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Molecularly imprinted polymer microprobes for manipulating neurological function by regulating temperature-dependent molecular interactions☆

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ABSTRACT

We describe molecularly imprinted polymer (MIP) microprobes for manipulating neurological function by the thermoregulatory process. Some target and disease areas show raised temperature than normal temperature which are involved in neuronal diseases, such as Alzheimer's, Parkinson's and Huntington's diseases. As well thermodynamic parameters can be obtained in the environments *in vitro*, which that the assay involves a temperature variable, at or beyond body temperature. We applied for studying molecular aspects of hypothalamus regulated physiology and behaviors. Steady-state binding was detected by fluorescence and Boltzmann energies-based fluorescence emission showed interactions of clozapine with altered energy levels. Dynamic spectra revealed dramatic effects on the fluorescence of the drug in dopamine imprint of MIPs exposed rat hypothalamic membranes at different temperatures. The MIPs that cooperatively occupied and provided the response to the neural proteins were not likely to be involved in the binding of clozapine within the cavity. The results indicated that the dynamics on the surface of proteins exposed to the drug–MIP with assembly, mediated specific function through temperature-dependent molecular interaction in the hypothalamus. Thus, this approach is applicable to processes of key importance of controlling the energy homeostasis and, plausibly, to selective signaling can affect in plasticity of the neural response.

1. Introduction

Drug or substrate interactions at the binding pocket of a specifically functional protein in the human brain is one of the essential processes that can be manipulated to selectively separate the therapeutic effects of drugs or drug candidates from their unwanted side effects. Their molecular and cellular interactions are central to studying how biologically active compounds exert their efficacy related to their distributions and structural dynamics in their molecular environments. Such approaches have potential importance for medicine and pharmaceutics [1,2]. By taking the feature of a unique interface produced by assembly that occurs normally in the function of a biological system that gives rise a desirable interaction of the delivered molecule [3]. The interacting of specific protein receptor can sense to the extracellular environments producing the developmental signals and improved responses [4]. The tremendous complexity of a molecular interaction in a living system has led to the evolution of physiological signaling molecules that evolve the receptor-mediated neuron in brain. Technology of a single molecule signal passing through a periodic mesoporous material where the specific structural feature influenced on the mobility of the desired molecule [5], this will be able to provide studying for particular cellular functions and even transducing for downstream signals in body. Of even more importance of the specialized responses of the receptors influence from the substrate-binding of anti-psychotic drugs for the psychotic responses and the behaviors and that were dependent on the active functional protein are associated with the physical environments that can affect the actual organization of protein binding sites and assemblies [6].

☆ This article is dedicated to Prof. Dr. Vimon Tantishaiyakul for the occasion of her 60th birthday.

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The self-organization involved in the cellular processes of the neural system, though at a relative minor level, can alter the conformational segregation through the ability of the network's that relies on the selective recognition of biomolecules. Signaling molecules within synapse give temporarily or emerging responses in the brain to minimize damage of the cells [7]. The homeostasis control, to circuits, centrally, primarily controlled by the biological activity of the hypothalamus which attenuating the dopamine and serotonin neurotransmitters release by regulating the social behaviors and influenced the balance of emotion and the post-traumatic disorder. The differences of therapeutics traverse the dopaminergic system that can be important to the drug sensitization in the behavioral phenomena, is not discovered, although the possibility it precisely identifiable different intrinsic activities of highly specialized neural proteins in the hypothalamus [8]. This neural system regulates basic function triggered other neurons and that evolve a cluster of populous receptors [9], or the coordination of the functional domains or counterparts [10], that affected to the function. They also regulated the body by respond to minor external stimuli such as motion, pH, carbon dioxide, light and temperature. There have been reported with the reinforced physiological effects and behaviors by the drug and the addiction related to dopamine D_1 receptor reward signaling in the nervous system. The successful development of a drug candidate that required the optimization of selective compound affected to the neural system regulating body to external stimuli. Some previously methods provided for further understanding of the specific aspects of the neurotransmitter receptors. Advance knowledge of the refined binding site is crucial for probing functionally modify selective therapeutics and manipulate biased signaling pathways [11,12]. Previously reported work focused on the role of the human immune systems interacting with specific receptors ultimately regulating responses in the neural system that how they are involved to the neural function have been not widely known [13]. Methodologies enable the study of different functional processes involving on the feedback methods within the neural systems and have been applied in the field of neurosciences in either cells or animals [14]. The recently reported involvement of stimuli on the dopaminergic receptor induced the local protein synthesis implicate the neurotransmitter receptors in the process of the neural circuit and neural plasticity in the hippocampus about which is very little was known [15].

Prescription costs for antipsychotic drugs in the United States totaled about $18.2 billion USD in 2011 and has since increased by 13%. Clozapine was chosen for this study because it is an atypical antipsychotic agent that acts as an antagonist for dopamine D_1, D_2, and D_4 receptors and serotonin HT_{2A} and 5-HT_{2B} receptors, and as a potent agonist at serotonin 5-HT_{1A}. It also affects epinephrine, acetylcholine and histamine receptors [16]. Clozapine has been used to treat clinical disorders including schizophrenia, Parkinson's disease, other neuroleptic disorders, attention deficit hyperactivity disorder and different spectrums of autism [17]. This dug had evolved to the extrapyramidal side effects, yet the risk of the mortality for long term use has not yet known [18]. If we can understand fundamental ability of receptor response regulating the function it might be interested in developing precisely manipulate specific molecular interactions that mediate nervous system function.

Molecular imprinting technology have aroused extensive attention and been widely applied in many fields and have drawn much attention in recent years [19,20]. The molecular imprinting technique is a well-known emerging approach allowing targeting of crucial components by its ability to recognize naturally occurring templates [21]. Upon polymerization, a three-dimensional network can be generated with selective recognition sites for a template. These biological analogous systems provided the opportunities to customize a preferred selectivity of the recognition element [22]. A microprobe is a molecule that has the ability to measure the properties of other structures or even to activate them to trigger signaling that causes interactions in many fields. They can be used to study the functional selectivity of targets or diseased cells for use in medical diagnostic and biosensor applications [23]. The monolithic polymer obtained by bulk polymerization has to be debrided, ground and sieved to an appropriate size, a process that is time consuming and would reduce the functional properties of the polymer product. In addition, the grinding operation results in producing irregular particles in shape and size, and some high affinity binding sites are destroyed and changed into low affinity sites. Suspension polymerization, emulsion polymerization, seed polymerization and precipitation polymerization has been developed to overcome these drawbacks and surface imprinted polymers are now widely used. In this work microprobes have been prepared based on molecular imprinting technology that allows the synthesis of MIPs by bulk polymerization [24]. We think that the bulk polymerization method offers a high yield of product and that whole template imprinted within the surrounding polymer matrix.

Nonetheless, the reactive substrate binding domain of a neural protein receptor in the hypothalamus is even more complex. While this question has been approached, the present approach offers a better potential as the molecularly imprinted polymers offer a great potential for probing manipulation by the pharmacological effects of antipsychotic drugs. Clozapine can be used as a fluorescent tag exquisite specificity and affinity for selective receptor. Investigating a drug-related structure where the effects in modulating the neural activity of the specific functional protein responses to stimuli, it is possible that this could allow for the improved survival or a reduced serious side effect of anti-psychotic drugs, this has been carried out by present method using MIPs. Use of a MIP is of interest because of the polymers' enhanced chemical stability, superior to that of their natural counterparts and which possibly enabling use for longer periods of time. Indeed we have recently applied this approach to discover potent dopaminergic receptor for treatment of patient suffering from Parkinson's disease [25]. The initial results suggest that MIPs would be highly useful in studying the receptor subtypes able to bind the coupling substances which enable of combining organizing or synthesizing the information of brain cells. This makes it possible to correlate both the structure and the drug-associated proteins through the modulation in a neural sensing protein ensure the linkage between a molecular subtype of the hypothalamus membrane and the neural activity that enable a fluorescent response, that changed in the presence of thermal dynamic, needed to be clarified [26]. One of the major issues of concern is use of specific probe in neuroscience evolved to a few molecules present. As tunable macromolecular interaction relies on the isolation of proteins, for overcoming the diffusion of coexisting matrixes *in vitro* has to be considered [27]. This is reflected by the alteration of the particle size and mass, density and shape which had the properties of extinction of the different components [28]. Also, a highly complex interaction, neural activity and that some drugs, *e.g.* food, vitamin, drug and water, that imbue external environment with significance and increase the behavioral activate the neurotransmitter activities [29]. Thus it is of great interest to understand how the plastic change can be elicited in response to exogenous Zn^{2+} biomolecule that may provide the specialized properties of the drug-related structure for the thermosensitivity.

In this study, we have evaluated the use of MIP microprobes for studying drug or substrate binding associated with neurological function through temperature-dependent molecular interactions. The relationship between fluorescence decay and phase contrast microscopy was determined and physical characterization was examined by ^{1}H NMR and X-ray microanalysis. With all these methods, the MIPs were compared with the corresponding non-imprinted polymer (NIP). Alterations of protein function can lead