Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 80 (2005) 151–159

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Effects of chlordiazepoxide on single-unit activity in the septal region of the freely moving rat: aversive vs. non-aversive contexts

Earl Thomas*, Craig E. Strickland, Elna Yadin, Debra A. Burock

Department of Psychology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010, United States

Received 10 March 2004; received in revised form 22 October 2004; accepted 29 October 2004
Available online 25 November 2004

Abstract

Evidence suggests that stimuli that have the property of inhibiting fear in a Pavlovian fear conditioning paradigm increase cellular activity in the lateral septum, a result consistent with the idea that the lateral septum is actively involved in the inhibition of fear. The experiments reported here were designed to determine if an anxiolytic drug with fear-inhibiting properties would also increase neuronal activity in the lateral septum in a manner that might relate to its mechanism of action as an anxiolytic. An experiment was performed to compare the effects of the benzodiazepine anxiolytic chlordiazepoxide (CDP) upon single-unit activity in the septal region of the rat brain during Pavlovian aversive conditioning with the effects of CDP in a non-aversive context. During Pavlovian conditioning there was a decrease in unit activity in the more lateral regions of the septum, the dorsolateral and ventrolateral nuclei, when a stimulus signaling footshock (CS+) was presented. This conditioned suppression of unit activity was blocked by an intraperitoneal injection of CDP. Additionally, CDP increased baseline unit activity in these regions in the absence of conditioned stimuli. In the more medial regions of the septum, the intermediate lateral septum, we observed few consistent changes either to the conditioned stimuli or to the drug. In a non-aversive context CDP had either no effect at low to moderate doses, or a suppressant effect at a higher dose. The results support a fear-relief hypothesis of lateral septal functioning and suggest the lateral septum as a possible site for the anxiolytic action of benzodiazepines.

Keywords: Single unit; Benzodiazepine; Chlordiazepoxide; Pavlovian conditioning; Fear; Septum

1. Introduction

There is accumulating evidence that the lateral septum plays an important role in the inhibition of fear and anxiety. For example, in common laboratory tests of anxiety such as the Vogel (Vogel et al., 1971) water conflict test, electrical stimulation of the lateral septum has the same anti-conflict action as the anxiolytic benzodiazepines (BZDs) (Yadin et al., 1993), suggesting an anxiolytic action of septal stimulation. Electrical stimulation of the lateral septum also has a suppressing effect on species-specific defense responses, indicative of fear, induced in rats by lesions of the ventromedial nucleus (Brayley and Albert, 1977). Lesions of the lateral septum, on the other hand, appear to be pro-conflict in the conflict test, suggesting an anxiogenic effect of the lesion (Yadin et al., 1993). Consistent with an anxiogenic effect of lateral septal lesions is the finding that such lesions facilitate contextual fear conditioning in a Pavlovian model of fear conditioning (Sparks and LeDoux, 1995).

Single- and multiple-unit recording experiments in the septum also support a role for the septum in the modulation of fear conditioning (Thomas and Yadin, 1980; Thomas et al., 1991; Yadin and Thomas, 1981). The recording data are consistent with a fear-relief role for the lateral septum and a possible fear excitatory function for the medial septal regions. When animals were tested in a Pavlovian differential conditioning paradigm with an aversive US, cells in the lateral septum increased their rates of firing in the presence of stimuli that signaled relief or safety and inhibited their rates of firing in the presence of stimuli that signaled fear. Most medial cells in this study were not sensitive to the conditioning contingencies. This accords

* Corresponding author. Tel.: +1 610 526 5013; fax: +1 610 526 7476.
E-mail address: ethomas@brynmawr.edu (E. Thomas).

0091-3057/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.10.018

with data from other experiments which found virtually no associative conditioning in medial septal cells in appetitive conditioning in rats reinforced with a food US (Segal, 1973) or conditioning of the nictitating membrane response in the rabbit with an air-puff US (Berger and Thompson, 1978).

However, in contrast to the data from other laboratories we did find that there are at least a proportion of medial septal cells that do show evidence of associative conditioning. In such cells, the preponderant response appeared to be the reverse of what we observed in the lateral septum. These medial septal cells were activated by a conditioned exciter of fear. It is possible therefore that there is some contribution by the medial septum to fear conditioning and the expression of fear.

Since cells in the septum respond in a consistent manner to stimuli associated with fear and fear-relief, it would be of interest to determine if the same cells respond appropriately to pharmacological agents which affect fear. Specifically, since lateral septal cells are activated by stimuli which have fear- relief properties, it seems reasonable that anxiolytic agents such as the BZDs might have a similar effect on cells in the lateral septum. The lateral septum contains moderately high densities of GABA-linked benzodiazepine receptors (Speth et al., 1980). These receptors may be of importance in mediating the behavioral effects of BZDs since both the GABA agonist muscimol and the BZD anxiolytics midazolam and chlordiazepoxide have anxiolytic effects when administered directly into the septum (Drugan et al., 1986; Grishkat, 1991).

The behavioral effects of BZDs are substantially different in the aversive context compared to the non-aversive context. So, for instance, moderate doses of BZDs increase behavior suppressed by conditioned fear but tend not to affect behavior that hasn't been suppressed (Cook and Davidson, 1978). Accordingly, one of the key goals of the present study was determine if the effects of BZDs on septal unit activity in the two contexts parallel their effects on behavior in a manner that might account for the behavioral effects of the drug. For the aversive context we chose a Pavlovian fear conditioning paradigm where it is possible to assess the effect of BZDs both upon baseline spontaneous unit activity as well as the effect on conditioned changes in unit activity. For the non-aversive context animals were placed in the conditioning chamber but no conditioned or unconditioned stimuli were presented.

2. Methods

2.1. Subjects

Subjects were male Sprague–Dawley rats. Animals were between 90 and 110 days old and weighed approximately 350–400 g at the time of surgery. Animals were individually housed in a light and temperature controlled animal colony. The animals were maintained on a 12-h dark and 12-h light cycle and were provided with ad lib food and water throughout the experiment. Care and use of animals were approved by the Institutional Animal Care and Use Committee and experiments were carried out in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

2.2. Apparatus

The recording chamber was constructed of clear Plexiglas with a metal grid floor. The chamber measured 25×22×34 cm and was located inside an electrically shielded, wooden, sound-attenuating cubicle. The cubicle contained a 7.5-W houselight, a 15-W light used as a visual conditioned stimulus (CS), and a loudspeaker, all of which were located on the cubicle wall opposite the uncovered Plexiglas wall. The house light remained on at all times. White noise (70 db) was presented continuously except during the auditory CS which was an 800 Hz, 70 db tone. The unconditioned stimulus (US) was a 1-s footshock delivered through the grid floor. It consisted of a 100 pulse/s square wave of 1 ms duration. The US was delivered by Grass stimulator (model S44) connected in series with a constant current unit (Grass model CCU1).

Recording electrodes consisted of a bundle of eight nichrome wires each 50 microns in diameter and Teflon insulated to the tip. These wires were soldered to female Amphenol pins which were inserted into a plug on the animal's head. The wires were twisted together and cut on a 45° angle before being implanted. This configuration yields a distance of approximately 50 μm between the shortest and longest electrode tips and simulates a microdrive allowing units to be sampled at slightly different dorso-ventral coordinates.

2.3. Surgery

The animals were anesthetized with sodium pentobarbital (42 mg/kg, i.p.). All electrodes were chronically implanted using standard stereotaxic procedures. Coordinates for the lateral septum were taken from Paxinos and Watson (1998) and were as follows: 0.6 mm anterior to bregma, 0.4 mm lateral to the midline, and 6.0 mm ventral to the skull surface. The plug included a ground wire which was wrapped around one of four stainless steel screws inserted in the skull. The screws and the plug were secured to the skull surface using dental cement.

2.4. Recording procedure

Single-unit activity was recorded using a high input impedance amplification system. Field-effect transistors were cemented to the plug on the animal's head. The headstage amplifier was connected in a voltage follower configuration and recording was done differentially through electrode pairs in the site. This configuration minimizes