Effect of GABAergic neurotransmission in globus pallidus and its involvement in neurologic disorders

CHEN Lei, YUNG Wing-Ho

Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Abstract: The globus pallidus occupies a critical position in the ‘indirect’ pathway of the basal ganglia and, as such, plays an important role in the modulation of movement. In recent years, the importance of the globus pallidus in the normal and malfunctioned basal ganglia is emerging. However, the function and operation of various transmitter systems in this nucleus are largely unknown. GABA is the major neurotransmitter involved in the globus pallidus. By means of electrophysiological recording, immunohistochemistry and behavioral studies, new information on the distribution and functions of the GABAergic neurotransmission in the rat globus pallidus has been generated. Morphological studies revealed the existence of GABA_A receptor, including its benzodiazepine binding site, and GABA_B receptor in globus pallidus. At subcellular level, GABA_A receptors are located at the postsynaptic sites of symmetric synapses (putative GABAergic synapses). However, GABA_B receptors are located at both pre- and postsynaptic sites of symmetric, as well as asymmetric synapses (putative excitatory synapses). Consistent with the morphological results, functional studies showed that activation of GABA_B receptors in globus pallidus reduces the release of GABA and glutamate by activating presynaptic auto- and heteroreceptors, and hyperpolarizes pallidal neurons by activating postsynaptic receptors. In addition to GABA_B receptor, activation of GABA_A receptor benzodiazepine binding site and blockade of GABA uptake change the activity of globus pallidus by prolonging the duration of GABA current. In agreement with the in vitro effect, activation of GABA_A receptor, GABA_B receptor benzodiazepine binding site and blockade of GABA uptake cause rotation in behaving animal. Furthermore, the GABA system in the globus pallidus is involved in the etiology of Parkinson’s disease and regulation of seizures threshold. It has been demonstrated that the abnormal hypoactivity and synchronized rhythmic discharge of globus pallidus neurons associate with akinesia and resting tremor in parkinsonism. Recent electrophysiological and behavioral studies indicated that the new anti-epileptic drug, tiagabine, is functional in globus pallidus, which may present more information to understand the involvement of globus pallidus in epilepsy.

Key words: globus pallidus; GABA; Parkinson’s disease; seizure

苍白球γ-氨基丁酸能神经传递及其与神经系统疾病的关系

陈蕾, 容永豪

香港中文大学医学院生理系, 香港

摘要: 苍白球是基底神经节间接环路的重要核团, 在机体运动功能调节中发挥重要作用。近年来, 苍白球在基底神经节正常及异常功能调节中的重要性已开始受到重视。然而, 目前对苍白球内各种神经递质系统的功能活动了解较少。GABA 是苍白球主要的神经递质。采用电生理记录、免疫组织化学及行为测试等实验方法, 人们对大鼠苍白球GABA能神经递质系统的分布与功能活动有了新的认识。形态学研究显示, 苍白球存在GABA_a受体及其苯二氮卓结合位点和GABA_b受体。在亚细胞水平, GABA_a受体主要位于对称性递质(GABA能突触)的突触后膜, 而GABA_b受体则位于对称性递质和非对称性递质(兴奋性递质)的突触前膜及突触后膜。功能学研究进一步揭示, 激活苍白球突触前膜GABA_b受体, 安息香酰胺释放, 激活突触后膜GABA_a受体, 可引起苍白球神经元超级化。除GABA_a受体外, 激活苍白球GABA_b受体苯二氮卓结合位点及阻断GABA, 重摄取可延长GABA电流持续时间, 从而改变苍白球神经元兴奋性。与实验结果相一致, 激活苍白球GABA_a受体和苯二氮卓结合位点及阻断GABA重摄取可引起整体动物旋转行为。苍白球GABA能传递系统与帕金森病病因学及癫痫发病有关。已证实, 左右球神经元放电频率的降低及其失电位的产生与帕金森病运动减少
Motor control is one of the best-known functions of the basal ganglia. This fact is revealed by the spectrum of motor disorders originating from the basal ganglia, including Parkinson’s disease, Huntington’s disease and tardive dyskinesia. Within the basal ganglia, the globus pallidus has long been regarded merely as a relay station between the striatum and subthalamic nucleus in the so-called ‘indirect pathway’[1]. However, as the widespread connections between the globus pallidus and other nuclei within and outside the basal ganglia have been revealed, this nucleus is now believed to be a critical and strategically placed component that can integrate the actions of the inhibitory inputs from the striatum and the excitatory inputs from the subthalamic nucleus, neocortex and thalamus[2-4]. In turn, the globus pallidus controls the activity of the whole basal ganglia[5].

By influencing the output of the basal ganglia, the globus pallidus plays a significant role in mediating movement in health and in diseased state. It is well known that the abnormal activity of the globus pallidus is involved in the manifestation of Parkinson’s disease, which includes decreased activity and increased rhythmic burst firing[6]. In addition, the globus pallidus has also been implicated in the control of epileptic seizure[7,8] and drug-induced tardive dyskinesia[9,10]. GABA is the major neurotransmitter used in the globus pallidus. In order to fully understand the functions of the globus pallidus in the basal ganglia, one must have a detailed knowledge of the GABAergic neurotransmission in the globus pallidus. This brief review will first describe the nature of this GABA system, highlighting some of our efforts that combine morphological, electrophysiological and behavioral approaches. Then, the contemporary view of the role of the globus pallidus in some specific neurological disorders and the involvement of the GABA system will be described. In the course of discussion, some interesting but still unanswered questions will be mentioned.

**GABAergic innervation of globus pallidus neurons**

The globus pallidus receives GABAergic innervation mainly from the striatum and local axon collaterals. In the squirrel monkey, the local axon collaterals have been estimated to represent 10% of the terminals in contact with the perikarya of external globus pallidus neurons[11,12]. Previous ultrastructural studies indicated that striatal terminals are located more distally on the dendritic trees, whereas pallidal terminals form a typical perineuronal nets covering the soma and proximal dendrites of adjacent neurons. Consistent with this anatomical observations, electrophysiological studies showed that pallidal stimulation induced inhibitory postsynaptic currents (IPSCs) not only with a shorter latency, but also a faster rise time and a different reversal potential compared with those obtained by striatal stimulation, suggesting that the pallidal inputs were evoked in more proximal regions of the neurons[13]. The effects of GABA are mediated by two receptor subtypes: GABA A and GABA B receptors. While the functions of the GABA A receptors in globus pallidus and other areas have been recognized for a long time, detailed characterization of the GABA B systems was enabled only relatively recently, following the cloning of the GABA B receptor[14]. As a result, much less had been known about the distributions and functions of the GABA B receptors in the globus pallidus.

**GABA A neurotransmission**

GABA A receptors are assembled from various subunits including α1~6, β1~4, γ1~3, δ, ε and ρ1~3, which are differentially expressed throughout the brain[15]. By using subunit-specific antibodies, an extremely diverse expression of GABA A receptor subunits in the globus pallidus has been demonstrated[16-19]. For example, previous studies indicated that there is distinct γ subunit labelling in the globus pallidus, subunits γ1 and γ3 staining were observed on the soma while subunits γ1 and γ2 were found on the dendrites[20,21]. Similarly, in human external globus pallidus, α3 subunit has been shown to be restricted to soma and proximal dendrites in high level, but not distal dendrites[22]. Most GABAergic symmetric synapses in globus pallidus are labeled for α1[23]. Manipulation or change of the GABA A receptor system has been shown to affect motor functions. It has been revealed that intrapallidal injection of bicuculline into the
external globus pallidus in monkeys induced dyskinesia which is induced by hyperactivity of pallidal neurons\textsuperscript{[24,25]}. Microinjection of GABA\textsubscript{A} receptor antagonist, bicuculline, into the globus pallidus had marked antiparkinsonian effects\textsuperscript{[26]}. In MPTP or 6-OHDA-induced parkinsonism, the level of GABA\textsubscript{A} receptor in the globus pallidus was significantly decreased\textsuperscript{[27-29]}. By using the specific \(\alpha_1\) subunit antibody, Caruncho\textsuperscript{[30]} reported that the expression of \(\alpha_1\) subunits was reduced significantly in globus pallidus early after 6-OHDA lesion.

**Benzodiazepine modulation site on GABA\textsubscript{A} receptor**

In addition to GABA binding site, GABA\textsubscript{A} receptors contain many other binding sites that interact with a diverse range of compounds such as benzodiazepines, barbiturates, anesthetics and zinc. The benzodiazepine binding site within the GABA\textsubscript{A} receptor is a modulation site of significant clinical interest\textsuperscript{[31]}. Upon binding to this site, benzodiazepine potentiates the GABA currents, leading to anxiolytic, anticonvulsant and sedative effects\textsuperscript{[32,33]}. Since autoradiographic studies revealed a relatively high binding density for zolpidem in globus pallidus\textsuperscript{[34]}, the electrophysiological effects of zolpidem on globus pallidus neurons has been studied recently, in order to better understand the significance of this modulation site. Patch-clamp recordings from the \textit{in vitro} brain slices showed that zolpidem enhances the action of GABA on postsynaptic GABA\textsubscript{A} receptors by prolonging the half decay time of IPSCs\textsuperscript{[35]}. The effect of zolpidem is sensitive to the benzodiazepine antagonist flumazenil, which had no effect on its own. The \textit{in vitro} effect of zolpidem implies that modulation of the benzodiazepine site \textit{in vivo} would enhance the inhibition on pallidal neurons. In this regard, it has also

---

**Fig. 1.** Double immunolabelling for GABA\textsubscript{B1} or GABA\textsubscript{B2} (immunogold) and PHA-L (immunoperoxidase) in the globus pallidus. *A, B:* Presynaptic (large arrow) and postsynaptic (small arrows) GABA\textsubscript{B1} immunogold particles at symmetric synapses formed by striatal boutons (b) anterogradely labelled with PHA-L. *C, D:* Presynaptic (large arrow) and postsynaptic (small arrows) GABA\textsubscript{B2} immunogold particles at symmetric synapses formed by PHA-L labelled striatal boutons (b). PHA-L labelled axons (ax) were visible in *B, C* and *D*. b, bouton; d, dendrite. Scale bars, 0.25 \(\mu\)m.
been shown that microinjection of zolpidem into the globus pallidus resulted in ipsilateral rotation in the behaving animals[35], consistent with inhibitory action on pallidal neurons.

Subcellular localization of pre- and postsynaptic GABA<sub>B</sub> receptors

GABA<sub>B</sub> receptors belong to G-protein coupled receptors and are divided functionally into pre- and postsynaptic receptors. There is abundant evidence from autoradiographic studies that GABA<sub>B</sub> receptors are expressed in the globus pallidus[36,37]. More recent studies by in situ hybridization and immunocytochemistry revealed the regional and cellular distribution of GABA<sub>B</sub> receptor subunits and their splice variants in the globus pallidus[38-41]. Recently, by means of pre-embedding immunogold labeling, a detailed description of the subcellular localization of both GABA<sub>B1</sub> and GABA<sub>B2</sub> receptor subunits in rat globus pallidus[42] has been achieved. At symmetric synapses, including those formed by anterogradely-labelled striatopallidal terminals, most GABA<sub>B1</sub> and GABA<sub>B2</sub> immunogold labelling was found in the main body of pre- and postsynaptic sites (Fig.1). However, at asymmetric synapses, mainly formed by vesicular glutamate transporter 2 (VGLUT2)-positive terminals, most GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits were found at the edges of both pre- and postsynaptic sites (Fig.2). These results demonstrate the existence of presynaptic

Fig. 2. Double immunolabelling for GABA<sub>B1</sub> or GABA<sub>B2</sub> (immunogold) and VGLUT2 (immunoperoxidase) in the globus pallidus. A, B: Presynaptic (large arrow) and postsynaptic (small arrow) GABA<sub>B1</sub> immunogold particles at asymmetric synapses formed by VGLUT2-labelled boutons (b). In B, a gold particle was located within the bouton (arrowhead). Note a presynaptic gold particle (large arrow) in the main body of a symmetric synapse formed by an unlabelled bouton (*). C, D: Presynaptic (large arrows) and postsynaptic (small arrow) GABA<sub>B2</sub> immunolabelling at asymmetric synapses formed by VGLUT2-labelled boutons (b). In C, the postsynaptic density was not prominent in the plane of this section, but subjunctional dense bodies were visible. Note an unlabelled bouton (*) formed symmetric synapse with the same dendrite (d). b, bouton; d, dendrite. Scale bars, 0.25 μm.
GABA<sub>B</sub> auto- and hetero- and postsynaptic GABA<sub>B</sub> receptors. This distribution pattern for GABA<sub>B</sub> receptors is markedly different from that observed for GABA<sub>A</sub> receptors in the globus pallidus, in which most GABA<sub>A</sub> receptors are located at symmetric synapses and rarely, if ever, occur in the presynaptic site.<sup>22,23</sup> Functions of pre- and postsynaptic GABA<sub>B</sub> receptors Consistent with the morphological observations, patch-clamp recordings revealed that the presynaptic GABA<sub>B</sub> auto-, hetero- and postsynaptic GABA<sub>B</sub> receptors are functional in globus pallidus. Thus, activation of presynaptic GABA<sub>B</sub> receptors inhibits the release of GABA as well as glutamate, while activation of postsynaptic GABA<sub>B</sub> receptors hyperpolarizes the pallidal neurons<sup>43,44</sup>. All these effects are sensitive to the potent and specific GABA<sub>B</sub> receptor antagonist CGP55845. Furthermore, activation of pallidal GABA<sub>B</sub> receptors by unilateral microinjection of the agonist baclofen induced ipsilateral turning in awake animals<sup>44</sup>. These results suggest that GABA<sub>B</sub> receptor in the globus pallidus plays an important role in the regulation of movement.

The morphological observation that some postsynaptic GABA<sub>B</sub> receptors were found at perisynaptic site of the glutamatergic synapses is intriguing. This observation raises the possibility that, in addition to activating pre- and postsynaptic GABA<sub>B</sub> receptors at GABAergic synapses, GABA released from GABA terminals may spill out to activate GABA<sub>B</sub> receptors at the glutamatergic synapses. Whether GABA does modulate glutamate transmission on the postsynaptic site, the mechanism involved and the significance of this process, are questions worth further pursuing.

Globus pallidus and neurological disorders Parkinson’s disease Parkinson’s disease is an age-related neurodegenerative disorder, characterized by resting tremor, rigidity and bradykinesia. Abnormal activity of the globus pallidus has been demonstrated to be involved in the manifestation of parkinsonian motor symptoms. In Parkinson’s disease and its animal models, it is widely believed that depletion of dopamine in basal ganglia leads to overactivity of the striatopallidal pathway. This results in the abnormal hypoactivity of the globus pallidal neurons, and then the decreased GABAergic output of the globus pallidus contributes to excessive inhibition of basal ganglia targets, leading to akinesia and hypokinetic symptoms of Parkinson’s disease<sup>46,47</sup>. Furthermore, in the absence of normal dopaminergic innervation, there is increased synchronized rhythmic discharge and burst firing in the globus pallidus, which may underlie resting tremor in parkinsonism<sup>46,47</sup>. Similar firing pattern in the globus pallidus neurons has also been reported in human suffering form Parkinson’s disease<sup>48</sup>. Recent studies on the firing properties of neurons from organotypic culture of the globus pallidus-subthalamic nucleus network showed that the excitatory subthalamic nucleus and the inhibitory globus pallidus spontaneously produce synchronized oscillating bursts, and pallidal lesion abolishes this bursting<sup>49</sup>. However, from the in vivo brain, Magill et al.<sup>50,51</sup> reported that the rhythmic oscillatory activity in the subthalamic nucleus and the globus pallidus network in Parkinson’s disease states might be driven by the cortex. More recently, Stanford<sup>52</sup> demonstrated that the bursting firing appears to arise due to the presence of intrinsic voltage- and sodium-dependent subthreshold membrane oscillations. Taken together, the intrinsic properties of the globus pallidus neurons and/or the extrinsic cortical inputs are important in the generation of these rhythmic firings. In addition, the firing variability of the globus pallidus neurons has been found to be associated with the severity of Parkinson’s disease<sup>53</sup>, together with a significant relationship between the neuronal activity and tremor as well as dyskinesia<sup>47,48</sup>. All these findings suggest that the modification of the firing patterns of the globus pallidus neurons constitutes the central origin of parkinsonian symptoms.

Recently, a therapeutic effect of the zolpidem on some groups of Parkinson’s patients has been reported<sup>54,55</sup>. Quantitative autoradiography revealed that the binding for zolpidem is reduced significantly following lesions of the nigrastrital tract<sup>56</sup>. Taken together these observations and the electrophysiological data described above, it is likely that the reduction of zolpidem binding in globus pallidus may reflect a compensatory mechanism for Parkinson’s disease. The beneficial effect of zolpidem administration in some groups of Parkinson’s patient may therefore derives from the interaction of its effects on various basal ganglia nuclei including the internal globus pallidus/entopeduncular nucleus and substantia nigra. Thus, more information derived from experiments is needed before one can fully understand the in vivo effects of zolpidem. Anyhow, this study suggests that the benzodiazepine binding site in globus pallidus is a possible drug target for the management of basal ganglia motor symptoms.

If GABA neurotransmission is important for the function of the basal ganglia, selective modulation of the GABA pathways by neuromodulator is expected to alter motor
function under normal or pathological conditions. 5-HT is a good example. It has been shown by Chadha et al.\(^{37}\) that administration of 5HT\(_{1A}\) agonist inhibits [\(^3\)H]-GABA release from rat globus pallidus and reverses akinesia following intrapallidal injection in reserpine-treated rat. This finding is consistent with the concept that disinhibition or excitation of the pallidal neurons would lead to decreased inhibitory output from the basal ganglia to target areas, and in line with our own observation that activation of presynaptic 5HT\(_{1A}\) receptors on striato-pallidal nerve terminals leads to decreased frequency of miniature IPSCs (unpublished observation).

**Epileptic seizures**

The basal ganglia are considered to be involved in the genesis and/or spread of epileptic activity. It has been demonstrated that the neocortical epileptiform activity is modulated by the basal ganglia.\(^{58-60}\) Among the nuclei in basal ganglia, the substantia nigra pars reticulata has been shown to be involved in epilepsy control in different animal models of epilepsy through its GABAergic projections.\(^{61,62}\). In the case of globus pallidus, early studies reported that the globus pallidus lesions prevented the generalized convulsions induced by cerebral cortex application of nicotine.\(^{63}\). Electrical stimulation of the globus pallidus enhanced the neocortex interictal seizure activity, proceeding to generalized seizure activity.\(^{60,64}\). Recently, Sawamura\(^{65}\) reported that kainic acid injection into the globus pallidus induced transient epileptogenesis, presumably due to the transient enhancement of the globus pallidus-substantia nigra circuit or epileptic excitation of the cortex.

A link between epileptic seizures and GABA neurotransmission in the globus pallidus is suggested by the following findings. First, the globus pallidus displays a very high density of binding site for tiagabine,\(^{66}\), a selective blocker of the GAT-1 GABA transporter,\(^{67}\) and a drug used clinically to treat epilepsy. Second, systemic administration of tiagabine significantly prolonged the decay kinetics of the GABA\(_{A}\) receptor-mediated IPSCS. The latter effect was reversed by the GABA\(_{A}\) receptor antagonist CGP55845, indicating the involvement of presynaptic GABA\(_{A}\) receptors.\(^{69}\). These data suggest that overspill of GABA, for instance, under intense presynaptic activity, could activate the presynaptic GABA\(_{B}\) receptors on the terminals to maintain the excitability of the pallidal neurons. At the same time, there is prolonged inhibition on postsynaptic GABA\(_{A}\) receptors. Behavioral studies showed that intrapallidal microinjection of tiagabine caused ipsilateral rotation, arguing that prolonged action of GABA on GABA receptors would dominate over its inhibitory effect on GABA release.\(^{69}\). Second, intrapallidal administration of tiagabine could inhibit significantly the occurrence of pentylenetetrazol (PTZ)-induced tonic seizure significantly.\(^{65}\). The additional finding that baclofen microinjection into the globus pallidus completely suppresses PTZ-induced tonic seizure suggests that GABA\(_{B}\) receptors play a significant role in modulating the threshold of seizure activity.\(^{70}\).

**Concluding remarks**

The importance of the globus pallidus in the basal ganglia circuit is emerging in recent years. By combining morphological, electrophysiological and behavioral studies, our laboratory has contributed some novel information on GABA neurotransmission in globus pallidus and its involvement in neurological disorders. However, the functioning of the globus pallidus also depends on other neurotransmitter/neuromodulator systems including, notably, glutamate, dopamine, enkephalins, neurotensin and 5HT. The functions and interplay between this rich repertoire of neuroactive compounds must be elucidated in detail before one could better understand the role of the globus pallidus.

**REFERENCES**


34 Duncan GE, Breese GR, Criswell HE, Mccown TJ, Herbert JS, Devaud L, Morrow AL. Distribution of \(^{3}H\) \(z\)-zolpidem binding sites in relation to messenger RNA encoding the \(\alpha_1, \beta_2\) and \(\gamma_2\) subunits of GABA\(_A\) receptors in rat brain. Neuroscience 1995;64: 1113-1128.


44 Chen L, Chan SCY, Yung WH. Rotational behavior and electrophysiological effects induced by GABA\(_A\) receptor activation in rat globus pallidus. Neuroscience 2002; 114:417-425.


49 Plenz D, Kitai ST. A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. Nature 1999; 400:677-682.


33-52.


