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Inhibitory effects of donepezil hydrochloride (E2020) on cholinesterase activity in brain and peripheral tissues of young and aged rats

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Abstract

Donepezil hydrochloride (donepezil: E2020: (±)-2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-indan-1-one monohydrochloride)) is a centrally acting acetylcholinesterase inhibitor developed for the treatment of Alzheimer's disease. In the present study, its inhibitory effect on the activity of cholinesterase ex vivo was evaluated in the brain, plasma, erythrocytes, heart, small intestine, liver and pectoral muscle of young adult as well as aged rats, in comparison with that of tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride). In aged animals, cholinesterase activity in heart, small intestine and pectoral muscle was lower, whereas that in plasma and liver was higher than in young rats. Both groups showed the highest levels in the brain. Donepezil, at doses of 1.25, 2.5 and 5 mg/kg, p.o., inhibited brain, plasma, erythrocyte, liver and pectoral muscle cholinesterase activity in young rats in a dose-dependent manner but had less effect on cholinesterase activity in heart and small intestine. In aged animals, inhibition of cholinesterase activity in the brain, erythrocytes and pectoral muscle by donepezil was more potent than that in young animals. Tacrine, at doses of 5, 10 and 20 mg/kg, p.o., dose-dependently inhibited cholinesterase activity in all tissues of both young and aged animals, but most potently in heart, small intestine and liver. The inhibition of cholinesterase activity by tacrine in the brain, plasma, erythrocytes, heart and liver was more potent in aged rats than in tissues of young rats. Brain and plasma concentrations of unchanged donepezil and tacrine were measured in the same animals as used for the cholinesterase inhibition study. Brain and plasma concentrations of donepezil and tacrine were higher in aged than in young animals. It is concluded that the inhibitory effects of donepezil and tacrine on cholinesterase activity are greater in aged than in young rats, owing to differences in the tissue concentrations of these compounds between young and aged animals. It is also suggested that the effect of donepezil on cholinesterase activity is more tissue-selective than that of tacrine. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Donepezil hydrochloride; E2020; Tacrine; Cholinesterase inhibitor; Brain; Peripheral tissue; (Rat, young); (Rat, Aged)

1. Introduction

Alzheimer's disease is an age-related and progressive neurodegenerative disease, characterized by deficits in memory and cognitive function. Remarkable dysfunction of the cholinergic system has been observed in several brain regions of patients suffering from Alzheimer's disease (Bowen et al., 1976; Davis and Maloney, 1976; Perry et al., 1977; Whitehouse et al., 1982), and was shown to be correlated with the severity of cognitive impairment (Perry et al., 1978). These pathological findings, in addition to the fact that the cholinergic system plays a role in memory functions (Drachman, 1977), have led to the hypothesis that enhancing cholinergic neurotransmission with cholinergic agents may ameliorate the cognitive impairment in Alzheimer's disease. Although many attempts have been made to reverse the cognitive impairment by using cholinergic agents, cholinesterase inhibitors are the only class of drugs currently approved for the treatment of Alzheimer's disease in Europe and by the US Food and Drug Administration (FDA).

We have developed a novel, piperidine-based, acetylcholinesterase inhibitor, donepezil hydrochloride (donepezil: E2020: (±)-2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-indan-1-one monohydrochloride)), for the treatment of Alzheimer's disease (Sugimoto et al., 1995). Donepezil was approved by the US FDA in 1996, and is now being prescribed worldwide. A large-scale multicenter, double-blind clinical study (Rogers and Friedhoff, 1998; Rogers et al., 1996, 1998a,b) has demonstrated that

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donepezil is a well-tolerated drug that improves cognitive performance and global function in patients with mild to moderate Alzheimer's disease. On the other hand, tacrine, which was the first drug approved by the US FDA for the treatment of Alzheimer's disease, has adverse effects related to its actions on the peripheral nervous system and to hepatic toxicity (Beermann, 1993). Since cholinesterase shows a widespread distribution, not only in the brain, but also in peripheral tissues, peripheral symptoms produced by inhibition of cholinesterase in several peripheral tissues are inevitable adverse effects of using non-selective cholinesterase inhibitors in the treatment of Alzheimer's disease. That is, a cholinesterase inhibitor which inhibits cholinesterase in the brain selectively in preference to cholinesterase in peripheral tissues is expected to be a superior drug for the treatment of Alzheimer's disease.

Pharmacological studies in vitro have revealed that donepezil is a reversible and non-competitive cholinesterase inhibitor, and is a far more selective inhibitor of acetylcholinesterase than of butyrylcholinesterase than is tacrine. It produces marked and long-lasting inhibition of brain cholinesterase without marked effects on cholinesterase in peripheral tissues and increases the brain content of acetylcholine in vivo. Moreover, in rats donepezil significantly ameliorates performance deficits in several learning and memory tasks including 8-arm radial maze impairments after scopolamine, and passive avoidance deficits produced by lesions of the nucleus basalis magnocellularis (Ogura et al., 1988; Yamanishi et al., 1988; Yamanishi et al., 1990; Rogers et al., 1991). However, these observations were made in young adult animals, and it is essential to investigate the pharmacological properties of this drug in aged animals as well, considering that this drug is administered to aged patients.

In the present study, we examined the inhibitory effect of donepezil on the activity of cholinesterase in the brain, blood (plasma and erythrocytes) and peripheral tissues (heart, small intestine, liver and pectoral muscle) of aged as well as young adult rats, in comparison with that of tacrine.

2. Materials and methods

2.1. Subjects

Young (8 weeks of age) and aged (26 months of age) male Fischer rats (Charles River Japan, Kanagawa, Japan) were used in the experiments. They were housed at a room temperature of 23 ± 1°C and relative humidity of 55 ± 10%, under a 12-h light/dark cycle (start at 7:00) for at least 1 week before experiments. All experiments were approved by the Animal Care and Use Committee of Eisai.

2.2. Drugs

Donepezil hydrochloride (E2020: (±)-2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-indan-1-one monohydrochloride) was supplied by Eisai Chemicals, (Ibaraki, Japan), and tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride) by Sigma (St. Louis, MO, USA). All other chemicals were commercial products of reagent grade. Donepezil and tacrine were dissolved in distilled water and administered by gavage.

2.3. Measurement of cholinesterase activity

The animals of each group were given donepezil (1.25, 2.5 and 5 mg/kg), tacrine (5, 10 and 20 mg/kg) or distilled water (control) orally. One hour after the administration of the test compounds, the animals were anesthetized by inhalation of a mixture of halothane (2%), nitrous oxide (70%) and oxygen (Balance). Blood was withdrawn and the whole brain and peripheral tissues (heart, small intestine, liver and pectoral muscle) were excised. The brain was split into two hemispheres for measurement of cholinesterase activity and drug concentration. Cholinesterase activity in plasma, erythrocytes and tissues was measured using the radiometric method of Thomsen et al. (1989) as modified by Sherman (1991). [^{3}H]acetylcholine iodide (New England Nuclear, Boston, MA, USA) was used as a substrate. In order to minimize the dilution effect which occurs in ex vivo assays of inhibition by reversible cholinesterase inhibitors, the tissues were homogenized and plasma and erythrocytes were diluted in 4 volumes of assay buffer, then finally 10 mg of each tissue or 10 μl of plasma or erythrocytes was diluted in 100 μl of assay solution.

2.4. Measurement of brain concentrations of cholinesterase inhibitors

Donepezil and tacrine concentrations in brain tissue and plasma were measured with a high-performance liquid chromatograph equipped with an ultraviolet spectrophotometer (HPLC-UV).

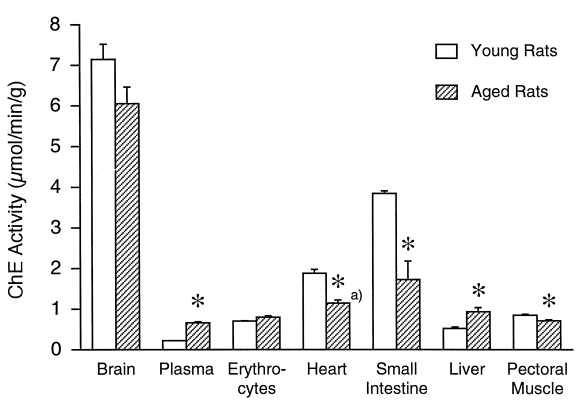

Fig. 1. Comparison of cholinesterase activity in the brain, plasma, erythrocytes, heart, small intestine, liver and pectoral muscle of young and aged rats. Values are means ± S.E. $n = 5$ (a) $n = 4$. $^*P < 0.05$ vs. the corresponding young rat group (Student's *t*-test).

2.5. Statistical analysis

The data were analyzed with Dunnett's multiple comparison test or Student's *t*-test. A *P* value of less than 0.05 was considered significant. Statistical analysis was conducted using the software package SAS ver.6.12® (SAS Institute Japan, Tokyo, Japan), available on the statistical analysis support system.

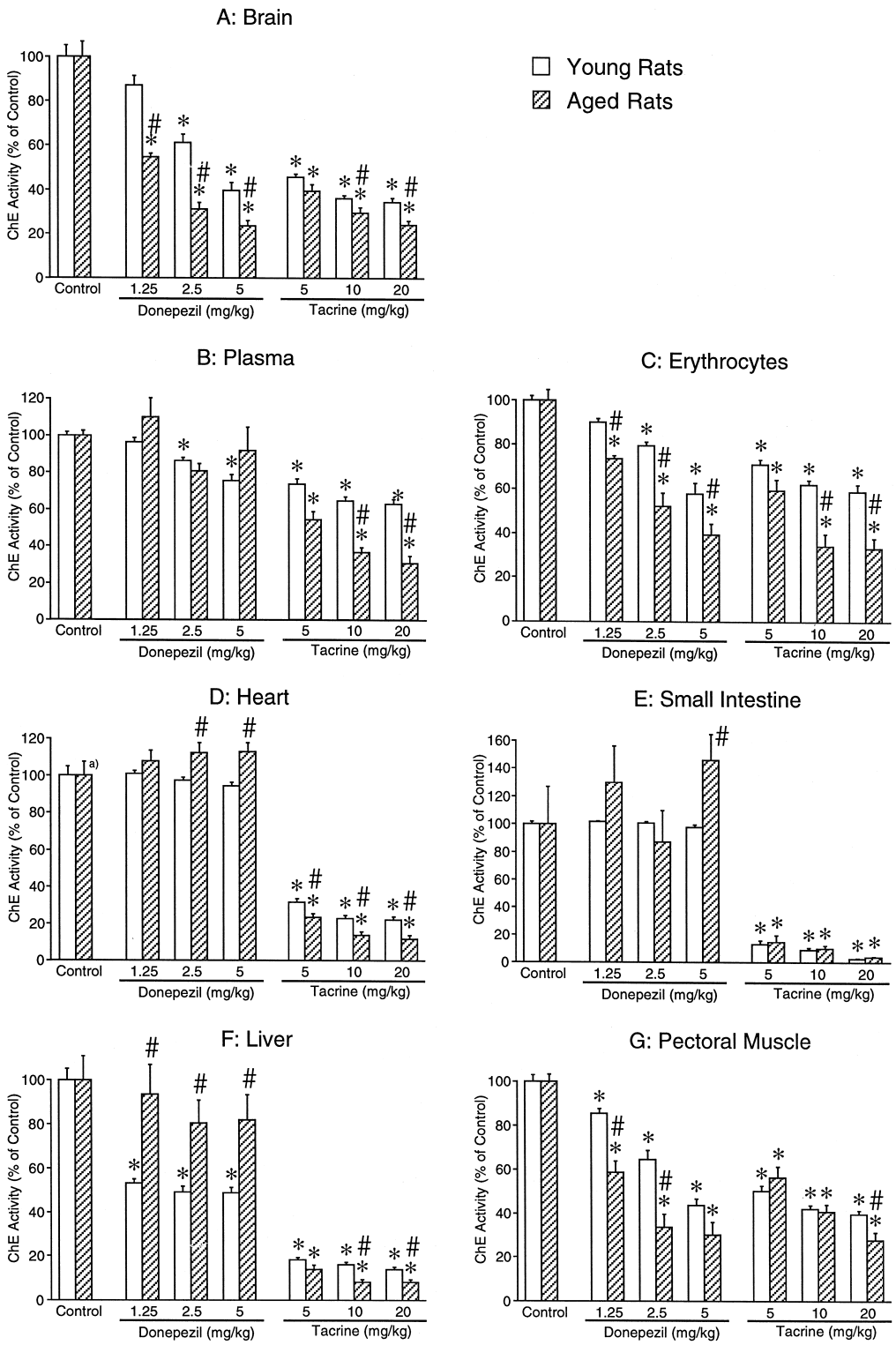

Fig. 2. Inhibitory effects of donepezil and tacrine on cholinesterase activity in the brain (A), plasma (B), erythrocytes (C), heart (D), small intestine (E), liver (F) and pectoral muscle (G) of young and aged rats. Values are means ± S.E. $n = 5$ (a) $n = 4$. $^{*}P < 0.05$ vs. respective control (Dunnett-type multiple comparison). $^{\#}P < 0.05$ vs. the corresponding young rat group (Student's *t*-test).

3. Results

3.1. Comparison of cholinesterase activities in the tissues of young and aged rats

Fig. 1 shows cholinesterase activity in the brain, plasma, erythrocytes, heart, small intestine, liver and pectoral muscle of young and aged rats. Cholinesterase activity in the brain was higher than that in blood or peripheral tissues. Among peripheral tissues, the small intestine had the highest activity followed by heart. Cholinesterase activity in plasma was the lowest among the samples examined. In aged animals, cholinesterase activity in heart, small intestine and pectoral muscle was lower, while that in plasma and liver was higher, than that of young rats.

3.2. Effects of donepezil and tacrine on cholinesterase activity in the tissues of young and aged rats

The effects of donepezil and tacrine on cholinesterase activity in the tissues of young and aged rats are shown in Fig. 2. Donepezil, at doses of 1.25, 2.5 and 5 mg/kg, inhibited brain, plasma, erythrocytes, liver and pectoral muscle cholinesterase activity in young rats. The inhibition was dose-dependent in the brain, plasma, erythrocytes and pectoral muscle, but plateaued in the liver. However, donepezil exerted no effect on cholinesterase activity in the heart and small intestine. In aged animals, inhibition of cholinesterase activity in brain, erythrocytes and pectoral muscle by donepezil was more potent than that in young animals. On the other hand, tacrine, at doses of 5, 10 and 20 mg/kg, dose-dependently inhibited cholinesterase activity in all tissues examined whether of young or aged animals, and the extent of the inhibition was greater in heart, small intestine and liver. The inhibition of cholinesterase activity by tacrine in all tissues examined, except for small intestine where cholinesterase activity was almost completely inhibited at all doses tested, was more potent in tissues of aged rats than of young rats.

3.3. Brain and plasma concentrations of donepezil and tacrine in young and aged rats

Brain and plasma concentrations of unchanged donepezil and tacrine are shown in Table 1. Both brain and plasma concentrations of donepezil and tacrine were higher in aged than in young animals at all doses tested.

4. Discussion

The inhibitory effects of donepezil and tacrine on cholinesterase activity in the brain and peripheral tissues were substantially more potent in aged than in young rats. In aged rats, the effect of donepezil was most potent in three tissues, brain, erythrocytes and pectoral muscle, of five tissues in which a significant inhibitory effect of the drug had been observed. In the case of tacrine, more potent inhibition of cholinesterase activity in aged than in young rats was observed in all tissues examined, except for small intestine where cholinesterase activity was almost completely inhibited at all doses tested. Pharmacokinetic data showed that concentrations of donepezil and tacrine in the brain and plasma were higher in aged than in young rats. This is considered to be due to the selective decrease in hepatic drug-metabolizing enzyme activity which is seen

Table 1
Brain and plasma concentrations of donepezil and tacrine in young and aged rats

		Brain concentration		Plasma concentration	
	Dose (mg/kg)	Mean ± S.E. (ng/g)	Ratio (A/Y)	Mean ± S.E. (ng/ml)	Ratio (A/Y)
Donepezil					
Young	1.25	45.9 ± 4.4	–	6.5 ± 0.6	–
	2.5	116.6 ± 9.5	–	15.7 ± 1.4	–
	5	281.5 ± 38.7	–	39.0 ± 6.0	–
Aged	1.25	151.1 ± 7.6[a]	3.3	16.6 ± 1.9[a]	2.6
	2.5	504.9 ± 115.8[a]	4.3	54.7 ± 13.7[a]	3.5
	5	777.5 ± 162.7[a]	2.8	94.4 ± 20.2[a]	2.4
Tacrine					
Young	5	944.0 ± 82.9	–	36.4 ± 6.4	–
	10	1367.7 ± 85.0	–	77.2 ± 8.0	–
	20	1471.4 ± 144.6	–	103.0 ± 19.6	–
Aged	5	1538.5 ± 243.8[a]	1.6	93.6 ± 22.0[a]	2.6
	10	2760.2 ± 398.7[a]	2.0	240.4 ± 35.1[a]	3.1
	20	4006.1 ± 549.4[a]	2.7	389.6 ± 76.3[a]	3.8

[a] $P < 0.05$ vs. the corresponding young group (Student's t-test). $n = 5$.
Ratio (A/Y): ratio of brain or plasma concentration of aged rats to that of the corresponding young rats.

exclusively in male rats (Kitagawa et al., 1985) and/or to an increase in the ratio of drug dose to liver weight in aged rats. These results suggest that the more potent inhibition of cholinesterase activity by these cholinesterase inhibitors in aged than in young rats does not result from pharmacodynamic changes, e.g., alteration of the nature of cholinesterase action as a function of aging, but from pharmacokinetic changes in aged rats.

An age-related decrease of cholinesterase has been reported for whole brain (Sastry et al., 1983) or for several brain regions of rats (Michalek et al., 1989; Pintor et al., 1990; Meneguz et al., 1992). The age-related change has been considered to consist in a selective loss of the enzymatic activity of the G4 (globular tetrameric) but not the G1 (monomeric) molecular form of acetylcholinesterase (Meneguz et al., 1992). The present finding that cholinesterase activity in the brain was lower in aged than in young rats, even though the difference was not statistically significant, may support those results. The present study demonstrates that donepezil inhibits cholinesterase activity in the brain in both young and aged rats even if an age-related decrease of cholinesterase activity in the brain occurs.

Donepezil did not inhibit plasma and liver cholinesterase activity in aged rats, although the effect was significant in young rats. This result was not consistent with the pharmacokinetic data. In plasma and liver, the cholinesterase activity of control aged animals was higher than that of control young animals. In our experiments, we measured total cholinesterase activity, which consists of acetylcholinesterase and butyrylcholinesterase activity. The fact that a selective acetylcholinesterase inhibitor, donepezil, did not inhibit cholinesterase of plasma and liver suggests that cholinesterase in plasma and liver of aged rats is mainly butyrylcholinesterase. Butyrylcholinesterase is predominantly synthesized in the liver and secreted into the plasma, supporting the idea that the increase in cholinesterase activity of these tissues in aged rats is due to an increase in butyrylcholinesterase activity. Thus, the disappearance of the effect of donepezil in these tissues of aged rats may result from the increase in the ratio of butyrylcholinesterase to acetylcholinesterase. Leeuwin and co-workers (Leeuwin, 1965; 1970; Leeuwin and Groenewoud, 1970) have reported that gonadal hormones play an inhibitory role in the regulation of liver and serum butyrylcholinesterase activity in male rats. Hormonal changes with aging may contribute to the increase in cholinesterase activity and the disappearance of the effect of donepezil in plasma and liver of aged male rats.

Differences in the intensity of the inhibitory effects of donepezil and tacrine on cholinesterase activity were observed among tissues in the present study. In young rats donepezil potently inhibited brain, erythrocyte, liver and pectoral muscle cholinesterase, and weakly inhibited plasma cholinesterase, but did not inhibit heart and small intestine cholinesterase. On the other hand, inhibition of cholinesterase by tacrine was potent in all regions examined, especially in heart, small intestine and liver. These results are in agreement with those of previous studies, in which young animals were used (Yamanishi et al., 1990; Rogers et al., 1991). It is considered that the difference in tissue selectivity between donepezil and tacrine depends on the difference in their selectivity for acetylcholinesterase, relative to butyrylcholinesterase, as suggested in these reports, since the brain/plasma penetration ratio of donepezil was somewhat lower than that of tacrine in the present study. Donepezil is a selective inhibitor of acetylcholinesterase, relative to butyrylcholinesterase, whereas tacrine is a non-selective inhibitor (Yamanishi et al., 1988; 1990; Rogers et al., 1991). Acetylcholinesterase is predominant in the nervous system, muscles and erythrocytes, where it is usually accompanied by a lower level of butyrylcholinesterase, and butyrylcholinesterase is expressed in other tissues (Edwards and Brimijoin, 1982). The activity ratio of butyrylcholinesterase to acetylcholinesterase is higher in heart (Michalek et al., 1983) and intestine (Ambache et al., 1971). Thus, the selective acetylcholinesterase inhibitor, donepezil, showed tissue selectivity as a consequence of its selective acetylcholinesterase inhibition, whereas the non-selective inhibitor, tacrine, inhibited cholinesterase activity in a broader range of tissues.

We anticipated that the preferential inhibition by donepezil of brain cholinesterase over heart and small intestine cholinesterase would be clinically advantageous from a safety point of view. Indeed, recent clinical studies with donepezil have demonstrated that donepezil is a well-tolerated drug, and no evidence of clinically significant adverse effects was observed (Rogers and Friedhoff, 1998; Rogers et al., 1996, 1998a,b). Donepezil had no hepatic toxicity, and side-effects owing to its cholinergic actions on the peripheral nervous system were transient and generally mild. Several researchers have indicated that donepezil is easier to use and causes fewer side-effects than tacrine (Barner and Gray, 1998; Giacobini, 1998; Tune and Sunderland, 1998; Whitehouse, 1998). The relatively low incidence and intensity of side-effects related to the actions of donepezil on the peripheral nervous system in clinical use may be due in part to the higher tissue selectivity for cholinesterase inhibition observed in our studies. However, these favorable characteristics are considered to depend mainly on the pharmacokinetic properties (Mihara et al., 1993; Ohnishi et al., 1993), which make it possible to maintain a stable plasma concentration of the drug in patients. It remains unclear whether the specificity of cholinesterase inhibitors for acetylcholinesterase over butyrylcholinesterase, which is the reason for the tissue-specific cholinesterase inhibition by donepezil, is relevant to the treatment of Alzheimer's disease, since the physiological role of butyrylcholinesterase is not yet established (Brufani et al., 1997; Giacobini, 1997). Nevertheless, we can say that a cholinesterase inhibitor that has a weaker

effect on tissues other than the target tissue, brain, is less likely to produce unpredictable adverse events related to its action.

In summary and conclusion, the findings of this study demonstrated that the inhibitory effects of donepezil and tacrine on cholinesterase were more marked in aged than in young rats, in agreement with the finding that the brain and plasma concentrations of these compounds are higher in aged than in young rats. It is further concluded that donepezil exhibits a more potent inhibitory activity on cholinesterase in the brain and pectoral muscle than in other peripheral tissues in both young and aged rats, whereas potent inhibition of cholinesterase by tacrine is seen in heart, small intestine and liver. These in vivo characteristics of cholinesterase inhibition suggest that donepezil may be a useful drug for the treatment of Alzheimer's disease.

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