Chronic Clomipramine and Triiodothyronine Increase Serotonin Levels in Rat Frontal Cortex In Vivo: Relationship to Serotonin Autoreceptor Activity

EITAN GUR, BERNARD LERER and MICHAEL E. NEWMAN

Biological Psychiatry Laboratory, Department of Psychiatry, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Accepted for publication July 20, 1998

ABSTRACT

Augmentation of tricyclic antidepressant (TCA) treatment with triiodothyronine (T3) has been shown to potentiate the therapeutic effect of TCA drugs in depressed patients. We have attempted to elucidate the mechanism of this potentiation by determining the effects of T3 alone and together with a TCA on serotonin (5-HT) levels in living rats, using in vivo microdialysis. A single s.c. injection of T3 at 0.1 mg/kg had no effect on 5-HT levels in frontal cortex or hippocampus. Chronic administration of clomipramine (10 mg/kg i.p. daily for 4 weeks) to rats resulted in increased basal 5-HT levels in the frontal cortex. Administration of T3 daily for 7 days at 0.1 mg/kg s.c. also resulted in elevated 5-HT levels, whereas in rats administered both clomipramine and T3, cortical 5-HT levels were significantly elevated compared with the levels in rats that had received only one treatment. Basal levels in hippocampus were unaffected by these treatments. Subcutaneous injection of the 5-HT-1a receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (0.2 mg/kg) resulted in a decrease in 5-HT levels in both cortex and hippocampus. In frontal cortex of animals that had received T3 or a combination of clomipramine and T3, the extent of the decrease was significantly reduced compared to that seen in control animals. The extent of the decrease in hippocampus was not affected by any of the treatments. Subcutaneous injection of the 5-HT-1b/1d antagonist GR 127935 (5 mg/kg) resulted in an increase in 5-HT levels in both brain areas. The extent of the increase was not affected by any of the treatments in either brain area. It is concluded that the action of T3 in potentiating the clinical response to TCA drugs may be due to its effect on 5-HT levels in the frontal cortex, which is due to desensitization of the presynaptic 5-HT-1a autoreceptors.

There is much evidence for a serotonergic dysfunction in major depression and for an involvement of increased serotonergic transmission in the mechanism of action of antidepressant drugs (ADs). Blier and de Montigny (1994) have proposed on the basis of electrophysiological evidence that each major class of AD increases serotonin (5-HT) neurotransmission by a distinct mechanism involving either presynaptic or postsynaptic 5-HT-1a receptors. Both tricyclic ADs (TCAs) and chronic electroconvulsive shock (ECS) had no effect on presynaptic (somatodendritic) 5-HT-1a receptors but increased postsynaptic receptor sensitivity, a finding supported by some reports of increased [3H]8-OH-DPAT binding at 5-HT-1a receptors in 5-HT terminal areas after chronic ECS or TCA administration, but not confirmed in others (for review, see Newman et al., 1993). The specific 5-HT reuptake inhibitors (SSRIs), exemplified by paroxetine, fluoxetine, fluvoxamine, and citalopram, which raise 5-HT synaptic levels when given acutely, were shown on repeated administration to increase 5-HT transmission by inducing desensitization of both the presynaptic 5-HT-1a somatodendritic autoreceptors in the raphe nuclei and the nerve terminal 5-HT-1b autoreceptors, which normally mediate feedback inhibition of 5-HT release. These effects lead to a slow increase in 5-HT levels, corresponding to the delayed clinical effect of these drugs.

Microdialysis experiments in which 5-HT levels are measured in vivo have provided partial support for this theory, especially regarding the SSRIs (Invernizzi et al., 1994; Kreiss and Lucki, 1995), although other studies have failed to provide such support (Hjorth and Auerbach, 1994; Bosker et al., 1995a,b; Auerbach and Hjorth, 1995; Moret and Briley, 1996). For the TCAs, two studies using microdialysis (Sleight et al., 1989; Kreiss and Lucki, 1995) have shown that these drugs induce no change in presynaptic 5-HT-1a or 5-HT-1b receptor sensitivity, which is consistent with the theory of

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; ANOVA, analysis of variance; ECS, electroconvulsive shock; HPLC, high-performance liquid chromatography; AD, antidepressant drug; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; T3, triiodothyronine.
Blier and de Montigny (1994). We have recently shown that chronic ECS had no effect on 5-HT-1a receptor sensitivity as measured by the action of 8-OH-DPAT to reduce 5-HT levels in either cortex or hippocampus (Gur et al., 1997).

Clomipramine is chemically a member of the TCA group of drugs but is characterized by a very high affinity for the 5-HT uptake site, of the same order of magnitude as that of the SSRIs. It is thus conceivable that clomipramine might operate by both of the mechanisms mentioned above. Clomipramine is one of the most effective ADs known and is regarded as the drug of choice for treatment of severely depressed hospital in-patients. There have been relatively few studies of the effects of clomipramine on 5-HT levels in living animals. Adell and Artigas (1991) found that a single injection of clomipramine at 10 or 20 mg/kg i.p. increased 5-HT levels in the dorsal raphe but had no effect in frontal cortex. However, local application of 10 or 40 μM clomipramine in the raphe resulted in a decrease in 5-HT levels in frontal cortex, due to activation of the somatodendritic 5-HT-1a autoreceptors. Carboni and di Chiara (1989), however, found that clomipramine at 20 mg/kg s.c. did increase 5-HT levels in rat frontal cortex, and a more recent study by Artigas’ group (Romero et al., 1996) found that 10 mg/kg clomipramine i.p. increased cortical 5-HT levels when given on its own and also potentiated the effects of the 5-HT-1a receptor antagonist WAY- 100635. No studies of the effects of chronic clomipramine on 5-HT levels in intact animals have been performed.

Very few studies have investigated the effects of chronic clomipramine at the electrophysiological level. de Montigny and Aghajanian (1978) found a selective increase in the inhibitory response of forebrain neurons to 5-HT applied by microiontophoresis, indicative of increased postsynaptic receptor sensitivity, after administration of clomipramine (5 mg/kg i.p.) for 14 days. Contreras et al. (1993) found that administration of clomipramine at 1.25 mg/kg i.p. b.i.d. for 30 days increased the duration of the suppression of cortical neuron firing induced by dorsal raphe stimulation, indicative of desensitization of the raphe 5-HT-1a autoreceptors. Similarly, in a study by Maudhuit et al. (1995), clomipramine given to neonatal rats at 15 mg/kg s.c. b.i.d. for 14 days had no effect on basal neuronal firing in the raphe nucleus but decreased the inhibitory response to i.v. citalopram, indicating that the somatodendritic receptors had become desensitized.

Several agents have been shown to potentiate the actions of TCAs when administered clinically as a supplement to existing drug therapy in patients who did not respond to an AD alone. These agents include lithium and triiodothyronine (T3), for which strong evidence of clinical efficacy exists, and pindolol, for which preliminary evidence is available. T3 supplementation has been shown to have a beneficial effect on depression in several studies. In a recently conducted meta-analysis, Aronson et al. (1996) aggregated eight studies with a total of 292 patients refractory to TCA therapy and found that patients treated with T3 augmentation were twice as likely to respond as controls. This corresponded to a 23.2% absolute improvement in response rates, with moderate to large improvements in depression scores. T3 has also been shown to be effective in animal models of depression such as the learned helplessness test (Brochet et al., 1987). The mechanism by which T3 augments the AD response is unknown. The most logical mechanism would involve correction of a putative abnormality of the thyroid axis in depression. However, the evidence for this is weak because there is no consistent relationship between abnormalities of basal thyroid hormone levels and responses to T3 augmentation (Joffe et al., 1995). Another possible mechanism involves a pharmacological effect of T3 on one of the neurotransmitter systems involved in depression, such as the noradrenergic or serotonergic system. Very few studies of the effects of T3 on 5-HT levels in animals have been performed, and none has been done using the in vivo microdialysis technique. Heal and Smith (1988) showed that a single injection of T3 at 0.1 mg/kg to mice had no effect on the concentration of 5-HT in fore-, mid-, or hindbrain, and also did not affect 8-OH-DPAT-induced hypothermia or the locomotor response induced by the 5-HT-1b agonist RU-24969. Repeated injections of T3 for 10 days reduced these responses and also induced a rise in 5-HT levels in midbrain and hindbrain. Sandrini et al. (1996) found that T3 given either acutely or for 3 to 7 days to rats at 0.1 mg/kg s.c. raised 5-HT levels in the cortex but not the hippocampus. 5-HT-1a receptor number was unchanged but there was a decrease in the amount of 5-HT-2 receptors in the cortex. In both these studies, 5-HT levels were measured in brain homogenates after killing of the animals. No studies have been reported in which the effects of T3 on 5-HT levels in vivo were measured.

In our work the effects of chronic administration of clomipramine on 5-HT levels in vivo were determined in two brain areas: frontal cortex and hippocampus. The effects of chronic clomipramine on the sensitivities of the somatodendritic 5-HT-1a autoreceptors and the nerve terminal 5-HT-1b autoreceptors were also determined by measuring the changes in 5-HT levels in both areas after peripheral administration of the 5-HT-1a agonist 8-OH-DPAT and the 5-HT-1b antagonist GR 127935, respectively. Although GR 127935 administered peripherally may also block the 5-HT-1da receptors present in dorsal raphe (Davidsson and Stamford, 1995), these autoreceptors control the amount of 5-HT released in cell body regions but do not directly modulate the firing activity of 5-HT neurons, which control the amount of 5-HT released at nerve terminal areas (Gobert et al., 1997). The effect of peripheral GR 127935 on 5-HT release from cortex and hippocampus may therefore be regarded as due to its actions on the 5-HT-1b receptors situated at the nerve terminals. The effect of a short (7-day) period of administration of T3, both alone and as an adjunct to chronic clomipramine treatment, on all of the above parameters was also determined.

**Materials and Methods**

**Drug Administration.** Albino rats (Sabra strain) were used in all experiments. The rats were housed by treatment group in a temperature-controlled environment (24°C) with a regular 12-h light/dark cycle. Food and water were freely available. Clomipramine was administered i.p. at a dose of 10 mg/kg dissolved in normal saline. T3 was administered s.c. at a dose of 0.1 mg/kg dissolved in 0.02 M NaOH. In the experiments involving chronic drug administration, there were four groups of rats. Rats in group A received daily injections of 0.9% saline for 4 weeks; rats in group B received saline injections daily for 4 weeks and also received injections of T3 for the last 7 days of the treatment period; group C received daily clomipramine injections for 4 weeks; and group D received daily clomipramine for 4 weeks and additional T3 injections for the last 7 days of...
the period. Treatment periods were arranged so that two rats completed the schedules on each experimental day. The two rats were either taken from groups A and C or from groups B and D. Microdialysis experiments were performed on two rats on each experimental day. The experimental design was intended to mimic the therapeutic use of T3 in depressed patients, where it is given to augment treatment with an already existing TCA drug.

**Implantation and Perfusion of the Microdialysis Probe.** Animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) approximately 2 h after the last treatment and mounted in a stereotaxic apparatus. Guides for dialysis probes (Bioanalytical Systems) were implanted into the ventral hippocampus at posterior 5.0 mm from bregma, 4.5 mm lateral, and 8.0 mm vertical, and into the frontal cortex at anterior 3.2 mm from bregma, 2.5 mm lateral, and 6.0 mm vertical, according to the atlas of Paxinos and Watson (1986). Ventral hippocampus was selected because T3 levels are higher in this area than in dorsal hippocampus. Rats were maintained under anesthesia for approximately 1 h, after which they were free-moving and had unlimited access to food and water. Dialysis probes (4 mm) were inserted into the guides toward the end of the period of anesthesia. The inlet of the probe was connected, through plastic tubing with an internal volume of 12 μl/m, to a 5-ml gas-tight syringe mounted on a microinfusion pump. The inlet and outlet tubing of the probe was mounted to a flexible cable running from the head of the rat to a liquid swivel, allowing the animal to rotate and rear without entangling the fluid tubing. The probes were perfused with Ringer’s solution containing 3 mM CaCl₂, 4 mM KCl, 130 mM NaCl, and 10 μM citrulina, pH 6.5, at 0.1 μl/min overnight. The next morning the flow rate was increased to 0.5 μl/min, and 30-min fractions were collected. After each experiment, the dialysis probes were removed under anesthesia, sterilized in alcohol, and if still intact reinserted into new animals. These animal procedures received the approval of the Institutional Animal Care and Use Committee of the Hebrew University Faculty of Medicine and Dental Medicine and Hadassah Medical Organization.

**5-HT Receptor Challenges.** 5-HT-1a receptor function was determined by s.c. injection of 0.2 mg/kg 8-OH-DPAT, and 5-HT-1b receptor function was determined by s.c. injection of 5 mg/kg GR 127935. Challenges were administered once stable baseline 5-HT levels had been obtained, usually after collecting four or five experimental samples. The two challenges were given to each animal on separate experimental days, so that animals received either a 5-HT-1a challenge 24 h after implantation of the probe and a 5-HT-1b challenge 48 h after implantation, or a 5-HT-1b challenge after 24 h and a 5-HT-1a challenge after 48 h. The order of the challenges was randomized so that all of the experimental groups received both sequences.

**Determination of 5-HT Levels.** Concentrations of 5-HT were determined by a Bioanalytical Systems (West Lafayette, IN) high-performance liquid chromatography (HPLC) system. Samples were injected immediately after collection using a Rheodyne 9125 injector with a 10-μl injection loop. The mobile phase was made up of 90 mM sodium dihydrogen phosphate, 10 mM NaCl, 0.5 mM EDTA, 0.15 g/liter sodium octyl sulfate, and 10.5% acetonitrile, pH 5, and was from Sigma Chemical Co. (St. Louis, MO). GR 127935 was a gift from Glaxo Wellcome (Stevenage, UK). Citalopram was a gift from H. Lundbeck A/S (Copenhagen, Denmark). HPLC grade acetonitrile was from Frutarom Ltd. (Haifa, Israel). All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany).

**Data Analysis.** Basal 5-HT levels were tested using one-way analysis of variance. The effects of 5-HT receptor challenges were tested using 5-HT levels expressed as percentages of the initial levels for each animal, and were analyzed over the whole time course by two-way analysis of variance, with treatment as a “between groups” variable and time (fraction number) as a “within groups” variable, i.e., as a repeated measure. Because the design of the experiment was factorial, planned comparisons were also performed using the appropriate contrasts to generate a main (overall) effect for T3, a main (overall) effect for clomipramine, and an interaction effect. In addition individual planned comparisons of the (T3 + clomipramine) combined group with the other groups were carried out.

**Results**

**Basal 5-HT Levels.** Acute administration of T3 by s.c. injection had no effect on 5-HT levels measured for 3 h after the injection in either cortex or hippocampus (data not shown). Table 1 shows basal 5-HT levels in frontal cortex and hippocampus of rats given each of the four chronic treatments. One-way ANOVA showed a significant effect of treatment on basal values in the cortex (F(df 3,35) = 4.67, P = .0075). Planned comparisons tests showed a significant main effect of T3 (F(df 1,35) = 7.42, P = .01), and a significant main effect of clomipramine (F(df 1,35) = 9.11, P = .0047), but no significant interaction (F(df 1,35) = 0.026, P = .87), indicating that the effects of T3 and clomipramine were additive, but not subadditive or synergistic. Individual planned comparisons between the (T3 + clomipramine) combined group and the other groups gave the following results: versus saline alone, F(df 1,35) = 13.9, P = .00068; versus T3 alone, F(df 1,35) = 4.64, P = .038; versus clomipramine alone, F(df 1,35) = 3.95, P = .054, indicating that cortical 5-HT levels in rats which received the combination treatment were significantly higher than levels in rats which had received one treatment only. In the hippocampus there was no overall difference in basal levels between the different groups (F(df 3,32) = 1.11, P = .355).

**5-HT-1a Receptor Challenges.** Fig. 1 shows the effects of s.c. injection of 0.2 mg/kg 8-OH-DPAT on 5-HT levels in frontal cortex. The values for each animal were expressed as percentages of the mean values obtained in the four fractions before administration of 8-OH-DPAT in the same animal. An overall ANOVA gave an almost significant effect of treatment (F(df 3,18) = 2.94, P = .06), a highly significant effect of time (F(df 7,126) = 17.44), and a significant interaction between treatment and time (F(df 21,126) = 1.736, P = .033). Planned comparison tests showed a significant main effect of T3 (F(df 1,35) = 13.9, P = .00068).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frontal Cortex</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 4 wk</td>
<td>8.64 ± 1.35 (9)</td>
<td>14.51 ± 3.25 (9)</td>
</tr>
<tr>
<td>Saline 4 wk + T3 7 days</td>
<td>16.77 ± 1.69 (11)</td>
<td>17.21 ± 2.58 (10)</td>
</tr>
<tr>
<td>Clomipramine 4 wk</td>
<td>17.70 ± 2.68 (12)</td>
<td>16.30 ± 2.33 (11)</td>
</tr>
<tr>
<td>Clomipramine 4 wk + T3 7 days</td>
<td>26.87 ± 6.72 (7)</td>
<td>23.60 ± 6.06 (6)</td>
</tr>
</tbody>
</table>

Results are expressed in fmol/5 μl dialysate and are mean ± S.E.M. of the number of observations in parentheses.
1,18\] = 5.65, \( P = .029 \) but no main effect of clomipramine \((F[df 1,18] = 3.08, \ P = .096)\) or interaction \((F[df 1,18] = 0.61, \ P = .44)\). The planned comparisons of the \((T3 + clomipramine)\) combined group with the other groups showed a significant effect \((F[df 1,18] = 7.93, \ P = .011)\) versus saline only.

Figure 2 shows the effects of 8-OH-DPAT administration in the hippocampus. The reduction in 5-HT levels was smaller and of shorter duration than the equivalent effect in frontal cortex (cf., maximum reduction in saline-treated rats to 10% of basal in frontal cortex, 50% of basal in hippocampus; minimum value maintained for 30 min in cortex but not hippocampus). The overall ANOVA showed an effect of time \((F[df 7,98] = 9.24, \ P = .005)\) but no effect of treatment \((F[df 2,14] = 0.159, \ P = .85)\) and no interaction between time and treatment. Tests of planned comparisons did not yield significant effects.

Discussion

Our results show increased extracellular basal levels of 5-HT in frontal cortex after chronic administration of clomipramine. Increased basal levels may be due to the continued presence of the uptake inhibitor, desensitization of either or both of the 5-HT autoreceptors that regulate synthesis and release of 5-HT, or a decrease in the number of transporter sites. All of these factors have been implicated in the changes in basal levels seen after chronic drug treatment by a number of investigators, and will now be considered in turn with regard to our experiments.

The half-life of clomipramine is 4.4 h in the rat (Fujita et al., 1991) and has been estimated at between 22 and 84 h in humans. Because in our work experiments were performed either 24 or 48 h after the last injection of clomipramine, it is unlikely that residual clomipramine accounted for the increased basal 5-HT levels. It has been suggested that the increases in basal 5-HT levels seen in several in vivo experiments in the apparent absence of changes in autoreceptor sensitivity, e.g., after chronic fluoxetine when measurements were made 24 h, but not 96 h, after the last injection (Invernizzi et al., 1996) were due to the presence of residual drug. This factor is especially important in experiments.
Fig. 4. Effect of systemic GR 127935 (5 mg/kg s.c.) on 5-HT levels in hippocampus. Results are means of observations from seven animals treated with saline, three animals treated with T3, five animals treated with clomipramine, and four animals treated with a combination of clomipramine and T3. S.E.s are omitted for clarity.

in both blood and brain was increased after chronic administration of the drug compared to the level after a single injection (Friedman and Cooper, 1983; Fujita et al., 1991). However, during a 14-day period of chronic administration, the concentration of clomipramine in brain remained constant (Fujita et al., 1991). It would therefore seem likely that the 5-HT uptake blockade induced by clomipramine remains constant over a period of chronic administration. Although this parameter has not been investigated in rat brain, administration of clomipramine to human volunteers resulted in a progressive inhibition of 5-HT uptake in platelets, reaching full inhibition after 1 week (Poirier et al., 1987).

Chronic clomipramine in our experiments did not alter the ability of a challenge dose of 8-OH-DPAT to lower 5-HT levels in the cortex, making it unlikely that desensitization of the 5-HT-1a autoreceptors in the dorsal raphe accounts for the increased basal levels in cortex. A reduction in the ability of a challenge dose of 8-OH-DPAT to lower 5-HT levels was found in cortex after chronic administration of citalopram at 10 mg/kg (Invernizzi et al., 1994), although Hjorth and Auerbach (1994) found no difference in this effect in either cortex or dorsal hippocampus at a dose of 5 mg/kg. Few reports have investigated the effects of chronic administration of TCA drugs on 5-HT levels in the living brain. Kreiss and Lucki (1995) found that chronic desipramine increased basal 5-HT levels in striatum but not ventral hippocampus of chronically treated rats. The response to a challenge dose of 8-OH-DPAT, however, was only slightly reduced in hippocampus and unaffected in striatum. Bel and Artigas (1996) found that imipramine given for 2 weeks by osmotic minipumps increased basal 5-HT levels in frontal cortex. Although the minipumps were left in place during the experiment, it is unlikely that the effect was due to the continued presence of drug, because the dose of imipramine used did not affect 5-HT levels in cortex when given as a single injection. Our results suggest that although the effects of clomipramine in cortex parallel those of imipramine in cortex or desipramine in the striatum, in none of these cases was the effect due to desensitization of the presynaptic 5-HT-1a receptors.

The effects of the 5-HT-1b/1d antagonist GR 127935 on 5-HT release in the brain of living animals have been examined in several studies. In the study by Hutson et al. (1995), neither peripheral administration of GR127935 at doses of 1 or 5 mg/kg i.p. nor local administration by infusion via the probe of GR 127935 at concentrations of 10, 33, or 100 μM affected 5-HT levels in guinea pig cortex. In two studies in which measurements were performed in guinea pig cortex (Skingle et al., 1995; Roberts et al., 1997), direct administration of GR 127935 via the microdialysis probe led to an increase in 5-HT levels. However, systemic administration of GR 127935 led to a decrease in 5-HT levels, which was explained by the antagonism of the compound at raphe 5-HT-1d receptors leading in turn to an increase in raphe 5-HT levels and consequent activation of 5-HT-1a somatodendritic autoreceptors, which then induced a decrease in cell firing and decreased release of 5-HT at the nerve terminals. In another two studies, however, systemic administration of GR 127935 resulted in increased 5-HT levels at nerve terminal areas. Rollema et al. (1996) found that GR 127935 at 0.3 mg/kg s.c. had no effect on 5-HT levels in guinea pig hypothalamus but potentiated the action of the SSRI sertraline to increase 5-HT, whereas GR 127935 alone at 5 mg/kg
s.c. increased 5-HT levels by 35%. Gobert et al. (1997) similarly found 2.5 mg/kg GR 127935 s.c. to potentiate the action of fluoxetine to increase 5-HT levels in rat frontal cortex. These authors concluded that 5-HT-1b autoreceptors exert only a slight and variable degree of tonic inhibitory feedback of 5-HT release under normal physiological conditions, but are activated when 5-HT levels are increased by uptake blockade. The increase in 5-HT levels observed in both frontal cortex and hippocampus after systemic administration of GR 127935 in our experiments is thus most likely a consequence of the presence of citalopram in the perfusion fluid. This interpretation is confirmed by the results of two more recent microdialysis studies, in rat ventral lateral geniculate nucleus and rat frontal cortex, respectively, in which increases in 5-HT levels after administration of GR 127935 were only observed when the SSRI paroxetine was present in the perfusion medium (Davidson and Stamford, 1997; Sharp et al., 1997).

Desensitization of nerve terminal 5-HT-1b autoreceptors or their species homologs in the guinea pig, 5-HT-1d autoreceptors, after chronic administration of 5-HT uptake blocking drugs has been shown electrophysiologically (Blier and de Montigny, 1994), and in experiments in which 5-HT release was measured in incubated slice preparations (Blier and Bouchard, 1994; El Mansari et al., 1995). However, microdialysis experiments in which 5-HT levels are measured in vivo have provided very little evidence for this effect. Moret and Briley (1996) found that chronic citalopram treatment increased the effect of the autoreceptor antagonist methiothepin to raise 5-HT levels in rat hypothalamus by blocking the tonic activity of 5-HT-1b receptors, when no washout period was given. After a 24-h washout period, however, the action of methiothepin to increase 5-HT release was no different in control and citalopram-treated animals. The effect was therefore most likely due to residual citalopram and not to a change in autoreceptor sensitivity. Similarly, Bosker et al. (1995a,b) obtained no change in the effect of the 5-HT-1b agonist RU 24969 applied locally in rat hippocampus to reduce 5-HT levels after chronic administration of fluvoxamine. A similar finding was obtained by Auerbach and Jhorth (1995) using citalopram. Although the effect of chronically administered clomipramine on this parameter has not previously been measured, Montero et al. (1991) showed that the number of 5-HT-1b receptors in rat frontal cortex as measured by [125I]cyanoindolol binding was unaffected by either acute or chronic treatment with clomipramine (10 mg/kg i.p. for 15 days). Our results provide a functional counterpart for this finding as the effect of GR 127935 to increase 5-HT levels was not modified by clomipramine in either cortex or hippocampus.

Blier and colleagues (Blier et al., 1990; Pineyro et al., 1995) have postulated that regulation of 5-HT levels by the nerve terminal 5-HT-1b autoreceptors occurs in the hippocampus but not in the cortex, because tonic activity at these receptors was observed in hippocampus but not cortex. Supporting evidence for this came from brain slice experiments in which hippocampal but not cortical 5-HT-1b receptors were desensitized after chronic antidepressant treatment (Blier and Bouchard, 1994; El Mansari et al., 1995). However, our results and the effects described above of either locally applied or systemic GR 127935 to increase cortical 5-HT levels when given alone (Skingle et al., 1995; Roberts et al., 1997) or in conjunction with an SSRI (Gobert et al., 1997; Sharp et al., 1997) argue against this.

Another factor that may contribute to changes in extracellular 5-HT levels is the number of 5-HT transporter or uptake sites. Graham et al. (1987) observed no change in binding of [3H]paroxetine to rat cortical or hippocampal membranes after administration of 10 mg/kg clomipramine i.p. b.i.d. for 19 days when the animals were sacrificed 72 h after the last dose. However, in the human studies of Poirier et al. (1987) the B_max for [3H]imipramine binding to platelets was decreased after 1 week of clomipramine administration and remained significantly below normal values after 1 week of washout, indicating down-regulation of the number of transporter sites. The down-regulation of the 5-HT transporter site in rat frontal cortex observed after chronic administration of the SSRI paroxetine (Pineyro et al., 1994) was indeed proposed to account for the increased 5-HT release observed after this treatment, because no changes in 5-HT autoreceptor activity could be shown (Blier and Bouchard, 1994; El Mansari et al., 1995). Because the changes observed in 5-HT-1a autoreceptor activity observed in our study after chronic clomipramine did not reach statistical significance, a change in the number of 5-HT transporter sites may also occur in brain and account for the increased basal 5-HT levels observed. Supporting evidence for this is provided by the findings of Lesch et al. (1993), who observed a reduction in the mRNA level for the 5-HT transporter in dorsal raphe after chronic administration of clomipramine to rats via osmotic minipumps.

Using in vivo methodology, our results confirm the results of Heal and Smith (1988), who found no effect of a single injection of T3 on 5-HT levels in either cortex or hippocampus. Our results also agree with those of Sandrini et al. (1996) who found increased 5-HT levels in rat cerebral cortex but not hippocampus after administration of T3 for 7 days. However, these authors observed no change in 5-HT-1a receptor number as measured by [3H]8-OH-DPAT binding after prolonged T3 administration, although we observed a decrease in 5-HT-1a receptor functioning as determined by the ability of a challenge dose of 8-OH-DPAT to elicit a decrease in 5-HT levels in frontal cortex. This finding is in keeping with the results of Heal and Smith (1988) who observed a reduction in the hypothymic response to 8-OH-DPAT in mice after administration of T3 for 10 days. However, these authors also observed a decreased locomotor response to the 5-HT-1b receptor agonist RU 24969 after T3. This observation was not paralleled in our studies, because T3 did not affect the response to GR 127935. Addition of T3 to clomipramine treatment in the last week of injections resulted in a further increase in basal 5-HT levels in the cortex, so that the levels became significantly greater than those obtained with either clomipramine alone or T3 alone. In animals given the combination treatment the response to 8-OH-DPAT was also significantly reduced compared to the response in animals given saline only, although the degree of reduction was not significantly greater than that in animals receiving either clomipramine alone or T3 alone. Desensitization of somatodendritic 5-HT-1a receptors therefore appears to account for the increased basal 5-HT levels seen after T3 administration in the cortex. The additional increase in 5-HT levels obtained on addition of T3 to an already existing TCA drug treatment.
may underlie the increase in therapeutic efficacy seen when this combination treatment is given clinically.

References


Send reprint requests to: Dr. M. E. Newman, Biological Psychiatry Labo ratory, Hadassah-Hebrew University Medical Center, P.O.B. 12000, Jerusalem 91120, Israel. E-mail: newman@hadassah.org.il