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Review

Binding of some antidepressants to the 5-hydroxytryptamine transporter in brain and platelets

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Abstract. Antidepressant agents with properties to inhibit 5-hydroxytryptamine (5–HT, serotonin) uptake in brain tissue and platelets bind with high affinities to neuronal and platelet membranes. [^{3}H]Imipramine, [^{3}H]paroxetine and [^{3}H]citalopram label specific binding sites related to the 5–HT transporter. [^{3}H]Paroxetine and [^{3}H]citalopram appear to be better ligands than [^{3}H]imipramine. The former label a homogenous population of binding sites, whereas the displaceable binding of [^{3}H]imipramine is heterogenous. Recent observations in several laboratories, which have taken the heterogeneity of [^{3}H]imipramine binding into account, indicate that the binding of antidepressants to the 5–HT transporter probably occurs to the same site that binds 5–HT for transport and not to a separate site as previously suggested. Additional bonds to subsites in close vicinity to the 5–HT recognition site may contribute to the binding. No convincing evidence has been presented of the existence of an endogenous ligand other than 5–HT itself that binds to the [^{3}H]imipramine binding site. Recent studies also suggest that repeated treatment of rats with antidepressant agents does not produce any alterations of the binding of [^{3}H]imipramine or [^{3}H]paroxetine to membranes of cerebral cortex. It is also doubtful whether the density of the 5–HT uptake site in platelets measured with these ligands is decreased in affective disorders as first reported.

Key words: Antidepressants – 5–HT uptake – Binding – Brain – Platelets

It is now more than 10 years since the important discovery was made that the antidepressant agent imipramine binds with high affinity to platelet plasma membranes (Rudnick and Talvenheimo 1978; Briley et al. 1979; Talvenheimo et al. 1979) and membrane fragments of brain (Raisman et al. 1979; Paul et al. 1980). This binding of imipramine was found already in the first studies to be associated with the 5-hydroxytryptamine (5–HT, serotonin) carrier mechanism (transporter) for which imipramine is a potent inhibitor both in platelets (see Stahl 1985 for references) and brain slices (Blackburn et al. 1967; Ross and Renyi 1969). Since the antidepressant action of imipramine and other tricyclic agents has been suggested to be at least partly due to the inhibition of the re-uptake of 5–HT into the nerve terminals (Lapin and Oxenkrog 1969; Carlsson et al. 1969), the high affinity binding of [^{3}H]imipramine to brain membranes became a new technique to study the 5–HT transporter. Over the last few years new data have emerged not only from studies on the [^{3}H]imipramine binding but also from those using more selective 5–HT uptake inhibitors as ligands. Our aim in this review is to discuss available research data on the 5–HT transporter in brain tissue and platelets.

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The 5–HT transporter in brain tissue and platelets

The 5–HT transporter associated with the membranes of the 5–HT neurons and platelets is a very efficient system for the reuptake of the released 5–HT molecules. Like the other amine transporters it has the characteristics of a secondary carrier system, i.e. it is saturable, requires energy, is temperature and Na^+ dependent, is inhibited by ouabain and by specific uptake inhibitors (Ross 1982). Since it is possible to obtain a homogenous preparation of platelets, the mechanism of the transport of 5–HT across the membrane has been extensively studied in platelets (for review see Given and Longenecker 1985; Stahl 1985). Of particular interest are the studies with plasma membrane vesicles of porcine platelets because the transport of 5–HT across the membrane can be examined separately from intracellular events (Rudnick 1977; Rudnick and Nelson 1978; Nelson and Rudnick 1979; Talvenheimo et al. 1979, 1983; Humphreys et al.

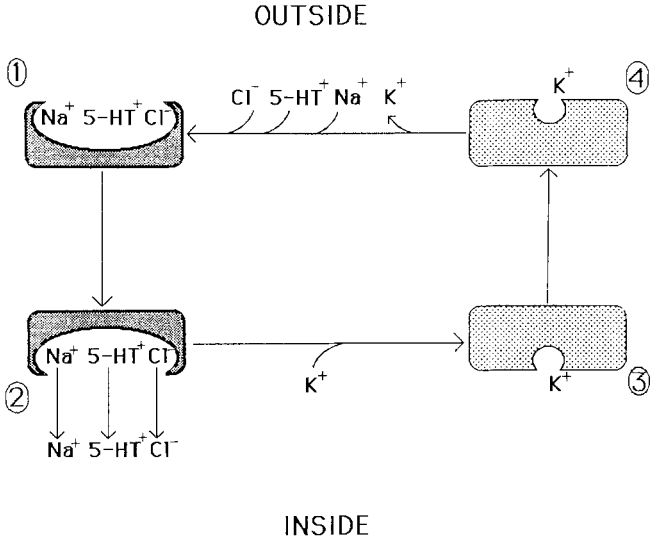

Fig. 1. Scheme of the carrier mediated transport of 5–HT through the neuronal or platelet membrane. *1* Na^+ and the protonated form of 5–HT in this order bind to the proteinaceous carrier. Cl^- is not required for the binding but is necessary for the net transport. *2* The translocation involves a conformation change of the carrier whereby Na^+, 5–HT and Cl^- are dissociated inside the membrane. *3* K^+ binds to the carrier and *4* the complex is translocated back to the exterior side where the binding of Na^+ to the carrier makes it ready for a new translocation cycle

1988). These studies have given the following picture of the 5–HT transporter in the platelet plasma membrane that is also valid for the transporter in the 5–HT neuron (O'Reilly and Reith 1988): one Na^+ in platelets or two Na^+ in neurons and the protonated form of 5–HT in this order bind to the proteinaceous carrier. Cl^- is not required for the binding but is necessary for the net transport. The translocation involves a conformational change of the carrier whereby Na^+, 5–HT and Cl^- are dissociated inside the membrane. K^+ binds to the carrier and the complex is translocated back to the exterior side where the binding of Na^+ to the carrier makes it ready for a new translocation cycle (Fig. 1). From measurements of V_{max} of the 5–HT transport and B_{max} of the binding of labelled 5–HT uptake inhibitors to this transporter (see below) one such translocation cycle has been estimated to take 0.1 s in human platelets (Ross, unpublished observations) and 0.2 s in porcine platelets (Talvenheimo et al. 1979) and 1 s in synaptosomes from rat cerebral cortex (Ross and Hall 1983). If we can assume that one molecule of imipramine or paroxetine binds to one 5–HT transporter, there are 300–500 5–HT transporters (estimated by density of [^{3}H]imipramine or [^{3}H]paroxetine binding sites) in one human platelet (Hrdina 1989; Andersson and Marcusson 1989).

Binding sites for 5–HT uptake inhibitors in brain tissue

The uptake site for 5–HT has been studied in binding experiments with several different radioligands, which are believed to bind to the exterior part of the 5–HT transporter. In addition to the radioligands reviewed below, i.e. [^{3}H]imipramine, [^{3}H]paroxetine and [^{3}H]citalopram, the 5–HT uptake site has been studied in binding experiments with [^{3}H]norzimeldine (Hall et al. 1982), [^{3}H]cyanoimipramine (Dumbrille-Ross and Tang 1983), [^{3}H]cocaine (Reith et al. 1984), [^{3}H]indalpine (Bénavidès et al. 1985) and [^{125}I]iodoimipramine (Humphreys et al. 1989).

[^{3}H]Imipramine binding

The most commonly used radioligand is [^{3}H]imipramine, which was found to label with nanomolar affinity binding sites related to the 5–HT uptake process in rat brain tissue (Raisman et al. 1979; Langer et al. 1980). The [^{3}H]imipramine binding in rat brain is sodium dependent (Briley and Langer 1981), correlates with the regional distribution of endogenous 5–HT (Palkovits et al. 1981) and is at least partly located on 5–HT terminals (Sette et al. 1981; Groß et al. 1981; Paul et al. 1981; Sette et al. 1983a; Hrdina 1985).

In later studies it was demonstrated that both high and low affinity [^{3}H]imipramine binding sites exist and that only the high affinity sites are related to the 5–HT uptake system (Reith et al. 1983; Hrdina 1984). Inhibition studies with 5–HT and 5–HT uptake inhibitors confirmed the heterogeneity of [^{3}H]imipramine binding (Sette et al. 1983b). The heterogeneity of the binding appears to be due to at least two separate binding sites; one high affinity (1 nM) site of protein nature that correlates with the regional distribution of 5–HT uptake and one low affinity (> 100 nM) site that is not of protein nature and does not correlate with the regional distribution of 5–HT uptake (Marcusson et al. 1985). Only the high affinity [^{3}H]imipramine binding site is sodium dependent and located on serotonergic nerve terminals (Marcusson et al. 1986; Severson et al. 1986; Hrdina 1987a).

A proper pharmacological definition of the specific [^{3}H]imipramine binding is of course crucial. High-affinity binding of [^{3}H]imipramine can be distinguished by using non-tricyclic selective 5–HT uptake inhibitors as displacing agents (e.g. paroxetine, citalopram, norzimeldine) (Mellerup and Plenge 1986; Marcusson et al. 1986) or 5–HT itself (Marcusson et al. 1986; Bäckström and Marcusson 1987). The use of desipramine-sensitive [^{3}H]imipramine binding, however, will result in binding not only to 5–HT uptake sites, but also to non-specific binding sites that may represent 40–50% in rat cortex and 80–90% in human cortex of the desimipramine displaceable binding (Marcusson et al. 1986; Bäckström and Marcusson 1987). This may sometimes result in erronous results in applied studies, e.g. desipramine-defined [^{3}H]imipramine has been found to show a marked age-related increase (Severson et al. 1985; Marcusson et al. 1987), whereas 5–HT-sensitive [^{3}H]imipramine binding remains unaltered or decreases with age (Marcusson et al. 1987).

Since the high affinity fraction of [^{3}H]imipramine binding was competitively inhibited by 5–HT and 5–HT uptake inhibitors, it was suggested that this fraction

represents binding to the substrate recognition site for 5–HT uptake and a "single-site model" of the [^{3}H]imipramine binding and 5–HT uptake site was proposed (Marcusson et al. 1986).

One major argument for the hypothesis that 5–HT and imipramine bind to different sites has been the observation that the K_m value of 5–HT uptake is considerably lower than the K_i value of 5–HT in inhibiting the [^{3}H]imipramine binding. This discrepancy between the numeric value of the kinetic constants is, however, to be expected in the single site model, since the rate limiting step in the transport of 5–HT is not the formation of the 5–HT-carrier complex but the translocation step, especially the reorientation of the unloaded transporter (cf Marcusson et al. 1986). Thus, a considerable part of the transporter is not available for binding 5–HT at the outside of the membrane but is on transportation service within or inside the membrane, resulting in saturation at lower concentration than the true equilibrium dissociation constant for the binding of 5–HT to the transporter. From mathematics of a model of the analogous noradrenaline transport, based on a steady-state assumption, Schömig et al. (1988) have concluded that the half-saturating substrate concentration (K_m) is not identical with the dissociation constant for the binding of a substrate to the substrate recognition site (K_d) and that the discrepancy between K_m and K_d is expected to be negatively correlated with the maximal initial transport rate of the substrate (V_{max}). However, in a study of the inhibition by 5–HT of the sodium-dependent [^{3}H]imipramine binding to crude membranes from rat cerebral cortex, Hrdina (1988) reported a K_i value (45 ± 18 nM) well within the range of its reported K_m values (32–102 nM) for uptake by brain synaptosomes.

In human brain tissue, the initial studies also pointed to a homogenous population of [^{3}H]imipramine binding sites (Rehavi et al. 1980; Langer et al. 1981). However, more recent studies have demonstrated both high and low affinity sites where only the high affinity sites are related to the 5–HT uptake site (Cash et al. 1985; Bäckström and Marcusson 1987; Marcusson et al. 1987; Plenge et al. 1990).

[^{3}H]Paroxetine binding

In comparison with imipramine, paroxetine is a more potent and selective inhibitor of 5–HT uptake (Hyttel 1982). The [^{3}H]paroxetine binding in rat brain exhibits a 50–100 fold higher affinity (K_d 0.05–0.15 nM) than [^{3}H]imipramine binding (Habert et al. 1985; Mellerup and Plenge 1986). In contrast to [^{3}H]imipramine binding, most of the [^{3}H]paroxetine binding is abolished after destruction of 5–HT terminals, suggesting a selective localization to 5–HT neurites (Habert et al. 1985; Marcusson et al. 1988a). Drug competition studies using 5–HT and 5–HT uptake inhibitors produced competitive inhibition patterns (Marcusson et al. 1988a; Marcusson et al. 1989). In drug-induced dissociation experiments when comparatively low concentrations of competing drugs were used, the [^{3}H]paroxetine binding appeared to be homogenous (Marcusson and Eriksson 1988). However, when 200–1000 times higher concentrations of competing drugs were used, non-homogenous dissociation patterns were obtained (Plenge and Mellerup 1985).

These dissociation data were recently confirmed for both [^{3}H]paroxetine and [^{3}H]imipramine, where low concentrations (0.01–1 μM) of, e.g. citalopram-induced competitive dissociation patterns, whereas higher concentrations slowed the dissociation (Schoemaker et al. 1989). Similar findings were noted for [^{3}H]paroxetine in the study by Graham et al. (1989). [^{3}H]Paroxetine binding has also been performed in vivo. In the study by Scheffel et al. (1989), it was shown that the in vivo distribution of [^{3}H]paroxetine binding in mouse brain correlated well with the known in vitro distribution and that the binding is closely related to the 5–HT uptake site.

In the human brain, [^{3}H]paroxetine binding exhibits the same pharmacological characteristics as in rat brain (Bäckström et al. 1989; Marcusson et al. 1989). The potencies of various compounds in inhibiting [^{3}H]paroxetine binding in human brain seem to correlate with their potencies in inhibiting 5–HT uptake in rat brain (Laruelle et al. 1988). However, the amount of specific binding is lower; for example in cortical areas the specific binding represents about 40% of total binding in human brain in comparison with 90% in rat brain (Marcusson et al. 1988a; Bäckström et al. 1989). In a recent report on human brain tissue by Plenge et al. (1990), it was concluded that [^{3}H]paroxetine and [^{3}H]citalopram in comparison with [^{3}H]imipramine allowed a more precise determination of 5–HT uptake sites (Plenge et al. 1990).

[^{3}H]Citalopram binding

In comparison with the labeling of multiple binding sites by [^{3}H]imipramine, [^{3}H]citalopram appears to be a selective radioligand for the 5–HT uptake site with a K_d of 0.8 nM in rat brain (D'Amato et al. 1987a). The binding is almost completely abolished after lesioning of 5–HT neurones in rat brain and potencies of the drugs for the inhibition of [^{3}H]citalopram binding and 5–HT uptake is closely correlated (D'Amato et al. 1987a). Similarly to [^{3}H]paroxetine binding, the [^{3}H]citalopram binding is competitively inhibited by 5–HT and imipramine and the dissociation rate is not altered by the addition of 5–HT or 5–HT uptake inhibitors (D'Amato et al. 1987a).

The [^{3}H]citalopram binding in human brain tissue is different from [^{3}H]paroxetine binding on one important aspect: the 5–HT sensitive [^{3}H]citalopram binding in human cortex represents 80%, whereas that of [^{3}H]paroxetine represent only about 40–50% (D'Amato et al. 1987b; Bäckström et al. 1989). This suggests that [^{3}H]citalopram may be a better radioligand for studying the 5–HT uptake site.

In summary, when considering the most recent studies on [^{3}H]imipramine, [^{3}H]paroxetine and [^{3}H]citalopram binding, the radioligand binding to the 5–HT uptake site is consistent with a single-site binding model. Of course, this requires an adequate definition of the specific binding. The single-site model of the 5–HT uptake site

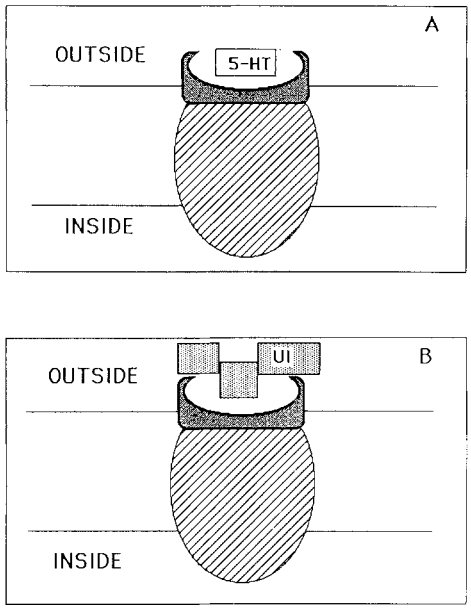

Fig. 2A, B. Single-site model of the 5–HT uptake site/antidepressant binding site. **A** 5–HT is bound to the substrate recognition site of the 5–HT carrier and transported through the carrier (see also Fig. 1 for details). **B** The 5–HT uptake inhibitor *(UI)* has bound to the 5–HT carrier and is at least partly overlapping the 5–HT substrate recognition site. The uptake inhibitor also has other bonds outside the substrate recognition site, which are unique to each uptake inhibitor. These bonds contribute to the 5–HT uptake inhibiting properties

proposes a common binding site for 5–HT and its uptake inhibitors. But the 5–HT uptake inhibitors probably have additional bonds outside the substrate recognition site, contributing to their uptake inhibiting properties (Fig. 2). The 5–HT uptake site is subject to allosteric alterations during the translocation of 5–HT. The dissociation studies cited above raise the possibility that 5–HT uptake inhibitors at high concentrations also may cause allosteric alterations of the 5–HT uptake site. Whether these alterations involve a separate low-affinity site for 5–HT and 5–HT uptake inhibitors or non-specific membrane effects remains to be clarified.

Regional distribution of 5–HT uptake sites by autoradiography

Studies of the regional distribution of 5–HT uptake sites by autoradiography have been performed with several radioligands. The first studies on rat brain used [^{3}H]imipramine with 100 μM imipramine or 100 μM desipramine to define non-specific binding (Biegon and Rainbow 1983; Dawson and Wamsley 1983; Grabowsky et al. 1983). These studies reported on high binding densities in the raphe area, superior colliculi and substantia nigra and several-fold lower densities in cortical areas, striatum and thalamus. Lesions with 5,7-dihydroxytryptamine (5,7–DHT) resulted only in a 50% decrease of [^{3}H]imipramine binding in rat brain (Dawson and Wamsley 1988). This is likely to be explained by the fact that desipramine-sensitive [^{3}H]imipramine binding also represent binding to non-serotonergic sites (see above). In an autoradiographic study on rat brain with [^{3}H]paroxetine dispiaced by 1 μM citalopram, lesions of serotonergic neurons with 3,4-methylenedioxyamphetamine resulted in a > 70% loss of binding, suggesting a more selective labeling of 5–HT uptake sites (De Souza and Kuyatt 1987). In this study the highest binding densities were found in the brain stem nuclei, moderate binding in prefrontal, frontoparietal and primary olfactory cortex, basal ganglia, thalamus, hypothalamus and hippocampus, whereas low densities were noted in the remaining neocortical areas. Insignificant densities were detected in white matter and cerebellum (De Souza and Kuyatt 1987). In the study by Cortés et al. (1988) the autoradiographic distribution of 5–HT uptake sites was studied both with [^{3}H]imipramine (sensitive to 100 μM desipramine) and [^{3}H]paroxetine (sensitive to 10 μM fluoxetine), and these radioligands were reported to label about the same population of binding sites. However, in this study the concentration of [^{3}H]paroxetine was 10^4 times higher than the apparent K_d for [^{3}H]paroxetine binding to human brain tissue, which makes binding to sites other than 5–HT uptake sites likely. For example, the [^{3}H]paroxetine binding to cortical areas is several-fold higher than reported B_{max} values for human frontal cortex in homogenate studies (Bäckström et al. 1989). In a very recent autoradiographic study on rat brain, [^{3}H]imipramine (sensitive to 100 μM desipramine) and [^{3}H]paroxetine (sensitive to 10 μM fluoxetine) binding, with radiologand concentrations around K_d, respectively, was compared (Hrdina et al. 1990). This study by Hrdina et al. clearly demonstrated that desipramine sensitive binding was more diffuse with a relatively high cortical binding density that was only partially decreased after lesion with parachloroamphetamine. However, the [^{3}H]paroxetine binding exhibited a more heterogenous distribution and was almost completely abolished by lesioning. A selective labeling of 5–HT uptake sites in autoradiographic studies on rat brain seems to occur also with [^{3}H]citalopram (D'Amato et al. 1987a). [^{3}H]Cyanoimipramine may also be a suitable ligand for the 5–HT uptake sites, since quantitative autoradiography showed similar B_{max} values to those reported for [^{3}H]citalopram and lesions with 5,7-dihydroxytryptamine resulted in an almost complete elimination of the binding (Kovachich et al. 1988).

Binding sites for 5–HT uptake inhibitors in platelets

[^{3}H]Imipramine binding

The mechanism of the binding of [^{3}H]imipramine to platelet membranes has been extensively studied by Rudnick and collaborators (Talvenheimo et al. 1979, 1983; Talvenheimo and Rudnick 1980; Humphreys et al. 1988). Although the binding of one imipramine molecule requires two Na^+ ions, whereas only one Na^+ ion is necessary for the 5–HT transport, the results obtained

are in accordance with the view that imipramine binds to the 5–HT transport site. The competitive inhibition of the [^{3}H]imipramine binding by 5–HT itself and by the 5–HT uptake inhibitors fluvoxamine, norzimeldine and alaproclate indicates that these compounds bind to the same site (Humphreys et al. 1988). These authors propose that the differing effect of Na^+ on the affinity of these compounds for the binding site is explained by the effect of Na^+ on the ligand binding subsites. Because 5–HT and the tested antidepressants have markedly different structures, they probably bind to different subsites in or near the substrate recognition site (cf Marcusson and Tiger 1988).

There appear to be no differences between specific [^{3}H]imipramine binding defined as that sensitive to 100 μM 5–HT and that sensitive to 100 μM desipramine in human platelets (Marcusson and Tiger 1988). However, high and low affinity components of [^{3}H]imipramine binding to platelets have been demonstrated (Ieni et al. 1984; Phillips et al. 1984; Biessen et al. 1988). In a recent study by Hrdina (1989), the specific [^{3}H]imipramine binding defined by 100 μM desipramine was found to consist of 30% sodium-independent binding, suggesting the presence of at least two subsites.

The observation that 5–HT and some (but not all) 5–HT uptake inhibitors at high concentrations (10–500 μM) strongly retard the dissociation of [^{3}H]imipramine from human platelet membranes has been taken as evidence for a model in which 5–HT is proposed to control [^{3}H]imipramine dissociation from an allosterically coupled site (Wennogloe and Meyerson 1983, 1985; Plenge and Mellerup 1985; Segonzac et al. 1985a, b). Humphreys et al. (1988) confirmed the slowing effect by high concentration of 5–HT on the dissociation of [^{3}H]imipramine from membrane vesicles of human platelets. This effect, however, was not Na^+ dependent. No corresponding effect was observed in vesicle membranes of porcine platelets (Humphreys et al. 1988). These authors concluded that because of 1) the required concentration of 5–HT for retarding the dissociation of imipramine is about 200 times higher than that required for competition with imipramine binding at equilibrium, 2) the species specificity and 3) the Na^+ independence, it is doubtful that the 5–HT site that inhibits imipramine dissociation is identical to the substrate recognition site for transport. In a recent study Humphreys et al. (1989) analyzed the dissociation of the very tightly bound ^{125}I–2-iodoimipramine from human platelet membranes. The dissociation upon dilution in the presence of Na^+ was very slow but increased in the absence of Na^+. The findings obtained indicate that the persistent binding is caused by a time dependent increase in the affinity of the transporter-iodoimipramine complex.

[^{3}H]Paroxetine binding

[^{3}H]Paroxetine binding to human platelets was characterized by Mellerup et al. (1983), who reported on a single class of binding sites with an apparent affinity, K_d, of 0.08 nM. Similarly to the findings on platelet [^{3}H]imipramine binding (Plenge and Mellerup 1985), the dissociation of platelet [^{3}H]paroxetine binding was clearly influenced by various antidepressants (Plenge and Mellerup, 1985). However, the concentrations of uptake inhibitors needed to alter the [^{3}H]paroxetine dissociation were high (200 μM) and it is not clear whether these alterations are due to non-specific membrane effects or to interaction with with specific binding sites of low affinity.

In addition to the choice of radioligand and pharmacological definition of specific binding, it appears that consideration has to be taken of platelet studies regarding the expression of B_{max}. For human control samples B_{max} values for [^{3}H]imipramine binding can range between 300 and 1600 fmol/mg protein (e.g. Weizman et al. 1986; Nemeroff et al. 1988; Hrdina 1989; Theodorou et al. 1989). For [^{3}H]paroxetine binding to human controls, the platelet B_{max} value varies between 900 and 1600 fmol/mg protein (Mellerup et al. 1983; D'Haenen et al. 1988; Andersson and Marcusson 1990).

Several factors may contribute to these differences. Seasonal changes of [^{3}H]imipramine binding with B_{max} ranging from 400 to 800 fmol/mg protein have been reported (Whitaker et al. 1984). Light-dark differences in B_{max} around 50% have also been described (Rocca et al. 1989), but usually such differences do not exist in human studies where the samples are mostly taken in the morning. Differences in population mean age are not likely to explain differences in B_{max}, since no age-related changes in B_{max} for [^{3}H]paroxetine binding to platelets were reported (Andersson and Marcusson 1990).

A more likely explanation for the broad range of B_{max} values is differences in protein concentrations in the platelet concentrate. Indeed, protein content in platelet membranes related to the number of platelets is highly variable (D'Haenen et al. 1988). I.e., variability of protein content may result in large variations in B_{max} expressed per mg protein (D'Haenen et al. 1988; Theodorou et al. 1989), and expressing B_{max} per number of platelets may decrease this variability (D'Haenen et al. 1988). However, a fairly good correlation between these two expressions of B_{max} exists ($r = 0.64$, $P < 0.01$, $n = 45$) (Andersson and Marcusson 1990).

Characterization and purification of the 5–HT transporter

Brain tissue

Solubilization of [^{3}H]paroxetine labeled 5–HT transporter has been performed on rat cortex, showing an intact pharmacological profile in comparison with intact membranes (Habert et al. 1986). In a later study, analysis of the purified preparation revealed a major polypeptide with a molecular weight of 110000 (Langer et al. 1989). Most recently Chang et al. (1989) reported on a genetically reconstituted high-affinity system for 5–HT transport in mouse fibroblast L–M cells transfected with human genomic DNA. The characteristics of the 5–HT uptake in these cells were similar to those reported on

synaptosomes. It is possible that these cell lines in the future can be used in the cloning of the 5–HT transporter gene.

Platelets

Solubilization by digitonin of the 5–HT transporter labeled with [^{3}H]imipramine left the pharmacological characteristics of the binding unchanged in comparison with intact membranes in porcine platelets (Talvenheimo and Rudnick 1980) and in human platelets (Davis et al. 1983). Gel filtration of solubilized [^{3}H]imipramine binding protein of human platelets gave a molecular weight of 300000–400000 (Cesura et al. 1983). Mellerup et al. (1984) reported on [^{3}H]imipramine and [^{3}H]paroxetine binding polymers with molecular weights of 90000 and 70000, respectively, in human platelets. These molecular weights are in the same range as those reported for the neuronal [^{3}H]paroxetine binding polymere (Langer et al. 1989).

Inhibition of 5–HT uptake

The tricyclic antidepressant imipramine, like most other 5–HT uptake inhibitors, inhibits the 5–HT transport competitively in brain synaptosomes (Marcusson et al. 1986) and in platelets (Humphreys et al. 1988, and references therein). As discussed by Wölfel et al. (1988) the reports of non-competitive inhibition of the platelet 5–HT uptake by imipramine are probably due to failure to measure the initial rates of uptake. Humphreys et al. (1988) examined the Na^+ dependence of the 5–HT transport inhibition by imipramine, fluvoxamine, norzimeldine, and alaproclate. The inhibitory potency for imipramine increased dramatically with increasing concentrations of Na^+ (from 20 to 200 meq/l). The increase in potency for norzimeldine and alaproclate was much less pronounced and the potency of fluvoxamine tended to decrease with increased Na^+ concentration. Thus, the Na^+ dependence for transport inhibition varies widely from one uptake inhibitor to another. The V_{max} of the 5–HT transport varies only 2- to 3-fold over the same Na^+ concentration range (Talvenheimo et al. 1983). Differences in Na^+ dependence between uptake inhibitors have also been observed in synaptosomal preparations of rat cerebral cortex (Wood 1987). The simplest explanation of the mode of inhibitory action of the uptake inhibitors is that while they bind to the 5–HT recognition site at the transporter, they also have other bonds outside the substrate recognition site which may differ in their Na^+ requirement.

Topography of the active centre of the 5–HT transporter

By comparing the inhibitory potencies of stereoisomers of 5–HT uptake inhibitors, attempts have been made to map the active area of the 5–HT transporter (Tuomisto 1978; Lindberg et al. 1978; Smith 1986; Plenge et al. 1987; Wägner and Fork 1987). From data of stereoisomers of 14 compounds and 12 other drugs, Smith (1986) arrived at a model for the topography of the putative 5–HT uptake area consisting of four binding sites located on three planes. One site is believed to have a negative charge and to be capable of forming a ionic bond with protonated amino groups. A second binding site is thought to be at the centre of a flat hydrophobic region, capable of van der Waals interactions with aromatic groups. The third site is proposed to be in a region of partial positive charge capable of localized charge transfer. These three binding sites are the same as proposed by Lindberg et al. (1978) and Wägner and Fork (1987) based on the stereoisomers of alaproclate. The fourth binding site introduced by Smith is believed to consist of a region of partial positive charge capable of electropositive or hydrogen bonding. This site may form a bond with the hydroxyl group of 5–HT. Using molecular graphic techniques as used by Wägner and Fork (1987) may further increase our knowledge on the topography of the active centre of the 5–HT transporter.

Endogenous ligand for the 5–HT uptake site

The natural endogenous ligand for the 5–HT transporter is 5–HT itself. However, based on the suggestion that [^{3}H]imipramine binds to a site close to but not identical with the 5–HT uptake site (Sette et al. 1983b) it was speculated that an endogenous ligand for [^{3}H]imipramine binding site would exist, with the capacity to regulate the 5–HT transporter (Barbaccia et al. 1983a, b; Langer et al. 1984). In fact, an endogenous factor extracted from rat brain inhibiting [^{3}H]imipramine binding was reported (Barbaccia et al. 1983). This was confirmed by Rehavi et al. (1985), who found that the extracted factor inhibited the binding in a competitive manner and that it was not protein in nature; Langer et al. (1984) suggested that 5-methoxytryptoline might be such an endogenous ligand for the [^{3}H]imipramine binding site in brain tissue and platelets (Segonzac et al. 1985), but this was questioned by Barbaccia et al. (1986). Proteins from human plasma, e.g. alpha-1-acidic glycoprotein, have been found to inhibit the binding of [^{3}H]imipramine in a competitive manner (Barkai et al; 1986; Abraham et al. 1987; Strijewski et al. 1988). Since plasma alpha-1-acidic glycoproteins, which have not been demonstrated in brain tissue, bind with high affinity various pharmacological agents (Müller et al. 1983), they probably compete with the 5–HT transporter to bind imipramine.

In summary, although there are several factors in plasma and homogenized brain tissue which can inhibit the binding of [^{3}H]imipramine to the 5–HT transporter, it is doubtful if these factors exert any effect in the brain in vivo.

Regulation of 5–HT uptake and binding sites for 5–HT uptake inhibitors in brain and platelets

5–HT uptake. Barbaccia et al. (1983a) reported an increased V_{max} value for the uptake of 5–HT in hippocampal minces after repeated treatment of rats with 10 mg/kg intraperitoneally imipramine twice daily for 21 days and 2–3 days washout period. Other studies with imipramine

and the 5–HT uptake inhibitors citalopram, imipramine, and norzimeldine do not reveal any changes in the kinetic parameters of the 5–HT uptake in the rat brain (Hyttel et al. 1984; Marcusson et al. 1988b). Hrdina (1987b) noted a decrease V_{max} for 5–HT uptake in rat brain after 3 weeks, treatment with fluoxetine (10 mg/kg by gastric intubation). Brunello et al. (1987) found an increased V_{max} of the 5–HT uptake in rat cerebral cortical slices after repeated treatment twice daily with 10 mg/kg IP with desipramine or fluvoxamine but not with citalopram. It is therefore obvious that if any "upregulation" of the 5–HT uptake mechanism occurs by repeated treatment with some antidepressant drugs this is not related to the 5–HT uptake inhibition.

Binding sites. The first studies on the effect of repeated treatment of rats with the antidepressants imipramine and desipramine on [^{3}H]imipramine binding in brain tissue and platelets reported a decreased number of [^{3}H]imipramine binding sites (Kinnier et al. 1980; Raisman et al. 1980; Briley et al. 1982; Barbaccia et al. 1983; Arora and Meltzer 1986). Other studies reported on no alterations of the imipramine binding in rodent brain tissue after chronic imipramine or desipramine treatment (Plenge and Mellerup 1982; Gentsch et al. 1984; Abel et al. 1985; Biegon et al. 1986). However, reduced binding (and higher K_d values) due to inhibition by residual drug in the tissue preparation was observed (Plenge and Mellerup 1982; Wilson and Roy 1985). All these studies failed to take into consideration the heterogeneity of the imipramine binding. In a recent study where only the high affinity [^{3}H]imipramine binding site related to the 5–HT uptake mechanism was studied, repeated treatment of rats with imipramine or norzimeldine did not produce any change in the imipramine binding in cerebral cortex (Marcusson et al. 1988b). A study of both high and low affinity [^{3}H]imipramine binding sites after chronic treatment of rats with antidepressant drugs (nortriptyline, fluoxetine, iprindole, maprotiline, and phenelzine) did not reveal any changes in binding density but only in affinity (Hrdina 1987b). Graham et al. (1987) found no changes in the kinetic parameters of [^{3}H]paroxetine binding in rat cerebral cortex affer repeated treatment with the 5–HT uptake inhibitors citalopram and clomipramine and with the monoamine oxidase inhibitors clorgyline and deprenyl. Neither did repeated treatment with electroconvulsive shock change [^{3}H]paroxetine binding in rat cortical membranes (Gleiter and Nutt 1988). It can be concluded that if chronic treatment of rats with antidepressants produces any decrease in the number of imipramine binding sites at all, this effect does not seem to have any relation to the number of substrate recognition sites for 5–HT uptake.

5–HT uptake and binding sites for 5–HT uptake inhibitors in brain and platelets in affective disorders

[^{3}H]Imipramine binding to platelets has been extensively studied in depression and this subject has been covered in recent review articles. Langer et al. (1987) showed reductions in B_{max} (10–50%) in 15 out of 25 studies on untreated depressed patients compared with controls. A WHO sponsored multicenter study on [^{3}H]imipramine binding to platelets in depression versus control failed to detect any significant differences in binding between the two groups (Potkin et al. 1988). In the first study on platelet [^{3}H]paroxetine binding in depression, no differences in B_{max} or K_d in comparison with controls were obtained (D'Haenen et al. 1988). The results from the above multicenter study and from the study using [^{3}H]paroxetine underline that the previous reports on decreased density of 5–HT uptake sites in depression can be questioned (see also Plenge and Mellerup 1988).

In post-mortem human brain tissue, the number of cortical and hippocampal [^{3}H]imipramine binding sites were reduced in suicide (Stanley et al. 1982) and depression (Perry et al. 1983). Since both these studies were carried out with a definition of specific binding that included a large proportion (at least 50%, see Bäckström et al. 1987) of binding not related to 5–HT uptake sites, the results are difficult to evaluate, since they depend on which fraction (specific or non-specific) of the binding that has been affected.

Antidepressant mechanisms related to 5–HT uptake inhibition

Several studies have described the biochemical and electrophysiological effects that occur with treatment with 5–HT uptake inhibitors and other antidepressant agents and their possible relation to antidepressant effect (e.g. Barbaccia et al. 1983b; Racagni et al. 1983; Van Praag 1983; Willner 1985; Blier et al. 1987) and it is not our aim to give a detailed review on this topic here. However, some important aspects will be discussed.

The 5–HT uptake inhibition occurs acutely during treatment with 5–HT uptake inhibitors (Ross and Rényi 1969), whereas the clinical effect occurs after 2–3 weeks of treatment, suggesting that adaptive mechanisms are involved. Studies of isolated binding parameters have tried to explain this delayed therapeutic effect, but there is no uniform picture on how monoamine uptake inhibitors affect these parameters.

Few studies involve electrophysiological studies of the effects of 5–HT uptake inhibition. Blier et al. (1987) review most of their own data on this topic concerning the 5–HT system and relate their findings to the therapeutic response in major depression. They report that 5–HT uptake inhibition results in a decrease of firing rate of 5–HT neurons after 2 days of treatment, but that that firing activity recovers and is back to normal by day 14. This progessive adaptation may be due to a desensitization of the somatodendritic 5–HT autoreceptor. However, 5–HT uptake inhibition leads to an enhanced efficacy of the stimulation of the 5–HT pathway (causing a suppression of firing of the post-synaptic neurons). This is due to a desensitization of the terminal 5–HT autoreceptor, allowing a greater amount of the neurotransmitter to be released per impulse. They concluded that sustained 5–HT uptake inhibition will not result in an

increased 5–HT neurotransmission until 5–HT neurons regain their normal firing activity at the same time as the capacity of the terminal 5–HT autoreceptor to inhibit the release of 5–HT is decreased (Blier et al. 1987).

However, even though these alterations of the 5–HT system occur, it is likely that they are only part of a more complex neurobiological response, where other systems, e.g. noradrenaline (Sulser et al. 1983) and GABA (Lloyd et al. 1985) are involved, resulting in an antidepressive effect. The finding that monoamine uptake inhibitors regulate glucocorticoid receptor messenger RNA concentrations, which may lead to a normalization of hyperactivity of the hypothalamic-pituitary-adrenal axis often seen in major depression (Pepin et al. 1989), underlines that the neurobiological response is complex and that the mechanisms responsible for the antidepressive effect will be difficult to identify.

Conclusion

In the light of recent observations of the heterogeneity of the binding of [^{3}H]imipramine to membranes of brain tissue and platelets and of the binding of other 5–HT uptake inhibitors to these membrane sites, it seems plausible to conclude that the binding sites for 5–HT uptake inhibitors associated with the 5–HT nerve terminals overlap the binding site for 5–HT on the transporter, with additional bonds to subsites which might be different for the various uptake inhibitors. Although a two-site model, in which the uptake inhibitor and 5–HT would bind to different sites (proteins) allosterically interacting with each other, also explains the observations obtained, these separate binding sites will have to be identified before the acceptance of a two-site model. No convincing evidence has been presented of the existence of an endogenous ligand other than 5–HT itself that binds to the [^{3}H]imipramine binding site and regulates the 5–HT tansporter. Since contradictory results have been obtained concerning the lowered B_{max} of [^{3}H]imipramine or [^{3}H]paroxetine binding to platelets in affective disorders, it is doubtful whether this binding can be used as a biological marker of depression.

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