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INHIBITION OF SYNAPTOSOMAL HIGH-AFFINITY UPTAKE OF DOPAMINE AND SEROTONIN BY ESTROGEN AGONISTS AND ANTAGONISTS

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Abstract—High-affinity uptake of dopamine and serotonin into a synaptosomal preparation from rat cerebral cortex was inhibited by a number of estrogen agonists and antagonists *in vitro* in a stereoselective and competitive manner. The most potent estrogenic inhibitors in the dopaminergic and serotonergic system were ethinylestradiol (K_I = 558 nM) and 2-hydroxyethinylestradiol (K_I = 226 nM), respectively. Structure–activity relationships are discussed and compared with the effects of estrogens on noradrenaline uptake. However, as all physiologically occurring estrogens inhibited amine uptake only in the micromolar concentration range it seems unlikely that this direct interaction of estrogens with the amine carrier is responsible for the changes in dopamine and serotonin uptake observed during the estrous cycle or after *in vivo* administration of estrogens and/or progesterone.

Estrogens act on the central nervous system controling various events such as the release of gonadotropins from the pituitary gland [1] or sexual behaviour [2]. The mechanisms by which the sex steroids modulate nerve cell activity in the brain are still obscure. Classical concepts assume modifications in the expression of the genome via activation of intracellular hormone receptors [3]. The resulting estrogen effects are slow in onset and long-lasting. On the other hand, Kelly *et al.* [4] reported that iontophoretic application of estradiol causes immediate changes in neuronal activities. The rapid onset and the short duration of this effect provided evidence for a non-genomic, presumably membrane-mediated process [5]. However, little is known about the biochemical nature and the physiological role of the non-genomic effects.

One important membrane-mediated effect in the central nervous system is the reuptake of released neurotransmitters which is the main mechanism of inactivation of the catecholamines noradrenaline and dopamine and of serotonin [6]. Changes of noradrenaline, dopamine and serotonin uptake into synaptosomes have been observed during the estrous cycle and after administration of estradiol and/or progesterone [7–11].

Recently, we have shown in synaptosomal preparations from rat cerebral cortex and hypothalamus that estrogens can inhibit noradrenaline uptake *in vitro* in a competitive manner [12]. Further investigations, however, revealed that most of the noradrenaline applied to a synaptosomal preparation from rat cerebral cortex is accumulated in dopaminergic nerve terminals [13].

Thus, the aim of the present investigation was to study the effects of estrogen agonists and antagonists on the synaptosomal uptake of dopamine and serotonin. Structure–activity relationships for the inhibition of dopamine and serotonin uptake by steroid hormones are discussed and compared with the effects of these compounds on noradrenaline uptake.

MATERIALS AND METHODS

Chemicals. 3,4[Ring-2,5,6-^{3}H]dihydroxyphenylethylamine (dopamine, spec. act. 40–60 Ci/mmole) and 5-[1,2-^{3}H]hydroxytryptamine (serotonin, spec. act. 26 Ci/mmole) were obtained from New England Nuclear.

Desmethylimipramine (DMI, mono-*N*-desmethylimipramine, hydrochloride) and clomipramine (hydrochloride) were donated by Ciba Geigy (Basel, Switzerland), amitriptyline (hydrochloride) and nortriptyline (hydrochloride) by Tropon and nomifensine (hydrogenmaleinate) by Hoechst (Frankfurt/Main, F.R.G.). Estradiol-17β (1,3,5(10)-estratriene-3,17β-diol), estrone (3-hydroxy-1,3,5(10)-estratriene-17-one), testosterone (17β-hydroxy-4-androsten-3-on), cortisone (17α,21-dihydroxy-4-pregnen-3,11,20-trione) and cortisol (11β, 17α,21-trihydroxy-4-pregnen-3,20-dione) were obtained from Merck (Darmstadt, F.R.G.), progesterone (4-pregnen-3,20-dione), 5α-dihydroprogesterone (5α-pregnan-3,20-dione) and diethylstilbestrol (*trans*-3,4-bis(*p*-hydroxyphenyl)-3-hexene) from Schering (West Berlin). 5α-Dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) was purchased from Sigma (Munich, F.R.G.) and estradiol-17α (1,3,4(10)-estratriene-3,17α-diol) and 17α-ethinylestradiol (17α-ethinyl-1,3,5(10)-estratriene-3,17β-diol) from Steraloids (Paesel, Frankfurt/Main, F.R.G.). Moxestrol (17α-ethynyl-11β -methoxy-1,3,5(10)-estratriene-3,17β-diol) was donated by Roussel UCLAF (U.S.A.), enclomiphene (RMI 16,289, *trans*-2-[*p*-(2-chloro-1, 2-diphenylvinyl)phenoxyl-triethylamine citrate) by Merrel Dow Pharmaceuticals (Cincinnati, OH), monohydroxytamoxifen (ICI 79,820, *trans*-1-(*p*-β-dimethylaminoethoxyphenyl)1 - (*p*-hydroxy-

phenyl)-2-phenyl-bet-1-ene) by Imperial Chemical Industries (Macclesfield, Cheshire, England) and nitromifene (CI 628, 1-([2-(*p*-methoxaphenyl)-β-nitrostyryl]phenoxyethyl)pyrrolidine, citrate, mixture of *cis* and *trans* isomers) by Parke-Davis & Co. Research Labs (Ann Arbor, MI).

The following catecholestrogens were kindly donated by Dr R. Knuppen (Institut für Biochemische Endokrinologie, Medizinische Hochschule, Lübeck, F.R.G.). They had been synthesized according to the method of Stubenrauch and Knuppen [14] with special precautions taken to ensure the absence (<50 ppm) of monophenolic estrogens: 2-hydroxyestradiol-17β(1,3,5(10)-estratriene-2,3,17β-triol); 2-hydroxyestradiol-17α(1,3,5(10)-estratriene-2,3,17α-triol); 4-hydroxyestradiol-17β-(1,3,5(10)-estratriene-3,4,17β-triol); 4-hydroxyestradiol-17α-(1,3,5(10)-estratriene-3,4,17α-triol); 2-hydroxyestrone(2,3-di-hydroxy-1,3,5(10)-estratriene-17-one); 4-hydroxy-estrone (3,4-dihydroxy-1,3,5(10)-estratriene-17-one); 2-hydroxyethinylestradiol(17α-ethinyl-1,3,5(10)-estratriene-2,3,17β-triol); 4-hydroxyethinylestradiol(17α-ethinyl-1,3,5(10)-estratriene-3,4,17β-triol); 2-methoxyestradiol(1,3,5(10)-estratriene-2,3,17β-triol-2-methyl ether); 2-methoxyestrone (2,3-dihydroxy-1,3,5(10)-estratriene-17-one-2-methyl ether). The following methyl-substituted estrogens, also donated by Dr Knuppen, had been prepared as described by Ball *et al.* [15]: 2-methylestradiol (2-methyl-1,3,5(10)-estratriene-3,17β-diol); 4-methylestradiol (4-methyl-1,3,5(10)-estratriene-3,17β-diol).

Measurement of dopamine and serotonin uptake. Rats of either sex of the strain Han:SPRD were obtained from the Zentralinstitut für Versuchstiere (Hannover, F.R.G.). Synaptosomes from cerebral cortex were prepared according to Whittaker and Barker [16] with slight modifications. For measurement of synaptosomal amine uptake the synaptosomal suspension was incubated in the absence and presence of competitors and the tritiated amine (50 nM unless otherwise indicated) in a total volume of 1 ml for 3 min at 37°. The incubation was stopped by rapid vacuum filtration over Whatman GF/C filters. Radioactivity accumulated by synaptosomes at 0–4° was routinely subtracted as a blank. At an amine concentration of 50 nM blanks were in the range of 5% of totally accumulated radioactivity. Uptake was calculated as pmole/min/mg synaptosomal protein. Protein was determined according to the method of Lowry *et al.* [17] with bovine serum albumin as a standard. Details of the procedure have been described previously [12].

Kinetic constants K_M and V_{max} of synaptosomal amine uptake were calculated from Lineweaver–Burk plots. Inhibitor constants (K_I) were estimated from the apparent K_M values ($K_{Mapp.}$) in the presence of 10^{-6} M inhibitor ([*I*]) by use of the equation given by Cheng and Prusoff [18] for competitive inhibition:

$$K_I = K_M^* [I]/(K_{Mapp.} - K_M)$$

Unless otherwise stated, results are given as means of a fivefold determination from at least two independent experiments. Results of the inhibition experiments were compared to controls by Student's *t*-test.

Table 1. Inhibition of dopamine and serotonin uptake in synaptosomes from rat cerebral cortex by andidepressants

	IC_{50} for dopamine uptake (μM)	IC_{50} for serotonin uptake (μM)
Amitriptyline	5	0.1
Clomipramine	9	0.02
Imipramine	1.6	0.1
Mianserin	–	5
Nomifensine	0.02	3
Nortriptyline	4	0.5

IC_{50}-values were determined as 50% inhibition of uptake of dopamine (50 nM) or serotonin (50 nM) into synaptosomes from rat cerebral cortex. Antidepressants were tested in concentrations ranging from 10^{-10} to 10^{-4} M. Given are means of at least two independent experiments.

RESULTS

Dopamine uptake

The kinetic constants of high affinity, low capacity uptake of dopamine into synaptosomes from cerebral cortex found in the present investigation were: K_M 254 ± 74 nM and V_{max} 50.9 ± 14.9 pmole/min/mg protein (N = 5).

Synaptosomal dopamine uptake was inhibited by antidepressants with the following order of potency: nomifensine ≫ nortriptyline > amitriptyline > clomipramine > imipramine (Table 1). Dopamine uptake was inhibited by some of the estrogen agonists

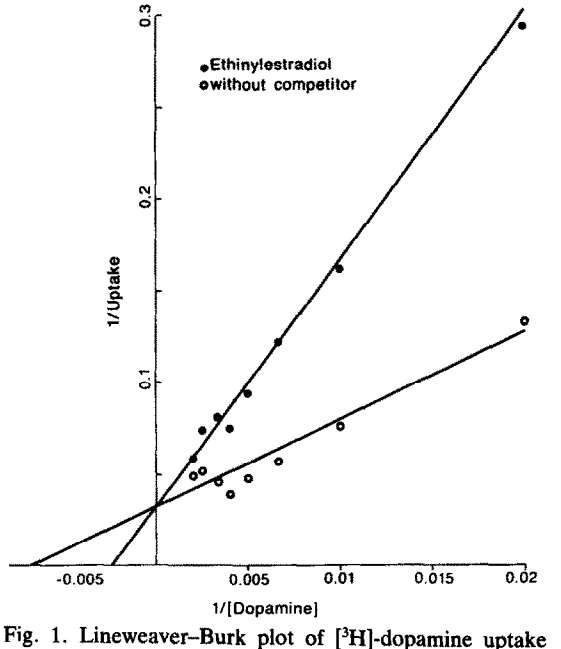

Fig. 1. Lineweaver–Burk plot of [^{3}H]-dopamine uptake kinetics in the absence (no competitor) and presence of 10^{-6} M 17α-ethinylestradiol. Uptake (pmole/min/mg protein) into synaptosomes from cerebral cortex was determined as described in the text. Each point represents the mean of a 5-fold determination in two independent experiments (SD less than 15%).

and antagonists included. Ethinylestradiol was the most potent inhibitor which reduced dopamine uptake by 60% in a concentration of 10^{-6} M and 90% in a concentration of 10^{-5} M. Lineweaver–Burk plots of dopamine uptake obtained in the absence and presence of 10^{-6} M ethinylestradiol revealed increased K_M- and unchanged V_{max}-values (Fig. 1). This points to competitive rather than non-competitive inhibition of dopamine uptake by estrogens. A K_I value of 558 nM was estimated from these plots.

2-Hydroxyethinylestradiol was less potent and inhibited dopamine uptake by 30% (10^{-6} M) and 70% (10^{-5} M). A similar potency was observed for 4-hydroxyethinylestradiol (Table 2). All other steroids were less potent. At a concentration of 10^{-5} M estradiol-17β inhibited dopamine uptake by 40%; its catechol derivatives (2-hydroxyestradiol-17β and 4-hydroxyestradiol-17β) were slightly less effective. Methylation of the 2-hydroxygroup (2-methoxyestradiol-17β) completely abolished the inhibitory effect. Similarly, the 11β-methoxy derivative of ethinylestradiol (moxestrol) did not affect synaptosomal dopamine uptake (Table 2). While 2-methylestradiol-17β decreased dopamine uptake to the same extent as 2-hydroxyestradiol-17β, 4-methylestradiol-17β had no effect (Table 2).

Estrone reduced dopamine uptake only slightly. No inhibitory effect could be shown neither for 2-hydroxyestrone nor for 2-methoxyestrone (Table 2). Stereospecificity of the inhibitory effect of estrogens was indicated by the finding that the 17α-epimers of estradiol-17β and 2-hydroxyestradiol-17β were much less potent or even ineffective. On the other hand, 4-hydroxyestradiol-17α inhibited dopamine uptake to a similar extent as 4-hydroxyestradiol-17β (Table 2).

The non-steroidal synthetic estrogen agonist diethylstilbestrol inhibited dopamine uptake to a similar extent as estradiol-17β. The non-steroidal estrogen antagonists nitromifene, monohydroxytamoxifen or enclomiphene did not affect dopamine uptake at concentrations of 10^{-6} M, but were equipotent with ethinylestradiol, the strongest estrogenic inhibitor, at a concentration of 10^{-5} M (Table 2). The non-estrogenic steroids progesterone, 5α-dihydroprogesterone, testosterone, 5α-dihydrotesto-

Table 2. Competition by estrogens agonists and antagonists for [^{3}H]-dopamine (50 nM) and [^{3}H]-serotonin (50 nM) uptake into synaptosomes from rat cerebral cortex expressed as per cent of uptake in the absence of inhibitor

	Dopamine-uptake			Serotonin-uptake		
	Concentration of inhibitor			Concentration of inhibitor		
Inhibitor	10^{-7} M	10^{-6} M	10^{-5} M	10^{-7} M	10^{-6} M	10^{-5} M
Ethinylestradiolderivatives						
Ethinylestradiol	84 ± 7.3	40 ± 0.4*	8 ± 1.7*	104 ± 11.6	59 ± 4.4*	16 ± 1.8*
2-Hydroxyethinylestradiol	92 ± 3.4	69 ± 7.3*	31 ± 1.6*	66 ± 3.8*	26 ± 1.9*	10 ± 1.0*
4-Hydroxyethinylestradiol	92 ± 6.8	80 ± 11.9*	31 ± 2.0*	90 ± 7.5	64 ± 8.1*	21 ± 2.3*
Moxestrol	91 ± 5.4	106 ± 4.0	93 ± 6.4	n.d.	n.d.	n.d.
Estradiolderivatives						
Estradiol-17β	101 ± 8.7	85 ± 5.9	64 ± 5.1*	104 ± 12.5	89 ± 4.4	57 ± 3.5*
2-Hydroxyestradiol-17β	109 ± 7.1	101 ± 8.6	66 ± 3.9*	96 ± 2.5	69 ± 3.8*	21 ± 3.8*
4-Hydroxyestradiol-17β	102 ± 10.6	91 ± 3.5	72 ± 4.4*	98 ± 7.4	95 ± 8.1	58 ± 4.4*
2-Methoxyestradiol-17β	108 ± 9.2	96 ± 8.6	85 ± 7.5	n.d.	n.d.	102 ± 3.8
2-Methylestradiol-17β	108 ± 5.8	102 ± 9.5	67 ± 7.3*	n.d.	n.d.	n.d.
4-Methylestradiol-17β	106 ± 9.8	111 ± 10.5	97 ± 3.5	n.d.	n.d.	n.d.
Estradiol-17α	103 ± 13.5	95 ± 11.8	80 ± 3.8*	100 ± 9.9	88 ± 5.0	81 ± 5.3*
2-Hydroxyestradiol-17α	101 ± 9.6	92 ± 9.9	93 ± 7.0	n.d.	102 ± 8.9	71 ± 7.5*
4-Hydroxyestradiol-17α	95 ± 10.2	81 ± 6.7*	58 ± 4.0*	n.d.	96 ± 10.0	79 ± 8.3*
Estronederivatives						
Estrone	96 ± 9.6	97 ± 6.4	79 ± 6.3*	103 ± 11.3	85 ± 8.9*	67 ± 7.8*
2-Hydroxyestrone	97 ± 7.7	87 ± 7.7	85 ± 3.9*	89 ± 7.5	68 ± 7.5*	23 ± 1.9*
4-Hydroxyestrone	100 ± 4.5	87 ± 12.8	83 ± 12.2	n.d.	104 ± 7.5	69 ± 4.6*
2-Methoxyestrone	n.d.	n.d.	90 ± 12.9	n.d.	n.d.	105 ± 11.3
Non-steroidal estrogen agonists and antagonists						
Diethylstilbestrol	102 ± 8.9	79 ± 5.4*	42 ± 6.1*	90 ± 2.5	85 ± 3.8*	68 ± 9.0*
Monohydroxytamoxifen	97 ± 7.8	91 ± 6.0	17 ± 1.3*	86 ± 5.3	82 ± 6.0*	56 ± 3.1*
Nitromifene	102 ± 3.7	98 ± 12.4	10 ± 1.7*	n.d.	101 ± 5.3	39 ± 3.8*
Enclomiphene	110 ± 9.8	101 ± 13.1	15 ± 1.4*	n.d.	101 ± 12.0	54 ± 1.5*
Non-aromatic steroid hormones						
Progesterone	106 ± 8.8	95 ± 4.5	89 ± 6.5	n.d.	n.d.	104 ± 8.1
5α-Dihydroprogesterone	n.d.	n.d.	96 ± 4.0	n.d.	n.d.	n.d.
Testosterone	n.d.	n.d.	112 ± 8.2	n.d.	n.d.	106 ± 4.0
5α-Dihydrotestosterone	n.d.	105 ± 11.4	93 ± 8.8	n.d.	n.d.	96 ± 8.5
Cortisone	n.d.	96 ± 5.7	94 ± 9.2	n.d.	n.d.	97 ± 3.8
Cortisol	n.d.	105 ± 7.0	98 ± 9.5	n.d.	n.d.	102 ± 8.6

Given are means ± SD of 2–4 independent experiments with a five-fold determination. n.d.: not determined.
*$P < 0.05$ vs control.

sterone, cortisone and cortisol had no inhibitory effect on synaptosomal dopamine uptake.

Serotonin uptake

The uptake of serotonin into synaptosomes from cerebral cortex had an affinity constant (K_M of 76 ± 12 nM and a capacity (V_{max} of 5.9 ± 0.7 pmole/min/mg protein (N = 9). It was inhibited by antidepressants with the well known [6] order of potency: clomipramine > imipramine = amitriptyline > nortriptyline > nomifensine > mianserin (Table 1).

Like dopamine uptake, serotonin uptake could be inhibited by some estrogen agonists and antagonists as shown in Fig. 2 and Table 2. The strongest estrogenic inhibitor was 2-hydroxyethinylestradiol. In a concentration of 10^{-6} and 10^{-5} M it inhibited serotonin uptake by 75 and 90%, respectively (Table 2). Ethinylestradiol and 4-hydroxyethinylestradiol reduced serotonin uptake by about 40% (10^{-6} M) and 80% (10^{-5} M, Table 2). Lineweaver–Burk plots of serotonin uptake data obtained in the absence and presence of 10^{-6} M of these inhibitors revealed unchanged V_{max}- and increased K_M-values pointing to competitive rather than non-competitive inhibition (Fig. 2). K_I values calculated from these plots were 226 nM for 2-hydroxyethinylestradiol, 1086 nM for ethinylestradiol and 1520 nM for 4-hydroxyethinylestradiol. Among other estrogens used (at a concentration of 10^{-5} M) 2-hydroxyestradiol-17β had a similar inhibitory potency as ethinylestradiol and 4-hydroxyethinylestradiol, inhibiting serotonin uptake by 80%. Estradiol-17β and 4-hydroxyestradiol-17β inhibited the uptake by about 40% while 2-methoxyestradiol-17β was ineffective (Table 2). 2-Hydroxyestrone reduced synaptosomal serotonin uptake by 75% and estrone and 4-hydroxyestrone by only 30% whereas 2-methoxyestrone had no inhibitory effect (Table 2). Estrogenic inhibition of synaptosomal serotonin uptake was stereospecific effect as the 17α-epimers of estradiol-17β and its catechol derivatives had a much weaker inhibitory potency reducing serotonin uptake by only 20–30% (Table 2). The synthetic estrogen agonist diethylstilbestrol was about equipotent with estrone (Table 2). The non-steroidal estrogen antagonists reduced synaptosomal serotonin uptake by 45–60% (Table 2). None of the non-estrogenic steroids progesterone, testosterone, 5α-dihydrotestosterone, cortisone or cortisol affected serotonin uptake (Table 2).

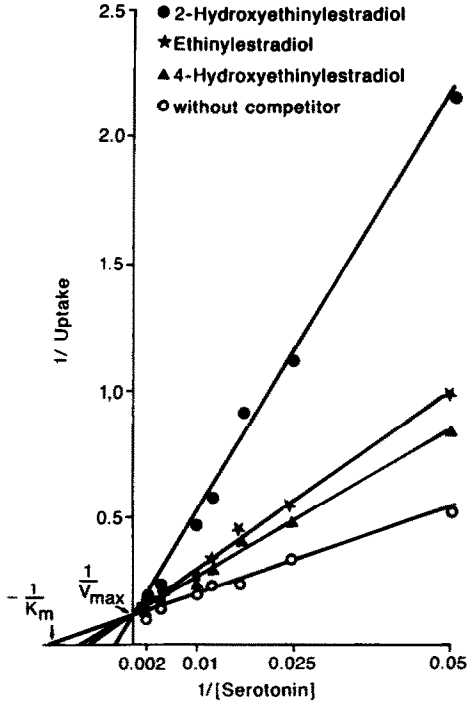

Fig. 2. Lineweaver–Burk plot of [^{3}H]-serotonin uptake kinetics in the absence (no competitor) and presence of 10^{-6} M 2-hydroxyethinylestradiol, 17α-ethinylestradiol, and 4-hydroxyethinylestradiol, respectively. Uptake (pmole/min/mg protein) into synaptosomes from cerebral cortex was determined as described in the text. Each point represents the mean of a fivefold determination in two independent experiments (SD less than 15%).

DISCUSSION

The kinetic constants for dopamine and serotonin accumulation into rat cerebral cortex synaptosomes revealed high-affinity, low capacity uptake with K_M-values of 254 nM for dopamine and 76 nM for serotonin. These affinity constants are in good agreement with values for dopamine [19, 20] and serotonin uptake [21–23] reported in the literature. From the inhibition of amine uptake by antidepressants (Table 1) an order of potency could be derived that is characteristic for the specific uptake into dopaminergic and serotoninergic neurons (for a review see ref. 6), respectively.

Dopamine and serotonin uptake were inhibited *in vitro* by a number of estrogen agonists and antagonists in a concentration dependent manner. The most potent estrogenic inhibitors were the synthetic estrogens ethinylestradiol (dopamine uptake) and 2-hydroxyethinylestradiol (serotonin uptake) with K_I-values of 558 and 226 nM, respectively. Lineweaver–Burk plots of the amine uptake data obtained in the absence and presence of 10^{-6} M of estrogens pointed to competitive rather than non-competitive inhibition (Figs. 1 and 2). Thus, estrogens seem to interact directly with the active center of the carrier molecules of dopamine and serotonin uptake. Moreover, inhibition of dopamine and serotonin uptake was stereospecific. The 17-β epimers of estradiol and its catechol derivatives (Table 2) were much more potent than the 17α-epimers of these hormones (Table 2). This is in good agreement with the classical estrogenic effects such as uterus weight gain or decrease in body weight and also with the short term effects observed by Kelly *et al.* [4].

The inhibitory effects of estrogens on dopamine uptake were very similar to those on noradrenaline uptake previously reported from our laboratory [12]. According to the classification of Koe [24], however, the estrogen agonists and antagonists are only weak inhibitors of noradrenaline uptake in the dopaminergic system they are even weaker inhibitors as indicated by the K_I-value of 558 nM (Fig. 1) for ethinylestradiol compared to 144 nM for the inhibition of noradrenaline uptake in the hypothalamus

[12]. Progesterone, 5α-dihydroprogesterone, testosterone, 5α-dihydrotestosterone, cortisone and cortisol did not interfere with catecholamine uptake (Table 2) suggesting that an aromatic ring A is needed for inhibition. Surprisingly, formation of a catechol structure by introduction of a second hydroxygroup in position 2 or 4 of the steroid did not enhance the inhibitory effect (Table 2). On the other hand, a catechol structure increases the inhibitory potency of inhibitors of the phenylethylamine type in the noradrenergic (for review see ref. 25) and in the dopaminergic system (for review see ref. 26). Methylation of the additional hydroxygroups to a methoxyfunction as well as a methoxygroup in position 11β of ethinylestradiol completely abolished the inhibitory effect (Table 2). Similar structure–activity relations have been described for the antidepressant nomifensine [27]. The inhibition of catecholamine uptake by this competitive antagonist decreases slightly after introduction of a hydroxygroup in the aromatic ring and markedly after methylation of the added hydroxyfunction. Introduction of methylgroups instead of hydroxygroups had a similar effect (Table 2).

Pronounced effects on the inhibitory potency were observed after modifications in position 17 of ring D of the estrogens. Compared to estradiol-17β, an additional 17α-ethinyl-group markedly enhanced the inhibitory effect (Table 2) whereas oxidation of the 17β-hydroxygroup (esterone, Table 2) reduced it.

In contrast to estrogens, the non-steroidal antiestrogens were more potent inhibitors of dopamine (Table 2) than of noradrenaline uptake. In radioligand binding studies antiestrogens had inhibited binding to dopamine receptors but neither to α- or β-adrenergic nor to serotonin receptors [28].

In contrast to the dopaminergic and noradrenergic uptake systems, a number of estrogens including the naturally occurring 2-hydroxyestradiol were strong inhibitors of synaptosomal serotonin uptake according to the classification of Koe [24]. As seen for the interference with catecholamine uptake, an aromatic ring A was a prerequisite for inhibition of serotonin uptake by steroid hormones.

The substituents on ring A had an overwhelming importance for the inhibitory potency whereas modifications on ring D lead to minor changes only. The substituents in position 17 showed a similar order of potency as for inhibition of catecholamine uptake where 17α-ethinylestrogens were more potent than estradiol-17β derivatives (Table 2) which were more potent than estrone derivatives (Table 2).

However, alterations of substituents on ring A differed from that in the catecholaminergic systems: Introduction of an additional hydroxygroup in position 2 markedly increased the inhibitory effect whereas an additional hydroxylation in position 4 slightly reduced it (Table 2). Introduction of additional hydroxygroups into the serotonin molecule reduces its affinity to the serotoninergic uptake system [29, 30]. On the other hand, methylation of the additional hydroxygroups to methoxyfunctions completely abolished the inhibitory effect (Table 2). The same was observed for 5-methoxytryptamine [31] and 4,5-dimethoxytryptamine [29]. The non-steroidal synthetic estrogen diethylstilbestrol and the non-steroidal anti-estrogens were only weak inhibitors of serotonin uptake.

In conclusion, estrogen agonists and antagonists can inhibit synaptosomal dopamine and serotonin uptake. However, since the inhibitory effects of the estrogens were only elicited in concentrations in the micromolar range, it seems unlikely that the alteration in synaptosomal dopamine and serotonin uptake observed after *in vivo* administration of estrogens and/or progesterone are due to a direct effect of the steroids on the uptake system.

The structure–activity relationships for the inhibition of dopamine uptake by estrogens differ markedly from those for the inhibition by phenylethylamine derivatives whereas those for the interference with serotonin uptake are in good agreement with those described for tryptamine derivatives.

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