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Modeling the onset of drug dependence: A consideration of the requirement for protein synthesis

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Abstract

It has been proposed, with some supporting evidence, that development of opiate tolerance and dependence requires protein synthesis. However, a quantitative, biologically based model within which to analyse and support the data has been lacking. Utilizing such a framework or model, we recently compared the time course of onset of opiate dependence in laboratory animals, with the mathematical time course of general changes in protein levels. Not only did the time course of onset of dependence parallel the time course of increasing levels of a protein, but also the half-life of the putative protein required by the model was very similar to those of many brain proteins. In this study, we have more extensively tested the model by producing and examining a much more detailed and surprisingly complex time course of the onset of dependence. Applying the protein synthesis time course model to the data suggested the presence of two distinct components of dependence, an early transient component and a later long-lasting component. These components appear to correspond to acute and chronic dependence, respectively. The protein synthesis hypothesis more readily applies to the chronic dependence portion. Because consideration of the model can generate components that correspond to accepted and well-known components of dependence, both the utility of the model as well as the hypothesis that opiate dependence at least partially requires protein synthesis are supported. It is also possible that individual components of the withdrawal syndrome have individual and unique rate limiting mechanisms. In any case, time course analysis may be helpful in revealing underlying mechanisms of change.

Keywords: Opiate withdrawal; Protein synthesis; Morphine; Naloxone; Opiate dependence

1. Introduction

Protein synthesis has been implicated as mandatory in the development of some neuronal processes and behaviors. Indeed, it has been observed that a behavior can apparently depend on the presence of a single protein. Several recent examples of this can be found in the literature on knockouts and gene expression. Either blocking or knocking out the mGluR5 receptor eliminates the rewarding effects of cocaine (Chiamulera et al., 2001; McGeehan and Olive, 2003). Enhanced expression of a single gene, the V1a receptor, increases affiliative behavior in vols (Lim et al., 2004), and a mutation in the CART peptide gene is associated with severe early onset obesity in humans (del Giudice et al., 2001). A number of studies show changes in levels of various proteins and mRNAs after administration of opiates (Berke and Hyman, 2000; Jacobs et al., 2002; Kuhar et al., 2001; Nestler and Aghajanian, 1997). Moreover, it has also been demonstrated that opiate-induced reward, tolerance to physical dependence and dependence on opiates require various proteins and mRNA synthesis (Cohen et al., 1965; Contarino et al., 2002; Cox, 1973; Cox and Osman, 1970; Loh et al., 1969; Nitsche et al., 2002; Terman et al., 2004). Given these and many more examples, the proposal that, at

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least in some cases, a protein can mediate a behavior has clear support, but application of a rationally based model and quantitative test of the concept has been lacking. What is new in the following approach is that a biologically-founded and quantitative model of the time course of protein synthesis is applied to the problem.

We recently used a mathematical modeling approach to test the possibility that opiate dependence required protein synthesis (Kuhar and Joyce, 2001, 2003). We tested if the time course of onset of drug dependence was similar to the general time course of changes in protein levels. Indeed, not only did the time courses parallel each other, but also the required half-lives of the hypothetical proteins were realistic in that they were typical of many known brain proteins (Kuhar and Joyce, 2001). While all modeling is at least somewhat speculative in that it requires selecting and testing one particular model over many others, a model has value in providing insight to data or in suggesting new experiments. Mathematical modeling has proven useful in a number of neuropsychiatric problems (Kuhar and Pogun, 2003). In this study we provide the most stringent test of the model in that we generate and use a far more detailed and complex time course than previously considered.

The multilevel analysis used here can be summarized as follows. In the simplest first case, a single protein is assumed to mediate some behavior, and changes in the protein level drive parallel changes in behavior. If a protein is given an impetus such that its levels must change, it is known that the levels will vary over time according to the relationship:

$$P_t/P_{max} = (1 - e^{-0.693t/h}), \quad (1)$$

where P_t = is protein level at time t, P_{max} is the maximal level of the protein, and h is the half-life of the protein (21). This follows from the known findings that protein synthesis is a zero-order process ($dP/dt = r$), while protein degradation is first order ($-dP/dt = kP$) (Berlin and Schimke, 1965; Hargrove, 1993; Schimke, 1973). The time course of onset of some signs of drug dependence, as well as the onset of antipsychotic effects, can fit this simple first case (Eq. (1)) (Kuhar and Joyce, 2001).

Perhaps a more realistic situation, one more applicable to drug dependence, is where there is a sequence of events or proteins where each is compatible with Eq. (1). This case assumes that a second protein or process, $P2$, depends on the presence of a first protein or process, that is $r2 = b \times P1$, where $r2$ is the rate of synthesis of the second protein and b is a proportionality constant. This could reflect the case where $P1$ is, for example, a transcription factor, and $P2$ is some protein expressed by the action of $P1$. Generalizing to N sequential proteins or processes, each dependent on the previous ones, we have

$$Pn_t/Pn_{max} = (1 - e^{-0.693t/h1}) \times (1 - e^{-0.693t/h2}) \ldots (1 - e^{-0.693t/hn}). \quad (2)$$

This more complex case is compatible with data some showing a time course of onset with a slight lag phase, for example (Kuhar and Joyce, 2001). An important feature of Eq. (2) is that if one of the half-lives is much longer than all of the others, then that associated term dominates and Eq. (2) reduces to Eq. (1). Thus Eq. (1) is a reasonable first tool with this approach. If the rate of onset of a behavior or physiological state fits the exponential Eq. (1) with an apparent half-life, h, it could be hypothesized that a key, dominant and rate-limiting protein mediates the process and has a half-life of h. Additional proteins or processes governed by Eq. (2) could be involved, but if their half-lives were much smaller than h they would have minimal relative effects on the time course of the overall process. The latter is key to our interpretation of the results from application of the model. Drug dependence is likely to require many changes in brain, perhaps changes in many proteins. But if one of these proteins is rate limiting, with a longer half-life than the other steps and proteins, then it should fit the model of Eq. (1) as a simplification of Eq. (2). Other interpretations are of course possible; for example, a fit to Eq. (1) could also result from several different but functionally additive proteins with similar or the same half-lives. While this overall approach can be criticized as elementary, it has met with some success (Kuhar and Joyce, 2001) and should be considered at least as a first stage of analysis with greater complexity to be added as needed.

Having reviewed the overall problem, its support, and the model that was utilized previously with some success, we now state the purpose of this paper. In our previous modeling studies (Kuhar and Joyce, 2001, 2003), we relied on very limited data from the literature. While the data was of some utility, we felt that having many more data points, particularly at earlier times during the onset of dependence, would provide a better test of the model. Accordingly, we have now measured opiate withdrawal at a large number of time points in an established rat model of opiate dependence (Holtzman, 2003). In order to test our hypothesis that opiate dependence requires protein synthesis, we have tested if the time course of onset of dependence is similar to the time course of onset of protein levels.

2. Methods

2.1. Subjects

Eight adult male Sprague-Dawley rats (Charles-River, Wilmington, MA) for each time point group and each saline group, a total of 296 animals, served as study subjects. They were individually housed in standard polycarbonate cages with free access to food and water. The colony room was maintained under a 12-h/12-h light/dark cycle, with lights on at 6:00 a.m. Each rat weighed 250–370 g at the time of osmotic pump implantation.