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RESPONSIVENESS OF BRAIN MUSCARINIC ACETYLCHOLINE RECEPTORS IN AGED RATS

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Summary. - *In the first part of the investigation the age-related changes in cortical, hippocampal and striatal cholinesterases (ChE), choline acetyltransferase (ChAT) and muscarinic acetylcholine receptors (mAChRs, measured as Bmax of ^{3}H-QNB binding) were compared in male Wistar, Fischer 344 and Sprague-Dawley rats aged 3 and 24 months. Fischer rats exhibited 1.5 fold higher levels of ChAT and mAChRs than Wistar and Sprague-Dawley rats in the areas analyzed. The age-related decline of cortical ChAT and mAChRs was present in Fischer rats but not in those of the other two strains, i.e. was strain-dependent. There were no age-related changes in hippocampal ChAT in any strain. The decline of striatal ChAT, hippocampal and striatal mAChRs and ChE in the three regions analyzed were present in Wistar, Fischer and Sprague-Dawley aged rats. Thus, these deficits (or lack of changes) appeared to be not strain-dependent. The overall data indicate the importance of genotype in the rate of aging, and an accelerated aging for Fischer 344 rats. In the second part of the investigation the responsiveness of cortical mAChRs, ChE and ChAT to a repeated treatment with an indirect cholinergic agonist (an antiChE agent, diisopropyl fluorophosphate-DFP) was studied in two independent experiments on Fischer 344 and Sprague Dawley rats aged 3 and 24 months. The s.c. administration of DFP (first dose 1.6, subsequent six doses of 1.1 mg/kg on alternate days) to senescent Fischer rats caused a considerably more pronounced syndrome of cholinergic stimulation than in young ones, resulting in 60 and 14% mortality, respectively. In spite of age-related decline of cortical ChE and mAChRs, the effects of DFP at the end of treatment did not differ between surviving senescent and young rats in terms of percentage inhibition of ChE (about 85%) and responsiveness of mAChRs, with their considerable down-regulation (about 40%). The s.c. administration of lower doses of DFP (first dose 1.1, subsequent two doses of 0.7 and four doses of 0.35 mg/kg on alternate days) to senescent Sprague-Dawley rats caused again higher mortality rate than in young ones (40 and 14%, respectively). The surviving rats of the two age-groups exhibited similar percentage inhibition of cortical ChE (about 70%) and similar responsiveness of mAChRs in terms of down-regulation (about 30%). The overall data indicate a great variability in the response of senescent rats to repeated cholinergic stimulation, from total failure of compensatory mechanisms resulting in death to considerable mAChRs plasticity. This plasticity was present in the cerebral cortex in two strains of rats having different absolute levels of mAChRs, both exhibiting (Fischer 344) and not exhibiting (Sprague-Dawley) age-related deficit, and independently of the degree of cholinergic stimulation. Therefore, the ability of central cholinergic systems to compensate for the antiChE insult and to preserve responsiveness of mAChRs is an essential part of the aging process.*

Riassunto (Responsività dei recettori muscarinici cerebrali nell'invecchiamento di ratto). - *Nella prima parte della ricerca sono state studiate le modificazioni delle colinesterasi (ChE), della colinacetiltransferasi (ChAT), e dei recettori muscarinici (mAChRs, misurati come Bmax del legame con ^{3}H-QNB) correlate all'invecchiamento. Tali parametri sono stati misurati nella corteccia cerebrale, ippocampo e striato di ratti maschi Wistar, Fischer 344 e Sprague-Dawley all'età di 3 e 24 mesi. I ratti Fischer presentavano livelli di ChAT e di mAChRs di circa 1,5 volte superiori rispetto a quelli degli altri due ceppi in tutte le regioni esaminate. La riduzione della ChAT e dei mAChRs nella corteccia cerebrale era presente nei ratti vecchi Fischer, ma non nei Wistar e Sprague Dawley, dipendeva quindi dal ceppo. L'attività della ChAT ippocampale non era modificata nel corso dell'invecchiamento in nessuno dei ceppi. Vi era inoltre una riduzione della ChAT striatale, della densità recettoriale nell'ippocampo e nello striato, e della ChE nelle tre aree cerebrali dei ratti vecchi dei tre ceppi. I dati indicano l'importanza del genotipo nell'invecchiamento cerebrale ed un accellerato processo di invecchiamento nei ratti Fischer*

344. Nella seconda parte della ricerca è stata studiata la responsività dei mAChRs e delle ChE al trattamento ripetuto con un agonista colinergico indiretto (diisopropil fluorofosfato, DFP). Tali parametri sono stati misurati nella corteccia cerebrale di ratti giovani e vecchi di ceppo Fischer 344 e Sprague-Dawley. La somministrazione s.c. di DFP (prima dose 1,6, sei dosi di 1,1 mg/kg, a giorni alterni) causava nei ratti vecchi Fischer una sindrome di stimolazione colinergica notevolmente più grave rispetto a quella osservata nei ratti giovani, con mortalità, rispettivamente del 60 e 14%. L'inibizione delle ChE (di circa l'85%) e la responsività dei recettori muscarinici (riduzione di Bmax di circa il 40%) nei ratti vecchi sopravvissuti non differivano da quelle dei giovani. La somministrazione s.c. di dosi più basse di DFP (prima dose 1,1, due dosi di 0,7 e quattro dosi di 0,35 mg/kg, a giorni alterni) causava nei ratti vecchi Sprague-Dawley una mortalità maggiore di quella dei giovani (rispettivamente del 40 e 14%). L'inibizione delle ChE (di circa il 70%) e la risposta recettoriale (riduzione di Bmax di circa il 30%) erano simili nei ratti giovani e vecchi sopravvissuti. I dati indicano una notevole variabilità della risposta alla stimolazione colinergica nei ratti vecchi. I sistemi colinergici cerebrali in alcuni ratti vecchi mantengono notevoli capacità di adattamento, con responsività recettoriale uguale a quella dei ratti giovani.

Introduction

It is well known that administration of multiple doses of direct and indirect cholinergic agonists [e.g. oxotremorine and anticholinesterase agents (antiChE)] to young or adult rodents induces the development of tolerance. In the early eighties the decrease in the density of muscarinic acetylcholine receptors (mAChRs) was recognized as the main adaptive mechanism to overstimulation by the agonists [1-3]. Subsequently, a lack of compensatory down-regulation of brain cortical mAChRs was observed in senescent rodents following repeated administration of oxotremorine [4-5]. Conversely, recent investigations in this laboratory showed an adaptive down-regulation of mAChRs in the cerebral cortex, hippocampus and striatum of senescent Fischer 344 rats following subacute treatment with the sublethal doses of the antiChE, diisopropyl fluorophosphate (DFP). This down-regulation induced by a strong cholinergic stimulation was present in the brain areas where marked age-related decline of mAChRs occurred [6]. Such age-related decline of mAChRs in rats has been described by most authors for the striatum and hippocampus, while controversial data (decline or no changes) have been obtained for the cerebral cortex [7-8]. It can be hypothesized that the responsiveness of cortical mAChRs to agonists in senescent animals is related to their age-related changes.

Direct comparison of age-related changes in Wistar and Fischer 344 rats performed recently in this laboratory showed that the decline of cortical mAChRs is strain-dependent, present in Fischer 344 but not in Wistar senescent rats [9]. There are no comparative data relative to Sprague-Dawley rats, a strain frequently used in laboratory investigations.

The purpose of the first part of the present study was to compare the age-related changes in brain mAChRs, cholinesterases (ChE) and choline acetyltransferase (ChAT) in Fischer, Wistar and Sprague Dawley rats. Since evident differences with regard to the cerebral cortex emerged between the strains analyzed, the second part of the study dealt with the effects of repeated administration of DFP (at very high doses) on cortical mAChRs, ChE and ChAT of young and senescent Fischer 344 rats. Finally, the effects of a similar treatment, but with considerably lower doses of DFP, were evaluated in the two age-groups of Sprague-Dawley rats. This made possible to assess whether the responsiveness, in terms of down-regulation, of brain mAChRs to an indirect cholinergic agonist is a more general phenomenon that is independent of age-related changes in mAChRs and of the degree of cholinergic stimulation.

Materials and methods

Animals

Male Wistar, Sprague-Dawley and Fischer 344 rats, aged 3-4 months (young) and 24-26 months (senescent) were purchased from Charles River Breeding Laboratories, Calco, Como. Information provided by the breeders showed that the 50% survival rate of the rats belonging to the three strains was very similar, namely 29-30 months. The mean ± SEM body weights (in g) for young and aged rats were 290 ± 9 and 720 ± 15 for Wistar, 242 ± 6 and 436 ± 10 for Fischer, and 300 ± 3 and 602 ± 3 for Sprague Dawley. The rats were housed 3 per cage in large metal cages in an air-conditioned room (21 ± 1 °C and 50 ± 10% relative humidity), with light from 7 AM to 7 PM and allowed free access to standard chow (containing 21% protein) and tap water.

Treatments

In the first experiment on Fischer 344 rats the administration of DFP was performed according to a schedule previously standardized to induce up to 80% inhibition of total brain ChE in adult males [10]. The first s.c. dose of 1.6 mg/kg (in arachis oil) and subsequent six doses of 1.1 mg/kg were injected on alternate days for 2 weeks.

In the second experiment on Sprague Dawley rats the same schedule was utilized, but the DFP doses were lower: first s.c. dose of 1.1 mg/kg, subsequently

two doses of 0.7 mg/kg, and four doses of 0.35 mg/kg.

Young and senescent control animals of the two strains were treated with the vehicle. All rats were killed by decapitation 48 h after the last treatment.

Preparation of biological material

The brain was dissected on wet ice and the cerebral cortex, hippocampus and striatum were removed and homogenized in 0.32 M sucrose solution at the ratio 1:10, using Polytron (Kinematica Gmbh), setting 5, for 30 s. The homogenates in two aliquots were frozen and stored at - 80 °C up to 4 weeks before analysis of ChAT and muscarinic receptor sites, while ChE were determined immediately.

Enzyme and receptor analyses

Cholinesterases. – ChE were measured according to a colorimetric method [11], as previously described [6, 12]. The samples (corresponding to 2-2.5 µl of homogenate) were incubated in 39 mM phosphate buffer, pH 7.2, containing 0.21 mM 5,5′ dithiobis-2 nitrobenzoic acid in a final volume of 1.4 ml, at 25 °C for 30 min (in duplicate). Acetylthiocholine (ATC) 0.56 mM was used as substrate. The absorbance at 412 nm was measured in a Gilford 2400 spectrophotometer. The enzymatic activity was expressed as nanomol of ATC hydrolyzed per min per mg of protein.

Choline acetyltransferase. – ChAT was measured according to the radiometric method [13] as previously described [6, 12] using ^{3}H-acetylcoenzyme A as substrate. Aliquots of brain homogenates were activated with a solution containing 0.32 M sucrose, 0.9% NaCl and 0.75% Triton X-100 to obtain 0.8 to 1 µg protein for each sample (2 µl). The incubation mixture contained 20 mM EDTA, 50 mM sodium phosphate buffer, pH 7.2, 0.10 mM eserine sulfate, 8 mM choline chloride, 0.3 M NaCl and 0.2 mM acetylcoenzyme A. The samples (in triplicate), in a final volume of 7 µl, were incubated in microtubes at 37 °C for 15 min. The reaction was stopped by washing twice with 4 ml of ice-cold buffer and addition of 0.4 ml of tetraphenylboron in heptanone (15 mg/ml) into vials. Eight ml of scintillation fluid (1 l of toluene, 4 g PPO, 100 mg POPOP, 50 g naphtalene) were added and vials shaken lightly. The radioactivity was measured in a Packard-Tricarb 4640 at a counting efficiency of 50%. The enzymatic activity was expressed as nanomol of acetylcholine synthetized per h per mg protein.

Muscarinic receptor binding. – Muscarinic receptor binding sites in the brain homogenates were determined by the rapid filtration method [14] as previously described [6, 9], using the specific ligand ^{3}H-quinuclidinyl benzilate (QNB). The samples (50 µl, corresponding to 20-50 µg protein) were incubated in 50 mM phosphate buffer, pH 7.4, containing six different concentrations of ^{3}H-QNB, from 0.05 to 1.5 nM, in a final volume of 0.2 ml (in triplicate). Parallel tubes which also contained 1 µM atropine sulfate were prepared to determine non-specific binding. After incubation at 37 °C for 45 min the reaction was terminated by the addition of 4 ml of ice-cold phosphate buffer and immediate filtration through Whatman GF/B filters. The tubes were washed twice with an additional 4 ml of buffer, the filters placed in vials with 10 ml of Filter Count (Packard) and radioactivity measured as described above. The specific binding was calculated as total minus non-specific binding. The Scatchard analyses of specific ^{3}H-QNB binding were carried out on six points with computer fitted regression lines (correlation coefficients $r > 0.80$), and results expressed as Bmax in femtomol per mg protein, and Kd (nM).

Proteins. – Protein content was determined with a colorimetric method [15] using bovine serum albumin as standard. Since no statistically significant age-related differences in protein content were found either for Fischer 344, Wistar or Sprague Dawley rats, all data were expressed only per mg of protein.

Materials. – DFP was obtained from Fluka, Bucks, Switzerland. ^{3}H-acetylcoenzyme A (4.4 Ci/mmol) and (–) ^{3}H-QNB, (46 Ci/mmol) were purchased from New England Nuclear Corporation, Boston (USA).

Statistics. – The data on 3-and 24-month rats of the three strains were evaluated by ANOVA. Post-hoc analysis was performed by Student's t-test, the accepted level of significance was $p < 0.05$. The effects of repeated DFP treatments were processed by factorial analysis of variance (2 ages × 2 treatments ANOVA), degrees of freedom 1, 20.

Results

Comparison of age-related differences in ChE, ChAT and mAChRs in the cerebral cortex, hippocampus and striatum of Wistar, Fischer 344 and Sprague-Dawley rats

The data on ChE and ChAT in the rats of the three strains are presented in Fig. 1. ChE levels in each brain area were similar in young rats of different strains. The analysis of variance showed significant age-related changes in enzymatic activity in the regions analyzed. The decline varied from 20 to 40% and was not strain-dependent. In the case of ChAT, Fischer 344 rats exhibited about 1.4 fold higher levels of activity than age-matched Wistar or Sprague Dawley rats in all the analyzed regions. As regards age-related differences, the decrease of ChAT in the cerebral cortex was observed only in Fischer rats. There were no age-related changes in the hippocampus of the three rat strains. On the contrary, a significant deficit of ChAT was observed in the

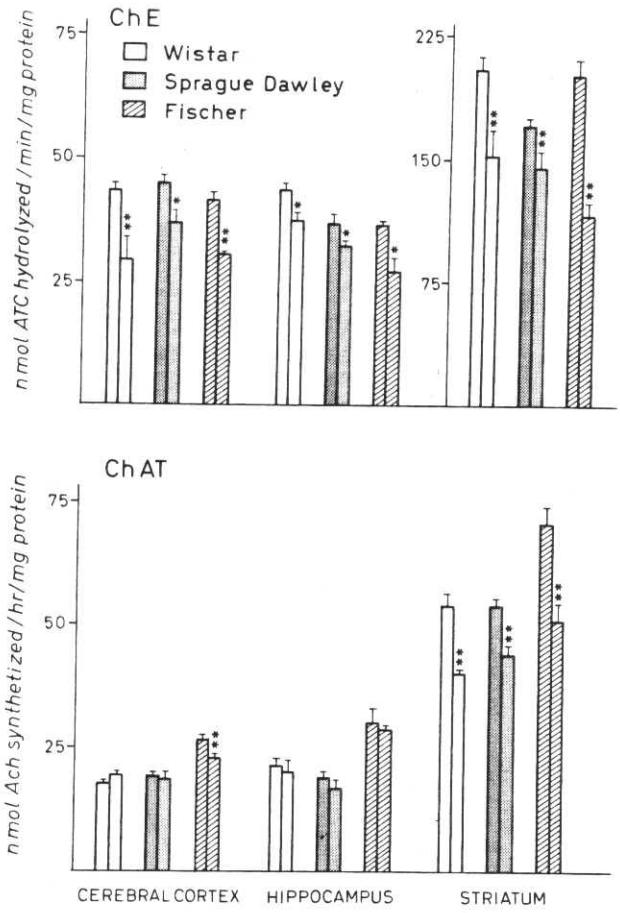

Fig. 1 – Age-related changes in cortical, hippocampal and striatal ChE and ChAT in Wistar, Sprague Dawley and Fischer 344 rats. The left and right columns of each pair represent values for 3- and 24-month old rats, respectively. Note the different scale used for ChE data in the striatum. Means ± SEM from six rats. Asterisks indicate significant age-related differences for each brain area (** $p < 0.01$, * $p < 0.05$) as assessed by Student's t-test.

Table 1. – *Age-related differences in mAChRs densities in brain regions of Wistar, Sprague-Dawley and Fischer 344 rats*

Brain Region	Age (months)	Bmax (fmol/mg protein) Wistar	Sprague Dawley	Fischer 344
Cerebral cortex	3	1207 ± 35	1173 ± 85	1970 ± 75
	24	1106 ± 6	1146 ± 33	1409 ± 36*
Hippocampus	3	1111 ± 76	1128 ± 37	1723 ± 20
	24	682 ± 55*	861 ± 21*	1326 ± 88*
Striatum	3	1292 ± 59	1260 ± 20	1874 ± 116
	24	856 ± 32*	908 ± 33*	1211 ± 72*

mAChRs were measured as specific ^{3}H-QNB binding. ^{3}H-QNB concentrations varied from 0.05 to 1.5 nM. Bmax and Kd were calculated from Scatchard analyses performed on six points with computer-fitted regression lines (correlation coefficients $r > 0.80$). Means ± SEM from six rats. Asterisks indicate significant differences from previous age (* $p < 0.01$), as assessed by Student's t-test. Kd varied from 0.18 to 0.28 nM and did not vary substantially according to brain area, strain, or age.

striatum of senescent Wistar, Sprague Dawley and Fischer rats (25, 18 and 28%, respectively).

The data on mAChRs are shown in Table 1. Bmax of ^{3}H-QNB binding were about 1.5 fold higher in Fischer 344 rats than in age-matched rats of the other two strains. As regards age-related changes in the cerebral cortex, there was a significant decline of mAChRs (by about 30%) in Fischer rats, while in Wistar and Sprague-Dawley rats the levels of mAChRs were not modified by aging. A significant decline of hippocampal and striatal mAChR densities was observed in the three strains (from 25 to 40%). The mAChRs affinities, measured as K_D, were similar in various brain areas and not substantially influenced by strain and age (data not reported).

Responsiveness of cortical ChE and mAChRs to a strong cholinergic stimulation by an indirect agonist, DFP, in young and senescent Fischer 344 rats

In the first week of treatment with sublethal doses of DFP both young and senescent rats exhibited a typical syndrome of central and peripheral cholinergic stimulation, lasting for many hours after each injection. Both the severity and the duration of the toxic syndrome appeared markedly more pronounced in senescent rats than in young ones, resulting in considerable loss of body weight and a high mortality rate (60 and 14%, respectively for the two age-groups). At the end of the treatment gross symptoms of cholinergic stimulation attenuated both in young and senescent rats. The effects of DFP on cortical ChE, ChAT and mAChRs are presented in Fig. 2. DFP induced a strong ChE inhibition both in young and senescent rats. In fact, no substantial differences were observed in the percentage inhibition of ChE due to DFP in the two age-groups. DFP treatment did not significantly modify the ChAT activity. Therefore the enzymatic activity in DFP treated young and senescent rats reflected the age-related differences.

As had to be expected, Bmax of mAChRs (^{3}H-QNB binding) in untreated animals declined significantly (by about 30%) with advancing age (compare Table 1). In young rats prolonged cholinergic stimulation caused a marked down-regulation of mAChRs. The senescent rats, in spite of age-related reduction of ^{3}H-QNB binding, showed an ulterior decrease of receptor density (without substantial changes in affinity) induced by the treatment. There were no differences in their percentage decrease between the two age-groups. This implies that the responsiveness of cortical mAChRs to an indirect cholinergic agonist, in spite of reduced levels, was well preserved in aged Fischer 344 rats.

Responsiveness of cortical ChE and mAChRs to a less strong cholinergic stimulation by DFP in young and senescent Sprague-Dawley rats.

At the beginning of the treatment the toxic syndrome appeared again considerably more pronoun-

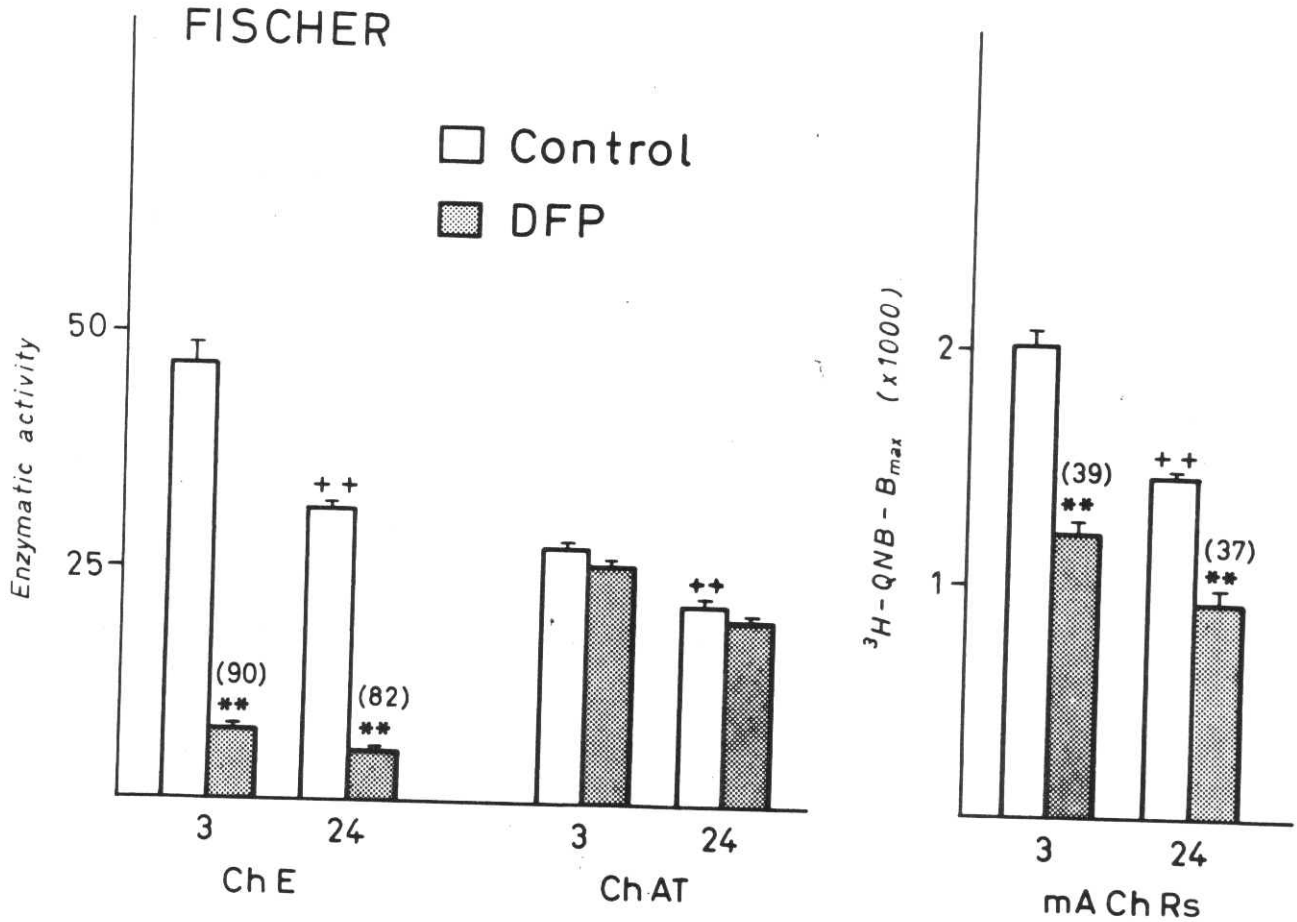

Fig. 2. – Effects of repeated administration of DFP (high dosage) on cortical ChE, ChAT and mAChRs of young and senescent Fischer 344 rats. Treatment on alternate days: DFP (in arachis oil) first s.c. dose 1.6 mg/kg, subsequently six doses of 1.1 mg/kg for 2 weeks. Rats were killed 48 h after the last injection. ChE activity is expressed in nmol of acetylcholine hydrolyzed/min/mg protein. ChAT activity is expressed in nmol of acetylcholine synthetized/h/mg protein. For mAChRs legend see Table 1. Numbers below the columns indicate age in months. Numbers in parentheses show percentage reduction of ChE or mAChRs of age-matched controls.
Means ± SEM from six rats. Asterisks indicate significant differences for age (++ $p<0.001$) and treatment (** $p<0.001$) as assessed by factorial analysis of variance (2 ages × 2 treatments ANOVA); degrees of freedom 1.20. ChE: F (age) 18.2; F (treatment) 314.0; F (age × treatment interaction) 7.4; ChAT: F (age) 54.9; F (treatment) 5.9; F (age × treatment interaction) 2.1; mAChRs: F (age) 62.6; F (treatment) 171.3; F (age × treatment interaction) 7.6; for $F>14.82$, $p<0.001$; for $14.82>F>8.10$, $p<0.01$. The data adopted from [6].

ced in senescent than in young rats. In order to avoid high mortality rate in the second week of DFP administration the doses were reduced by 50%. This stopped the loss of body weight and reduced mortality (40 and 14%, respectively, for senescent and young rats). The effects of DFP on cortical ChE, ChAT and mAChRs are presented in Fig. 3. The reduced dosage of DFP induced somewhat lesser inhibition of ChE, with no differences in the percentage inhibition between the two age-groups. Here again, DFP treatment did not modify ChAT activity.

The data confirmed the lack of age-related decline of mAChRs in the cerebral cortex in this strain of rats (Table 1). Prolonged cholinergic stimulation, on the other hand, caused a marked down-regulation of mAChRs both in young and senescent rats which was rather similar in the two age-groups.

With a view to ascertaining, whether the death of the animals during the treatment depended on the lack of down-regulation of cortical mAChRs, two moribund senescent rats were killed 48 h after the third DFP injection. The Bmax for cortical ^{3}H-QNB binding amounted to 625 and 544 fmol/mg protein. These values were similar to those observed in surviving senescent rats at the end of DFP treatment and considerably lower than those of age-matched controls (Fig. 3).

Discussion

Most previous investigations dealing with biochemical changes of cholinergic neurotransmission systems in aging were performed on rats of one particular strain, i.e. without comparing strains in any one experiment. In a recent paper from this laboratory such changes in Fischer 344 and Wistar rats were evaluated in parallel [9]. The present study extends the comparison to Sprague-Dawley rats, a frequently utilized strain in neurochemical studies.

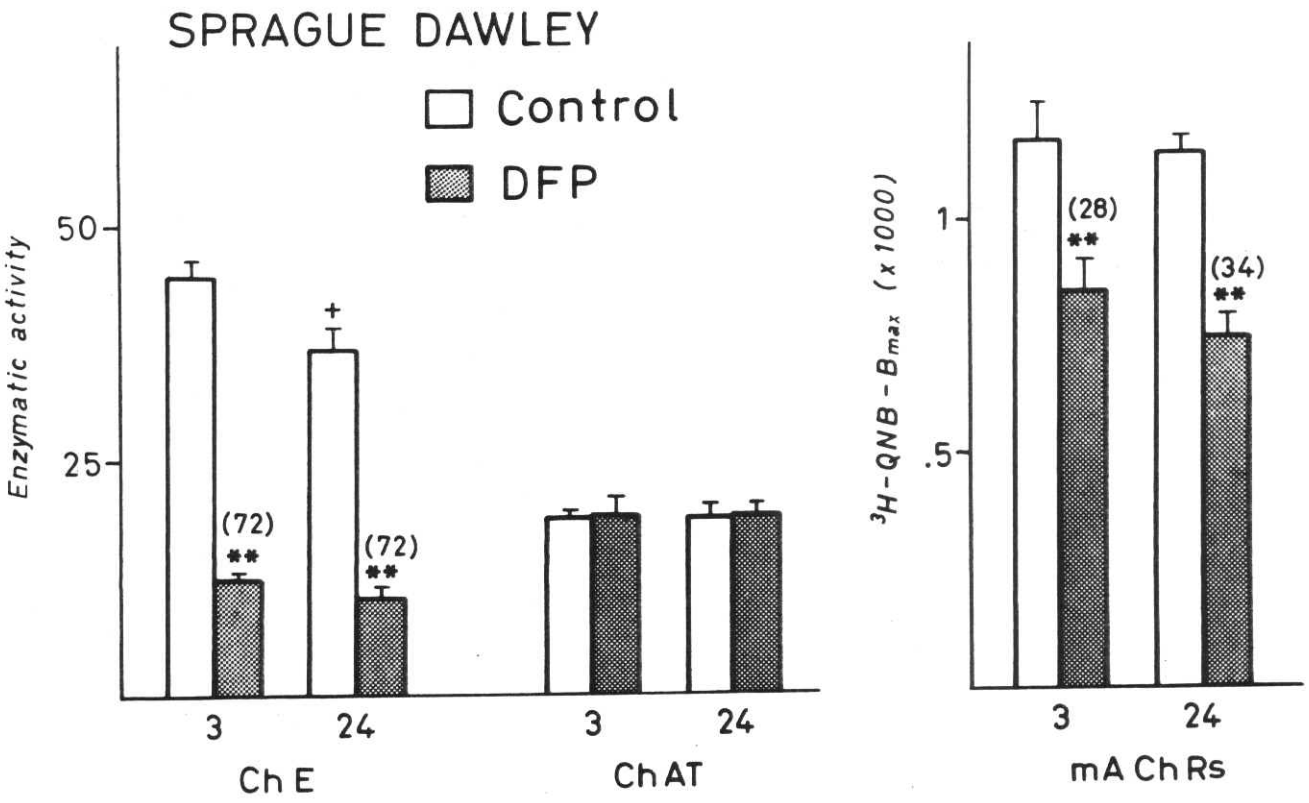

Fig. 3. – Effects of repeated administration of DFP (low dosage) on cortical ChE, ChAT and mAChRs of young and senescent Sprague-Dawley rats. Treatment on alternate days: DFP (in arachis oil) first s.c. dose 1.1 mg/kg, subsequently 2 doses of 0.7 mg/kg and 4 doses of 0.35 mg/kg for 2 weeks. Rats were killed 48 h after the last injection. For legend relative to ChE and ChAT see Fig. 2, for that of mAChRs see Table 1.

Means ± SEM from six rats. Asterisks indicate significant differences for age (+ $p < 0.01$) and treatment (** $p < 0.001$) as assessed by factorial analysis of variance (2 ages × 2 treatments ANOVA); degrees of freedom 1.20. ChE: F (age) 26.1; F (treatment) 109.1, F (age × treatment interaction) 12.9; ChAT: F (age) 0.4, F (treatment) 0.3; F (age × treatment interaction) 0.2; mAChRs: F (age) 13.5; F (treatment) 38.0; F (age × treatment interaction) 0.1.

The age-related changes are compared in rats having the same age, environment, a similar 50% survival rate (28-29 months), and using the same methods for preparation of biological material and neurochemical analyses. The data clearly indicate strain-dependent differences both for ChAT activity and mAChRs. In fact, Fischer 344 rats exhibit about 1.5 fold higher ChAT and mAChRs levels than Wistar and Sprague-Dawley rats in all brain areas analyzed. As concerns the age-related changes, the decline of cortical ChAT and mAChRs is present in Fischer 344, but not in Wistar or Sprague-Dawley rats, i.e. is strain-dependent. There are no age-related changes in hippocampal ChAT in the three strains of rats. The decline of striatal ChAT, hippocampal and striatal mAChRs and ChE in the three regions analyzed are present in Fischer, Wistar and Sprague-Dawley rats. Thus, these variations do not appear to be strain-dependent.

Most of the above age-related modifications (or lack of them) were also demonstrated by other authors working with Fischer, Wistar or Sprague-Dawley senescent rats of the same sex [4, 7, 16-19]. The few apparent discrepancies between the literature data and the present findings consist of a significant age-related decrease of cortical ChAT in Wistar [18] and cortical ChAT and mAChRs in Sprague-Dawley rats [20]. Available data suggest that Long Evans rats are more like Wistar (or Sprague-Dawley) than Fischer 344 rats, with no age-related deficit in cortical ChAT [21] and mAChRs [22]. This suggests that senescent Fischer 344 rats, with deficit of the cholinergic parameters measured in the three brain areas, exhibit an accelerated rate of aging.

This was an important reason for performing the first series of experiments on responsiveness to an indirect agonist, DFP, on senescent Fischer rats. Another reason was the recent observation indicating in this strain a lack of down-regulation of cortical mAChRs (measured also as Bmax of ^{3}H-QNB binding) following repeated intracerebroventricular administration of a direct cholinergic agonist, oxotremorine [4]. The data presented here show that, in spite of increased overall vulnerability of senescent rats to DFP, some of them activate compensatory mechanisms antagonizing the cholinergic stimulation and exhibit a marked down-regulation of

mAChRs, similar to that present in young animals. Therefore, our data are apparently discrepant with previous findings [4]. There may be several causes for the discrepancy including differences in the methodology of drug administration (intracerebroventricular and systemic), with consequent differences in the degree of cholinergic, also peripheral, stimulation producing 10 and 60% mortality. The discrepancy may also depend on the fact that oxotremorine is a direct and DFP an indirect cholinergic agonist, thus implying involvement of different receptor subtypes. Recent findings show that two muscarinic receptor populations (M1 and M2) are differently distributed at the cholinergic synapses in the cerebral cortex and have different mechanisms and functions [23]. It is very likely that the treatment with oxotremorine affects mainly the M2 receptors. These receptors account for about 40% of total in the rat frontoparietal cortex [23] and their partial loss might not be detected when all the muscarinic receptors are jointly determined. On the other hand DFP treatment results in a marked increase of acetylcholine, the endogenous agonist of both types of cholinergic receptors (M1 and M2). Thus, while the experiment with oxotremorine could involve just one of muscarinic receptor subtype, DFP treatment would affect both subtypes. The plausibility of this explanation is enhanced by the fact that in 3-month rats the decline in the Bmax of ^{3}H-QNB binding sites in the cerebral cortex in our experiments was greater (about 40%) than in rats treated with oxotremorine (about 27%). If this explanation is correct, it might be also hypothesized that M1 receptors retain the ability to down-regulate in senescence better than M_2 receptors.

The purpose of the second series of experiments with DFP was to assess whether the observed responsiveness of mAChRs in senescent rats was a more general phenomenon, i.e. present also in a strain which does not exhibit age-related decline of cortical mAChRs, and after weaker cholinergic stimulation.

The data show that aged Sprague-Dawley rats also exhibited considerable vulnerability and marked mortality even after weaker stimulation. The mortality (40%) was somewhat below that observed in aged Fischer rats (60%), but greater than that of young animals of the two strains (14%). The surviving Sprague-Dawley rats exhibited again persistent plasticity of mAChRs in terms of down-regulation. Thus in two series of experiments there was a great variability in the response of aged rats to DFP treatment, from total failure of adaptive mechanisms resulting in death to considerable cortical mAChRs plasticity. This plasticity was present even in the dying animals. This suggests that death depended on the failure of compensatory mechanisms pertaining to the peripheral rather, than the central, toxic effects of DFP.

Beside the plasticity of mAChRs, the present study also evaluated potential age-related differences in the response to DFP in terms of cortical ChE inhibition and ChAT activity. In the two series of experiments no differences were found in the ChE inhibition (independently of its degree) between young and senescent rats. This implies that there were no age-related alterations in the permeability of the blood-brain-barrier which could modify the access of DFP to the CNS. Similarly, no differences in ChAT activity due to DFP treatment were observed in the CNS of senescent Fischer and Sprague-Dawley rats. It is of interest that a marked increase of this enzymatic activity was detected in the peripheral nervous system (ileum strip) of the same senescent Sprague-Dawley rats [24].

In conclusion, the ability of central neurotransmitter systems to compensate for pathological or xenobiotic induced insult is an essential part of the aging process.

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