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4R-cembratrienediol protects against diisopropylfluorophosphate-induced neurodegeneration with a long window of therapeutic opportunity

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4R-cembratrienediol (4R) is a neuroprotective compound that protects rat brains against diisopropylfluorophosphate (DFP) induced neurodegeneration. DFP is a surrogate for organophosphate chemical warfare nerve agents. The objective of this work was to determine the window of therapeutic opportunity for the neuroprotective effect of 4R against DFP-induced neurodegeneration. Male adult rats were pretreated with ancillary drugs that decrease mortality and allow for DFP to cause brain damage. For that purpose rats were injected with 0.1 mg/kg pyridostigmine (i.m.), 20 min later with 3 mg/kg ipratropium or methyl atropine. After 10 min 8.5 mg/kg DFP was injected (i.p.). 4R, 6 mg/kg in DMSO, was injected (s.c.) 1 h before DFP or either 5 or 24 after DFP. Animals assigned to the 1 h before application of 4R group received 4R in (s.c.) followed 30 min later by the pyridostigmine and 10 min later with the atropine analogue. DFP was injected 10 min after the atropine analogue. Controls were treated as the experimentals but instead of 4R were injected with vehicle. Other subjects received 4R 5 or 24 h after DFP. Behavioral convulsions but not central seizures were video recorded and quantified with a Racine scale for 4½ hours after DFP injection. Forty eight hours after DFP injection, the animals were anesthetized and euthanized by perfusion with formaldehyde. The sectioning of most brains to 40 μM slices and the de Olmos silver staining and nestin immunolabeling were done by NeuroscienceAssociates. For Cresyl violet staining the brains were cryosectioned to 17 μm thick slices. The mortality of rats receiving the pyridostigmine-atropine analogue followed by DFP was 5–20%. Behavioral convulsions lasted for the whole observation period. Treatment with 4R did not alter the Racine scores or mortality. Densitometric analysis of hippocampal pyramidal neurons revealed a 25% reduction in the CA1 region by DFP rats that did not received 4R. 4R treatment significantly decreased neuronal loss. 4R robustly decreased the DFP caused neuronal damage with a 24 h long window of therapeutically opportunity. In vitro studies suggest that neuroprotection by 4R is mediated by nicotinic receptors and the underlying mechanism is based on 4R anti-inflammatory and anti-apoptotic properties. In conclusion, our results show that the above describe DFP treatment decreased the number of live neurons in the CA1 hippocampal area and that a single injection of 4R applied up to 24 h after DFP prevents neuronal death. 4R could be developed as a neuroprotective treatment to be used after the classical antidotes were administered in the ER.

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N-methyl-D-aspartate receptor-mediated neurotransmission in the nucleus accumbens shell or core regulates nicotine reinforcement and nicotine-seeking behavior in rats

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The reinforcing effects of nicotine are partly mediated by dopaminergic neurons which originate in the ventral tegmental area (VTA) and terminate in the nucleus accumbens (NAc). Both the VTA and the NAc receive excitatory glutamatergic terminals which originate from the prefrontal cortex and other corticolimbic nuclei. Glutamatergic neurotransmission in the VTA and NAc is mediated by several glutamatergic receptors, including the N-methyl-D-aspartate (NMDA) receptors. Nicotine increases the activity of the dopaminergic neurons predominantly by increasing glutamatergic transmission in the VTA. Blockade of NMDA receptors in the VTA decreases nicotine self-administration. Nicotine also increases glutamate levels in the NAc. However, little is known about the role of NMDA receptors in the NAc shell and core in nicotine intake and nicotine-seeking behavior. Thus, the present study assessed the effects of bilateral administration of the competitive NMDA receptor antagonist LY235959 (0, 0.1, 1 and 10 ng/0.5 μl/side) into the NAc shell or core on intravenous nicotine self-administration, in separate cohorts of rats for each brain site. Additional groups of rats were used to assess the effects of the same microinjections on cue-induced reinstatement of nicotine seeking. LY235959 (10 ng/0.5 μl/side) microinjections into the NAc shell (n = 7–8/group), but not the core (n = 7–10/group), significantly increased nicotine self-administration under both fixed- and progressive-ratio schedules of reinforcement. Furthermore, LY235959 microinjections (10 ng/0.5 μl/side; n = 8/group) into either the NAc core or shell significantly increased reinstatement of cue-induced nicotine-seeking behavior compared to reinstatement after vehicle administration, with the effects being more prounouced in the core. Taken together, these data suggest that blockade of NMDA receptors in either the shell or core increases nicotine intake and/or nicotine-seeking behavior. NMDA receptors in the NAcc are predominantly located on cell bodies of inhibitory GABAergic neurons and a few of these neurons project to the VTA. Blockade of these NAc NMDA receptors diminishes the inhibitory output from the NAcc to the VTA. Therefore, a possible mechanism for the findings of the present study could be that blockade of NAc NMDA receptors increases the activity of VTA dopaminergic neurons by decreasing the inhibitory output from the NAc, an action that ultimately leads to increased nicotine intake and nicotine-seeking behavior. In conclusion, NMDA-mediated glutamatergic transmission in the NAc shell and core critically regulates nicotine intake and nicotine-seeking behavior in rats.

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