Cell, Vol. 98, 551–554, September 3, 1999, Copyright ©1999 by Cell Press

How Do Antidepressants Work? Prospects for Genetic Analysis of Drug Mechanisms

Minireview

William R. Schafer
Department of Biology
University of California, San Diego
La Jolla, California 92093-0349

Fluoxetine and the Serotonin Model for Depression

Fluoxetine (a.k.a. Prozac) is well known for its ability to treat clinical depression, one of the most prevalent of all psychiatric disorders. Yet the mechanism by which fluoxetine actually functions to relieve depression is not well understood. Fluoxetine is the best-known member of a class of antidepressant drugs known as serotonin-selective reuptake inhibitors (SSRIs). In popular accounts, their antidepressant action is usually explained in the context of a long-standing model of depression called the serotonin hypothesis. According to this model, levels of serotonergic neurotransmission in the forebrain are a key determinant of mood: high activity results in euphoria, low activity results in dysphoria. Depression is said to be caused by chronically low levels of serotonergic transmission. SSRIs interfere with the activity of the serotonin transporter (5-HTT), a reuptake molecule that removes serotonin from the synapse; thus, the putative low levels of synaptic serotonin in the depressed patient are elevated, and depression is relieved. Consistent with this model, many other antidepressants, including tricyclics (e.g., clomipramine) and monoamine oxidase inhibitors, also potentiate serotonergic transmission by interfering with serotonin reuptake or degradation.

Variations on this serotonin model represent the most widely accepted explanation for antidepressant action and are consistent with most experimental data (Leonard, 1994; Potter, 1996). However, a number of facts are difficult to reconcile with the serotonin model, at least in its simplest form. For example, if levels of serotonergic transmission were directly correlated with mood, one might predict that normal individuals treated with serotonin reuptake blockers would experience euphoria, and that dietary serotonin depletion would induce depression. In fact, these manipulations of serotonin levels have little effect on mood except in individuals who are depressed or recently recovered from depression (McAllister-Williams and Young, 1998). Moreover, even in depressed patients the effects of serotonin reuptake blockers on mood do not correlate temporally with their effects on serotonergic transmission; whereas SSRIs and most tricyclics elevate synaptic serotonin levels within hours, their effects on mood are not apparent for 2–6 weeks. Finally, although many drugs used to treat mood disorders block uptake or degradation of serotonin, a number of effective antidepressants clearly do not, including selective norepinephrine reuptake inhibitors (SNRIs) such as desipramine and atypical antidepressants such as tianeptine, which enhances rather than inhibits serotonin reuptake, MK869, which antagonizes substance P receptors, and bupropion, whose target is unknown (Baldessarini, 1996; Kramer et al., 1998).

Together, these observations indicate that any connection between serotonin and mood is likely to be more complicated and perhaps more indirect than a simplistic version of the serotonin model would imply. Consequently, most current versions of the serotonin model hypothesize that SSRIs are effective against depression not because of their acute effects on serotonergic transmission, but because of long-term adaptive changes in

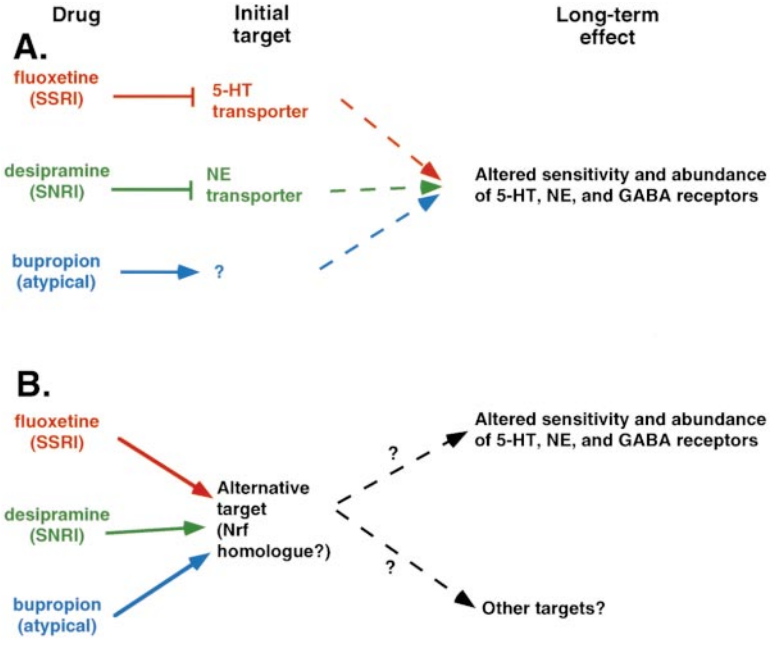

Figure 1. Models for How Chemically Diverse Antidepressants Might Alleviate Mood Disorders

monoamine neurotransmission that arise from chronic inhibition of serotonin reuptake (Leonard, 1994). According to this hypothesis (Figure 1A), long-term activation of different direct targets by different classes of antidepressants (the serotonin transporter by SSRIs, other targets by atypical antidepressants) leads to a common set of adaptive responses that include changes in the sensitivity or expression of various monoamine and GABA receptors in the brain. However, it is also possible that the antidepressant properties of fluoxetine and other antidepressants might be mediated, at least in part, by common, serotonin-independent targets (Figure 1B).

The identification of possible serotonin-independent targets of fluoxetine (and other antidepressants) could be important for another reason. The major advantage of SSRIs over older antidepressant drugs is their relative lack of adverse side effects. Even so, patients taking fluoxetine often experience nausea, headaches, motor agitation, sleep disorders, and sexual dysfunction (Baldessarini, 1996). Thus, it is obviously of great interest to identify new antidepressant drugs that are more potent and specific in their alleviation of depression, yet cause fewer side effects. At present, however, it is not clear which side effects are a consequence of serotonin-reuptake inhibition and which are due to the action of the drug at other potential targets. It is known that cytochrome P450 isoenzymes in the liver, which are important for excretion of many drugs, are directly inhibited by fluoxetine, and the adverse interactions between SSRIs and other drugs may result in part from these serotonin-independent actions of fluoxetine (Ereshefsky et al., 1996). It is conceivable therefore that serotonin-independent targets in the nervous system might underlie some of its behavioral and neurological side effects as well.

Thus, both the antidepressant activity and the adverse side effects of fluoxetine could, at least in principle, involve serotonin-independent target molecules. Yet because it is difficult to guess what these molecules might be, few attempts have been made to identify 5-HTT-independent targets of fluoxetine, or even to determine if they exist. In a way, the problem is reminiscent of the situation faced by the proverbial inebriate who only searches for his lost keys under the streetlight because the light is better there. In this case, investigations of fluoxetine's mechanism of action have been limited by the assumption that the only important target of fluoxetine with respect to depression is the serotonin transporter. Of course, this might very well be true. However, if the antidepressant mechanism of fluoxetine in fact involves alternative, serotonin-independent target molecules, their identification will be essential for understanding how antidepressants work. Yet given the absence of a convincing animal model for depression, it has been difficult to devise a strategy to identify such targets.

Fluoxetine Resistance Genes in C. elegans

In a paper published in the August issue of *Molecular Cell*, Choy and Thomas describe a novel approach they have used to identify serotonin-independent targets of fluoxetine and other antidepressants (Choy and Thomas, 1999). This study applies to pharmacology a time-honored principle of molecular and developmental biology: since cellular signaling pathways are often highly conserved across evolution, the information gained from studying simple organisms is likely to provide critical insights into how the same process works in more complex organisms. In this case, they decided to study fluoxetine responses in a simple, experimentally accessible organism, the nematode *C. elegans*, with the notion that human homologs of these molecules might be relevant to the clinical activities of antidepressants. Although this paper represents only a first step in these investigations, it clearly demonstrates the enormous potential that genetic analysis offers as a way to investigate the molecular basis for drug responses. And though it may be too soon to know whether this sort of pharmacological genetics will ultimately provide an explanation of how antidepressants work in humans, past history (along with some encouraging news from the worm genome) suggests that the prospects are quite good.

Choy and Thomas first demonstrated that fluoxetine has serotonin-independent as well as serotonin-dependent effects on worm behavior. Exposure to fluoxetine has two major effects on worms: stimulation of egg laying and hypercontraction of muscles in the nose. Previous work had indicated that the effect of fluoxetine on egg laying was probably due to potentiation of serotonergic transmission (Weinshenker et al., 1995). This conclusion rests on the observations (1) that serotonin, like fluoxetine, stimulates egg laying , and (2) that mutant animals that can not synthesize endogenous serotonin fail to lay eggs in response to fluoxetine. Thus, the relevant molecular target for fluoxetine with respect to egg laying is most likely the serotonin transporter. In contrast, the effect of fluoxetine on the nose muscles appears to be completely independent of serotonin. Even high serotonin concentrations do not cause nose contraction, and the ability of fluoxetine to induce nose contraction is unaffected by mutations that block serotonin synthesis. Remarkably, treatment with other antidepressant drugs, including the chemically unrelated tricyclic antidepressant clomipramine, induced contraction of the nose muscles in a manner indistinguishable from fluoxetine. Thus, the ability to contract the nose muscles represents a common, serotonin-independent property of multiple antidepressant drugs and suggests that these agents might share common serotonin-independent molecular targets.

Next, Choy and Thomas screened for mutant animals that were resistant to fluoxetine-induced nose contraction. They identified fluoxetine resistant mutations in seven genes, which they designated Nrf genes, for *n*ose *r*esistant to *f*luoxetine. Since all the Nrf mutations were recessive, the seven genes identified in this initial screen appear to be good candidates for mediating the serotonin-independent effects of fluoxetine. Particularly encouraging is the fact that all the Nrf mutations confer resistance to several chemically diverse molecules that share the ability to alleviate depression. Nonetheless, the Nrf mutants retain sensitivity to other drugs, such as nicotinic agonists, that can also induce nose contraction. It therefore appears unlikely that the Nrf genes prevent fluoxetine-induced nose contraction in a nonspecific way, such as by crippling the contractile activity of the nose muscles. Thus, the Nrf genes appear to

encode a set of functionally related molecules that mediate the response of the nose muscles to fluoxetine and other antidepressants.

How Do NRF-6 and NDG-4 Promote Fluoxetine Response?

The ultimate elucidation of the molecular basis for the fluoxetine response of the nose muscles will require the cloning and molecular characterization of the Nrf genes. So far two of the Nrf genes have been cloned, *nrf-6* and *ndg-4*. These two genes define the first members of a novel gene family, and thus the biochemical activities of their gene products are not yet known. Both encode predicted multipass integral membrane proteins that are expressed not in the nose muscle themselves, but in the nasal epidermis. Given their expression patterns and putative structure, perhaps the most logical explanation of their role in fluoxetine response is that they might transport the drug across the nasal epidermis, where it could access the nasal neuromusculature. Consistent with this hypothesis, other aspects of the *nrf-6* and *ndg-4* mutant phenotype strongly suggest defects in the transport of molecules across cellular boundaries. In *nrf-6* or *ndg-4* mutants yolk proteins, which are normally transported from the intestine to the gonad across a barrier of *nrf-6*/*ndg-4*-expressing cells, accumulate in the gut, suggesting that the NRF-6 and NDG-4 proteins facilitate transport of yolk proteins across the intestine. If NRF-6 and NDG-4 do in fact function as fluoxetine transport molecules, the NRF-6 and NDG-4 vertebrate homologs might not represent fluoxetine targets per se, but might rather function in transport of antidepressants across the blood–brain barrier.

However, if NRF-6/NDG-4 function in drug transport, it is curious that such a transporter would specifically transport multiple structurally dissimilar antidepressant molecules. Thus, an alternative possibility must be considered: that despite their localized expression in the nasal epidermis, NRF-6 and NDG-4 might actually encode targets of fluoxetine. In a simple version of this hypothesis, activation of NRF-6/NDG-4 by fluoxetine could promote the release of factors from epidermal cells that induce nose muscle contraction. In fact, the nematode nasal epidermis is known to contain acetylcholine, a neurotransmitter implicated in excitation of the nose muscles (Johnson and Stretton, 1985). However, compelling evidence for involvement of acetylcholine or other epidermally released factors in the response to fluoxetine is currently lacking. At present, the data are insufficient to distinguish between these and other models for how NRF-6 and NDG-4 promote fluoxetine-induced nose contraction; the resolution of this question probably awaits the cloning and molecular analysis of the remaining Nrf genes.

Will Mutant Worms Help Us Understand Depression?

This study clearly demonstrates that in nematodes, fluoxetine has effects on the neuromuscular system that are mediated by serotonin-independent target molecules. Although it is not clear if similar target molecules are present in humans, the existence of expressed sequence homologs of *nrf-6*/*ndg-4* indicates that their function is not restricted to nematodes. This study also effectively illustrates the power of using a phenotypically based genetic screen to identify the molecular targets of drugs, even if they are novel or unsuspected. It seems certain that the Nrf screen will be an effective way to identify additional molecules that mediate serotonin-independent responses to fluoxetine in *C. elegans*, and given the facility of nematode genetics, it is probably only a matter of time before these responses are understood at the molecular level.

However, a skeptic might ask whether understanding how fluoxetine makes a worm's nose contract will provide meaningful insight into how it alleviates mood disorders in humans. Of course, it is possible that the molecules involved in fluoxetine-induced nose contraction will have no direct connection to the clinical mechanisms of antidepressants; this question can not be resolved definitively until human Nrf gene homologs are identified and characterized. However, the nearly complete *C. elegans* genome sequence indicates that many (though not all) molecules involved in neurotransmission and neuronal excitability are conserved between nematodes and vertebrates (Bargmann, 1998), several of which have been first identified in *C. elegans* (Culotti, 1994; Rand and Nonet, 1997). It should also be noted that recent genetic studies of lithium response pathways in *Dictyostelium*, an organism which diverged from the human line far earlier than nematodes, have identified genes previously linked to bipolar depression (Williams et al., 1999). Thus, it is reasonable to expect that at the molecular level, fluoxetine response pathways might show similarly impressive conservation.

Prospects for Genetic Analysis of Other Drug Mechanisms

A more specific question regarding the Nrf screen is whether the serotonin-independent fluoxetine targets defined by the Nrf mutants will be the ones most relevant to understanding depression.

Since the Nrf screen assayed relatively rapid paralysis by toxic concentrations of fluoxetine, genes specifically required for the long-term adaptive pathways would probably not be identified by the Nrf screen. However, the adaptive downregulation of egg-laying behavior after that prolonged exposure to serotonin (Schafer and Kenyon, 1995) might be used as a basis for a genetic screen to identify genes involved in long-term serotonin-dependent responses to fluoxetine. Alternatively, since the *C. elegans* genome sequence is nearly complete, the entire genome could be comprehensively surveyed using DNA microarrays to identify genes that are induced or repressed by prolonged exposure to fluoxetine (DeRisi et al., 1997). Of course, eventually this approach can be used to identify fluoxetine-regulated human genes as well.

Essentially all of the techniques described here could be applied equally well to other neuroactive substances that affect humans and worms (or flies) in a conserved fashion. For example, the mechanisms of action of therapeutic agents such as antipsychotics and anaesthetics (Kayser et al., 1999; van Swinderen et al., 1999) may ultimately be understood in part through genetic studies of resistant or hypersensitive mutants. Likewise, many drugs of abuse have dramatic effects on worm and fly behavior (McClung and Hirsch, 1998; Moore et al., 1998); genetic analysis of the long-term responses to these

agents may provide important insights into the mechanisms of addiction. Drugs have historically been effective tools for investigating how worm neurons work; worm neurons may prove equally effective for investigating how drugs work.

Selected Reading

Baldessarini, R.J. (1996). In The Pharmacological Basis of Therapeutics, J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, and A.G. Gilman, eds. (New York: McGraw Hill).

Bargmann, C.I. (1998). Science *282*, 2028–2033.

Choy, R.K.M., and Thomas, J.H. (1999). Mol. Cell *4*, 143–152.

Culotti, J.G. (1994). Curr. Opin. Genet. Dev. *4*, 587–595.

DeRisi, J.L., Iyer, V.R., and Brown, P.O. (1997). Science *278*, 680–686.

Ereshefsky, L., Riesenman, C., and Lam, Y.W. (1996). J. Clin. Psych. *57* (Suppl. *8*), 17–24.

Johnson, C.D., and Stretton, A.O. (1985). J. Neurosci. *5*, 1984–1992.

Kayser, E.-B., Morgan, P.G., and Sedensky, M.M. (1999). Anesthesiology *90*, 545–554.

Kramer, M.S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J.J., Reines, S.A., Liu, G., Snavely, D., Wyatt-Knowles, E., et al. (1998). Science *281*, 1640–1645.

Leonard, B.E. (1994). In Psychopharmacology of Depression, S.A. Montgomery and T.H. Corn, eds. (Oxford, UK: Oxford University Press), pp. 19–30.

McAllister-Williams, R.H., and Young, A.H. (1998). In New Models for Depression, D. Ebert and K.P. Ebmeier, eds. (Basel: Karger).

McClung, C., and Hirsch, J. (1998). Curr. Biol. *8*, 109–112.

Moore, M.S., DeZazzo, J., Luk, A.Y., Tully, T., Singh, C.M., and Heberlein, U. (1998). Cell *93*, 997–1007.

Rand, J.B., and Nonet, M.L. (1997). In *C. elegans* II, D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Schafer, W.R., and Kenyon, C.J. (1995). Nature *375*, 73–78.

van Swinderen, B., Saifee, O., Shebester, L., Roberson, R., Nonet, M.L., and Crowder, C.M. (1999). Proc. Natl. Acad. Sci. USA *96*, 2479–2484.

Weinshenker, D., Garriga, G., and Thomas, J.H. (1995). J. Neurosci. *15*, 6975–6985.

Williams, R.S.B., Eames, M., Ryves, W.J., Viggars, J., and Harwood, A.J. (1999). EMBO J. *18*, 2734–2745.