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Baclofen disrupts passive avoidance retention in rats

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Abstract. Baclofen (Lioresal, Ciba-Geigy) is an analog of the inhibitory neurotransmitter GABA and is used clinically to control spasticity. Recent studies have demonstrated that this compound produces a marked inhibition of synaptically evoked responses in area CA3 of the hippocampal slice, suggesting that this drug could influence behavior mediated by the limbic system. In the present study, male rats of the Fischer-344 strain were trained on a one-trial passive avoidance task and tested for retention 1 week later. After the training trial, separate groups of rats received either 5 or 10 mg/kg/4 ml IP of baclofen or the distilled H_2O vehicle immediately, 10 min, or 60 min after training. One week later, the rats that received baclofen immediately after training reentered the test chamber with a significantly higher frequency than controls, although no differences in vacillatory responses were observed between groups. Similar effects were observed following posttrial administration of chlordiazepoxide. In a separate experiment rats were tested for locomotor activity after receiving the same doses of baclofen. Although baclofen decreased activity during a 30-min period after dosing, rats exposed to baclofen showed no significant change in activity relative to controls 1 week later. These data are consistent with the interpretation that baclofen may interfere with memory consolidation or retention.

Key words: Baclofen – Memory – Passive avoidance – GABA receptors

γ-Aminobutyric acid (GABA) is a potent inhibitory neurotransmitter in the central nervous system (CNS). Activation of $GABA_a$ receptors increases chloride conductance, resulting in membrane hyperpolarization and inhibition of the production of the action potential. γ-(p-chlorophenyl)-GABA (baclofen) is an analog of GABA which, although inactive at $GABA_a$ receptors, mimics the effects of GABA on $GABA_b$ receptors (Hill and Bowery 1981). $GABA_b$ receptors have been shown to exist in the mammalian brain and can be differentiated from the bicuculline-sensitive $GABA_a$ receptors (Bowery 1982).

Baclofen has been used clinically as a centrally active muscle relaxant to control spasticity. Considerable evidence exists indicating that baclofen inhibits excitatory neurotransmission in the spinal cord, probably by blocking the release of neurotransmitter from synaptic terminals (Fox et al. 1978; Ault and Evans 1981). Recent work (Ault and Nadler 1983; Blaxter and Carlen 1985) has shown that baclofen has pharmacological activity in the CNS, particularly in the hippocampus. Our previous work has shown, moreover, that baclofen effectively blocks plasticity-related evoked and spontaneous activity in area CA3 of the hippocampal slice (Swartzwelder et al. 1985, 1986). It is significant that this inhibition is accomplished with concentrations of baclofen considerably below those measured in the CSF of patients receiving the drug for the control of spasticity (Knutsson et al. 1974).

The potency with which baclofen attenuates burst firing of CA3 cells led to the hypothesis that this compound may disrupt processes dependent upon the functioning of the hippocampus. To explore this possibility, we designed the present experiments to assess the effects of baclofen upon retention of passive avoidance learning. Deficits in a one-trial inhibitory avoidance task have been reported in animals having loss of CA3 pyramidal or dentate granule cells following trimethyltin (Walsh et al. 1982) or colchicine (Walsh et al. 1986), respectively.

Materials and methods

Subjects and apparatus. Male rats of the Fischer-344 strain (Charles River Breeding Co., Wilmington, Ma.) were housed in groups of four with free access to food and water throughout the experiments. The animals were divided into groups of 12 each for passive avoidance experiments. A two-compartment trough-shaped alley [51 L × 30/6.8 W (top/bottom) × 15 cm H] was used for passive avoidance training and retention testing (see Jarvik and Kopp 1967). The alley was constructed of black Plexiglas and consisted of two compartments, 17 and 34 cm in length. The compartments were separated by a door which could be raised and lowered by a pullchain. A single 15-W incandescent bulb was positioned 10 cm above the lid of the smaller compartment. The floor of each compartment consisted of parallel stainless steel plates, separated along their legnth by 2 cm. Constant current electric footshock (0.3 mA) from a Coulbourn solid state shocker (Model E13–16) was administered through the floor plates of the larger compartment. The passive avoidance apparatus was housed in a larger sound- and light-attenuating chamber, with background illumination and masking noise provided by the chamber exhaust fan.

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Procedure and dosing. On the day of testing, rats were placed into the illuminated compartment facing the door. Ten seconds later the door was raised and the latency to cross from the smaller to the larger compartment was recorded. Contingent on crossing to the darkened compartment within 120 s, the door was closed and footshock was delivered for 1 s. Rats not making this response were not used further.

Rats were removed from the test chamber and given baclofen (Ciba-Geigy Pharmaceuticals) by IP injection in a dose of 5 or 10 mg/kg in a volume of 4 ml/kg body weight. Control animals were given the same volumes of distilled water vehicle. Three separate experiments determined the effect of varying the time between training and injection of baclofen. In these studies, injections were made immediately, 10 min, or 60 min after the training session. In another experiment, rats were given 10 mg/kg chlordiazepoxide hydrochloride, IP, in 1 ml/kg distilled water. This drug was chosen as a comparison compound because it is known to compromise the ability of animals to consolidate memory (Oishi et al. 1972), although it is used clinically for very different purposes than is baclofen. In addition, chlordiazepoxide is thought to have a GABA-ergic component associated with its anxiolytic effects (Tsuchiya and Fukushima 1978; Schlosser and Franco 1979; Valdes et al. 1981). There were 12 rats per group.

Retention was assessed 7 days later using a procedure identical to that for training, except that footshock was not delivered. For up to 300 s, the latency to cross from the small to the large compartment was recorded. In addition, as a measure of vacillatory responding (Mactutus et al. 1982), the total number of head-pokes (head, including ears) and half-crosses (head and two forepaws) were recorded.

Motor activity. As a measure for possible nonspecific effects of baclofen on behavior, loss of righting reflex was assessed approximately 30 min after injection by placing the animals on their backs and observing their ability to right themselves. In order to assess potential residual effects of baclofen injections during training upon the retention test 1 week later, we measured the locomotor activity of rats trained in the passive avoidance apparatus and given baclofen (10 mg/kg) or the distilled water vehicle. Five minutes after injection, the animals were placed individually into one of four rectangular chambers measuring 43 (L) × 21 (W) × 18 (H) cm and having two rows of photodetectors positioned 5.5 or 13 cm from the floor. The lower cells measured horizontally directed motor activity, while the upper detectors measured rearing. Activity was measured for 30 min after injection. The rats were retested for 30 min 1 week later. There were ten rats per group.

Statistical analysis. Latency measures from the passive avoidance task were considered to be not normally distributed and were analysed for overall statistical significance by a Kruskall-Wallace one-way analysis of variance. Differences between groups were assessed using Mann-Whitney *U*-tests when appropriate (Siegel 1956). Data for vacillatory responses were normally distributed and were analysed with parametric analyses of variance followed by pairwise *t*-test where appropriate. Motor activity data were analysed for overall statistical significance using a four (treatment) by two (test day) repeated measures analysis of variance

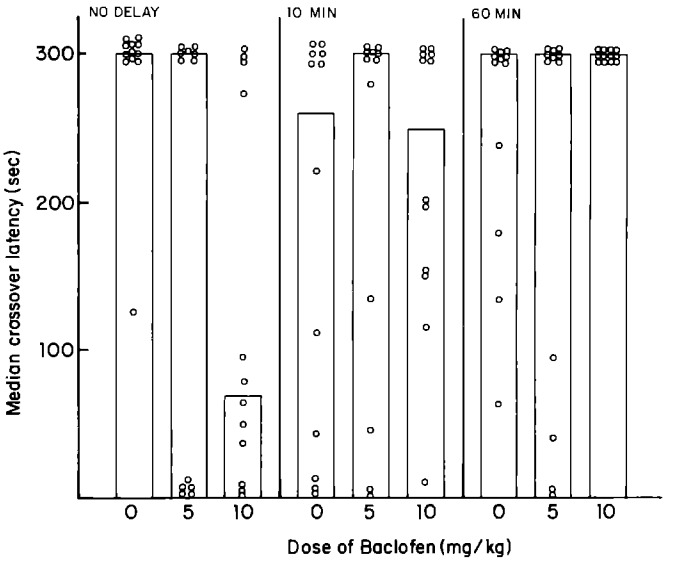

Fig. 1. Effect of baclofen on crossover latencies during retest for passive avoidance performance 1 week after training. Rats were trained in a step-through passive avoidance procedure and given 0, 5, or 10 mg/kg (±)-baclofen, IP, immediately, 10 min, or 60 min after training. Testing occurred 1 week later. Data are median latencies (with individual data points shown) of 12 rats per group

(Winer 1971). Post-hoc comparisons between groups were made using Fisher's Least Significant Difference test.

Results

The animals receiving baclofen immediately after the passive avoidance training trial showed significantly decreased latencies to cross into the larger chamber upon retesting 1 week later, relative to controls ($H=9.5$, $P<0.01$). Mann-Whitney *U* tests indicated that the latencies of rats receiving 5 or 10 mg/kg baclofen decreased relative to vehicle controls ($P<0.05$, Fig. 1). However, there were no significant effects of baclofen upon latencies if injected 10 ($H=0.3$) or 60 ($H=2.7$) min after the training trial. Vacillatory responses did not differ between groups when baclofen was injected immediately after training. However, the rats which received 10 mg/kg baclofen 10 min after training showed fewer nose pokes and partial crossovers during retention testing (Table 1, $P<0.05$). Chlordiazepoxide had a similar effect on passive avoidance, decreasing crossover latencies from 272 ± 13 s for controls to 130 ± 38 for the treated rats ($P<0.05$). Chlordiazepoxide had no effect on vacillatory responses (Table 1).

Although rats treated with baclofen showed suppression of activity in their home cages 30 min after injection, there was no loss of righting reflex. The total number of activity counts obtained 30 min after 0 mg/kg baclofen (106 counts per 30 min ± 27) was significantly depressed relative to controls (1070 ± 70), but upon retesting 1 week later, the animals having received baclofen (1295 ± 110) did not differ from controls (1275 ± 98).

Chlordiazepoxide-exposed animals did not differ significantly from controls.

Discussion

The results of this study demonstrate that baclofen, in doses which leave the righting reflex intact, interfered with the

Table 1. Effects of baclofen and chlordiazepoxide on vacillatory responses 7 days after passive avoidance training

Time after training-treatment	Average responses ± SE[a]	
	Nose pokes	Partial cross overs
No delay		
Control	1.8 ± 0.3	0.7 ± 0.2
Baclofen – 5 mg/kg	2.0 ± 0.6	0.8 ± 0.3
Baclofen – 10 mg/kg	2.0 ± 0.5	1.1 ± 0.4
10 min delay		
Control	2.5 ± 0.4	1.2 ± 0.2
Baclofen – 5 mg/kg	1.8 ± 0.3	1.8 ± 0.6
Baclofen – 10 mg/kg	1.3 ± 0.4[b]	0.5 ± 0.3[b]
1 hour delay		
Control	1.2 ± 0.4	1.3 ± 0.3
Baclofen – 5 mg/kg	1.8 ± 0.4	1.2 ± 0.4
Baclofen – 10 mg/kg	2.1 ± 0.4	0.8 ± 0.3
No delay		
Control	2.0 ± 0.5	1.2 ± 0.4
CDZ – 10 mg/kg	1.7 ± 0.4	0.9 ± 0.3

[a] There were 12 rats per group
[b] Differs significantly from controls ($P < 0.05$)

retention of one-trial passive avoidance. One week after training, baclofen produced a dose-dependent decrease in latencies to enter the side of the test chamber in which the rats had previously been shocked. This response was not associated with changes in vacillatory behaviors, suggesting that prior exposure to baclofen did not alter the reactivity or emotionality of the rats. Baclofen significantly reduced motor activity for up to 30 min after the injection, but this effect had dissipated after 1 week.

Baclofen is known to produce inhibition within the CNS by stimulating the $GABA_b$ receptor (Bowery 1982). At low concentrations, baclofen facilitates a potassium conductance which results in a net postsynaptic hyperpolarization of the neuron. This effect has been demonstrated in hippocampal pyramidal cells in vitro (Newberry and Nicoll 1984, 1985). However, the most thoroughly studied effect of baclofen is the suppression of synaptic transmission. Studies of spinal cord as well as a variety of cerebral regions have demonstrated this effect (Davidoff and Sears 1974; Fox et al. 1978; Ault and Evans 1981; Ault and Nadler 1982). The mechanism of this effect is the inhibition of transmitter release from presynaptic terminals (Fox et al. 1978; Bowery et al. 1980; Olpe et al. 1982), possibly through action at a calcium channel (Desarmenien et al. 1984; Dunlap 1984). This effect occurs at higher concentrations than required for the postsynaptic effects alone. The doses used in the present experiments were sufficiently high to induce this effect.

Of particular relevance to the present results are demonstrations that baclofen potently inhibits the release of excitatory amino acids (Collins et al. 1982; Ault and Nadler 1982; Olpe et al. 1982). One interpretation of our results is that baclofen may reduce the release of such excitatory neurotransmitters involved in the development of memory. The neuroanatomical locus of such an action is, of course, open to speculation, since both $GABA_b$ receptors and excitatory amino acids are distributed widely throughout the brain. However, several lines of evidence suggest the possibility of hippocampal involvement in this process. First, of course, the hippocampus, and other closely connected limbic structures have been implicated in memory consolidation in general and passive avoidance learning in particular (Lidsky and Slotnick 1971; Chung Shin-Ho 1977; Gray and McNaughton 1983). Excitatory amino acids are known to act as neurotransmitters within the hippocampus, and are thought to support plasticity in this area which is related to learning and memory (Baudry et al. 1980; Swanson et al. 1982; Fagni et al. 1983; Wieraszko 1983). Finally, baclofen has extremely powerful inhibitory effects on both the normal activity of hippocampal pyramidal cells (Ault and Nadler 1982; Newberry and Nicoll 1984, 1985) as well as on plasticity-related activity in this structure (Swartzwelder et al. 1986). Thus it seems likely that a decrease in excitatory neurotransmitter release related to activation of the $GABA_b$ receptor by baclofen may account for the ability of this drug to disrupt passive avoidance learning.

It is also possible that baclofen may have interfered with retention of the passive avoidance task by a mechanism tangential to its effect on the $GABA_b$ receptor. One possibility is that baclofen may have interfered with nonassociative physiological processes involved in the development of memory. For instance, it is now widely believed that neurohumoral responses, i.e., release of hormones such as epinephrine, ACTH, vasopressin and glucocorticoids are intricately involved in memory formation (Gold and McGaugh 1977). Such hormones may act by providing an internal cue that contributes to retrieval of memory (McGaugh 1983). Therefore, pharmacological alteration of such neurohumoral influences by baclofen may have contributed to the observed behavioral effects. In fact, studies showing disruption of performance of shock-motivated tasks by post-trial injections of chlorpromazine (Johnson 1969), amobarbital (Steinberg and Tomkiewicz 1968) or secobarbital (Pare 1961) might be explained as a reduction of the arousing events associated with footshock. Such a mechanism could explain the effects of both baclofen and chlordiazepxide upon passive avoidance performance 1 week after training.

The doses of baclofen used in these experiments are significantly higher than those used clinically to control spasticity (see Knutsson et al. 1974), although 5 mg/kg baclofen (IP) has been used to control seizures produced by kainic acid administered intracerebroventricularly (Gruenthal et al. 1984). Therefore, these results do not directly suggest that patients undergoing baclofen therapy at the usual doses would experience consolidation deficits at the magnitude we have observed in this study. However, it should be noted that disruption of passive avoidance retention, as we have used it, is the disruption of memory consolidation for a very extreme situation, viz., a strong footshock in a completely novel environment. Baclofen's disruption of memory for this event at 5 and 10 mg/kg reflects a very strong effect. Although it is not clear that lower doses of baclofen would disrupt passive avoidance learning in the rat, it is possible that therapeutic concentrations could disrupt more subtle and labile processes involved in learning and memory in the human. For example, clinical case reports have shown that memory deficits were induced when baclofen was given in conjunction with pre-existing antidepressant medication (Sandyk and Gillman 1985). When the baclofen treatment was terminated, the memory deficits were ameliorated. Although these reports do not

address the issue of possible drug interaction effects, they do establish some evidence of memory deficit associated with typical therapeutic doses of baclofen. This possibility is further suggested by the fact that hippocampal excitability is makedly decreased by concentrations of baclofen which are below those observed in the CSF of patients undergoing antispastic therapy with baclofen (Knutsson et al. 1974; Swartzwelder et al. 1986).

The results of our experiments suggest that in addition to its antispastic effects, baclofen may have psychopharmacological activity as well. However, additional studies will be necessary to identify the mechanism by which, and extent to which, baclofen affects retention of passive avoidance behavior.

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