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Effects of Tamoxifen on Striatal Dopamine and 5-Hydroxytryptamine Release in Freely Moving Male Rats: An In-vivo Microdialysis Investigation

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Abstract

Recent studies indicating interaction of oestrogens with central cholinergic, dopaminergic and 5-HTergic systems have led to the assumption of a protective role of oestrogens in certain neurodegenerative disorders. The non-steroidal drug tamoxifen, a mixed oestrogen agonist–antagonist, has been shown to modulate central nervous system functions in the corpus striatum. In this study we used a microdialysis technique to examine the effects of tamoxifen upon the striatal dopaminergic and 5-HTergic systems in intact freely moving male rats.

The extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid were measured after intraperitoneal administration of either the control or tamoxifen, and were compared with their corresponding baseline levels. Significant 25–35% increases in the baseline levels of dopamine and 3,4-dihydroxyphenylacetic acid were observed after the highest doses of tamoxifen ($1{\cdot}5\,mg\,kg^{-1}$ and $3{\cdot}0\,mg\,kg^{-1}$, respectively), whereas the lowest dose of tamoxifen ($0{\cdot}3\,mg\,kg^{-1}$) elevated dopamine and 3,4-dihydroxyphenylacetic acid levels by a detectable 15% of the basal. In addition, the ratio of 3,4-dihydroxyphenylacetic acid-to-dopamine remained unchanged in comparison with that of the pretreatment levels. Whereas no change in the striatal 5-hydroxyindoleacetic acid concentrations was seen with the lowest and highest dose regimen, the intermediate dose elicited a moderate increase (20%) in basal 5-hydroxyindoleacetic acid levels.

The pharmacological relevance of the effects of tamoxifen on the dopaminergic and 5-HTergic systems, as a prelude to the development of non-steroidal oestrogenic compounds in reducing the risk of neurodegenerative disorders such as Alzheimer's disease, is discussed.

The influence of the gonadal hormone oestrogens on central nervous system functions has been widely reported. Whereas most work in this area has focused on hypothalamic effects, recent advances in neuroscience have revealed extra-hypothalamic roles of oestrogens on central dopaminergic, 5-hydroxytryptaminergic (5-HTergic) and cholinergic functions. For example, oestrogens have been shown to alter brain dopamine metabolism (Euvrard et al 1980; Alderson & Baum 1981) and dopamine receptor sensitivity (Hruska et al 1980; Hruska et al 1982; Roy et al 1982). Oestrogens have also been implicated in dopamine-regulating activity (Menniti & Baum 1981; Fields & Gordon 1982; Joyce 1983), and dopamine-associated affective disorders (Koller et al 1976). Likewise, alterations in oestrogen levels have been shown to effect changes in 5-hydroxytryptamine (5-HT) release and 5-HT receptor density (Kanga 1992). Recent work in some laboratories has also suggested the involvement of oestrogens in the maintenance and proper function of basal forebrain cholinergic neurons (Rhodes et al 1996; Gibbs et al 1997).

Brain dopaminergic, 5-HTergic and cholinergic systems have been widely implicated in age-related neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease and a marked

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decrease in brain oestrogen levels with age has been observed for both men and women (Miranda & Sohrabji 1996). These observations—i.e. interaction of oestrogens with central dopamine, 5-HT and acetylcholine systems and age-related depletion of this gonadal hormone—have led to the hypothesis of the involvement of oestrogens in the pathophysiology of Alzheimer's disease. Indeed, oestrogen-replacement therapy is currently being considered as a possible method for prevention and treatment of Alzheimer's disease in subjects believed to be predisposed to these conditions (e.g. postmenopausal women). However, an increased risk of breast cancer has been reported with long-term oestrogen use. In males prominent oestrogen side-effects are associated with development of feminine characteristics such as enlargement of mammary glands and changes in the vocal cords. Because the undesirable side-effects of oestrogens are believed to be related to their steroidal nature, several laboratories have recently focused efforts towards the development of non-steroidal oestrogens for use in oestrogen-replacement therapy. The non-steroidal oestrogens, also known as selective oestrogen-receptor modulators, are defined as compounds that have oestrogen agonism in the targeted non-reproductive tissues (such as brain, bone and liver) and oestrogen antagonism or minimum agonism (i.e. clinically insignificant), or both, in reproductive tissues such as the breast and uterus (Muchmore, D. B., Eli Lilly, Indianapolis, IL; unpublished data). The non-steroidal anti-oestrogenic drug tamoxifen (*trans*-1-(4-β-dimethyaminoethoxyphenyl)-1,2-diphenylbut-1-ene) is the first compound for which clinical data demonstrate the feasibility of achieving a selective oestrogen-receptor modulator profile (Greese & Dodge 1996).

Tamoxifen was originally developed as an anti-oestrogenic agent and is used clinically to treat breast cancer (Legha et al 1978; Lerner & Jordan 1990). However, some recent studies have shown tamoxifen to have mixed oestrogen agonist–antagonist properties in the mammalian central nervous system. For example, McDermott et al (1995) have reported a significant increase in basal dopamine levels in the mouse striatum after tamoxifen or oestrogen treatment. Also, like oestrogens, tamoxifen has been shown to inhibit, competitively, binding of dopamine to striatal membrane-bound dopamine D_2 receptors (Hiemke & Ghraf 1984) and Gray & Ziemian (1992) have reported a high-affinity binding site ($K_d = 1-3 \times 10^{-9}$ M) for tamoxifen in several areas of rat brain (e.g. the hypothalamus, preoptic, amygdala, cortex and cerebellum).

Most work directed toward understanding the relationships between non-steroidal and steroidal oestrogenic compounds and their influence on extrahypothalamic functions has been focused on females; similar studies of such compounds on males have received relatively little attention, presumably because of the conception of oestrogens as a predominantly female gonadal hormones. Nevertheless, studies from some laboratories do suggest a protective role of oestrogens in males also. For example, chronic oestrogen treatment has been found to increase the levels of striatal dopamine receptor in intact male rats (Paolo et al 1982), and to reduce tardive dyskinesia significantly in male patients (Villeneuve et al 1980). Likewise, association of higher plasma oestradiol with significantly improved visual memory in young healthy men (Kampen & Sherwin 1996) and improved memory function in male rats after intrahippocampal injection of oestradiol have been recently reported (Packard et al 1996). In another recent study oestrogens have been shown to be important in the normal skeletal growth and development of males (Smith et al 1994). Collectively, these observations imply important involvement of oestrogen—specifically, with respect to its extrahypothalamic functions—in males, similar to those in their female counterparts.

Because of the growing interest in the development of non-steroidal selective oestrogen receptor modulators as protective agents in the prevention of Alzheimer's disease, we focused our attention on tamoxifen (the supposedly first-generation selective oestrogen-receptor modulator). To date, all studies indicating the oestrogen-like mimicking effects of tamoxifen in the CNS have been exclusively reported for females. In addition, the pharmacodynamic effects of tamoxifen on brain catecholamine and 5-HT levels have not yet been reported for any in-vivo system. This investigation was undertaken to evaluate the effects of tamoxifen on extracellular levels of dopamine and 5-HT, and their major metabolites 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid, in the corpus striatum of awake freely moving male rats. The analytical technique used was on-line HPLC microdialysis.

Materials and Methods

Drugs and reagents

Tamoxifen was a gift from Zeneca Pharmaceuticals, Wilmington, DE. Dopamine, 5-HT, 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid were obtained from

Research Biochemical International, Natick, MA. Other reagents were of analytical grade.

Animals

Adult male Sprague–Dawley rats, 275–350 g, were obtained from Taconic Farm (German Town, New York). All animals were housed individually in clear 45 × 22 × 20 cm acrylic cages under a 12-h light–dark cycle with lights on at 0600 h. The animals had free access to Purina lab chow and water. All treatment in this experiment was reviewed and approved by the Animal Care Committee at St John's University, and adheres to the NIH Guidelines for the Care and Use of Laboratory Animals. All animals were adapted to the laboratory for 2 weeks before experimentation.

For all surgical procedures, rats were anaesthetized by intraperitoneal administration of chloral hydrate (400 mg kg^{-1}) 15 min before surgery, and were placed in a stereotaxic frame (David Kopf Instrument). A midline incision was made and the skull was exposed and cleaned aseptically. A small hole was drilled into the right frontal bone on the exposed area by using coordinates (A, +0·5; L, −2·5; V, −2·5 relative to the bregma) for implanting a siliconized guide cannula into the striatum. The coordinates were chosen according to the stereotaxic atlas of Paxinos & Watson (1986). Three stainless steel bone-screws were introduced for no more than 1 mm in depth into three holes drilled into two parietal bones and the left frontal bone of the exposed area. The whole exposed area and guide cannulae were cemented with dental acrylic. The rats were returned to their cages for at least 3 days to recover with free access to food and water during the recovery period.

Microdialysis

On the day of the microdialysis study rats were placed in a hemispherical bowl (polypropylene chamber, 30 × 60 cm) for at least 30 min before probe insertion. The animals remained in this bowl until the study was complete (up to 10 h). A microdialysis probe (CMA/12, 4 mm long, CMA/Microdialysis, Acton, MA) was positioned within the guide cannulae, and held in place by means of a pressure-fitting connector. Artificial cerebral spinal fluid (155·0 mM Na^+, 1·1 mM Ca^{2+}, 2·9 mM K^+, 132·8 mM Cl^-, and 0·83 mM Mg^{2+}, pH 6·7, unadjusted) was administered through the microdialysis probe at a flow rate of 2·0 μL min^{-1} by means of polyethylene tubing and a liquid swivel connected to a microinfusion pump (CMA/100, CMA/Microdialysis). Dialysate fractions (40 μL) were collected every 20 min within an injection loop (50 μL), injected on-line (CMA/160, CMA/Microdialysis) on to an HPLC column (see below), and analysed to determine dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 5-HT and 5-hydroxyindoleacetic acid levels. The in-vitro recoveries of the microdialysis probes were within the range 19–21% for dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 5-HT and 5-hydroxyindoleacetic acid.

Histology

Upon completion of the study, rat brains were sectioned to verify probe placement. The rats were deeply anaesthetized (chloral hydrate 500 mg kg^{-1}, i.p.) and perfused with 300 mL saline then 300 mL 10% formalin through the arch of aorta. The brains were removed, and probe placement was verified histologically on serial coronal sections stained with cresyl violet. Data obtained from incorrectly implanted probes were excluded.

Analysis of dopamine, 5-hydroxytryptamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid

Separation and detection of dopamine, 5-HT and their metabolites in standard and microdialysate samples was achieved by HPLC equipped with dual potentiostat electrochemical detectors (BAS LC-4C; Bioanalytical System, Lafayette, IN) connected in parallel. The dual glassy carbon electrodes were set at an applied potential of 650 mV relative to Ag/AgCl. The range (sensitivity) of LC-4C was set at 0·1 μA for 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid, and 0·5 nA for dopamine and 5-HT. Compounds were separated on a BAS reversed-phase column (C-18, Phase II ODS-3, 100 mm × 3·2 mm). The mobile phase was 9·5% methanol, 50 mM monobasic sodium phosphate, 1·0 mM sodium octyl sulphate and 0·1 mM EDTA, pH 4·0 at a flow rate of 1·0 mL min^{-1}. The chromatograms were recorded on a dual-pen chart recorder.

Drug treatment

All pharmacological treatment was performed after stabilization of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid levels in the microdialysate. A stable baseline, defined as the three consecutive samples in which dopamine, 3,4-dihydroxy-phenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid levels varied by 10% or less, was generally obtained 2–3 h after the beginning of the probe placement. Upon establishment of the baseline measurement, vehicle (vegetable oil) or one of the three doses of tamoxifen (0·3, 1·5 or

3·0 mg kg^{-1}) was administered by intraperitoneal injection.

Data analysis

The levels of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid in each microdialysate sample in response to treatment with drug or vehicle were expressed as percentages of the average baseline level (as defined above). Chromatographic peak identification for each compound was based upon its retention time in comparison with that from an authentic standard. A group of five or six rats was used for each treatment regimen. Data for dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid after vehicle or drug treatment are reported as mean values ± s.e.m. of the percentage obtained in n independent measurements. Data for multiple observations over time were analysed by one-way analysis of variance with repeated measures for overall treatment effects. Scheffe's post-hoc test was used for multiple pairwise comparisons of treatment groups for each 20-min time interval. Statistical analysis was performed with GB-Stat 5.0.6 software (Silver Spring, MD).

Results

The effects of intraperitoneal administration of vegetable oil (control) and tamoxifen on the concentrations of extracellular dopamine measured by microdialysis in the rat striatum are compared in Figure 1. In replicate 20-min samples, dopamine concentrations were stable during a 2-h period after probe implantation and before injection of drug or vehicle. Although there was no significant change in dopamine concentration, compared with pretreatment concentrations, after vehicle injection, a significant increase ($P < 0{\cdot}01/0{\cdot}05\%$) in the dopamine level was observed within 1 h of drug treatment (all three dose levels) with the maximum increase appearing 60 min after treatment. The increase in dopamine levels was dose-dependent, in that the lowest dose of tamoxifen (0·3 mg kg^{-1}—equivalent to a one-off dose for man) produced a subtle maximum increase of 15% ($P < 0{\cdot}05\%$) over pretreatment dopamine levels, whereas moderate maximum increases of 25% ($P < 0{\cdot}01\%$) and 35% ($P < 0{\cdot}01\%$), respectively, were observed after the 1·5 and 3·0 mg kg^{-1} doses at the same time-point (i.e. 60 min after drug treatment).

The effects of tamoxifen on the concentrations of 3,4-dihydroxyphenylacetic acid and homovanillic acid are shown in Figures 2 and 3, respectively. Significant ($P < 0{\cdot}01\%$ and $P < 0{\cdot}05\%$) increases in 3,4-dihydroxyphenylacetic acid levels were

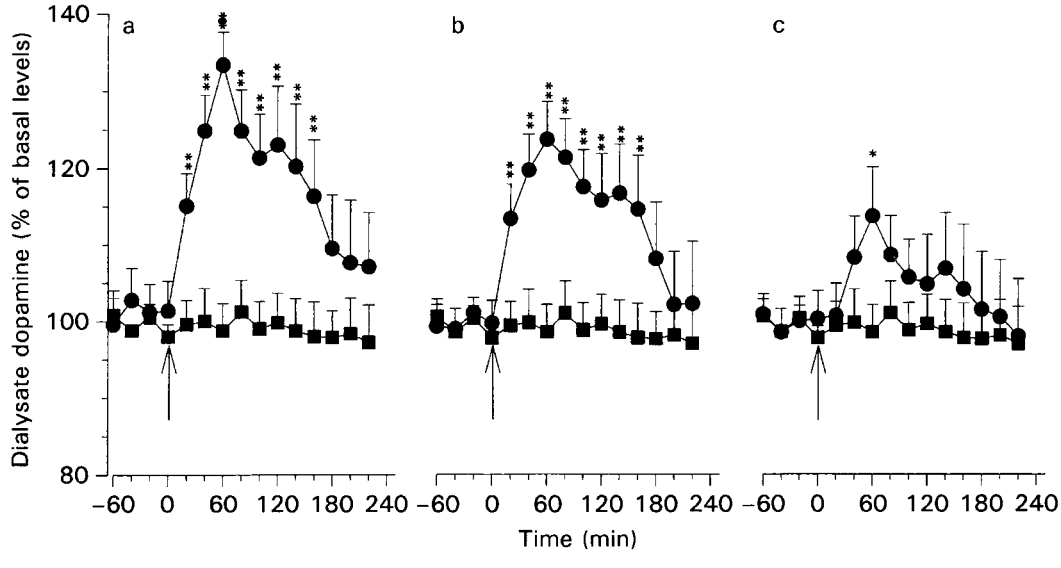

Figure 1. Effects of tamoxifen on extracellular levels of dopamine in perfusates from the striatum of freely moving rats. The flow rate of perfusate through the dialysis tubing was 2 μL min^{-1}. Dopamine was measured in 20-min samples (40 μL). Values are expressed as a percentage of the basal level of dopamine. The basal values used for comparison are the average of the last three sequential measurements (between −60 and 0 min samples) differing by < 10% before treatment with drug or vehicle during the 60 min baseline period. Each value is the mean ± s.e.m. (indicated by the vertical lines) of results from five experiments from a group of five rats. Tamoxifen (●) or vehicle (■) was administered after sample collection at time zero (point indicated by arrow: a, 3·0 mg kg^{-1} tamoxifen; b, 1·5 mg kg^{-1} tamoxifen; c, 0·3 mg kg^{-1} tamoxifen). For comparison, the control included similar measurements after the administration of vehicle (vegetable oil) only. *$P < 0{\cdot}05$, **$P < 0{\cdot}01\%$, significantly different from result from control (vehicle-treated) group (one-way analysis of variance with Scheffe's post-hoc test).

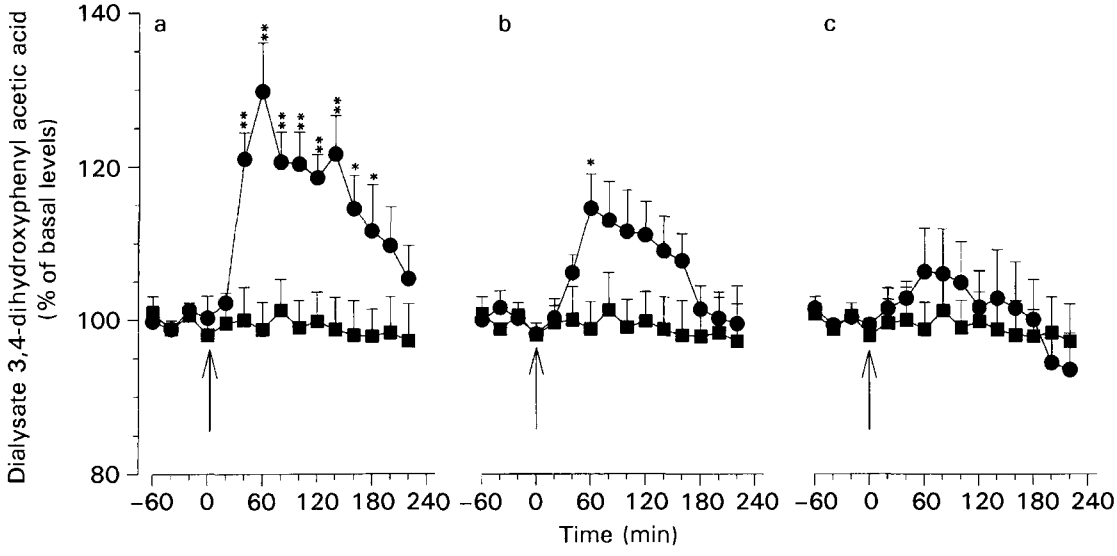

Figure 2. Effects of tamoxifen on extracellular levels of 3,4-dihydroxyphenylacetic acid in perfusates from the striatum of freely moving rats. The flow rate of perfusate through the dialysis tubing was 2 μL min^{-1}. 3,4-Dihydroxyphenylacetic acid was measured in 20-min samples (40 μL). Values are expressed as a percentage of the basal level of dopamine. The basal values used for comparison are the average of the last three sequential measurements (between −60 and 0 min samples) differing by < 10% before treatment with drug or vehicle during the 60 min baseline period. Each value is the mean ± s.e.m. (indicated by the vertical lines) of results from five experiments from a group of five rats. Tamoxifen (●) or vehicle (■) was administered after sample collection at time zero (point indicated by arrow: a, 3·0 mg kg^{-1} tamoxifen; b, 1·5 mg kg^{-1} tamoxifen; c, 0·3 mg kg^{-1} tamoxifen). For comparison, the control included similar measurements after the administration of vehicle (vegetable oil) only. *P < 0·05, **P < 0·01%, significantly different from result from control (vehicle-treated) group (one-way analysis of variance with Scheffe's post-hoc test).

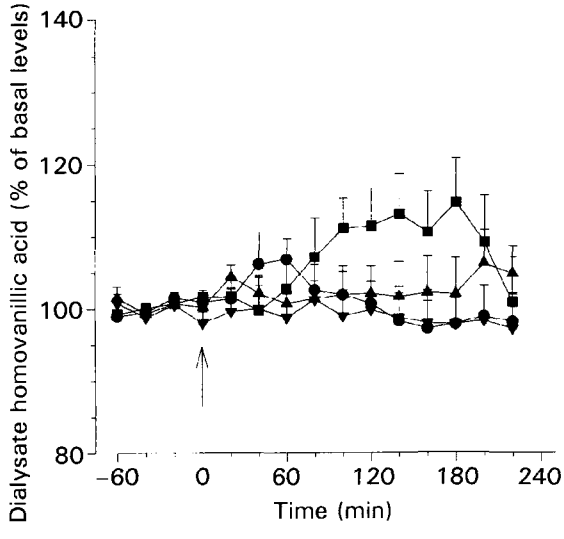

Figure 3. Effects of tamoxifen on extracellular levels of homovanillic acid in perfusates from the striatum of freely moving rats. The flow rate of perfusate through the dialysis tubing was 2 μL min^{-1}. Homovanillic acid was measured in 20-min samples (40 μL). Values are expressed as a percentage of the basal level of dopamine. The basal values used for comparison are the average of the last three sequential measurements (between −60 and 0 min samples) differing by < 10% before treatment with drug or vehicle during the 60 min baseline period. Each value is the mean ± s.e.m. (indicated by the vertical lines) of results from five experiments from a group of five rats. Tamoxifen (●, 3·0 mg kg^{-1}; ■, 1·5 mg kg^{-1}; ▲, 0·3 mg kg^{-1}) or vehicle (▼) was administered after sample collection at time zero (point indicated by arrow). There was no statistically significant difference from basal homovanillic acid concentrations as a function of tamoxifen treatment at any dose level.

observed in the 3·0 mg kg^{-1} and 1·5 mg kg^{-1} treatment groups, respectively, 1 h after drug administration. Indeed, the pattern of the increase was parallel to that of the dopamine levels as shown in Figure 1. Thus the group treated with the highest dose of tamoxifen (3 mg kg^{-1}) elicited a maximum increase of 25% (P < 0·01%) in pre-treatment 3,4-dihydroxyphenylacetic acid concentrations 60 min after drug administration, whereas the lowest increase (statistically not significant) in the 3,4-dihydroxyphenylacetic acid concentrations was seen after the 0·3 mg kg^{-1} dose. Interestingly, during the same time period there were no significant changes in the levels of homovanillic acid in any of the tamoxifen-treatment groups (Figure 3).

An attempt to measure 5-HT and dopamine and its metabolites simultaneously in the rat striatum dialysate samples was not successful, probably, because of the low levels, beyond the sensitivity range of our detection system, of 5-HT in this region of the brain. Nevertheless, we were able to measure 5-hydroxyindoleacetic acid, a principal 5-HT metabolite, in the same dialysate sample along with dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid. As shown in Figure 4, there was no significant change in 5-hydroxyindoleacetic acid level compared with the basal level after the lowest and the highest tamoxifen doses. However, a

Table 1. Effects of tamoxifen on the ratio of 3,4-dihydroxyphenylacetic acid-to-dopamine in comparison with the ratio before treatment.

Treatment	Dose	Time after treatment (min)	
		60	180
Control (pretreatment)	–	1·02 ± 0·05	1·02 ± 0·05
Tamoxifen	0·3 mg kg^{-1}	1·08 ± 0·09	1·05 ± 0·08
	1·5 mg kg^{-1}	1·13 ± 0·08	1·08 ± 0·07
	3·0 mg kg^{-1}	1·14 ± 0·09	1·09 ± 0·06

The 3,4-dihydroxyphenylacetic acid-to-dopamine ratio was estimated from the concentrations of 3,4-dihydroxyphenylacetic acid and dopamine indicated in Figures 1 and 2, respectively, for the 60- and 180-min time-points. The control is the 3,4-dihydroxyphenylacetic acid-to-dopamine ratio obtained from the basal values of 3,4-dihydroxyphenylacetic acid and dopamine. Each value is the mean ± s.e.m. of results from five experiments on a group of five rats. There was no significant difference between the 3,4-dihydroxyphenylacetic acid-to-dopamine ratio for the control and any of the tamoxifen-treatment groups.

significant 20% ($P < 0·01\%$) maximum increase in the 5-hydroxyindoleacetic acid level was observed with the intermediate dose range (1·5 mg kg^{-1}), 60 min after treatment.

The 3,4-dihydroxyphenylacetic acid-to-dopamine ratio after vehicle or drug treatment was compared with the value before treatment. As shown in Table 1, there was no significant change in the ratio in any of the tamoxifen treatment groups up to 3 h post-treatment.

Discussion

In this study microdialysis was used to investigate the pharmacodynamic effects of the prototype non-steroidal oestrogenic agent tamoxifen on central dopaminergic and 5-HTergic systems in a freely moving animal model. Tamoxifen treatment elicited a moderate, but significant, dose-dependent change in the steady-state concentrations of dopamine in the intact male rats. Whether tamoxifen alters dopamine levels directly by its interaction with dopamine receptors or by modulating oestrogen receptors in the brain, or both, is not known. It is noteworthy that brain uptake of tamoxifen has been demonstrated in mice (Wilking et al 1982), rats and man (Lien et al 1991a, b). In addition, binding of tamoxifen to the dopamine D_2 receptor (Hiemke & Ghraf 1984) and alteration of dopamine levels by tamoxifen (Baksi et al 1981, 1985) have been reported. However, these studies have been performed in in-vitro systems in which the overall in-vivo pharmacodynamic effects mediated by other neuronal mechanisms are totally excluded. In the current investigation, treatment with tamoxifen after raclopride (a dopamine D_2 antagonist) did not inhibit the tamoxifen-induced increase in dopamine concentrations in the striatum (results known shown). Although this does not disprove direct interaction of tamoxifen with the dopamine receptor, other possibilities, such as modulation of dopamine by interaction of tamoxifen with other neurotransmitter(s) might be speculated—especially considering that in-vivo microdialysis enables the monitoring of basal and drug-induced modulatory changes in neurotransmission.

Measurement of 3,4-dihydroxyphenylacetic acid concentrations and the ratio of 3,4-dihydroxyphenylacetic acid to dopamine levels is considered to be a sensitive and valid index of changes in the rate of dopamine utilization (Lavielle et al 1978). Several laboratories have reported a high rate of dopamine utilization based on the evidence of an increased 3,4-dihydroxyphenylacetic acid-to-dopamine ratio (Roth et al 1974; Korf et al 1976). Baksi et al (1985) have shown a reduced turnover of dopamine in the striatum of female rabbits subjected to tamoxifen treatment. In our studies, although a significant concentration-dependent increase in extracellular 3,4-dihydroxyphenylacetic acid levels was observed in the striatum of rats receiving tamoxifen, the increase in 3,4-dihydroxyphenylacetic acid levels was parallel to that of dopamine levels in the corresponding dose-treatment groups. Furthermore, compared with before treatment with the drug there was no significant change in the 3,4-dihydroxyphenylacetic acid-to-dopamine ratio in any of the corresponding drug-treatment groups (Table 1). In accordance with similar observations reported elsewhere (Lavielle et al 1978), our results indicate no alteration in either dopamine synthesis or dopamine utilization after tamoxifen treatment, and indirectly provide evidence of non-interference of tamoxifen in the dopamine uptake process.

Although direct measurement of 5-HT in the striatum with dopamine and its metabolites in the same dialysate sample could not be achieved, we were able to measure 5-hydroxyindoleacetic acid (a principal 5-HT metabolite) levels simultaneously. Meeusen et al (1996) have demonstrated increased levels of this 5-HT metabolite, without changes in 5-HT levels, after treatment of ovariectomized rats with oestradiol; they postulated that the increased level of the metabolite signified elevated 5-HTergic activity. Other researchers have also used plasma and cerebrospinal fluid 5-hydroxyindoleacetic acid to assess the changing activity of central 5-HT neurons in a living man, and to predict drug

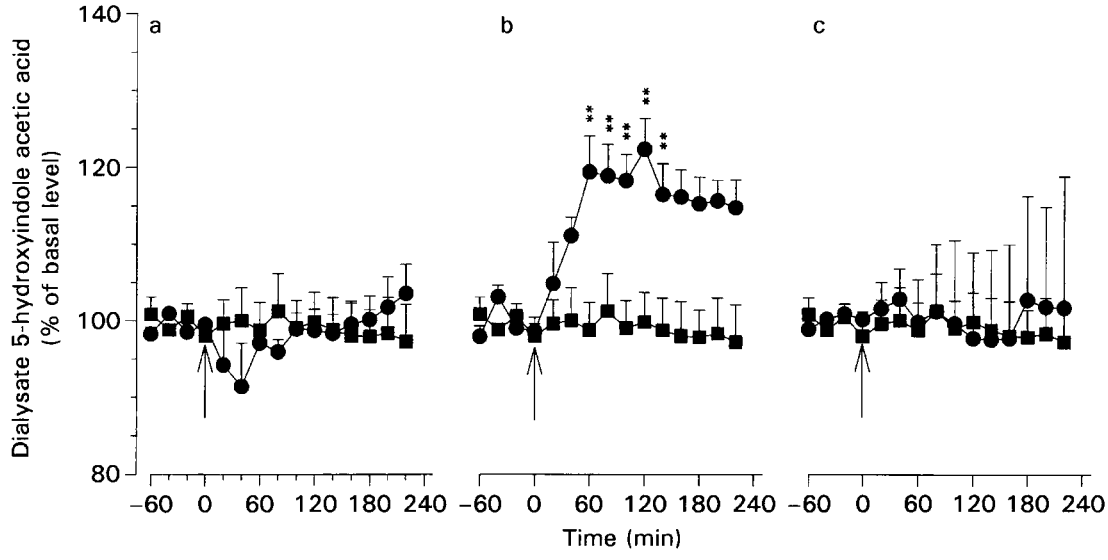

Figure 4. Effects of tamoxifen on extracellular levels of 5-hydroxyindoleacetic acid in perfusates from the striatum of freely moving rats. The flow rate of perfusate through the dialysis tubing was 2 μL min^{-1}. 5-Hydroxyindoleacetic acid was measured in 20-min samples (40 μL). Values are expressed as a percentage of the basal level of dopamine. The basal values used for comparison are the average of the last three sequential measurements (between −60 and 0 min samples) differing by < 10% before treatment with drug or vehicle during the 60 min baseline period. Each value is the mean ± s.e.m. (indicated by the vertical lines) of results from five experiments from a group of five rats. Tamoxifen (●) or vehicle (■) was administered after sample collection at time zero (point indicated by arrow: a, 3·0 mg kg^{-1} tamoxifen; b, 1·5 mg kg^{-1} tamoxifen; c, 0·3 mg kg^{-1} tamoxifen). There was no statistically significant difference from basal 5-hydroxyindoleacetic acid levels as a function of tamoxifen treatment at the highest and the lowest doses.

response or clinical response in neuropsychopharmacology and neuropsychiatry research (Cheng et al 1993). In the current work, although the lowest and highest doses of tamoxifen did not affect 5-hydroxyindoleacetic acid levels, we observed a significant increase ($P < 0{\cdot}01\%$) in 5-hydroxyindoleacetic acid concentrations with the intermediate dose range of tamoxifen. This result, in the absence of relevant data—such as a direct effect of tamoxifen on the extracellular 5-HT levels, or binding of tamoxifen to 5-HT receptor—is difficult to explain. Nevertheless, in accordance with a similar reported observation (Meeusen et al 1996), like oestrogen, tamoxifen seems to subtly modulate striatal 5-HTergic systems.

McDermott et al (1995) showed a marked similarity between the basal dopamine release-rate profiles after tamoxifen- and oestrogen-treatment in the female, indicating a complete oestrogen agonist action of tamoxifen at nigrostriatum dopamine functions. Our results are consistent with their reports of oestrogen-like agonist action of tamoxifen in the male rats. This observation is significant considering that oestrogen affects neurochemistry of dopamine in the striatum (Van Hartesveldt & Joyce 1986), and that the striatum contains the second highest brain concentration of this neurosteroid (Bixo et al 1986), approximately 80% of the dopamine in the central nervous system (McDermott 1993).

Although dopamine is believed to be the principal neurotransmitter in the aetiology of Parkinson's disease, it has also been implicated in the age-related neurologic dysfunction leading to dementia. Thus, an overlap of the pathophysiology of Alzheimer's and Parkinson's diseases has been reported (Ditter & Mirra 1987) and people with Alzheimer's disease have been shown to develop Parkinsonian symptoms, presumably as a result of alteration of nigrostriatum dopamine functions (Mayeux & Stern 1988). In addition, an attention- and award-mechanistic role of dopamine has been suggested in the interaction between cholinergic and dopaminergic systems in learning and memory behavior (Reiderer & Wuketich 1976; Beninger 1983). That the dopaminergic and 5-HTergic systems, perhaps in association with the cholinergic systems, are involved in the aetiology of Alzheimer's disease is further evidenced by the elevating effects of the cholinesterase inhibitor tacrine on extracellular levels of central dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid (Soininen et al 1990; Warpman et al 1996). It is to be noted that tacrine is used clinically in the treatment of Alzheimer's disease. It is perhaps worth pointing out that oestrogen replacement has been shown to increase choline acetyltransferase activity (Luine 1985; Singh et al 1994) in the hippocampal formation, cortex and basal forebrain of ovariectomized rats.

In addition, recent studies by Gibbs (1996) have identified equal numbers of cholinergic neurons containing oestrogen receptors in different regions of the brain (e.g. within the medial septum, horizontal limb of the diagonal band of Broca, nucleus basalis mangnocellularis, and striatum) in gonadectomized male and female rats.

As stated above, long-term clinical use of oestrogen is currently being considered in the prevention and treatment of Alzheimer's disease, and efforts are in progress in a search for a non-steroidal oestrogenic agent devoid of oestrogenic side-effects. The results of our current investigation substantiate an oestrogen-like action of tamoxifen on central dopaminergic and, perhaps, 5-HTergic systems. Because of the nature of Alzheimer's disease, a long-term treatment strategy (perhaps throughout a patient's life) will be required if any agent is to prevent this neurodegenerative disorder. Further, a delicate balance between the brain level of different neurotransmitters often becomes very critical in the drug-treatment strategy adopted in many neurologic disorders—a point well illustrated by increased levels of acetylcholine (an unwanted side-effect) when dopamine antagonists are used as antipsychotic agents. In the light of this, it is not merely important, but imperative, to assess the interaction of non-steroidal oestrogenic compounds with different neurochemical systems to obtain a broad profile of the effects of these agents on acetylcholine, dopamine and 5-HT levels. In this regard, we expect our results to serve as a prelude to future work towards the development of non-steroidal oestrogenic compounds as protective agents against Alzheimer's disease.

Finally, the technique of microdialysis measures relevant extracellular chemical events in an intact tissue environment, and thus enables assessment of the overall in-vivo pharmacodynamic effects mediated by other neuronal mechanisms after drug administration. Our current project shows the relevance of this technique in the pharmacodynamic assay of non-steroidal oestrogenic compounds. Current investigations in this laboratory include comparison of the pharmacodynamic effects of oestrogen, tamoxifen, droloxifene (a second-generation selective oestrogen-receptor modulator) and raloxifene (a third-generation selective oestrogen-receptor modulator) on dopamine, 5-HT and acetylcholine systems in awake freely moving animals.

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