Biochemical Pharmacology, Vol. 38, No. 21, pp. 3819–3826, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Pergamon Press plc

A COMMON BINDING SITE FOR TRICYCLIC AND NONTRICYCLIC 5-HYDROXYTRYPTAMINE UPTAKE INHIBITORS AT THE SUBSTRATE RECOGNITION SITE OF THE NEURONAL SODIUM-DEPENDENT 5-HYDROXYTRYPTAMINE TRANSPORTER

DAVID GRAHAM, HUGUETTE ESNAUD, ESTELLE HABERT and SALOMON Z. LANGER*
Department of Biology, Synthélabo Recherche (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

(*Received* 6 *January* 1989; *accepted* 18 *May* 1989)

Abstract—An investigation of the site of interaction of a variety of tricyclic and nontricyclic 5-HT uptake inhibitors with the neuronal sodium-dependent 5-HT transporter was undertaken. The dissociation of [^{3}H]paroxetine binding induced by indalpine (10 μM), SL 81.0385 (10 μM), fluoxetine (10 μM), citalopram (10 μM), paroxetine (0.15 μM), imipramine (10 μM) and 5-HT (50 μM) produced monophasic dissociation curves and gave $t_{1/2}$ values of dissociation similar to that induced by dilution alone. In inhibition studies of [^{3}H]paroxetine binding with citalopram, imipramine and 5-HT, increases in the concentration of [^{3}H]radioligand used led to parallel rightward shifts of the inhibition curves with no diminution of the maximum degree of inhibition (I_{max}). "Schild-type" analyses of the data obtained from the inhibition curves with these 3 compounds gave slopes close to unity. In chemical modification studies, treatment of membrane fractions with *N*-ethylmaleimide led to a pronounced reduction in specific [^{3}H]paroxetine binding. Preincubation of these membranes with SL 81.0385, fluoxetine, imipramine, tryptamine and 5-HT provided significant protection against this NEM-induced inactivation. The above findings are interpreted to provide evidence for a common or at least overlapping binding site for the tricyclic and nontricyclic 5-HT uptake inhibitors with the substrate recognition site of the neuronal sodium-dependent 5-HT transporter.

The mechanism of 5-hydroxytryptamine (5-HT)† uptake by the sodium-dependent 5-HT transporter into platelets and brain tissue preparations has been widely studied using both direct uptake assays and radioligand binding techniques (for review see Ref. 1). This uptake process probably consists of at least three separate steps, namely binding, translocation across the membrane and internal release of substrate. Binding assays with radiolabelled 5-HT uptake inhibitors have been employed to examine the first step of this uptake process and to study structure–activity relationships of binding to the substrate recognition site and the nature of the interaction of 5-HT uptake inhibitors with the transporter. [^{3}H]Imipramine has principally been used as a marker of both the platelet and neuronal sodium-dependent 5-HT transporter and on the basis of some of these studies this tricyclic antidepressant has been reported to interact allosterically with the substrate recognition site of this macromolecule [2, 3].

The existence of [^{3}H]imipramine binding site heterogeneity, particularly in brain membrane preparations, has, however, subsequently been revealed [4–8]. As such the validity of earlier findings on the site(s) of interaction of various classes of 5-HT uptake inhibitors with the sodium-dependent 5-HT transporter needs to be re-evaluated. In the present report we have examined the topography of 5-HT uptake inhibitor binding sites on this transporter using the much more selective 5-HT uptake inhibitor ligand [^{3}H]paroxetine [9] in conjunction with competitive inhibition, dissociation kinetic and chemical modification studies. The effect of the sulphydryl group alkylating agent *N*-ethylmaleimide (NEM) on [^{3}H]paroxetine binding to the sodium-dependent 5-HT transporter of rat cerebral cortical membranes was studied in the presence and absence of several classes of compounds known to exhibit affinity for the transporter. Also, in addition to competitive inhibition experiments the dissociation of [^{3}H]paroxetine binding to rat cerebral cortical membranes from equilibrium conditions was evaluated in the presence of various 5-HT uptake inhibitors. The current findings provide evidence for a common or at least overlapping binding site for the tricyclic and nontricyclic 5-HT uptake inhibitors with the substrate recognition site of the neuronal sodium-dependent 5-HT transporter.

MATERIALS AND METHODS

Materials. [^{3}H]Paroxetine (19 Ci/mmole) was purchased from New England Nuclear (Boston, MA); *N*-ethylmaleimide (NEM) was obtained from Fluka (Buchs, Switzerland). The following drugs were supplied by the Chemistry Department from L.E.R.S.-Synthélabo (Paris): SL 81.0385 benzoate, imi-

* Author to whom correspondence should be addressed.
† Abbreviations used: DTT: dithiothreitol; 5-HT: 5-hydroxytryptamine; NEM: *N*-ethylmaleimide.

pramine HCl, fluoxetine HCl and citalopram HCl. The following drugs were kindly donated: spiperone from Janssen Pharmaceutica (Beerse, Belgium); and maprotiline HCl from Ciba–Geigy (Basel, Switzerland). Tryptamine HCl and 5-HT creatinine sulphate were purchased from Sigma (St Louis, MO).

Preparation of rat cerebral cortical membranes. Membranes of cerebral cortex of male Sprague–Dawley rats were prepared as outlined previously [9]. Briefly, cerebral cortex was homogenized in 50 volumes of 50 mM Tris–HCl buffer, pH 7.4, containing 120 mM NaCl and 5 mM KCl and centrifuged at 30,000 *g* for 10 min. The resulting pellet was resuspended in 50 volumes of buffer and recentrifuged. This final pellet was resuspended at a protein concentration of 1 mg/ml as measured by the method of Peterson using bovine serum albumin as standard [10].

[^{3}H]*Paroxetine binding.* Routinely, aliquots (100 μg protein) of membranes were incubated with 0.2 nM [^{3}H]paroxetine at 25° in a final volume of 2 ml for at least 60 min. Incubations were then terminated by rapid filtration using a Brandwell cell harvester through Whatman GF/B glass fibre filters (filters were pretreated with 0.05% polyethylenimine before use). The filters were washed with 4 × 4 ml of ice-cold buffer, dried and the radioactivity measured by liquid scintillation spectrometry. The specific binding of [^{3}H]paroxetine was defined as the difference between the total binding and that remaining in the presence of 10 μM fluoxetine.

For the kinetic dissociation experiments similar [^{3}H]paroxetine binding assays were set up at 25° in the presence and absence of 10 μM fluoxetine. After 60 min of incubation, the dissociation of [^{3}H]paroxetine binding from equilibrium conditions was initiated by the addition of 20 μl of each of the displacing agents tested and then dissociation followed for at least 4 hr. At each time-point incubations were terminated by filtration as described above. Dissociation kinetics of [^{3}H]paroxetine binding were subsequently analysed as described previously [9] in which first-order rate dissociation constants were calculated from the relationship $l_n(B_t/B_0) = -k_{-1}t$, where B_0 represented the amount of [^{3}H]paroxetine specifically bound at equilibrium and B_t the amount specifically bound at time t.

Chemical modification with NEM. Typically, chemical modification using 10 mM NEM was carried out on 5 ml aliquots of membranes. After incubation for 60 min at 25° the treatment was terminated by diluting the reaction mixture with 10 ml of ice-cold buffer containing 5 mM dithiothreitol (DTT), followed by centrifugation at 48,000 *g* for 15 min at 4°. The resulting pellet was resuspended and centrifuged four more times using 20 ml of buffer at each stage. The final pellet was resuspended in 5 ml of buffer for [^{3}H]paroxetine binding assays. Experiments in which protection against effects of NEM alkylation of membranes was investigated included a 15-min preincubation period at 25° with various agents before addition of NEM.

Table 1. Dissociation from equilibrium conditions of [^{3}H]paroxetine binding to rat cerebral cortical membranes by 5-HT and various 5-HT uptake inhibitors

Displacing agent	Concentration (μM)	$t_{1/2}$ (min)
Indalpine	10.0	82.0 ± 17.5
SL 81.0385	10.0	92.0 ± 2.0
Fluoxetine	10.0	94.0 ± 16.0
Citalopram	10.0	98.2 ± 2.0
Paroxetine	0.15	104.0 ± 12.0
Imipramine	10.0	111.0 ± 1.2
5-HT	50.0	100.0 ± 1.1
5-HT	500.0	185.0 ± 15**
Imipramine	100.0	202.0 ± 13**
Paroxetine + 5-HT	0.15 500.0	162.0 ± 24**
Dilution		97.0 ± 8.0

Rat cerebral cortical membranes were incubated with 0.2 nM [^{3}H]paroxetine. Upon attainment of equilibrium conditions dissociation of [^{3}H]paroxetine binding was initiated by addition of the various displacing agents listed above. First-order dissociation kinetic plots were used to calculate $t_{1/2}$ values. Data represent the means ± SE of at least 3 experiments (**$P < 0.01$ compared to $t_{1/2}$ value for dilution-induced dissociation; Duncan test).

RESULTS

Dissociation of [^{3}H]*paroxetine binding*

In a previous report on the properties of [^{3}H]paroxetine binding to rat cerebral cortical membranes we have shown that at 22° equilibrium binding of this ligand is attained by 45 min [9]. For the present study therefore dissociation of [^{3}H]paroxetine binding to rat cerebral cortical membranes by various agents was initiated after a [^{3}H]paroxetine binding preincubation period of 60 min. Addition of a large excess of unlabelled paroxetine produced monophasic dissociation of [^{3}H]paroxetine binding from equilibrium conditions with a first-order dissociation rate constant (k_{-1}) of 0.008/min and a $t_{1/2}$ value of dissociation of 104 ± 12 min (Table 1). Similar monophasic dissociation curves were displayed in chase experiments on [^{3}H]paroxetine binding in which the selective 5-HT uptake inhibitors indalpine, SL 81.0385, fluoxetine and citalopram gave $t_{1/2}$ values of dissociation of between 82–98 min (Fig. 1 and Table 1). These $t_{1/2}$ values of dissociation were in close agreement with the rate of dissociation of [^{3}H]paroxetine binding from equilibrium conditions as induced by dilution (Table 1).

The tricyclic antidepressant, imipramine (10 μM), and 5-HT (50 μM) gave dissociation rates of [^{3}H]paroxetine binding similar to those observed with the selective 5-HT uptake inhibitors (Table 1). However, significant decreases in the dissociation rate of [^{3}H]paroxetine binding from rat cerebral membranes occurred with the use of high micromolar concentrations of either imipramine (100 μM) or 5-HT (500 μM) as displacing agents. A high micromolar concentration of 5-HT (500 μM) in com-

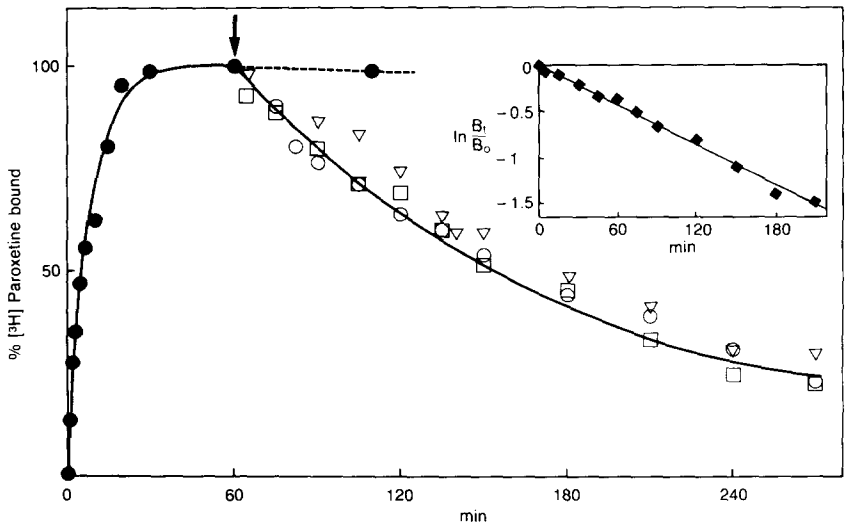

Fig. 1. Association and dissociation of [^{3}H]paroxetine binding. Rat cerebral cortical membranes were incubated with 0.2 nM [^{3}H]paroxetine at 25° in the presence and absence of 10 μM fluoxetine for the times indicated. Dissociation was started by the addition (↓) of 10 μM citalopram (□——□), 10 μM imipramine (▽) or 50 μM 5-HT (○) at 60 min, by which time the association time-course had reached equilibrium (indicated by dashed line). The amount of specific [^{3}H]paroxetine binding, expressed as a percentage of that obtained at 60 min, was calculated as a function of time (the dissociation curve plotted represents the data obtained in a typical citalopram dissociation experiment). Inset: first-order kinetic dissociation plot of [^{3}H]paroxetine binding upon the addition of 10 μM citalopram.

bination with paroxetine also produced a significant decrease in the dissociation rate of [^{3}H]paroxetine binding compared to that observed with paroxetine alone (Table 1).

Inhibition of [^{3}H]paroxetine binding

The inhibition of [^{3}H]paroxetine binding by citalopram, imipramine and 5-HT was examined at different [^{3}H]paroxetine concentrations. With each drug increases in the concentration of [^{3}H]paroxetine used resulted in rightward shifts in the inhibition curves with no diminution of I_{max} (Fig. 2A, B, C). Moreover, "Schild-type" analyses of the various curves produced by each drug revealed slopes close to unity (Fig. 3). In addition, derivation of K_i values from the various experimental IC_{50} values using the Cheng–Prusoff correction gave K_i values which were similar within each set of drug inhibition curves (citalopram, 0.99–1.41 nM; imipramine, 23.5–33.0 nM; 5-HT, 1.27–1.72 μM).

Effect of NEM treatment on [^{3}H]paroxetine binding activity

In preliminary experiments a concentration-dependent inactivation of [^{3}H]paroxetine binding activity in rat cerebral cortical membranes was noted upon pretreatment of the membranes with the sulphydryl alkylating agent, NEM, for 2 hr at 25° (Fig. 4A). The time-course of this inactivation showed a maximal effect by 1 hr (Fig. 4B) and thus in subsequent studies membranes were treated for 1 hr using 10 mM NEM.

The effect of NEM on [^{3}H]paroxetine binding activity was investigated in the presence of compounds known to have affinity for the neuronal sodium-dependent 5-HT transporter. Preincubation of membranes with the 5-HT uptake inhibitors SL 81.0385, fluoxetine and imipramine at concentrations which produce complete inhibition of 5-HT uptake gave significant protection against the reduction in [^{3}H]paroxetine binding activity induced by NEM treatment (Table 2). Also, a similar protective effect was noted in the presence of excess 5-HT and tryptamine (Table 2). In contrast, maprotiline and spiperone (compounds which do not possess affinity for the 5-HT transporter) failed to offer protection against NEM treatment.

DISCUSSION

The selective 5-HT uptake inhibitors, [^{3}H]paroxetine, [^{3}H]citalopram and [^{3}H]indalpine have been used as specific markers of both the neuronal and platelet sodium-dependent 5-HT transporter [9, 11, 12]. In these studies the binding of each of these selective 5-HT uptake inhibitors not only occurred to a single class of noninteracting sites, but it was also inhibited by 5-HT and various 5-HT uptake blockers with Hill coefficients close to unity [9, 11, 12]. As such this data would be consistent with a single site model for the binding of the substrate, 5-HT, and various tricyclic and nontricyclic 5-HT uptake inhibitors to the sodium-dependent 5-HT transporter. For the present study a more rigorous evaluation of the nature of the interaction of these 5-HT uptake inhibitors with the neuronal sodium-dependent 5-HT transporter of rat cerebral cortex was undertaken.

In one series of experiments the dissociation kinetics of [^{3}H]paroxetine binding from equilibrium

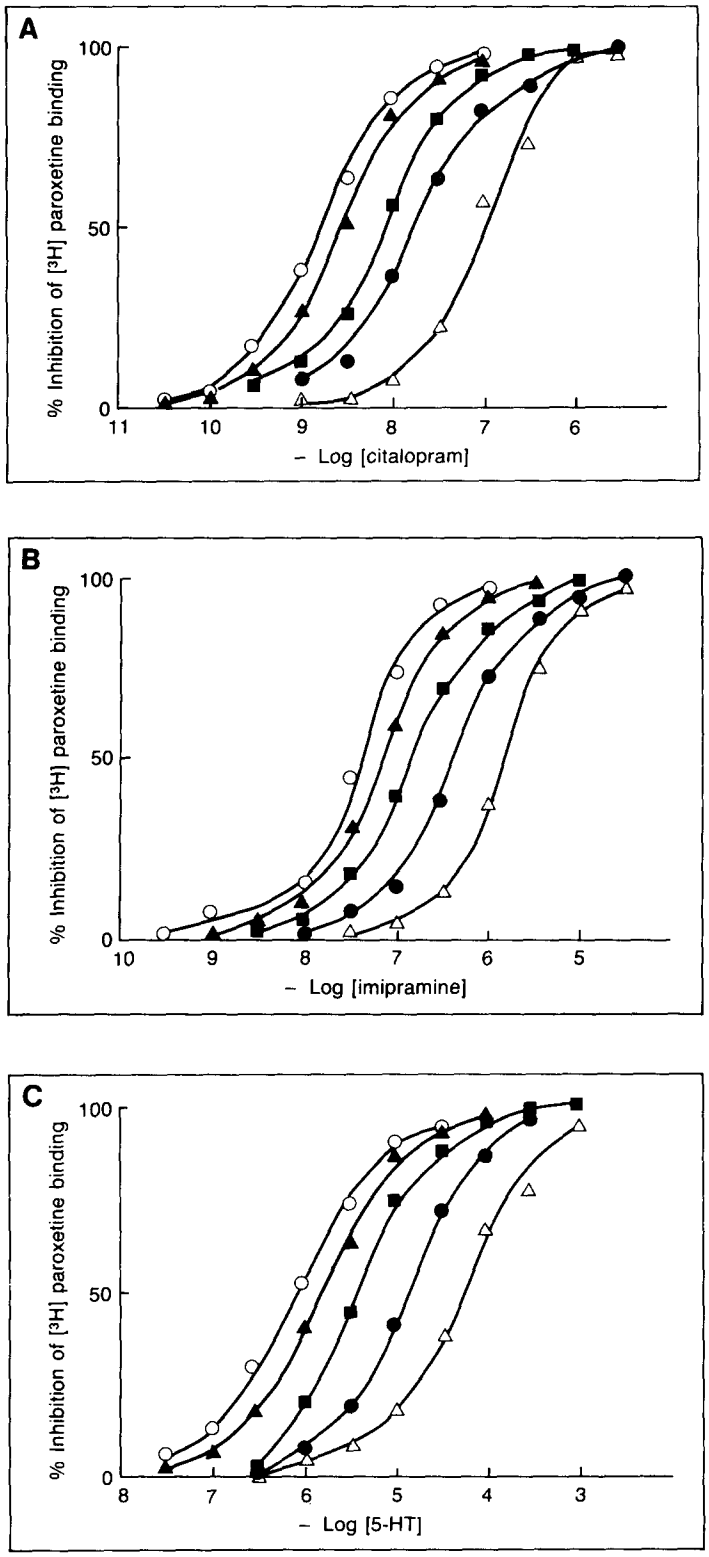

Fig. 2. Competitive inhibition of [^{3}H]paroxetine binding by citalopram, imipramine and 5-HT. Competitive inhibition experiments of [^{3}H]paroxetine binding to rat cerebral cortical membranes were set up with the indicated concentrations of citalopram (A), imipramine (B) or 5-HT (C) at different [^{3}H]paroxetine concentrations (○–○, 0.05 nM; ▲–▲, 0.1 nM; ■–■, 0.3 nM; ●–●, 1.0 nM; △–△, 3.0 nM). The inhibition experiments with 0.05 nM and 0.1 nM [^{3}H]paroxetine were performed using 200 μg of membrane protein in a final assay volume of 4 ml, whereas the experiments with 0.3, 1.0 and 3.0 nM [^{3}H]paroxetine were carried out in a final assay volume of 2 ml with 100 μg of membrane protein.

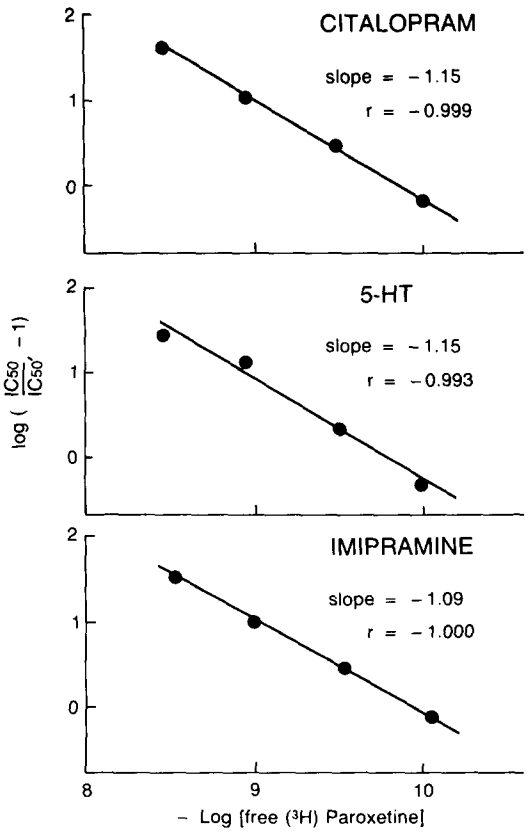

Fig. 3. "Schild-type" analysis of the competitive inhibition curves of [^{3}H]paroxetine binding by citalopram, imipramine and 5-HT. From the competitive inhibition experiments of [^{3}H]paroxetine binding shown in Fig. 2, the concentration of drug in each curve that inhibited specific [^{3}H]paroxetine binding by 50% (IC_{50} value) was calculated using the nonlinear regression analysis programme ALLFIT [25]. The various IC_{50} values obtained for each drug were then analysed using the plot log $[IC_{50}/IC'_{50}]V$ − log free [^{3}H]paroxetine concentration, where IC'_{50} represented the IC_{50} value for each drug obtained using 0.05 nM [^{3}H]paroxetine.

conditions were examined. The monophasic dissociation curve produced by the addition of an excess of unlabelled paroxetine gave a $t_{1/2}$ value of dissociation that was in close agreement with that of the rate of dilution-induced [^{3}H]paroxetine dissociation, thus indicating a lack of cooperativity in the binding of [^{3}H]paroxetine to the sodium-dependent 5-HT transporter. Moreover, the similarity of the $t_{1/2}$ values of dissociation of [^{3}H]paroxetine binding obtained in the chase experiments with 5-HT and a number of structurally distinct 5-HT uptake inhibitors suggested the absence of allosterism in the interactions of these compounds with the transporter. In this context, it is interesting to note that an investigation of the dissociation kinetics of [^{3}H]citalopram binding induced by dilution in the presence or absence of 5-HT and imipramine did not reveal allosteric interactions [11]. Evidence from kinetic dissociation experiments using selective 5-HT uptake inhibitors as radioligands would therefore favour the proposal that 5-HT and the tricyclic and nontricyclic 5-HT uptake inhibitors bind to a mutually exclusive site on the neuronal sodium-dependent 5-HT transporter.

In an early report on [^{3}H]imipramine binding to rat cerebral cortical membrane preparations a complex pattern of inhibition of the binding of this radioligand was produced by 5-HT and nontricyclic 5-HT uptake blockers, whereas imipramine gave competitive inhibition [2]. The nature of these inhibition curves was suggested to indicate allosterism between the binding site for this tricyclic antidepressant and the 5-HT substrate recognition site of the transporter. However, recent reports showing heterogeneity of binding sites for [^{3}H]imipramine could also explain these findings of complex inhibition curves [4–8], and indeed studies on protease-sensitive [^{3}H]imipramine binding to rat cerebral cortical membranes [6] or [^{3}H]imipramine binding to platelets [13] have revealed monophasic displacement curves in competition inhibition experiments with 5-HT and tricyclic and nontricyclic 5-HT uptake inhibitors. Nevertheless, kinetic studies on the dissociation of [^{3}H]imipramine binding in the presence of high micromolar concentrations of 5-HT or tryptamine produced a decrease in the rate of dissociation of this radioligand [13–15]. These latter findings have been used to suggest that the sodium-dependent 5-HT transporter might indeed contain an allosteric binding site for imipramine separate from the substrate recognition site [13–15].

The present study revealed an interesting parallel to the findings noted with [^{3}H]imipramine dissociation in that the rate of dissociation of [^{3}H]paroxetine binding was decreased in the presence of high micromolar concentrations of 5-HT (500 μM) or imipramine (100 μM). Also, the dissociation rate of [^{3}H]paroxetine binding induced by unlabelled paroxetine was significantly reduced when performed in combination with 500 μM 5-HT. In chase experiments using lower concentrations of displacing ligand, however, 10 μM imipramine and 50 μM 5-HT (concentrations which are nevertheless 200- and 100-fold higher than their K_i values on [^{3}H]paroxetine binding, respectively, see Ref. 9) gave $t_{1/2}$ values of dissociation of [^{3}H]paroxetine binding similar to that produced by dilution. As such these latter kinetic data indicate that the primary site of interaction of imipramine and 5-HT with the sodium-dependent 5-HT transporter is mutually exclusive with the paroxetine binding site. A reduction in the dissociation rate of [^{3}H]imipramine and [^{3}H]paroxetine binding in the presence of high micromolar concentrations of displacing agents could nevertheless suggest that such compounds have secondary binding sites of lower affinity on the transporter. Alternatively, these compounds might exert a nonspecific effect at high concentrations on the membrane environment surrounding the transporter which as a result perturbs [^{3}H]radioligand binding. This latter explanation could well be the more probable as high micromolar concentrations of 5-HT induce a more dramatic reduction in the rate of [^{3}H]imipramine dissociation in human or mouse platelet compared to rat cerebral cortex preparations [13, 15 and present study]. Moreover, high micro-

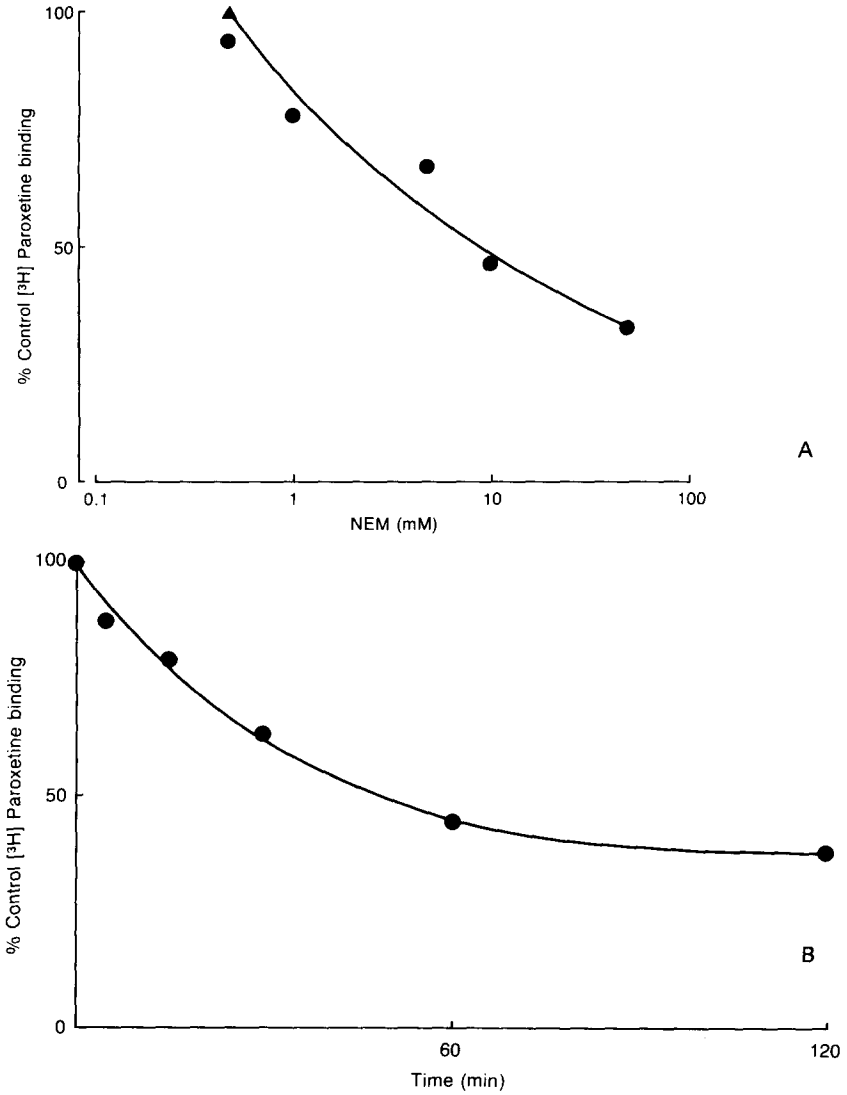

Fig. 4. (A) Effect of NEM pretreatment on [^{3}H]paroxetine binding. Rat cerebral cortical membranes were pretreated with different concentrations of NEM for 60 min at 25°. The membranes were then washed extensively with ice-cold buffer containing 5 mM DTT as described in Materials and Methods. Binding assays were performed using 0.2 nM [^{3}H]paroxetine. Binding in the absence of NEM is represented by the solid triangle. The data represent a typical experiment replicated twice. (B) Time-course of inactivation by NEM pretreatment of [^{3}H]paroxetine binding activity. Incubation mixtures were set up in which rat cerebral cortical membranes were pretreated with NEM (final concentration 10 mM) at 25° for the indicated time-points. The incubation mixtures were then washed extensively with ice-cold buffer containing 5 mM DTT as described in Materials and Methods. Binding assays were performed using 0.2 nM [^{3}H]paroxetine. The data represent a typical experiment replicated twice.

molar concentrations of serotonin have been reported to have no effect on dilution-induced [^{3}H]imipramine dissociation using porcine platelet preparations [16]. Opposite effects on the rate of dissociation of [^{3}H]imipramine binding have also been noted in the presence of high micromolar concentrations of the selective 5-HT uptake inhibitor antidepressants, paroxetine and citalopram [14]. As such the use of high micromolar concentrations of these displacing ligands in dissociation kinetics and the significance that might be attributed to their effects should be interpreted with extreme caution.

In an additional series of experiments the interaction of citalopram, imipramine and 5-HT with the neuronal sodium-dependent 5-HT transporter was analysed from the different inhibition curves of [^{3}H]paroxetine binding produced by these compounds at several [^{3}H]paroxetine concentrations. For each compound increases in the concentration of [^{3}H]paroxetine used led to parallel rightward shifts in the inhibition curves generated with no decrease in the maximal amount of inhibition. Moreover, "Schild-type" analyses of these data revealed slopes close to unity. In accordance with the [^{3}H]paroxetine

Table 2. Protection by various compounds against the effect of NEM on [^{3}H]paroxetine binding activity

Compound	% Control [^{3}H]paroxetine binding +NEM	−NEM
None	44 ± 4	100 (control)
SL 81.0385 (500 nM)	89 ± 9**	107 ± 8
Fluoxetine (500 nM)	73 ± 7**	85 ± 2
Imipramine (1 μM)	85 ± 12**	102 ± 5
5-HT (100 μM)	92 ± 10**	104 ± 9
Tryptamine (100 μM)	69 ± 1**	96 ± 8
Maprotiline (500 nM)	55 ± 3	98 ± 3
Spiperone (500 nM)	49 ± 3	105 ± 8

Rat cerebral cortical membranes were incubated for 15 min at 25° in the presence and absence of the various compounds listed. NEM was added to give a final concentration of 10 mM and the incubation continued for 1 hr at 25°. After extensive washing, membranes were assayed for [^{3}H]paroxetine binding activity using 0.2 nM [^{3}H]paroxetine. Data represent the means ± SE of at least 3 experiments. Significantly different from NEM alone treated value: ** $P < 0.01$ (Duncan test).

dissociation experiments, therefore, these experiments indicate that the paroxetine binding site is mutually exclusive with the binding site for citalopram, imipramine and 5-HT and rule out the existence of allosteric interactions between these compounds [17].

Chemical modification experiments using functional group agents have been used extensively to derive information about protein active centres [18–21]. Previous studies on [^{3}H]imipramine binding have postulated that —SH groups could play an important role in the binding of this tricyclic antidepressant to the transporter [22, 23]. Moreover, in one of these studies fluoxetine was reported to prevent the decrease in imipramine binding activity induced by NEM [22]. In the present study, a decrease in [^{3}H]paroxetine binding activity was noted upon pretreatment of rat cerebral cortical membranes with the sulphydryl group alkylating agent, NEM, suggesting that at least one —SH group also plays an important role in the binding of [^{3}H]paroxetine to the neuronal sodium-dependent 5-HT transporter. This effect of NEM on [^{3}H]paroxetine binding activity could be largely prevented upon incubation of the membranes with SL 81.0385, fluoxetine, imipramine, tryptamine and 5-HT. As such, the results of these protection experiments provide supporting evidence for the model of the transporter proposed from the [^{3}H]paroxetine dissociation and competitive inhibition studies in which the tricyclic and nontricyclic 5-HT uptake inhibitors bind to common or overlapping domains at the indolealkylamine recognition site. One possible explanation for the protective effects observed against NEM-inactivation of [^{3}H]paroxetine binding could be that the uptake inhibitors and the indolealkylamines sterically exclude NEM from alkylating a functional cysteine group located at the [^{3}H]paroxetine binding site. Nevertheless, if this were the case the high concentrations of alkylating agent needed would suggest a relatively chemically inert —SH group(s). Alternatively, the relatively slow effect of NEM might result from attack of a —SH group situated in a rather inaccessible and perhaps conformationally-unfavourable position. In this latter instance the binding of the protective agents at or in close proximity to the substrate recognition domain might stabilize the transporter in its NEM-insensitive conformation.

Recently, the neurochemical profile of 4-[(2-naphthalenyl)methoxy]piperidine, SL 81.0385, a novel highly selective and potent 5-HT uptake inhibitor chemically unrelated to the tricyclic antidepressants, has been reported [24]. The uptake of 5-HT into rat hypothalamic synaptosomes and human platelets was described to be inhibited competitively by SL 81.0385 [24]. In the present study the effects of SL 81.0385 on [^{3}H]paroxetine dissociation kinetics and in the NEM-induced [^{3}H]paroxetine binding inactivation experiments confirm the competitive nature of this 5-HT uptake inhibitor.

In conclusion, the present results on inhibition of [^{3}H]paroxetine binding, [^{3}H]paroxetine dissociation kinetics and protection against NEM-induced [^{3}H]paroxetine binding inactivation concur with a model of the sodium-dependent 5-HT transporter in which the tricyclic and nontricyclic 5-HT uptake inhibitors bind to common or overlapping domains located at the substrate recognition site.

Acknowledgements—The authors wish to thank Mrs G. Darles for technical assistance and Miss F. Péchoux for secretarial assistance in preparing the manuscript.

REFERENCES

1. Graham D and Langer SZ, The neuronal sodium-dependent serotonin transporter: studies with [^{3}H]imipramine and [^{3}H]paroxetine. In: *Neuronal Serotonin* (Eds. Osborne NN and Hamon M), pp. 367–391. Wiley, Chichester, 1988.
2. Sette M, Briley M S and Langer SZ, Complex inhibition of [^{3}H]imipramine binding by serotonin and non-

tricyclic serotonin uptake blockers. *J Neurochem* **40**: 622–628, 1983.
3. Barbaccia ML, Gandolfi O, Chuang DM and Costa E, Modulation of neuronal serotonin uptake by a putative endogenous ligand of imipramine recognition sites. *Proc Natl Acad Sci USA* **80**: 4134–4138, 1983.
4. Reith MEA, Sershen H, Allen D and Lajtha A, High and low affinity binding of [^{3}H]imipramine in mouse cerebral cortex. *J Neurochem* **40**: 389–395, 1983.
5. Hrdina PD, Differentiation of two components of specific [^{3}H]imipramine binding in rat brain. *Eur J Pharmacol* **102**: 481–488, 1984.
6. Marcusson J, Fowler CJ, Hall H, Ross SB and Winblad B, Specific binding of [^{3}H]imipramine to protease-sensitive and protease-resistant sites. *J Neurochem* **44**: 705–711, 1985.
7. Marcusson J, Bäckström IT and Ross SB, Single site model of the neuronal 5-hydroxytryptamine uptake and imipramine-binding site. *Mol Pharmacol* **30**: 121–128, 1986.
8. Moret C and Briley MS, High- and low-affinity binding of [^{3}H]imipramine in rat hypothalamus. *J Neurochem* **47**: 1609–1613, 1986.
9. Habert E, Graham D, Tahraoui L, Claustre Y and Langer SZ, Characterization of [^{3}H]paroxetine binding to rat cortical membranes. *Eur J Pharmacol* **118**: 107–114, 1985.
10. Peterson GL, A simplification of the protein assay method of Lowry *et al* which is more generally applicable. *Anal Biochem* **83**: 346–348, 1977.
11. D'Amato R, Largent BL, Snowman AM and Snyder SH, Selective labelling of serotonin uptake sites in rat brain by [^{3}H]citalopram contrasted to labelling of multiple sites by [^{3}H]imipramine. *J Pharmacol Exp Ther* **242**: 364–371, 1987.
12. Bénavidès J, Savaki HE, Malgousis C, Laplace C, Margelidon C, Daniel M, Courteix J, Uzan A, Guérémy G and Le Fur G, Quantitative autoradiography of [^{3}H]indalpine binding sites in the rat brain. I. Pharmacological characterization. *J Neurochem* **45**: 514–520, 1985.
13. Segonzac A, Raisman R, Tateishi T, Schoemaker H, Hicks PE and Langer SZ, Tryptamine, a substrate for the serotonin transporter in human platelets, modifies the dissociation kinetics of [^{3}H]imipramine binding: possible allosteric interaction. *J Neurochem* **44**: 349–356, 1985.
14. Wennogle LP and Meyerson LR, Serotonin uptake inhibitors differentially modulate high affinity imipramine dissociation in human platelet membranes. *Life Sci* **36**: 1541–1550, 1985.
15. Kim SS and Reith MEA, Effect of serotonin on the dissociation of high-affinity binding of [^{3}H]imipramine in mouse cerebral cortex. *Neurosci Lett* **67**: 123–128, 1986.
16. Humphreys CJ, Levin J and Rudnick G, Antidepressant binding to the porcine and human platelet serotonin transporters. *Mol Pharmacol* **33**: 657–663, 1988.
17. Birdsall NJM, Hulme EC, Stockton J and Zigmong MJ, Allosteric interactions in receptor binding studies. In: *Investigation of Membrane-located Receptors* (Eds. Reid E, Cook GMW and Morré DJ), pp. 481–486. Plenum Press, New York, 1984.
18. Annamalai AE and Colman RF, Reaction of the adenine nucleotide analog 5′-p-fluorosulfonyl benzoyl adenosine at distinct tyrosine and cystein residues of rabbit muscle pyruvate kinase. *J Biol Chem* **256**: 10276–10283, 1981.
19. Shorr RGL, Minnich MD, Gotlib L, Varrichio A, Strohsacker W and Crooke ST, Evidence for tyrosine at the ligand binding site center of beta-adrenergic receptors. *Biochem Pharmacol* **35**: 3821–3825, 1986.
20. Quennedey MC, Bockaert J and Rouot B, Direct and indirect effects of sulphydryl blocking agents on agonist and antagonist binding to central α_1- and α_2-adrenoceptors. *Biochem Pharmacol* **33**: 3923–3928, 1984.
21. Nakata H, Regan JW and Lefkowitz RJ, Chemical modification of α_2-adrenoceptors. *Biochem Pharmacol* **35**: 4089–4094, 1986.
22. Davis A, Temperature-sensitive conformational changes in [^{3}H]imipramine binding sites and the involvement of sulfur-containing bonds. *Eur J Pharmacol* **102**: 341–347, 1984.
23. Biassoni R and Vaccani A, Selective effects of thiol reagents on the binding sites for imipramine and neurotransmitter amines in the brain. *Brit J Pharmacol* **85**: 447–456, 1985.
24. Scatton B, Claustre Y, Graham D, Dennis T, Serrano A, Arbilla S, Pimoule C, Schoemaker H, Bigg D and Langer SZ, SL 81.0385: a novel selective and potent serotonin uptake inhibitor. *Drug Dev Res* **12**: 29–40, 1988.
25. De Lean AP, Munson PJ and Rodbard D, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am J Physiol* **235**: E97–E102, 1978.