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Evidence for the involvement of neuronal nitric oxide synthase and soluble guanylate cyclase on cognitive functions in rats

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ABSTRACT

Aims: The influence of 3-bromo-7-nitroindazole (3-Br 7-NI), a potent and selective neuronal nitric oxide synthase (nNOS) inhibitor, and [1H-[1,2,4]-oxadiazole[4,3a]-quinoxaline-1-one] (ODQ), a highly selective, irreversible inhibitor of soluble guanylate cyclase (sGC), on working and reference memory and emotional learning was investigated in rats.
Main methods: The effects were assessed in the three-panel runway and step-down passive avoidance task, respectively.
Key findings: 3-Br 7-NI (5, 10, and 20 mg/kg) and ODQ (5, 10, and 20 mg/kg) significantly increased the number of errors and latency of both working and reference memory performance of rats and impaired retention for the passive avoidance task. The effect of 3-Br 7-NI was reversed by L-arginine (250 mg/kg).
Significance: Findings of the study supported the hypothesis that nNOS inhibition disrupts reference and working memory processes in terms of an impairment in the strategies used for solving learning tasks, and, according to these results, nNOS-sGC may be required for emotional learning and both reference and working memory.

Introduction

Learning and memory are the most unique mental processes studied in neuroscience. A variety of pharmacological experiments have been performed to understand the underlying mechanisms. The use of nitric oxide synthase (NOS) inhibitors in many different kinds of learning paradigms has shown that nitric oxide (NO) acts as a retrograde messenger in regulating the memory process (Medina and Izquierdo, 1995). NO is an intercellular messenger in the central nervous system and is formed on demand through the conversion of L-arginine to L-citrulline via the enzyme NOS. There are three isoforms of NOS: the brain or neuronal form (nNOS), the endothelial form (eNOS) and the inducible form (iNOS). As it is known, both nNOS and eNOS can be expressed in neurons (Prast and Philippu, 2001). Studies using 7-nitroindazole (7-NI), a neuronal and eNOS inhibitor (Meyer et al., 1998), and knockout mice (Prast and Philippu, 2001) have suggested that eNOS may play an important role in regulating learning and memory processes and nNOS. 3-Bromo-7-nitroindazole (3-Br 7-NI) is relatively more selective inhibitor of nNOS than the other two NOS isoforms and is clearly a more appropriate tool to describe the role of nNOS in the central nervous system (Bland-Ward and Moore, 1995). Therefore, in the current study, we have investigated the effect of a relatively specific nNOS inhibitor 3-Br 7-NI on emotional learning and both reference and working memory processes.

It is well known that soluble guanylate cyclase (sGC), a heterodimeric enzyme that converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP), is a critical component of NO-cGMP signaling pathway. cGMP is a second messenger nucleotide that has been strongly implicated in the process of learning and memory (Prickaerts et al., 2005). There are studies about modulation of synaptic function by cGMP, which shows that it might be using multiple mechanisms to modulate synaptic efficacy and its actions, including regulation of synaptic plasticity (Barnstable et al., 2004). There is also evidence for sGC activation in memory formation in a number of studies (Chien et al., 2005, 2008; Zhuo et al., 1994). Taken together, as a next step, we investigated the effect of 3-Br 7-NI, a potent and relatively selective nNOS inhibitor, and [1H-[1,2,4]-oxadiazole[4,3a]-quinoxaline-1-one] (ODQ), a highly selective, irreversible inhibitor of soluble guanylate cyclase (sGC), on working and reference memory and emotional learning.

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Materials and methods

Animals

Adult male Wistar rats (Kocaeli University, Experimental Medical Research and Application Center, Turkey) weighing 200–300 g were kept in an animal colony at a density of approximately 5–6 per cage for 2 weeks before the experiments. The experiments were conducted between 9:00 a.m. and 12:00 p.m. under standard laboratory conditions, which were maintained (22 ± 2 °C room temperature; 12-h light/dark cycle with lights on at 7:00 p.m.). Tap water and food pellets were provided ad libitum. All animals used in this study were naive to the experimental tests. Different rat groups were used in each experiment.

The experiments reported in this study were conducted in accordance with the Regulation of Animal Research Ethics Committee in Turkey (6 July 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey, Project number: HAEK 24).

Drugs

3-Br 7-NI, ODQ and L-arginine HCl were purchased from Sigma-Aldrich (USA). ODQ and 3-Br-7-NI were dissolved in dimethyl sulfoxide (DMSO), whereas L-arginine was dissolved in saline. Behavioral tests were performed 30 min after 3-Br-7-NI (5, 10 and 20 mg/kg) treatment and 20 min after ODQ (5, 10, and 20 mg/kg) treatment. In a separate experiment, L-arginine (250 mg/kg) was injected 20 min prior to NOS inhibitors. All drugs were prepared immediately prior to use and given intraperitoneally (i.p.) in a volume of 0.1 ml per 100 g body weight of rats. Doses of drugs were selected according to behavioral and neurochemical studies to show that the drugs have the intended effects (Fidecka, 2003; Heiberg et al., 2002) and to confirm the selected doses on locomotor activities; all results were measured.

Apparatus and procedures

Passive avoidance learning

In this type of avoidance learning test, the animals were refraining from making the measured response. A step-down variant passive avoidance apparatus was used (Ugo Basile model 7551, Italy). The apparatus (measuring 22 × 21 × 22 cm) consisted of two compartments: a light and dark compartment separated by a guillotine door. On day one (training trial), the rats were placed individually into the light compartment and allowed to explore the boxes to become aware of the environment.

1. Pre-acquisition trial: After 30 s, the door between the two boxes was opened, and the animal moved into the dark compartment freely.
2. The acquisition (training) trial was conducted 15 min after the pre-acquisition trial. Rats were placed in the light compartment, and after a 30 s adaptation period, the door between the compartments was opened. Having completely entered the dark compartment, the door was automatically closed, and an electric foot-shock (0.5 mA) of 3 s duration was delivered to the animal via the grid floor. The time taken to reenter the dark compartment was recorded (training latency). Any animal failing to cross from the light to the dark compartment within 300 s was discarded from the experiment. Animals were then removed from the dark compartment and returned to their home cages. Between each training session, both chamber compartments were cleaned to remove any confounding olfactory cues.
3. Retention trial: Recall of the inhibitory stimulus was evaluated 24 h post-training by returning the animals to the light compartment and recording their latency to enter the dark compartment (four paws in). No foot-shock was applied in this trial. If the animal did not enter the dark compartment within 300 s, it was returned to its cage and a maximum latency of 300 s was recorded. This latency served as a measure of retention performance of step-down avoidance responses (retention latency).

In the present study, rats received 3-Br 7-NI and ODQ 30 min and 20 min before foot-shock training, respectively. Also, L-arginine was injected 20 min before NOS inhibitor.

Three-panel runway test

A three-panel runway test was used to evaluate reference and working memory performances of the rats according to the method described in previous studies by Furuya et al. (1988) and Ohno et al. (1992). The three-panel runway apparatus (175 × 36 × 25 cm, length × width × height) was composed of a start box, a goal box, and four consecutive intervening choice points. Each choice point consisted of a gate with three panels (12 × 25 cm, width × height). Rats were prohibited from passing through two of the three panels in the gate by front stoppers and were also prohibited from returning either to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all gates. When rats reached the goal box, they received food pellets as positive reinforcement (Fig. 1).

At the beginning of the test, all front stoppers were removed so that at each choice point, a rat could pass through any one of the three-panel gates. The rats were forced to repeatedly run the task until the elapsed time from leaving the start box to reaching the goal box consistently fell below 20 s. Once the rats reached this state, they were forced to run the task when the front stopper of only one of the three-panel gates (the correct panel gate) was removed at each choice point.

In the working memory task, six consecutive trials were performed each day at 2 min intervals (one session), and water was freely available between trials in the home cage. The locations of the correct panel gates were held constant within a session but were changed from one session to the next. Thus, 12 different patterns of correct panel gate locations were used in this experiment, as previously described (Furuya et al., 1988; Ohno et al., 1992). In the reference memory task, six consecutive

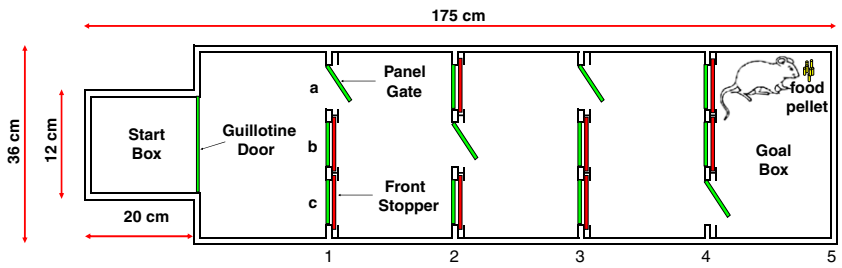

Fig. 1. Schematic drawing of the three-panel runway apparatus. Rats were allowed to perform the task after the guillotine door was opened in front of the start box. Rats had to pass through four consecutive choice points to obtain food pellets placed in the goal box. Each choice point consisted of a gate with three panels (a, b and c). The rats could pass through only one of the three-panel gates (the correct panel gate) at each choice point.