Published in final edited form as:

Pharmacol Res. 2011 October ; 64(4): 323–326. doi:10.1016/j.phrs.2011.05.019.

Pharmacological Regulation of Gene Expression

Italo Mocchetti

Department of Neuroscience, Georgetown University Medical Center Washington DC

Keywords

Diazepam Binding Inhibitor; Dopamine; Opioid peptides; NGF; GM1; Trk

1. Introduction

I was at Columbia University in New York City in August 1985 when Dr. Costa called me and asked me if I was interested in joining him in his new neuroscience institute at Georgetown University in Washington DC. I accepted his invitation without hesitation. Previously, I had the honor of working with him as a postdoctoral fellow for more than two years at the Laboratory of Preclinical Pharmacology (LPP), St. Elizabeths Hospital, Washington DC. I then learned that the institute would be called the Fidia-Georgetown Institute for the Neurosciences (FGIN). A few months later on a train for Washington DC, I still remember the great excitement I felt at the prospect of continuing to work with Dr. Costa and I will be always grateful to him for that opportunity.

The major emphasis of the neuroscience program at FGIN was drug discovery, which ultimately requires learning more about drug targeting and receptor specificity. Although Dr. Costa had developed and proved several new concepts in the pharmacology of receptors, including the allosteric modulation of neurotransmitter receptors, he was concerned about the paucity of knowledge on how drugs affecting neurotransmitter receptors could permanently change the expression of genes relevant to neuronal survival and plasticity. Based on my previous experience as a molecular biologist at the LPP and Columbia University, Dr. Costa asked me to direct a research program on transmitter regulation of gene expression. Two main research areas were investigated: how drug abuse influences the expression of genes and how neuronal plasticity could be induced pharmacologically. Neuronal plasticity, as he often pointed out, indicates a number of neuronal mechanisms that are coordinately triggered to overcome functional impairment due to damage evoked by diseases and trauma. During my time at FGIN, I had the privilege of working with a number of highly motivated and exceptionally bright researchers. Here, I will briefly describe the contribution of these scientists from the official opening of FGIN (November 1985) to 1990, when, due to my new position as Assistant Professor in the Department of Anatomy and Cell Biology, the last collaborator from FGIN left my laboratory. I will be always grateful to Dr. Costa for his mentorship and guidance not only during my direct association with him but also after I became an independent investigator.

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Correspondence should be sent to: Italo Mocchetti, Georgetown University Medical Center, Department of Neuroscience New Research Building, WP13 3970 Reservoir Rd, N.W., Washington DC, 20057 mocchetti@georgetown.edu.

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2. Drug abuse and gene expression

2.1. Opioid peptides

Back in the late 80s, the endogenous opioid system was the most extensively characterized of all neuropeptides, and thus it was used as a valuable experimental model to investigate how changes in synaptic activity induced by pharmacological means or electrical stimulation affect the biosynthesis and availability of neuropeptides. This work was a continuation of studies initiated at LPP, where we used various biological measurements of peptide synthesis to determine the dynamic state that regulates neuropeptide expression and availability. Such measurements included DNA transcription, mRNA levels, peptide precursors and end products [1]. With the help of Dr. J.R. Naranjo (a PhD from the Instituto Cajal, Madrid, Spain), we established that catecholamines, and in particular dopamine, play a major role in the regulation of enkephalin expression in the striatum and adrenal chromaffin cells. We were able to characterize that the two major subtypes of dopamine receptors, D1 and D2, modulate positively and negatively, respectively, the turnover of enkephalins in the striatum [2]. Furthermore, Dr. Naranjo, in collaboration with Dr. M. Iadarola (NIH), a former alumnus of Dr. Costa, established that increased synaptic activity, such as that obtained by convulsive seizures generated by electrical stimulation of the amygdala, up-regulates the expression of mRNA coding for proenkephalin, a precursor protein that contains several enkephalin sequences, and the synthesis of enkephalins. Such up-regulation was observed mainly in brain areas in which seizures propagate. Overall, this research project provided support to the concept of activity-dependent regulation of opioid peptide expression.

The area-selective regulation of enkephalin expression after neurotransmitter receptor activation prompted us to examine the hypothesis that drugs of abuse could change the availability of endogenous opioid peptides. With the help of a PhD student (Ms. A. Ritter), we searched for mechanisms that could account for morphine tolerance and dependence. Surprisingly, we found that chronic morphine treatment does not change proenkephalin or enkephalin levels. However, by using a cDNA probe for the proopiomelanocortin (POMC) gene and specific antibodies that recognize the end products, β -endorphin and corticotrophin-like intermediate lobe peptide (CLIP), we discovered that a chronic morphine treatment leading to tolerance and dependence decreases POMC expression and processing in the hypothalamus, suggesting that opiate abuse decreases synthesis and utilization of endorphins [3].

A similar approach was used to study the effect of cocaine and dopamine on peptides expressed in the nigro-striatal system and, in particular, cholecystokinin (CCK). In collaboration with Dr. X-Z Ding (a Fogarty International Program scholarship fellow from China) we observed a selective increase in striatal CCK expression by cocaine and other dopaminergic agonists. The opposite effect was elicited by dopaminergic receptor antagonists. Overall, we demonstrated that dopamine exerts a modulatory role on the expression of several neuropeptides. These data confirmed the validity of measuring the dynamic state of neuropeptide stores to investigate how changes in neuronal activity triggered by pharmacological means alter gene expression.

2.2. Diazepam Binding Inhibitor

The information obtained by studying enkephalin expression after chronic drug treatments prompted us to speculate that drugs that elicit dependence and tolerance could also change the dynamic state of endogenous modulators. The negative allosteric modulator of GABA_A receptors, Diazepam Binding Inhibitor (DBI), was discovered by Drs. Costa and Guidotti along with other collaborators at LPP. The histological localization of DBI in the brain was

later confirmed by Dr. H. Alho (a visiting Associate from Finland). While at Columbia University, I had the honour and privilege of working in Dr. J. Brosius' laboratory to clone DBI from a library of rat hypothalamus. We obtained the full length sequence of rat DBI mRNA [4] and confirmed that DBI is a peptide of 86–87 amino acids. A DBI cDNA was then prepared to determine the distribution and expression of DBI in rat brain by Northern blot analysis and *in situ* hybridization. Expression of DBI was found in the brain both in neuron and glial cells, as well as other peripheral tissues, including liver [4,5], but DBI is poorly expressed in spleen, muscles and lung. Dr. Alho continued this work with DBI in Finland and he demonstrated that DBI is also expressed in human brain tumours [6]. Interestingly his team found a correlation between cell proliferation and increased DBI expression in brain tumours [7]. These data suggested that DBI may have different roles in different tissues. To learn more about the role of DBI in the central nervous system, studies were undertaken to characterize its expression. Using a specific DBI antibody generated by Dr. Guidotti and a DBI cDNA clone, Drs. M. Miyata (an MD from Japan) and C. Ferrarese (a PhD from Brescia, Italy) measured the synthesis and turnover of DBI after chronic treatment with two well-known benzodiazepines, diazepam and chlordiazepoxide. We discovered that these treatments increase DBI synthesis and processing in selected brain areas [8]. Because such treatment also elicits tolerance, it was inferred that DBI could play a key role in benzodiazepine tolerance and anxiety disorders. To gain insights into a possible relation between DBI expression and pathogenesis of anxiety disorders, we studied the chromosomal localization of human DBI. This work, carried out in collaboration with Drs. M.A. DeBernardi and G. Brooker at FGIN and R. Crowe (Department of Psychiatry, University of Iowa), established that the DBI gene is located on the q12–21 region of the long arm of chromosome 2 [9]. Further studies revealed that DBI expression follows a developmental pattern in both the brain and peripheral tissues. Later on, Dr. M.R. Santi (an MD from the University of Modena, Italy) showed that the content of DBI mRNA in rat liver was significantly higher at postnatal day 1 than in adult animals, consistent with the role of DBI in the liver as a modulator of cell metabolism. Indeed, it was subsequently discovered by different groups that DBI is involved in mitochondrial steroidogenesis, glucose-stimulated insulin secretion, and intracellular transport of acyl-CoA esters.

3. Pharmacological regulation of Nerve Growth Factor biosynthesis

3.1. Beta-adrenergic receptors

The scientific background and interest of Dr. Costa was continuously evolving from neurotransmitters to neuropeptides and then neurotrophic factors. In 1986, experimental evidence favored the hypothesis that the neurotrophic factor Nerve Growth Factor (NGF) could modulate the survival of cholinergic neurons suggesting that NGF might be used in the treatment of Alzheimer's disease or other neurodegenerative disorders. Because neurotrophic factors are polypeptides that do not efficiently cross the blood brain barrier, we developed a new area of research aimed at increasing the availability of neurotrophic molecules in the central nervous system by using pharmacological tools. The idea was to enhance production of NGF as a preventive treatment in populations at risk for Alzheimer's disease. At that time, information about NGF gene regulation was subtle at best. Accordingly, Dr. Costa used to argue that before embracing such a pharmacological approach to prevent neurodegenerative diseases, more information on the mechanisms regulating NGF gene expression and biosynthesis in the brain was needed. Moreover, while NGF expression was detected in the peripheral tissue, little was known about NGF expression in the central nervous system.

An early report from Costa's group had suggested that NGF could be regulated by intracellular levels of cAMP. Therefore, we first used a series of pharmacological stimuli known to increase cAMP levels to enhance NGF gene expression. The investigation began

with the help of Drs. R. Dal Toso (a senior scientist from FIDIA, Italy) and M.A. DeBernardi, who developed a cRNA probe that allowed measuring NGF mRNA in C6-2B cells, a glioblastoma cell line. It was discovered that NGF transcription was up-regulated by stimuli that activate the cAMP and protein kinase A pathway [10]. These results were followed by the first demonstration that the early inducible gene c-fos was involved in the cAMP-dependent activation of NGF gene expression [11]. These findings, obtained in collaboration with Dr. A.M. Szekely (an MD from Semmelweis University in Budapest-Hungary) and H. Alho, were instrumental in the characterization of cAMP responsive cis-elements in the NGF promoter and putting forward the hypothesis that a similar mechanism occurs also *in vivo*. Indeed, with the help of Dr. M. Fabrazzo, an MD from the University of Naples, Italy, who joined FGIN as a PhD student in Pharmacology, we were able to demonstrate that increases in cAMP levels, such as those obtained by activation of beta-adrenergic receptors, evoke NGF mRNA accumulation in rat cerebral cortex. This discovery was the conceptual basis of my first RO1 grant award. Some years later, Dr. Costa who had already moved his group to the Department of Psychiatry at the University of Illinois, Chicago, was informed that we had identified the cAMP and c-fos binding sites in the NGF promoter. This work reported for the first time that the proximal NGF promoter contains a CCAAT/enhancer binding protein site and an AP-1 element that activate NGF transcriptional expression in response to beta-adrenergic receptor stimulation [12].

3.2. Glucocorticoids

In a parallel series of experiments, Drs. M. Fabrazzo and F-Y Sun (from a Fogarty International Program award from China), were able to demonstrate that adrenal corticosteroid can increase the expression of NGF in the hippocampus and cerebral cortex [13]. In these studies, using reserpine (a drug that was originally characterized by B.B. Brody, Dr. Costa's mentor) or bicuculline, it was shown that the hypothalamic/pituitary/adrenal axis controls the expression of NGF in the brain. These data fostered the discovery that stress influences NGF expression in the brain. Similarly, Dr. A. Yakovlev (a senior investigator from the Russian Academy of Medical Sciences, USSR) conducted follow-up experiments to examine whether glucocorticoids and cAMP change the expression of the receptors for NGF. We found that these stimuli increase the expression of the low (p75NTR) and high affinity TrkA NGF receptors in glial cells and neurons, respectively, suggesting that NGF receptors can be differentially regulated by environmental clues. These findings were followed some years later by the functional demonstration that low doses of glucocorticoids increase NGF and TrkA *in vivo* and induce hypertrophy of TrkA positive medial septal cholinergic neurons [14]. This was the first evidence that pharmacological tools can be used to modulate expression of NGF *in vivo* to improve neuronal plasticity. A number of papers have appeared since then demonstrating that glutamate and other neurotransmitters modulate the expression of a variety of neurotrophic factors. Thus, our work was crucial for the understanding of the molecular mechanisms underlying neurotransmitters-mediated neuronal plasticity.

3.3. Cytokines

Additional studies conducted at FGIN revealed that NGF transcription is not regulated only by beta-adrenergic receptors, protein kinase A or steroids. In fact, in Dr. Wise's group, Dr. J. Alberch (a postdoctoral fellow from the University of Barcellona, Spain) showed that cholinergic activity in the septal forebrain area modulates NGF expression. In addition, Dr. Wise and his group (Drs. Carman-Krzan, Vige' and Tang) provided evidence that NGF expression in astrocytes is increased by interleukin-1 beta or basic fibroblast growth factor [15] and other cytokines; this increase in NGF expression is followed by an accumulation of NGF secretion. Moreover, they established the primary signaling mechanisms that regulate

interleukin-mediated NGF secretion. These include Ca^{2+} , protein kinase C, and the phospholipase A2-lipoxygenase pathway.

To shed further light into mechanisms of NGF regulation, Drs. Wise and Pshenichkin exposed astrocytes to okadaic acid, an inhibitor of phosphoprotein phosphatases. Such agents dramatically increased both NGF mRNA content and NGF secretion in astrocytes, suggesting important roles for phosphoprotein phosphatases in regulating NGF production/secretion. It was concluded that glial cell production of NGF following neuronal injury might limit the extent of neuronal loss and promote regenerative processes to restore neuronal function.

4. Concluding remarks

The most important lesson that I learned from Dr. Costa was that, in science, no hypothesis is too wild as long as it can be tested. I have followed his advice many times in my career. For instance, several years after Drs. Costa, Guidotti, Manev and Favaron demonstrated that gangliosides are neuroprotective drugs against glutamate and ischemia [16], my group attempted to establish their mechanism of action. Here, the wild hypothesis was that GM1 activates a neuroprotective second messenger. We tested this hypothesis and it turned out that Dr. Costa was again right because GM1 and other gangliosides activate Trk, the high affinity receptor for the neurotrophins and its associated signal mechanisms [17]. I had to tell him that. Thus, I went to visit him at the University of Illinois in Chicago and present my data. In keeping with the tradition of the Monday morning meetings, he asked several questions but overall he was happy to hear my story. The fondest memory I have of that day was Dr. Costa's kind comment: "You came to LPP as a young and naïve scientist, but now I see that you are an accomplished investigator". To you Dr. Costa, my deepest gratitude for helping me make this transition.

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