Biochemical Pharmacology 79 (2010) 665–668

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Measuring *levels* of proteins by various technologies: Can we learn more by measuring turnover?

Michael J. Kuhar *

Yerkes National Primate Research Center of Emory University, 954 Gatewood Rd NE, Atlanta, GA 30329, USA

ARTICLE INFO

Article history:
Received 8 September 2009
Accepted 30 September 2009

Keywords:
Protein levels
Protein turnover
Protein half-lives
Western blotting
PET scanning

ABSTRACT

In routine experiments, scientists measure the *levels* of various substances such as proteins after various treatments. Detection of a change in levels suggests an impact of treatment on that particular protein. However, we sometimes forget the importance of turnover in this process. Proteins have half-lives that may change in response to treatments (which is in fact why *levels* may change), and an examination of half-lives may yield better clues as to how treatment affects the protein. After an exploration of the quantitative aspects of protein turnover, several interesting conclusions may be drawn. (1) Even though levels of some proteins may NOT change after treatments, their half-lives and turnovers do change, and these may be more sensitive indicators of the impact of treatments on the proteins of interest. (2) Treatments can affect protein *levels* because they alter either the synthesis or degradation of the protein or both. But, the rate of change of the levels depends on the half-life of the protein. If the experimenter waits only a fraction of a half-life of the protein after treatment, no significant change in level may be found since it can take up to 5 half-lives for the protein level to adjust to about 97% of its new level after treatment. (3) Half-lives of the same protein can vary in different species and experimental conditions may have to be altered if using different species. These factors suggest that a consideration of protein turnover and half-lives will be useful for future studies of this type.

1. Introduction

In many kinds of routine studies, pharmacologists and other scientists measure proteins by various technologies that include Western blotting, immunocytochemistry, mass spectrometry, etc. mRNAs have been measured by using microarray technology and RT-PCR. Proteins in living brains, for example, can be examined using PET scanning. These technologies have one thing in common, which is that they measure *levels* of proteins rather than some other index which might be a better measure of the utilization or activity of the protein. The proposal of this commentary is that a measurement of the *turnover or half-life* is a better indicator of the activity or utilization of these proteins. This is not a proven, general rule, but rather it is something worthwhile to consider.

It is known that proteins (and other molecules such as mRNAs) turn over, that is, they are degraded or removed and replaced by newly synthesized proteins. It is known that turnover rates of different proteins vary, and often depend on physiologic conditions. The concept of turnover is presented in Fig. 1, where there are two compartments. We can think of each of these compartments as being a brain region or a cell compartment or any entity where a measurement is made. Moreover, we can think of the dots in each compartment as protein molecules of interest. Note that if we measure the levels of the proteins in each box, we find 11 protein molecules, which is the same. However, there is a striking difference between the proteins in the top vs. the bottom boxes or compartments. The top compartment has one protein molecule that degrades per unit time, and it is replaced by one new molecule that is synthesized during that time. The second compartment is quite different. It degrades or loses 10 protein molecules per unit time, and 10 are synthesized to replace them. So, if we measure the levels of proteins, we see that there are 11 molecules in each compartment. However, that clearly does not provide an adequate description of the proteins in those compartments. The top compartment has proteins turning over much more slowly, while the bottom compartment has proteins turning over much more rapidly.

In the 1960s and 1970s, it was realized, in the field of neurotransmitter research, that simply looking at neurotransmitter levels alone did not adequately reflect the activity of the neurons containing those neurotransmitters. Rather, turnover of neurotransmitters became, and still is, a better measure, and

* Tel.: +1 404 727 1737; fax: +1 404 727 3278.
E-mail addresses: mkuhar@emory.edu, michael.kuhar@emory.edu.

0006-2952/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2009.09.029

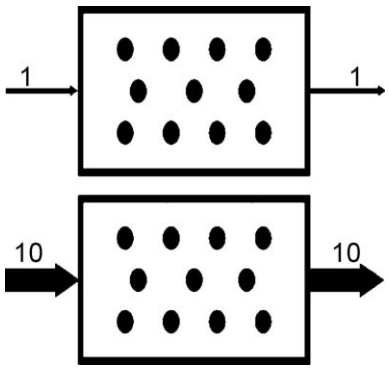

Fig. 1. Schematic illustrating the relative importance of turnover vs. levels of a protein. The concept and relative importance of turnover is illustrated by this figure, where there are two boxes or compartments. Either of these compartments could be a brain region or a cell compartment or any entity where a measurement is made. Let us think of the dots in each compartment as the protein molecules of interest. Note that if we measure the levels of the proteins in each box, we find 11 protein molecules, which is the identical. However, there is a striking difference between the top vs. the bottom boxes or compartments. The top compartment has one protein molecule that degrades per unit time, and it is replaced by one new molecule that is synthesized at about the same time. The second compartment degrades or loses 10 protein molecules per unit time, and 10 are synthesized to replace them. So, if we measure the levels of proteins, we see that there are 11 molecules in each compartment. However, that clearly does not provide an adequate description of the proteins in those compartments. The top compartment has proteins turning over much more slowly, while the bottom compartment has proteins turning over 10 times more rapidly. See text for further discussion.

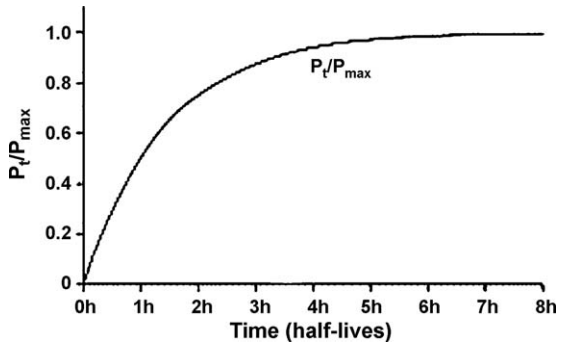

Fig. 2. The time for a protein to reach a new level after a change in synthesis rate or degradation rate depends on the half-life of the protein. The curve is generated from Eq. (3). If a treatment causes a change in synthesis or degradation, then the time it takes to reach about 97% of its new levels is 5 half-lives. See text for additional details. Adapted from Kuhar and Joyce [12].

perhaps the best measure, of neuronal activity and utilization of those neurotransmitters. Thus, ideas utilized in the past for other molecules and systems have relevance to current measurements of proteins and many other molecules such as mRNAs.

2. Half-lives of proteins

Examining Fig. 1 again, we can consider the idea of half-life which is well known to be the time that it takes for half the molecules in a compartment to be replaced. Half-lives have been measured for various substances for many years, and the quantitative formulation describing protein turnover and half-lives has been described in several places [12,14,16].

The equations relevant for describing these half-lives are as follows, and more of the details of the derivations can be found elsewhere. The basic equation for the change in protein levels over time is

$$P_t = (r/k)(1 - e^{-kt}) \quad (1)$$

where r is the rate of protein synthesis, and k is the degradation rate constant. P_t is the protein level at time t and P_{max} is the maximal level of the protein. t is the time after some event such as a change in synthesis or degradation, and as t becomes large, P_t reaches its maximal level, and we get

$$P_{max} = r/k. \quad (2)$$

Note that if either (or both) of the synthesis rate or degradation rate constant change, P_{max} will change unless the ratio of r/k remains about the same even after the change. This is really one of the points of this article. r and k can change together in the same fractional way without any significant or measureable change in P_{max} (levels). Therefore, simply measuring P_{max} does not provide more subtle information about the protein under study, which is how quickly it is synthesized or degraded.

Eq. (2) can be revised to

$$\frac{P_t}{P_{max}} = (1 - e^{-0.693t/h}) \quad (3)$$

where the half-life, h, is introduced. This is done because protein half-lives can be measured in the laboratory. The half-life of the protein is

$$h = \frac{0.693}{k} \quad (4)$$

It is intuitively clear that a faster turnover corresponds to a shorter half-life, while a slower turnover corresponds to a longer half-life.

A plot of P_t/P_{max} vs. h is shown in Fig. 2, where the exponential rise is evident. But if there is a sequence of proteins (say a total of "n" proteins in sequence) where the new level of a subsequent protein depends on the level of the previous protein, then we have:

$$\frac{Pn_t}{Pn_{max}} = (1 - e^{-0.693t/h1}) \times (1 - e^{-0.693th2}) \cdots (1 - e^{-0.693t/hn}), \quad (5)$$

where Pn_t is the level if protein "n" at time t, Pn_{max} is the maximal level of protein "n", $h1$ is the half-life of protein 1, and hn is the half-life of protein "n". After some thought and analysis of Eq. (5), the overall time for a change in levels of protein "n" will depend on the protein in the sequence with the slowest turnover or longest half-life [12].

Considering the case of a single protein as in Fig. 2, it is seen that after some treatment or change that alters synthesis or degradation, it will take 5 half-lives to reach about 97% of the new level. The change that alters synthesis or degradation could be the administration of some drug, either acute or chronic. Note that Fig. 2 suggests that the change observed is an increase because the curve rises, but it just as well could be a decrease with the level falling below the x-axis. The equation is the same but the sign of the change would be different.

This author realizes that reading equations can generate a strong urge to do something else. However, please read on.

3. Applications and examples

A well-known case where a protein level is changed and is associated with disease is the levels of D2 dopamine receptor proteins in brains of patients who have taken cocaine repeatedly for substantial periods of time. Fig. 3 shows that the levels of dopamine receptor proteins are decreased in cocaine abusers and return very slowly, over a period of months, towards normal levels [17]. Such a slow return suggests a relatively long half-life of D2 receptor proteins in humans, but of course other explanations are

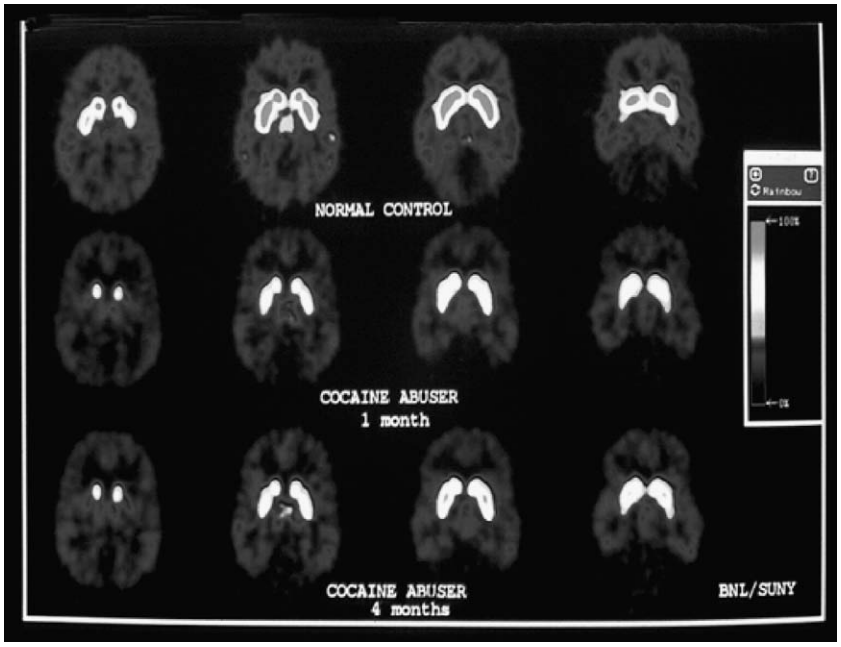

Fig. 3. Changes in levels of dopamine D2 receptors is associated with chronic exposure to cocaine. This is a case where measuring *levels* of D2 receptors provided interesting data. After cessation of drug use, it takes months for the levels of D2 receptors inhuman brain to return to normal. The change in the levels of the D2 receptors is due to either an increase of synthesis rate or a decrease in the degradation rate constant. But for some proteins, under certain conditions, r and k may change similarly in the same direction and there would be no apparent difference in protein levels, which could mislead us to infer that the proteins were not especially involved in the action of the drug. See Table 1 and the text for explanations and discussion. Reproduced from Volkow et al. [17] with permission.

possible. So here is a case where measuring levels alone provides interesting information. But it is not known if r, or k or both are changed in the addicts.

There are studies with animals where the levels of proteins are unchanged, but the turnover and half-life are altered. Consider the turnover and half-life of dopamine transporters in the rat brain. In these experiments, dopamine transporter (DAT) ligand binding was blocked by an irreversible agent, RTI-76. The turnover of these DAT proteins was determined by measuring the rate of return of DAT binding after RTI-76 administration. It is assumed that the rate of return of binding reflects new synthesis, rather than the chemical separation and loss of RTI-76 blockade. Studies by Kimmel et al. [10,11] have shown (Table 1), that acute cocaine administration can cause a significant increase in the half-life of DATs, while the change in B_{max} (or level) of the transporters is not significantly different. Similarly, withdrawal from repeated cocaine results in a significant decrease in half-life, while again there is no change in the B_{max} or levels of DATS in treated animals vs. control animals. Thus, it is clear that turnover and half-lives can be different after a treatment, while levels are not significantly different. If we considered only levels (P_{max}), we would be tempted to say that the drug had no or little effect on the DAT protein; but by examining the turnover and half-lives, we can see significant effects of the drug.

Another example where measuring only levels has lead to confusion, is the story about the levels of CART peptides in the rat nucleus accumbens after administration of cocaine or amphetamine. Douglass et al. [2] found an increase in CART mRNA levels after acute drug administration, and this is in fact how CART peptide was fully discovered. While some workers were able to reproduce this increase [1,4,7], many others were not [9,13,18], creating doubt about the cocaine-CART interaction. Could it be that measuring levels alone was not precise enough because r and k were changing similarly (see Eq. (2) above)? Because there was no established way to measure the half-life or turnover of CART peptides, another approach was taken by Hubert and Kuhar [6] who showed that administration of cocaine did increase c-fos levels in CART-containing neurons in the nucleus accumbens. Thus, the activity of CART neurons was increased as the work of Douglass suggested, but it took another approach, other than just measuring levels, to provide a more definitive answer. It is likely that situations – where there are conflicting reports in measurements of levels – can be helped by an assessment of turnover.

Does increased turnover reflect an increase in the utilization of the protein? Well, this is a matter for further study, but consider the work of Ehlers [3] for example. This study showed that AMPA receptors for glutamate were increasingly internalized by endocytosis and degraded with increased stimulation and activity of the receptors. Use of a receptor antagonist reduced internalization. After endocytosis some of the AMPA receptors were recycled to the cell surface and a portion were degraded. An interpretation is that the use of the protein results in an increased turnover of the protein. Another example is the work shown in Table 1. When cocaine is administered acutely, the transporter is blocked and its utilization is reduced; note there is a concomitant increase in the half-life of the transporter.

Of course it is possible that attempts to measure half-lives according to the model and equations given above are overly simplistic. There may be isoforms of proteins with different half-lives, or r and k may not be constant throughout the experiment. Nevertheless, understanding the factors underlying protein dynamics can be helpful.

Table 1
Changes in DAT in rat striatum and nucleus accumbens after treatment with cocaine.

	Change in $t_{1/2}$	Change in B_{max}
Acute cocaine (Nuc Acc)	+43%	Not significant
Withdrawal (striatum)	−55%	Not significant

Data from Table 3 in Kimmel et al. [10], and from Table 1 in Kimmel et al. [11].

4. Is measuring levels useless?

Obviously not. Many, many studies of many proteins indicate changes in levels after treatments with important implications, and the dopamine D2 receptor story is described above. The point here is that measuring levels is not the whole story.

The reason one finds a change in levels after treatments is because that there is in fact a turnover of proteins, and when r or k changes, P_{max} will change. But it is useful to understand that the half-life of a protein determines the time needed to find the maximal change in levels. Eq (3) and Fig. 2 show that it takes about 5 half-lives to attain about 97% of the maximal change in P_{max} after some treatment. But if your experiment does not allow enough time to observe the change, say you are only allowing a fraction of a half-life, then you may erroneously conclude there is no change in levels. For example, if your protein of interest has a half-life of say 6 h, but you examine levels only 1 h after treatment, then you can only expect to see 11% of the new level, which may not be enough of a change to measure. Fig. 2 shows that the half-life determines how quickly the new level is reached. Moreover, it has been pointed out that half-lives for the same protein can vary in different species (see Refs. [12]) and you may have to adjust experimental times when changing species. There may be other situations where half-lives are different such as in transgenic mice. Thus, just being informed about turnover and half-lives can improve your perspective on experiments where you are only measuring *levels* of proteins or other substances. Even if changes in levels are found, it can be more informative to measure r and k and turnover as well.

5. Possible mechanisms of changes in protein turnover

It is well known that G-protein-coupled receptors (GPCRs) are connected to signaling pathways within the cell and nucleus. Changes in the activity of neurotransmitters at these GPCRs can result in changes in gene expression or mRNA translation. Changes in gene expression or translation will in time result in changes in levels of various proteins in a cell. An example of GPCR regulation is provided by Park et al. [15]. mGluRs were activated in hippocampal cultures and the levels of the Arc/Arg3.1 protein, a protein involved in synaptic plasticity, were increased within 5 min. This increase was prevented by a protein synthesis inhibitor indicating that de novo translation took place. While this could be due to an increased rate of translation, or by a stable rate of translation together with reduced degradation of mRNAs, the authors seemed to favor an increased rate of translation.

Of course, there are other mechanisms that control the synthesis and degradation of proteins, and the half-life of mRNA molecules is one of them. It is possible that half-lives or mRNAs change under various physiological conditions (e.g. [5]). Moreover, the internalization of proteins can be regulated by molecules such as ubiquitin (e.g. [8]). Thus, there are several factors that could influence the half-lives of proteins. But a consideration of the utilization and power of turnover and half-life measurements does not necessarily require an in-depth understanding of the mechanisms producing such changes.

6. Conclusion

It is proposed that measuring *levels* of proteins does not provide a full picture of their possible activity and use. Measuring half-lives and turnover of proteins is presumably a better indicator of activity and use. Given the potential increase in sensitivity and understanding of cellular dynamics, these efforts seem worthwhile.

Acknowledgment

NIH grants RR00165, DA15162, and support from the Georgia Research Alliance.

References

[1] Brenz Verca MS, Widmer DA, Wagner GC, Dreyer J. Cocaine-induced expression of the tetraspanin CD81 and its relation to hypothalamic function. Mol Cell Neurosci 2001;17:303–16.
[2] Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci 1995;15:2471–81.
[3] Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. Neuron 2000;28:511–25.
[4] Fagergren P, Hurd YL. Mesolimbic gender differences in peptide CART mRNA expression: effects of cocaine. Neuroreport 1999;10:3449–52.
[5] Hamilton TA, Novotny M, Datta S, Mandal P, Hartupee J, Tebo J, et al. Chemokine and chemoattractant receptor expression: post-transcriptional regulation. J Leukoc Biol 2007;82:213–9.
[6] Hubert GW, Kuhar MJ. Cocaine administration increases the fraction of CART cells in the rat nucleus accumbens that co-immunostain for c-Fos. Neuropeptides 2008;42:339–43.
[7] Hunter RG, Vicentic A, Rogge G, Kuhar MJ. The effects of cocaine on CART expression in the rat nucleus accumbens: a possible role for corticosterone. Eur J Pharmacol 2005;517:45–50.
[8] Hutt DM, Powers ET, Balch WE. The proteostasis boundary in misfolding diseases of membrane traffic. FEBS Lett 2009;583:2639–46.
[9] Jones DC, Kuhar MJ. Cocaine-amphetamine-regulated transcript expression in the rat nucleus accumbens is regulated by adenylyl cyclase and the cyclic adenosine 5′-monophosphate/protein kinase a second messenger system. J Pharmacol Exp Ther 2006;317:454–61.
[10] Kimmel HL, Carroll FI, Kuhar MJ. Withdrawal from repeated cocaine alters dopamine transporter protein turnover in the rat striatum. J Pharmacol Exp Ther 2003;304:15–21.
[11] Kimmel HL, Joyce AR, Carroll FI, Kuhar MJ. Dopamine D1 and D2 receptors influence dopamine transporter synthesis and degradation in the rat. J Pharmacol Exp Ther 2001;298:129–40.
[12] Kuhar MJ, Joyce AR. Slow onset of CNS drugs: can changes in protein concentration account for the delay? Trends Pharmacol Sci 2001;22:450–6.
[13] Marie-Claire C, Laurendeau I, Canestrelli C, Courtin C, Vidaud M, Roques B, et al. Fos but not Cart (cocaine and amphetamine regulated transcript) is overexpressed by several drugs of abuse: a comparative study using real-time quantitative polymerase chain reaction in rat brain. Neurosci Lett 2003;345:77–80.
[14] Norman AB, Battaglia G, Creese I. Differential recovery rates of rat D2 dopamine receptors as a function of aging and chronic reserpine treatment following irreversible modification: a key to receptor regulatory mechanisms. J Neurosci 1987;7:1484–91.
[15] Park S, Park JM, Kim S, Kim JA, Shepherd JD, Smith-Hicks CL, et al. Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron 2008;59:70–83.
[16] Sladeczek F, Bockaert J. Turnover in vivo of alpha 1-adrenergic receptors in rat submaxillary glands. Mol Pharmacol 1983;23:282–8.
[17] Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, et al. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 1993;14:169–77.
[18] Vrang N, Larsen PJ, Kristensen P. Cocaine-amphetamine regulated transcript (CART) expression is not regulated by amphetamine. Neuroreport 2002;13:1215–8.