SHORT COMMUNICATION

Christian Hölscher 7-Nitro Indazole, a Neuron-specific Nitric Oxide Synthase Inhibitor, Produces Amnesia in the Chick

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Abstract

7-Nitro indazole (7-NI), which is selective for the neuronal isoform of nitric oxide synthase (NOS), was tested in a passive avoidance task in the chick. Injection of 50 mg/kg i.p. pretraining had amnesic effects for the task when tested 30 min, 2 or 24 hr after training. Injections post-training had no effect. Because 7-NI does not inhibit the endothelial isoform of NOS, it does not affect blood vessel relaxation, as nonspecific inhibitors do. This effect on blood vessels could explain the amnesic effects produced by nonspecific NOS inhibitors. The results support the theory that NO is a neuronal transmitter that is important in processes of synaptic plasticity and learning.

The messenger nitric oxide (NO) is considered to play a vital role in synaptic plastic events and in learning and memory formation. The enzyme NO synthase (NOS) has been found in rat neurons (Bredt and Snyder 1992; Vincent and Kimura 1992), and endogenous NO production occurs in neuronal cultures (Ma et al. 1991) and in cerebellar slices (Shibuki and Odada 1991). In the chick, the localization of NO synthase was investigated employing the NADPH diaphorase technique (Bredt and Snyder 1992; Vincent and Kimura 1992), and neurons and a dense meshwork of neurophil in areas that are of importance in learning of the passive avoidance task employed here were found to stain for NADPH diaphorase (Brüning 1993). Inhibition of NO synthesis prevents long-term potentiation or long-term depression in slice preparations (Böhme et al. 1991; Schuman and Madison 1991; Bon et al. 1992), but see Linden and Connor (1992). Such changes in synaptic transmission are thought to be necessary processes that occur during learning and memory formation (for discussion, see Bliss and Collingridge 1993).

To show that NO actually plays a role in learning and memory formation, the inhibitors that prevent long-term potentiation and long-term depression had to be tested in learning tasks. In previous studies, inhibitors of NOS proved to impair learning in the chick (Hölscher and Rose 1992, 1993) or rat (Chapman et al. 1992; Böhme et al. 1993). However, NO also plays a role in dilating blood vessels. It is produced in the endothelial tissue by a NOS isoform that is specific to this tissue (Garthwaite et al. 1988; Lambert et al. 1991). It cannot be ruled out that inhibition of NOS and prevention of blood vessel relaxation are the reason for the amnesic effects, as the NOS inhibitors used in learning tasks in previous investigations also have effects of blood pressure (Macrae et al. 1993). The novel inhibitor 7-nitro indazole (7-NI) is selective for the neuron isoform of NOS and does not affect blood pressure (Moore et al. 1993).

To investigate whether the amnesia observed after injection of nonspecific NOS inhibitors is attributable to prevention of blood vessel relaxation and increase of blood pressure and not to a block of synaptic

plastic changes that underlie learning and memory formation, 7-NI was injected i.p. into day-old chicks.

Ross chunky chicks (24 ± 4 hr old) were injected i.p. with either 0.5 ml of sesame oil or 10, 20, 40, or 80 mg/kg of 7-NI (Affiniti, UK) suspended in 0.5 ml of sesame oil. Each group consisted of 11–15 chicks. One hour after injection, each chick was pretrained by three presentations of a 2.5-mm-diam. white bead and pecking behavior recorded. There was no difference between test and control groups. For training, birds were presented with a 4-mm-diam. chrome bead dipped in methylthranilate (for details, see Lössner and Rose 1983). More than 90% of birds pecked the bead and showed a disgust response (shaking of the head, wiping of the beak). These birds were then tested by a person blind to treatment of chicks by presentation of a dry chrome bead once only, with each time point in the graph representing a different group. Birds were scored in real time for peck (amnesia) or avoid (recall). Each test for each bird was regarded as a single observation, and data were evaluated statistically by χ^2 .

As shown in Figure 1, 40 mg/kg injected 1 hr pretraining showed amnesic effects when tested 30 min, 2 hr, or 24 hr post-training but not when tested 5 min post-training. 7-NI injected 30 min post-training had no effect (72% avoidance in control group, 64% in test group, $n = 34$). To test whether the drug might affect general motor skills or orientation of chicks, 50 mg/kg of 7-NI was injected 1 hr pretraining, and chicks were trained on a bead dipped in water. Both groups pecked the bead when tested 30 min post-training (80% pecked in control, 72% in test group, $n = 32$). This suggests that 7-NI does not impair motor performance, motivation, or general orientation of the animals.

To assess the concentration range in which 7-NI impairs memory formation, one of four different concentrations of 7-NI was injected 1 hr pretraining. As Figure 2 shows, a significant difference between test and control group can be seen at the concentrations 40 and 80 mg/kg, though a trend towards impairment can be seen after injecting 20 mg/kg.

Figure 1: Amnesic effect of the NOS inhibitor 7-NI in a one-trial passive avoidance task. (Open bars) Saline; (hatched bars) 7-NI. When injected before training (2 mg/kg), 7-NI produced amnesic effects in chicks when tested later than 5 min after training. The eight groups consisted of 11–15 chicks. Each group was tested only once. (*) $P < 0.05$.

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Figure 2: Dose-response curve for 7-NI in the chick. (Open bars) Saline; (hatched bars) 7-NI. Four doses were tested in a one-trial passive avoidance task. Injection was before training, and testing was 2 hr after training. The eight groups consisted of 14–16 chicks. Each group was tested only once. (*) $P < 0.05$; (**) $P < 0.01$.

The results obtained with 7-NI are comparable to the results of previous experiments using *N*-nitro-L-arginine (Hölscher and Rose 1992, 1993), a drug that also inhibits endothelial NOS (Lambert et al. 1991). This indicates that the amnesic effect of *N*-nitro-L-arginine is primarily the result of inhibiting the neuronal isoform of NOS.

Both NOS inhibitors act rapidly when injected pretraining but are without effect when injected post-training. This suggests that NO plays a role in the early stages of learning and does not play an essential role after that. This is in agreement with the theory that the fast diffusing and fast decaying molecule NO is an early messenger that is essential for induction but not for maintenance of synaptic plastic changes (Bliss and Collingridge 1993).

The results further support the theory that NO plays a role in synaptic plastic events and in learning and memory.

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