Research

Enhancement of Inhibitory Avoidance and Conditioned Taste Aversion Memory With Insular Cortex Infusions of 8-Br-cAMP: Involvement of the Basolateral Amygdala

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There is considerable evidence that in rats, the insular cortex (IC) and amygdala are involved in the learning and memory of aversively motivated tasks. The present experiments examined the effects of 8-Br-cAMP, an analog of cAMP, and oxotremorine, a muscarinic agonist, infused into the IC after inhibitory avoidance (IA) training and during the acquisition/consolidation of conditioned taste aversion (CTA). Posttraining infusion into the IC of 0.3 μg oxotremorine and 1.25 μg 8-Br-cAMP enhanced IA retention. Infusions of 8-Br-cAMP, but not oxotremorine, into the IC enhanced taste aversion. The experiments also examined whether noradrenergic activity in the basolateral amygdala (BLA) is critical in enabling the enhancement of CTA and IA memory induced by drug infusions administered into the IC. For both CTA and IA, ipsilateral infusions of β-adrenergic antagonist propranolol administered into the BLA blocked the retention-enhancing effect of 8-Br-cAMP or oxotremorine infused into the IC. These results indicate that the IC is involved in the consolidation of memory for both IA and CTA, and this effect requires intact noradrenergic activity into the BLA. These findings provide additional evidence that the BLA interacts with other brain regions, including sensory cortex, in modulating memory consolidation.

Extensive evidence indicates that the insular cortex (IC) and amygdala are involved in the acquisition of aversively motivated tasks. Lesions or functional inactivation of either the IC or amygdala impair acquisition of conditioned taste aversion (CTA), a paradigm in which animals learn to avoid a taste paired with a visceral malaise, as well as the consolidation of memory for inhibitory avoidance (IA) training, in which animals avoid an apparatus context previously associated with footshock (Dunn and Everitt 1988; Bermudez-Rattoni and McGaugh 1991; Bermudez-Rattoni, et al. 1991; Parent et al. 1992, 1995). The IC and amygdala are functionally and reciprocally interconnected (Lasiter and Glanzman 1985; Escobar et al. 1989), and there is a direct projection between the amygdala and the IC via the internal capsule (Norgren and Wolf 1975; Kiefer 1985).

Findings of many studies indicate that the basolateral nucleus of the amygdala (BLA) is involved in mediating the effects of many drugs and hormones on memory consolidation (McGaugh et al. 1996). Such findings provide strong support for the hypothesis that the BLA plays a critical role in regulating the consolidation of lasting memories of significant experiences (McGaugh 2002). Moreover, there is extensive evidence that lesions or inactivation of β-adrenoreceptors in the BLA block the memory-enhancing effect of drugs infused into other brain regions, including the hippocampus (Roozendaal and McGaugh 1997; Roozendaal et al. 1999), caudate nucleus (Packard and Teather 1998), nucleus basalis (Power et al. 2002), and entorhinal cortex (Roesler et al. 2002).

The IC is an area spanning from the lateral frontal cortex to the perirhinal cortex in the rostrocaudal direction, and from the ventral edge of the somatomotor cortex to the pyriform cortex in the dorsoventral direction (Saper 1982). The IC is also referred to as the visceral cortex, as it receives visceral information from the thalamus and is known to be involved in visceral reactions and stress (van der Kooy et al. 1984; Krushel and van der Kooy 1988). Moreover, the IC receives convergent limbic and primary sensory inputs that are not found within any other area in the cortex (Saper 1982). Studies over the past decade have widened the role of the insula to include its designation as a somatosensory area, a multifaceted sensory area, and a component of limbic integration cortex, consistent with a role for the IC in cognitive associative processes. Several studies have confirmed that the IC is involved in taste processing and memory formation and, particularly, mediation of associative aspects of taste responses (Bures et al. 1998, Berman et al. 2000).

Previous studies from our laboratory (Dalmaz et al. 1993; Power and McGaugh 2002; Roesler et al. 2002), as well as other laboratories (Baratti and Kopf 1996; Barros et al. 2000), have reported that posttraining infusions of the muscarinic agonist oxotremorine or 8-bromo-adenosine 3′-5′ cyclic monophosphate (8-Br-cAMP), an analog of the second messenger cAMP, enhance memory consolidation for IA training when injected into several brain regions, including cortical areas. Studies have not, as yet, investigated the effects of infusions administered into the IC, an important cortical area related to spatial and nonhippocampal-dependent tasks. The first aim of the present study was to determine whether 8-Br-cAMP and oxotremorine infused into the IC after training enhance the consolidation of memory for IA, a hippocampal-dependent aversive task, and whether these drugs also modulate the acquisition/consolidation of CTA, an aversive task that is known not to be hippocampus dependent. A second

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aim was to investigate whether an intact and functioning BLA is critical in enabling the enhancement of CTA and IA memory induced by infusions of 8 Br-cAMP and oxotremorine administered into the IC. To investigate this issue, we infused the β-adrenoreceptor antagonist propranolol into the BLA concurrently with infusions of 8-Br-cAMP or oxotremorine into the IC.

RESULTS

Histology

Figure 1 shows a schematic drawing illustrating the area targeted for localization of cannulae tips in the IC and BLA. Forty-two animals were excluded from the final analysis because of inappropriate localization of needle tips in the IC or BLA.

Posttraining Infusion of 8-Br-cAMP Analog or Oxotremorine in IC Enhances IA Consolidation

This experiment examined the effect of 8-Br-cAMP or oxotremorine during IA memory consolidation. The drugs were infused into the IC (unilaterally) immediately after IA training (Fig. 2A,B): 0.25 µg of 8-Br-cAMP (cAMP .25), 1.25 µg of 8-Br-cAMP (cAMP 1.25), 0.06 µg of oxotremorine (OXO .06), and 0.3 µg of oxotremorine (OXO .3). A control group received saline infusions (CON) or drugs but not footshock: 1.25µg, (cAMP 1.25 NS) or 0.25 µg (cAMP .25 NS) of 8-Br-cAMP or 0.3 µg of oxotremorine (oxo .3 NS). There were no significant differences among these groups in training entrance latencies (data not shown). However, there was a significant difference between the latencies in control group (CON) during the training and the test day, which could be interpreted as evidence of learning in control group (paired *t*-test training versus test latency, t value = -2.39; $P < 0.05$). Groups given 1.25 or 0.25 µg of 8-Br-cAMP or 0.3 µg of oxotremorine after being placed in the training apparatus but not given footshock showed no significant increases in response latencies on a retention test ($P > 0.05$).

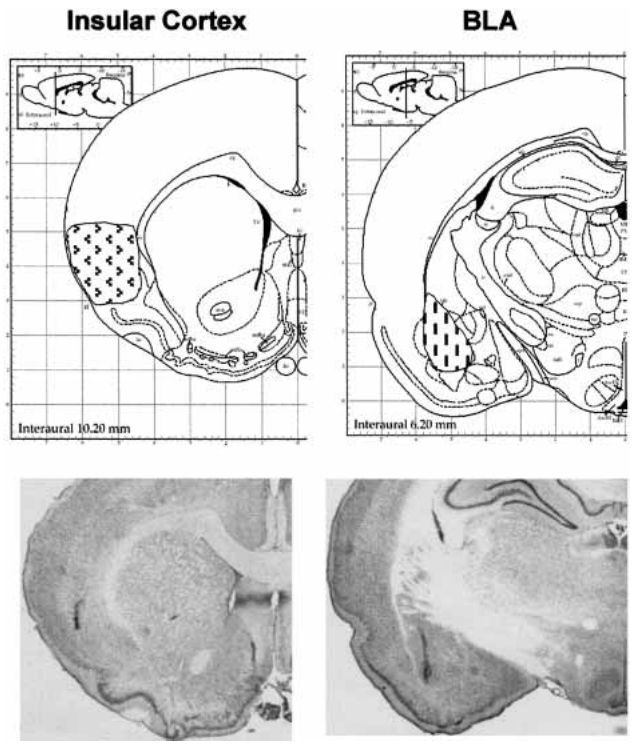

Figure 1 Schematic drawing and micrographs illustrating the area considered for a good localization of tip injectors into the IC and BLA.

An ANOVA of the retention latencies revealed a significant treatment effect ($F_{7,55} = 6.88$; $P < 0.001$; Fig. 2B). Fisher's post hoc analyses showed that a posttraining infusion into the IC of 1.25 µg 8-Br-cAMP, but not 0.25 µg, enhanced retention latencies compared with those of controls ($P < 0.05$). In addition, the retention latencies of the OXO .06 and OXO .3 were significantly longer than those of the controls (OXO .06, $P < 0.001$; OXO .3, $P < 0.05$). The retention latencies of the OXO .06 group were significantly longer than those of the cAMP .25, cAMP 1.25, and OXO .3 groups ($P < 0.01$).

Unilateral Infusions of 8-Br-cAMP, But Not Oxotremorine, Into the IC Enhance CTA Memory Formation

For the CTA experiment, we infused the doses found to be effective in the IA experiment (1.25 µg 8-Br-cAMP and 0.3 µg oxotremorine) into the IC immediately before the IP injections of a low dose of 0.075 M LiCl that we have previously found not to produce CTA (Fig. 2C; Miranda et al. 2002). Controls received either a low dose (LL-CON) or a high dose (0.4 M, 9.5 mL/kg; CTA-CON) of LiCl. There was no significant difference among groups in baseline water intake or during acquisition saccharin consumption (data not shown). There was, however, a significant difference between groups on the test day (Fig. 2D), as revealed by ANOVA ($F_{3,21} = 13.96$; $P < 0.001$). Post hoc analysis showed that IP injection of a high dose of LiCl (CTA-CON) significantly reduced saccharin consumption compared with the consumption by the control group given a low dose of LiCl (LL-CON; $P < 0.01$). The group given 8-Br-cAMP in the IC together with low doses of LiCl (cAMP 1.25) showed reduced saccharin consumption that was significantly different from that of the control groups given low doses of LiCl (LL-CON $P < 0.01$). However, the 0.3 µg dose of oxotremorine (OXO .3) did not significantly affect saccharin consumption ($P > 0.05$). Groups given 1.25µg 8-Br-cAMP, but not LiCl after 30 min of saccharin consumption, showed no significant effect (data not shown), indicating that 8-Br-cAMP by itself does not affect subsequent saccharin intake. These findings indicate that 8-Br-cAMP infused into the IC just before low doses of LiCl enhances taste aversion memory. Oxotremorine infused into the IC, using one of the doses found to enhance IA retention, did not significantly affect CTA retention.

Propranolol Infusions Administered Into the Basolateral Amygdala Block 8-Br-cAMP and Oxotremorine Enhancement of IA

Recently, we found that bilateral infusions of the β-adrenoreceptor antagonist propranolol into the BLA, just before LiCl injections, disrupted CTA memory consolidation (Miranda et al. 2003). To study the BLA interactions with the IC, we infused propranolol (1.0 µg) unilaterally into the BLA concurrently with 8-Br-cAMP (1.25 µg) or oxotremorine (0.06 µg) infused into the ipsilateral IC immediately after IA training (procedures shown in Fig. 2A).

During IA training, there were no significant differences among the groups in their entrance latencies (data not shown). However, on the IA retention test, the groups differed significantly in retention latencies ($F_{5,59} = 5.17$; $P =$; 0.001; Fig. 3A). Further analysis with Fisher's post hoc tests showed that posttraining infusion of 1.25 µg dose of 8-Br-cAMP administered into the IC and saline infused into the BLA (SAL-8Br) enhanced retention latencies compared with the control group (SAL-SAL; $P < 0.01$). Propranolol infusions of the BLA and saline infusions

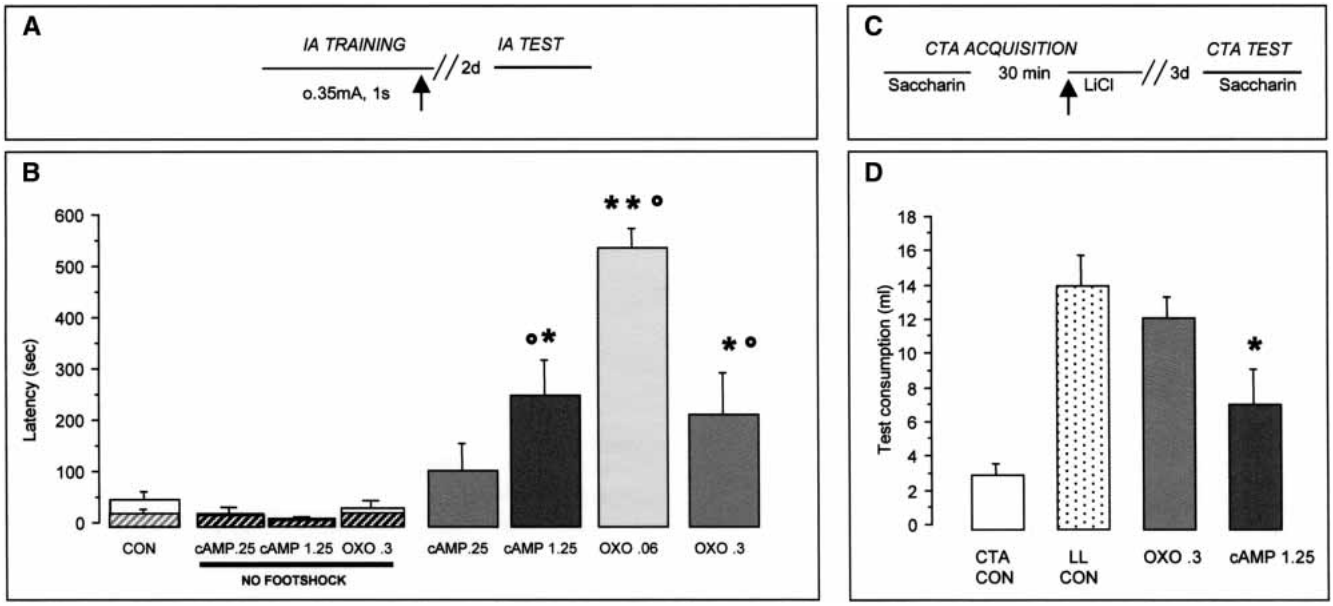

Figure 2 Protocol of infusions during inhibitory avoidance training (*A*) and during conditioned taste aversion acquisition (*C*); arrows indicate the moment when the drugs were infused. (*B*) Time latency during the IA test for control received saline infusions (CON; n = 16), 0.25 µg of 8-Br-cAMP (cAMP .25; n = 10), 1.25 µg of 8-Br-cAMP (cAMP 1.25; n = 13), .06 µg of oxotremorine (OXO .06; n = 6), and 0.3µg of oxotremorine (OXO .3; n = 9) groups. $^{*}P < 0.05$ and $^{**}P < 0.001$, from control; $^{\circ}P < 0.01$ from OXO .06. The shaded box inside CON and NO FOOTSHOCK groups indicates the entrance latency during the training day (mean of CON group = 12.38; paired *t*-test training versus test latency in CON, *t* value = −2.39; $P < 0.05$, NO FOOTSHOCK groups showed any significant differences). (*D*) Consumption of saccharin during CTA test day for IP injection of a high dose of LiCl (CTA-CON, n = 9), IP injection of a low dose of LiCl-control group (LL-CON, n = 7), 1.25 µg 8-Br-cAMP (cAMP 1.25, n = 5), and 0.3 µg oxotremorine (OXO .3, n = 5). $^{*}P < 0.01$ from CTA-CON.

of the IC (PROP-SAL) did not affect retention ($P > 0.05$). However, propranolol infused into the BLA blocked the retention-enhancing effect of 8-Br-cAMP infused into the IC (PROP-8Br; $P < 0.01$). In addition, posttraining infusion of 0.06 µg dose of oxotremorine infused into the IC along with saline infused into the BLA (SAL-OXO) enhanced retention latency compared with

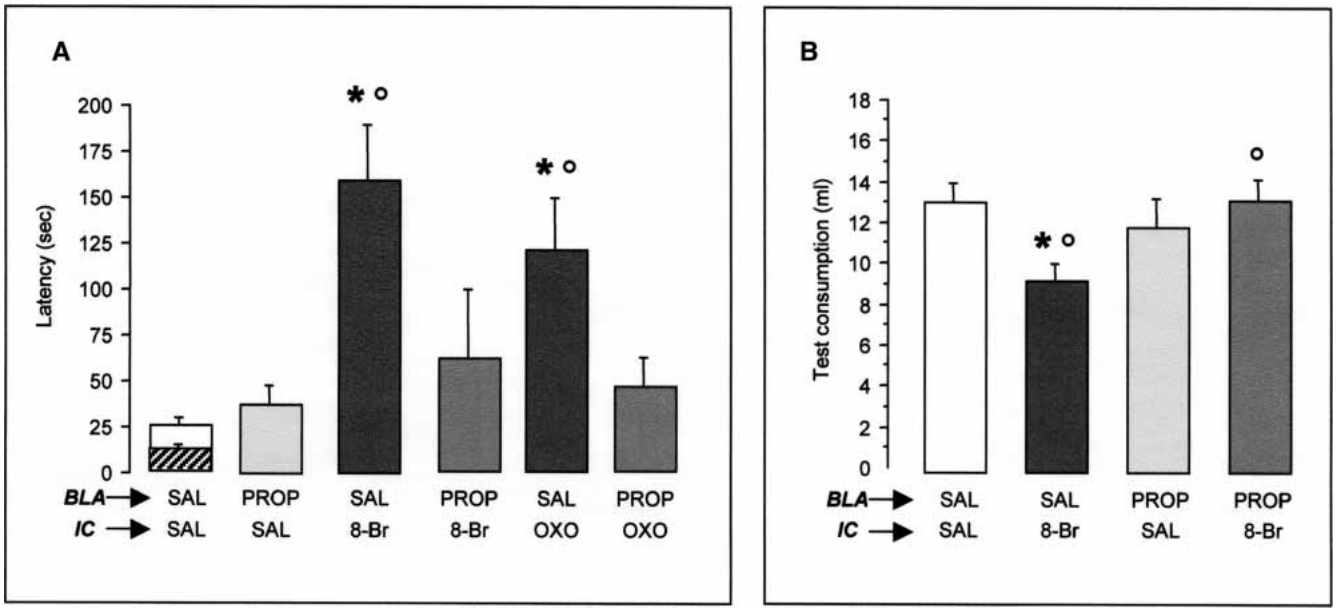

Figure 3 (*A*) Time latency during IA retention test. Groups with posttraining infusion of saline administered into the BLA and saline infused into the IC (SAL-SAL, n = 14), infusions of saline into the BLA and 8-Br-cAMP into the IC (SAL-8Br, n = 13), propranolol infusions of the BLA and saline infusions of the IC (PROP-SAL, n = 11), propranolol into the BLA and 8-Br-cAMP into the IC (PROP-8Br, n = 10; $P < 0.01$), saline infused into the BLA and oxotremorine into the IC (SAL-OXO, n = 6), and propranolol into the BLA and 0.6 µg of oxotremorine into the IC (PROP-OXO, n = 11). $^{*}P < 0.01$ versus SAL-SAL, $^{\circ}P < 0.05$ versus PROP-8Br or PROP-OXO. The shaded box inside SAL-SAL group indicates the entrance latency of the SAL-SAL group during the training day (mean, 17.43). (Paired *t*-test training versus test latency, *t* value = −2.14; $P = 0.05$). (*B*) Saccharine consumption on the CTA retention test for control (SAL-SAL, n = 17), saline infused into the BLA and 8-Br-cAMP into the IC (SAL-8Br, n = 19), propranolol into the BLA and saline into the IC (PROP-SAL, n = 13), and propranolol into the BLA and 8-Br-cAMP into the IC (PROP-8Br, n = 15). $^{*}P < 0.01$ versus SAL-SAL group; $^{\circ}P < 0.05$ versus SAL-8-Br group.

that of the control group (SAL-SAL; $P < 0.05$). Propranolol infused into the BLA blocked the memory-enhancing effects of oxotremorine into the IC (PROP-OXO; $P > 0.05$ versus SAL-SAL). These findings indicate that unilateral propranolol infusions administered into the BLA did not impair IA retention but blocked the memory enhancement induced by 8-Br-cAMP or oxotremorine infused into the IC immediately after training.

Propranolol Infusions Administered Into the Basolateral Amygdala Block 8-Br Enhancement of CTA Memory

To study the BLA interactions with the IC during CTA, we infused propranolol into the BLA at the same time we injected 8-Br-cAMP into the ipsilateral IC, 30 min after saccharin presentation/just before injecting low doses of LiCl (Fig. 2C).

There were no significant differences among the groups during baseline or saccharin acquisition consumption (data not shown). An ANOVA analysis revealed significant differences among groups in saccharine consumption on the CTA retention test ($F_{3,60} = 3.45$; $P = 0.02$; Fig. 3B). Further analysis with Fisher's post hoc tests indicated that 1.25 µg dose of 8-Br-cAMP infused into the IC and saline infused into the BLA (SAL-8Br, n = 19) significantly decreased saccharine consumption compared with that of the control group (SAL-SAL, n = 17); that is, enhanced aversive memory ($P < 0.01$). Propranolol infusions administered into the BLA (PROP-SAL, n = 13) did not alter consumption ($P > 0.05$) but blocked the aversion-enhancing effect of 8-Br-cAMP infused into the IC (PROP-8Br, n = 15; $P < 0.05$ compared with SAL-8-Br group).

Considered together, these findings indicate that drug infusions administered into the IC can modulate memory formation for both IA and CTA, and such effects require intact noradrenergic activity in the BLA.

DISCUSSION

Posttraining infusions of 8-Br-cAMP or oxotremorine infused into the IC enhanced IA retention. Infusions of 8-Br-cAMP into the IC just before LiCl administration also enhanced CTA retention, whereas oxotremorine infusions, in the low dose used, were ineffective. Infusions of oxotremorine or 8-Br-cAMP administered into the IC did not affect retention performance of animals that did not receive footshock or LiCl during the training session. These findings are consistent with previous evidence indicating an involvement of the IC in the acquisition and consolidation memory for CTA and IA training (Bures et al. 1998; Mello e Souza et al. 2001) as well as water-maze spatial training (Bermudez-Rattoni et al. 1991).

The present results are also in agreement with the extensive evidence that posttraining infusions of norepinephrine or 8-Br-cAMP enhance IA memory when administered into other brain regions, including the dorsal hippocampus, entorhinal, parietal, and cingulate cortex (Barros et al. 2000; Izquierdo and McGaugh 2000; Roesler et al. 2002). It is well established that cAMP activates cAMP-dependent protein kinase (PKA) and phosphorylation of the transcription factor cAMP-response element-binding (CREB) protein (Ferrer et al. 1996), proteins implicated directly in long-term neuroplasticity and memory formation (Bernabeu et al. 1997; Guzowski and McGaugh 1997).

The enhancement observed in IA memory consolidation with posttraining IC infusions of oxotremorine suggests an involvement of the cholinergic system in the IC during memory consolidation and is in agreement with previous reports suggesting an important role of cholinergic activation during consolidation of IA and other tasks (Introini-Collison et al. 1989; Power and McGaugh 2002; Power et al. 2002). Recently, it has been suggested that modulation of memory consolidation induced by cholinergic influences within the BLA requires activation of both m1 and m2 receptor synapses (Power et al. 2003). Posttraining intra-BLA infusions of the nonselective muscarinic agonist oxotremorine immediately after IA training enhance performance in a 48-h retention test. However, intra-BLA coinfusions of oxotremorine with either the selective m1 antagonist telenzipine or the selective m2 antagonist methoctramine blocked the oxotremorine-induced enhancement. Combinations of these antagonists did not act additively to block memory enhancement by oxotremorine. This indicates that the oxotremorine effect observed is due to a specific muscarinic effect on m1 and m2 receptors (Power et al. 2003), which may be showing their effects via activation of tyrosin kinases and phospholipases during the increase in memory consolidation reported here.

In the CTA experiments, the drug infusions were administered 30 min after the presentation of the novel taste. This delayed infusion, in contrast to the immediate posttraining infusion given in the IA experiments, may have been administered too long after the taste experience to affect the cholinergic-sensitive period of taste-memory consolidation. Cortical cholinergic activity appears to be involved during early taste memory formation as ACh is released in the IC by presentation of a novel taste, but not by LiCl injections (Miranda et al. 2000). Furthermore, other studies have reported that both cholinergic and noradrenergic activity in the IC are involved during the early stage of CTA acquisition (Berman et al. 2000; Ramirez-Lugo et al. 2003). However, the possibility that the differential effect of oxotremorine on IA and CTA seen in the present studies was due to task-dependent shifts in dose response effects cannot be excluded.

A second important finding reported here is that unilateral infusions of the BLA did not impair IA or CTA retention but blocked the memory enhancement induced by 8-Br-cAMP and oxotremorine, as well as the enhancement of CTA memory induced by 8-Br-cAMP infused into the IC. The findings suggest that bilateral depletion of noradrenergic activity into the BLA is necessary to disrupt taste aversion memory formation, as reported previously (Miranda et al. 2003).

Our results are in agreement with previous evidence that infusions of norepinephrine or β-adrenoreceptor agonists into the amygdala after training enhance memory consolidation of several tasks (Liang et al. 1986, 1990; Ferry and McGaugh 1999; Hatfield and McGaugh 1999). They also concur with prior findings that lesions or infusions of a β-adrenoreceptor antagonist into the BLA block the memory-modulating effects of drugs administered directly into the hippocampus (Roozendaal and McGaugh 1997; Roozendaal et al. 1999) or caudate nucleus (Packard et al. 1994; Packard and Teather 1998). Our results provide additional evidence that the BLA is involved in regulating the consolidation and storage of memory in other regions of the brain, including the IC, and that an intact BLA is essential to enable the effects of drugs on memory consolidation (McGaugh et al. 1996; McGaugh 2000; Roozendaal et al. 2001).

Recently, we found that the noradrenergic antagonist propranolol microinjected bilaterally into the BLA just before the presentation of LiCl disrupts taste aversive memory (Miranda et al. 2003). Other investigators (Bahar et al. 2003) reported that local blockade of β-adrenergic receptors in the CeA blocks acquisition but not extinction of CTA, and a similar intervention in the BLA blocks extinction but not acquisition. Furthermore, recent findings indicate that the BLA regulates the development of learning-induced plasticity in the medial geniculate nucleus (Maren et al. 2001; Poremba and Gabriel 2001).

The interaction between IC and BLA found in the present experiments fits well with the previous finding that in rats with *N*-methyl-D-aspartate–induced lesions of the BLA, as well as lesions of the nucleus basalis magnocellularis induced by anti-

cholinergic immunotoxin, 192 IgG saporin disrupted CTA acquisition, whereas similar lesions made separately did not affect CTA memory (Gutierrez et al. 1999).

In summary, the present findings indicate the IC is involved in modulating the consolidation of memory for both IA and CTA training and that the modulatory influences require intact noradrenergic activity into the BLA. Thus, the findings provide additional evidence that the BLA interacts with other brain regions in modulating the consolidation of different forms of memory (McGaugh 2002).

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats (225 to 250 g at the time of arrival, n = 266) were used. Animals were housed individually under a standard 12-h light/12-h dark cycle at a room temperature of 22°C with food and water available ad libitum. Training and testing were performed during the light phase of the cycle. All methods used were in compliance with National Institutes of Health guidelines for care of laboratory animals and were approved by the University of California, Irvine Institutional Animal Care and Use Committee.

Surgery

After arrival, the animals were adapted to the vivarium for 1 week before surgery. They were anaesthetized with sodium pentobarbital (50 mg/kg body weight, IP) and given atropine sulphate (0.4 mg/kg, IP) to maintain respiration. Animals were implanted via stereotaxic frame (Kopf Instruments) unilaterally with a guide cannula (23 gauge, 15 mm) aimed at the right IC (coordinates: anteroposterior [AP], 1.2 mm; mediolateral [ML], −5.5 mm; dorsoventral [DV], 4.0 mm from bregma) or with two cannulae directed to the right IC and to the right BLA (AP, −2.5 mm from bregma; ML, −5.0 mm from midline; DV, −6.5 mm from bregma) based on the atlas of Paxinos and Watson (1998). The cannulae were attached to the skull with two anchoring screws and dental cement. A stylet (15-mm-long 00 insect dissection pin) inserted into the cannulae to maintain patency was removed only for the drug infusion. After surgery, the rats received a subcutaneous 3.0-mL injection of saline to prevent dehydration and were retained in an incubator until recovery from anesthesia was complete before being returned to their home cages. The rats were allowed to recover for 7 d before initiation of training and were handled three times for 1 min during this period to accustom them to the infusion procedures.

IA Apparatus and Procedures

The IA apparatus was a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top, 6.4 cm wide at the floor) divided into two compartments separated by a sliding door that opened by retracting into the floor. The starting compartment (31 cm long) was illuminated, and the shock compartment (60 cm long) was darkened. The rat was placed in the starting compartment and allowed to enter the dark (i.e., shock) compartment. After the rat stepped completely into the dark compartment, the door was closed and a mild inescapable footshock (0.35 mA, 1.0 sec) was delivered. The rat was removed from the dark alley 15 sec after the termination of the footshock and immediately given a unilateral infusion of vehicle or drug into the BLA and/or IC. On the retention test 48 h after training, the rat was placed in the starting compartment and the latency to enter the dark compartment (maximum of 600 sec) was recorded and taken as a measure of retention. Shock was not administered on the retention test trial.

Conditioned Taste Aversion

One week after surgery, the animals were deprived of water for 24 h and then habituated to drink water from a graduated cylinder for 20 min each day for 5 d, until a stable water consumption baseline was attained. On the next day, animals were randomly assigned to groups, and acquisition of CTA was performed. Water was substituted with a 0.1% saccharin solution, and 30 min later the animals were IP injected either with 0.4 M LiCl (9.5 mL/kg), which induces a robust CTA, or with 0.075 M LiCl (9.5 mL/kg) which does not produce a clear CTA (Miranda et al. 2002). Drug infusions into the IC and BLA were given simultaneously just before the IP injection of LiCl (Fig. 2C). For the next 3 d, water baselines were recorded. On the next day, the water was substituted with a 0.1% saccharin solution to test for taste aversion.

Drugs and Infusion Procedures

The cAMP analog, 8-Br-cAMP (Sigma; 0.25 or 1.25μg), oxotremorine (Sigma; 0.06 or 0.3 μg), and propranolol (1.0 μg) were dissolved in isotonic saline. Unilateral infusions of saline, 8-Br-cAMP, or oxotremorine were given into the IC, and saline or propranolol was given to the BLA, using 30-gauge injection needles connected to a 10 μL Hamilton microsyringe by polyethylene tubing. The injection needle protruded 2 mm beyond the cannula tip to reach the IC or amygdala. A 0.5-μL infusion volume of vehicle or drug was given into the IC and a 0.2-μL infusion volume was given into the BLA, for 30 sec by an automated syringe pump, simultaneously (Sage Instruments). The injection needle was kept in place for an additional 30 sec after the infusion. The animals were gently held during the infusions. Doses were selected on the basis of previous findings (Packard et al. 1994; Roesler et al. 2002; Miranda et al. 2003). In the IA experiments, the infusions were administered posttraining. In the CTA experiments, the infusions were administered prior to the LiCl injections (Fig. 2A,C).

Histology

The rats were anaesthetized with an overdose of sodium pentobarbital (~100 mg/kg) and perfused intracardially with 0.9% saline (w/v) followed by 4% formaldehyde (w/v). The brains were removed and placed in 4% formaldehyde. Two days later, the brains were placed in a 20% sucrose solution for cryoprotection. Sections of 50μm were made by using a freezing microtome and stained with cresyl violet. The sections were examined under a light microscope, and the location of IC and BLA cannulae tips was determined.

Statistics

Data were analyzed with a two-way ANOVA with drug treatment as between-subjects variables, followed by Fisher's post hoc tests to determine the source of the significance. Probability levels of <0.05 were considered significant.

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