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Changes in brain striatum dopamine and acetylcholine receptors induced by chronic CDP-choline treatment of aging mice

Rosa Giménez, Josep Raïch & [1]Juan Aguilar

Department of Biochemistry, Faculty of Pharmacy, University of Barcelona, Diagonal 643, 08028 Barcelona, Spain

1 Spiroperidol binding (dopamine D_2 receptors) and quinuclidinyl benzilate binding (muscarinic receptors) in striata of 19-month old mice was analyzed for animals that had received chronic administration of cytidine 5'-diphosphocholine (CDP-choline) incorporated into the chow consumed (100 or 500 mg kg^{-1} added per day) for the 7 months before they were killed.

2 Treated animals displayed an increase in the dopamine receptor densities of 11% for those receiving 100 mg kg^{-1} and 18% for those receiving 500 mg kg^{-1} as compared to the control aged animals that had received no CDP-choline. Control animals showed, from 2 months to 19 months of life, a 28% decrease in the receptor density. No change in the affinity of the receptors for spiroperidol was found in the treated or untreated animals.

3 Muscarinic acetylcholine receptor densities were also partially recovered by the same treatment in aged animals that showed a 14% decrease of these receptors in this case. The muscarinic receptor density increased 6% for the animals that received 100 mg kg^{-1} and 17% for the animals that received 500 mg kg^{-1} without any change in the affinity of the receptor for quinuclidinyl benzilate.

4 Aged animals displayed a slight increase in brain membrane fluidity as indicated by a decrease in the polarization value of the non-polar fluorophore 1,6-diphenyl-1,3,5-hexatriene. Interestingly, in the treated animals a greater increase in membrane fluidity was determined and found to be very similar for the two doses.

5 It is concluded that chronic administration of CDP-choline to aged animals promoted a partial recovery of the striatum dopamine and acetylcholine receptor function normally reduced with aging, which might be explicable in terms of mechanisms involving fluidity of the brain neuronal membrane.

Keywords: Muscarinic receptor; dopamine D_2 receptor; aging; striatum; choline donor; CDP-choline (cytidine 5'-diphosphocholine); membrane fluidity

Introduction

Cytidine 5'-diphosphocholine (CDP-choline) has been widely used for the treatment of different diseases of the central nervous system (Manaka *et al.*, 1974; Salvadorini *et al.*, 1975; Cohadon *et al.*, 1982; Lecuire & Duplay, 1982). Membrane diffusion of this compound, after intravenous or oral administration, requires splitting of the molecule into CMP and phosphocholine, which are later metabolized to cytidine and choline (Yashima *et al.*, 1975; Lopez-G. Coviella *et al.*, 1987). These two major circulating products are then incorporated into their respective cellular pools and their increased concentrations would account for the pharmacological properties of CDP-choline.

Choline, as phosphocholine, is a precursor in the synthesis of phosphatidylcholine (PC), and cytidine, as CTP, is also an intermediary in the synthesis of PC and other membrane phospholipids. Administration of CDP-choline reportedly affects brain phospholipid biosynthesis (Dorman *et al.*, 1983), membrane structure (Horrocks *et al.*, 1981) and, both *in vitro* and *in vivo*, the metabolism and release of certain brain and peripheral neurotransmitters (Martinet *et al.*, 1981; Agut *et al.*, 1984; Lopez-G. Coviella *et al.*, 1986).

The question of how neurotransmitter binding can be affected by this treatment seemed of primary importance in understanding the mechanisms of the pharmacological action of CDP-choline. Some reported therapeutic effects of choline donors on several diseases that go together with aging such as Parkinson's disease (Agnoli *et al.*, 1982), Huntington's chorea (Growdon *et al.*, 1977) or Alzheimer's disease (Etienne *et al.*, 1979), focused our attention on the neurotransmitters dopamine and acetylcholine as good markers of possible neurotransmission changes in these cases. Several authors have reported that the density of either dopamine receptors (Marquis *et al.*, 1981; Morgan *et al.*, 1987) or muscarinic cholinoceptors (Biegon *et al.*, 1988) decreases with aging of animals without any change in affinity. Morgan *et al.* (1987) have found that the decrease in dopamine receptor density is mostly found in the D_2 type, which led us to analyze a D_2 ligand such as spiroperidol. Furthermore, in man, Seeman *et al.* (1987) have reported a decrease of D_1 and D_2 dopamine receptors of 3.2% and 2.2% per decade respectively.

It has been shown that the phospholipid content of various regions of the human brain diminishes with age and that the cholesterol content displays, in these same samples, an even more dramatic decrease that results in a decrease in the cholesterol/phospholipid ratio (Söderberg *et al.*, 1990). In another context, measurements of phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine have been shown to increase by 15% to 20% in membrane preparations of aging mice treated with CDP-choline (Lopez-G. Coviella *et al.*, 1988). These changes in the lipid composition of the membrane may cause modifications in the membrane fluidity according to descriptions of several authors, showing that methylation of membrane phospholipids affect the membrane fluidity (Mio *et al.*, 1984).

In this context it seemed of interest to determine in aging animals the effect of the administration of CDP-choline on the possible modulation of the number of receptors as well as on their brain membrane fluidity.

Methods

Animals

Female CD-1 mice were housed at constant room temperature (21°C), relative humidity (60%) and with 12:12 h light:dark cycle, with free access to food and water. At 12 months of age animals were assigned randomly to one of three

[1] Author for correspondence.

dietary groups and fed either regular (0 mg kg^{-1} day^{-1}), or CDP-choline supplemented 100 mg or 500 mg kg^{-1} day^{-1}) chow for another 7 months. From the average animal weight and food intake, amounts of CDP-choline to be added to the diet were calculated to adjust a mean dose and the adjustment was repeated every two weeks. A group of young animals consisting of untreated two month-old mice kept under the same conditions were used for comparison. The group of aged animals fed with the regular diet was used as control animals.

Tissue preparation

Mice were decapitated, and the corpus striatum was immediately removed and immersed in 50 mM Tris-HCl buffer (pH 7.1) containing (mM): NaCl 120, KCl 5, Ca_2Cl 2, $MgCl_2$ 1 and pargyline 10 μM. The striatum of each mouse was homogenized in 2 ml of buffer for 15 s with a Polytron (PCU Kinematica Model GmbH) at setting 7. The homogenate was then centrifuged at 27,000 g for 20 min and the pellet resuspended in the same volume of buffer, and centrifuged twice more. The final pellet was resuspended in 2.5 ml of the same buffer.

When the preparation was used for the measurement of membrane fluidity, cerebral hemispheres were homogenized in 2 ml of 10 mM Tris-HCl (pH 7.4) and the homogenate centrifuged at 12,000 g for 30 min. The pellet was discarded and the supernatant centrifuged at 48,000 g for 30 min. The pellet, resuspended in the homogenizing buffer, was used as plasma membrane fraction for use in fluidity measurements.

Binding assay

The D_2 receptor densities were measured by saturating the tissue with increasing concentrations of [^{3}H]-spiroperidol. The binding of [^{3}H]-spiroperidol was determined by a modification of the assay described by Leysen *et al.* (1978). Membranes from the striatum of the mice were added to test tubes containing [^{3}H]-spiroperidol. Chlorpromazine at 10 μM concentration was used to define specific binding. Concentration kinetics of the binding were performed at concentrations of [^{3}H]-spiroperidol ranging from 24 pM to 820 pM.

Muscarinic cholinoceptor densities were measured by saturating the tissue with increasing concentrations of [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB). The binding of [^{3}H]-QNB was measured by a modification of the assay described by Baron & Siegel (1989). The test tubes contained [^{3}H]-QNB in Tris-buffered Krebs (pH 7.4) containing (mM): NaCl 118, KCl 5, $MgCl_2$ 2, $CaCl_2$ 1.9, Tris 25 and glucose 10. Nonspecific binding was defined as that remaining in the presence of 10 μM atropine. Concentration kinetics were performed between 75 pM and 3026 pM of [^{3}H]-QNB.

Binding assays were carried out in duplicate at 25°C for 40 min and membrane-bound ligand was collected *in vacuo* onto GF/B filter strips with a Brandel Cell Harvester, followed by three washes of 5 ml of cold buffer. Radioactivity in each filter was determined after 24 h in 5 ml of scintillation fluid consisting of 0.8% 2,5-diphenyl oxazole in toluene at a counting efficiency of 35–40%.

Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin used as standard.

Fluorescence polarization

Fluorescence polarization to estimate membrane fluidity was performed as described by Wilson *et al.* (1988) except that crude membrane preparations contained 80 μg of protein that were diluted to 2 ml of the original homogenizing buffer were used. This suspension was added to a test tube with the non-polar fluorophore, 1,6-diphenyl-*trans*-1-3,5-hexatriene (DPH) at a DPH to lipid molecular ratio of 1:400.

Statistical analysis

Saturation binding data were obtained by Scatchard plot. The data are reported as arithmetic means ± s.e.mean. Significance tests were performed by Student's t test. Differences between means were regarded as statistically significant when $P < 0.02$.

Chemicals

[^{3}H]-spiroperidol (72.9 Ci mmol^{-1}) was obtained from New England Nuclear, U.K. and [^{3}H]-QNB (44.3 Ci mmol^{-1}), from Amersham, U.K. Chlorpromazine, pargyline and DPH were from Sigma, St. Louis, MO. U.S.A. Atropine was purchased from Merck, Darmstadt, Gemany and CDP-choline from Ferrer Internacional, S.A. Barcelona, Spain.

Results

Spiroperidol binding

Our reference values of spiroperidol binding of striata showed, as has already been reported (Morgan *et al.*, 1987), clear differences between the groups of young and old animals. In our experimental conditions the receptor density of old animals presented a significant 28% decrease ($P < 0.01$) compared to the value obtained with young animals (Table 1). The K_d displayed a slightly increased value for the old animals as compared to the young ones (non-significant).

The B_{max} values obtained with treated animals always lay between the reference values of the young and old animals. As shown in Table 1, the average value determined for the group of animals treated with 100 mg kg^{-1} of CDP-choline was 11% higher than that of the group of non-treated aged animals. The average value for the group of animals treated with 500 mg kg^{-1} was even higher, with an increase reaching 18% of the average value corresponding to the group of non-treated aged animals. This second value differed significantly ($P < 0.02$) from that of the aged control group. The average values of the K_d for both groups of treated animals were slightly higher than the average values found for both groups of untreated animals (old and young); however, the differences were not statistically significant.

Quinuclidinyl benzilate binding

Our results indicated that the difference in binding between old and young mice is smaller for QNB than for spiroperidol. The decrease, although statistically significant ($P < 0.01$), was in this case a mere 14% (Table 2). The values of K_d determined for QNB, as those found for spiroperidol, did not differ significantly, displaying a slight increase.

The data obtained with the treated aged animals followed the same trends as were found for spiroperidol. Thus the treatment of aged animals with CDP-choline at 100 mg kg^{-1} increased the B_{max} by 6% while treatment with 500 mg kg^{-1} increased the B_{max} by 17%; in this second case with a high statistical significance ($P < 0.02$). The average value of K_d corresponding to either of the two groups of treated animals dis-

Table 1 Effect of chronic administration of cytidine 5'-diphosphocholine (CDP-choline) on spiroperidol binding constants

Animal group	B_{max} (fmol mg^{-1})	K_d (pM)	n
Aged control	306 ± 14.8	49 ± 2.9	20
Aged + 100 mg kg^{-1}	340 ± 17.9	55 ± 3.4	20
Aged + 500 mg kg^{-1}	361 ± 16.8*	53 ± 2.6	16
Young	423 ± 40.7**	43 ± 9.1	5

Results are presented as means ± s.e.mean. Untreated aged animals are taken as control and the differences between means analysed by Student's unpaired test. Statistically significant differences from the control group are shown as * $P < 0.02$ and ** $P < 0.01$.

Table 2 Effect of chronic administration of cytidine 5'-phosphocholine (CDP-choline) on the quinuclidinyl benzilate (QNB) binding constants

Animal group	B_{max} (fmol mg^{-1})	K_d (pM)	n
Aged control	1871 ± 63	299 ± 19	12
Aged + 100 mg kg^{-1}	1988 ± 61	306 ± 25	12
Aged + 500 mg kg^{-1}	2190 ± 70*	286 ± 29	11
Young	2168 ± 66*	332 ± 30	12

Results are presented as means ± s.e.means. Untreated aged animals are taken as control and the differences between means analysed by Student's unpaired test. Statistically significant differences from the control group are shown as * $P < 0.01$.

played no difference when compared to the reference value of the non-treated animals (Table 2).

Membrane fluidity

Studies of brain membrane fluidity were performed by measuring the polarization changes of the non-polar fluorophore DPH used as a probe in our membrane preparations. Membrane fluidity for old animals was shown to be slightly higher than that corresponding to membranes of young animals as indicated by the decrease of 0.55 in the steady state polarization value (Table 3). Chronic administration of CDP-choline clearly diminished the polarization and hence increased the membrane fluidity. The values determined differed significantly from those of the aged control animals regardless of the dose administered, with a change of 1.13 ($P < 0.02$) for the animals treated with 100 mg kg^{-1} of CDP-choline and a change of 1.08 ($P < 0.005$) for the animals treated with 500 mg kg^{-1}. No clear distinction could be established between the two groups of treated animals, which had a very close polarization average value.

Discussion

Phospholipid methylation and PC membrane enrichment has been correlated with increases in β-adrenoceptors by Hirata & Axelrod (1980). Increase in receptor densities by administration of CDP-choline has also been reported for the cholinergic system in young animals (Petkov & Popova, 1987). In this paper we demonstrate that D_2 dopamine receptor densities, as indicated by the spiroperidol binding, which are normally reduced with aging (Seeman *et al.*, 1987; Morgan *et al.*, 1987) are partially recovered by administration of a choline and cytidine donor such as CDP-choline, known to increase the membrane levels of PC (Lopez-G. coviella *et al.*, 1988). Similarly, the decline in muscarinic cholinoceptors also found with aging is clearly blunted by CDP-choline treatment.

In the two cases studied, changes in receptor densities as a consequence of CDP-choline administration seemed not to affect the affinity constant of the receptor for their ligands, which is consistent with the fact that in general, aging induced a decrease in receptor densities without any change in affinity (Pradhan, 1980). Thus, CDP-choline treatment seems to improve the dopamine receptor and also the muscarinic cholinoceptor function through increases in the receptor number.

With the information available to date, it has been difficult to propose a molecular mechanism to explain this phenomenon, especially for the simultaneous effect on both systems. Some authors have tried to assign the parallel modification of dopamine and choline receptors by CDP-choline administration to a specific mechanism of increase in dopamine content of the striatum after treatment, which would cause a decline in the acetylcholine content leading to an increase in the cholinoceptor densities (Petkov & Popova, 1987). The observations described here of spiroperidol and QNB binding modification could also be explained by a less specific effect of the membrane structure on the receptor function, either through receptor availability or through changes in receptor conformation (Loh & Law, 1980; Henis, 1989).

It is well known that in most of the regions of the human brain, including the striatum, the phospholipid content declines with age and that cholesterol declines even more, resulting in an important decrease in the cholesterol to phospholipid ratio (Söderberg *et al.*, 1990). It has also been shown that the decrease in cholesterol content of the membranes enhances their fluidity (Wilson *et al.*, 1988). Therefore, aging would lead to an increase in membrane fluidity, as is indicated by our experiments showing a lower polarization value (higher membrane fluidity) for the old animals compared to the young animals. As CDP-choline administration increases brain phospholipid content (Lopez-G. Coviella *et al.*, 1988), the cholesterol to phospholipid ratio would also decrease with the corresponding membrane fluidity increase (see Table 3). How these changes in membrane structure modify receptor function cannot be unambiguously explained at present. It should be borne in mind that receptor density measurements are a consequence of receptor number and optimal receptor structure and that the interaction of membrane phospholipids and proteins with receptor protein may affect receptor mobility as well as receptor structure.

Chronic treatment of aged mice with CDP-choline recovers the reduced density of dopamine and acetylcholine receptors of the striatum. This beneficial effect of the choline donor might perhaps be related, through more or less complex mechanisms, to an increase in brain membrane fluidity observed in these animals.

Table 3 Effect of chronic administration of cytidine 5'-phosphocholine (CDP-choline) on the fluorescence polarization value of cerebral membranes

Animal group	*Polarization × 100*	n
Aged control	30.63 ± 0.28	17
Aged + 100 mg kg^{-1}	29.50 ± 0.37*	14
Aged + 500 mg kg^{-1}	29.54 ± 0.19**	16
Young	31.18 ± 0.19	17

Results are presented as means ± s.e.mean. Untreated aged animals are taken as control and the differences between means analysed by Student's unpaired test. Statistically significant differences from the control group are shown as * $P < 0.02$ and ** $P < 0.005$.

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