Review Article

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Involvement of nitric oxide in learning & memory processes

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Nitric oxide (NO), synthesized from the amino acid, L-arginine by nitric oxide synthase (NOS) has received attention as a neurotransmitter in the brain. NO has been found to induce cognitive behaviour in experimental animals. In order to show evidence for the involvement of NO in learning and memory processes, the reports indicating the effects of its precursor, donors, and inhibitors of its synthesis in mammals, birds, fishes and invertebrates have been reviewed. Further, learning and memory impairment occurring in man and animals due to defective NO activity in the brain due to pathological conditions such as epilepsy, stress, diabetes and side effects of therapeutic agents and reversal of this condition by L-arginine and NO donors have been included. In addition, the reports that indicate ageing-induced impairment of cognition that is known to occur in Alzheimer's disease due to deposition of the toxic protein, beta amyloid and the effect of L-arginine and NO donors in preventing dementia in these patients have been reviewed.

Key words L-arginine - learning and memory processes - nitric oxide (NO) - NOS

Introduction

It has been known for many years that NO which is a gas made up of two most common gases in the atmosphere, occurs in the biological system. NO crosses cell membranes freely and plays a role as a neurotransmitter in the brain. The function of NO in the hypothalamus has largely been implicated in learning process and in memory formation[1]. In order to establish evidence for the involvement of NO in learning and memory processes, the experimental findings that demonstrated synthesis of NO and the neuronal action of NO at the time when experimental animals were trained to learn and then to remember a specific task, were reviewed in this article. In addition, the cognitive effects of agents that increase or decrease NO concentration in the hypo-thalamus were also included. Further, the reports indicating the beneficial effects of NO elevating agents in alleviating cognitive disorder caused by pathological conditions, by the deposition of endogenous substance like beta amyloid in the brain of patients with Alzheimer's disease (AD) and the toxicities of therapeutic agents were also included.

NO as a neurotransmitter and its involvement in learning and memory processes

Studies in experimental animals have well documented the synthesis of NO in the brain, and its

role in a variety of neuronal functions including learning and memory processes, cortical arousal, nociception, food intake, penile erection, yawning, blood vessel dilatation and immune response[1]. NO is synthesized in the brain upon demand as in cognitive condition for which NO activity is required. Neurons synthesize NO as a response to the activation of N-methyl-D-aspartate (NMDA) receptors by the excitatory amino acid glutamate. NO is generated in the neuronal cells as a co-product of the conversion of the semi-essential amino acid L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS) with calcium and calmodulin as cofactors. Three distinct NOS have been identified in the hippocampus, cortex, cerebellum, corpus striatum and medulla of rat brain. NOS from endothelial cells (eNOS) and neurons (nNOS) are constitutively expressed and the action of these enzymes are stimulated by an increase in intracellular calcium. NO produced by these enzymes act as a neuronal messenger. NO synthesized by calcium-independent induction NOS (iNOS) mediates immune function[1].

Although it plays an important role in cell signaling in the brain, NO has been described as an unconventional neurotransmitter, because it is not stored in synaptic vesicles and not released upon membrane depolarization but released as soon it is synthesized. NO does not mediate its action by binding to membrane associated receptors but diffuses from one neuron to another and acts directly on intracellular components. NO function as a neurotransmitter by stimulating soluble guanylyl cyclase to form the second messenger molecule, cyclic guanosine monophosphate (cGMP) in the target cells[1]. Studies on various forms of synaptic plasticity in the brain have provided insight into the cellular and molecular mechanisms for learning and memory processes. Long-term potentiation (LTP), a homosynaptic plasticity[2] and long-term depression (LTD), a heterosynaptic plasticity[3] are two major forms of activity dependent synaptic plasticity in the brain. NO-cGMP pathway has been implicated in the induction of hippocampal LTP and LTD which are known to be the predominant mechanisms of learning and memory processes. LTP in the hippocampus is the primary experimental model for investigating the synaptic basis of learning and memory in vertebrates[2]. Expression of LTD-like synaptic plasticity in the hippocampus has been suggested to underlie certain forms of motor learning and visual recognition memory[4]. NO acts as a retrograde messenger for the induction of LTP and LTD in the hippocampus[3].

NO formed in the hippocampus has been suggested to have a role in learning and memory processes because the activity of NMDA receptor which is an initiator of the reaction that produces NO from L-arginine, has been activated in this region of the brain at the time of consolidation of the acquired avoidance task in chicks[5] and in rats[6]. For further study, the rates of synthesis of NO and induction of LTP and LTD have been determined in the hippocampus of experimental animals that have been trained to learn and then to consolidate the acquired maze traversing, exploring, avoidance and object recognizing tasks. Since neuronal NO has a very short half-life (5 sec), the level of its metabolite, nitrite or the activity of NOS has been measured in the hippocampus of animals during learning process. Learning of spatial task by rats was found to be accompanied by an elevation of nitrite in the hippocampus[6]. The activity of NOS was found to be increased by 45 per cent in the hippocampus immediately after acquisition of an avoidance task in rats[7]. Further, spatial memory was accompanied by an increase in the activity of NOS in the hippocampus of rats[8]. These findings and an increased expression of nNOS in the hippocampus during learning of odour in sheep[9] and in mice[10] provide evidence for a correlation between learning process and an activation of nNOS in the hippocampus. In addition, an increased formation of NO at the time when rats were learning foot-shock avoidance task was accompanied by an induction of LTP and LTD in the hippocampus[7].

Alteration in learning and memory processes by agents that increase/decrease NO concentration in the brain

In order to demonstrate adequate evidence for the involvement of NO in learning and memory processes, the effects of the agents that are known to increase/decrease NO concentration in the brain regions have been tested in experimental animals. L-arginine, the precursor of NO[11], and the donors ofNO such as sodium nitro-prusside(SNP)[12], S-nitroso-N-acetylpenicillamine (SNAP)[13] and molsidomine[14] are known to increase the concentration of NO in the brain regions of rats. The antagonists of NMDA receptors, dizocipline (MK-801) and AP5 have been found to inhibit NO synthesis in the brain[15]. The synthetic analogues of L-arginine that are known to decrease NO concentration by inhibiting nNOS and eNOS are N-nitro-L-arginine methyl ester (L-NAME) and N-nitro-L-arginine (L-NA), N-monomethyl-L-arginine (L-NMMA)[1], and the nitro-indazole compound, 7-nitroindazole (7-NI)[16] are

known to inhibit NO synthesis in the brain. The results of the studies carried out with these compounds on the cognitive behaviour of different species of animals have been shown in the Table.

These findings provide strong support to the concept that NO plays a vital role in both learning process and memory of the learnt task.

Since NO is known to relax blood vessels and to increase blood supply to the brain[1], this action of NO can also be assessed to have a role in inducing neuronal activity. In this context, a decrease in NO synthesis following an inhibition of NOS activity is likely to result in vasoconstriction and a decrease in perfusion into the brain. This effect of NOS inhibitors can be proposed for an impairment of learning and memory processes in animals treated with these compounds. Interestingly, 7-NI which failed to affect cerebral blood flow because of its inhibitory action on neuronal specific NOS[16], impaired learning and memory processes in rats[28], chicks[31] and in fishes[9]. These findings clearly show that not an inhibition of cerebral blood flow but an impairment of neuronal action of NO is responsible for the cognitive deficits produced by the inhibitors of NO synthesis.

The physiological effects of NO predominate when NO is produced in sufficient concentration. Interestingly, a several fold increase by L-arginine of NO concentration in the brain has resulted in an impairment of retention of acquired task in rats[32]. Larger doses of SNP[7] and molsidomine[14] also impaired avoidance and maze learning tasks, respectively in rats. Excess formation of NO has been suggested for these effects of NO donors, because an increased synthesis of NO has been found to produce neurotoxicity due to accumulation of its toxic metabolite, peroxynitrite[1]. In support of this finding, the memory impairing action of higher doses of L-arginine was prevented by L-NAME and 7-NI[32].

Table. Effect of agents that increase/decrease NO concentration in the brain

Drug	Animal	Experiment	Result	Reference
1. Precursor of NO				
L-arginine	Rats	Learning and memory of avoidance task	Facilitated	11, 12
2. NO donors				
SNP	Rats	Learning and memory of avoidance task	Facilitated	12
	Chicks	Learning and memory of avoidance task	Facilitated	5
	Rats	Ageing-induced memory impairment	Prevented	12
SNAP	Rats	Memory of avoidance task	Facilitated	7
Molsidomine	Rats	Learning of maze performance and object recognition	Facilitated	14
3. Inhibitors of NO synthesis				
MK-801, AP5	Gold fish	Learning of avoidance task	Impaired	15
MK-801	Mice	Learning of avoidance task	Impaired	17
L-NAME	Rats	Learning and memory of maze performance	Impaired	18, 19
	Rabbit	Eye-blink conditioning	Impaired	18
	Chicks	Learning of avoidance task	Impaired	20
	Rats	Learning and memory of avoidance task	Impaired	21
	Rats	Learning of avoidance task	Impaired	34
	Octopus vulgaris	Learning of avoidance task	Impaired	22
	Aplysia	Learning of avoidance task	Impaired	23
	Honey bee	Learning of olfactory discrimination	Impaired	24
L-NA	Rats	Learning and memory of avoidance task	Impaired	25
	Rats	Learning and memory of maze performance	Impaired	26
	Rats	Learning and memory of maze performance	Impaired	29
L-NMMA	Rats	Memory of avoidance task	Impaired	16, 27
7-NI	Rats	Learning and memory of avoidance task	Impaired	28
	Rats	Learning and memory of maze performance	Impaired	29
	Chicks	Learning of avoidance task	Impaired	31
4. Polychlorinated biphenyls	Rats	Memory of avoidance task	Impaired	30

The effects of L-arginine and NO donors have been tested against learning and memory impairment caused by the inhibitors of NO synthesis. In this study, the memory impairing action of MK-801 was prevented by both L-arginine and SNP in mice[17]. L-arginine reverted learning and memory impairment produced by L-NA[22,25] and 7-NI[28]. Further, molsidomine prevented L-NA from impairing acquisition and retention of avoidance task in rats[14]. SNAP prevented L-NAME from impairing long-term memory in aplysia[23]. The effects of NOS inhibitors have also been tested on the cognitive action of L-arginine. L-NAME prevented L-arginine from facilitating learning and memory processes of avoidance task in rats[21]. Moreover, L-arginine-induced facilitation of spatial learning was prevented by L-NAME[33]. All these experimental findings confirm that the mechanism mediated by NO is involved in learning and memory processes.

Association between cholinergic neurons and NO in learning and memory processes

NO mediated mechanisms have been assigned a role in cortical perfusion and cognitive function. Cholinergic transmission has also been associated with cerebral blood flow and performance in learning and memory tasks suggesting a link between cholinergic and NO-mediated mechanisms[34]. In view of this finding an interaction is likely to occur between cholinergic and NO activities in learning process and memory formation. Inhibition of NO synthesis has been shown to cause a decrease in acetylcholine (ACh) release and an impairment of retention of conditioned response in rats[35]. Further, antagonism of nicotinic receptors and NOS activity has been found to impair formation of tactile associative long-term memory in honey bees[36]. Further, activation of muscarinic acetylcholine receptors has been found to induce NO-dependent LTP in rat medial prefrontal cortex[37]. The combined action of the inhibitors of NO and Ach have also been tested, and it was observed that the combined action of L-NAME and scopolamine, the muscarinic receptor antagonist resulted in an impairment of maze learning in rats[38]. Further, NO donor molsidomine has antagonized scopolamine and L-NAME-induced memory impairment in rats[39]. These findings clearly indicate that cholinergic activity has an involvement in cognitive effect of NO. However, NO formed as a result of iNOS upregulation during hypoxia has been found to interrupt memory process by inhibiting acetylcholinergic activity[40].

Involvement of other neurotransmitters in the action of NO in learning and memory processes

The interaction of NO with dopamine, noradrenaline (NA), gamma aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT) has also been investigated. Dopamine and its agonist have enhanced cognitive behaviour and have prevented L-NAME from impairing learning and memory process in rats[41]. Alpha adrenergic antagonist, phentolamine and 6-hydroxydopamine, a depletory of NA, prevented SNP from promoting memory formation in mice[42]. Blockade of GABA activity has resulted in an impairment of olfactory discrimination in honey bees[24]. Memory impairment by L-NAME has been found to be accompanied by a decrease in the conversion of 5-HT to its metabolite, 5-hydroxyindole acetic acid in the hippocampus of rats[43].

Endogenous substances and pathophysiological factors that alter the action of NO on learning and memory processes

The endogenously occurring analogues of L-arginine such as methyl-L-arginine,dimethyl-L-arginine and agmatine which are normally present in the nervous tissue, have been found to inactivate both nNOS and eNOS and to decrease NO production in the brain[1]. The levels of these analogues are increased in pathological conditions like chronic renal failure and essential hypertension resulting in a decrease in the production of NO and cGMP in the brain regions of rats[44]. However, evidence for the role of these endogenous substances on cognitive behaviour of experimental animals is yet to be investigated.

Melanin concentrating hormone has been found to increase NOS activity and the levels of NO and cGMP in the hippocampus to prevent L-NAME from producing amnesia in rats[45]. The neurosteroids, such as pregnonolone and dehydrepiandrosterone have been found to improve avoidance task and maze performance and to inhibit MK-801 and ageing-induced memory impairment in rats by increasing NO synthesis in the brain[46].

Diabetes has been found to decrease synthesis of NO, induction of LTP and synaptic plasticity in the hippocampus of rats[47], suggesting that insulin deficiency and occurrence of blood sugar greater than normal level can result in an inhibition of NO synthesis and an impairment of cognitive behaviour. Administration of insulin results in an induction of NO synthesis in

the hippocampus and improvement of learning and memory processes in rats[48]. In this study, L-NAME prevented insulin-induced memory improvement suggesting further that NO has a role in the cognitive action of insulin.

The production of NO by nNOS was found to be diminished in the hippocampus of stress-induced rats. This was accompanied by a deficit in learning and memory processes in these animals[49]. Chronic brain hypoperfusion decreased NOS activity, NO synthesis and impaired memory formation in rats[50]. Hypoxia-ischaemia was found to decrease the activity of nNOS in the hippocampus and to delay acquisition of avoidance task in rats[51].

Memory impairment has been found to occur in patients with epilepsy[52]. Epidemiological studies have shown that several children with epilepsy have learning difficulties and memory impairment soon after recovery from seizures[53]. Convulsion disorder induced experimentally by picrotoxin (PCT) has been found to impair the ability of rats to learn and to remember shock-avoidance task[28]. Since, sustained clonic convulsions are known to produce hypotension and ischaemia resulting in neuronal death[54], this abnormality seems to be responsible for an impairment of learning and memory processes in these animals. However, there is evidence for a decreased synthesis of NO in the hypothala-mus of rats that convulsed after administration of PCT[10]. In this study, PCT-induced convulsions was accompanied by memory impairment and a decrease in the concentration of NO in the brain, and NO increasing dose of L-arginine restored NO concentration in the brain and reverted the memory impairment in these animals.

Ageing on NO synthesis and learning and memory processes

Comparative study carried out in young adult (3-4 month old) and aged (13-17 month old) rats have shown that NO production is decreased with ageing because serum L-arginine level and urinary excretion of nitrite and nitrate have been found to be decreased (30-50%) in aged rats in comparison to that of young rats[55]. Behavioral study also showed that aged rats required significantly more trials than young ones to learn maze task[56]. In this study, the function of glutamate-NO-cGMP pathway was lesser in older animals than that was measured in young animals suggesting that a decrease in NO activity was responsible for impairment of learning process in older animals. Further, the activity of cGMP hydrolyzing enzyme, phosphodiesterase (PDE) was found to be greater in the brain of aged (24 month old)in comparison to that was measured in young (3 month old) rats[57]. This factor seems to be responsible for a decreased activity of both cGMP and NOS and a lesser synthesis of NO in the brain of aged rats. Interestingly, sildenafil, an inhibitor of PDE, has been found to enhance memory for mation and to prevent L-NAME from impairing foot-shock avoidance and maze learning tasks in rats[58]. This finding has led these investigators to suggest that sildenafil may prevent ageing-induced cognitive decline by modulating NO-cGMP pathway.

AD is known to be associated with progressive neuro-degeneration, resulting in disturbance of learning, memory, thought, orientation, judgement and eventually dementia. Neuronal damage that occurs in AD has been found to result in an impairment of NO synthesis and a decrease in NO containing neurons in the hippocampus[59]. An inhibition of NO synthesis has been found in this study, to impair vasodilatation resulting in a decrease in blood flow and a reduction in the availability of glucose and other nutrients which are necessary for the continued neuronal activity in the brain. Further, deposition of the neurotoxic proteins such as microglia, apolipoprotein[60], and beta amyloid plaque[61] in the brain tissue has been found to be characteristic of AD. Although, accumulation of microglia and apolipoprotein seems to have an involvement in dementia associated with AD, these agents are unlikely to impair cognition in these patients by inhibiting the activity of NO in the brain[60]. On the other hand, ageing-induced deposition of a toxic protein beta amyloid, has been proposed to destroy brain cells[61], and to inhibit NO synthesis, and induction of NMDA receptor-dependent LTP[62] and to disrupt synapses of the nerve cells which are responsible for learning process and memory formation[63]. The protein fragment, beta amyloid that occurs in the brain tissue of individuals with AD tends to accumulate into clumps in the brain[61] and to impair LTP and synapses of the nerve cells that are responsible for information processing and memory formation by disrupting the activity of NO in the brain[64]. Thus damage to LTP by beta amyloid results in synaptic dysfunction, neuronal injury and inhibition of long-term memory in these patients. Since beta amyloid disrupts synaptic function, deposition of amyloid plaques has been considered as a key player in the development and progress of AD. In view of this finding, agents that increase NO concentration in the

brain are likely to prevent impairment of cognition in AD patients. In support of this suggestion, L-arginine, the precursor of NO[59] and NO donors[65] have been found to produce therapeutic effects in patients with age-related degenerative disease such as AD.

Involvement of NO in learning and memory impairment caused by side effects of therapeutic agents

The antiepileptic drugs such as phenobarbitone, phenytoin and carbamazepine are known to produce cognitive deterioration as a side effect[66]. The anticonvulsant dose of phenobarbitone impaired retention of acquired pole-climbing shock-avoidance task in rats[67]. In this study, NO increasing dose of L-arginine prevented the memory impairing action of the anticonvulsant. This finding together with the anticonvulsant effect of L-arginine and SNP against PCT-induced convulsions in rats[68] has led these investigators to suggest that administration of L-arginine or SNP along with phenobarbitone is likely to result in a potent antiepileptic effect as well as prevention of cognitive deficit produced by both epilepsy and the anticonvulsant.

Another therapeutic agent, morphine has been found to impair learning and memory of avoidance task in mice by decreasing NO synthesis in the brain[69]. In this study, L-arginine prevented morphine from producing cognitive deficit.

Conclusion

The experimental findings reviewed provide sufficient evidence that NO activates the computational ability of the brain. These findings provide sufficient support to the report that L-arginine, the precursor[59] and donor[65] of NO may play a prominent role in the treatment of age-related degenerative disease such as AD. L-arginine and NO donors may also be effective in preventing cognitive disorder produced by epilepsy, antiepileptic drugs, and diabetes. For further investigation in the mechanism of action of NO[70], its interaction with enzymes, ion channels and receptors may have to be investigated, to explore new prospective on the mechanism of its cognitive action in the brain.

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