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Apparent down-regulation of rat brain μ- and κ-opioid binding sites labelled with [^{3}H]cycloFOXY following chronic administration of the potent 5-hydroxytryptamine reuptake blocker, clomipramine

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Abstract—This study examined the effect of chronic clomipramine administration on opioid μ- and κ-binding sites. Clomipramine (5 mg kg^{-1} day^{-1}) or saline was administered to rats via osmotic minipumps for 3 days or 28 days. Lysed-P2 brain membranes were prepared and preincubated for 60 min without (control membranes) or with 1 μM of the μ-selective acylating agent, 2-(4-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidazole-HCl (BIT), to deplete membranes of μ-binding sites. [^{3}H]6-Desoxy-6β-fluoronaltrexone ([^{3}H]cycloFOXY) was used to label μ and κ-binding sites. Weighted nonlinear least squares analysis of cycloFOXY binding surfaces permitted determination of the K_d and B_{max} values of μ- and κ-binding sites in control and treated rats. Subacute (3 days) administration of rats with clomipramine had no significant effect on [^{3}H]cycloFOXY binding. Chronic (28 days) administration of clomipramine produced a small (approximately 10%) but statistically significant decrease in the B_{max}. These findings are discussed in reference to other studies that have examined the effect of chronic antidepressant administration on opioid receptors, and speculate that the endogenous opioid systems may play a role in obsessive-compulsive disorder.

Clomipramine is a tricyclic antidepressant with powerful therapeutic properties in obsessive-compulsive disorder (Thoren et al 1980a). Generally, it requires at least 8–12 weeks of chronic daily administration at doses close to, and sometimes higher than, 200 mg day^{-1} to develop its therapeutic antiobsessional effect (Asberg 1982). Its mechanism of action as an antiobsessional agent is known to result from blockade of 5-hydroxytryptamine (5-HT) reuptake in the central nervous system (CNS) (Thoren et al 1980b; Zohar et al 1988; Benkelfat et al 1989).

Clomipramine is also known to interact in the CNS with several other neural pathways and receptor binding sites (Hyttel & Larsen 1985). In particular, acute pretreatment with clomipramine enhances analgesia following intrathecal administration of morphine. This effect is not present after chronic administration of clomipramine, suggesting that chronic administration may produce a functional opioid receptor subsensitivity in the spinal cord (Kellstein et al 1988). In addition, the analgesic effect in the tail flick test observed following clomipramine administration is blunted by the opioid receptor antagonist, naloxone (Sacerdote et al 1987), indicating that clomipramine acts directly on the endogenous opioid system.

Despite these convincing behavioural studies, only scarce and somewhat puzzling data are available on the effects of chronic clomipramine and other selective 5-HT reuptake inhibitors on opioid binding sites. When clomipramine is combined with another selective 5-HT reuptake blocker, nisoxetine, it reduces the affinity of opioid receptors for [^{3}H]naloxone in the spinal cord without altering receptor density (B_{max}) (Kellstein et al 1988). Conversely, the highly selective 5-HT reuptake blocker, citalopram, moderately elevates the density of [^{3}H]naloxone binding sites in the rat cortex after chronic administration (Antkiewicz-Michaluk et al 1984).

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Because no studies have examined the effects of chronic clomipramine on rat brain opioid receptors, we decided to investigate this issue using the opioid antagonist, [^{3}H]cycloFOXY. The present study examined the effects of 3-day and 28-day clomipramine administration, using implanted osmotic minipumps, on μ- and κ-opioid binding sites in rat brain.

Materials and methods

Clomipramine treatment. Male Wistar rats ca 250 g at the beginning of the study were housed in a temperature-controlled (24 ± 1°C) room with a 12-h light/dark cycle (lights on at 07:00 h). Animals had free access to Purina rat chow and water.

Two groups of 18 animals each were implanted with osmotic minipumps (Alza Corp., Palo Alto, CA) under pentobarbitone anaesthesia. Group A received clomipramine (5 mg kg^{-1} day^{-1}) and group B received 0·9% NaCl (saline). Incisions were made on the back of the neck of each animal. The minipumps were then placed under the skin and the incisions were clipped. In group A, 10 animals received clomipramine for 3 days (subacute) and the remaining 8 animals received clomipramine for 28 days (chronic). In group B, 10 animals received saline for 3 days (subacute) and 8 animals received saline (chronic) for 28 days. In animals receiving clomipramine or saline for 28 days, minipumps were replaced after 2 weeks of treatment. After 28 days of treatment, animals were decapitated; brains were quickly removed, and membranes prepared as described below.

[^{3}H]cycloFOXY binding assay. Two rats were killed and lysed-P2 membranes were prepared from whole brain (without cerebellum). The homogenate was then split into two equal portions, which were incubated in the absence (termed CNT-P2) and presence (termed BIT-P2) of 1 μM of the μ-selective acylating agent, 2-(4-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidazole-HCl (BIT), as previously described (Rothman et al 1988). Because BIT-P2 are membranes depleted of μ-binding sites, their use permits the selective labelling of κ-binding sites using [^{3}H]cycloFOXY. When CNT-P2 is used, [^{3}H]cycloFOXY labels both μ- and κ-binding sites. Membranes from 8 rats treated with placebo and 8 rats treated chronically with clomipramine for 28 days were prepared in this manner to yield four separate membrane preparations for each group. The same procedure was used for the subset of 10 rats treated with placebo or clomipramine for 3 days to yield five separate membrane preparations for each group. The [^{3}H]cycloFOXY binding assay was performed for 4 to 6 h at 0°C in 50 mM TRIS-HCl, 100 mM NaCl, pH 7·4, as previously described (Rothman & McLean 1988). [^{3}H]CycloFOXY (20·5 Ci mmol^{-1}) was prepared as described (Ostrowski et al 1987).

The method of binding surface analysis used in this study was described previously (Rothman 1986; Rothman & McLean

1988; Rothman et al 1988). Each membrane preparation was assayed using the following technique: two concentrations of [^{3}H]cycloFOXY (0·42 nM and 2·14 nM) were each displaced by 8 concentrations of cycloFOXY between 0·5 and 128 nM, thereby generating 18 data points per binding surface (16 displacement points and 2 "specific binding" points). Binding surfaces were fitted to various binding equations using MLAB (Knott & Reece 1972), which uses a weighted, non-linear least squares curve fitting algorithm.

Statistical differences were determined using Student's t-test.

Results

The first part of the experiment was designed to examine the effects of subacute clomipramine treatment on the specific binding of [^{3}H]cycloFOXY. In this experiment, 3-day administration of clomipramine did not significantly affect the specific binding of 2 nM of [^{3}H]cycloFOXY to κ- and μ-binding sites (data not shown).

As reported in Table 1, chronic 28-day administration of clomipramine produced a small (7%) but statistically significant decrease in the number of κ-binding sites, and a significant 15% decrease in the number of μ-binding sites labelled by [^{3}H]cycloFOXY. No significant change was found in K_d values.

Table 1. Effects of chronic 28-day clomipramine treatment on μ- and κ-opioid binding sites labelled by [^{3}H]cycloFOXY.

Group	Binding Site	B_{max} (fmol(mg protein)$^{-1}$)	K_d (nM)	r^2
Placebo BIT-P2	κ	218±4·8	1·27±0·04	0·99
Clomipramine BIT-P2	κ	204±6·0*	1·22±0·06	0·99
Placebo CNT-P2	μ	55·4±3·4	0·22±0·04	0·99
Clomipramine CNT-P2	μ	46·0±3·2*	0·23±0·04	0·99

As described in Methods, the combined BIT-P2 binding surfaces of each experimental group (72 data points) were fit to the one site binding model for the best-fit parameter estimates of the κ-binding site. To obtain the best fit parameter estimates of the μ-binding site, the combined CNT-P2 binding surfaces of each experimental group (72 data points) were fitted to the two-site binding, with the K_d and B_{max} of the κ-binding sites fixed to that determined above, for the best-fit parameter estimates of the μ-binding site. Each value reported above is ±s.d. (n=4). * $P<0·01$ when compared with control.

Discussion

The present study shows a modest, but significant decrease in the density of μ- and κ-binding sites labelled with [^{3}H]cycloFOXY after long-term, but not short-term, clomipramine administration. This direct effect of clomipramine on opioid receptor subtypes is consistent with data obtained from in-vitro displacement studies of tritiated opioid ligands. In a recent report, clomipramine was compared with imipramine and amitriptyline and shown to be the only tricyclic antidepressant to interact directly with the μ-, δ-, and κ-opioid binding sites in the bovine adrenal medulla, with K_i values in the micromolar range (Carydakis et al 1986).

Several studies have shown chronic administration of tricyclic antidepressants or second generation antidepressants, or chronic electroconvulsive shock (ECS) treatment, to alter B_{max} and/or K_ds of various opioid binding sites in rat brain, although there has been some controversy (Reisine & Soubrie 1982; Antkiewicz-Michaluk et al 1984, 1987; Christensen et al 1986; Stengaard-Pedersen & Schou 1986; Hamon et al 1987) related to the experimental conditions of these studies (e.g., type of drug and rat brain region). As the present study was carried out in whole rat brain, it is possible that the changes observed in the density of opioid binding sites resulted from changes in various magnitudes and/or changes in different brain regions. Thus, these results suggest that more pronounced changes might be observed in discrete brain regions. Therefore, when the present data are compared with data from other studies, these results should be interpreted cautiously because different antidepressant drugs were used in the studies and no regional comparisons were made in the present study.

Nevertheless, we found at least one comparable study of [^{3}H]naloxone binding sites in whole rat brain homogenates during antidepressant therapy (Baraldi et al 1983) that revealed a marked increase (50%) in opioid binding sites in the rat brain after chronic 21-day administration of imipramine (5 mg kg^{-1} day^{-1} i.p.) However, it is difficult to interpret such a large difference between changes in the density of μ-binding sites after chronic administration of clomipramine or imipramine. This discrepancy in drug effect could be explained by different methodologies. In the imipramine study, animals were treated i.p. with high doses of imipramine (20 mg kg^{-1} day^{-1}) and were decapitated 48 h after the last imipramine dose. In the clomipramine experiment, doses were much lower (5 mg kg^{-1} day^{-1}) and drugs were administered continuously by means of a minipump, and animals were decapitated while at a steady drug level. These differences might indicate that the increase in opioid binding sites density following chronic imipramine could be related to a withdrawal phenomenon.

However, it is tempting to suggest that the reported differences in the effects of chronic imipramine and clomipramine administration on opioid receptors in the rat brain may have some therapeutic implications for the treatment of obsessive-compulsive disorder. Indeed, clomipramine and imipramine have comparable antidepressant effects, but here dramatic differences in their antiobsessional properties, with imipramine being a much weaker antiobsessional agent (Foa et al 1987). The present findings could suggest that some of the effects of clomipramine on the opioid system may be related to its antiobsessional properties. Although the present study does not intend to address this issue directly, the demonstrated occurrence of adaptive processes of the various brain opioid receptors during long-term clomipramine administration would be of value in supporting a potential role for the endogenous opioid system in the pathophysiology of obsessive-compulsive disorder.

Other studies have also suggested that the endogenous opioid system has a role in obsessive-compulsive disorder. One study showed that opioid receptor blockade with naloxone can exacerbate obsessive-compulsive symptoms (Insel & Pickar 1983). Recently, non-medicated obsessive-compulsive disorder patients were shown to have significantly higher titres of serum antibodies against pro-dynorphin, compared with normal volunteers, or patients with schizophrenia, Alzheimer's disease, or multiple sclerosis (Roy et al 1989). Other studies have shown CSF concentrations of dynorphin A [1–8] to be increased in patients with Gilles de la Tourette syndrome, a disorder known to be genetically and clinically linked to obsessive-compulsive disorder (Leckman et al 1988). In this study, CSF dynorphin levels correlated positively in Tourette patients with clinical measures of coexisting obsessive-compulsive symptoms. If confirmed and replicated, this finding could be significant, because clomipramine has been reported recently to decrease another

opioid peptide, met-enkephalin, in a dose-dependant fashion in regional brain levels of rats (Kurumaji et al 1988).

In conclusion, it is conceivable that the down-regulation of μ-opioid receptors observed with chronic clomipramine (although of modest magnitude) may play a role in the therapeutic profile of clomipramine in obsessive-compulsive disorder. Additional animal studies examining in more detail changes in rat brain opioid receptors occurring during chronic administration of clomipramine, as well as clinical studies such as PET studies in obsessive-compulsive disorder patients using the available opioid ligand, [^{3}H]cycloFOXY, and neurochemical and pharmacological challenge studies with selective opioid agonists (off and on clomipramine) are necessary to determine the role of the central opioid system in the pathophysiology of obsessive-compulsive disorder.

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