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Effect of Chronic Treatment with Piracetam and Tacrine on Some Changes Caused by Thymectomy in the Rat Brain

CAI SONG,[1] BERNADETTE EARLEY[2] AND B. E. LEONARD

Department of Pharmacology, University College Galway, Galway, Ireland

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SONG, C., B. EARLEY AND B. E. LEONARD. *Effect of chronic treatment with piracetam and tacrine on some changes caused by thymectomy in the rat brain.* PHARMACOL BIOCHEM BEHAV **56**(4) 697–704, 1997.—Thymectomized rats, 5 weeks after surgery, showed a significant impairment in learning and memory as shown by deficits in passive avoidance and in the Morris water maze test. The behaviour of the thymectomized rats in the "open field" apparatus was largely unchanged. Following treatment for 20 days with either piracetam (500 mg/kg) or tacrine (3.0 mg/kg), the deficit in passive avoidance learning was largely reversed. Chronic treatment with tacrine also reversed the deficit in the behaviour of the thymectomised rats in the Morris water maze. The effects of thymectomy on the biogenic amines and some of their metabolites in the amygdaloid cortex, hypothalamus, striatum, and olfactory bulbs were also determined. Relative to the sham-operated controls, thymectomy resulted in a reduction in the noradrenaline concentration in the amygdala, hypothalamus, and olfactory bulbs. This effect was reversed by chronic piracetam and tacrine treatments. The concentration of dopamine was also reduced in the olfactory bulbs after thymectomy, whereas in the striatum the concentration of 5-hydroxytryptamine (5-HT; serotonin) was increased. The concentration of gamma amino butyric acid (GABA) was determined in amygdaloid cortex and hippocampus only. The only significant change occurred following chronic treatment of thymectomized rats with tacrine, when a significant elevation of GABA was found. Neither piracetam nor tacrine produced any change in the amines of their metabolites in the sham-operated controls. Tacrine, however, elevated the dopamine and reduced the 5-HT content of the hypothalamus and increased the 3,4-dihydroxylphenylacetic acid concentration of the striatum of thymectomized rats. Examination of the differential white blood cell count of the thymectomized rats showed that the percentage of lymphocytes was decreased, and the percentage of neutrophils increased, relative to the sham-operated controls. Chronic tacrine, but not piracetam, treatment reversed the lesion-induced changes. © 1997 Elsevier Science Inc.

Thymectomy Biogenic amines Memory Piracetam Tacrine

THE INTERACTION between the central nervous system (CNS) and the immune system is well established (11,17,34). The thymus gland is generally considered to be one of the most important lymphoid organs in the modulation of neuroendocrine and immune systems in ageing (20,69). In both human and rat, noradrenergic and cholinergic nerves have been found to innervate the cortex and medulla of the thymus gland (30,32,42). In addition, the thymus gland produces cytokines and other peptides that affect the differentiation, growth, and ageing of neurons in the CNS (16,35,69). Immune, neuroendocrine, and somatic mutation theories have been proposed to account for the changes associated with ageing (5). The immune theory of ageing hypothesizes that the functional activity of the immune system decreases with age, the greatest changes being in thymus-dependent immune function (25,63). These changes include a reduction in lymphocyte proliferation and a defect in cytokine release (45,65). In addition, a number of studies have reported that the concentrations of monoamine metabolites and the activity of acetylcholinesterase are increased, whereas concentrations of noradrenaline (NA), acetylcholine, and dopamine (DA) are decreased in the ageing brain (21,36,50); the concentration of serotonin (5-HT) is increased or unchanged (21,60).

There is evidence to suggest that ageing is associated with an impairment in learning and memory, which is proposed to result from changes in the concentrations of specific neurotransmitters (27,65). In 1991, Song and Bao (56) reported that changes in the concentrations of neurotransmitters and their

[1] Requests for reprints should be addressed to: Cai Song at her present address: Life Sciences Research Centre, 1125 Colonel By Drive, Carleton University, Ottawa, Ontario K1S 5B6, Canada.
[2] Present address: Teagasc, Grange Research Centre, Dunsany, County Meath, Ireland.

metabolites after thymectomy are similar to those occurring in the ageing brain (45). Other investigators have reported that thymectomised mice may be considered to be a model of accelerated ageing for immunopharmacological studies (9). However, little is known about the role of the thymus in learning and memory. Therefore, the present study was undertaken to investigate the relationship between changes in behaviour and central neurotransmitters following thymec-tomy. Possible changes in cellular immunity were assessed by means of the differential white blood cell (WBC) count. In addition, changes after chronic treatment with the anticholinesterase tacrine and the nootropic agent piracetam were also investigated.

MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats (200–230 g) were obtained from Harlan Olac (Bicester, Oxon, UK). The animals were housed four per cage (40 × 28 × 20 cm) on a 12 L:12 D cycle and at a room tem-perature of 21 ± 1°C. Water and food were available ad lib. Piracetam was obtained from UCB Beerce (Belgium) and tacrine from the Aldrich Chemical Co. (London, UK). Both drugs were dissolved in physiological saline and administered for 20 days. Doses of the drugs were selected according to published literature: piracetam was injected at a dose of 500 mg/kg (33,47) and tacrine at a dose of 3 mg/kg (48).

Surgical Methods

Thymectomy was performed under tribromoethanol (250 mg/kg IP) anaesthesia (61) using the surgical method described by Waynfoth and Flecknell (64). Briefly, the anaesthetised rat was placed on its back with the head toward the investigator. A midline incision was made in the skin from the base of the neck posteriorly over the thorax, and the connective tissue between the two submandibular salivary glands was separated. After opening the sternohyoid muscle, the trachea and thymus gland were exposed and the thymus gland carefully removed. During the procedure, care was taken to prevent a pneumothorax and damage to the carotid artery. Sham-operated rats were treated in the same way, but the thymus gland was not removed. After surgery, the animals were treated with penicillin (50,000 I/rat) daily for 5 days and were allowed to recover for 14 days.

Procedures

Two separate experiments were undertaken. In the first experiment, the passive avoidance and "open field" tests were performed. For these tests, the animals were assigned to six groups of 10 as follows: a) sham-operated rats injected with saline, b) sham-operated rats injected with piracetam (500 mg/kg IP), c) sham-operated rats injected with tacrine (3.0 mg/kg IP), d) thymectomised rats injected with saline, e) thymectomised rats injected with piracetam (500 mg/kg IP), and f) thymectomised rats injected with tacrine (3.0 mg/kg IP). After the recovery period, the animals were treated with the two drugs for 20 days. On day 15 of drug administration, each rat was placed singly in the "open field." On day 16, the rats were subjected to a one-trial passive avoidance test that lasted 3 days. Finally, on day 21, the animals were sacrificed and the concentrations of biogenic amines and some of their metabolites were determined by high performance liquid chromatography (HPLC). Trunk blood was collected for corticosterone assay and a blood smear for a differential WBC count.

In a second experiment, the Morris water maze test was performed. For this experiment, the animals were divided into four groups of nine as follows: a) sham-operated rats treated with saline, b) sham-operated rats treated with tacrine (3 mg/kg IP), c) thymectomised rats treated with saline, and d) thymectomised rats treated with tacrine (3 mg/kg IP). After 14 days of recovery, the animals were treated with saline or tacrine for 20 days. On day 15 of tacrine administration, rats were singly subjected to the Morris water maze; the test lasted 5 days. Brain and blood samples were obtained between 0900 and 1100 h. After sacrifice, analyses were conducted only on those experimental animals from which the thymus gland had been completely removed.

Behavioural Tests

The "open field" apparatus and experimental procedure were as described by Gray and Laljee (26). In brief, the rats were placed singly in the centre of a brightly illuminated open field apparatus. A 60-W bulb was positioned 90 cm above the white floor of the open field; the base of the open field was divided into 60 squares of 10 $cm.^2$ Ambulation, rearing, grooming, and defaecation scores were recorded in a 3-min period of observation.

The passive avoidance apparatus consisted of a box divided by a wall into two compartments of equal size (24 × 16 × 20 cm). The front white chamber, illuminated from above by a 40-W incandescent light bulb 30 cm above the floor, was connected to the rear dark chamber, which was equipped with a grid floor; the two chambers were separated by a guillotine door (13). The rats were subjected to a single adaptation trial (T1) 24 h prior to the acquisition trials (learning trials; T2). The adaptation trial consisted of placing the rat in the brightly lit compartment, opening the guillotine door, timing the latency to enter the dark compartment, leaving the rat in the dark compartment for 60 s, and then removing the animal from the apparatus. The acquisition trial was performed in the same manner, except that as soon as the rat had moved into the dark compartment, the guillotine door was shut and the rat was subjected to an unavoidable 1-mA foot shock of 3 s duration. After 24 h, the retention test (T3) consisted of placing the rat in the brightly lit compartment; the latency to enter the dark compartment was recorded during a 5-min period of observation.

The Morris water maze test was carried out as described (40). On day 12, rats were placed in a pool of water (1 m diameter, 80 cm high, at 26 ± 1°C) and allowed to swim freely for 1 min with no opportunity for escape. On day 2, a platform (10 cm in diameter) was positioned in one of the quadrants of the maze, and the latency to locate the platform was recorded. Each rat was subjected to five trials per day with an intertrial interval of 5 min in five different starting positions for each trial. On day 3, the platform was in the same position and the test was the same as on day 2. On day 4, the platform was repositioned to another quadrant of the maze and the latency to locate the platform was recorded over five trials. On day 5, the position of the platform and the test were the same as on day 4.

Differential White Cell Count

Blood-smeared slides were stained with Wright's stain. A WBC count was performed on each sample, using standard hospital procedures, by means of a microscope (58).

Brain Dissection and Neurotransmitter Assay

Following decapitation, the brains were rapidly removed. Five brain regions (hypothalamus, amygdala, olfactory bulbs, striatum, and hippocampus) were dissected on ice and weighed. HPLC with electrochemical detection was used for the estimation of NA, DA, 5-HT, and their metabolites (51).

Concentrations of gamma amino butyric acid (GABA) in the amygdala and hippocampus were measured by the method described by Earley and Leonard (14). Results are expressed as nanograms (for monoamine neurotransmitters) and micrograms (for GABA) per gram fresh weight, mean ± SEM.

Assay of Corticosterone Concentrations

Serum corticosterone levels were measured by a modification of the method of Glick et al. (23). Briefly, 100 μl of serum sample was mixed with 600 μl of chloroform for 15 s. Next, 500 μl of the chloroform extraction phase was transferred into a tube containing 400 μl of ethanol:sulphuric acid and mixed for 15 s. Samples were then placed in the dark for 45 min. A 300-μl aliquot from the lower acid phase was removed, and fluorescence was measured in a spectrophotofluorimeter at an excitation wavelength 474 nm and an emission wavelength 518 nm. A standard curve was prepared using corticosterone concentrations ranging from 10 to 80 μg/dl. Results are expressed as micrograms of corticosterone per deciliter of serum.

Statistical Analysis

For the behavioural results, a Kruskal–Wallis nonparametric one-way analysis of variance (ANOVA), followed by a two-tailed Mann–Whitney *U*-test, was used (54,66). For the differential WBC count and the neurotransmitter determinations, a one-way (ANOVA) was used, followed by an a posteriori least significant difference (LD) multiple comparison procedure. The results for the biochemical studies and the differential WBC counts are expressed as mean ± SEM. The results for the behavioural studies are expressed as median ± median deviation. The significance level for differences between groups was set at $p < 0.05$ for both statistics.

Ethical Approval

All experiments were approved by the Committee for Biological Procedures of the University and in strict accordance with the Cruelty to Animals Act of the State.

RESULTS

Behavioural Tests

In the open field test, neither thymectomy nor piracetam nor tacrine significantly affected the ambulation, grooming, or defaecation scores. Thymectomy caused a slight increase in the rearing score (sham control 5 ± 2, thymectomy 18 ± 3), which was reduced by both piracetam (2 ± 2) and tacrine (2.1 ± 2).

In the passive avoidance test, thymectomised and sham-operated animals did not show any difference on days T1 and T2. However, on day T3, the latency to enter the dark compartment for the thymectomised rats was significantly shorter than that for sham-operated animals ($U = 15.37$, df = 5, $p < 0.05$). Chronic administration of piracetam ($U = 19.63$, df = 5, $p < 0.01$) and tacrine ($U = 14.78$, df = 5, $p < 0.05$) markedly prolonged the latency time of thymectomised rats (Fig. 1).

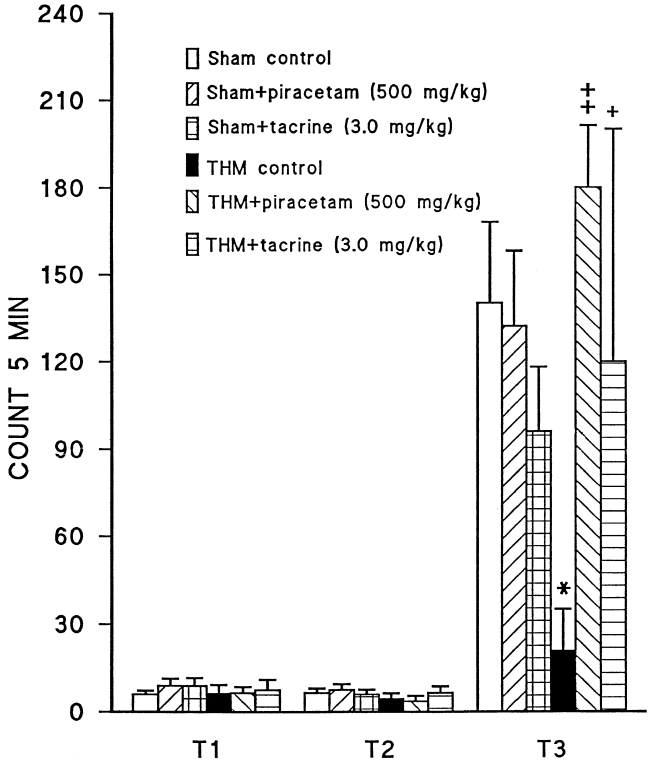

FIG. 1. Passive avoidance performance in sham-operated control and thymectomised (THM) rats after chronic treatment with piracetam and tacrine for 20 days. Results are expressed as median ± median deviation, $n = 10$. *$p < 0.05$ vs. sham control; +$p < 0.05$ vs. thymectomised control; ++$p < 0.01$ vs. thymectomised control.

Neither drug significantly affected the behaviour of the sham-operated rats.

Thymectomised rats also showed a significant learning impairment in the Morris water maze ($U = 16.43$–17.52, df = 3, $p < 0.05$), but the increase in escape latency on days 2 and 3 was largely reduced by tacrine treatment $U = 16.34$–16.75, df = 3, $p < 0.05$) (Fig. 2). Tacrine had no apparent effect on the behaviour of the sham-operated rats in the Morris water maze.

Changes in the Concentrations of Neurotransmitters

In the amygdala of thymectomised rats, the concentration of NA was significantly reduced relative to the sham-operated controls [$F(5, 60) = 2.81$, $p < 0.025$; Table 1]. Both piracetam and tacrine treatment significantly elevated NA concentrations relative to the thymectomised controls [$F(5, 60) = 2.37$, $p < 0.05$ and $F(5, 58) = p < 0.025$, respectively; Table 1].

In the hypothalamus of untreated thymectomised rats, the decrease in the NA concentrations nearly reached significance ($p = 0.06$). After piracetam administration, the concentration of NA was significantly elevated [$F(5, 58) = 2.38$, $p < 0.05$] and the increase in DA concentration nearly reached significance ($p = 0.06$). Tacrine treatment markedly increased the DA [$F(5, 60) = 2.70$, $p < 0.05$] and reduced the 5-HT [$F(5, 58) = 2.74$, $p < 0.05$] concentrations in the hypothalamus of treated thymectomised rats (Table 2).

A nonsignificant decrease in the concentrations of NA, DA, and 3,4-dihydroxylphenylacetic acid (DOPAC) and a

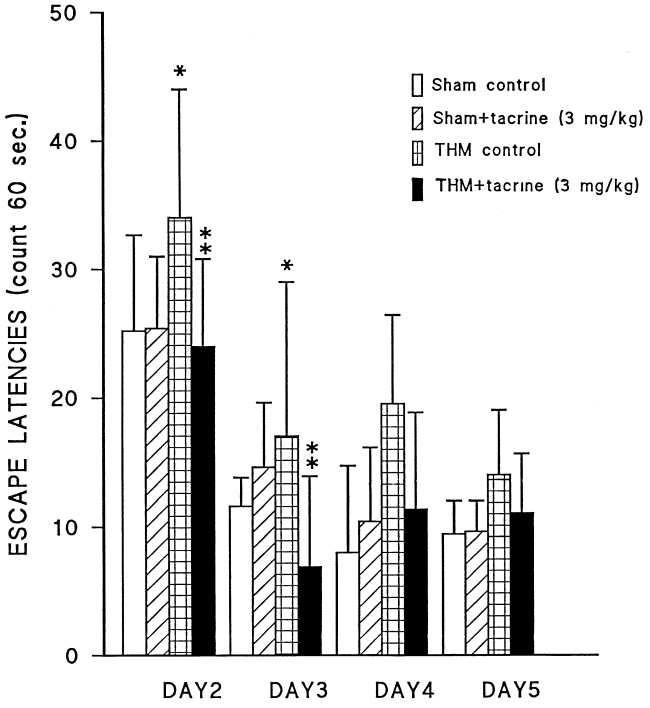

FIG. 2. Behaviour of sham-operated control and thymectomised (THM) rats in the Morris water maze after chronic treatment with tacrine for 20 days. Results are expressed as median ± median deviation, $n = 9$. *$p < 0.05$ vs. sham controls; **$p < 0.05$ vs. THM controls.

slight increase in the 5-HT concentration [$F(5, 58) = 2.31$, $p < 0.057$] were found in the striatum of thymectomised rats (Table 3). After piracetam administration the NA content was slightly increased [$F(5, 58) = 2.33$, $p < 0.058$], and tacrine treatment significantly increased the DOPAC concentration [$F(5, 58) = 2.47$, $p < 0.05$].

Table 4 shows that there was a significant decrease in the concentrations of NA [$F(5, 58) = 3.39$, $p < 0.025$] and DA [$F(5, 58) = 2.36$, $p < 0.05$] in the olfactory bulb following thymectomy. Tacrine treatment significantly increased the NA concentration [$F(5, 58) = 2.64$, $p < 0.05$; Table 4].

After thymectomy, the concentration of GABA was unchanged in the amygdala and hippocampus. However, chronic treatment with tacrine significantly raised the GABA concentration in the hippocampus of the thymectomised rat [$F(5, 56) = 3.57$, $p < 0.01$; Table 5].

Neither piracetam nor tacrine treatment significantly changed the amine concentrations of the sham-operated rats in any of the brain regions studied.

Immunological Changes

After thymectomy, the percentage of lymphocytes was markedly decreased [$F(5, 60) = 3.55$, $p < 0.01$] and the percentage of neutrophils was increased [$F(5, 60) = 3.47$, $p < 0.01$; Table 6]. Piracetam treatment reduced the percentage of lymphocytes and increased neutrophils in the sham-operated rats [$F(5, 60) = 2.62$, $p < 0.05$]. In contrast, after tacrine administration, the percentage of lymphocytes was increased and the percentage of neutrophils was decreased in the thymectomised rats [$F(5, 60) = 3.62$, $p < 0.01$; Table 6].

Corticosterone Concentrations

There was no significant change in the concentration of corticosterone following thymectomy or drug treatment. The concentration of corticosterone in the serum of sham-operated rats was 18.39 ± 3.03 μg/dl, in sham-operated rats injected with piracetam was 15.04 ± 1.34, and in those injected with tacrine was 22.43 ± 2.32. The corticosterone concentration in thymectomised rats was 18.71 ± 3.72, in thymectomised rats treated with piracetam was 13.76 ± 1.33, and in thymectomised rats treated with tacrine was 15.42 ± 1.82 μg/dl. None of these changes was statistically significant.

DISCUSSION

It is apparent from these studies that thymectomy results in significant changes in memory and learning, as indicated by the deficits in both the passive avoidance and the Morris water maze tests. However, when the rats were subjected to the stressful novel environment of the "open field," the only difference between the thymectomised animals and their sham-operated controls was an increase in the rearing scores. Because there was no evidence of an increase in the serum corticosterone concentration in the thymectomised rats following exposure to the open field, it seems unlikely that this increase in the rearing score is a reflection of an acute stress response.

Ageing is generally accompanied by an impairment of learning and memory (1,46,53). Several rodent models of ageing, such as methylazoxymethanol-treated rats and aged rodents, are known to show impairments of learning and memory in the passive avoidance and Morris water maze tests (20,31) that qualitatively resemble the changes seen following thymectomy. It may be hypothesised that thymectomy also pro-

TABLE 1

EFFECTS OF CHRONIC TREATMENT WITH PIRACETAM AND TACRINE FOR 20 DAYS ON THE LEVELS OF BIOGENIC AMINES AND RELATED COMPOUNDS IN THE AMYGDALA OF SHAM-OPERATED AND THYMECTOMISED (THM) RATS

Biogenic Amine	Sham Control	Sham + Piracetam (500 mg/kg)	Sham + Tacrine (3.0 mg/kg)	THM Control	THM + Piracetam (500 mg/kg)	THM + Tacrine (3.0 mg/kg)
VMA	399.28 ± 58.16	329.95 ± 22.25	443.97 ± 45.20	281.63 ± 38.34	298.68 ± 10.17	416.51 ± 47.22
NA	1601.33 ± 45.49	1598.46 ± 92.69	1720.89 ± 102.24	1349.69 ± 71.60*	1531.23 ± 48.64†	1636.00 ± 69.20†
DA	693.15 ± 132.43	692.40 ± 188.78	544.78 ± 53.37	622.98 ± 130.02	622.98 ± 130.02	470.36 ± 65.82
5-HIAA	1662.11 ± 132.43	1292.40 ± 188.79	1437.76 ± 85.94	1845.35 ± 423.93	1314.56 ± 89.51	1275.55 ± 88.90
5-HT	1769.48 ± 123.62	1586.84 ± 126.99	1844.39 ± 100.75	1664.33 ± 92.24	1725.55 ± 118.71	1568.75 ± 93.61

Results are expressed as mean ± SEM, ng per g fresh weight, $n = 10$. NA, noradrenaline; DA, dopamine; 5-HIAA, 5-hydroxy indole acetic acid; 5-HT, serontonin. *$p < 0.05$ vs. sham control (NA); †$p < 0.05$ vs. THM control (NA).

TABLE 2

EFFECTS OF CHRONIC TREATMENT WITH PIRACETAM AND TACRINE FOR 20 DAYS ON THE LEVELS OF BIOGENIC AMINES AND RELATED COMPOUNDS IN THE HYPOTHALAMUS OF SHAM-OPERATED AND THYMECTOMISED (THM) RATS

Biogenic Amine	Sham Control	Sham + Piracetam (500 mg/kg)	Sham + Tacrine (3.0 mg/kg)	THM Control	THM + Piracetam (500 mg/kg)	THM + Tacrine (3.0 mg/kg)
NA	7334.51 ± 177.01	6658.03 ± 549.55	7334.51 ± 7478.08	6377.57 ± 422.79*	7567.16 ± 255.35†	7071.64 ± 277.29
DA	1080.77 ± 55.44	1132.03 ± 122.61	1207.20 ± 128.42	959.10 ± 65.84	1162.65 ± 77.94	1261.88 ± 103.38‡
5-HIAA	1148.01 ± 53.13	1111.34 ± 107.07	1449.48 ± 413.91	1239.13 ± 161.18	1160.55 ± 39.54	1045.81 ± 52.51
5-HT	1920.75 ± 155.93	2119.16 ± 213.35	1860.10 ± 116.73	2237.31 ± 150.06	2156.14 ± 60.39	1827.03 ± 82.68§

Results are expressed as mean ± SEM, ng per g fresh weight, $n = 10$. Abbreviations for amines as in Table 1. *$p < 0.06$ vs. sham control (NA); †$p < 0.05$ vs. THM control (NA); ‡$p < 0.05$ vs. THM control (DA); §$p < 0.05$ vs. THM control (5-HT).

duces changes that are predictive of premature ageing in rodents. It is known that the thymus gland is one of the first organs to age (4) and that the ageing of other organs may occur as a consequence of decreased secretion of cytokines and thymus peptides (68). In the brain, these immune modulators are known to affect neuronal differentiation and growth such that a premature deficit in these factors may cause early neuronal degeneration (16,35,49). It has been observed that castration can reverse the age associated changes in the thymus of experimental animals (29) although the mechanism whereby this occurs is unclear. In humans who have undergone thymectomy or have a thymoma, adaptation to stress is impaired and emotional lability (as shown by agitation, fear, and anger) is a frequent feature (2,4,37,41). Thus, it may be hypothesised that the behavioural changes seen in the thymectomised rats are partly a reflection of maladaption due to premature neuronal ageing.

In previous studies in which the neurotoxicant trimethyltin was used to produce hippocampal damage leading to an impairment of passive avoidance and Morris maze behaviour (12), we have shown that the plasma corticosterone concentration is also significantly raised. However, in the present study, the plasma corticosterone concentration was unchanged despite the behavioural deficits that occurred following thymectomy. It is known that thymulin stimulates ACTH secretion and thereby increases adrenal glucocorticoid secretion (24,39), whereas another thymus peptide, thymosin, has the opposite effect on ACTH and steroid secretion (10). Thus, total removal of the thymus, as occurred in the present study, would be expected to reduce the activity of the pituitary–adrenal axis and thereby attenuate the ACTH-induced stress response. This could account for the lack of change in the corticosterone concentration even when the thymectomised rats were subject to a stressful environment, which would be expected to increase corticotropin releasing factor release and to produce the increased rearing behaviour observed in the open field apparatus (52,57). It is also of interest that no significant change in the plasma ACTH concentration has been observed in humans following thymectomy (10).

Several investigations have demonstrated that thymectomy causes a defect in aspects of immune function (79,44). Such changes include a decrease in mitogen-stimulated lymphocyte proliferation and suppression of lymphocyte production by the bone marrow (38). In the present study, thymectomy was found to reduce the percentage of lymphocytes and increase the percentage of neutrophils without affecting the percentages of monocytes and eosinophils. Clearly, such changes are indicative of a significant change in cellular immunity after thymectomy. The extent of these changes, and the possible mechanisms whereby they occur, will be the subject of further investigations.

The most significant change in brain biogenic amine concentrations that occurred following thymectomy was the decrease in the concentration of noradrenaline, which was significantly reduced in the amygdaloid cortex, hypothalamus, and olfactory lobes. Apart from a rise in the concentration of 5-HT in the striatum, and a fall in the dopamine concentration in the olfactory lobes, no other significant changes in the amines or their metabolites occurred in the brain regions that were analysed after thymectomy. Clearly, the discrete changes in brain amines following thymectomy do not compare with the robust changes in these transmitters that have been reported to occur in the ageing rodent brain (6,21,50). Nevertheless, the change in the NA concentration after thymectomy may be of biological significance in explaining some of the observed behavioural changes. It is known that NA regulates

TABLE 3

EFFECTS OF CHRONIC TREATMENT WITH PIRACETAM AND TACRINE FOR 20 DAYS ON THE LEVELS OF BIOGENIC AMINES AND RELATED COMPOUNDS IN THE STRIATUM OF SHAM-OPERATED AND THYMECTOMISED (THM) RATS

Biogenic Amine	Sham Control	Sham + Piracetam (500 mg/kg)	Sham + Tacrine (3.0 mg/kg)	THM Control	THM + Piracetam (500 mg/kg)	THM + Tacrine (3.0 mg/kg)
NA	198.92 ± 77.15	259.28 ± 84.76	323.71 ± 58.57	127.70 ± 38.44	293.70 ± 83.24*	169.08 ± 47.51
DA	36,638.98 ± 3376.36	32,896.10 ± 2103.98	37,615.28 ± 2020.25	34,911.53 ± 1839.91	34,362.32 ± 1663.15	40,189.10 ± 2546.78
DOPAC	5025.37 ± 551.54	4166.54 ± 303.40	5457.67 ± 359.59	4602.49 ± 178.60	4244.00 ± 436.42	5752.03 ± 439.06*
5-HIAA	2120.39 ± 185.25	2101.08 ± 152.21	2215.97 ± 200.77	2310.10 ± 143.94	2703.32 ± 367.19	2501.54 ± 237.15
5-HT	1005.09 ± 58.14	1204.70 ± 82.06	1108.81 ± 99.93	1194.83 ± 72.93*	1361.43 ± 105.91	1230.12 ± 120.33

Results are expressed as mean ± SEM, ng per g fresh weight, $n = 10$. DOPAC, 3,4-dihydroxylphenylacetic acid; other abbreviations for amines as in Table 1. *$p < 0.058$ vs. control (5-HT); †$p < 0.05$ vs. THM control (NA); ‡$p < 0.05$ vs. THM control (DOPAC).

TABLE 4

EFFECTS OF CHRONIC TREATMENT WITH PIRACETAM AND TACRINE FOR 20 DAYS ON THE LEVELS OF BIOGENIC AMINES AND RELATED COMPOUNDS IN THE OLFACTORY BULB OF SHAM-OPERATED AND THYMECTOMISED (THM) RATS

Biogenic Amine	Sham Control	Sham + Piracetam (500 mg/kg)	Sham + Tacrine (3.0 mg/kg)	THM Control	THM + Piracetam (500 mg/kg)	THM + Tacrine (3.0 mg/kg)
VMA	271.51 ± 30.78	281.74 ± 50.18	589.46 ± 136.46	282.04 ± 20.95	336.71 ± 21.86	307.19 ± 67.68
NA	761.18 ± 17.31	753.94 ± 55.59	699.70 ± 46.18	663.31 ± 22.98*	725.81 ± 18.59	768.55 ± 33.21§
DA	315.86 ± 21.23	241.10 ± 14.47	253.35 ± 31.58	274.53 ± 731‡	251.90 ± 17.59	261.43 ± 12.16
5-HIAA	387.23 ± 34.98	386.41 ± 39.11	281.22 ± 48.84	402.00 ± 31.34	382.10 ± 11.97	378.44 ± 34.61
5-HT	585.29 ± 27.87	565.98 ± 45.42	499.43 ± 54.18	564.92 ± 32.43	496.30 ± 23.52	569.77 ± 53.31

Results are expressed as mean ± SEM, ng per g fresh weight, $n = 10$. Abbreviations for amines as in Table 1. *$p < 0.05$ vs. sham control (NA); ‡$p < 0.05$ vs. sham control (DA); §$p < 0.05$ vs. THM control (NA).

alertness and the sleep–wake cycle, maintains attention, and is involved in memory and learning (28). A reduction in the concentration of NA has been associated with an impairment of learning and memory (28), an effect that may be enhanced by the increased serotonergic function that has been reported to occur in ageing (43,67). Although the increase in serotonergic activity found in the present study after thymectomy reached significance only in the striatum, it may have contributed to the adverse effect of the NA deficit on memory and learning.

Many of the behavioural and neurotransmitter changes that occurred following thymectomy were reversed by piracetam and tacrine, the effect of the latter drug being particularly marked. Piracetam is the first of the so-called "nootropic" drugs and has been widely used in some countries in the treatment of senile dementia and impairment of learning and memory (8,33). The mechanism of action of piracetam is thought to involve changes in the cholinergic, dopaminergic, noradrenergic, and serotonergic systems (15,18,47). Chronic treatment with piracetam has been shown to significantly improve learning and memory in the passive avoidance, conditioned avoidance, water maze, and T-maze tests (62). In the present study, chronic treatment with piracetam significantly reversed learning impairment of thymectomised rats in the passive avoidance test. Chronic piracetam administration was also shown to significantly reverse the reduction in the concentration of NA in thymectomised rats. However, piracetam did not normalise the changes in the lymphocyte or neutrophil differential WBC counts that occurred after thymectomy. In fact, piracetam was shown to decrease the percentage of lymphocytes and to increase the percentage the neutrophils in the sham-operated rats. Thus, the beneficial effects of piracetam on the behaviour and neurotransmitter changes elicited by thymectomy can be distinguished from its lack of effect on this aspect of the immune system. It is noteworthy that piracetam alone had no significant effect on any of the neurotransmitter or behavioural parameters determined in the sham-operated animals.

The central anticholinesterase tacrine, unlike piracetam, was found to largely reverse all the deficits caused by thymectomy. Thus, tacrine was found to significantly improve the learning and memory of thymectomised rats in both the passive avoidance and the Morris water maze tests. Other investigators have reported that tacrine administration improved learning and memory in a minority of patients with Alzheimer's disease and in ageing rats in the passive avoidance test (22,48). In the present study, the changes in the concentrations of neurotransmitters were also similar to those found by other investigators, namely an increase in the concentrations of the catecholamines after tacrine administration, at least in some of the brain regions studied. In addition, the present study shows that tacrine significantly raised the level of GABA in the hippocampus of thymectomised rats. The hippocampus has been closely related to learning and memory (19), and there is experimental evidence that NA can augment the action of GABA and improve learning in ageing rats (3). However, whether tacrine directly or indirectly affects the GABAergic system via the noradrenergic or cholinergic system is unclear. Following thymectomy, the activity of acetylcholinesterase is increased (59), and the activity of choline acetyltransferase is reduced after thymic atrophy (55), changes that are similar to those occurring in the cholinergic system of the ageing brain (27). Thus, changes in the cholinergic system following thymectomy may contribute to the memory and learning impairment found in the present study. The beneficial effect of tacrine on the behavioural, neurotransmitter, and immune changes caused by thymectomy may be at least partly due to its central anticholinesterase activity (22). It is worthy of note that tacrine had no effect on any of the behavioural, neurotransmitter, or immune parameters determined in the sham-operated rats and would therefore appear to be specific in correcting the deficits caused by thymectomy.

TABLE 5

EFFECTS OF CHRONIC TREATMENT WITH PIRACETAM AND TACRINE FOR 20 DAYS ON THE LEVELS OF GABA IN THE AMYGDALA AND HIPPOCAMPUS OF SHAM-OPERATED AND THYMECTOMISED (THM) RATS

	Sham Control	Sham + Piracetam (500 mg/kg)	Sham + Tacrine (3.0 mg/kg)	THM Control	THM + Piracetam (500 mg/kg)	THM + Tacrine (3.0 mg/kg)
Amygdala	810.17 ± 19.24	781.75 ± 36.71	858.54 ± 51.93	779.15 ± 31.72	802.71 ± 39.66	862.49 ± 36.36
Hippocampus	608.07 ± 33.09	581.27 ± 42.02	620.11 ± 16.79	567.19 ± 28.22	556.47 ± 37.14	682.44 ± 26.75*

Results are expressed as mean ± SEM, μmg per g fresh weight, $n = 10$. *$p < 0.01$ vs. THM control.

TABLE 6

DIFFERENTIAL WHITE CELL COUNTS IN SHAM-OPERATED AND THYMECTOMISED (THM) RATS FOLLOWING CHRONIC TREATMENT WITH PIRACETAM AND TACRINE FOR 20 DAYS

	Sham Control	Sham + Piracetam (500 mg/kg)	Sham + Tacrine (3.0 mg/kg)	THM Control	THM + Piracetam (500 mg/kg)	THM + Tacrine (3.0 mg/kg)
% Lymphocytes	77.00 ± 1.56	68.25 ± 2.97*	77.44 ± 1.18	67.10 ± 2.26†	61.00 ± 2.97	73.59 ± 1.83§
% Neutrophils	19.62 ± 1.55	28.56 ± 2.23*	19.00 ± 1.39	30.20 ± 4.86†	34.33 ± 2.92	22.77 ± 1.93§
% Monocytes	3.75 ± 0.64	5.13 ± 0.76	4.22 ± 0.32	3.50 ± 0.21	6.44 ± 0.22‡	5.00 ± 0.44
% Eosinophils	1.50 ± 0.13	1.31 ± 0.16	1.00 ± 0.22	1.05 ± 0.21	1.00 ± 0.27	1.64 ± 0.45

Results are expressed as mean ± SEM, $n = 10$. *$p < 0.05$ vs. sham control; †$p < 0.01$ vs. sham control; ‡$p < 0.05$ vs. THM control; §$p < 0.01$ vs. THM control.

In conclusion, the present study demonstrates that thymectomy results in a significant change in the brain neurotransmitters and impairment of learning and memory that stimulate changes found in normal ageing. This suggests that the thymectomised rat may be a useful model for studying the relationships among ageing, neurodegeneration, and memory. Furthermore, the results show that piracetam and particularly tacrine are effective agents in the improvement of learning and memory following thymectomy. The mechanisms whereby piracetam and tacrine improve learning and memory may involve changes in brain noradrenergic function.

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