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Baclofen prevents rapid amygdala kindling in adult rats

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Abstract. This study investigates the effect of the gamma-aminobutyric acid ($GABA_B$) agonist, baclofen, on amygdala kindling in adult rats. Baclofen has been reported to be anticonvulsant in a variety of seizure models and prevents kindling in immature rats. These experiments describe the effects of baclofen (2, 5 and 10 mg/kg, i.p.) on the afterdischarge threshold and kindling rate. Baclofen, 10 mg/kg, significantly increased the afterdischarge threshold in the amygdala. Baclofen at 5 and 10 mg/kg, retarded the rate of kindling as measured by the number of stimuli required to advance to subsequent seizure stages. These results suggest that baclofen may decrease the local excitability of the amygdala and retard the rate of seizure spread (or generalization) throughout the brain. Baclofen, acting at $GABA_B$ receptors exerts an anticonvulsant effect on amygdala kindling in these experiments.
Key words. Baclofen; kindling; amygdala; $GABA_B$ receptors.

The physiological effect(s) of central gamma-aminobutyric acid ($GABA_B$) receptors are not clearly understood. The $GABA_B$ receptors are located both postsynaptically and as presynaptic receptors which regulate GABA release[1] and cause hyperpolarization of postsynaptic cells by activation of potassium channels[2]. The $GABA_B$ agonist, baclofen, is a muscle relaxant acting at the spinal level[3], yet some of the physological/pharmacological effects of baclofen are poorly understood. The function of $GABA_B$ receptors is unclear, since there is some question whether GABA released from the neuron binds to pre- or postsynaptic receptors[4]. It was previously demonstrated that baclofen can prevent seizures evoked by electrical stimulus (i.e. kindling) of the amygdala in 16-day-old rats[5]. To extend our knowledge of $GABA_B$ receptor function in kindling of adult rats, baclofen was administered to adult rats and kindling performed. Kindling is a model of epilepsy in which three aspects of seizure development may be addressed: local events, seizure propagation and postictal refractoriness[6]. The term kindling refers to the progressive development of behavioral and electroencephalographic signs of seizures, which are elicited by repetitive subconvulsant stimuli[7]. Kindling progresses through a characteristic series of stages which are easily and clearly recognized[8]. Local seizure events are those related to the initiation of seizures at the epileptic focus (i.e. amygdala, in these experiments). An approximation of the local excitability of the amygdala can be obtained by measuring the afterdischarge threshold (ADT) which is defined as the lowest current capable of inducing a high amplitude, synchronized waveform – an afterdischarge (AD)[5]. Seizure propagation (generalization) involves the spread of epileptic activity from the focus to distant sites in the CNS and can be quantified by determing the number of stimuli necessary to progress to each seizure stage as well as the emergence of generalized seizures[5]. Kindling mechanisms are not fully understood, but GABA, the major inhibitory neurotransmitter of the CNS, appears to have an important impact on seizures evoked by kindling and a variety of other seizure models. Drugs which augment brain GABA levels (e.g. gamma-vinyl GABA[9]) and uptake inhibitors (e.g. SKF 89976-A[10]) retard the rate of kindling. Drugs potentiating the function of the $GABA_A$ receptor complex are also reported to retard kindling development[11]. Recently, Karlsson et al., have demonstrated an acceleration of amygdala kindling associated with blockade of the $GABA_B$ receptor by the $GABA_B$ antagonist – CGP 35348 in adult rats[12]. Drugs decreasing GABA synthesis (3-mercaptopropionic acid) or antagonists of the $GABA_A$ receptor (bicuculline) also accelerate kindling[13].

GABAergic compounds have been reported to have anticonvulsant actions in some seizure models[14–16], yet some are reported to be proconvulsant in flurothyl-induced seizures of immature rats[17,18]. Thus, these experiments were carried out to further characterize the role of $GABA_B$ receptors in the kindling model of epilepsy and to evaluate the anticonvulsant potential of baclofen against amygdala kindling in adult rats.

Materials and methods

Male rats obtained from Taconic Farms (Germantown, NY) were housed in the Animal Care Center of St. John's University, an AAALAC accredited facility, in an environmentally controlled room (20–23 °C; 35–55% humidity; 12 hour light:dark cycle, lights on 07:00) with food and water available ad libitum until the day of surgery. Prior to surgery the rats were anesthetized

with an intramuscular injection of ketamine:xylazine (70:7 mg/kg) and placed in a stereotaxic frame (Stoelting) with the skull set flat (incisor bar −0.5 to −1.0 mm). Bipolar electrodes (MS 303/2; Plastics One, Roanoke, Virginia, USA) were placed in the left or right amygdala (15° forward angle; coordinates: A/P +0.05 cm, lateral +0.35 cm, depth −0.98 cm) and two stainless steel screws fixed to the skull. The electrode and screws were secured in place with dental acrylic. After a two week recovery period the rats were used for experiments of kindling.

The ADT was determined as follows: each rat received an initial 30 microampere (μA) stimulus and was stimulated with 30 μA increments/minute until an AD was noted. The stimulus intensity was then reduced by 15 μA and the rat restimulated. The lowest current intensity which elicited an AD was the afterdischarge threshold. The kindling paradigm consisted of stimuli (400 μA; 60 Hz, sinusoidal current; 1 sec duration) delivered hourly up to a maximum of 30 stimulations, or until the rat was fully kindled (3 consecutive stage 5 seizures). The seizure stages evoked by amygdala kindling are clearly defined and easily recognized. They include:

- stage 1, facial movements
- stage 2, rhythmic head movements and turning of the body
- stage 3, alternating forelimb clonus (or unilateral forelimb clonus)
- stage 4, bilateral forelimb clonus, and
- stage 5, bilateral forelimb clonus with rearing and falling[8].

All rats were stimulated over a 5-hour period until the maximum number of stimulations (≤ 30) was reached. Baclofen (the (-) isomer; Sigma Chemical Co., St. Louis, Missouri, USA) or saline was injected intraperitoneally (10 ml/kg) at doses of 2, 5 or 10 mg/kg to separate groups of rats randomly selected following electrode implantation. Injections were made 1/2 hour prior to delivery of the first kindling stimulus of each experimental regimen. All rats were observed prior to and during the experimental period in order to determine what (if any) behavioral effects may be elicited by baclofen at the doses used in these studies.

Electrode placement in the amygdala was confirmed by inspection of stained histological sections. Frozen sections of the brain (20 micron) were made on a freezing microtome (Slee–Hacker Instruments, Hackensack, New Jersey, USA) and stained with thionin. Electrode placement was confirmed by microscopic examination of brain sections. Any animal in which placement of the electrode in the amygdala was questionable or incorrect was not included in the analysis of the data. The failure rate for electrode placement was approximately 15% of all animals implanted. Data is expressed as the mean ± SE and was compared by analysis of varience (ANOVA) with post hoc testing for significance (Newumann–Keul's test). Probabilities of $\leq 5\%$ were considered statistically significant.

Results

Baclofen increased the afterdischarge threshold of the amygdala and retarded the rate of kindling. The ADT was elevated by baclofen in a dose-dependent manner (fig. 1). The increase in ADT was statistically significant in rats receiving 10 mg/kg (189 ± 3.7 μA; $n = 7$; $p < 0.05$, ANOVA), baclofen – 2 mg/kg and 5 mg/kg increased the ADT, although these increases were not significant (165 ± 5.1 μA; $n = 8$ and 178 ± 4.1 μA; $n = 7$, respectively). The ADT for vehicle-infused (saline) control rats was 156 ± 3.9 μA ($n = 6$). Baclofen 5 and 10 mg/kg increased the number of stimulations required to achieve increasing stages of seizure severity during kindling (fig. 2). Baclofen 5 mg/kg, i.p. significantly increased the number of stimuli required to achieve seizure stages 3, 4 and 5 as compared to vehicle-infused rats (Bac 5 ($n = 8$) vs control ($n = 9$); $p < 0.05$, ANOVA). Baclofen 10 mg/kg, i.p., significantly increased the number of stimulations required to reach seizure stages 2, 3, 4 and 5 as compared to controls (Bac 10 ($n = 7$) vs control; $p < 0.05$, ANOVA). Baclofen 2 mg/kg, i.p., ($n = 6$) did not increase the number of stimulations required to progress through the seizure stages as compared to vehicle-infused control rats.

Baclofen (2, 5 and 10 mg/kg, i.p.) did not adversely affect the animals. Except for slight sedation at the 5 and 10 mg/kg doses the rats were freely moving and

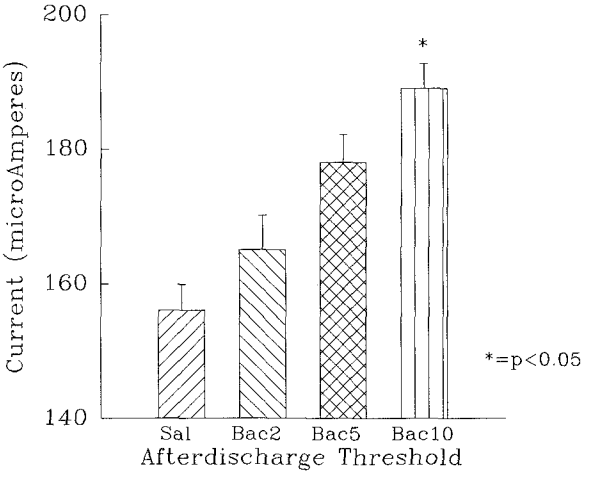

Figure 1. Afterdischarge threshold of the amygdala in adult rats. Baclofen (2, 5 or 10 mg/kg) or saline were administered i.p. to separate groups of rats and the ADT determined as described in 'Materials and methods'. Baclofen appeared to increase the ADT in a dose-dependent manner. The baclofen – 10 mg/kg dose significantly increased the ADT as compared to saline-injected control rats; $p < 0.05$ (one-way ANOVA, Neumann-Keul's post hoc test for significance). Number of rats/group: saline, $n = 6$; baclofen 2 mg/kg, $n = 8$; baclofen 5 mg/kg, $n = 7$; baclofen 10 mg/kg, $n = 7$.

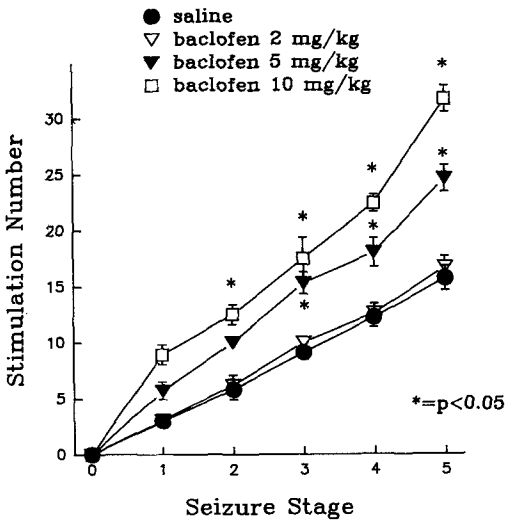

Figure 2. Rate of kindling in adult rats. Baclofen (2, 5 or 10 mg/kg) or saline were administered i.p. prior to the kindling paradigm. Baclofen, 5 mg/kg, significantly increased the number of stimulations required to reach stage 3, 4 and 5 seizures. Baclofen, 10 mg/kg, significantly increased the number of stimulations required to reach stage 2, 3, 4 and 5 seizures. Data were analyzed by a one-way ANOVA-repeated measures with Neumann-Keul's post hoc testing for statistical significance. Probabilities of 5% or less were considered significant. Number of rats/group: saline, n = 9; baclofen 2 mg/kg, n = 6; baclofen 5 mg/kg, n = 8; baclofen 10 mg/kg, n = 7.

responsive to their environment; sedation was judged subjectively. Thus, muscle relaxation or motor incoordination do not appear to be factors which might influence these results.

Discussion

These studies demonstrate an anticonvulsant effect of baclofen on kindling in adult rats and are in agreement with earlier studies performed in immature (16-day-old) rats[5]. Baclofen has been reported to be either anticonvulsant or proconvulsant in a variety of seizure models. In rodents, a number of studies have described an anticonvulsant effect on chemically induced seizures, including: thiosemicarbazide, pentylenetetrazole, isoniazid, hyperbaric O_2 and muscimol-induced myoclonic jerks in mice[3,19–22], allylglycine, 3-mercaptopropionic acid and kainic acid in rats[19,23–25]. Baclofen has been reported to exacerbate spontaneous generalized spike-wave discharges in epileptic rats[26], and to enhance myoclonic jerks in 'well-kindled' adult rats[27]. The paper of Cottrell and Robertson[27] describes a proconvulsant effect of l-baclofen on cysteamine-induced myoclonic jerks when injected intraperitoneally in previously kindled rats. These authors also found a convulsant effect of baclofen when administered alone to the kindled rats. The apparent epileptic effect of baclofen may be related to the previous electrical kindling of these animals. Our results with baclofen are in close agreement with Karlsson et al.[12], who obtained similar results using a single, i.p., dose of baclofen (6 mg/kg). The only differences in these studies are that the present study used an hourly stimulus to induce kindling, while Karlsson et al., stimulated their animals once daily.

Amygdala kindling allows two processes to be studied: local seizure activity and propagation (or spread). Stage 1–2 seizures are local seizures confined to the amygdala, while stage 4–5 seizures are generalized convulsions[28]. Thus the kindling rate reflects the rate of seizure genesis and propagation throughout the brain. This study demonstrates that baclofen retards the rate of kindling by increasing the number of stimuli required to enter seizure stages 3, 4 and 5. This suggests that baclofen (in adult rats) may retard the generalization or spread of epileptic activity from the focus throughout the brain. Baclofen, at 10 mg/kg, increased the ADT as compared to saline-infused controls suggesting a decrease in the local excitability of the focus (i.e. amygdala). These results taken together suggest baclofen reduces both the spread of epileptic discharges in the brain, as well as reducing the local excitability of the amygdala, the site from which the seizure arises.

The difference in the doses which are effective at retarding kindling rate in immature rats[5] and adult rats may be explained by several factors (age, weight, developmental, pharmacokinetic or pharmacodynamic). An interesting possibility is that this difference may be due to differences in GABA receptor expression in adult and immature rats. $GABA_B$ receptor number (B_{max}) in the substantia nigra is greater in immature rats than adults[29]. Previous work has shown an age-related difference in $GABA_A$ receptors in the substantia nigra of immature rats[30]. Both populations of GABA receptors display differences in ontogeny which may influence GABAergic function of the substantia nigra. The substantia nigra is critical to the control and expression of generalized seizures[31], and is an important site controlling the expression of kindled seizures[32,33]. Baclofen infusions into the substantia nigra of immature rats exerts an anticonvulsant effect on fluorthyl-induced generalized seizures[14], thus baclofen may elicit its anticonvulsant action (at least in part) at the substantia nigra. Baclofen exerts an anticonvulsant effect on kindling in adult animals, but appears to require higher doses to display anticonvulsant effects similar to those seen in immature animals.

In conclusion, baclofen has an anticonvulsant effect in the kindling model of epilepsy in adult rats. The dose of baclofen required to exert this effect is greater than in immature rats and may reflect age-related differences seen in $GABA_B$ receptors including those found in the substantia nigra.

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