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# Nongenomic steroidal modulation of high-affinity serotonin transport

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**Abstract**

The ability of steroids to modulate high-affinity 5-HT transport was investigated using cell-based models which stably manifest all known properties of this transport system. β-Estradiol (E2) exhibited noncompetitive, and possibly allosteric, inhibition of both radiolabeled serotonin ([$^3$H]5-HT) transport by, and radiolabeled cocaine congener ([$^3$H]CFT) binding to, this system. Such inhibitory effects were observed within short time courses and unlikely to result from genomic effects normally ascribed to estrogen action. Rather, such nongenomic effects on 5-HT uptake were more akin to modulatory effects of select steroid metabolites on other plasma membrane systems such as neurotransmitter receptors and ionic channels. Beyond E2, preliminary examination of other steroid metabolites and synthetic steroid receptor agonists/antagonists revealed that inhibition of 5-HT transport is additionally attributable only to estriol (E3, an E2 metabolite) and tamoxifen (a nonsteroidal, E2 receptor antagonist). These findings indicate that the present form of transport modulation is only rendered by select compounds and not a general property of steroidal and related agents. Assessments of covalent conjugates of E2 suggested that E2 interacts with the transporter protein at allosteric site(s) inaccessible from the extracellular domain. These findings collectively suggest that steroid-mediated regulation of 5-HT transport may be a physiologically relevant mechanism, and that antidepressant as well as psychostimulant effects in vivo may contain a steroidal component. 

*Keywords:* Cocaine congener; β-Estradiol; Tamoxifen; Retinoid

## 1. Introduction

A key regulatory process of chemical neurotransmission is the inactivation of neurotransmitters following their stimulated release. This inactivation process is mediated by membrane-integral, high-affinity transport/reuptake systems which are relatively specific for individual neurotransmitters. Acetylcholine is the only known exception to this paradigm because extracellular acetylcholine is first hydrolyzed into acetate and choline, and choline is then sequestered by a specific transport mechanism. Such transport systems have access to the extracellular domains of neuronal synaptic and somatic regions, as well as of glial elements surrounding the synaptic clefts. They share a number of functional similarities including relatively high affinities for their ligands (when compared with the affinities of amino acid and sugar uptake systems), and dependence on extracellular $Na^+$ and $Cl^-$ ions [1]. These plasma membrane systems are structurally and pharmacologically distinct from intracellular transport systems which sequester neurotransmitters into synaptic vesicles

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from cytoplasmic domains. In addition, some of these plasma membrane transport systems are intimately involved with neuropsychiatric disorders. Most notably, the 5-HT transport system is a principal site of action of both antidepressants (tricyclics and nontricyclics) and psychostimulants (e.g., cocaine, amphetamine).

Exogenous steroid/retinoid hormones are known to profoundly affect a variety of neuronal effector mechanisms. These hormones can enter cells via diffusion across the plasma membrane, and subsequently activate appropriate nuclear receptors to function as transcriptional factors which regulate the expression of target gene sequences. Such nuclear effects are generally detectable on the time scale of hours or longer following hormone administration (presumably due to rate limitations in conveying transcriptional alterations to posttranslational effector changes). Another form of steroid/retinoid hormone action particularly pertinent to synaptic neurotransmission has been exemplified by a variety of steroid metabolites/analogs exerting regulatory effects on membrane effector systems such as ion channels (e.g., $Ca^{2+}$ channels) and neurotransmitter receptors (including nAChR, $GABA_A$ receptor, NMDA receptor) with time courses (seconds to minutes) far too brief to invoke significant transcriptional/translational effects [2,3]. These near-instantaneous, nongenomic effects of hormones on neural activities are not likely to be simply alterations of plasma membrane biophysical properties that subsequently impinged on ion channels or receptor complexes [4,5]. Rather, these effects are more likely to result from steroid association with specific plasma membrane sites [6], which may be structurally inherent in the target channels/receptors. Beyond neurotransmitter receptors and ion channels, the findings in this report will illustrate that neurotransmitter transport systems are also subject to hormonal regulation in vitro via nongenomic mechanism(s).

## 2. Materials and methods

### 2.1. Cell models of 5-HT uptake

Previous studies in this laboratory, on the structural and mechanistic properties of 5-HT transport, have relied extensively upon cell-based models which either endogenously or exogenously express this system. These models provide significant experimental advantages by their relative uniformity in activity, ease of maintenance and continuous propagation. More significantly, the 5-HT transport systems stably expressed in these cell lines share essentially identical kinetic, pharmacological and ion-dependence properties, and have demonstrated comparabilities with CNS-derived models of 5-HT transport [7–10]. A human placental trophoblastic cell line, JAR, was previously shown to exhibit 5-HT transport activities [11]. A transfectant cell line, L-14, was derived from random transfection of human genomic DNA into mouse L-M fibroblasts [7], and has been useful in defining the common structural motifs of antidepressants which are critical for interaction with the 5-HT transporter [9]. L-14 cells and JAR cells were maintained in continuous culture and used for transport assays by following procedures previously described. Monkey kidney COS-1 cells transiently expressing the cloned mouse brain SERT (mSERT) cDNA (GenBank accession no. AF013604), following the lipofection-mediated transfection procedure as previously described, also manifest the same transport properties as the other two cell lines [10].

### 2.2. [$^3$H]5-HT transport

Cultured cells were trypsinized and then plated into 96-well plates for measurements of initial transport velocities. Two to 3 days post-plating, when the cells reached confluence, they were rinsed twice with oxygenated Krebs' Ringer buffer (128 mM NaCl, 5.2 mM KCl, 2.1 mM $CaCl_2$, 2.9 mM $MgSO_4$, 5 mM glucose, 10 mM Hepes, pH 7.4; supplemented with 0.5 mM pargyline and 0.5 mM ascorbate) and then incubated at 37°C with [$^3$H]5-HT. At the end of the incubation, excess radioligands were removed and the cell-monolayers were washed three times with excess Ringer's buffer at room temperature. Incubations in the presence of 100 μM imipramine served to measure nonspecific uptake levels, and yielded the same uptake levels as measured in untransfected cells. Incubated cells were then lysed with 1% SDS and transferred into counting vials for liquid scintillation spectrometry. Additional wells were processed in parallel and used for protein determination by the

BCA method, in order to normalize the observed uptake activities.

### 2.3. [$^3$H]Cocaine congener binding

Initial studies indicated that radiolabeled 3β-(4-fluorophenyl)tropan-2β-carboxylic acid methyl ester ([$^3$H]CFT) bound to the 5-HT transporter in both mSERT-transfected COS-1 cells and L-14 cells in concentration-saturable manners. Curiously, binding activities were also detected in untransfected COS-1 and L-M cells, at levels substantially lower than those of the transfected cells, and characterized by nonsaturability (with increasing [$^3$H]CFT concentrations up to 50 nM) as well as both imipramine and cocaine sensitivities (data not shown). The nature of such binding sites in the untransfected cells were unclear. Specific levels of [$^3$H]CFT binding were operationally defined as differences in binding activities measured in both transfected and correspondingly untransfected cells. Confluent cell monolayers in 96-well plates were used for equilibrium [$^3$H]CFT binding by the same procedure used for transport studies, but with the following modifications: incubations were at 4°C for 2 h (in order to reach equilibrium); post-binding washes used ice-cold Ringer's buffer. By this approach, mSERT-transfected COS-1 cells exhibited a saturable binding profile best characterized by one binding site with an equilibrium $K_d$ of 21.3 nM, $B_{max}$ of 2.47 pmol/mg protein and Hill coefficient of 1.01. This binding activity was also competitively inhibited by antidepressants and other cocaine analogs. Similar findings were also attained from L-14 cells (when using L-M cells for measuring nonspecific binding activities).

### 2.4. Materials

The expression vector used for heterologous expression of mSERT, pBK-CMV, was purchased from Stratagene. Dulbecco's modified Eagle medium, lipofectamine and Opti-MEM medium were from Gibco/BRL. Defined calf serum was obtained from HyClone. [$^3$H]5-HT (20–35 Ci/mmol), [$^3$H]CFT (60–87 Ci/mmol), [α-$^3$H]AIB (10–25 Ci/mmol), D-[$^3$H]glucose (20–30 Ci/mmol) and [$^3$H]cocaine (25–50 Ci/mmol) were from New England Nuclear. All hormone analogs/metabolites/conjugates were from Sigma. The BCA protein quantitation kit was purchased from Pierce.

## 3. Results

### 3.1. Acute hormone administration

L-14 cells, when exposed to various hormones concurrently with [$^3$H]5-HT for only 10 min, exhibited significant inhibition of 5-HT transport by E2 between $10^{-4}$ and $10^{-8}$ M (Fig. 1), with an apparent $IC_{50}$ of 4.4 μM. The potency of E2 in this case was quite similar to that of an earlier study demonstrating E2's inhibition of 5-HT transport as discerned in brain synaptosomes [12]. The other tested hormones exhibited only modest inhibition even at 100 μM, and therefore deemed not as effective as E2. Studies with JAR and mSERT-transfected COS-1 cells also revealed that E2 was the only effective hormone at inhibiting 5-HT transport and with $IC_{50}$ values comparable to that discerned from L-14 cells. The apparent transport inhibition by E2 was unlikely to be strictly a nonspecific or 'global' membrane effect

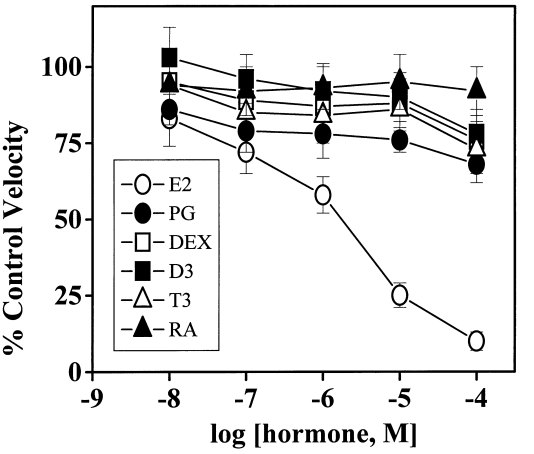


Fig. 1. Acute hormone effects on [$^3$H]5-HT uptake by L-14 cells. Initial uptake velocities were measured as the differences between [$^3$H]5-HT (0.1 μM) incubations (at 37°C for 10 min) in the presence and absence of 100 μM imipramine. Cells were cultured in normal media/sera. Hormones at the indicated concentrations were added when the uptake measurements were initiated in oxygenated Ringer's buffer. Initial velocity (pmol/mg per min) was calculated for each hormone treatment, and normalized by control uptake velocity. Comparable findings were also attained from JAR and mSERT-transfected COS-1 cells. Each point is mean ± S.D. ($n = 8$).

(e.g., alterations of membrane fluidity caused by steroid diffusion into the lipid bilayer) since all of the hormones tested, including E2, have similar hydrophobic tendencies and comparable lipid-partitioning capacities. Furthermore, in the same cells used for 5-HT transport studies, we have also observed that E2 was less inhibitory on glucose uptake activities (as measured by specific uptake of D-[$^3$H]glucose), and not detectably inhibitory on system A of amino acid uptake (as measured by specific uptake of [α-$^3$H]AIB).

The apparently limited efficacies of the other hormones in transport inhibition initially suggested the possibility that E2 might be mediating its inhibitory effect either via membrane-bound or intracellular estrogen receptors. Examination of estrogenic metabolites (E1 and E3) and an estrogen receptor antagonist (tamoxifen) revealed that, while estrone was ineffective, estriol (E3) and tamoxifen both inhibited SERT activity with relatively comparable inhibitory potencies ($IC_{50}$ of E3 is 25.5 μM; that of tamoxifen is 16.9 μM). Based on these findings, it seemed unlikely that conventional estrogen receptors mediated the observed effects, since they manifest widely varying affinities for these estrogenic agents [13,14]. Additionally, the time course (10 min) of hormone treatment in these studies were deemed inadequate for activation of nuclear receptor pathways.

### 3.2. Chronic hormone administration

The effects on 5-HT transport of chronically administering these hormones in culture were also examined. Only JAR cells were suitable for this particular study since the endogenous human SERT gene is expressed through its appropriate, *cis*-regulatory elements. Because of the short-term estrogenic effects on 5-HT transport (as shown in Fig. 1), chronic hormone studies required that the JAR cells be maintained in specialized media devoid of estrogenic substances. It is well known that phenol red, which serves as pH indicator in most culture media formulations, is highly estrogenic [15]. The calf sera normally used to supplement the culture media also contain steroids/retinoids and their metabolites which can be efficiently removed by charcoal stripping as previously described [16]. To avoid interference from these sources, JAR cells were maintained in culture media deprived of phenol red, and supplemented with charcoal-stripped calf sera for at least 48 h prior to experimental use.

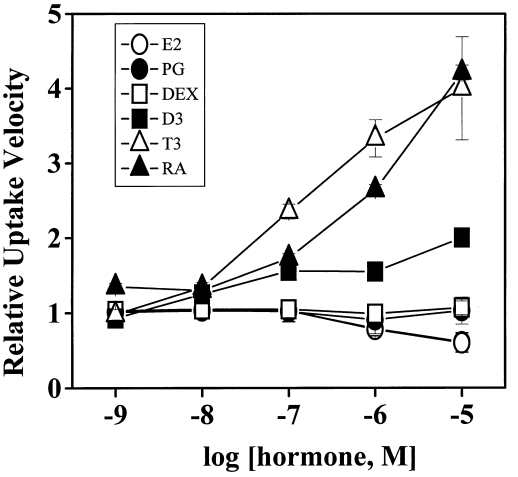


Fig. 2. Chronic hormone effects on [$^3$H]5-HT uptake activity in JAR cells. Cells were cultured in medium deprived of phenol red, and charcoal-stripped sera for at least 48 h prior to uptake assay (to minimize cell exposure to estrogenic agents). Hormones at the indicated concentrations were added to cells in the modified culture medium. Uptake velocity was measured for 48 h following hormone treatment by incubating cells with 0.1 μM [$^3$H]5-HT for 10 min at 37°C. Nonspecific uptake level was measured by inclusion of 100 μM imipramine. Measured velocities were normalized by control levels measured in the absence of hormone addition. The same hormones were tested as in Fig. 1. Each point is mean ± S.D. ($n = 8$).

Under these conditions, progesterone and dexamethasone exhibited minimal effects on 5-HT transport activities; at concentrations above 1 μM, E2-mediated inhibition became evident, consistent with E2's rapid inhibition of this system (as seen in Fig. 1). The reduced levels of E2-mediated inhibition observed in this case, relative to those seen during the acute administration studies, may have resulted from hormone metabolism during the prolonged incubation. 3,3′,5-triiodo-L-thyronine ($T_3$) and *all-trans* retinoic acid (RA) were highly stimulatory, and in dose-dependent manners; vitamin D was intermediate between the other steroids and $T_3$/RA (Fig. 2). These observed effects were not reflective of changes in cell number following hormone treatments. Because the time course of hormone exposure in this study was of sufficient length to allow activation of the respective nuclear hormone receptors, the observed transport-stimulatory effects in this case might be attributable to hormone-induced transcriptional changes of hu-

man SERT in JAR cells. The long-term hormone effects were also notably distinct from those of the short-term study, thereby further strengthening the notion that the effects seen in Fig. 1 represented nongenomic regulation of 5-HT transport.

### 3.3. E2 modulation of [$^3$H]5-HT transport

The nongenomic mechanism of transport inhibition attributed to E2 was further analyzed. Using all three cell lines, varying concentrations (up to 30 μM) of E2 were incorporated into kinetic analysis with differing [$^3$H]5-HT concentrations (0.1–0.4 μM). Dixon plot of the results attained under these conditions revealed that the inverse-velocity profiles all intersected on the *x*-axis, and not above said axis, indicating that E2 inhibited transport via a noncompetitive (possibly allosteric) mechanism (Fig. 3). This finding was consistent for all three cell lines, and at variance with a competitive mechanism of transport inhibition attributed to E2 in a prior study [12]. The novelty of this finding is that the vast majority of known inhibitors for this transport system function via competitive mechanism(s) and mutually compete for common or overlapping binding site(s) on the transporter [9]. The possibility of an allosteric site on the transporter that modulates 5-HT transport readily suggested that E2 might also modulate transport binding of cocaine derivatives and antidepressant agents [17–20].

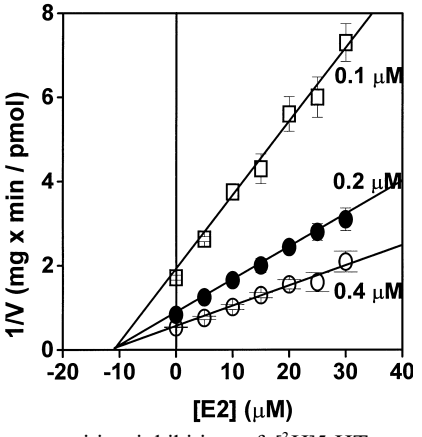


Fig. 3. Noncompetitive inhibition of [$^3$H]5-HT uptake by E2. Dixon plot of initial uptake velocities measured in L-14 cells using [$^3$H]5-HT concentrations of 0.1, 0.2 and 0.4 μM, and each [$^3$H]5-HT concentration in the presence of E2 concentrations ranging from 0 to 30 μM. Convergence of the inverse-velocity profiles on the *x*-axis indicates a noncompetitive mechanism of transport inhibition. Transport velocities were measured at 37°C for 10 min; inclusion of 100 μM imipramine served to measure nonspecific transport velocities. The same inhibitory mechanism was discerned in JAR and mSERT-transfected COS-1 cells. Each value is mean ± S.D. ($n = 6$).

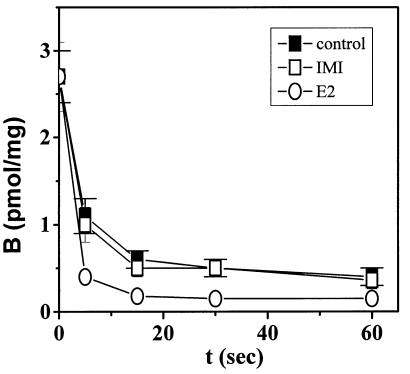


Fig. 4. Estrogenic modulation of [$^3$H]CFT dissociation from SERT binding. After binding of [$^3$H]CFT (100 nM) to L-14 cells reached equilibrium (at 4°C for 2 h), unbound radioligand was removed and the cells were exposed at room temperature to Ringer's buffer alone (Control), Ringer's plus 100 μM E2 (E2), or Ringer's plus 100 μM imipramine (IMI) for the indicated time before quantitation of bound [$^3$H]CFT levels. The differential decreases of specific binding activities, as functions of time, were indicative of E2's ability to increase the off-rate of bound [$^3$H]CFT, an ability attributable to a noncompetitive inhibitor acting through allosteric mechanism(s). Comparable results were observed with mSERT-transfected COS-1 cells. Each point is mean ± S.D. ($n = 6$).

### 3.4. E2 modulation of [$^3$H]CFT binding

E2 was also examined for its ability to modulate transport-inhibitor binding to the transport system. After [$^3$H]CFT binding to L-14 cells was allowed to reach equilibrium (at 4°C), the bound ligand was allowed to dissociate into excess Ringer's buffer at room temperature for varying periods of time before the levels of SERT-specific [$^3$H]CFT binding were quantitated. As shown in Fig. 4, the presence of E2 (100 μM) in the Ringer's buffer during the dissociation phase clearly altered the level of [$^3$H]CFT remaining bound to the 5-HT transporter, presumably because E2 allosterically increased the binding off-rate. Imipramine, however, did not exhibit significant effect on the off-rate of bound [$^3$H]CFT, consistent with its action as a competitive inhibitor of 5-HT uptake and [$^3$H]CFT binding. These findings suggested that E2 can allosterically and noncompeti-

tively inhibit cocaine analogs from binding to the 5-HT transporter, in keeping with kinetic results indicating that E2 noncompetitively and allosterically inhibited 5-HT uptake. In parallel binding studies, E2 exerted similar effects on [$^3$H]CFT binding to mSERT-transfected COS-1 cells, while having no effect on binding to the untransfected COS-1 cells. The presence of E2 reduced the level of bound [$^3$H]CFT in L-14 cells to almost that of background by 5 min, when the binding dissociation was conducted at room temperature. The same study, when conducted at 4°C, revealed no discernible differences in specific binding levels until after 15 min, strongly indicating that the mechanism of E2-mediated binding modulation was also temperature dependent. The same binding/dissociation paradigm, when applied to [$^3$H]cocaine binding to the transport system, yielded qualitatively comparable results. Thus, uptake and binding studies both point to a consistent mechanism whereby E2 allosterically modulates this transport system, and such modulation presumably arises from E2 interacting with the transporter at a site distinct from the site shared by 5-HT and other transport inhibitors.

### 3.5. Survey of steroid analogs/metabolites

As mentioned previously, E2, E3 and tamoxifen exhibited inhibitory, nongenomically mediated activities toward 5-HT transport. Estrone, progesterone and dexamethasone were relative ineffective in mediating such activity. The extent to which other steroid metabolites (in the biosynthetic pathways of progestins, androgens and estrogens) might impact this transport system was also examined. In addition, synthetic steroid analogs (beclomethasone and diethylstilbestrol) and covalent E2 conjugates were also tested. The findings (summarized in Table 1) indicated that of all of the steroids/analogs tested, only E2, E3 and tamoxifen were capable of rendering discernable transport inhibition at concentrations below 100 μM. Thus, inhibition of 5-HT transport is not a general property of steroidal compounds and subtle structural variations have dramatic impacts on transport-inhibitory potencies. Lack of transport modulation by diethylstilbestrol is further confirmation that nuclear estrogen receptors are not likely to be involved in the observed transport effects, since diethylstilbestrol possesses high affinity for the estrogen receptor and mimics E2's biological effects in a variety of systems. Sulfate and glucuronide conjugates of E2 also presented no inhibitory activity toward transport. Since such covalent modifications of E2 impede its partitioning into the membrane lipid bilayer, it is tempting to speculate that E2's site of action is not directly accessible from the extracellular domain. Consistent with this notion, we have also observed that an E2:BSA conjugate (which contains about 30 moles of E2 per mole of BSA) possessed no inhibitory activity at concentrations the equivalent of 500 μM E2. These observations provide further distinction between the E2-binding site on the transporter from the site for which 5-HT and antidepressants/cocaine congeners mutually compete (since the latter is accessible from the extracellular domain).

## 4. Discussion

The in vitro findings presented herein collectively indicate that select, estrogen-related steroids are ca-

Table 1
Inhibitory potencies of steroid metabolites/analogs

| Steroid | $IC_{50}$ (μM) |
|---|---|
| Pregnenolone | >100 |
| Pregnenolone sulfate | >100 |
| 5α-Dihydropregnanolone | >100 |
| Testosterone | >100 |
| Dehydroisoandrosterone | >100 |
| 17β-Estradiol | 4.4 ± 0.3 |
| ß-Estradiol-17-glucuronide | >100 |
| 17β-Estradiol-3-sulfate | >100 |
| 17β-Estradiol-3-glucuronide | >100 |
| Estrone | >100 |
| Estriol | 25.5 ± 2.1 |
| Diethylstilbestrol | >100 |
| Tamoxifen | 16.9 ± 1.4 |
| Corticosterone | >100 |
| Deoxycorticosterone | >100 |
| Allotetrahydrodeoxy-corticosterone | >100 |
| Progesterone | >100 |
| Dexamethasone | >100 |
| Beclomethasone | >100 |

Each metabolite/analog was tested at several concentrations ranging from 0.1 to 100 μM for inhibition of [$^3$H]5-HT (0.1 μM) uptake velocity. Velocity measurements were conducted at 37°C for 10 min; nonspecific velocity was measured by inclusion of 100 μM imipramine. Each value is mean ± S.D. ($n$ = 6).

pable of inhibiting 5-HT transport via a nongenomic mechanism. The steroid-mediated effect on 5-HT transport extend beyond ligand translocation to interaction with competitive inhibitors as well. Consistent manifestations of this form of transport modulation in three distinct cell models rule out the possibility of cell-specific factor(s) that contributed to the observed transport effects. Rather, this form of transport inhibition is mediated by a noncompetitive and possibly allosteric mechanism which is clearly distinct from those of antidepressants and psychostimulants, and likely to involve direct interaction between the steroids and the 5-HT transporter. Both tricyclic and nontricyclic antidepressants competitively inhibit 5-HT transport and share with 5-HT common or overlapping binding sites on the transport system. Molecular modeling of the nontricyclic antidepressant structures led to a prior proposal that interaction with the 5-HT transporter at the ligand-binding site required a specific spatial separation of an amino group and a phenyl moiety, acting as a transport pharmacophore [9]. Cocaine congeners function as competitive inhibitors of both 5-HT transport and antidepressant binding to the system, and presumably share identical or overlapping binding sites with antidepressants and 5-HT. These congener structures also contain the previously described pharmacophore (Chang, unpublished results). The antidepressants and psychostimulants all interact with this transport system at domains accessible from the extracellular domain. The structural lack of the proposed pharmacophore in E2 (as well as in E3), the apparent interaction of E2 with the transporter at site(s) inaccessible from the extracellular domain, plus the noncompetitive nature of transport/binding inhibition by E2 are all consistent with its mechanistic distinction from other inhibitors of 5-HT transport.

Other studies have suggested that the central 5-HT uptake system fluctuates during the estrus cycle [21]. Exogenous administrations of steroids such as β-estradiol (E2) and progesterone (PG) can also effect brain region-specific changes in 5-HT uptake. [22–25]. Peripheral gonadectomies have been shown to also alter the activity of this transport system in brain region-specific manners [26–29]. In light of the present findings, such hormone effects on 5-HT transport in vivo are likely to represent both transcriptional changes of the transporter gene (as elicited by nuclear steroid hormone receptors) and nongenomic modulations of transport functions (as elicited by the steroid hormones directly). It is therefore of interest, in future studies, to re-examine hormonal effects on 5-HT uptake in vivo in terms of delineating the relative contributions of both genomic and nongenomic regulatory mechanisms.

The described findings are also consistent with nongenomic, steroidal regulation of ion channel and receptor activities in sharing several functional commonalities. First, such steroid effects tend to be mediated by allosteric modulation of membrane effector systems [2,3,30–33]. Second, the time course of such modulatory effects are near-instantaneous; in the case of ion channels, steroid-mediated effects can be observed within seconds of steroid application [4,34]. Third, covalent conjugations (with sulfate, glucuronide or lipid moieties) and even subtle structural alterations (as occur during steroid metabolism) can dramatically and unpredictably alter the potency of neuroactive steroids in this particular mechanistic context [12,31,35,36]. Fourth, unlike steroid affinities for their respective nuclear receptors, half-maximal concentrations of steroids in modulating channel/receptor activities often fall within 1–10 μM [5,37–39]. While this apparently low range of steroid potencies on membrane effector systems supports the notion for lack of genomic involvement, the physiological relevance of such modulatory phenomena is uncertain since steroid/metabolite concentrations in vivo are thought to rarely exceed the nM range (or at least so in the general circulation).

Several possible explanations have been advanced in order to preliminarily reconcile the apparent inconsistency between endogenous steroid concentrations and discernable potencies in imparting nongenomic regulatory effects on membrane effector systems. Steroid metabolite levels in the brain has been shown to be significantly higher than those quantitated in peripheral circulation [40] and exhibit significant region-specific variations [41,42]. CNS steroid metabolite levels are subject to significant and potentially rapid fluctuations during menstrual/estrous cycles and in response to induced stress [43]. Collectively, these observations suggest that CNS levels of steroid metabolites have the potential to reach levels capable of modulating channels and re-

ceptors. Steroid metabolites are often sequestered by binding proteins in both cytoplasmic and extracellular domains (which could render region-specific variations in steroid concentrations in the CNS), and a large portion of each metabolite may also be covalently conjugated into water- or lipid-soluble forms [44]. Such sequestrations and covalent conjugations can effectively reduce the active or available concentrations of each metabolite at or near each membrane effector system, possibly allowing the local concentration of each steroid to be drastically underestimated [32]. The overall structural similarities between the tested steroidal compounds suggest that subtle changes in the basic steroid structure can dramatically alter the potency of 5-HT transport inhibition. It is possible, therefore, that endogenous steroids exist in the CNS which might be more potent inhibitors of 5-HT transport than E2. Relevance of these possible explanations to steroid-mediated regulation of 5-HT transport in vivo requires additional study.

Nongenomic mechanism of 5-HT transport modulation by endogenous steroids, as elucidated from the studies described herein, might be better understood with additional insights into the structural attributes of this system. The noncompetitive and possibly allosteric effects of E2 on both ligand transport and cocaine binding processes, which are likely to extend to antidepressant interactions with SERT as well, might be mediated through E2-induced conformational changes in the transporter. The exact quaternary structure of the active transport complex, and its possible perturbations by E2, also remain to be defined. Further, it will be of interest to examine the general applicability of this form of transport modulation to other neurotransmitter transport systems. Given the intimate link between the 5-HT transport system and several neuropsychiatric disorders (e.g., depression, substance abuse and addiction), the present findings also suggest the provocative possibility of endocrine involvement in the underlying etiology or progression of these disorders, and may preliminarily substantiate the long-held belief that gender-specific distinctions exist in such disorders. Further, steroid-mediated modulation of inhibitor-binding indicates that endocrine functions may impact the efficacy of antidepressant therapies targeted specifically toward this transport system, the psychostimulant effects mediated by 5-HT transport, as well as individual susceptibilities to substance abuse/addiction. These issues will be better assessed through in-depth understanding of the physiological significance of steroid-mediated regulatory impacts, both genomically and nongenomically, on 5-HT transport.

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