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^{3}H-Imipramine High-Affinity Binding Sites in Rat Brain. Effects of Imipramine and Lithium

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Abstract. The specific high-affinity binding of ^{3}H-imipramine to rat brain membranes was investigated. Five weeks of lithium treatment decreased the number of binding sites, but had no effect on the affinity constants. Long-term imipramine treatment had no effect on the number of binding sites but apparently decreased the affinity. The latter effect was probably due to imipramine remaining in the membrane preparation.

Key words: ^{3}H-imipramine binding site – Imipramine – Lithium – Rat

Imipramine, as well as other tricyclic antidepressants, binds at several neurotransmitter-receptor binding sites. The affinity between imipramine and these sites may vary a great deal, with dissociation (K_D) or inhibitory (K_I) constants in the range of 20–40,000 nM. The interaction between imipramine and these receptors may be important for both antidepressant effects and side effects, e.g., anticholinergic effects.

Recently, specific, saturable, high-affinity binding sites for imipramine have been described, in brain and platelets, with K_D in the range 2–6 nM (Raisman et al. 1980; Rehavi et al. 1980; Langer and Briley 1981). The functional or pharmacological significance of these binding sites is unclear, but evidence is accumulating to indicate that they are related to the re-uptake sites for serotonin (Paul et al. 1980; Langer et al. 1980; Sette et al. 1981). Another observation of clinical relevance is the decrease in the number of specific imipramine binding sites in rat brain during prolonged treatment with imipramine (Raisman et al. 1980).

The effect of lithium has been studied on a number of receptors in several systems and it has been reported that lithium treatment reduced the number of dopamine receptors (Verimer et al. 1980; Rosenblatt et al. 1980), serotonin receptors (Treiser and Kellar 1980), and acetylcholine receptors (Pestronk and Drachman 1980).

In the present study the effects of imipramine and lithium have been studied on the high affinity binding of imipramine in rat brain.

Material and Methods

Female Wistar rats (of initial weight about 200 g) were used. Rats treated with imipramine received 3 mg per rat per day in the drinking water. Rats treated with lithium received 600 µmole (45 mg Li_2Co_3) per rat per day in the diet. The treatment period was 5 weeks.

The rats were decapitated, and the brain removed and placed on ice. The cerebral cortex was dissected and homogenized (Ultra-turrax, 30 s high speed) in 15 ml ice-cold buffer (50 mM Tris, HCl, 120 mM NaCl, 5 mM KCl, pH 7.5). The homogenate was centrifuged at 30,000 *g* for 10 min, and the pellet was resuspended and centrifuged twice. After the third resuspension, protein concentration was determined (Peterson 1977) and buffer was added to each sample to give a concentration of 2 mg membrane protein/ml. The membranes were stored at −80° C. ^{3}H-imipramine binding was determined by incubation of the membranes with radioactive imipramine, 0.2 mg membrane protein, 0.5–7 nM ^{3}H-imipramine (specific activity 23 Ci/mmol, Radiochemical Centre, Amersham, England), final volume 300 µl. Non-specific binding was determined in paired samples containing 10 µM desimipramine. After a 1-h incubation at 0° C, 5 ml ice-cold buffer was added and the samples were rapidly filtered through Whatman GF/C glass fibre filters. The filters were washed three times with 5 ml ice-cold buffer, dried and counted in 3 ml Picofluor 15 in a Packard 460 CD liquid-scintillation counter.

Specific binding was calculated as the difference between the binding with and without 10 nM desipramine.

The specific bound imipramine, *B*, expressed as femtomoles imipramine bound/mg protein, at the various imipramine concentrations in the incubation medium. *F* nmole/l, were used to construct Scatchard plots by means of linear regression of two variables. Mean values were compaired using Student's *t*-test.

Results

Lithium. After 5 weeks of lithium treatment, ^{3}H-imipramine binding was analysed in rat brain cortex. Using ten concentrations of radioactive imipramine in the range 0.5–7 nM, a Scatchard plot for each rat was constructed, and the dissociation constant, K_D, and the maximal number of binding sites, B_{max}, were determined. In the control rats ($n = 12$) the mean value for K_D was 5.5 ± 0.6 nM (SEM), and mean value for B_{max} was 810 ± 34 (femtomol/mg protein). In the lithium-treated rats ($n = 12$) K_D did not change significantly (5.3 ± 0.6 nM) whereas the number of binding sites decreased by about 15 % (B_{max} = 690 ± 22 femtomol/mg protein). This decrease was statistically significant, $P < 0.01$. In Fig. 1 the mean values for bound ^{3}H-imipramine at the

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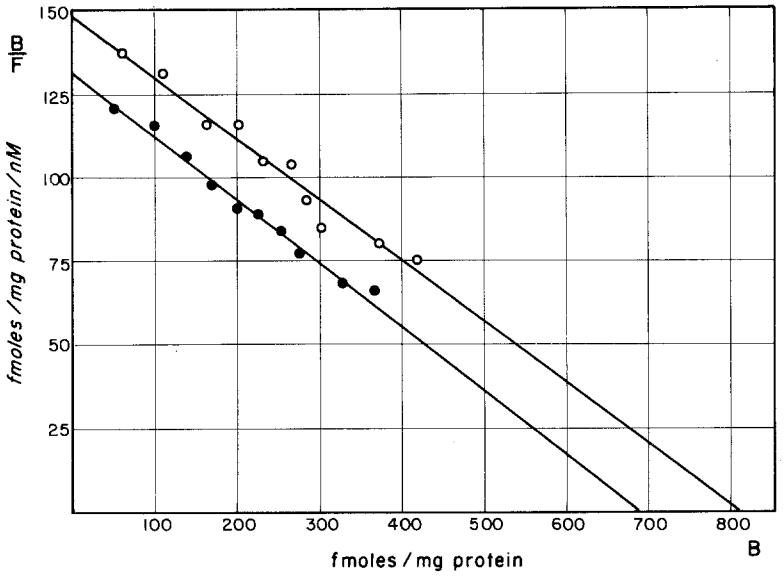

Fig. 1
Scatchard plots for control rats (○——○) and lithium-treated rats (●——●)

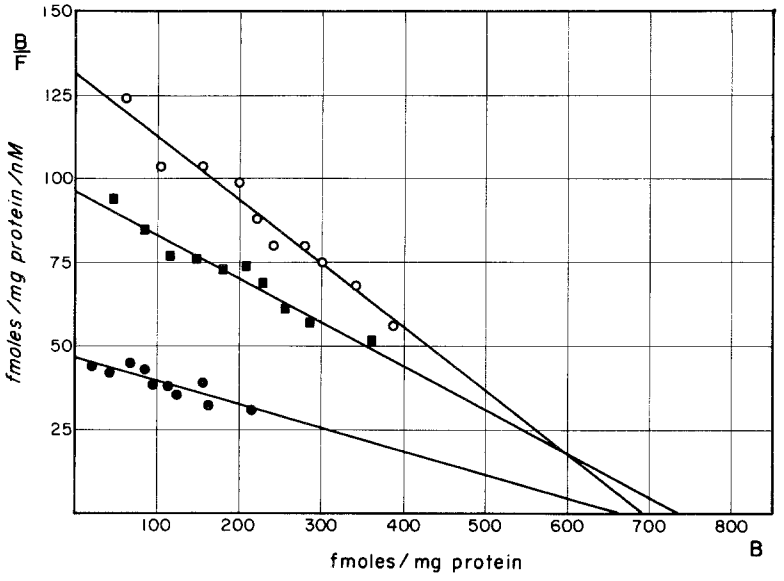

Fig. 2
Scatchard plots for control rats (○——○), imipramine-treated rats without a withdrawal period before decapitation (●——●), and imipramine-treated rats decapitated after a 24-h withdrawal period (■——■)

various concentrations of ^{3}H-imipramine are used to construct a Scatchard plot illustrating these findings.

Imipramine. After 5 weeks of imipramine treatment ^{3}H-imipramine binding was analysed in rat brain cortex. The imipramine-treated rats were divided into three groups (each $n = 3 \times 12$), which were decapitated at various time intervals after administration of imipramine had been terminated. One group was decapitated immediately after withdrawal of imipramine, a second group 24 h later, and a third group after 48 h. Figure 2 shows Scatchard plots for the first two groups and the control group. Data for the 48-h group were almost identical to those of the control group, and thus are not shown in the figure. The *B*-values used in the figure are mean values of the 12 animals in each group for bound ^{3}H-imipramine at the various concentrations of free ^{3}H-imipramine. The K_D value for the control group found in the Scatchard plot was 5.3 nM, and the rats which were decapitated 48 h after imipramine withdrawal had a mean K_D value of 5.2. In contrast, the K_D for the imipramine-treated rats which were decapitated 24 h after imipramine withdrawal was 7.9 nM and in the group which were decapitated immediately following imipramine withdrawal the K_D value was further increased to 13.9 nM. The respective B_{max} values for the four groups were 690, 715, 740 and 660 femtomoles/mg protein.

Figure 3 shows the results of an experiment in which imipramine-treated rats were given additional radioactive imipramine during the last 2 days of the treatment periods. It is seen that radioactivity still remained in the brain 72 h after withdrawal of imipramine and radioactive imipramine. Furthermore, it is seen also that the washed membranes contained radioactivity for up to 36 h.

Discussion

Lithium treatment was found to reduce the number of binding sites in rat brain cortex, without changing the affinity

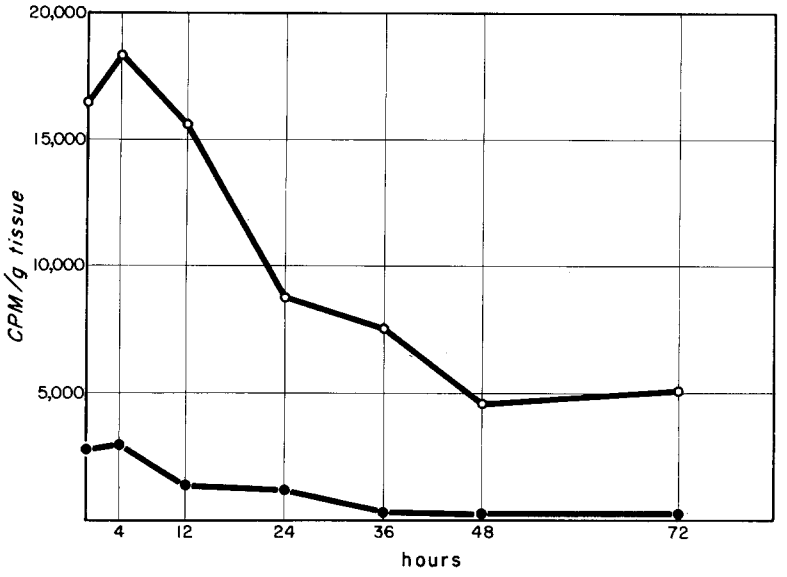

Fig. 3
Radioactivity in whole rat brain (○——○), and in washed membranes (●——●) after 2 days of administration of radioactive imipramine to long-term imipramine-treated rats. Zero time is the time of withdrawal of both imipramine and radioactive imipramine

between the receptor and imipramine, as B_{max} decreased from 800 – 690 femtomol/mg protein, whereas K_D remained unchanged. A similar effect of lithium on other receptors has previously been reported; chronic treatment with lithium was found to decrease dopamine receptor density in rat corpus striatum (Rosenblatt et al. 1980), and reduce the number of serotonin receptors in rat hippocampus (Treiser and Kellar 1980). In addition, changes in receptor concentration and function induced by various treatments may be modified by lithium treatment, e.g., haloperidol-induced dopamine receptor supersensitivity is prevented by lithium treatment (Vermier et al. 1980), and the increase in acetylcholine receptors that occurs in denervated skeletal muscle is inhibited by lithium (Pestronk and Drachman 1980). It is interesting to speculate whether these effects on receptor density are independent specific lithium effects, or whether they are caused by an unspecific lithium effect on, e.g., membrane structure.

Imipramine treatment had no effect on the number of ^{3}H-imipramine binding sites or on the affinity constant when imipramine was withdrawn 48 h before decapitation. One reason for this lack of effect may be that possible changes had been normalized within the 48 h. When the rats were decapitated without any withdrawal period from the imipramine treatment, no obvious change in B_{max} was observed, whereas K_D showed an increase compared with the control rats. The same results were found using a withdrawal period of 24 h, except that the increase in K_D was much smaller. If this increase in K_D was true, the latter finding would indicate that the effect of imipramine progressively disappeared during the withdrawal period. However, a much more likely explanation is that the results are caused by competition during the assay procedure by imipramine remaining in the membrane preparation. This possibility was tested in rats by adding radioactive imipramine to the daily dose of imipramine during the last days of the treatment. It was found that the brain contained detectable radioactivity even 72 h after the withdrawal of imipramine. However, more important was the observation that the washed membrane preparations also contained radioactivity up to 36 h after the last intake of imipramine. Further washings of the membranes did not significantly reduce the amount of radioactivity. During the first 36 h of the withdrawal period, radioactivity in the crude brain homogenate decreased by about 50 %, whereas radioactivity in the membrane preparation decreased more than 90 %, indicating that the imipramine metabolites in the brain during this period became increasingly hydrophilic.

Chronic treatment with desipramine has been reported to decrease high-affinity ^{3}H-imipramine binding about 40 %, with no change in the affinity constant in rats killed without a withdrawal period after the treatment with desipramine (Raisman et al. 1980).

We have observed that desipramine is eliminated from the brain as slowly as imipramine, so that even if the K_D value for desipramine is higher than for imipramine (Langer et al. 1980) the possibility exists that the effect of desimipramine may also be partly due to desipramine remaining in the membrane preparation.

Provided that the present results were also valid for the ^{3}H-imipramine binding sites in human brain, imipramine would reduce the affinity by competition, whereas lithium would reduce the number of binding sites.

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