Pharmacology, Biochemistry and Behavior 98 (2011) 539–543

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Stress-dependent impairment of passive-avoidance memory by propranolol or naloxone

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ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 1 March 2011
Accepted 4 March 2011
Available online 11 March 2011

Keywords:
Propranolol
Naloxone
Forced swim
Passive-avoidance training
Stress
Memory modulation

ABSTRACT

Previous work has shown that the effect of opioid-receptor blockade on memory modulation is critically dependent upon the intensity of stress. The current study determined the effect of adrenergic-receptor blockade on memory modulation under varied levels of stress and then compared the effect of adrenergic-receptor blockade under intense stress to that of a) opioid-receptor blockade and b) concurrent opioid- and adrenergic-receptor blockade. In the first experiment, the β-adrenergic-receptor blocker propranolol impaired retention in the passive-avoidance procedure when administered immediately after exposure to intense stress (passive-avoidance training followed by swim stress) but not mild stress (passive-avoidance training alone). In the second experiment, while separate administration of either propranolol or the opioid-receptor blocker naloxone immediately after exposure to intense stress impaired retention, the combined administration of propranolol and naloxone failed to do so. These findings demonstrate that the effect of β-adrenergic-receptor blockade or opioid-receptor blockade on memory modulation in the passive-avoidance procedure is dependent upon the intensity of stress, and suggest that concurrent inactivation of endogenous adrenergic- and opioid-based memory modulation systems under stressful conditions is protective of memory.

1. Introduction

Evidence that an adrenergic-mediated system modulates memory under stressful conditions comes primarily from studies in which adrenergic agonists, administered shortly after passive-avoidance training, enhance retention (Ferry et al., 1999; Ferry and McGaugh, 2008; Liang et al., 1986, 1990). Less consistent are the results of studies investigating the effect on memory modulation of adrenergic blockers; for example, the ß-adrenergic antagonist propranolol, administered systemically shortly after training, impairs retention in a spatial water maze (Cahill et al., 2000) but fails to impair retention in a passive-avoidance procedure (Decker et al., 1990; McGaugh, 1989; Saha et al., 1991). Immersion in water that accompanies spatial water maze learning, however, is extremely stressful and markedly activates the endogenous adrenergic system (Cahill et al., 2000; Mabry et al., 1995). Consequently, a possible explanation for these conflicting results is that the intensity of stress from immersion in water (that accompanies spatial water maze learning) is greater than that of stress from mild foot-shock (used in passive-avoidance training), suggesting that the effectiveness of ß-adrenergic receptor blockade in impairing retention depends on the level of stress (and thus the degree of stress-induced adrenergic system activation) that accompanies its administration. This hypothesis was tested in the first experiment by determining if propranolol impairs retention in the passive-avoidance procedure when animals are exposed to an additional stressor (forced swim) known to activate memory modulation systems, including the endogenous adrenergic system (Gotoh et al., 1998; Jordan et al., 1994), subsequent to passive-avoidance training.

A similar hypothesis has previously been tested regarding the effect on retention of blockade of another stress-related memory-modulation system, the opioid system (Schneider et al., 2009). The findings indicated that the effectiveness of opioid-receptor blockade in impairing retention depended on intensity of stress. Consequently, in the second experiment, the effect of pharmacological blockade of the adrenergic system on retention under highly stressful conditions was compared to that of blockade of the opioid system, while the potential interaction between the two memory modulation systems was investigated through concurrent ß-adrenergic receptor and opioid-receptor blockade.

2. Material and methods

2.1. Subjects

The subjects (n = 69) were male Long-Evans hooded rats weighing 240–280 g at the start of the experiment. The rats were housed two to

Abbreviations: HPA, hypothalamo-pituitary-adrenal; Nal, naloxone; NE, norepinephrine; Pro, propranolol; STL, step-through latency; Veh, vehicle.

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doi:10.1016/j.pbb.2011.03.005

a cage with access to food and water ad libitum. The colony room was maintained at 20 °C and was illuminated on a 12-h light–dark cycle (lights on at 9:00 a.m.). All experiments were conducted between 10:00 a.m. and 12:00 p.m. The experimental protocol was approved by Swarthmore College's *Institutional Animal Care and Use Committee* and was in compliance with the National Research Council *Guide for the Care and Use of Laboratory Animals*.

2.2. Apparatus

The rats were trained in a standard trough-shaped passive-avoidance apparatus that consisted of a small lighted compartment (20 W × 28 H × 18 L cm at the top; 8 W × 28 H × 18 L cm at the base), illuminated by a 95-W bulb, connected to a larger dark compartment (20 W × 28 H × 42 L cm at the top; 8 W × 28 H × 42 L cm at the base). A manually operated sliding door separated the two compartments. The top of each compartment was hinged and the floor of each compartment was made of stainless steel plates. A constant-current Lafayette Master Shocker (Model 2400SS; Lafayette, IN) was connected to the floor of the large compartment. The apparatus was located in a quiet, dimly illuminated room.

2.3. Drug administration and drug doses

The rats were injected intraperitoneally with vehicle (0.9% saline, 2 ml/kg), the ß-adrenergic antagonist dl-propranolol hydrochloride (10 mg/kg, 2 ml/kg, Sigma Chemical), the opioid antagonist naloxone hydrochloride (3 mg/kg, 2 ml/kg, Sigma Chemical) or a mixture of propranolol (10 mg/kg) and naloxone (3 mg/kg) in a 2 ml/kg injection volume. The dose of propranolol chosen was similar to doses that have previously been shown to be effective in adrenergic studies on memory modulation (Cahill et al., 2000; Przybyslawski et al., 1999). The dose of naloxone has been used in previous passive-avoidance studies including a dose–response study (Schneider et al., 2006) using the procedures employed in the present study to produce both enhancement and impairment of retention under mild and intense stress, respectively (McGaugh et al., 1988; Schneider et al., 2009).

2.4. Forced swim procedure

The forced swim procedure (15 min in duration) was administered in a quiet, dimly lit room and consisted of placing rats in a cylindrical tank (46-cm tall × 20-cm diameter) with water (~22 °C) filled to a depth of 30 cm (Porsolt et al., 1978; Schneider et al., 2009). The water depth of 30 cm forced the rats to swim or float without their tails touching the bottom of the tank.

The use of a compound stressor (i.e., foot-shock from passive-avoidance training followed by forced swim) to augment the intensity of stress exposure has been validated in previous neurochemical studies using activation of the hypothalamo-pituitary-adrenal (HPA) axis as a physiological index of stress intensity. In these studies, not only has swim stress been shown to elevate levels of plasma corticosterone (Kirby et al., 1995) and alter norepinephrine (NE) and opioid (dynorphin) levels in limbic nuclei, including the amygdala (Gotoh et al., 1998; Jordan et al., 1994; Land et al., 2008), but the HPA response to swim stress has been shown to be augmented by prior exposure to shock (Christianson et al., 2003). Thus, exposure to forced swimming – with or without exposure to shock – not only meets the criteria of a stressor but produces neurochemical effects (particularly with respect to adrenergic and opioid action) consistent with a potential modulator of retention under highly stressful conditions.

2.5. Experimental procedure

Two experiments were conducted. The timeline for each experiment was as follows: rats underwent passive-avoidance training, in which they received a single foot-shock for stepping from a lighted to dark compartment, followed immediately by the experimental treatment (see below); 24 h later, a retention test was administered.

2.5.1. Passive-avoidance training

The training procedure consisted of the following: each rat was placed in the lighted compartment facing away from the sliding door. After 15 s the door was raised, the animal was allowed to step into the dark compartment, the door was lowered and shock (0.5 mA, 0.5 s) was delivered to the floor of the compartment. The animal remained in the dark compartment for 15 s and was then removed and *immediately administered the experimental treatment*. After each animal completed the trial the apparatus was cleaned.

2.5.2. Experimental treatment

In the first experiment, *immediately* after training, the animals were randomly assigned to one of two groups: swim and no swim. Animals in the swim group were exposed to forced swim stress (15 min in duration); animals in the no swim group were, in lieu of exposure to forced swim, placed in a quiet, dimly lit room (15 min in duration). Immediately thereafter each group was divided into two subgroups and was administered either the adrenergic blocker propranolol (Pro) or the vehicle yielding the following 4 groups: *No Swim-Vehicle*, *No Swim-Pro*, *Swim-Vehicle* and *Swim-Pro*.

In the second experiment, *immediately* after training, all animals were exposed to forced swim (15 min in duration) and then were randomly assigned to one of four groups – *Swim-Vehicle, Swim-Pro*, *Swim-Nal* and *Swim-Pro + Nal* – that received vehicle, propranolol (Pro), naloxone (Nal), or a combination of propranolol and naloxone, respectively.

2.5.3. Testing

The next day, animals received a retention test (identical to the training trial except shock was omitted and the experimental treatment was not administered) in which step-through latencies (STLs) served as the measure of retention (i.e., as STLs increased, retention was taken to increase). If STLs reached 600 s, the trial was terminated.

2.6. Statistical analysis

Data were analyzed with one-way analyses of variance which, if statistically significant, were followed by protected-*t* multiple comparisons tests. P values (two-tailed) of less than or equal to .05 were taken as statistically significant.

3. Results

The results of the first experiment, as shown in Fig. 1, indicate that propranolol administered immediately after exposure to the compound stressor (that is, foot-shock from the training procedure followed by forced swim) impaired retention: mean STLs in the *Swim-Pro* group (97.0 ± 50.8) were markedly lower than mean STLs in the *Swim-Vehicle* group (342.6 ± 96.1) ($t(14) = 2.41$, $p < 0.05$). On the other hand, propranolol administered in the absence of forced swim (i.e., when the stressor was foot-shock alone from the training procedure) did not impair retention: the difference in mean STLs between the *No Swim-Pro* group (334.8 ± 83.1) and the *No Swim-Vehicle* group (368.2 ± 76.4) did not reach statistical significance ($t(15) = 0.29$, $p = 0.77$). The finding that propranolol impaired retention only when administered after an intense stressor (specifically, after the combined stressor of foot-shock from passive-avoidance training followed by exposure to forced swim)