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Baclofen Infused in Rat Hippocampal Formation Impairs Spatial Learning

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ABSTRACT: Recent studies show that baclofen, a selective $GABA_B$ agonist, impairs different kinds of learning. In the present study we investigated the effect of microinfused baclofen into the hippocampus of male Wistar rats, on the performance in the Morris water maze. Rats of 8–10 weeks of age were implanted with cannulae aimed bilaterally at the hippocampal formation. Baclofen (1 µl of 0.2 mM, 2.0 mM, and 20.0 mM) or sterilized saline was microinfused 1 h before each daily session (3 trials/session, 1 session/day) for 4 days. On the fifth day, the animals did not receive drug or saline injections and the retention of the location of the escape platform was tested in a 30 s free swim trial. Results from the free swim trial indicate that the doses of baclofen used during training affected the ability of the rats to swim to the target quadrant. Although no significant difference compared with the saline group was observed, the experimental rats showed a more generalized swim trajectory in the area of the target and both adjacent quadrants. Moreover, 1µl of 20.0 mM baclofen also impaired the acquisition. We suggest that baclofen has an impairing action on spatial learning, although more studies should be conducted to reach a more precise conclusion. *Hippocampus 1998;8:109–113.*

KEY WORDS: Morris water maze; hippocampus; memory; $GABA_B$ agonists

INTRODUCTION

Many studies have demonstrated the participation of the hippocampus in learning and memory processes. In the mammalian hippocampus, the phenomenon of long-term potentiation (LTP), a stimulation-induced form of synaptic plasticity, has been hypothesized to reflect a potential neural mechanism for memory storage (Douglas and Goddard, 1975; Swanson et al., 1982; McNaughton, 1983; Teyler and DiSena, 1984; Eccles, 1986; McNaughton and Morris, 1987; Matthies, 1989).

Neuroanatomically, $GABA_B$ receptors can be subclassified according to their signal transduction mechanisms. $GABA_B$ receptors are known to be located pre- and post-synaptically and they can be electrophysiologically identified (Dutar and Nicoll, 1988; Bowery, 1989; Paredes and Agmo, 1992). The activation of post-synaptic $GABA_B$ receptors causes an increase in the K^+ conductance, thus hyperpolarizing the post-synaptic cell. Activation of pre-synaptic $GABA_B$ receptors is associated with Ca^{2+} channels and causes a decrease in neurotransmitter release. Different lines of evidences support a $GABA_B$ modulatory role on hippocampus LTP induction (Mott et al., 1990; Olpe and Karlsson, 1990; Burgard and Sarvey, 1991; Davies et al., 1991; Brucato et al., 1995). Moreover, Burgard and Sarvey (1991) have described a dual effect of baclofen, an agonist of $GABA_B$ receptors, on the dentate gyrus of rat hippocampal slices. Thus, low doses of baclofen induce an excitatory effect on dentate gyrus while high doses produce an inhibitory action on it.

The results concerning the behavioral effects of baclofen are at present not clear. Laboratory animal studies reported impairment (Swartzwelder et al., 1987; Castellano et al., 1989, 1993; Castellano and McGaugh, 1991; DeSousa et al., 1994; Stackman and Walsh, 1994) or facilitation (Georgiev et al., 1988; Saha et al., 1993) in the acquisition of a conditioned response. On the radial arm maze, pre-training administration of low doses of baclofen did not affect spatial learning (Sidel et al., 1988), but a reduced number of correct choices was also reported (Stackman and Walsh, 1994). Post-training administration of baclofen dose-dependently impaired retention of inhibitory avoidance in mice (Castellano et al., 1993). Also, Ramirez and co-workers (1997), have shown that baclofen microinfusions into the cerebellum completely prevent acquisition and block expression of the eyeblink response in rabbits. More recently, McNamara and Skelton (1996) demonstrated a dose-dependent baclofen-induced impairment of spatial learning in rats. Some authors proposed cholinergic mechanisms for the amnesic action of baclofen (Sidel et al., 1988; Nakagawa et al., 1995). Also, clinical studies demonstrate cases of baclofen-induced memory impairments in patients who were receiving baclofen as a treatment of some central nervous system (CNS) disturbance (Sandyk and Gillman, 1985; Lee et al., 1992).

Considering the participation of the hippocampal formation on spatial learning and the modulatory effects demonstrated by baclofen on hippocampus LTP induction, we decided to investigate the effect of baclofen

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microinfused into rats' hippocampus on spatial learning in the Morris water maze.

MATERIALS AND METHODS

Subjects

Male Wistar rats weighing between 200 and 250 g were subjects for the present experiment. Experimental groups were composed of six rats each, and the control group was composed of 10 animals. All rats were housed in groups of six, maintained on a 12:12 h light/dark cycle and given food and water ad lib. These conditions meet the standards for the care of laboratory animals outlined in the United States NIH "Guide for the Care and Use of Laboratory Animals."

Surgery

All surgical procedures were performed under aseptic conditions. Animals were anesthetized with chloral hydrate (400 mg/kg, ip). Bilateral guiding cannulae made of 23 gauge (11 mm in length) steel tubing were placed stereotaxically in an area near the hippocampus, approximately 5.0 mm posterior to bregma, 3.5 mm lateral to the midline, and 3.5 mm below the cortical surface (Paxinos and Watson, 1986). The cannulae were cemented into place with dental acrylic. Once cemented, stylets made from 00 insect pins, which fit snugly into the 23 gauge tubing, were placed into the cannulae. Rats were allowed to recover for 1 week prior to behavioral training.

Apparatus

The water maze used was a circular, galvanized-steel tank measuring 1.80 m in diameter and 0.50 m in height, filled up to a depth of 20 cm with 25 +/− 3°C water (Arolfo and Brioni, 1991). Four points equally spaced around the perimeter of the tank were arbitrarily designated to serve as starting locations, but only three of them were used in the present study. On this basis, the tank was divided in four equal quadrants in a clockwise order (target, adjacent, opposite, and adjacent). Located in the center of one of these quadrants was a 14 / 14 / 19 cm (w/l/h) Plexiglas platform (i.e., its surface was 1.0 cm below the water level).

Place Training Procedure

A trial was considered started when the rat was placed into the pool at one of the three chosen starting positions (W, E, S). These chosen starting positions were maintained during the 4 training days and for all the groups studied. They were given in the same sequence every day. The distance from the E position to the platform was shorter than the distance from positions W and S. Positions W and S were equally distant from the escape platform. Each rat was allowed to swim until it located and climbed onto the platform (maximum: 90 s). The animal remained on the platform for a period of 20 s before being removed and taken to a cage for an intertrial interval of 5–10 s. If the animal did not reach the platform in 90 s, it was gently guided to it and the latency for that trial was recorded as 90 s. At the end of the third trial, the rat was returned to its home cage. Rats were submitted to 12 trials during 4 consecutive days of training. The platform remained in a fixed location throughout the training period (reference memory). Escape latencies were measured as the time after being released in the pool until the rat escaped onto the platform.

Free Swim Trials

Twenty-four hours after the training phase a 30 s trial without the escape platform was carried out. The four possible positions of the platform and the limits of the four quadrants were marked on the video screen to indicate its exact surface area. From video tapes made during this free swim trial, it was possible to calculate the quadrant times (the number of seconds spent by the rat in the four quadrants). This trial was started from the E or W starting position in a counterbalanced order in each group.

Infusion Protocols

In the acquisition phase of the experiment, animals were intrahippocampally infused with 1 μl of baclofen (RBI), 0.2 mM, 2 mM, 20 mM or sterilized saline. These doses were selected according to previous studies in which Ramirez and co-workers (1997) observed that 3–5 μM of baclofen given to hippocampal slices in vitro facilitated the induction of LTP, whereas 10–12 μM of baclofen inhibited its induction in dentate gyrus. These doses are in the range used by Burgard and Sarvey (1991; 0.1, 0.5, and 5 μM). We simply used doses 1,000 times smaller for our in vivo studies. In the present experiment all animals received 1 μl of a specific drug dose via a 27 gauge, 25 mm long infusion cannula that protruded approximately 1 mm from the holding cannula (infusion rate 0.5 μl/min). The infusions were made possible using a microinfusion pump (Syringe Pump model 341A, Sage Instruments) that was manually turned on and off. The infusion time was controlled by a chronometer. Animals were then placed in their home cages. One hour after the infusion, the rats were brought back for the behavioral training described above. No drug or saline solution was infused prior to the free swim trial conducted 24 h after the acquisition phase.

Histology

At the end of training, each animal was sacrificed by an overdose ip injection of chloral hydrate. Each rat was then intracardially perfused with normal saline followed by 10% formalin. After fixation, brains were embedded in albumin, and 80 μm serial sections were taken and stained. Locations of the cannulae positions are shown in Figure 1.

Statistics

The data were analyzed by one-way and two-way analysis of variance (ANOVA) with repeated measures where appropriate, followed by the Fisher PLSD test for individual mean comparisons.

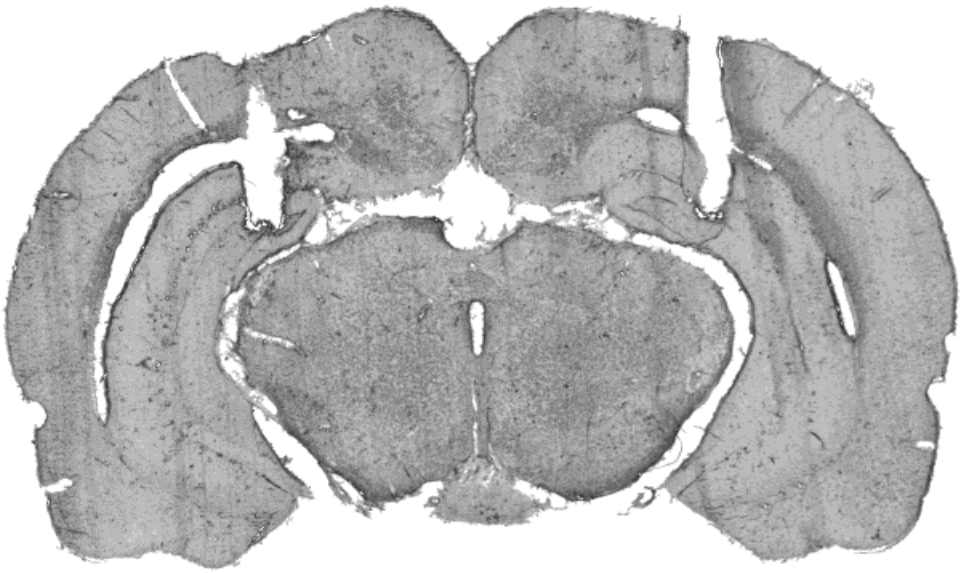

FIGURE 1. Brain scanned picture from a representative animal showing the cannulae location in the area of the hippocampus.

RESULTS

Figure 2 shows the mean escape latencies for control and baclofen (1 μl of 0.2 mM, 2 mM and 20 mM) treated rats. A two-way analysis of variance of the latencies with repeated measures on the trial variable shows a significant drug effect [$F(3,24) = 9.892$, $P < 0.0002$], a repeated measures effect [$F(11,264) = 38.90$, $P < 0.0001$], and a significant drug / trial interaction effect [$F(33,264) = 1.612$, $P < 0.03$]. A post hoc PLSD Fisher test revealed significant differences between saline animals and experimental groups in some trials, as shown in Figure 2.

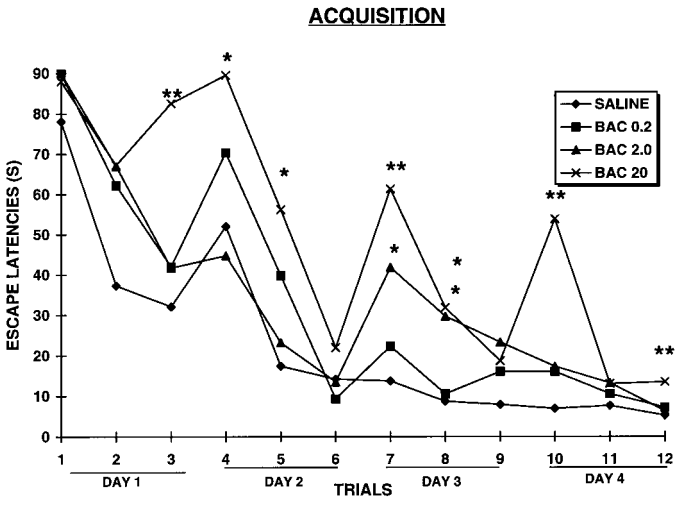

FIGURE 2. Effect of different doses of baclofen in the acquisition of spatial learning. Escape latencies, in seconds, to reach the hidden platform during each training trial (3 trials/day, 4 days of training). SALINE, saline group (n = 10); BAC 0.2, group injected with 1 μl of 0.2 mM baclofen (n = 6); BAC 2, group injected with 1 μl of 2 mM baclofen (n = 6); BAC 20, group injected with 1 μl of 20 mM baclofen (n = 6). Fisher PLSD test for individual mean comparisons: * $P < 0.05$; ** $P < 0.01$.

A free-swim trial was carried out 24 h after the 4-day training period to characterize the baclofen effect further. Animals were released into the pool under no saline or drug injection and in the absence of the escape platform. Although no significant difference between experimental and control rats was observed in the target quadrant time, [$F(3,27) = 0.52$, NS], the time spent by control rats in the target quadrant was significantly longer than the time they spent in any of the other quadrants [$F(3,36) = 8.15$, $P < 0.0005$] (Fig. 3). The time spent in the target quadrant by all baclofen groups (1 μl of 0.2 mM, 2 mM, and 20 mM), was not significantly different compared with the time in any of the other quadrants [$F(3,20) = 1.54$, NS; $F(3,20) = 2.82$, NS and $F(3,20) = 2.05$, NS, respectively]. They searched for the absent platform in a more extended area, including not only the target quadrant, but also both adjacent quadrants. Their search was not as accurate as that of the control rats.

DISCUSSION

The results of the present study demonstrate a dose-dependent impairment in the performance of the Morris navigation task, after intrahippocampal injection of baclofen. The escape latencies of rats that received 1 μl of 0.2 mM baclofen were similar to those of control animals during the 12 training trials. However, rats that received 1 μl of 2 mM baclofen showed a tendency toward longer escape latencies and were significantly different from control animals in trial 7 (the first trial of day 3). Animals that received 1

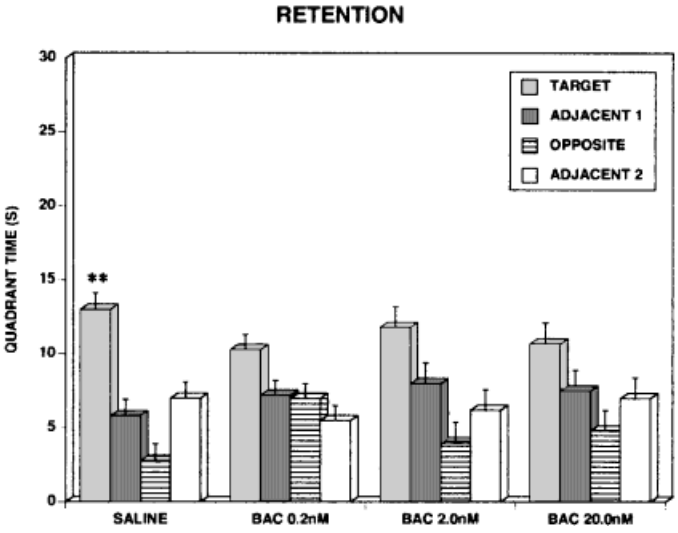

FIGURE 3. Effect of different doses of baclofen on the retention of spatial learning. Time in seconds spent by the animals in each of the four quadrants of the pool during a 30 s trial in the absence of the escape platform performed 24 h after the acquisition phase (QUADRANT TIME). SALINE, saline group (n = 10); BAC 0.2, group injected with 0.2 mM baclofen (n = 6); BAC 2, group injected with 2 mM baclofen (n = 6); BAC 20, group injected with 20 mM baclofen (n = 6). The animals received drug or saline injections (1μl) during the acquisition phase. In the retention phase, no injections were administered. TARGET, quadrant where the platform was located during the acquisition phase; ADJACENT 1, right adjacent quadrant to the target quadrant. ADJACENT 2, left adjacent quadrant to the target quadrant; OPPOSITE, opposite quadrant to the target quadrant. Fisher PLSD test for individual mean comparisons: ** $P < 0.01$.

μl of the higher dose of baclofen (20 mM) into the hippocampal formation, were impaired in their escape latencies on trials 3, 4, 5, 7, 8, 10, and 12. It is interesting to note that all experimental groups showed longer escape latencies than control animals in trials 4, 7, and 10, the first trials of days 2, 3, and 4, respectively. Although not all of them were significantly longer, it seems as if baclofen is affecting primarily the long-term memory of the platform location. The memory of this location seems not to be affected from trial to trial during the day, at least for the baclofen doses of 0.2 and 2.0 mM (short-term memory). A free swim trial carried out after the acquisition phase showed no significant spatial bias toward the target quadrant in all baclofen groups. Even though no significant difference was observed when we compared the time spent in the target quadrant of experimental animals with control rats, it is obviously demonstrated that control animals spent almost 50% of the time searching for the absent platform in the precise platform location. On the other hand, although experimental rats searched in the target quadrant, they also directed their search to both adjacent quadrants. Probably, in a way, baclofen affected the accurate retention of the platform location, allowing only a memory of an extended area where the platform was situated, as these rats used a larger area of searching in which both adjacent quadrants were included. It should be noted that none of the doses of baclofen used in this study were ataxic, as righting reflex and swimming ability were not compromised (data not shown).

Considering that electrophysiologically, low doses of baclofen facilitate hippocampal LTP induction (Burgard and Sarvey, 1991), and high doses block its generation (Mott et al., 1990), our original hypothesis was to demonstrate a dual effect of baclofen on rats' performance in the Morris water maze. Instead, baclofen impaired spatial learning in a dose-dependent manner. The post-synaptic actions of baclofen, on $GABA_B$ receptors, may account for these results, since a hyperpolarization of hippocampal neurons has been described for high doses of baclofen (Dutar and Nicoll, 1988). Presynaptically, baclofen acts on $GABA_B$ receptors. When the action of baclofen is on autoreceptors of hippocampal GABAergic neurons, a decreased inhibition, translated as an excitation, is observed, leading to the facilitation of the LTP induction (Burgard and Sarvey, 1991). Under our experimental conditions, 1 μl of low doses of baclofen (0.2 mM and 2 mM) locally infused in the hippocampus did not improve learning in the water maze, but instead, they impaired the retention of the accurate spatial location of the platform. One explanation for the lack of facilitating effects on the behavioral test may be a decreased induced excitation of hippocampal neurons, other than GABAergic inhibitory ones. An alternative explanation is a probable low sensitivity of the chosen test to demonstrate the improving effects of baclofen low doses. We could probably assume that the lowest dose chosen was not low enough to facilitate learning, but even a dose as low as 0.08 mM used in pilot experiments, did not show any effect (data not shown).

A possible role of $GABA_B$ autoreceptors in certain forms of synaptic plasticity has been demonstrated in the hippocampal formation. In conclusion, our results show an impairing dose-dependent effect of baclofen on spatial learning. Although experimental rats did not show a complete lack of retention of the platform location, they did not recall it as accurately as control animals (free swim trial). We also demonstrate a behavioral correlate of the electrophysiological actions of high doses of baclofen on the modulation of LTP induction in the hippocampus.

Other experiments should be conducted to characterize further the effect of microinfused baclofen in rat hippocampus on the performance of memory tasks such as the Morris water maze.

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