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SUBCELLULAR DISTRIBUTION OF THE ANTIDEPRESSANT DRUG DESIPRAMINE IN CULTURED HUMAN FIBROBLASTS AFTER CHRONIC ADMINISTRATION

DRUG-EFFECT ON THE SUBCELLULAR DISTRIBUTION OF ACCUMULATED PHOSPHOLIPIDS

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Abstract—Desipramine (DMI) is an important antidepressant drug and a lysosomotropic substance. In cultured fibroblasts it interferes with lysosomal functions, e.g. phospholipid degradation. Chronic exposure of cells with DMI induces storage of phospholipids. Subcellular fractionations of cultured human fibroblasts that had been exposed to a short pulse of ^{3}H-DMI showed accumulation of DMI in two acidic compartments, one of high density represented the lysosomes and one of much lower density may contain pinosomes. In chronically exposed cells DMI accumulated in the subcellular fractions of lower density only. DMI induced an important shift of lysosomal enzymes from vesicles of high density to the ones of lower density. Phospholipids were accumulating in those vesicles of lower density as well as in the fractions that contained plasma membranes. DMI also accumulated in one part of the Golgi vesicles of acute and chronically exposed cells. In the latter phospholipids and arylsulfatase A activity were also accumulating. DMI possibly interferes with membrane recycling. This eventually could induce changes in phospholipid content and composition in the plasma membrane which may have important implications for membrane functions.

Desipramine (DMI) is a clinically active tricyclic antidepressant. It is an amphiphilic cationic drug, which in its non-protonated lipophilic form, is easily taken up into the cells. At the acidic pH of the lysosomes it becomes protonated and thus water-soluble, and is therefore accumulated in the lysosomes since the hydrophilic form cannot penetrate the lysosomal membranes. This has been shown in previous work by Honegger *et al.* [1] investigating the uptake kinetics of the drug in cultured human fibroblasts.

Lysosomotropic drugs like DMI or chloroquine [2] can interfere with lysosomal functions in different ways. Direct inhibition of individual lysosomal enzymes or functional inhibition of lysosomal enzymes through a rise in the intralysosomal pH [3], as well as drug induced secretion of lysosomal enzymes from the cell and a consecutive decrease of intralysosomal enzyme activities [4] have been described.

In many animal and human tissues, and also in cultured cells, granular cytoplasmic inclusions are fomed after prolonged treatment with lysosomotropic drugs.

Fauster *et al.* [5] were able to show that chronic exposure of cultured human fibroblasts to DMI resulted in a perinuclear accumulation of granules, visible by phase contrast microscopy. Simultaneously a large increase of cellular phospholipids with an overproportionate rise of phosphatidylinositol has been seen after exposure to DMI. An effect on the phospholipid metabolism occurs even after a single dose of DMI. The degradation of [^{14}C]-glycerol-labeled phosphatidylinositol was greatly diminished while the degradation of the other phospholipids was less severely but also significantly reduced [5]. The present work was aimed at cellular localization of radiolabelled DMI and at investigation of the distribution patterns of total and individual phospholipids in subcellular fractions of cultured human fibroblasts after chronic treatment with DMI.

MATERIALS AND METHODS

Cell culture. Normal human fibroblasts were cultivated from skin biopsies obtained during minor surgery after informed consent was received. Fibroblast cultures were grown in Eagle's minimal essential medium supplemented with 10% of fetal calf serum and buffered at pH 7.4 with bicarbonate and 5% CO_2 in air. The media contained non-essential aminoacids, 50 units of penicillin and 10 μg of chlortetracycline per ml. Fibroblast stock cultures were maintained at 37° in 175 cm^2 Falcon culture flasks and the medium was changed twice a week.

Subcellular fractionation. Cells were grown in large 850 cm^2 roller bottles. Before havesting, the medium was discarded and the cultures were washed once with 50 ml of culture medium. Cells were scraped off with a "rubber policeman" in 50 ml of 0.25 M sucrose at 0°. After centrifugation at 1500 *g* for 5 min at 4° the cells were pooled and suspended in 5 ml of 0.25 M

sucrose at 0°. The cells were homogenized by trituration with a 10 ml Falcon pipet (50 times pipetting and blowing out with an automatic pipettor). The opening of the pipet was 1 mm.

The lysed cells were centrifuged at 1000 *g* for 20 min at 4° in a TFT 65.13 fixed angle rotor (26°) on a TGA/65 ultracentrifuge (Tegimenta/Kontron, Zuerich, Switzerland). A nuclear fraction (P1) and a postnuclear supernatant (SN1) were separated. The resulting postnuclear supernatant was then separated at 25,000 *g* for 1 hr into a mitochondrial/lysosomal fraction (P2) and into a postmitochondrial supernatant (SN2). After the quantative removal of the supernantant the sediment (P2) was suspended in ice-cold 0.25 M sucrose. The postmitochondrial supernatant (SN2) was further separated by centrifugation at 105,000 *g* for 1 hr into a particulate microsomal fraction (P3) and into a soluble cytosolic fraction (SN3).

Percoll isopyknic gradient fractionation. By mixing nine parts of commercially available Percoll (density (d) = 1.129 g/ml) with one part of 2.5 M sucrose (d = 1.316 g/ml) a stock isotonic Percoll solution (SIP) was obtained. By dilution of SIP with 0.25 M sucrose the desired densities of the isotonic Percoll solution were achieved.

Subfractionation of the mitochondrial/lysosomal fraction P2. Three millilitres of the P2 membrane fraction suspended in 0.25 M sucrose solution were layered on 27 ml of isotonic Percoll medium (d = 1.055 g/ml). The samples were centrifuged at 30,000 *g* for 1 hr in a 20° fixed angle rotor (TFT_{70}). Twenty fractions of 1.5 ml each were collected after puncturing the centrifugation tubes at the bottom and pumping 2.2 M sucrose at 4° in an ISCO/gradient fractionation apparatus (Instrumentation specialities W. Meyer, Luzern, Switzerland).

Subfractionation of the postmitochondrial supernatant (SN2). Three millilitres of the SN2 fraction was layered onto 27.0 ml of isotonic Percoll medium (d: 1.045 g/ml) and centrifuged at 30,000 *g* for 2 hr in a 20° fixed angle rotor (TFT_{70}). The gradient was collected in 20 fractions of 1.5 ml each. The density profile of the gradients was measured by using density marker beads from Pharmacia (Uppsala, Sweden).

Biochemical analyses. Protein was measured according to the method of Lowry *et al.* [6] using crystallized bovine serum albumin as a reference. Lipids were extracted according to the method of Folch *et al.* [7]. Total phospholipids were determined using the test kit combination from Boehringer (Mannheim, F.R.G.). Total phospholipid content of the fibroblast culture was also directly measured in the cell homogenate by fluorometry according to the method of Jouanel *et al.* [8]. Fibroblast phospholipids extracted from a large quantity of fibroblasts served as a reference.

Quantitative determination of desipramine (DMI). Fibroblasts were grown in 850 cm^2 roller flasks and incubated for 9 days with three changes of medium containing 10 μmM DMI. Control cells were grown in the absence of DMI. Both, control and test cells were labeled with (^{3}H)-DMI (4.17 μCi per 200 ml of medium per roller flask) for 2 hr prior to harvesting. (^{3}H)-DMI was determined in aliquots of the cell homogenate and of the subcellular fractions in a liquid scintillation spectrometer. Total DMI content was measured by fluorometry after acid extraction in total cell homogenate and in cellular fractions. The fluorescence of DMI was stimulated at 268 nm and measured at 425 nm in a Perkin–Elmer fluorometer (PE/2000).

Marker enzymes. Cytochrome-*c* oxidase (EC 1.9.3.1) was measured according to the method of Wharton and Tzagoloff [9]. Beta-D-glucosidase (EC 3.2.1.210) was measured fluorometrically as described by Barrett [10] using 4-methylumbelliferone as a reference. Arylsulfatase A (EC 3.1.6.1) was determined according to the method of Baum *et al.* [11]. 5′-Nucleotidase (EC 3.1.3.5) was measured according to the method of Dixon and Purdom [12] using a phosphate standard solution as a reference (5 mg phosphorus per 100 ml aqua bidest). Galactosyltransferase (EC 2.4.1.38) was measured according to the method of Brew *et al.* [13] as modified by Rome *et al.* [14].

Chemicals. MEM dry powdered media with Earl's salt were purchased from Seromed (Munich, F.R.G.). Fetal calf serum was from Boehringer (Mannheim, F.R.G.). Penicillin G was from Gist/Brokades (Delft, Holland), chlortetracycline from Gibco (Glasgow, Scotland). Bactotrypsin from Difco Laboratories (Detroit, MI). Crystalline bovine serum albumin was purchased from Sigma (St. Louis, MO), DNA from calf thymus from Serva (Heidelberg, F.R.G.). The phospholipid standards were purchased from Supelco (Gland, Switzerland). The enzyme substrates cytochrome *c* and paranitrocatechol sulfate were from Sigma (St. Louis, MO), 4-methylumbelliferyl-B-D-fucopyranoside and 4-methylumbelliferone from Koch-Light (Colnbrook, U.K.) and ovalbumin from Fluka (Switzerland).

5′-Nucleotidase activity was measured using a test kit from Sigma (St. Louis, MO). All other chemicals, solutions and solvents were of analytical purity grade and were purchased from Merck (Darmstadt, F.R.G.).

Radiochemicals. (^{3}H)-Desmethylimipramine hydrochloride (specific radioactivity 41.7 Ci per mmol) and (^{14}C)-uridine-diphosphate-galactose (specific radioactivity 302 mCi per mmol) were obtained from NEW (Dreieichenhain, F.R.G.).

RESULTS

Subcellular fractionation by differential centrifugation

The distribution and the relative enrichment of marker enzymes, protein, phospholipids and DMI in the fractions obtained after differential centrifugation are summarized in Table 1. In the postnuclear pellet (P_2) of control cells the specific activities of 5′-nucleotidase, beta-D-glucosidase, arylsulfatase and cytochrome-*c*-oxidase were increased 2–3-fold (Table 1a and 1b). This fraction also was enriched in mitochondrial plasma membranes and lysosomes. P3 the microsomal sediment, showed a 7-fold increase in the specific activity of galactosyltransferase. Very little of the galactosyltransferase activity was found in the soluble fraction SN3 and comparably small activities of the lysosomal enzymes were found. Homogenate of fibroblasts that

Table 1. Marker enzymes, protein, phospholipid, (^{3}H)-DMI and nonlabeled DMI in subcellular fractions after differential centrifugation
(a) Concentrations and specific enzymes activities

Fraction	Protein		5′-Nucleotidase		Cytochrom-*c* oxidase		Beta-D-glucosidase		Arylsulfatase A		Galactosyl transferase		Phospholipids		^{3}H-DMI		DMI non-labelled
	mg / ml homogenate		μ moles / hr mg Prot.		μ moles / min mg Prot.		nmoles / hr mg Prot.		nmoles / hr mg Prot.		dpm / mg Prot. 10^3				dpm / mg Prot. 10^3		nmole / mg Prot.
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C D
Homogen.	3.89	3.96	36.0	19.7	5.9	8.6	6.7	6.3	949	661	117	80	20.6	73.7	239	389	65.4
Ho		(102)		(55)		(147)		(93)		(70)		(68)		(353)		(163)	
Nuclear	2.35	1.92	30.2	26.5	6.6	11.3	8.1	5.2	1069	491	36	67	23.2	45.4	244	273	40.1
P_1		(82)		(88)		(173)		(64)		(46)		(186)		(195)		(112)	
Mit.-Lys.	0.47	0.94	96.4	42.1	21.3	18.1	16.2	11.6	2147	1313	289	48	47.0	98.9	717	820	129.–
P_2		(200)		(44)		(85)		(72)		(61)		(17)		(210)		(114)	
Microsom.	0.18	0.17	84.4	38.8	—	—	1.7	7.6	139	329	821	520	59.4	95.3	133	435	64.7
P_3		(94)		(46)				(459)		(237)		(63)		(160)		(327)	
Cytosol	0.71	0.79	0.6	1.8	—	—	0.7	3.8	116	506	19	27	2.4	58.5	20	237	63.3
SN_3		(111)		(315)				(539)		(438)		(141)		(244)		(1200)	

Protein values are expressed as mg per ml of the original homogenate. The results represent the average of the means from two independent experiments. The variation between the values was less than 10%. The numbers in brackets are the values of treated cells expressed in percent of control values.

(b) Relative distribution of protein, enzyme activities, phospholipids and of DMI in subcellular fractions. The results are derived from Table 1a. Recovery of the enzyme-activities in the individual fractions

	Protein		5′-Nucleotidase		Cytochrome-*c*-oxidase		Beta-D-glucosidase		Arylsulfatase A		Galactosyl-transferase		Phospholipids		^{3}H-DMI		DMI non-labeled
	%		%		%		%		%		%		%		%		%
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C D
Hom	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
P_1	60	48	51	64	68	63	73	40	68	37	19	40	68	30	62	34	30
P_2	12	24	32	50	44	50	29	44	27	46	30	14	28	32	36	90	47
P_3	5	4	11	9	—	—	1	5	1	3	32	27	13	5	3	5	4
SN_3	18	20	0.3	2	—	—	2	12	2	15	3	7	2	16	2	12	19

C = Control cells, receiving (^{3}H)-DMI in tracer dose 2 hr prior to cell harvesting. D = Cells chronically exposed for 9 days with 10 μM DMI and receiving (^{3}H)-DMI in tracer dose 2 hr prior to cell harvest.

were exposed to 10 μM DMI for 9 days contained equal amounts of protein and beta-D-glucosidase activity, about half of the 5'-nucleotidase and galactosyltransferase activities but significantly increased cytochrome-*c*-oxidase activity (Table 1) as compared to control cells. In the P2 sediment (mitochondrial–lysosomal pellet) more of the total protein was sedimented at 20,000 *g* and therefore the specific activities of all marker enzymes were lower than in the control cells.

Lysosomal enzyme activities were elevated in P3 and in the soluble fraction SN3. The phospholipid content was largely increased, compared to control cultures. In control cultures phospholipids were sedimentable with the P2-pellet and some with the P3 sediment. In DMI exposed cultures, however, a large proportion were non sedimentable phospholipids in the supernatant (SN2). Uptake of (^{3}H)-DMI into chronically DMI exposed cells was about 60% larger than the uptake into cells after single exposure. Most of this increase was accounted for by increased (^{3}H)-DMI in P3 and in SN3.

Subfractionation of P2 (mitochrondrial/lysosomal pellet)

Figure 1 shows the density distribution pattern of the protein and specific activities of the marker enzymes (activities are given as specific activities per

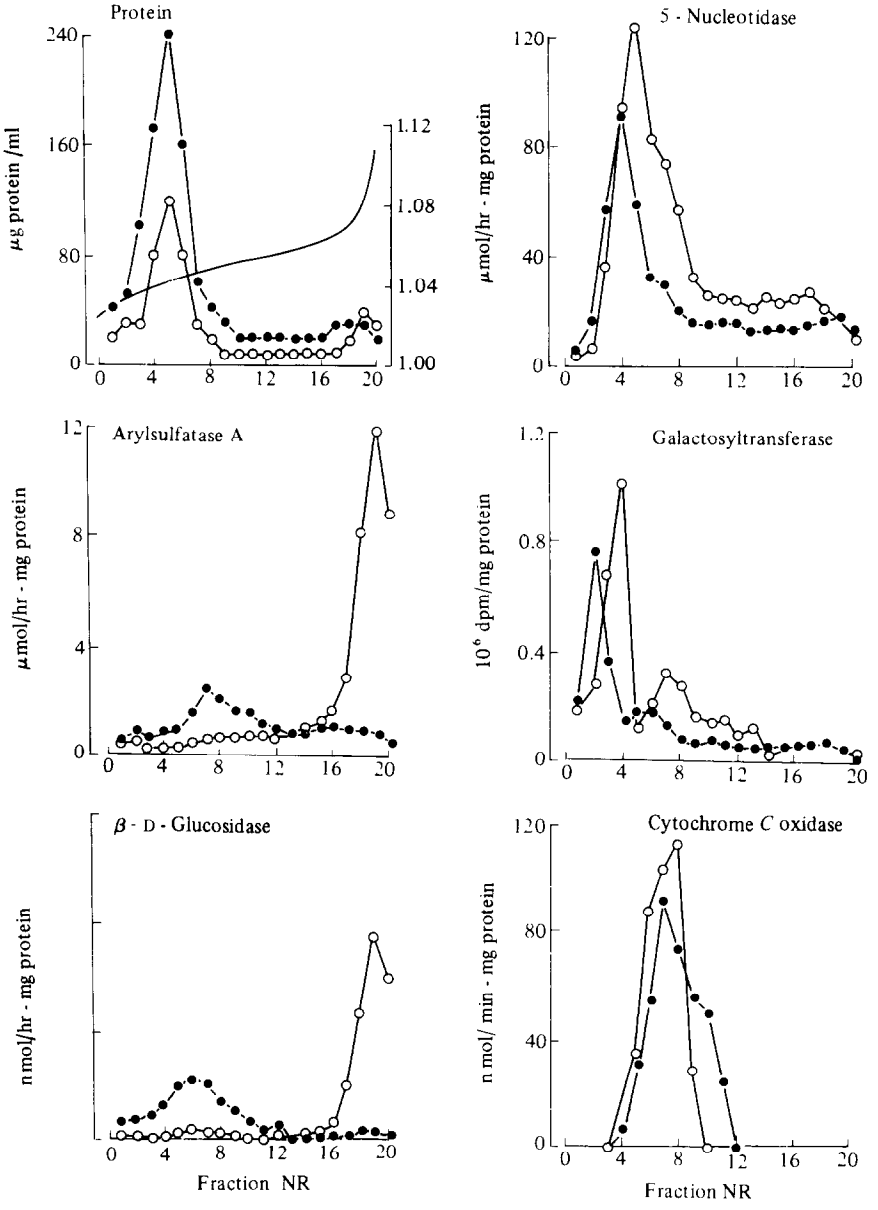

Fig. 1. Marker enzyme activities in the fractions after P2 Percoll gradient centrifugation. The enzyme activities are expressed per mg protein of the individual fractions (specific activities). They represent the mean of two independent experiments. The density pattern of the gradient is shown together with the protein distribution: ○, control cells; ●, cells exposed to 10 μM DMI for 9 days.

mg protein). The distribution of proteins, of 5'-nucleotidase and of cytochrome-*c*-oxidase was quite comparable between drug exposed and control cells. Galactosyl transferase of the exposed cells appeared to be associated with membranes which were slightly less dense than those of the control cells. Quite remarkable differences, however, were found with respect to the lysosomal enzymes. Most of the activities of arylsulfatase A and beta-D-glucosidase of control cells were at the densest portion of the gradient. In chronically DMI exposed cells, however, most of the lysosomal enzymes banded at fractions of considerable lower densities. At the bottom part of the gradient there were minimal lysosomal enzyme activities compared to the control cells.

Figure 2 shows the density distributions of phospholipids and of DMI. In control cells that had received (^{3}H)-DMI 2 hr before harvesting (Fig. 2b) two peaks of radioactivity were observed. One coincided roughly with cytochrome-*c*-oxidase activity (Fig. 1) and a second peak coincided with the activity of lysosomal enzymes at the bottom of the gradient. The phospholipid distribution also showed two peaks that were not correlated with the peaks of (^{3}H)-DMI. The first of the two phospholipid peaks around fraction 4 was at the same place as the peak for 5'-nucleotidase activity. The other peak of phospholipids between fractions 11 and 16 did not correlate with any of the marker enzymes. Only two minor shoulders corresponded each with the main location of the (^{3}H)-DMI.

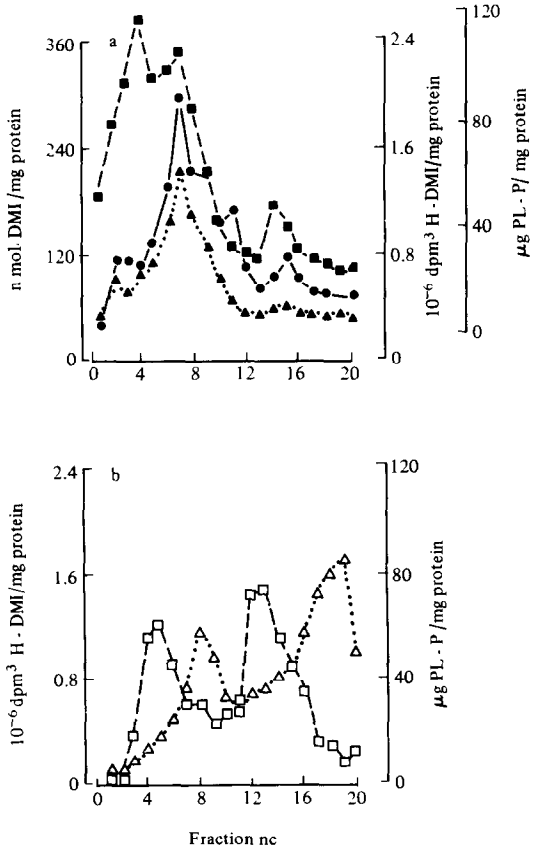

Fig. 2. Phospholipid, (^{3}H)-DMI and nonlabeled DMI distribution in the fractions after P2 Percoll gradient centrifugation. The results are expressed per mg of protein of the individual fractions. They represent the mean of two independent experiments. (a) Cells treated for 9 days with 10 µM DMI and receiving (^{3}H)-DMI in tracer dose 2 hr prior to cell harvesting. (b) Cells receiving (^{3}H)-DMI in tracer dose 2 hr prior to cell harvesting. □■, phospholipids; ●, nonlabeled DMI; △▲, (^{3}H)-DMI.

In cells chronically exposed to DMI the phospholipid distribution showed quantitative and qualitative differences from that observed in control cells (Fig. 2a). The distribution pattern of phospholipids showed three instead of two peaks. Two coincided with the two peaks observed in control cells. The first phospholipid peak, coinciding with 5'-nucleotidase was greatly elevated whereas the one around fractions 11–16 seemed to be decreased. The third rather pronounced peak of phospholipids appeared between fractions 6–8 where in normal cells only a shoulder was observed. The distribution of (^{3}H)-DMI and of nonlabeled DMI only showed one major peak coinciding with the middle peak of the phospholipids. Radioactive DMI distribution was very similar to that of the nonlabeled DMI measured by fluorometry.

Subfractionation of microsomal membranes and cytosol (SN2)

Figure 3 shows the density distribution of the cytosol and of the microsomal membranes. After chronic exposure to DMI there was a difference in the distribution of the proteins as well as a remarkable increase of the lysosomal arylsulfatase A activity throughout the gradient. This was partially due to an increase of the free cytosolic enzyme activity (fractions 1–4). There was also an increase of the membrane associated arylsulfatase A activity in the microsomal fractions. The activity of beta-D-glucosidase was at the lower limit of its detectability, cytochrome-*c*-oxidase was completely missing in the microsomal fractions (results not shown). 5'-Nucleotidase banded in the middle of the gradient and was quite well separated from the activity of the galactosyltransferase which showed two peaks of activity in control and in exposed cells.

Figure 4 shows the distribution of phospholipids and of the radiolabeled as well as of the nonlabeled DMI. The lower part of the figure shows the results in control cells which received (^{3}H)-DMI 2 hr before cell harvesting. There was only one out of three phospholipid peaks which corresponded to the peak of the 5'-nucleotidase activity. The upper part of Fig. 4 shows the results in chronically DMI exposed fibroblasts. A major peak of phospholipids appeared which banded at a somewhat lower density. It coincided with the second peak of galactosyltransferase as well as with the peak for arylsulfatase A. There was, however, a considerable portion of the lipids in the cytosolic supernatant. A second minor shoulder of phospholipids was in the area where the peak activity of 5'-nucleotidase was found. The distribution of (^{3}H)-DMI only showed one peak of activity at the place where the second peak of galactosyltransferase, the peak of arylsulfatase A as well as the major peak of phospholipids were found.

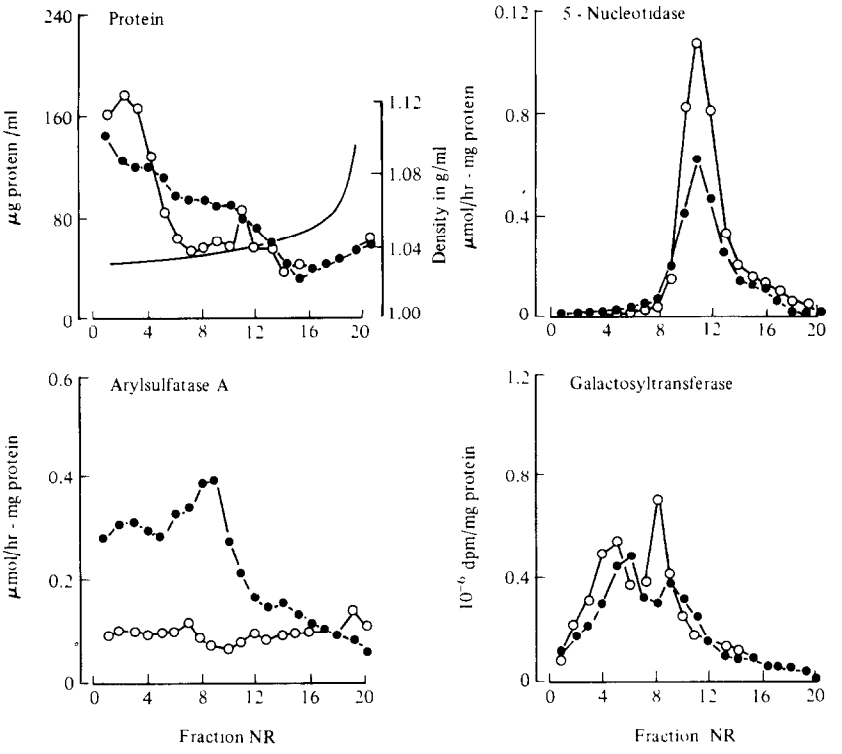

Fig. 3. Marker enzyme activities in the fractions after SN2 Percoll gradient centrifugation. The enzyme activities are expressed per mg of protein of the individual fractions (specific activities). They represent the mean of two independent experiments. The density pattern of the gradient is figured together with the protein distribution. ○——○, control cells; ●——●, cells exposed to 10 μM DMI for 9 days.

There was also considerable radioactivity of (^{3}H)-DMI in the cytosolic fraction. Nonlabeled DMI distribution similarly to the (^{3}H)-DMI with an additional small peak at the site of 5′-nucleotidase activity.

DISCUSSION

Desipramine (DMI) is widely used for its antidepressant action. From its chemical structure and its physicochemical characteristics, DMI is an amphiphilic-cationic compound that belongs to the group of lysosomotropic drugs like chloroquine. DMI is accumulated in cultured human fibroblasts when repeatedly added to the cultured medium [1]. It inhibits phospholipid degradation in cultured fibroblasts and thus causes phospholipid accumulation [5]. Recently we were able to show that chronic administration of DMI reduced the number of β-adrenoceptors in cultured cells to a similar extent as chronic exposure to DMI reduced β-adrenoceptor number in rat brain [15]. From microscopical pictures it appeared that phospholipid accumulation occurred in lysosomes or in lysosome like vesicles [5, 16].

The aim of this study was to localize DMI and the accumulated phospholipids and to assign them to defined subcellular fractions in fibroblasts that had been exposed to DMI either chronically or for short times. For this we developed a simple technique to homogenize comparably small amounts of fibroblasts. Trituration with a plastic Falcon pipet proved to be a suitable and reproducible method of cell homogenization. Differential centrifugation followed by analytical centrifugation in Percoll gradients gave fractions of distinct subcellular organelles identified by appropriate marker enzymes. Chronic DMI exposure seemed to affect the specific activity of the marker enzymes in the cultured fibroblasts. The observed rise in cytochrome-*c*-oxidase activity could indicate an increase in the number of mitochondria while the decrease of the 5′-nucleotidase activity in the cells could reflect a partial inactivation by relocation of plasma membranes, e.g. with lysosomes of DMI treated cells.

The decrease of arylsulfatase A activity also observed in chronically drug treated cells can be explained more precisely, since it is known that lysosomotropic drugs such as chloroquine induce the secretion of lysosomal enzymes into the culture medium [4, 17].

These secreted lysosomal enzymes are newly synthesized proforms deviated on their way to the lysosomes [18]. Only lysosomal enzymes whose transport from the Golgi to the lysosomes is mediated by a protein mannose-6-phosphate are secreted by the action of lysosomotropic drugs [19]. Lysosomal enzymes that are membrane bound, such as β-glucosidase, are not affected, explaining the normal activity of this enzyme in chronically DMI exposed cells.

DMI is a lysosomotropic drug and is accumulated by a trapping mechanism in acidic vesicles only [1].

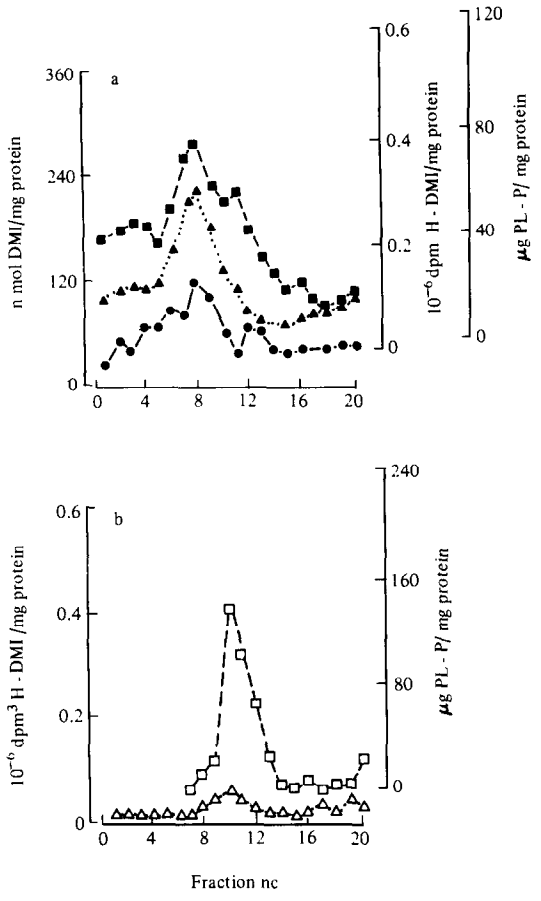

Fig. 4. Phospholipid, (^{3}H)-DMI and nonlabeled DMI distribution in the fractions after SN2 Percoll gradient centrifugation. The results are expressed per mg of protein of the individual fractions. They represent the mean of two independent experiments. (a) Cells chronically exposed for 9 days with 10 µM DMI and receiving (^{3}H)-DMI in tracer dose 2 hr prior to cell harvesting. (b) Control cells, receiving (^{3}H)-DMI in tracer dose 2 hr prior to cell harvesting. □■, phospholipids; ●, nonlabeled DMI; △▲, (^{3}H)-DMI.

Incubation of cultured fibroblasts with micromolar concentrations of DMI resulted in intracellular concentrations of DMI in the millimolar range [1]. This extent of accumulation is hardly compatible with a major intramembraneous distribution such as in the mitochondrial membranes. Not only lysosomes [3] but also pinosomes or endosomes [20, 21] are characterized by a low intravesicular pH of 4–5. Labeling of cultures with tracer amounts of DMI 2 hr before harvesting the cells revealed two peaks of DMI in the subcellular fractions of the P-2 gradient. One of the two peaks could be attributed to lysosomes by lysosomal enzyme activities located at high densities. The other one could tentatively be of pinosomal origin banding at intermediate densities [22, 23]. Another peak of DMI accumulation, observed in the microsomal subfractions coincided with one of the two packs of the Golgi-ER region.

Chronic exposure of cultured fibroblasts to DMI resulted in a subcellular redistribution of radiolabeled DMI, which now accumulated almost exclusively in fractions of intermediate density together with unlabeled DMI and the bulk activity of lysosomal enzymes. These fractions also contained excess phospholipids and could represent lysosomes that had acquired a lower density by storage of phospholipids. This is one possible assumption.

There is, however, an alternative hypothesis. DMI could disturb the exchange traffic between the pinocytic or endocytic vesicles and the lysosomes so that lysosomal enzymes, DMI and phospholipids would accumulate within the pinosomes, organelles which usually only contain small amounts of lysosomal enzyme activity [21]. In this case these vesicles of intermediate density containing phospholipids would rather be pinosomes or endosomes than lysosomes in spite of their high activity of arylsulfatase A.

This possibility would also explain why other cellular organelles such as the plasma membranes and the Golgi region that also show an increased phospholipid content in chronically DMI exposed cells did not significantly change their density and therefore their banding positions along the Percoll gradient. Recently it could be shown that chronic exposure to DMI not only increased total phospholipid content of cultured cells but also changed the composition of the phospholipids accumulated. The changes mainly consisted in an overproportionate increase of phosphatidylinositol due to a rapid turnover of this phospholipid [5].

Phospholipid analyses in the subcellular fractions have shown an altered phospholipid pattern in the light lysosomal enzyme containing fraction as well as in the plasma membrane fraction (Stoffel *et al.*, unpublished results) similar to that found in total phospholipids. This preliminary finding may be an important new aspect of the chronic DMI effect. Alterations in the phospholipid pattern of plasma- and other cellular membranes could change the properties and the function of membranes. In this respect, the recent finding that chronic exposure of cultured fibroblasts to DMI reduced the number of functional beta-adrenoceptors [15] could be due to a change in the plasma membrane phospholipid composition.

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