Quantitative autoradiographic localization of [³H]imipramine binding sites in the brain of the rat: Relationship to ascending 5-hydroxytryptamine neuron systems

(antidepressant/monoamine/receptor/image analysis/central nervous system)

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ABSTRACT Quantitative autoradiography shows that there is a close relationship between [³H]imipramine binding sites and the distribution of 5-hydroxytryptamine (5-HT) neurons in the rat brain. High labeling is observed in the midbrain raphe nuclei, the areas of the dopamine cell groups of the substantia nigra and of the ventral tegmental area of Tsai, the ventral amygdaloid nucleus, the midline thalamic area, and parts of the hypothalamus. Thus, antidepressant drugs that have high affinity for [³H]imipramine binding sites can exert an influence at the 5-HT cell body as well as at the 5-HT nerve terminal level. The present results underline the possibility that the 5-HT and dopamine hypotheses for the mechanism of action of antidepressant drugs are not mutually exclusive, because both 5-HT and dopamine neurons can be regulated by large numbers of [³H]imipramine binding sites.

During recent years, high-affinity binding sites for the antidepressant drug [³H]imipramine have been found in membrane fractions from various parts of the brain (1). The high-affinity [³H]imipramine binding sites have been shown to be located in the 5-hydroxytryptamine (5-HT) nerve cell membrane and seem to participate in regulation of the 5-HT uptake mechanism (2). Most of the currently used antidepressant drugs have a high affinity for this binding site, indicating its possible importance for the therapeutic activity of antidepressant drugs. These results also underline the importance of 5-HT neurons as targets for antidepressant action (3, 4) of imipramine and antidepressant drugs of the imipramine type.

Knowledge of the exact regional distribution of the [³H]imipramine binding sites may facilitate our understanding of the mechanism(s) underlying the therapeutic action of antidepressant drugs acting at these sites. In the present paper, we describe a quantitative receptor autoradiographical analysis of the regional distribution of [³H]imipramine binding sites and their location, especially in relationship to various ascending 5-HT pathways.

MATERIAL AND METHODS

Male specific-pathogen-free Sprague-Dawley rats (200 ± 20 g) (Anticimex, Stockholm, Sweden) were used. They were kept under standardized light/dark conditions (lights on at 08:00 and off at 20:00). They were given water and food pellets ad lib. Under Brietal anesthesia (Eli Lilly), the rats were killed by perfusion with phosphate-buffered saline/0.1% paraformaldehyde. Fifty minutes after perfusion, the brains were rapidly dissected out and frozen and various parts of the brain were sectioned in a cryostat (Reichert, Vienna, Austria). The 14-μm-thick cryostat sections were dried for 30 min in a stream of cold

air. After drying, the sections were incubated with 15 nM [³H]imipramine for 1 hr at 4°C. The binding procedure was based on that of Raisman *et al.* (1). Nonspecific binding was determined by incubation of adjacent sections with [³H]imipramine in the presence of 10 μM desipramine. After rapid washing with two 5-ml portions of 170 mM Tris·HCl/120 mM NaCl/5 mM KCl, pH 7.4, and one portion of distilled water, the various coronal sections of the brain were autoradiographed using ³H-sensitive film (Ultrofilm; 24 × 30 cm; LKB). Exposure was for 6 wk at -80°C. The receptor autoradiogram was developed at 22°C for 4 min with a 1:6 dilution of Gaevort 615 developer, washed, and fixed with a 1:5 dilution of Ilford Hypam rapid fixative for 2 min.

Quantitation of the receptor autoradiograms was carried out as described by Unnerstall *et al.* (5) with some modifications (see below). With this procedure and standards containing various concentrations of [³H]imipramine and considering the quenching of radioactivity that takes place in the grey matter (6), it is possible to calculate the concentration (in fmol) of [³H]imipramine bound per mm² of the structure under study. Briefly, the standards were prepared by using 10-μl droplets of Tris buffer containing [³H]imipramine in the concentration range 0.5–30 nM (eight concentrations). These droplets were applied to gelatine-coated object glasses, which were dried at reduced pressure and subsequently apposed to the tritium-sensitive film together with the object glasses with the brain sections. The following rostro-caudal levels of the brain were analyzed in coronal sections [according to the König and Klippel atlas (7)]: A 160, A 1,760, A 3,430, and A 5,910.

The optical density of the tritium-exposed film was measured by using an automatic image analyzer (TESAK system) connected to a PDP 11 computer. The neuroanatomical structures to be measured were selected by a light pen on a television screen showing the whole coronal section to be analyzed. This procedure was carried out by staining the section with cresyl violet after the exposure had been completed and then comparing the autoradiogram with the cresyl violet-stained section on the television screen.

To obtain specific [³H]imipramine binding, measurements were always made of two adjacent sections, one showing total binding and one showing nonspecific binding (i.e., that of sections that had been incubated with [³H]imipramine in the presence of 10 μM desipramine). The mean optical attenuation obtained in a selected area of the autoradiogram showing total binding was then reduced by the mean optical attenuation of the background (radioactivity overlying white matter) of the same autoradiogram, and the corresponding value (in fmol) was ob-

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Abbreviation: 5-HT, 5-hydroxytryptamine.

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tained from the standard curve. The same type of procedure was carried out for the receptor autoradiogram showing non-specific binding of [^3H]imipramine as well as for each concentration in the standard curve. In the latter cases, the background values were obtained by measuring the mean optical attenuation of droplets of buffer lacking [^3H]imipramine. The specific binding (in fmol/mm²) of an area was then obtained by calculating the difference between the total binding of the area and its nonspecific binding. Intraassay variability of the measurements was $\approx 5\%$, and interassay variability was $\approx 10\%$.

Results of the overall evaluation of optical density in the receptor autoradiograms, as determined by the use of the image analyzer, which codes the optical densities into 16 tones, are shown in Fig. 1 A–D. The absolute amounts of specific [^3H]imipramine binding in selected brain areas are given in Table 1.

RESULTS

In agreement with available biochemical results (2) on the distribution of [^3H]imipramine binding sites in the brain, the

Table 1. Quantitative autoradiographic analysis of specific [^3H]imipramine binding sites in various nuclei of the mesencephalon, diencephalon, and telencephalon of the normal male rat

Area		Binding, fmol/mm ²		Area	Binding, fmol/mm ²
Areas shown in Fig. 1A					
co	Cortex occipitalis	0.052 ± 0.002	sgcv	Substantia grisea centralis, pars ventralis	0.447 ± 0.002
s	Subiculum	0.135 ± 0.006			
so	Stratum opticum colliculi	0.234 ± 0.002	rd	Nucleus raphe dorsalis	0.660 ± 0.010
si	Colliculus inferior	0.101 ± 0.006	rm	Nucleus raphe medianus	0.393 ± 0.002
llv	Nucleus lemnisci late- ralis ventralis	0.009 ± 0.008	po	Nucleus pontis	0.116 ± 0.002
			for	Formatio reticularis	0.107 ± 0.001
sgcd	Substantia grisea cen- tralis, pars dorsalis	0.227 ± 0.002			
Areas shown in Fig. 1B					
	Cortex medialis	0.045 ± 0.002	op	Nucleus pretectalis profundis	0.221 ± 0.005
	Cortex dorsalis	0.057 ± 0.003	ip	Nucleus interpenduncularis	0.150 ± 0.006
	Cortex lateralis	0.115 ± 0.002	DT	Decussatio tegmenti (A10)	0.496 ± 0.004
ce	Cortex entorhinalis	0.196 ± 0.01	sgc	Substantia grisea centralis	0.222 ± 0.005
h	Hippocampus	0.192 ± 0.002	for	Formatio reticularis	0.139 ± 0.001
ccgm	Nucleus centralis cor- pori geniculati medialis	0.168 ± 0.002	snc	Substantia nigra, pars compacta	0.434 ± 0.005
apm	Area pretectalis medialis	0.258 ± 0.001	snr	Substantia nigra, pars reticulata	0.391 ± 0.008
Areas shown in Fig. 1C					
	Cortex medialis	0.061 ± 0.003	hl	Nucleus lateralis habenulae	0.132 ± 0.001
	Cortex dorsalis	0.047 ± 0.001	nhp	Nucleus hypothalamicus posterior	0.274 ± 0.006
	Cortex lateralis	0.035 ± 0.006			
pi	Cortex piriformis	0.065 ± 0.003	sut	Nucleus subthalamicus	0.106 ± 0.008
cl	Clastrum	0.082 ± 0.002	MFB	Medial forebrain bundle	0.362 ± 0.001
cp	Nucleus caudatus putamen	0.150 ± 0.001	hlh2	Forel H1-H2	0.126 ± 0.002
al	Nucleus amygdaloideus lateralis	0.257 ± 0.003	pvs	Nucleus premammilaris ventralis	0.291 ± 0.001
apo	Nucleus amygdaloideus posterior	0.128 ± 0.006	tpm	Nucleus posterior medialis thalami	0.302 ± 0.001
amp	Nucleus amygdaloideus medialis posterior	0.203 ± 0.005	tlp	Nucleus posterior lateralis thalami	0.119 ± 0.003
abp	Nucleus amygdaloideus basalis posterior	0.106 ± 0.002	t1	Nucleus lateralis thalami	0.112 ± 0.002
			tr	Nucleus reticularis thalami	0.053 ± 0.003
h	Hippocampus	0.078 ± 0.005	tv	Nucleus ventralis thalami	0.052 ± 0.001
gd	Gyrus dentatus	0.099 ± 0.001	tvb	Nucleus ventralis thalami pars dorsalis	0.036 ± 0.003
dcgl	Nucleus dorsalis corpori geniculata lateralis	0.135 ± 0.003			
			tpo	Nucleus posterior thalami	0.158 ± 0.008
hm	Nucleus medialis habenulae	0.144 ± 0.007			
Areas shown in Fig. 1D					
	Cortex medialis	0.167 ± 0.001	pt	Nucleus paratenialis	0.278 ± 0.001
	Cortex dorsalis	0.111 ± 0.003	pvs	Nucleus periventricularis stellatocellularis	0.383 ± 0.009
	Cortex lateralis	0.120 ± 0.002			
	Cortex supragenualis	0.239 ± 0.002	pvr	Nucleus periventricularis rotundocellularis	0.434 ± 0.001
pi	Cortex piriformis	0.187 ± 0.001			
cp	Nucleus caudatus putamen	0.203 ± 0.003	re	Nucleus reuniens	0.454 ± 0.003
gp	Globus pallidus	0.198 ± 0.001	npt	Nucleus periventricularis hypothalami	0.369 ± 0.002
tr	Nucleus reticularis thalami	0.251 ± 0.001			
			nha	Nucleus hypothalamicus anterior	0.251 ± 0.002
tam	Nucleus anterior medialis thalami	0.244 ± 0.002			

Results represent mean $\pm$ SEM ($n = 3$). Values in boldface type fall outside the overall mean by >1 SD.

FIG. 1. (Legend appears at the bottom of the next page.)

FIG. 2. [^3H]Imipramine receptor autoradiogram from a coronal section of rat brain at KK level 160. The densitometric map of this receptor autoradiogram is shown in Fig. 1A. ($\times 2$.)

present observations based on the autoradiographic technique reveal a marked difference in the distribution of the [^3H]imipramine binding sites at the four rostro-caudal levels analyzed. The quantitative analysis shows a >10 -fold difference in amounts of [^3H]imipramine per unit area of different brain areas (Table 1). The highest densities [i.e., densities higher than the overall mean $+ 1$ SD (boldface type in Table 1)] were observed within the nucleus raphe dorsalis and medianus, the substantia grisea centralis, pars ventralis (Figs. 1A and 2), the substantia nigra (Fig. 1B), the area medial of the substantia nigra (Fig. 1) containing the A10 dopamine cell group according to Dahlström and Fuxe (8), and the midline thalamic area [especially within the nucleus periventricularis stellato and rotundocellularis, nucleus reuniens and nucleus periventricularis hypothalami (Fig. 1C and D)] while the superficial layers of the superior colliculus (Figs. 1A and 2) and the lateral amygdaloid nucleus (Fig. 1C) had somewhat lower densities. Another consistent finding was the high degree of binding of [^3H]imipramine in areas in which the 5-HT axon bundles ascend (Figs. 1A–D and 2) such as the midline of the midbrain and the lateral hypothalamic area, which is traversed by the medial forebrain bundle. These areas also contain medium to high densities of 5-HT terminals (9). It should be noted that some hypothalamic areas (such as the nucleus anterior hypothalami) and thalamic nuclei (such as the nucleus paratenialis and nucleus reticularis thalami) exhibit high levels of [^3H]imipramine binding although the density of 5-HT nerve terminals in these areas is low (9). The dorsal and lateral cortical areas as a rule showed weak labeling with [^3H]imipramine with the exception of parts of cingulate cortex and supragenual cortex (Fig. 1D and Table 1), while the ventral hippocampal formation, the subiculum, and the entorhinal and piriform cortex showed moderate labeling with [^3H]imipramine (Fig. 1A–C and Table 1).

DISCUSSION

The present results, based on autoradiographic localization of [^3H]imipramine binding, indicate that a major action of the antidepressant drug imipramine could be exerted in certain subcortical areas as well as in the midbrain raphe nuclei and the

substantia nigra. The results suggest that the ascending 5-HT neurons are a major target for antidepressant action (3, 9–11) of imipramine-like drugs, because the distribution to a large extent coincided with the distribution of 5-HT cell bodies and nerve terminal systems (8). Of particular interest in this regard is the large amount of [^3H]imipramine binding found in the substantia nigra and in the ventral tegmental area containing the dopamine cell bodies giving rise to the meso-telencephalic dopamine systems, which are heavily innervated by 5-HT terminals. Although it remains to be established whether the [^3H]imipramine binding sites reside on 5-HT afferents or on post-synaptic elements within the ventral tegmentum, our findings suggest a possible role of imipramine binding sites in the modulation of dopamine neurotransmission in the ascending dopamine neuron system to the telencephalon (10, 13). Because the substantia nigra dopamine neurons and the ventral tegmental dopamine neurons project mainly to neostriatum and limbic areas, respectively (14, 15), antidepressant drugs can probably influence both striatal and limbic dopamine projections. Interesting in this regard is the observation that some depressed patients show a psychomotor retardation that could involve dysregulation of dopamine systems (10, 13).

Obviously, the large numbers of [^3H]imipramine binding sites present in the amygdaloid complex, especially in the lateral amygdaloid nucleus and in the midline thalamic area, are of substantial interest when looking for potential target areas of antidepressant drugs. The amygdaloid cortex has previously been considered an important area in the regulation of emotional and food-searching behaviors (16). As a rule, the limbic cortical areas were more heavily labeled by [^3H]imipramine than the neocortical areas, again underlining the old cortex with its 5-HT terminal systems as a target area for antidepressant drugs. The midline and intralaminar thalamic nuclei are known to send projections into many parts of the cerebral hemispheres, mainly affecting the activity of the associational cortex (17).

The high number of [^3H]imipramine binding sites in the midbrain raphe nuclei suggests new aspects on how [^3H]imipramine binding sites can regulate 5-HT neuronal function. At the cell body level, they may regulate the synthesis of 5-HT and 5-HT comodulators as well as the firing rate. The [^3H]imipramine binding sites are also clustered in certain areas (see above) that contain a high density of 5-HT nerve terminal systems, such as the substantia nigra, the superior colliculus, and the lateral amygdaloid cortex, which is in line with the view that the [^3H]imipramine site is a regulator of 5-HT uptake and release mechanisms (2). The present findings also indicate that [^3H]imipramine binding sites may also be located in the 5-HT nerve cell membrane of the nonterminal axons. This result is expected in view of the fact that the 5-HT reuptake mechanism is also in this part of the neuron (18). The relationship between 5-HT terminal networks and the [^3H]imipramine binding sites in some nuclei of the midline thalamic area and in anterior hypothalamus is unclear in view of the relatively low number of 5-HT terminal networks and the relatively high degree of [^3H]imipramine binding found in these areas. Therefore, the possibility must be considered that some [^3H]imipramine binding sites may be linked to other types of transmitter-identified neurons than the 5-HT neurons.

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FIG. 1 (on preceding page). Densitometric maps printed by a PDP 11 computer after image analysis of autoradiograms of [^3H]imipramine receptors by the TESAK system. Density maps were obtained by coding the original autoradiograms in 16 gray tones. Anatomical landmarks shown on the right were obtained by comparison of Nissl-stained sections with the corresponding plots of the König and Klippel (KK) atlas. (A) KK 160. (B) KK 1,760. (C) KK 5,910. (D) KK 3,430. The abbreviations reported are mainly according to the König and Klippel atlas; the complete list is given in Table 1.

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