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ORGANSPECIFIC, QUALITATIVE CHANGES IN THE PHOSPHOLIPID COMPOSITION OF RATS AFTER CHRONIC ADMINISTRATION OF THE ANTIDEPRESSANT DRUG DESIPRAMINE*

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Abstract—Rats were chronically treated with daily i.p. injections of 10 mg/kg desipramine for 21 days. A 30% decrease in the number of β-adrenoceptors was observed in brain. A receptor desensitization of similar extent was noted in submaxillary glands and lung. No change in β-adrenoceptor number was present in heart. Total phospholipid contents were not altered in these organs after chronic drug treatment. However, organ-specific changes were found in the phospholipid composition of submaxillary glands, lung and liver but not in whole brain and heart. The changes were variable but an increase in phosphatidylinositol and decreases in phosphatidylethanolamine and sphingomyelin were consistent. Possible alterations in the phospholipid composition of the brain might have been masked by the large and stable pool of myelin phospholipids. A causal relationship between changes in the phospholipid composition and β-adrenoceptor desensitization is discussed.

The classical tricyclic antidepressant drugs have been in clinical use for nearly three decades. Despite extensive efforts, the mechanism of action of these drugs has not been fully elucidated. Recently, antidepressant drug-induced changes in the functioning of different neurotransmitter systems have been described [1–3]. These include alterations in the density and affinity of pre- and postsynaptic receptors [4, 5] and effects on neurotransmitter turnover [6, 7]. All attempts to explain the mechanisms of antidepressant actions by a specific influence on one particular neurotransmitter system have yet been unsuccessful because of the heterogeneity and complexity of the brain. In addition, none of the current hypotheses is able to explain the time lag between the beginning of the therapy and the onset of clinical effectiveness. One effect common to all classical tricyclic and to most of the "atypical" antidepressants and even to electroconvulsive treatment is a reduction in β-adrenoceptor density, measured in brain of animals after chronic drug administration [8]. In rats chronically treated with desipramine (DMI) a reduction in β-adrenoceptor density has been found in brain but also in peripheral organs, as shown in a preliminary study [9]. Very recently we have described a reduction of β-adrenoceptor number in cultured cells chronically exposed to micromolar concentrations of DMI [10]. Additionally, effects of antidepressant drugs on cellular phospholipid (PL) metabolism have been observed in cultured cells [11]. These *in vitro* results encouraged us to verify the changes in PL-metabolism *in vivo* and to correlate them with alterations in the β-adrenoceptor density in various organs of rats chronically treated with DMI.

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MATERIALS AND METHODS

Treatment of animals. Male Sprague–Dawley rats weighing 180 g were chronically treated with desipramine (DMI) for 21 days. The drug was given once a day in doses of 10 mg/kg intraperitoneally (i.p.). Control animals received daily injections of saline (1.0 ml/kg). Injections were always given between 9 and 10 a.m.

During the experimental periods the animals had free access to standard laboratory diet and water. The rats were sacrificed at the same time of the day, 1, 2, 3 and 7 days respectively after the last injection. After decapitation, cerebrum, submaxillary glands, heart and lung were quickly removed, immediately frozen in liquid nitrogen and stored at −20°.

Preparation of tissue-homogenates and membrane fractions. Organs were homogenized using a Polytron tissue homogenizer and a glass-glass tissue grinder. The tissue concentrations were 5% (w/v) for the submaxillary glands and 10% for the other tissues in Tris–maleate buffer (TMB) (80 mM, pH 7.4) containing 6 mM $MgCl_2$ and 1 mM EGTA.

Crude membrane preparations were obtained by fractional centrifugation. Tissue homogenates were centrifuged at 200 *g* for 10 min. The supernatants were then centrifuged at 49,000 *g* for 20 min and the resulting pellets were suspended in TMB. The protein concentrations were adjusted to 2.5 mg/ml.

β-Adrenoceptor binding studies. Radioligand binding experiments were carried out according to Honegger *et al.* [9, 12]. Crude membrane suspensions were incubated with the beta-adrenolytic

drug (-)-^{3}H-dihydroalprenolol (^{3}H-DHA, 55.4 Ci/mMol, New England Nuclear, Dreieich, F.R.G.) at 37° for 20 min. The volume of the incubation mixture was 150 μl. Final concentrations of ^{3}H-DHA were 0.8, 1.6, 2.4, 4.8, 7.2 and 9.6 nM respectively. Non-specific binding was determined in the presence of 1 μM timolol, a potent beta-adrenoceptor blocking compound. This concentration was sufficient to quantitatively displace the specifically bound ^{3}H-DHA but it did not interfere with the nonspecific ^{3}H-DHA-binding. The equilibrium reaction was stopped by addition of 1.0 ml of ice-cold TMB to the incubation mixture. The samples were immediately filtered through presoaked glass fiber discs (GF/C Whatman, Maidstone, U.K.). Each filter was rinsed three times with 5.0 ml saline, dried and placed into a counter vial. 10.0 ml of Kontrogel liquid scintillation cocktail (Kontron, Zürich, Switzerland) was added and the radioactivity on the filter discs was determined in a liquid scintillation counter MR 300 (Kontron, Zürich, Switzerland).

Specific binding was calculated by subtracting the ^{3}H-DHA-binding in the presence of timolol from the total ^{3}H-DHA-binding in the absence of timolol. Binding parameters b_{max} and K_D were computed by nonlinear regression analysis on a desktop computer HP 9816 (Hewlett-Packard, Palo Alto, CA). MODFIT-programs were developed by A. Cachelin according to McIntosh and McIntosh [13].

In each binding experiment membrane preparations from at least two control animals were included. The specific ^{3}H-DHA-binding of DMI-treated animals was expressed as percent of control values.

Determination of total phospholipid content. Total phospholipids were determined according to a fluorimetric assay with 1,6-diphenyl-hexatriene (DPH) [14]. DPH was purchased from Fluka Chemical Co. (Buchs, Switzerland). The assay was done with 40 μl of the diluted tissue homogenates (2.5% w/v). For the calibration a lecithin-standard from Sigma Chemical Co. (St. Louis, MO) was used. Total phospholipid contents were expressed in nmoles of phospholipid per mg of protein [15].

Separation and determination of individual phospholipids. Phospholipids were extracted from tissue homogenates according to Folch *et al.* [16]. Chloroform–methanol extracts containing 300 μg of total phospholipids were stored at −20°. For the chromatographic separation, phospholipids were dissolved in a mixture of chloroform, methanol and water (80:14:1). 20 μl of this solution were applied on precoated thin-layer plates (HPTLC silica gel 60, Merck Darmstadt, F.R.G.) using a spray-on technique with nitrogen as carrier gas (Linomat III, Camag, Muttenz, Switzerland). On each plate phospholipid-extracts of control and DMI-treated tissues were co-chromatographed with a mixture of standard-phospholipids (Supelco SA, Gland, Switzerland). The plates were developed in a solvent mixture of chloroform, methanol, formamid and water (60:40:6:2.5) in a paper-lined chromatography tank. The plates were then dried at 160° for 10 min. The spots were visualized by dipping the plates into a 0.5% cupric (II)–acetate solution in absolute ethanol and into a methanolic phosphoric acid (8%) solution. Finally the plates were heated at 160° for 6 min [17].

A TLC scanner from Camag (Muttenz, Switzerland), interfaced with an electronic integrator HP 3390A (Hewlett-Packard, Palo Alto, CA) was used for the photodensitometric determination of the chromatograms. The detection of the chromatographic spots was performed at 546 nm (high-pressure Hg-lamp). The difference in the staining density of equimolar amounts of individual PL were corrected by means of factors derived from chromatograms of PL standard mixtures. This technique permits a one-dimensional separation of sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE).

Statistics. Results were expressed as mean ± standard deviation ($x \pm SD$). Significant differences were quantified by Student's t-test with $P < 0.05$ accepted as the level of significance.

Chemicals. All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, F.R.G.). Timolol was kindly provided by Merck, Sharp and Dohme (Rahwey, NJ). Desipramine was a generous gift of Ciba-Geigy (Basel, Switzerland).

RESULTS

Body weight

At the beginning of the treatment period the mean body weight of the rats was 178 ± 13 g (N = 39). After 21 days control rats weighed 321 ± 20 g (N = 24), while the DMI-treated rats were 278 ± 12 g (N = 15). The drug treatment reduced the daily weight increase by 30.1%. The difference in body weights between treated and control rats was statistically significant after 7 days of treatment.

β-Adrenoceptor-binding

Values of the ^{3}H-DHA binding parameters as maximal binding (b_{max}) and equilibrium dissociation constant (K_D) from crude membrane preparations of brain, submaxillary glands and heart and from lung homogenates of control and DMI-treated animals are summarized in Table 1.

Reductions in the number of β-adrenoceptor sites were seen only after long term DMI-treatment. Chronic saline i.p.-applications or single DMI-injections 24 hr before decapitation of the animals did not show any measurable effect on β-adrenoceptor density in the organs mentioned above. Chronic administration of DMI induced a significant decrease in β-adrenoceptor densities in brain, submaxillary glands and lung from animals killed 24 hr after the last injection. No apparent alterations in the number of β-adrenoceptors were seen in heart. Neither homogenates nor membrane preparations of liver showed any measurable specific ^{3}H-DHA binding.

K_D-values as a measure for binding affinities were not changed by the drug treatment in all the organs investigated.

Studies on the reversibility of the drug induced reduction in receptor sites revealed a recovery of the reduced receptor densities between 24 hr and 7 days after the last dose. b_{max}-Values determined 7 days

Table 1. ^{3}H-DHA-binding to crude membrane fractions and tissue homogenates of organs from control and DMI treated rats*

Organ	Control b_{max}§ [fmoles/mg protein]	Control K_D§ [nM]	DMI-treated b_{max}§ [fmoles/mg protein]	DMI-treated K_D§ [nM]
Brain†	82.8 ± 8.8	2.00 ± 0.11	56.6 ± 6.1‖	2.09 ± 0.38
Submax. glands†	214.6 ± 22.1	3.07 ± 0.40	167.6 ± 17.4‖	3.15 ± 0.75
Heart†	45.6 ± 5.2	2.08 ± 0.36	42.8 ± 3.3‖	2.11 ± 0.26
Lung‡	128.5 ± 15.2	0.93 ± 0.17	82.1 ± 16.2‖	0.97 ± 0.12

* Rats received daily i.p.-injections of saline or DMI (10 mg/kg) for 21 days.
† Crude membrane fractions.
‡ Tissue homogenate.
§ Values represent mean ± SD of 4–10 independent experiments.
‖ Statistically significant ($P < 0.01$) determined by Student's t-test.

after cessation of the treatment were similar to values of control animals, except for brain (Table 2).

Tissue content of total phospholipids

Phospholipid contents related to protein showed organ-specific differences (Table 3). Values in brain were 4–8 times higher than in the other investigated organs.

No changes in phospholipid contents could be observed following chronic DMI-treatment.

Phospholipid composition

The relative amounts of the individual phospholipids of brain, submaxillary glands, heart, lung and liver of control animals are shown in Fig. 1a. Each organ showed a characteristic phospholipid pattern, those of submaxillary glands and liver being similar.

Chronic DMI-treatment induced significant changes in the relative amounts of individual phospholipids of submaxillary glands, lung and liver (Fig. 1b). Also the changes in the phospholipid patterns of submaxillary glands and liver were similar. In brain and heart no effects of the DMI-treatment could be seen on the phospholipid pattern.

In submaxillary glands and liver an increase of PI and PS was noted, in the lung an increase of PC and PI. The percental increase of these PL was at the expense of PE and SM.

Table 2. Recurrence of β-adrenoceptor binding sites in different rat organs after cessation of chronic DMI-treatment*

Organ	b_{max} (in % of control values)† 24 hr‡	48 hr‡	72 hr‡	168 hr‡
Brain§	68.3 ± 7.4	76.6	82.4	83.2
Submax. glands§	78.1 ± 9.1	78.8	—	98.8
Heart§	93.9 ± 7.2	94.5	109.9	108.1
Lung‖	63.9 ± 12.6	62.3	90.5	98.9

* Rats were treated with daily i.p.-injection of saline (controls) on DMI (10 mg/kg) for 21 days.
† Values represent mean ± SD of 4–10 (24 hr) or 2 (48, 72 and 168 hr) independent experiments.
‡ Time after the last DMI-dose.
§ Crude membrane fractions.
‖ Tissue homogenate.

DISCUSSION

In vitro studies with cell culture systems in our laboratories have revealed that chronic exposure of cultured cells to desipramine leads to a reduction in the number of beta-adrenoceptors [10], to a PL-accumulation and to changes in the cellular PL-composition [11, 18]. The drug effects on receptors and on PL might be causally related since the functioning of membrane proteins as e.g. neurotransmitter receptors are depending on the PL-environment [19].

The present investigation was performed in order to verify these *in vitro* effects of chronic DMI application *in vivo*.

β-Adrenoceptors

In agreement with the *in vitro* findings and with preliminary *in vivo* data in rats [9] chronic treatment with DMI reduced the number of β-adrenoceptors in brain. This present study also showed a β-adrenoceptor desensitization of 20–35% in lung and submaxillary glands when compared to controls. In contrast, the receptor number of heart was not significantly altered by the drug treatment (Tables 1

Table 3. Total phospholipid contents of different tissues from desipramine-treated and control rats

Tissue	Total phospholipid contents* [nmoles/mg protein] Control rats†	DMI-treated rats‡
Brain	872 ± 46 (18)	824 ± 23 (5)
Submax. glands	201 ± 38 (4)	216 ± 43 (4)
Liver	153 ± 32 (10)	153 ± 36 (5)
Lung	216 ± 53 (12)	214 ± 17 (4)
Heart	106 ± 17 (5)	103 ± 15 (5)

* Phospholipids were determined according to Jouanel *et al.* [14]. The assay was performed with tissue homogenates in Tris–maleate-buffer (2.5% w/v). The values shown are expressed as mean ± SD. The number of animals in parenthesis.
† Saline-injections (1.0 ml/kg i.p.) for 21 days.
‡ Chronic DMI-treatment with daily i.p.-injections of 10 mg/kg for 21 days.

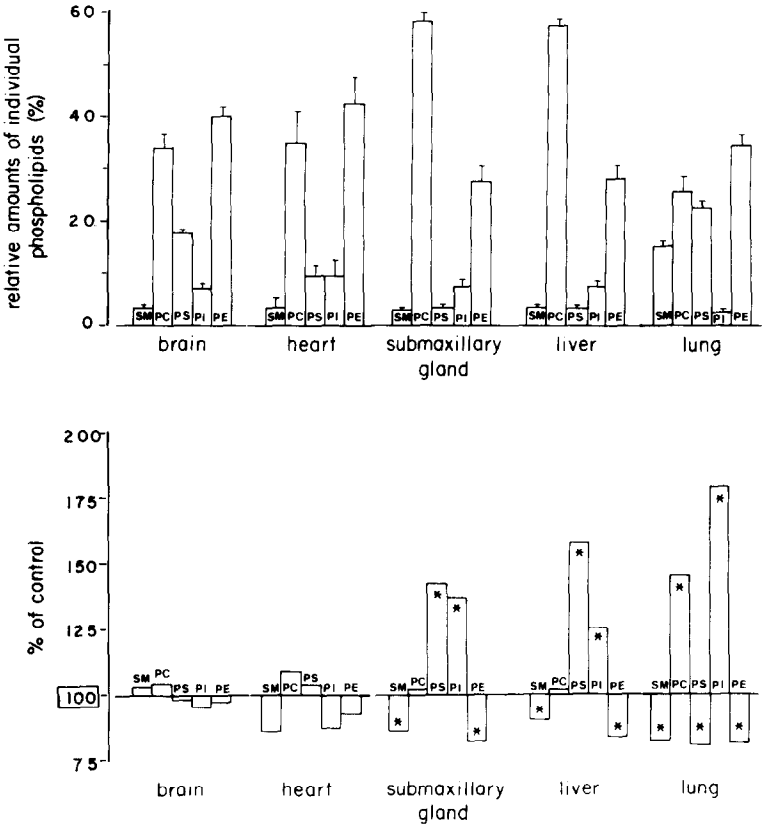

Fig. 1. Phospholipid (PL) composition of different rat tissues and effects of chronic desipramine (DMI)-treatment on individual PL-patterns. For the determination of the PL-composition, tissue homogenates were extracted according to Folch *et al.* [16] and the PL analyzed using a HPTLC method with photodensitometric detection. (A) PL-composition of tissues derived from control rats. The relative amounts of individual PL were indicated as mean ± SD of tissue samples from 5 animals. (B) Changes of the relative amounts of individual PL as a consequence of the chronic DMI-treatment. Statistical significance of differences from control data (*) were determined by Student's *t*-test ($P < 0.05$). SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

and 2), which confirms a previous report by Wolfe *et al.* [20]. The receptor numbers recovered with a half time of 24 hr and were back to control values 7 days after cessation of treatment with the exception of brain (Table 2). DMI elimination from the tissues was still more rapid; no DMI metabolites could be detected 48 hr after the last dose in the respective tissues (results not shown). Our findings do not allow to differentiate *de novo* synthesis of receptors from resensitization by recovery of hidden receptors.

Phospholipids

Total phospholipids from brain, lung, heart, submaxillary glands and liver of DMI treated rats were not significantly different from those in organs of control rats. Marked differences, however, were found in the phospholipid contents between various organs, confirming previous findings of other authors [21]. The absence of a PL-accumulation is in contrast to reported PL-accumulation in rats after chronic treatment with imipramine in doses between 60 and 120 mg/kg and day [22]. This apparent discrepancy can be explained by the much lower doses (10 mg/kg·day) applied in our experiments as well as to the difference in the intervals of drug application. The PL-accumulation observed in cell cultures is the result of a permanent exposure of the cells to constant drug levels [11, 18] comparable to an infusion. In the present experiments, however, rats received one single injection per day, which results in a short transient increase in plasma drug level followed by a long period of low plasma concentration of DMI.

Administration of antidepressants to patients by infusion has been shown to shorten the lag period between beginning of treatment and the onset of clinical effectiveness [23]. One wonders whether this also might increase the risk of phospholipidosis.

The PL-patterns of the individual organs investigated were remarkably different from each other. The two intestinal organs, submaxillary gland and liver, however, were indistinguishable. Changes in the organotypic PL-composition appeared to be a more sensitive indicator for DMI effects than total PL-content. They showed some correspondence with β-adrenoceptor desensitization. In the heart there was neither a change in the PL-pattern nor in β-

Table 4. Accordance of desipramine effects *in vitro* and *in vivo*

Chronic antidepressant effects	*In vitro*	Ref.	*In vivo*	Ref.
β-Adrenoceptor desensitization	+	10	+	5,9
Decrease in the isoproterenol sensitive cAMP stimulation	+	28	+ −	1 this paper
PL-accumulation	+	11,22	+	22
Changes in the PL-composition	+	11,18	+	this paper
Time lag	+	10	+	1

adrenoceptor density. In lung and submaxillary glands the decrease in β-adrenoceptor densities appeared to be related to the extent of PI-increase. There was, however, a discrepancy in brain where the drug induced receptor desensitization was not accompanied by a detectable change in the PL-composition. This can possibly be explained by the presence of a large stable pool of PL in myelin with a very slow turnover rate of several weeks to months [24, 25].

The present results are compatible with a causal relationship between β-adrenoceptor desensitization and changes in the PL-metabolism as could be deduced from previous findings in cell cultures [10, 11, 18]. DMI is a lysosomal drug which interferes with lysosomal PL-degradation presumably by an inhibition of phospholipases [26]. A major aspect of the altered PL-metabolism is the increase in percentage of PI, both in cultured cells and *in vivo*. Incorporation of exogenous PI into membranes of turkey erythrocytes has been shown to reduce the β-adrenergic sensitive cAMP-formation [27]. The accordance of the pertinent data from *in vitro* and *in vivo* experiments is documented in Table 4. It demonstrates an example of the significance of *in vitro* systems for its relevance *in vivo*.

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