Much evidence suggests that serotonergic (5-hydroxytryptamine, 5-HT) neurons are important targets for the action of antidepressant drugs. Thus treatment with various classes of antidepressant drugs produces multiple effects on 5-HT neurons and receptors in the brain. The implications of these changes for the mechanism of action of antidepressant drugs are presently not clear. Knowledge regarding the effects of antidepressant drugs is based on studies using gross biochemical techniques, e.g., biochemical binding studies, in large areas of the brain. It is likely that important information is missed by these methods, as selective changes in identified neurons are not detected. Morphometric and microdensitometric methods to analyze the effects of psychoactive drugs on transmitter-identified neurons, especially on the central monoamine neurons, have been developed. The procedures include techniques for the quantitative evaluation of coexistence in nerve cell bodies and nerve terminals as well as analysis of different receptors using quantitative receptor autoradiography in identified neurons in the brain. In this chapter we have used these methods to study the effects of acute and chronic oral treatment with imipramine.

Repeated (2 × 10 μmol/kg p.o. for 14 days) but not acute (10 μmol/kg p.o.) treatment with imipramine produced a 35% reduction of $^{3}H$-LSD (a radioligand for 5-HT$_2$ receptors) binding in layer IV in the frontal cortex and a 37% reduction in the occipital cortex. On the other hand, the binding of $^{125}$I-neuropeptide Y, a new important neuropeptide, was not changed in these regions. Interestingly, in other areas of the cortex (using a different way of cutting the brain) no changes in 5-HT$_2$ receptor binding were observed. These results suggest that imipramine produces selective changes in specific receptor populations in defined areas of the brain. It is notable that receptor autoradiographic studies have shown that most of the 5-HT$_2$ receptors in the cerebral cortex labeled by $^{125}$I-LSD are localized within layer IV. Layer IV is the major target for the specific afferent input to the cerebral cortex. The present data, if shown to be valid for other types of antidepressant drugs, suggest that antidepressant drugs may have an important modulatory influence on specific sensory input to the cerebral cortex.

The question arises whether such changes in 5-HT receptors, caused by chronic antidepressant treatment, are reflected in altered release or receptor activity of
5-HT and peptides known to coexist with 5-HT. Because coexistence presently is established only in the descending bulbospinal pathways, we have used the spinal cord as our model. Coexistence of at least three putative neurotransmitters—substance P (SP), thyrotropin-releasing hormone (TRH), and 5-HT—has been demonstrated in the spinal cord using immunohistochemical methods. The functional significance of the coexistence has not been clarified. However, chronic treatment with antidepressant drugs with monoamine uptake blocking properties has been shown to change the tissue levels of coexisting peptides. Because the radioimmunoassay (RIA) methods (analyzing large areas of the spinal cord) can give only indirect evidence that the observed changes occur in neurons with coexistence, the effect of acute and chronic imipramine treatment was studied using morphometric and microdensitometric techniques.

The microdensitometric studies showed that chronic but not acute imipramine treatment selectively increased SP immunoreactivity in the 5-HT/SP co-storing nerve terminals of the medial part of the ventral horn in both the cervical and the lumbar enlargements (Fig. 21.1). In contrast, the TRH immunoreactivity appeared to be unaltered in the 5-HT nerve terminal systems of the ventral horn by chronic imipramine treatment at the dose level used in this study (2 × 10 μmol/kg p.o.). Quantitative analysis of the entity of coexistence in the 5-HT nerve terminal networks of these areas showed that all the 5-HT nerve terminals in the medial part of the ventral horn of the cervical and lumbar enlargements contained SP and TRH immunoreactivities and that this phenomenon was not changed by acute and chronic treatment with imipramine. The biochemical studies demonstrated that chronic imipramine treatment selectively reduced 5-HT utilization in the ventral horn of the spinal cord as evidenced by a reduced 5-hydroxyindoleacetic acid (5-HIAA/5-HT) ratio in the rats treated chronically with imipramine (Fig. 21.1). It is possible that the selective increases of SP immunoreactivity following chronic imipramine treatment reflect a reduced release of SP from the co-storing nerve terminals secondary to a reduced firing rate in the bulbospinal 5-HT neurons.

![Graph](image.png)

Figure 21.1. Effects of chronic imipramine treatment (2 × 10 μmol/kg p.o. for 14 days) on SP immunoreactivity (IR) in nerve terminal profiles of the cervical enlargement of the spinal cord in the male rat. SP was analyzed in the medial (VM) and lateral (VL) parts of the ventral horn. Means ± SEM are shown (n = 5 rats). In addition, 5-HT and 5-HIAA levels were determined by HPLC in the ventral (VC) and dorsal (DC) horns of the cervical enlargement of the spinal cord. Means ± SEM are shown (n = 7–8 rats). *p<0.05. **p<0.01. (Data are modified from ref. 6.)