Chronic imipramine treatment-induced changes in acetylcholinesterase (EC 3.1.1.7) activity in discrete rat brain regions

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Abstract

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Cholinergic as well as monoaminergic neurotransmission seems to be involved in the etiology of affective disorders. Chronic treatment with imipramine, a classical antidepressant drug, induces adaptive changes in monoaminergic neurotransmission. In order to identify possible changes in cholinergic neurotransmission we measured total, membrane-bound and soluble acetylcholinesterase (Achase) activity in several rat brain regions after chronic imipramine treatment. Changes in Achase activity would indicate alterations in acetylcholine (Ach) availability to bind to its receptors in the synaptic cleft. Male rats were treated with imipramine (20 mg/kg, *ip*) for 21 days, once a day. Twenty-four hours after the last dose the rats were sacrificed and homogenates from several brain regions were prepared. Membrane-bound Achase activity (nmol thiocholine formed min^{-1} mg protein^{-1}) after chronic imipramine treatment was significantly decreased in the hippocampus (control = 188.8 ± 19.4, imipramine = 154.4 ± 7.5, P<0.005) and striatum (control = 850.9 ± 59.6, imipramine = 742.5 ± 34.7, P<0.005). A small increase in total Achase activity was observed in the medulla oblongata and pons. No changes in enzyme activity were detected in the thalamus or total cerebral cortex. Since the levels of Achase seem to be enhanced through the interaction between Ach and its receptors, a decrease in Achase activity may indicate decreased Ach release by the nerve endings. Therefore, our data indicate that cholinergic neurotransmission is decreased after chronic imipramine treatment which is consistent with the idea of an interaction between monoaminergic and cholinergic neurotransmission in the antidepressant effect of imipramine.

Introduction

Cholinergic neurotransmission is involved in many aspects of central nervous system function, such as learning and memory (1,2) and rapid eye movement sleep generation (3,4). It also seems to be involved in pathological conditions such as Alzheimer's disease (2) and depression (5,6).

Imipramine, one of the most extensively studied tricyclic antidepressants, inhibits 5-hydroxytryptamine (5-HT) uptake both *in vitro* and *in vivo* and chronic treatment induces adaptive changes in brain monoamin-

ergic neurotransmission (7-12). Janowsky et al. (6) proposed that depression in humans may be associated with overactive cholinergic and decreased monoaminergic neurotransmission. Data in the literature have shown that imipramine, besides having a serotonergic action, may also have a cholinergic effect since it binds to the muscarinic receptor (13,14). Therefore, it seems worthwhile to study the effect of imipramine on neurotransmitters other than monoamines.

Acetylcholinesterase (Achase, EC 3.1.1.7) is an important constituent of cholinergic neurotransmission (15) and hydrolyzes acetylcholine (Ach) in the synaptic cleft, thus terminating its action. The number of Achase molecules can be up-regulated (16) or down-regulated (17). Inhibition of Achase leads to a down-regulation of cholinergic receptors (18,19). The levels of Achase appear to be controlled by the interaction of Ach with its receptor, with an enhanced interaction increasing the levels of Achase (20). Therefore, Achase can be used as an index of cholinergic function, and changes in enzyme activity may indicate alterations in the availability of Ach at the level of its receptors.

A few investigators have studied the effect of antidepressants on Achase activity. To our knowledge, only Geoffroy et al. (21) have reported the effect of chronic imipramine treatment on Achase activity in brain regions. However, these authors assayed the enzyme activity in whole homogenates and in their experimental design added behavioral manipulations to drug treatment, making it difficult to interpret their results in terms of the effect of the drug alone on Achase activity.

In the present study we report a decrease in the activity of membrane-bound Achase in the striatum and hippocampus after chronic imipramine treatment. Our data suggest an interaction between monoaminergic-cholinergic neurotransmission in the brain which may be involved in the antidepressant effect of the drug.

Material and Methods

Subjects

Male Wistar rats, 3 months old, weighing 250-300 g, from our own breeding facilities were used in the experiment. After weaning, the rats were kept 3 to a wire cage under controlled temperature (22ºC) and on a 12-h light-dark cycle (lights on from 7:00 to 19:00 h). The animals had free access to food and water until sacrifice.

Imipramine hydrochloride (Ciba-Geigy Co., São Paulo) diluted in distilled water was administered intraperitoneally, once a day for 21 days (around 9:00 a.m.), at a dose of 20 mg/kg (0.1 ml/100 g body weight). Control rats received vehicle. Control and treated rats were sacrificed 24 h after the last dose. The dose of imipramine and the time-course of treatment were chosen on the basis of available data showing significant changes in brain neurotransmission.

Tissue preparation

After decapitation, the brains were excised and kept on a cooled Petri dish on crushed ice. Brains were washed superficially with isotonic saline to clear the tissue of blood. Different brain regions (total cerebral cortex, hippocampus, thalamus, striatum, pons and medulla oblongata) were dissected immediately. Tissue was weighed and kept in cold 0.32 M sucrose buffered with Tris to pH 7.4.

Homogenate preparation

Homogenates (5% w/v) were prepared in ice-cold 0.32 M sucrose using glass homogenizer tubes and a motor-driven Teflon™ pestle and tissue fractions were prepared according to Chubb and Smith (22). Homogenates were centrifuged at 900 g for 10 min at 0ºC. The supernatant was collected and centrifuged at 100,000 g for 60 min at 4ºC.

The resulting supernatant was the source of soluble Achase and the resulting pellet was resuspended in the original volume. This latter preparation was the source for membrane-bound Achase. Enzyme activity was also measured in the 900 *g* supernatant. Homogenates were kept at -20°C until assayed.

Determination of Achase activity

Achase activity was determined using the method of Ellman et al. (23) modified for microassays. Acetylthiocholine was used as substrate at a final assay concentration of 1 mM. All materials, including reagents and homogenates, were kept on crushed ice before incubation. Enzyme activity was determined in duplicate for both samples and blanks. One hundred μl of buffer-substrate (0.1 M sodium phosphate buffer, pH 8, plus acetylthiocholine iodide) was pipetted into a microtube and 5 μl of the homogenate was added. Blanks were obtained by adding 15 μl of 2.4 N perchloric acid to the tubes before incubation. The tubes were incubated for 30 min in a shaking water bath at 37°C. After the addition of perchloric acid the enzyme activity tubes were centrifuged at 2,250 *g* for 15 min at 0°C. An aliquot of 50 μl was pipetted into another tube and 500 μl of Ellman's reagent was added. After standing for 15 min at room temperature, samples were read in a Beckman DU-2 spectrophotometer using microcuvettes at 412 nm. Proteins were assayed by the method of Lowry et al. (24) using bovine serum albumin as standard.

The activity of Achase is reported as nmol thiocholine formed min^{-1} mg $protein^{-1}$. The assay was set up to allow the reaction to be linear for both tissue concentration and incubation time. To test for the contribution of butyrylcholinesterase to the hydrolysis of Ach, the enzyme activity was measured using butyrylthiocholine as the substrate at 1 mM final concentration. Since the activity was very low (<5%), as also shown by others (25), no inhibitor of butyrylcholinesterase was used.

Reagents

All reagents used were from Sigma Chemical Co. (St. Louis, MO) and were analytical grade. Twice-distilled water prepared with an all-glass apparatus was used throughout the assays.

Statistical analysis

The Student *t*-test was used to test for statistical differences (P≤0.05, two-tailed).

Results

The data in Table 1 show that chronic imipramine treatment induced a highly significant decrease in Achase activity in the hippocampus in the 900 *g* supernatant fraction (t = 3.04, df = 10, P<0.02) and in the 100,000 *g* pellet (t = 4.04, df = 10, P<0.005) but no change was observed in the 100,000 *g* supernatant. A highly significant decrease in Achase activity (Table 1) was also observed in the striatum after chronic imipramine in both the 900 *g* supernatant (t = 4.63, df = 10, P<0.001) and 100,000 *g* pellet (t = 3.87, df = 10, P<0.005). No difference was observed in

Table 1 - Achase activity in the hippocampus and striatum of rats chronically treated with imipramine.

Achase activity is reported as nmol thiocholine formed min^{-1} mg $protein^{-1}$. Results are reported as mean ± SD for 6 animals in each group. Final acetylthiocholine concentration in the assay was 1 mM. *P<0.02, **P<0.005, ***P<0.001 compared to control (Student *t*-test).

Brain region	Supernatant 900 *g*	Pellet 100,000 *g*	Supernatant 100,000 *g*
Hippocampus			
Control	141.2 ± 18.3	188.8 ± 19.4	101.6 ± 15.0
Imipramine	112.0 ± 14.6*	154.4 ± 7.5**	92.0 ± 12.5
Striatum			
Control	659.9 ± 28.9	850.9 ± 59.6	305.6 ± 23.1
Imipramine	581.5 ± 29.8***	742.5 ± 34.7**	274.8 ± 46.0

the 100,000 g supernatant.

Chronic imipramine treatment induced a small but significant increase in Achase activity in the medulla oblongata only in the 900 g supernatant fraction (control = 143.3 ± 8.2, imipramine = 157.6 ± 8.0; t = 3.04, df = 10, P<0.02). There was no statistically significant difference between the 100,000 g pellet and the supernatant. In the pons, a small but statistically significant increase was also observed only in the 900 g supernatant (control = 200.6 ± 7.9, imipramine = 213.3 ± 6.9; t = 2.90, df = 10, P<0.02) (data not shown). No statistically significant differences in Achase activity after chronic imipramine were observed for the thalamus or total cerebral cortex in any of the fractions assayed (data not shown).

Discussion

The main finding of the present study was a significant decrease in membrane-bound Achase activity in the hippocampus and striatum after chronic imipramine treatment. Although we also observed statistically significant differences in other brain regions (medulla oblongata and pons), they were obtained only in the total fraction (900 g) and were small in amplitude. Therefore, from a biological point of view, they may have no great significance for the effect of the drug.

The decrease in Achase activity may lead to changes in cholinergic receptors after chronic imipramine treatment. Data in the literature have shown that chronic imipramine treatment did not change the number of muscarinic receptors in the striatum (8,26) or hippocampus (26). However, there is a report showing an increased number of receptors in the striatum (27). Due to the known diversity of both muscarinic and nicotinic brain cholinergic receptors (28,29) the possibility of a change in these receptors cannot be ruled out.

The decrease in Achase activity in the striatum and hippocampus after chronic imipramine treatment may indicate a secondary change induced by the drug acting primarily on serotonergic synapses through serotonergic afferents to these regions. Cholinergic neurons in the striatum are mostly intrinsic to this brain region and afferent connections from serotonergic raphe nuclei to the striatum have been described (30). Cholinergic projections to the hippocampus through the fornix arise in basal forebrain neurons and the hippocampus also receives projections from serotonergic raphe nuclei (30). These anatomical data seem to favor an indirect action of imipramine on cholinergic neurotransmission.

The striatum is one of the richest cholinergic areas in the brain and all cholinergic markers are highly concentrated in this brain region (31,32). The striatum is involved in the control of motor behaviors but its involvement with affective behavior has not yet been described, although Graybiel (33) has raised this possibility. Therefore, the effect of imipramine on this brain region may be involved in its antidepressant effect. Cholinergic neurons in the striatum are under dopaminergic afferent control and chronic imipramine treatment has been shown to induce a decrease in D_1 dopaminergic receptors (8,11) and in B_{max} of [^{3}H]DTG binding to the haloperidol-sensitive sigma sites (34). On the other hand, imipramine binds with high affinity to striatum membranes and this site seems to be located on the 5-HT transporter (35). Moreover, imipramine decreases [^{3}H]5-HT binding to striatal membranes (9). These data support the view that the effect of imipramine on Achase activity is not a direct one.

Chronic imipramine has been shown to induce several effects in the hippocampus, including sensitization of hippocampal CA1 neurons to the inhibitory effect of 5-HT (36), potentiation of the increase in the neuronal firing rate induced by a D_2 dopaminergic agonist (37), an increase of the suppressant

effect of the electrical stimulation of the afferent 5-HT pathway on the firing activity of CA3 pyramidal neurons (38), a decrease in 5-HT2 receptor sites (7), a decrease in 5-HT and beta-adrenergic receptor sites (9), a decrease in 5-HT transporter sites (12), and a reduction of inhibition of forskolin-stimulated adenylate cyclase by 5-HT (10). These data indicate that, as observed in the striatum, the changes in Achase activity observed after chronic imipramine may also reflect alterations induced by the drug primarily on serotonergic neurotransmission. The hippocampus is a brain structure involved in emotions, being part of the limbic system (39), and changes in hippocampal cholinergic neurotransmission induced by chronic imipramine may be involved in the antidepressant effect of the drug.

Recent data in the literature using microdialysis techniques have shown an interaction between brain serotonergic and cholinergic neurotransmission. Drugs enhancing serotonergic neurotransmission when administered acutely increase the release of Ach *in vivo* in the hippocampus (40). We did not find data in the literature on the release of Ach after chronic imipramine treatment which could help establish a relationship between the decrease in Achase activity and Ach levels in the hippocampus.

The indirect effect of imipramine on cholinergic neurons suggested by our results may also involve its metabolite desipramine which is an antidepressant drug that inhibits the uptake of noradrenaline. Therefore, the changes in Achase activity may also involve noradrenergic neurotransmission.

In conclusion, our results show that chronic imipramine treatment changes membrane-bound Achase activity only in some brain regions, probably leading to a change in cholinergic neurotransmission. Our data obtained in rats seem to support Janowsky's proposal (6) of an involvement of cholinergic neurotransmission in human depression.

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