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Lidocaine attenuates anisomycin-induced amnesia and release of norepinephrine in the amygdala

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ABSTRACT

When administered near the time of training, protein synthesis inhibitors such as anisomycin impair later memory. A common interpretation of these findings is that memory consolidation requires new protein synthesis initiated by training. However, recent findings support an alternative interpretation that abnormally large increases in neurotransmitter release after injections of anisomycin may be responsible for producing amnesia. In the present study, a local anesthetic was administered prior to anisomycin injections in an attempt to mitigate neurotransmitter actions and thereby attenuate the resulting amnesia. Rats received lidocaine and anisomycin injections into the amygdala 130 and 120 min, respectively, prior to inhibitory avoidance training. Memory tests 48 h later revealed that lidocaine attenuated anisomycin-induced amnesia. In other rats, *in vivo* microdialysis was performed at the site of amygdala infusion of lidocaine and anisomycin. As seen previously, anisomycin injections produced large increases in release of norepinephrine in the amygdala. Lidocaine attenuated the anisomycin-induced increase in release of norepinephrine but did not reverse anisomycin inhibition of protein synthesis, as assessed by c-Fos immunohistochemistry. These findings are consistent with past evidence suggesting that anisomycin causes amnesia by initiating abnormal release of neurotransmitters in response to the inhibition of protein synthesis.

1. Introduction

The predominant model for the molecular basis of memory is that memory formation occurs in two stages: an early transient stage independent of protein synthesis and a long-term memory phase dependent on training-initiated *de novo* protein synthesis (cf.: Davis & Agranoff, 1966; Davis & Squire, 1984; Dudai, 2002; Izquierdo et al., 2002; Kandel, 2001; Nader, 2003; Schafe & LeDoux, 2000). Support for this model comes from numerous studies in which protein synthesis inhibitors such as anisomycin administered near the time of training have no effect on memory tested during the first few hours after training but impair memory tested at longer time intervals.

However, evidence obtained over the past 40 years suggests that drugs that inhibit protein synthesis may lead to secondary dysfunctions in neural activity that might produce amnesia. Several studies have suggested that protein synthesis inhibitors interfere with neurotransmitter synthesis and release. For example, early studies showed that protein synthesis inhibitors reduced the activity of tyrosine hydroxylase, the rate-limiting enzyme involved in the synthesis of norepinephrine and dopamine from tyrosine (Flexner & Goodman, 1975; Flexner, Serota, & Goodman, 1973; Goodman, Flexner, & Flexner, 1975). Recent studies found that intra-amygdala and intra-hippocampal injections of anisomycin induced abnormally large increases in the release of norepinephrine, dopamine, serotonin, and acetylcholine at the site of the injection (Canal, Chang, & Gold, 2007; Qi & Gold, 2009). Related to these findings is evidence that many neurotransmitter-related drugs and hormones reverse amnesias associated with protein synthesis inhibitors and do so without reversing the extent of inhibition of protein synthesis (cf.: Davis & Squire, 1984; Gold, 2008; Martinez, Jensen, & McGaugh, 1981).

The present study tested whether a local anesthetic, lidocaine, would attenuate anisomycin-related memory impairments by inhibiting the anisomycin-induced increases in norepinephrine release, though likely not baseline release (Pan, Chen, Tsai, & Liao, 1996). Since the time course for anterograde amnesia observed with lidocaine is shorter than that for anisomycin, we were able to adjust the timing of the injections such that lidocaine had no

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effect on memory while anisomycin retained its ability to impair memory. Our hypothesis was that lidocaine would reduce the magnitude of anisomycin-induced increases in norepinephrine release and would attenuate anisomycin-induced amnesia.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (4–5 months old; Harlan Labs, Madison, WI) were individually housed and maintained on a 12: 12-h light–dark cycle with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign and were in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Surgery

Rats (total $N = 51$) were anesthetized with isoflurane and placed in a stereotaxic apparatus with skulls arranged in a horizontal position. For the behavioral experiment, 23-gauge guide cannulae (Plastics One, Roanoke, VA) were aimed bilaterally at the basolateral amygdala (coordinates AP = −2.9 mm from bregma, ML = ±4.8 mm, DV = 5.4 mm below dura) and secured to the skull using dental cement and four small screws. Stylets ending flush with the tips of the cannulae were inserted. For the microdialysis experiment, an injection guide cannula was implanted as above on one side and a guide cannula for a combination microdialysis/injection probe was implanted on the contralateral side. The guide cannula for the SciPro probe was lowered until it was 3 mm above the amygdala (coordinates as above, except DV = −4.4 mm below dura). Rats were allowed to recover from surgery for at least one week prior to behavioral and microdialysis procedures. The rats were handled for 5 days for 3 min/day prior to the days of training and microdialysis sampling.

2.3. Inhibitory Avoidance Training and Testing

Rats were trained on a one-trial inhibitory avoidance task using a trough-shaped apparatus (91 cm long, 22.9 cm wide at top, 7.6 cm wide at bottom, and 15.2 cm deep) in which a door separated a well-lit start compartment (31 cm) from a dark shock compartment (60 cm). After placement of rats into the start compartment on the training day, the door was opened and the rats were allowed to cross into the dark compartment where they received a footshock (0.5 mA, 1.5 s). Forty-eight hour later, rats were again placed into the start compartment and the door was opened. The latency to enter the dark compartment on the test day (maximum = 180 s) was taken as the measure of memory.

2.4. Injections

For behavioral testing, anisomycin (Sigma, St. Louis, MO) was dissolved in 1 N HCl and brought to a final pH of 7.2 with 1 N NaOH. Phosphate-buffered saline (1 mM KH_2PO_4, 155 mM NaCl, 3 mM Na_2HPO_4) was added to obtain a final anisomycin concentration of 125 μg/μl; 0.5 μl (62.5 μg) was injected per side into the amygdala of each rat. Lidocaine was dissolved in 0.9% saline for a final concentration of 10 μg/0.5 μl/side. Rats received bilateral injections (0.25 μl/min for 2 min via CMA/100 microinjection pump) of saline or lidocaine 10 min prior to injections of PBS or anisomycin, the second injection coming 2 h prior to training. The timing of the injections was determined by pilot studies to ensure an absence of anterograde impairments of memory by lidocaine and a presence of memory impairments by anisomycin. We had found that anisomycin impaired 48-h memory if injected into the amygdala up to 2 h but not 6 h or more before inhibitory avoidance training (Canal et al., 2007). In pilot studies for the present experiment, lidocaine impaired memory if injected within 30 min of training but not if injected at longer pretraining intervals. Four groups were trained and tested in the behavioral experiment: saline + PBS ($n = 7$), lidocaine + PBS ($n = 4$), saline + anisomycin ($n = 9$), lidocaine + anisomycin ($n = 12$). Following injections, cannulae remained in place for an additional 1 min in order to allow diffusion of drugs. Rats were placed back into their home cages until they were trained on the inhibitory avoidance task.

2.5. Norepinephrine samples

In the microdialysis experiment, combination microdialysis/injection probes (MAB 9.14, SciPro, Sanborn, NY) were used to inject drugs and to collect microdialysis samples simultaneously. The probes consisted of a 2 mm membrane and an injection port that extended 1 mm below the microdialysis membrane. Combination probes were inserted into the guide cannulae and artificial cerebrospinal brain fluid (128 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$, 2.1 mM $MgCl_2$, 0.9 mM NaH_2PO_4, 2.0 mM Na_2HPO_4, 1.0 mM dextrose; pH = 6.5) was perfused through the brain at a rate of 0.6 μl/min. Each 45-min sample (27 μl) was collected in a tube containing 40 μl of 0.2 N acetic acid for a total volume of 67 μl. Samples collected during the first hour were discarded. Then, three baseline samples were collected before intra-amygdala bilateral injections of anisomycin ($n = 4$), lidocaine ($n = 3$), or both anisomycin and lidocaine ($n = 4$) at concentrations as above. Injection procedures followed those used in behavioral experiments and drugs were injected during the first 4 min of the corresponding microdialysis sample. Five post-injection samples were collected thereafter. Immediately following collection, samples were frozen at −20 °C until assayed. Microdialysis samples were analyzed for norepinephrine content using HPLC with electrochemical detection with procedures described previously (Canal et al., 2007). The detection limit for norepinephrine was 0.5 pg.

2.6. Histology

The locations of cannula placements were assessed histologically, using 50 μm frozen sections (Leica 1800 cryostat) through the amygdala and cresyl violet staining. Rats with cannulae placements outside the amygdala were excluded from data analyses. While only rats with bilateral cannula placements in the amygdala were included here, the localization of drug effects to the amygdala or to individual nuclei within the amygdala was not specifically determined. Drug diffusion estimates vary with method of assessment, e.g. tissue staining, autoradiography or neurophysiology (Edeline, Hars, Hennevin, & Cotillon, 2002). Lidocaine injections of 1 μl volumes, twice that employed here, exhibit spread of 1.7 mm using autoradiography measures (Martin, 1991), an extent of spread suggesting that the effects in the present experiment were largely confined to amygdala sites of action.

2.7. Immunohistochemistry

Separate groups of rats were used to examine c-Fos protein expression in the amygdala, used as a marker of protein synthesis inhibition as employed previously (e.g., Canal, Chang, & Gold, 2008; Canal & Gold, 2007; Canal et al., 2007; Inda, Delgado-García, & Carrión, 2005; Mamou, Gamache, & Nader, 2006). Three groups received concentrations and times of injections used in inhibitory avoidance testing. These groups included: saline + PBS ($N = 4$), saline + anisomycin ($N = 2$), and lidocaine + anisomycin ($N = 2$).