingomyelin to PC and cholesterol-to-phospholipid mole ratio reduces lateral heterogeneity, which by itself may affect many cell functions [14,15]

Another factor is that the aging-related alteration in lateral organization of membrane lipids may affect cell shape in the reaggregates similarly to our recent observation with cultured myocyte

monolayers [3] This could cause an increase in cell surface area and in its internal volume as was described for model systems [16,17], which in turn would cause the increase in creatine phosphokinase level Reversing the lipid composition to that obtained for reaggregates of newbron rats (Table I) reduces the enzyme level by the reverse process This hypothesis is supported by the observation that the increase in the surface area of the plasmalema and the organular endoplasmic reticulum of aortic smooth muscle cell increased during their aging in parallel to the increase of sphingomyelin and cholesterol levels [18]

This work suggests that cultured rat heart reaggregates can serve a a good model system to improve our understanding of aging processes, since cells which can be obtained from rats of any age can be studied in culture, and some of the aging-dependent changes can be manipulated through the growth medium Therefore, this system may simplify the present complexity of work with the whole animal, while having many of the advantages available for cultured monolayers

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References

1 Barenholz, Y (1984) in Physiology of Membrane Fluidity (Shinitzky, M , ed), Vol 1, pp 131–173 CRC Press, Boca Raton
2 Shinitzky, M (1984) in Physiology of Membrane Fluidity (Shinitzky, M , ed), Vol 1, pp 1–51, CRC Press, Boca Raton
3 Yechiel, E and Barenholz, Y (1985) J Biol Chem 260, 9123–9131
4 Yechiel, E , Barenholz, Y and Henis, Y I (1985) J Biol Chem 260, 9132–9136
5 Yechiel, E , Henis, Y I and Barenholz, Y (1986) Biochim Biophys Acta 858, 95–104
6 Rothstein M (1982) Biochemical Approach to Aging, Academic Press, New York
7 Sperelakis, N (1982) Cardiovascular Toxicology (Van Stel, E W , ed), pp 57–108, Raven Press, New York
8 Jourdon P and Sperelakis, N (1980) J Mol Cell Cardiol 12, 1441–1458
9 Hertz, R and Barenholz, Y (1975) Chem Phys Lipids 15, 138–156
10 Barenholz, Y , Gibbes, D , Litman, B J , Goll, J , Thompson, T E and Carlson, F D (1977) Biochemistry 16, 2806–1810
11 White D A (1973) in Form and Function of Phospholipids (Ansell, G B Hawthorne, J N and Dawson, R M C , eds), pp 441–482, Elsevier, Amsterdam
12 Mohamed, S N , Holmes, R and Hartzell, C R (1983) In Vitro 19, 471–478
13 Van Brussel, E , Yang, J J and Seradarian, M W (1983) J Cell Physiol 116, 221–226
14 Klausner, R D and Kleinfeld, A M (1984) in Cell Surface Dynamics (Perlson, A S , Delisi, C and Weigel, F W , eds), pp 23–58, Marcel Decker, New York
15 Sabine, J R (1982) Trends Biochem Sci 8, 234–236
16 Lichtenberg, D , Felgner, P L and Thompson, T E (1982) Biochim Biophys Acta 684, 277–281
17 Slater, J L , Lichtenberg D and Thompson, T E (1983) Biochim Biophys Acta 734, 125–128
18 Portman, O W (1966) Ann N Y Acad Sci 162, 120–136