Published in final edited form as:

J Chem Neuroanat. 2017 October ; 83-84: 50–58. doi:10.1016/j.jchemneu.2016.08.004.

Tamoxifen and amphetamine abuse: Are there therapeutic possibilities?

Sarah Mikelman, Natalie Mardirossian¹, and Margaret E. Gnegy*

Department of Pharmacology, 2220E MSRB III, 1150 West Medical Center Drive, University of Michigan Medical School, Ann Arbor, MI 28109-5632, United States

Abstract

Although best known as a selective estrogen receptor modulator (SERM), tamoxifen is a drug with a wide range of activities. Tamoxifen has demonstrated some efficacy has a therapeutic for bipolar mania and is believed to exert these effects through inhibition of protein kinase C (PKC). As the symptoms of amphetamine treatment in rodents are believed to mimic the symptoms of a manic episode, many of the preclinical studies for this indication have demonstrated that tamoxifen inhibits amphetamine action. The amphetamine-induced increase in extracellular dopamine which gives rise to the ‘manic’ effects is due to interaction of amphetamine with the dopamine transporter. We and others have demonstrated that PKC reduces amphetamine-induced reverse transport through the dopamine transporter. In this review, we will outline the actions of tamoxifen as a SERM and further detail another known action of tamoxifen—inhibition of PKC. We will summarize the literature showing how tamoxifen affects amphetamine action. Finally, we will present our hypothesis that tamoxifen, or an analog, could be used therapeutically to reduce amphetamine abuse in addition to treating mania.

Keywords

Tamoxifen; Amphetamine; Dopamine; Protein kinase C; Bipolar mania

1. Introduction

Nearly all addicting drugs, and notably amphetamine, increase dopamine in the nucleus accumbens. Deletion of dopamine or blockade of dopamine receptors abolishes self-administration of addictive drugs and electrical stimulation of dopamine neurons maintains brain stimulation reward (Xi and Gardner, 2008). The reinforcing properties of amphetamine depend on the enhanced levels of extracellular dopamine (Ranaldi et al., 1999), elicited by amphetamine binding to the dopamine transporter (Sulzer et al., 2005). If tamoxifen has utility for treatment of bipolar mania, it might also have a deterrent effect on amphetamine abuse. There is, at present, no therapeutic approved for the treatment of amphetamine use disorder and there is a huge unmet need. In 2013, the number of individual amphetamine-type stimulants reported to the United Nations Office of Drug Control reached 348, up from

*Corresponding author. pgnegy@umich.edu (M.E. Gnegy).

¹Present address: Meridian Rx, Detroit, MI 48226, United States.

251 in 2012. Methamphetamine is a markedly destructive drug that has been abused by >13,000,000 people, but recent abuse of the related prescription drug, Adderall® (amphetamine), is escalating (Brandt et al., 2014) as is use of cathinones, known as β -ketone-amphetamines or “bath salts” (Prosser and Nelson, 2012). Suicide as a risk of amphetamine dependence accounted for 854,000 drug abuse life years in the Global Burden of Disease Study 2010 (Degenhardt et al., 2013). Numerous drugs to treat this disorder have been tested in clinical trials: indirect dopamine agonists (low-dose amphetamine and modafinil), and partial agonists (aripiprazole), opioid antagonist (naltrexone), serotonin/norepinephrine reuptake inhibitors and, recently topiramate and bupropion. No single drug or drug combination has shown consistent efficacy in therapeutic trials for amphetamine use disorder (Brackins et al., 2011; Brensilver et al., 2013; Carson and Taylor, 2014; Heinzerling et al., 2014; Ling et al., 2012).

As a substrate of the dopamine and norepinephrine transporters, amphetamine blocks uptake of these catecholamines by the transporters and subsequently elicits a reversal of transport to release the catecholamines into the extracellular space (Sulzer et al., 2005). The increase in extracellular dopamine is the main mechanism by which amphetamine elicits hyperactivity in man and laboratory animals (Wise and Bozarth, 1987). The discovery that tamoxifen was useful as a therapeutic for bipolar mania (Bebchuk et al., 2000) led to the preclinical finding that tamoxifen would block hyperactive behaviors elicited by amphetamine in male rats, because amphetamine hyperactivity is a model for mania in laboratory animals (Einat et al., 2007). It is postulated that this action of tamoxifen is due to its ability to block protein kinase C (PKC), as opposed to its estrogen receptor binding activity. This hypothesis (Zarate and Manji, 2009) has merit due to the reported inhibition of amphetamine-stimulated dopamine efflux by inhibitors of PKC by our lab and others (Giambalvo, 1992; Gnegy, 2003). We posit that by knowing how tamoxifen works in the brain and affects the dopaminergic system, whether it is via PKC, binding to the estrogen receptor, or another previously undescribed mechanism, novel therapeutics may be created to prevent amphetamine abuse without causing serious adverse events.

We have long suggested that a PKC inhibitor may have potential as a therapeutic for amphetamine abuse. Though multiple PKC inhibitors have gone to clinical trials, with varying degrees of success, tamoxifen is the only PKC inhibitor exhibiting any kinase specificity that is capable of crossing the blood-brain barrier (Chico et al., 2009). The drug tamoxifen has a multiplicity of reported actions beyond its original clinical indication as an estrogen receptor modulator, including inhibition of PKC. Because of our interest in PKC inhibitors as a therapeutic for amphetamine abuse, here we present a review of the body of work which indicates a new potential therapeutic role for the old drug, tamoxifen.

2. Tamoxifen

Tamoxifen is a first generation non-steroidal selective estrogen receptor modulator (SERM) and is primarily used as the standard of care for the treatment and prevention of recurrence in pre- and post-menopausal women with estrogen receptor-positive breast cancer (Early Breast Cancer Trialists' Collaborative et al., 2011). In the United States, tamoxifen was the first anti-estrogen medication approved for clinical use to prevent and reduce breast cancer

occurrence (Jordan, 2003). Tamoxifen was initially referred to as an anti-estrogen as it blocks estrogen receptors in breast tissue, thus reducing effects caused by estrogen. However, tamoxifen also acts as an agonist at estrogen receptors in certain regions of the body such as the endometrium, liver, and bone; thus, its reclassification as a SERM. Tamoxifen also mimics the effect of estrogen in assessment of cardiovascular tone (Leung et al., 2007). Similarly, the drug raloxifene is estrogenic in bone tissue while being antiestrogenic in breast tissue, and as such has proven useful as a preventative treatment for osteoporosis in post-menopausal women, but which lacks the increased cancer risk and other unpleasant side effects of hormone replacement therapy (Dane et al., 2007; Pinkerton and Thomas, 2014). Tamoxifen is generally taken for five years followed by a different therapeutic depending on the patient's condition. Recent results from the Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial concluded that ten years of adjuvant tamoxifen therapy reduce mortality to a greater extent than five years (Davies et al., 2013). As such, it is important to consider the long-term effects of tamoxifen on CNS function.

Tamoxifen is a lipophilic compound that accumulates in tissue, including brain, (Lien et al., 1991a) giving it a high apparent volume of distribution of 50–60 liters (Lien et al., 1989). Despite the observation that tamoxifen and its metabolites are substrates for P-glycoprotein (Iusuf et al., 2011), tamoxifen readily crosses the blood brain barrier and concentrations in brain tissue can reach levels 40 times higher than concentrations in serum (Lien et al., 1991b). The elimination half-life of tamoxifen in humans ranges from 7 to 10 days (de Vos et al., 1992). There are three known active metabolites: N-desmethyltamoxifen, 4-hydroxytamoxifen, and 4-hydroxy-N-desmethyltamoxifen, also known as endoxifen (Fig. 1A). N-desmethyltamoxifen is the primary metabolite in humans, followed by endoxifen and then 4-hydroxytamoxifen. N-desmethyltamoxifen circulates at concentrations 2–3 fold higher than tamoxifen but is equipotent to tamoxifen at the estrogen receptor (Lien et al., 1991a). Both hydroxylated metabolites are 100-fold more active than tamoxifen and have activity at the estrogen receptor commensurate with estradiol (Furr and Jordan, 1984; Leung et al., 2007). Thus, tamoxifen is considered to be a prodrug. The microsomal enzyme CYP2D6 is responsible for the metabolism of tamoxifen to both 4-hydroxytamoxifen and endoxifen, such that drug–drug interactions are a consideration in patients taking tamoxifen (Jordan, 2007).

As mentioned above, tamoxifen can cross the blood brain barrier, yet its activity in the brain is not fully understood, nor has it been clearly delineated as an estrogen receptor agonist or antagonist in the relevant cells. Some studies have demonstrated agonist effects of tamoxifen in the CNS, particularly at G-protein coupled estrogen receptors (Gibbs et al., 2014). In contrast, recent research has led to the assumption that tamoxifen acts as an antagonist in the brain due to its opposite effect on cognition as estrogen (Chen et al., 2014). In order to properly understand the context of our investigation into the dopamine-modulating effects of tamoxifen, we must first explore the mechanisms of action of the drug. The first to be discussed is its role as an estrogen receptor modulator especially since estrogen itself has notable effects on the dopaminergic system.

3. Neurological effects of estrogen

Classified as the main female sex hormone, estrogen (17- β -estradiol) is involved in the growth of female sex organs, the distribution of body fat, and the regulation of various behaviors. In the central nervous system, estrogen improves cognition and improves memory, while offering protection against various neurotoxic insults (Engler-Chiurazzi et al., 2016).

Estrogen receptors have two forms, ER α and ER β , encoded by separate genes. Classically, estrogen exerts its effects by stimulating the translocation of estrogen receptors α or β into the nucleus where the receptors affect the expression of numerous genes. More recently, it has become clear that estrogen exerts a number of rapid, non-genomic effects, both through the classical estrogen receptors, which can translocate to the membrane to interact with membrane proteins, and through the G-protein coupled estrogen receptor (GPER1) originally known as GPR30 (Thomas et al., 2005).

Estradiol has notable effects on the dopamine system in the brain including increasing dopamine release. The effect on dopamine systems could underlie some of the sex differences in motivated behavior (Yoest et al., 2014). Estrogen receptor subtypes α and β as well as GPER1 are located in both nuclei and membranes in dopaminergic areas (Almey et al., 2015). While concentrations of ER α are low in dopamine neurons, ER β is relatively abundant in dopamine cell bodies in the substantia and ventral tegmental area in both male and female rats (Creutz and Kritzer, 2002). The membrane location of classical estrogen receptors plus existence of a GPER1 helps explain the rapid effects of estrogen on some neuronal parameters, such as the reduction in L-type Ca²⁺ currents on neostriatal neurons (notably, this effect was enhanced in neurons from female rats relative to those from male rats) (Mermelstein et al., 1996). In the striatum, ER α and GPER1 are localized to extranuclear sites on GABAergic neurons in female rats (Almey et al., 2016). Studies in laboratory animals demonstrate that estrogen acts directly in the dopamine terminal areas (striatum, nucleus accumbens) to induce changes in dopamine release and dopamine receptor activity (Becker, 1999; Thompson and Moss, 1994). In female, ovariectomized rats, physiological levels of estrogen directly increase exocytotic and amphetamine-stimulated dopamine release in striatum and nucleus accumbens (Becker, 1990; Thompson and Moss, 1994). In the female rat striatum, 17- β -estradiol increases dopamine release by inhibiting intrinsic GABA neurons that have recurrent collaterals onto dopamine terminals and by downregulating presynaptic D₂ dopamine receptor function directly on the dopamine terminal, leading to an enhancement in exocytotic dopamine release (Thompson and Certain, 2005; Yoest et al., 2014). Most striking is that estrogen is neuroprotective in many models of both dopaminergic and general neurotoxicity, including models for stroke (Shao et al., 2012), Parkinson's disease (Litim et al., 2016) and methamphetamine abuse (Dluzen and McDermott, 2006).

4. Selective estrogen receptor modulator activity of tamoxifen in the dopamine system

Investigation of the effects of tamoxifen on the dopaminergic system is complicated by the lack of knowledge concerning tamoxifen's specific effects on the estrogen receptor in the dopamine neuron; it is not always clear whether tamoxifen is functioning as an agonist or antagonist at the estrogen receptor. Additionally, tamoxifen has been identified as a potent agonist at GPER1, the G-protein coupled estrogen receptor (Thomas et al., 2005). Evidence that may clarify the SERM activity of tamoxifen in the dopamine neuron has proven contradictory. Estrogen increases amphetamine-stimulated dopamine efflux in female rat striatal synaptosomes (Xiao et al., 2003). Tamoxifen, at concentrations that will compete with estrogen for occupancy of the estrogen receptors, does not inhibit this effect of estrogen although the estrogen receptor antagonist ICI 182,780 does, indicating that tamoxifen is not an antiestrogen in this system. However, neither does tamoxifen appear to be estrogenic in this system, as tamoxifen by itself, at low levels that would occupy the estrogen receptor (370 nM), has no effect on amphetamine-stimulated dopamine efflux (Xiao et al., 2003). At higher levels (μ M concentrations), tamoxifen will inhibit this activity, as discussed below.

Both estrogen and tamoxifen are neuroprotective in dopaminergic models of neurotoxicity (Arevalo et al., 2011). Though the neuroprotective properties of tamoxifen are beyond the scope of this review, a significant portion of the work addressing tamoxifen and the dopaminergic system has been conducted within this field, and thus warrants a closer examination. Estrogen is protective in multiple models of dopaminergic neurotoxicity (Callier et al., 2001; Dluzen, 2000), and SERMS have been investigated as potential neuroprotective agents that lack some of the wide spread side effects of estrogen (Arevalo et al., 2011). Tamoxifen is protective against methamphetamine-induced neurotoxicity in males and females, though estrogen is neuroprotective in only females (Dluzen et al., 2001a). Though at first look this may seem to indicate an estrogenic role for tamoxifen in the dopamine neuron, the fact that tamoxifen was effective in males, where estrogen was not, may be the first indication of a non-estrogenic mechanism for the neuroprotective effects seen with tamoxifen. The same group later found that tamoxifen antagonized the neuroprotective action of estrogen in methamphetamine- (Gao and Dluzen, 2001) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in female mice (Dluzen et al., 2001b), indicating an anti-estrogenic role for tamoxifen. Another study reported that the neuroprotective mechanism of tamoxifen is mediated by classical estrogen receptors, since they are inhibited by estrogen receptor antagonists, such as ICI 182,780, in male rat hippocampal slices (Zhang et al., 2009). In this case, the neuroprotective signaling of tamoxifen may involve the activation or inhibition of signaling kinases which then control synaptic transmission and the expression of molecules involved in the regulation of cell death, oxidative stress, and inflammation. Elsewhere, tamoxifen prevented the formation of hydroxyl radicals following administration of the neurotoxin 1-methyl-4-phenyl-phridinium (MPP^+) to male rat striatum by retrodialysis. Interestingly, tamoxifen was effective when administered after the initial MPP^+ insult, indicating that tamoxifen was affecting the downstream neurotoxic pathways (Obata and Kubota, 2001). The antioxidant properties of tamoxifen are considered important in this study, rather than the estrogenic or anti-

estrogenic effects, thus providing a potential explanation for the observation that tamoxifen was neuroprotective in male mice (Dluzen et al., 2001a). Conversely, however, tamoxifen has also been determined to have an oxidative effect in the neuron (Gundimeda et al., 1996), further complicating our understanding of tamoxifen's effects in the dopaminergic system.

4.1. Non estrogen-receptor mechanisms of action of tamoxifen

At micromolar concentrations, ~10 to 100 fold higher than those at which it binds to the estrogen receptors, tamoxifen elicits numerous signaling mechanisms that are seemingly unrelated to its SERM activities, such as activation or inhibition of mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and Akt (Carthy et al., 2015; Lee et al., 2009a; Lee et al., 2009b), inhibition of protein kinase C (PKC) (O'Brian et al., 1985), inhibition of phosphorylation of CREB (Sharma et al., 2007), or inhibition of nuclear factor- κ B-induced transcription (Cerciat et al., 2010). Through various mechanisms and at micromolar concentrations, tamoxifen is also reported to inhibit the following: voltage-dependent Ca^{2+} channels (Song et al., 1996), ceramide glycosylation and hydrolysis (Morad and Cabot, 2015), hERG postassium channels (Chae et al., 2015), calmodulin-dependent cAMP phosphodiesterase (Rowlands et al., 1990) and glutamate uptake (Freund et al., 1995). All of these activities depend on the dose of tamoxifen and not all may bear physiological relevance.

The estrogen receptor-binding capacity of tamoxifen can be structurally separated from the other mechanisms. Tamoxifen belongs to the triphenylethylene class of compounds. The three phenyl groups, linkers and side chains provide a rich foundation for structure-activity relationships. de Medina et al. (2004a) performed a comprehensive structure-activity analysis of tamoxifen to determine those components of the molecule which are important for the estrogen receptor binding properties of the drug, versus those that are important for some of tamoxifen's other activities: inhibiting PKC and acyl coenzyme A: cholesterol acyl transferase (ACAT) and calmodulin binding. The affinity for tamoxifen for the estrogen receptor is in the high nanomolar range (189 nM, (Parker et al., 2000)) while the affinities for PKC, calmodulin and ACAT are in the low to high micromolar ranges (de Medina et al., 2004b; O'Brian et al., 1990, 1985). The existence of triphenylethylene moiety and side chain, versus the phenylpropene group and basicity of the side chain differentiate estrogen receptor binding activity from PKC inhibition, respectively (de Medina et al., 2004a, see Fig. 1C).

The mechanism by which tamoxifen inhibits PKC varies from that of the more traditional, primarily bisindolmaleimide, inhibitors. The bisindolmaleimide series, including Ro31-8220, inhibit PKC by competitively binding to the ATP substrate site (Davis et al., 1992). Tamoxifen weakly binds to the catalytic subunit of PKC ($K_i = \sim 200 \mu\text{M}$, (O'Brian et al., 1988a,b), but has higher affinity in the presence of Ca^{2+} , diacylglycerol and the regulatory subunit (Bignon et al., 1989; O'Brian et al., 1988b). Tamoxifen and structurally similar anti-estrogens inhibit PKC primarily by competitively inhibiting the binding of phosphatidylserine to the C2 regulatory subunit of PKC (Su et al., 1985) thus prohibiting the binding of the enzyme to the membrane (Edashige et al., 1991; Gundimeda et al., 1996). Tamoxifen potency is increased in the presence of diolein, Ca^{2+} and the PKC activator

phorbol myristate acetate but it is not competitive with them (Bignon et al., 1990). Tamoxifen also displays specificity in kinase inhibition. Though tamoxifen does not inhibit protein kinase A or protein kinase G, it does inhibit myosin light chain kinase due to its binding of calmodulin (CaM) (Su et al., 1985). The major metabolites of tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen all inhibit PKC with greater potency than tamoxifen (Ali et al., 2010; O'Brian et al., 1986).

5. Therapeutic utility of tamoxifen in inhibiting mania

An interest in the therapeutic utility of tamoxifen as a protein kinase C inhibitor was initiated in the field of bipolar mania research, when it was observed that valproate (Chen et al., 1994) and lithium (Lenox et al., 1992; Manji et al., 1993; Wang and Friedman, 1989), the only two drugs approved at the time for the treatment of mania, were both found to inhibit protein kinase C, despite their being structurally unrelated. Furthermore, the brains of bipolar patients exhibit enhanced protein kinase C activity (Wang and Friedman, 1996). A preliminary clinical study in male and female inpatients exhibiting mania found that tamoxifen, at doses ranging from 20 to 80 mg per day, reduced symptoms of mania (as measured by the Young mania Rating Scale (YMRS)) by 50% in 5 out of 7 patients, and that the drug was well tolerated (Bebchuk et al., 2000). Further studies with larger sample sizes have since been conducted. In one, 40 male and female patients were given lithium supplemented with 80 mg/day of tamoxifen or placebo for a 6 week double blind study, and tamoxifen with lithium was significantly more effective in reducing scores on the YMRS compared to lithium alone. Again, tamoxifen was generally well tolerated (Amrollahi et al., 2011). In a third trial, 66 male and female inpatients were given tamoxifen or a placebo for up to 3 weeks. Tamoxifen significantly lowered scores on both the YMRS and the Clinical Global Impressions Mania scale compared to placebo and patients taking tamoxifen required less sedation with lorazepam as the trial progressed. Interestingly, tamoxifen also caused a greater decrease in depressive symptoms compared to placebo, as measured by the Hamilton Depression Rating Scale and the Montgomery-Åsberg Depression Rating Scale, though these results did not reach significance (Yildiz et al., 2008). Three additional trials have been carried out to test the effectiveness of tamoxifen either as an adjunct- or mono- therapy. A meta-analysis of these three trials as well as the two described above observed that tamoxifen is an effective treatment for bipolar mania in both males and females, but that further studies utilizing a larger sample size and a longer follow up duration will be necessary to make any conclusions as to its safety and efficacy (Talei et al., 2016). It is also worth noting that in one trial, the efficacy of tamoxifen in treating bipolar mania was specific and not mimicked by the antiestrogen medroxyprogesterone which does not inhibit PKC (Kulkarni et al., 2006).

These early clinical results were encouraging with regards to both the efficacy and the safety of tamoxifen in this indication. However, the limitations of human research made further clarification of tamoxifen's mechanism difficult. Thus, researchers took a step back into preclinical rodent models of the disease, where they could better characterize the effects of tamoxifen on the brain at the molecular level.

6. Tamoxifen inhibits amphetamine action: evidence for a role of PKC

Many of the subsequent preclinical studies utilized amphetamine as a model of bipolar mania, since the hyperactivity in response to amphetamine in laboratory animals is considered to mimic many aspects of a manic episode (Flemenbaum, 1974). Small doses of tamoxifen (1–5 mg/kg) attenuate amphetamine-stimulated hyperactivity, risk taking behavior, and appetitive vocalization in male rats (Abrial et al., 2013; Cechinel-Recco et al., 2012; Einat et al., 2007; Pereira et al., 2014). On a cellular level, tamoxifen prevented and reversed amphetamine-induced mitochondrial dysfunction, oxidative damage, and changes in neuronal signaling activity in male rats (Cechinel-Recco et al., 2012; Moretti et al., 2011; Steckert et al., 2012; Valvassori et al., 2014). Tamoxifen (1 mg/kg) given for 2 days reduced hyperactivity stimulated by amphetamine in male rats (Einat et al., 2007). Interestingly, tamoxifen has proven equally effective in alternative models of bipolar mania, including paradoxical sleep deprivation in male rats, albeit with a much higher dose of tamoxifen (80 mg/kg) (Abrial et al., 2015), sleep deprivation in male mice (Armani et al., 2012), and methylphenidate induced hyperactivity in male mice (Pereira et al., 2011).

The effect of tamoxifen in models of bipolar mania is hypothesized to be the result not of its SERM activity, but its effects as a protein kinase C inhibitor. This assumption is supported by the observation that lithium, the most common treatment for mania, inhibits protein kinase C (Chen et al., 2000) and amphetamine-stimulated hyperactivity in male mice (Sabioni et al., 2008) while clomiphene, a SERM that is structurally related to tamoxifen but that lacks PKC inhibitory activity (Fig. 1B), has no effect on methylphenidate induced hyperactivity in male mice (Pereira et al., 2011). Similar to what was observed in human trials, medroxyprogesterone, a progestin with anti-estrogenic properties, was only minimally effective in inhibiting amphetamine-stimulated hyperactivity in male mice (Sabioni et al., 2008). The doses used in these studies are sufficient to inhibit PKC, as Einat et al. (2007) that pretreatment with tamoxifen inhibited amphetamine-stimulated increases in phosphorylation of the PKC substrate growth associated protein-43 (GAP-43). Published data on tamoxifen pharmacokinetics and brain tissue disposition indicate that concentrations near 1 mg/mg of tissue and higher are readily achieved in human brain at steady state; this is estimated at ~2–10 μ M or higher (Kisanga et al., 2003; Lien et al., 1991a,b). Therefore it is possible that tamoxifen could inhibit PKC at doses of tamoxifen used to treat mania, and even breast cancer. Unfortunately, the vast majority of the work done to date has focused on male animal models and they have used male animals. Thus it is difficult to speculate whether tamoxifen might behave differently in females. Surprisingly, although all of these models of bipolar mania are dependent on dysfunction of the dopaminergic system, and most specifically on the dopamine transporter, no work has been done to determine the effects of tamoxifen on dopamine transporter function.

7. PKC mediates amphetamine-stimulated dopamine efflux and amphetamine-stimulated locomotor behaviors but not dopamine influx

Our lab has published extensive data indicating a role for PKC, especially the β isozyme, in amphetamine action, both behaviorally and molecularly. We and Giambalvo (Giambalvo,

2004; Iwata et al., 1997a) showed that amphetamine increases PKC activity *in vivo* and *in vitro*. Either inhibition (Johnson et al., 2005; Kantor and Gnegy, 1998) or deletion of PKC β (Chen et al., 2009; Kantor and Gnegy, 1998; Zestos et al., 2016) reduces amphetamine-stimulated dopamine efflux in striatal slices from male and female rats with no change in dopamine influx. Suppression of amphetamine-stimulated dopamine efflux by PKC inhibitors was independent of extracellular Ca²⁺ (as is dopamine transporter function (Raiteri et al., 1979)), the chemical class of inhibitor used, or the inhibitor binding site on PKC (catalytic *vs* regulatory subunits) (Kantor and Gnegy, 1998). Both a general PKC inhibitor, Ro31-8220, (Loweth et al., 2009) and enzastaurin, which is selective for PKC α and PKC β , block amphetamine-mediated dopamine efflux in the nucleus accumbens when measured using *in vivo* microdialysis (Zestos et al., 2016). Molecularly, overexpression of PKC β but not PKC α increased amphetamine-stimulated efflux from human dopamine transporter-HEK293 cells (Johnson et al., 2005). Interestingly, tamoxifen and structurally similar triphenylethylenes show specificity for inhibition of the Ca²⁺ and diacylglycerol-dependent conventional isoforms of PKC (Bignon et al., 1990; Gundimeda et al., 1996), which include the α , β , and γ isoforms. In an examination of the inhibitory effect of tamoxifen against purified conventional isoforms of PKC, 4-hydroxytamoxifen showed greater potency in inhibiting the β isoforms as compared to the α and γ isoforms (Gundimeda et al., 1996).

Similarly, either PKC inhibitors or deletion of PKC β reduce locomotor activity (Browman et al., 1998; Chen et al., 2009) and conditioned place preference, a motivated behavior, in response to amphetamine (Aujla and Beninger, 2003). Repeated, intermittent amphetamine enhances amphetamine-stimulated locomotor behaviors and amphetamine-stimulated dopamine efflux (Robinson and Becker, 1986). This sensitization increases the pursuit and self-administration of amphetamine (Vezina, 2004). PKC inhibitors reduce the development (Narita et al., 2004) and expression of sensitization to repeated amphetamine (Kantor et al., 1999). We and others find that repeated amphetamine leads to an increase in striatal PKC activity, which is maintained throughout amphetamine administration and after weeks of withdrawal (Iwata et al., 1996, 1997b; Narita et al., 2004, 2005). Thus PKC plays a strong role in effects of acute and repeated amphetamine use, supporting the contention that tamoxifen is inhibiting amphetamine activity by inhibiting PKC.

PKC inhibitors have the advantage of primarily blocking amphetamine-stimulated reverse transport without blocking inward transport, owing, perhaps, to the multimeric structure of the dopamine transporter (Sitte and Freissmuth, 2015). A further indication for a PKC inhibitor as a therapeutic for amphetamine abuse rests in its effects on the D2 autoreceptor. PKC inhibitors increase the function of dopamine autoreceptors by increasing their surface availability (Luderman et al., 2015). Midbrain dopamine receptors (autoreceptors) are down-regulated as a result of amphetamine self-administration in rats (Calipari et al., 2014). Similarly, human amphetamine abusers have reduced availability of striatal D2/D3 dopamine receptors as measured by positron emission tomography (Chang et al., 2007). Unfortunately, due to a lack of pharmacological differentiation, any measurement of dopamine receptors *via* imaging in the human striatum will quantify both presynaptic autoreceptors and postsynaptic receptors. Down-regulation of autoreceptors enhances dopaminergic activity, which would contribute to the reinforcing activity of amphetamine. It is entirely possible

that inhibition of PKC would increase surface expression of postsynaptic D2/D3 receptors as well as presynaptic receptors (Namkung and Sibley, 2004) and also have therapeutic efficacy for that reason.

8. Tamoxifen as a potential treatment of amphetamine abuse?

As summarized above, extensive preclinical data demonstrate that tamoxifen and other PKC inhibitors will reduce amphetamine-stimulated molecular and behavioral activities. Although the site of action of tamoxifen in inhibiting amphetamine activity has not yet been delineated, evidence from other PKC inhibitors suggests that it could inhibit amphetamine-stimulated dopamine efflux through the dopamine transporter. Indeed, we have preliminary evidence that both 3–10 μ M tamoxifen and 4-hydroxytamoxifen inhibit amphetamine-stimulated dopamine efflux in male mouse and male rat striatal synaptosomes and human dopamine transporter-containing mouse neuroblastoma (N2A) cells (Mikelman et al., 2015). The inhibitory action of tamoxifen in the neuroblastoma cells was unaffected by antagonism of the estrogen receptors with ICI 182,780, supporting a role for PKC inhibition in the observed effects.

As any treatment for drug abuse is likely to require extended use, one has to be concerned with long-term effects of tamoxifen or its structural congener. Such a drug, however, might not have to be used for as long a duration in treating amphetamine abuse as that used to prevent recurrence of breast cancer. Doses of tamoxifen used to treat mania range from 20 to 80 mg/day while doses for treatment or prevention of breast cancer commonly are 20–40 mg/day (Shelly et al., 2008). Clinical trials for treatment of mania using higher-dose tamoxifen are reported as well-tolerated by both men and women but these trials were generally short-term (Zarate et al., 2007; Yildiz et al., 2008; Amrollahi et al., 2011). Many of the more serious side effects of tamoxifen, such as increase in endometrial thickness, risk of endometrial cancer and thromboembolic events, are due to its estrogen agonist effects (Shelly et al., 2008). Thus, design of a safe drug based on the tamoxifen scaffold that does not bind to estrogen receptors may alleviate many of the reported side effects of tamoxifen.

Another consideration in one taking tamoxifen is drug interactions based on metabolism. Both tamoxifen and amphetamine are substrates for CYP2D6 and so there could be competitive inhibition for the enzyme. It is unclear as to whether this would be a major problem clinically. If tamoxifen or a congener effectively reduces amphetamine/methamphetamine use, there may not be a serious metabolic interaction between the two drugs. The CYP2D6 phenotype affects the production of metabolites and physiological effects of both tamoxifen (Powers et al., 2016) and amphetamine (Bousman et al., 2009). Similarly, serious drug–drug interactions occur when CYP2D6 inhibitors, such as some selective serotonin reuptake inhibitors, are given with CYP2D6 substrates (Binkhorst et al., 2013). 3,4-Methylenedioxymethamphetamine (MDMA) inhibits CYP2D6 (Rietjens et al., 2012), but amphetamine does not (Ermer et al., 2015) and methamphetamine only weakly inhibits the enzyme (de la Torre et al., 2012).

There have been concerns about cognitive changes following tamoxifen treatment. There are reports of impairments in verbal fluency (Boele et al., 2015), decision making (Chen et al.,

2014), visuo-spatial ability and processing speed (Palmer et al., 2008) in breast cancer patients taking tamoxifen. Conversely, one study found that in postmenopausal women tamoxifen may act as an estrogen-like agonist to enhance cholinergic system activity and hippocampus-mediated learning (Newhouse et al., 2013). In the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (P-1) study 13,388 women at an increased risk of breast cancer were treated with tamoxifen for 5 years (20 mg oral daily). A cognitive assessment report from that study found that there was little difference between tamoxifen and placebo groups in cognitive dysfunction (Cella et al., 2008). In a study examining cognitive function in women who were current users and past users of tamoxifen, current users reported some memory impairment but there was no differences between past users of tamoxifen and those who had never used it (Paganini-Hill and Clark, 2000). It would seem that although tamoxifen can cause some cognitive issues, it is reversible. Because estrogen has positive effects on cognition (Engler-Chiurazzi et al., 2016), it is possible that tamoxifen is affecting cognition by acting at the estrogen receptor. A drug designed on the tamoxifen scaffold that does not bind to the estrogen receptor could have fewer effects on cognition.

Tamoxifen has already been demonstrated to have utility as a treatment for bipolar mania. Here, we have posited another therapeutic use for this old and established drug. Not only might tamoxifen serve as an effective therapeutic for amphetamine abuse, but the widespread use of tamoxifen also serves to demonstrate that a protein kinase C inhibitor can be administered without having deleterious effects on the patient. Future work to generate a drug related to tamoxifen that lacks its activity at the estrogen receptor may produce a novel therapeutic that aids in the management of substance abuse disorders, as well as bipolar mania. Some triphenylethylene analogs show this activity (Fig. 1C, Dore et al., 1992) and could prove to be clinically useful.

Acknowledgments

Funding

Supported by DA11697 (MEG), DA007267 (SKM), GM07767 (SKM).

References

- Abrial E, Etievant A, Betry C, Scarna H, Lucas G, Haddjeri N, Lambas-Senas L. Protein kinase C regulates mood-related behaviors and adult hippocampal cell proliferation in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. 2013; 43:40–48.
- Abrial E, Betourne A, Etievant A, Lucas G, Scarna H, Lambas-Senas L, Haddjeri N. Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania. *Int. J. Neuropsychopharmacol*. 2015:18.
- Ali SM, Ahmad A, Shahabuddin S, Ahmad MU, Sheikh S, Ahmad I. Endoxifen is a new potent inhibitor of PKC: a potential therapeutic agent for bipolar disorder. *Bioorg. Med. Chem. Lett*. 2010; 20:2665–2667. [PubMed: 20227879]
- Almey A, Milner TA, Brake WG. Estrogen receptors in the central nervous system and their implication for dopamine-dependent cognition in females. *Horm. Behav*. 2015; 74:125–138. [PubMed: 26122294]
- Almey A, Milner TA, Brake WG. Estrogen receptor alpha and G-protein coupled estrogen receptor 1 are localised to GABAergic neurons in the dorsal striatum. *Neurosci. Lett*. 2016

- Amrollahi Z, Rezaei F, Salehi B, Modabbernia AH, Maroufi A, Esfandiari GR, Naderi M, Ghebleh F, Ahmadi-Abhari SA, Sadeghi M, et al. Double-blind, randomized, placebo-controlled 6-week study on the efficacy and safety of the tamoxifen adjunctive to lithium in acute bipolar mania. *J. Affect. Disord.* 2011; 129:327–331. [PubMed: 20843556]
- Arevalo MA, Santos-Galindo M, Lagunas N, Azcoitia I, Garcia-Segura LM. Selective estrogen receptor modulators as brain therapeutic agents. *J. Mol. Endocrinol.* 2011; 46:R1–9. [PubMed: 21071476]
- Armani F, Andersen ML, Andreatini R, Frussa-Filho R, Tufik S, Galduroz JC. Successful combined therapy with tamoxifen and lithium in a paradoxical sleep deprivation-induced mania model. *CNS Neurosci. Therap.* 2012; 18:119–125. [PubMed: 22070228]
- Aujla H, Beninger RJ. Intra-accumbens protein kinase C inhibitor NPC 15437 blocks amphetamine-produced conditioned place preference in rats. *Behav. Brain Res.* 2003; 147:41–48. [PubMed: 14659568]
- Bebchuk JM, Arfken CL, Dolan-Manji S, Murphy J, Hasanat K, Manji HK. A preliminary investigation of a protein kinase C inhibitor in the treatment of acute mania. *Arch. Gen. Psychiatry.* 2000; 57:95–97. [PubMed: 10632242]
- Becker JB. Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. *Synapse.* 1990; 5:157–164. [PubMed: 2309159]
- Becker JB. Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol. Biochem. Behav.* 1999; 64:803–812. [PubMed: 10593204]
- Bignon E, Ogita K, Kishimoto A, Gilbert J, Abecassis J, Miquel JF, Nishizuka Y. Modes of inhibition of protein kinase C by triphenylacrylonitrile antiestrogens. *Biochem. Biophys. Res. Commun.* 1989; 163:1377–1383. [PubMed: 2783141]
- Bignon E, Pons M, Gilbert J, Nishizuka Y. Multiple mechanisms of protein kinase C inhibition by triphenylacrylonitrile antiestrogens. *FEBS Lett.* 1990; 271:54–58. [PubMed: 2226814]
- Binkhorst L, Mathijssen RH, van Herk-Sukel MP, Bannink M, Jager A, Wiemer EA, van Gelder T. Unjustified prescribing of CYP2D6 inhibiting SSRIs in women treated with tamoxifen. *Breast Cancer Res. Treat.* 2013; 139:923–929. [PubMed: 23760858]
- Boele FW, Schilder CM, de Roode ML, Deijen JB, Schagen SB. Cognitive functioning during long-term tamoxifen treatment in postmenopausal women with breast cancer. *Menopause.* 2015; 22:17–25. [PubMed: 24977455]
- Bousman CA, Glatt SJ, Everall IP, Tsuang MT. Genetic association studies of methamphetamine use disorders: a systematic review and synthesis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2009; 150B:1025–1049. [PubMed: 19219857]
- Brackins T, Brahm NC, Kissack JC. Treatments for methamphetamine abuse: a literature review for the clinician. *J. Pharm. Pract.* 2011; 24:541–550. [PubMed: 22095579]
- Brandt SA, Taverna EC, Hallock RM. A survey of nonmedical use of tranquilizers, stimulants, and pain relievers among college students: patterns of use among users and factors related to abstinence in non-users. *Drug Alcohol Depend.* 2014; 143:272–276. [PubMed: 25150402]
- Brensilver M, Heinzerling KG, Shoptaw S. Pharmacotherapy of amphetamine-type stimulant dependence: an update. *Drug Alcohol Rev.* 2013; 32:449–460. [PubMed: 23617468]
- Browman KE, Kantor L, Richardson S, Badiani A, Robinson TE, Gnegy ME. Injection of the protein kinase C inhibitor Ro31-8220 into the nucleus accumbens attenuates the acute response to amphetamine: tissue and behavioral studies. *Brain Res.* 1998; 814:112–119. [PubMed: 9838071]
- Calipari ES, Sun H, Eldeeb K, Luessen DJ, Feng X, Howlett AC, Jones SR, Chen R. Amphetamine self-administration attenuates dopamine D2 autoreceptor function. *Neuropsychopharmacology.* 2014; 39:1833–1842. [PubMed: 24513972]
- Callier S, Morissette M, Grandbois M, Pelaprat D, Di Paolo T. Neuroprotective properties of 17beta-estradiol, progesterone, and raloxifene in MPTP C57Bl/6 mice. *Synapse.* 2001; 41:131–138. [PubMed: 11400179]
- Carson DS, Taylor ER. Commentary on Heinzerling et al. (2014): a growing methamphetamine dependence therapeutics graveyard. *Addiction.* 2014; 109:1887–1888. [PubMed: 25297959]

- Carthy JM, Sundqvist A, Heldin A, van Dam H, Kleetsas D, Heldin CH, Moustakas A. Tamoxifen inhibits TGF-beta-Mediated activation of myofibroblasts by blocking non-Smad signaling through ERK1/2. *J. Cell. Physiol.* 2015; 230:3084–3092. [PubMed: 26096876]
- Cechinel-Recco K, Valvassori SS, Varela RB, Resende WR, Arent CO, Vitto MF, Luz G, de Souza CT, Quevedo J. Lithium and tamoxifen modulate cellular plasticity cascades in animal model of mania. *J. Psychopharmacol.* 2012; 26:1594–1604. [PubMed: 23076832]
- Cella D, Land SR, Chang CH, Day R, Costantino JP, Wolmark N, Ganz PA. Symptom measurement in the Breast Cancer Prevention Trial (BCPT) (P-1): psychometric properties of a new measure of symptoms for midlife women. *Breast Cancer Res. Treat.* 2008; 109:515–526. [PubMed: 17851765]
- Cerciat M, Unkila M, Garcia-Segura LM, Arevalo MA. Selective estrogen receptor modulators decrease the production of interleukin-6 and interferon-gamma-inducible protein-10 by astrocytes exposed to inflammatory challenge in vitro. *Glia.* 2010; 58:93–102. [PubMed: 19533603]
- Chae YJ, Lee KJ, Lee HJ, Sung KW, Choi JS, Lee EH, Hahn SJ. Endoxifen, the active metabolite of tamoxifen, inhibits cloned hERG potassium channels. *Eur. J. Pharmacol.* 2015; 752:1–7. [PubMed: 25680947]
- Chang L, Alicata D, Ernst T, Volkow N. Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. *Addiction.* 2007; 102(Suppl. 1):16–32. [PubMed: 17493050]
- Chen G, Manji HK, Hawver DB, Wright CB, Potter WZ. Chronic sodium valproate selectively decreases protein kinase C alpha and epsilon in vitro. *J. Neurochem.* 1994; 63:2361–2364. [PubMed: 7964759]
- Chen G, Masana MI, Manji HK. Lithium regulates PKC-mediated intracellular cross-talk and gene expression in the CNS in vivo. *Bipolar Disord.* 2000; 2:217–236. [PubMed: 11249800]
- Chen R, Furman CA, Zhang M, Kim MN, Gereau R.W.t. Leitges M, Gnegy ME. Protein kinase Cbeta is a critical regulator of dopamine transporter trafficking and regulates the behavioral response to amphetamine in mice. *J. Pharmacol. Exp. Ther.* 2009; 328:912–920. [PubMed: 19098163]
- Chen X, Li J, Chen J, Li D, Ye R, Zhang J, Zhu C, Tian Y, Wang K. Decision-making impairments in breast cancer patients treated with tamoxifen. *Horm. Behav.* 2014; 66:449–456. [PubMed: 25036869]
- Chico LK, Van Eldik LJ, Watterson DM. Targeting protein kinases in central nervous system disorders. *Nat. Rev. Drug Discov.* 2009; 8:892–909. [PubMed: 19876042]
- Creutz LM, Kritzer MF. Estrogen receptor-beta immunoreactivity in the midbrain of adult rats: regional, subregional, and cellular localization in the A10, A9, and A8 dopamine cell groups. *J. Comp. Neurol.* 2002; 446:288–300. [PubMed: 11932944]
- Dane C, Dane B, Cetin A, Erginbas M. Comparison of the effects of raloxifene and low-dose hormone replacement therapy on bone mineral density and bone turnover in the treatment of postmenopausal osteoporosis. *Gynecol. Endocrinol.* 2007; 23:398–403. [PubMed: 17701771]
- Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, Abraham M, Medeiros Alencar VH, Badran A, Bonfill X, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet.* 2013; 381:805–816. [PubMed: 23219286]
- Davis PD, Elliott LH, Harris W, Hill CH, Hurst SA, Keech E, Kumar MK, Lawton G, Nixon JS, Wilkinson SE. Inhibitors of protein kinase C. 2 Substituted bisindolylmaleimides with improved potency and selectivity. *J. Med. Chem.* 1992; 35:994–1001. [PubMed: 1552513]
- Degenhardt L, Whiteford HA, Ferrari AJ, Baxter AJ, Charlson FJ, Hall WD, Freedman G, Burstein R, Johns N, Engell RE, et al. Global burden of disease attributable to illicit drug use and dependence: findings from the Global Burden of Disease Study 2010. *Lancet.* 2013; 382:1564–1574. [PubMed: 23993281]
- Dluzen DE, McDermott JL. Estrogen, testosterone, and methamphetamine toxicity. *Ann. N. Y. Acad. Sci.* 2006; 1074:282–294. [PubMed: 17105924]
- Dluzen DE, McDermott JL, Anderson LI. Tamoxifen diminishes methamphetamine-induced striatal dopamine depletion in intact female and male mice. *J. Neuroendocrinol.* 2001a; 13:618–624. [PubMed: 11442776]

- Dluzen DE, McDermott JL, Anderson LI. Tamoxifen eliminates estrogen's neuroprotective effect upon MPTP-induced neurotoxicity of the nigrostriatal dopaminergic system. *Neurotoxic. Res.* 2001b; 3:291–300.
- Dluzen DE. Neuroprotective effects of estrogen upon the nigrostriatal dopaminergic system. *J. Neurocytol.* 2000; 29:387–399. [PubMed: 11424955]
- Dore JC, Gilbert J, Bignon E, Crastes de Paulet A, Ojasoo T, Pons M, Raynaud JP, Miquel JF. Multivariate analysis by the minimum spanning tree method of the structural determinants of diphenylethylenes and triphenylacrylonitriles implicated in estrogen receptor binding, protein kinase C activity, and MCF7 cell proliferation. *J. Med. Chem.* 1992; 35:573–583. [PubMed: 1738150]
- Early Breast Cancer Trialists' Collaborative, G. Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet.* 2011; 378:771–784. [PubMed: 21802721]
- Edashige K, Sato EF, Akimaru K, Yoshioka T, Utsumi K. Nonsteroidal antiestrogen suppresses protein kinase C—its inhibitory effect on interaction of substrate protein with membrane. *Cell Struct. Funct.* 1991; 16:273–281. [PubMed: 1838308]
- Einat H, Yuan P, Szabo ST, Dogra S, Manji HK. Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. *Neuropsychobiology.* 2007; 55:123–131. [PubMed: 17641532]
- Engler-Chiurazzi EB, Singh M, Simpkins JW. From the 90s to now: a brief historical perspective on more than two decades of estrogen neuroprotection. *Brain Res.* 2016; 1633:96–100. [PubMed: 26740397]
- Ermer J, Corcoran M, Martin P. Lisdexamfetamine dimesylate effects on the pharmacokinetics of cytochrome P450 substrates in healthy adults in an open-label, randomized, crossover study. *Drugs R D.* 2015; 15:175–185. [PubMed: 25862215]
- Flemenbaum A. Does lithium block the effects of amphetamine? A report of three cases. *Am. J. Psychiatry.* 1974; 131:820–821. [PubMed: 4834820]
- Freund WD, Grieshop B, Neumann U, Reddig S. Glutamate-induced calcium responses in rat primary cortical cultures are potentiated by co-administration of glutamate transport inhibitors. *Neurosci. Lett.* 1995; 188:61–64. [PubMed: 7783980]
- Furr BJ, Jordan VC. The pharmacology and clinical uses of tamoxifen. *Pharmacol. Ther.* 1984; 25:127–205. [PubMed: 6438654]
- Gao X, Dluzen DE. Tamoxifen abolishes estrogen's neuroprotective effect upon methamphetamine neurotoxicity of the nigrostriatal dopaminergic system. *Neuroscience.* 2001; 103:385–394. [PubMed: 11246153]
- Giambalvo CT. Protein kinase C and dopamine transport—2. Effects of amphetamine in vitro. *Neuropharmacology.* 1992; 31:1211–1222. [PubMed: 1361666]
- Giambalvo CT. Mechanisms underlying the effects of amphetamine on particulate PKC activity. *Synapse.* 2004; 51:128–139. [PubMed: 14618680]
- Gibbs RB, Nelson D, Hammond R. Role of GPR30 in mediating estradiol effects on acetylcholine release in the hippocampus. *Horm. Behav.* 2014; 66:339–345. [PubMed: 24928571]
- Gnegy ME. The effect of phosphorylation on amphetamine-mediated outward transport. *Eur. J. Pharmacol.* 2003; 479:83–91. [PubMed: 14612140]
- Gundimeda U, Chen ZH, Gopalakrishna R. Tamoxifen modulates protein kinase C via oxidative stress in estrogen receptor-negative breast cancer cells. *J. Biol. Chem.* 1996; 271:13504–13514. [PubMed: 8662863]
- Heinzerling KG, Swanson AN, Hall TM, Yi Y, Wu Y, Shoptaw SJ. Randomized, placebo-controlled trial of bupropion in methamphetamine-dependent participants with less than daily methamphetamine use. *Addiction.* 2014; 109:1878–1886. [PubMed: 24894963]
- Iusuf D, Teunissen SF, Wagenaar E, Rosing H, Beijnen JH, Schinkel AH. P-glycoprotein (ABCB1) transports the primary active tamoxifen metabolites endoxifen and 4-hydroxytamoxifen and restricts their brain penetration. *J. Pharmacol. Exp. Ther.* 2011; 337:710–717. [PubMed: 21378205]

- Iwata S, Hewlett GH, Ferrell ST, Czernik AJ, Meiri KF, Gnegy ME. Increased in vivo phosphorylation state of neuromodulin and synapsin I in striatum from rats treated with repeated amphetamine. *J. Pharmacol. Exp. Ther.* 1996; 278:1428–1434. [PubMed: 8819530]
- Iwata S, Hewlett GH, Gnegy ME. Amphetamine increases the phosphorylation of neuromodulin and synapsin I in rat striatal synaptosomes. *Synapse.* 1997a; 26:281–291. [PubMed: 9183817]
- Iwata SI, Hewlett GH, Ferrell ST, Kantor L, Gnegy ME. Enhanced dopamine release and phosphorylation of synapsin I and neuromodulin in striatal synaptosomes after repeated amphetamine. *J. Pharmacol. Exp. Ther.* 1997b; 283:1445–1452. [PubMed: 9400020]
- Johnson LA, Guptaroy B, Lund D, Shamban S, Gnegy ME. Regulation of amphetamine-stimulated dopamine efflux by protein kinase C beta. *J. Biol. Chem.* 2005; 280:10914–10919. [PubMed: 15647254]
- Jordan VC. Tamoxifen: a most unlikely pioneering medicine. *Nat. Rev. Drug Discov.* 2003; 2:205–213. [PubMed: 12612646]
- Jordan VC. New insights into the metabolism of tamoxifen and its role in the treatment and prevention of breast cancer. *Steroids.* 2007; 72:829–842. [PubMed: 17765940]
- Kantor L, Gnegy ME. Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices. *J. Pharmacol. Exp. Ther.* 1998; 284:592–598. [PubMed: 9454802]
- Kantor L, Hewlett GH, Gnegy ME. Enhanced amphetamine- and K⁺-mediated dopamine release in rat striatum after repeated amphetamine: differential requirements for Ca²⁺- and calmodulin-dependent phosphorylation and synaptic vesicles. *J. Neurosci.* 1999; 19:3801–3808. [PubMed: 10234012]
- Kisanga ER, Gjerde J, Schjott J, Mellgren G, Lien EA. Tamoxifen administration and metabolism in nude mice and nude rats. *J Steroid Biochem. Mol. Biol.* 2003; 84:361–367. [PubMed: 12711024]
- Kulkarni J, Garland KA, Scaffidi A, Headey B, Anderson R, de Castella A, Fitzgerald P, Davis SR. A pilot study of hormone modulation as a new treatment for mania in women with bipolar affective disorder. *Psychoneuroendocrinology.* 2006; 31:543–547. [PubMed: 16356651]
- Lee ES, Sidoryk M, Jiang H, Yin Z, Aschner M. Estrogen and tamoxifen reverse manganese-induced glutamate transporter impairment in astrocytes. *J. Neurochem.* 2009a; 110:530–544. [PubMed: 19453300]
- Lee ES, Yin Z, Milatovic D, Jiang H, Aschner M. Estrogen and tamoxifen protect against Mn-induced toxicity in rat cortical primary cultures of neurons and astrocytes. *Toxicol. Sci.* 2009b; 110:156–167. [PubMed: 19383943]
- Lenox RH, Watson DG, Patel J, Ellis J. Chronic lithium administration alters a prominent PKC substrate in rat hippocampus. *Brain Res.* 1992; 570:333–340. [PubMed: 1617424]
- Leung FP, Tsang SY, Wong CM, Yung LM, Chan YC, Leung HS, Yao X, Huang Y. Raloxifene, tamoxifen and vascular tone. *Clin. Exp. Pharmacol. Physiol.* 2007; 34:809–813. [PubMed: 17600563]
- Lien EA, Solheim E, Lea OA, Lundgren S, Kvinnsland S, Ueland PM. Distribution of 4-hydroxy-N-desmethyldtamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res.* 1989; 49:2175–2183. [PubMed: 2702659]
- Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res.* 1991a; 51:4837–4844. [PubMed: 1893376]
- Lien EA, Wester K, Lonning PE, Solheim E, Ueland PM. Distribution of tamoxifen and metabolites into brain tissue and brain metastases in breast cancer patients. *Br. J. Cancer.* 1991b; 63:641–645. [PubMed: 2021551]
- Ling W, Shoptaw S, Hillhouse M, Bholat MA, Charuvastra C, Heinzerling K, Chim D, Annon J, Dowling PT, Doraimani G. Double-blind placebo-controlled evaluation of the PROMETA protocol for methamphetamine dependence. *Addiction.* 2012; 107:361–369. [PubMed: 22082089]
- Litim N, Morissette M, Di Paolo T. Neuroactive gonadal drugs for neuroprotection in male and female models of Parkinson's disease. *Neurosci. Biobehav. Rev.* 2016; 67:79–88. [PubMed: 26708712]
- Loweth JA, Svoboda R, Austin JD, Guillory AM, Vezina P. The PKC inhibitor Ro31-8220 blocks acute amphetamine-induced dopamine overflow in the nucleus accumbens. *Neurosci. Lett.* 2009; 455:88–92. [PubMed: 19368852]

- Luderman KD, Chen R, Ferris MJ, Jones SR, Gnegy ME. Protein kinase C beta regulates the D(2)-like dopamine autoreceptor. *Neuropharmacology*. 2015; 89:335–341. [PubMed: 25446677]
- Manji HK, Etcheberrigaray R, Chen G, Olds JL. Lithium decreases membrane-associated protein kinase C in hippocampus: selectivity for the alpha isozyme. *J. Neurochem*. 1993; 61:2303–2310. [PubMed: 8245981]
- Mermelstein PG, Becker JB, Surmeier DJ. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J. Neurosci*. 1996; 16:595–604. [PubMed: 8551343]
- Mikelman S, Guptaroy B, Jutkiewicz E, Gnegy M. The protein kinase C inhibitor tamoxifen inhibits neurochemical and reinforcing behavioral effects of amphetamine. *FASEB J*. 2015; 29 (930.911).
- Morad SA, Cabot MC. Tamoxifen regulation of sphingolipid metabolism-therapeutic implications. *Biochim. Biophys. Acta*. 2015; 1851:1134–1145. [PubMed: 25964209]
- Moretti M, Valvassori SS, Steckert AV, Rochi N, Benedet J, Scaini G, Kapczinski F, Streck EL, Zugno AI, Quevedo J. Tamoxifen effects on respiratory chain complexes and creatine kinase activities in an animal model of mania. *Pharmacol. Biochem. Behav*. 2011; 98:304–310. [PubMed: 21281661]
- Namkung Y, Sibley DR. Protein kinase C mediates phosphorylation, desensitization, and trafficking of the D2 dopamine receptor. *J. Biol. Chem*. 2004; 279:49533–49541. [PubMed: 15347675]
- Narita M, Akai H, Nagumo Y, Sunagawa N, Hasebe K, Nagase H, Kita T, Hara C, Suzuki T. Implications of protein kinase C in the nucleus accumbens in the development of sensitization to methamphetamine in rats. *Neuroscience*. 2004; 127:941–948. [PubMed: 15312906]
- Narita M, Miyatake M, Shibasaki M, Tsuda M, Koizumi S, Yajima Y, Inoue K, Suzuki T. Long-lasting change in brain dynamics induced by methamphetamine: enhancement of protein kinase C-dependent astrocytic response and behavioral sensitization. *J. Neurochem*. 2005; 93:1383–1392. [PubMed: 15935054]
- Newhouse P, Albert K, Astur R, Johnson J, Naylor M, Dumas J. Tamoxifen improves cholinergically modulated cognitive performance in postmenopausal women. *Neuropsychopharmacology*. 2013; 38:2632–2643. [PubMed: 23867982]
- O'Brian CA, Liskamp RM, Solomon DH, Weinstein IB. Inhibition of protein kinase C by tamoxifen. *Cancer Res*. 1985; 45:2462–2465. [PubMed: 3157445]
- O'Brian CA, Liskamp RM, Solomon DH, Weinstein IB. Triphenylethylenes: a new class of protein kinase C inhibitors. *J. Natl. Cancer Inst*. 1986; 76:1243–1246. [PubMed: 3458960]
- O'Brian CA, Housey GM, Weinstein IB. Specific and direct binding of protein kinase C to an immobilized tamoxifen analogue. *Cancer Res*. 1988a; 48:3626–3629. [PubMed: 3378206]
- O'Brian CA, Ward NE, Anderson BW. Role of specific interactions between protein kinase C and triphenylethylenes in inhibition of the enzyme. *J. Natl. Cancer Inst*. 1988b; 80:1628–1633. [PubMed: 3264029]
- O'Brian CA, Ioannides CG, Ward NE, Liskamp RM. Inhibition of protein kinase C and calmodulin by the geometric isomers cis- and trans-tamoxifen. *Biopolymers*. 1990; 29:97–104. [PubMed: 2158363]
- Obata T, Kubota S. Protective effect of tamoxifen on 1-methyl-4-phenylpyridine-induced hydroxyl radical generation in the rat striatum. *Neurosci. Lett*. 2001; 308:87–90. [PubMed: 11457566]
- Paganini-Hill A, Clark LJ. Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. *Breast Cancer Res. Treat*. 2000; 64:165–176. [PubMed: 11194452]
- Palmer JL, Trotter T, Joy AA, Carlson LE. Cognitive effects of tamoxifen in pre-menopausal women with breast cancer compared to healthy controls. *J. Cancer Surviv*. 2008; 2:275–282. [PubMed: 18923905]
- Parker GJ, Law TL, Lench FJ, Bolger RE. Development of high throughput screening assays using fluorescence polarization: nuclear receptor-ligand-binding and kinase/phosphatase assays. *J. Biomol. Screen*. 2000; 5:77–88. [PubMed: 10803607]
- Pereira M, Martynhak BJ, Baretta IP, Correia D, Siba IP, Andreatini R. Antimanic-like effect of tamoxifen is not reproduced by acute or chronic administration of medroxyprogesterone or clomiphene. *Neurosci. Lett*. 2011; 500:95–98. [PubMed: 2169957]
- Pereira M, Andreatini R, Schwarting RK, Brenes JC. Amphetamine-induced appetitive 50-kHz calls in rats: a marker of affect in mania? *Psychopharmacology*. 2014; 231:2567–2577. [PubMed: 24414610]

- Pinkerton JV, Thomas S. Use of SERMs for treatment in postmenopausal women. *J. Steroid Biochem. Mol. Biol.* 2014; 142:142–154. [PubMed: 24373794]
- Powers JL, Buys SS, Fletcher D, Melis R, Johnson-Davis K, Lyon E, Malmberg EM, McMillin GA. Multi-g ene and drug interaction approach for tamoxifen metabolite patterns reveals possible involvement of CYP2C9, CYP2C19 and ABCB1. *J. Clin. Pharmacol.* 2016
- Prosser JM, Nelson LS. The toxicology of bath salts: a review of synthetic cathinones. *J. Med. Toxicol.* 2012; 8:33–42. [PubMed: 22108839]
- Raiteri M, Cerrito F, Cervoni AM, Levi G. Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor nomifensine. *J. Pharmacol. Exp. Ther.* 1979; 208:195–202. [PubMed: 216794]
- Ranaldi R, Pocock D, Zereik R, Wise RA. Dopamine fluctuations in the nucleus accumbens during maintenance, extinction, and reinstatement of intravenous D-amphetamine self-administration. *J. Neurosci.* 1999; 19:4102–4109. [PubMed: 10234038]
- Rietjens SJ, Hondebrink L, Westerink RH, Meulenbelt J. Pharmacokinetics and pharmacodynamics of 3,4-methylenedioxymethamphetamine (MDMA): interindividual differences due to polymorphisms and drug–drug interactions. *Crit. Rev. Toxicol.* 2012; 42:854–876. [PubMed: 23030234]
- Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res.* 1986; 396:157–198. [PubMed: 3527341]
- Rowlands MG, Parr IB, McCague R, Jarman M, Goddard PM. Variation of the inhibition of calmodulin dependent cyclic AMP phosphodiesterase amongst analogues of tamoxifen; correlations with cytotoxicity. *Biochem. Pharmacol.* 1990; 40:283–289. [PubMed: 2165403]
- Sabioni P, Baretta IP, Ninomiya EM, Gustafson L, Rodrigues ALS, Andreatini R. The antimanic-like effect of tamoxifen: behavioural comparison with other PKC-inhibiting and antiestrogenic drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 2008; 32:1927–1931. [PubMed: 18930105]
- Shao B, Cheng Y, Jin K. Estrogen, neuroprotection and neurogenesis after ischemic stroke. *Curr. Drug Targets.* 2012; 13:188–198. [PubMed: 22204318]
- Sharma K, Mehra RD, Dhar P, Vij U. Chronic exposure to estrogen and tamoxifen regulates synaptophysin and phosphorylated cAMP response element-binding (CREB) protein expression in CA1 of ovariectomized rat hippocampus. *Brain Res.* 2007; 1132:10–19. [PubMed: 17161830]
- Shelly W, Draper MW, Krishnan V, Wong M, Jaffe RB. Selective estrogen receptor modulators: an update on recent clinical findings. *Obstet. Gynecol. Surv.* 2008; 63:163–181. [PubMed: 18279543]
- Sitte HH, Freissmuth M. Amphetamines, new psychoactive drugs and the monoamine transporter cycle. *Trends Pharmacol. Sci.* 2015; 36:41–50. [PubMed: 25542076]
- Song J, Standley PR, Zhang F, Joshi D, Gappy S, Sowers JR, Ram JL. Tamoxifen (estrogen antagonist) inhibits voltage-gated calcium current and contractility in vascular smooth muscle from rats. *J. Pharmacol. Exp. Ther.* 1996; 277:1444–1453. [PubMed: 8667209]
- Steckert AV, Valvassori SS, Mina F, Lopes-Borges J, Varela RB, Kapczinski F, Dal-Pizzol F, Quevedo J. Protein kinase C and oxidative stress in an animal model of mania. *Curr. Neurovascular Res.* 2012; 9:47–57.
- Su HD, Mazzei GJ, Vogler WR, Kuo JF. Effect of tamoxifen, a nonsteroidal antiestrogen, on phospholipid/calcium-dependent protein kinase and phosphorylation of its endogenous substrate proteins from the rat brain and ovary. *Biochem. Pharmacol.* 1985; 34:3649–3653. [PubMed: 3840375]
- Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog. Neurobiol.* 2005; 75:406–433. [PubMed: 15955613]
- Talaei A, Pourgholami M, Khatibi-Moghadam H, Faridhosseini F, Farhoudi F, Askari-Noghani A, Sadeghi R. Tamoxifen: a protein kinase C inhibitor to treat mania: a systematic review and meta-analysis of randomized, placebo-controlled trials. *J. Clin. Psychopharmacol.* 2016; 36:272–275. [PubMed: 27088436]
- Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology.* 2005; 146:624–632. [PubMed: 15539556]

- Thompson TL, Certain ME. Estrogen mediated inhibition of dopamine transport in the striatum: regulation by G alpha i/o. *Eur. J. Pharmacol.* 2005; 511:121–126. [PubMed: 15792779]
- Thompson TL, Moss RL. Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. *J. Neurochem.* 1994; 62:1750–1756. [PubMed: 8158125]
- Valvassori SS, Bavaresco DV, Budni J, Bobsin TS, Goncalves CL, de Freitas KV, Streck EL, Quevedo J. Effects of tamoxifen on tricarboxylic acid cycle enzymes in the brain of rats submitted to an animal model of mania induced by amphetamine. *Psychiatry Res.* 2014; 215:483–487. [PubMed: 24359811]
- Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci. Biobehav. Rev.* 2004; 27:827–839. [PubMed: 15019432]
- Wang HY, Friedman E. Lithium inhibition of protein kinase C activation-induced serotonin release. *Psychopharmacology.* 1989; 99:213–218. [PubMed: 2508156]
- Wang HY, Friedman E. Enhanced protein kinase C activity and translocation in bipolar affective disorder brains. *Biol. Psychiatry.* 1996; 40:568–575. [PubMed: 8886289]
- Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol. Rev.* 1987; 94:469–492. [PubMed: 3317472]
- Xi ZX, Gardner EL. Hypothesis-driven medication discovery for the treatment of psychostimulant addiction. *Curr. Drug Abuse Rev.* 2008; 1:303–327. [PubMed: 19430578]
- Xiao L, Jackson LR, Becker JB. The effect of estradiol in the striatum is blocked by ICI 182,780 but not tamoxifen: pharmacological and behavioral evidence. *Neuroendocrinology.* 2003; 77:239–245. [PubMed: 12766324]
- Yildiz A, Guleryuz S, Ankerst DP, Ongur D, Renshaw PF. Protein kinase C inhibition in the treatment of mania: a double-blind, placebo-controlled trial of tamoxifen. *Arch. Gen. Psychiatry.* 2008; 65:255–263. [PubMed: 18316672]
- Yost KE, Cummings JA, Becker JB. Estradiol, dopamine and motivation. *Cent. Nerv. Syst. Agents Med. Chem.* 2014; 14:83–89. [PubMed: 25540977]
- Zarate CA, Manji HK. Protein kinase C inhibitors: rationale for use and potential in the treatment of bipolar disorder. *CNS Drugs.* 2009; 23:569–582. [PubMed: 19552485]
- Zarate CA Jr, Singh JB, Carlson PJ, Quiroz J, Jolkovsky L, Luckenbaugh DA, Manji HK. Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study. *Bipolar Disord.* 2007; 9:561–570. [PubMed: 17845270]
- Zestos AG, Mikelman SR, Kennedy RT, Gnegy ME. PKCbeta inhibitors attenuate amphetamine-stimulated dopamine efflux. *ACS Chem. Neurosci.* 2016
- Zhang H, Xie M, Schools GP, Feustel PF, Wang W, Lei T, Kimelberg HK, Zhou M. Tamoxifen mediated estrogen receptor activation protects against early impairment of hippocampal neuron excitability in an oxygen/glucose deprivation brain slice ischemia model. *Brain Res.* 2009; 1247:196–211. [PubMed: 18992727]
- de Medina P, Favre G, Poirot M. Multiple targeting by the antitumor drug tamoxifen: a structure-activity study. *Curr. Med. Chem. Anticancer Agents.* 2004a; 4:491–508. [PubMed: 15579015]
- de Medina P, Payre BL, Bernad J, Bosser I, Pipy B, Silvente-Poirot S, Favre G, Faye JC, Poirot M. Tamoxifen is a potent inhibitor of cholesterol esterification and prevents the formation of foam cells. *J. Pharmacol. Exp. Ther.* 2004b; 308:1165–1173. [PubMed: 14617686]
- de Vos D, Slee PH, Stevenson D, Briggs RJ. Serum elimination half-life of tamoxifen and its metabolites in patients with advanced breast cancer. *Cancer Chemother. Pharmacol.* 1992; 31:76–78. [PubMed: 1458563]
- de la Torre R, Yubero-Lahoz S, Pardo-Lozano R, Farre M. MDMA, methamphetamine, and CYP2D6 pharmacogenetics: what is clinically relevant? *Front. Genet.* 2012; 3:235. [PubMed: 23162568]

Fig. 1. Tamoxifen metabolism and related structures. (A) Tamoxifen metabolism in the human. (B) Clomiphene, a selective estrogen receptor modulator, that does not inhibit PKC activity. (C) A substituted triphenylethylene that inhibits PKC but poorly binds to estrogen receptors (Dore et al., 1992).