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Effect of an Adenosine A_1 Agonist Injected Into Substantia Nigra on Kindling of Epileptic Seizures and Convulsion Duration

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HERBERG, L. J., I. C. ROSE AND M. MINTZ. *Effect of an adenosine A_1 agonist injected into substantia nigra on kindling of epileptic seizures and convulsion duration.* PHARMACOL BIOCHEM BEHAV 44(1) 113–117, 1993.—The substantia nigra pars reticulata (SNr) has been reported to be critically involved in the development and propagation of epileptic seizures, while extracellular adenosine appears to be important for making seizures stop. In the present study, an adenosine A_1 receptor agonist [N^6-cyclohexyladenosine (CHA); 2.0 nmol/side, or vehicle] was injected bilaterally into the SNr shortly before each of the first five of a series of daily kindling stimuli delivered to the rat amygdala. Injections did not affect the acquisition of kindled afterdischarges or the rate at which seizures developed over subsequent kindling sessions, but convulsions occurring 48–72 h after treatment were significantly shortened. Thus, purinergic mechanisms in the SNr do not appear to be specifically involved in the acquisition of kindled seizures but may contribute to a postictal inhibitory process that shortens the convulsive component.

Adenosine analogs Convulsions Cyclohexyladenosine Epilepsy Kindling Postictal inhibition Substantia nigra

THE substantia nigra pars reticulata (SNr) is thought to play a crucial role in controlling the development and propagation of cerebral epileptic activity (26,28,29,32). It is the only midbrain structure that increases its glucose uptake after kindled convulsions (13), and it is also an especially effective target for anticonvulsant agents injected intracranially (20,21,23,25). However, the interpretation of these findings is not straightforward, lesion studies have been inconclusive [cf. (24,25)], and the nature of the actual role played by the SNr—inhibitory or disinhibitory—remains controversial [cf. (26,29)].

Anticonvulsant agents that have been investigated by injection into the SNr are, for the most part, compounds affecting glutamatergic or GABAergic transmission. Other studies, however, have recently suggested that heightened concentrations of extracellular adenosine, derived in part from the breakdown of adenosine triphosphate (ATP) in actively discharging neurons (40), may play an important role throughout the brain as an endogenous anticonvulsant, serving to prevent the recurrence of seizures or reduce their severity (10). Systemic injections (12,31,34,41) or intracranial injections (6,8,15) of metabolically stable adenosine analogs have been shown to inhibit the expression of seizures in a number of different experimental models. Adenosine antagonists, such as theophylline or caffeine, in general have the opposite effect (3,4,27).

An interesting question not yet looked at is whether intranigral injection of adenosine A_1 agonists, recently shown to attenuate established tonic-clonic seizures when produced by electroconvulsive shock (8), might inhibit also the acquisition of seizures through kindling. In the kindling procedure, a daily electrical stimulus, initially without overt effect, leads to progressively longer local afterdischarges, culminating in generalized tonic-clonic convulsions at each trial (16). Kindling also gives rise to a transient inhibitory process that tends to oppose seizure activity (39). The inhibitory component is especially prominent if kindling stimuli are given at brief (<60 min) intervals (2), after which it may be impossible to elicit seizures or resume successful kindling for several days (30,38).

In the present study, we compared the progress of kindling in two groups of rats, pretreated either with bilateral intranigral injections of an adenosine analog or with vehicle for the first five kindling sessions. Although the early kindling sessions of a series are in general without obvious convulsive effect, they cumulatively lead to increasingly pronounced epileptic manifestations in the sessions that follow; we examined whether the cumulative build-up from the first five sessions

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could be suppressed or delayed by intranigral injections of N^6-cyclohexyladenosine (CHA).

METHOD

Subjects

Male hooded rats (type PVG, Bantin & Kingman Ltd., Hull, UK) weighing 250–300 g were anesthetized with pentobarbitone (55 mg/kg, IP) and implanted with twisted bipolar stainless steel electrodes (Plastic Products Co., Roanoke, VA) aimed at the central amygdala, and with bilateral 21-g guide cannulae (Plastic Products) directed toward the substantia nigra. Electrode coordinates relative to bregma were A-1.0, 4.5 lateral, and 8.6 mm deep, with the incisor bar fixed 5.0 mm above the interaural plane (33). The guide cannulae were angled 15° rostrally to avoid the electrode assembly, and entered the skull 6.0 mm posterior and 2.5 mm lateral to bregma, reaching a nominal 5.0 mm below the skull surface. During injections, 28-g injection cannulae projected about 3.0 mm beyond the guide cannulae to terminate in the SNr. At the end of the experiment, 0.5 μl 0.5% pontamine sky-blue was injected through the cannulae under terminal anesthetic, and cannula and electrode coordinates were verified on photographic projections of 50-μm frozen sections.

Kindling

Kindling stimulation took place in a transparent observation cage with an electrically grounded conductive floor. Afterdischarge thresholds were not measured before commencement of kindling because it was necessary to ensure that initial kindling stimulations were uniformly covered by treatment with drug or vehicle. A maximally suprathreshold stimulus (a 200-μA constant-current 1-s 50-Hz sinewave train) was therefore used for kindling. This current intensity was well in excess of the initial afterdischarge thresholds recorded under previous similar conditions, which in general ranged between 8.0 and 50 μA. Kindling with maximal current intensities will be relatively insensitive to anticonvulsant agents that act primarily by elevating seizure thresholds; however, the antikindling action of adenosine does not appear to depend upon threshold changes (4,36). Seizure intensity was graded on Racine's standard six-point scale (35): 0, no response; 1, facial movement; 2, head nodding; 3, forelimb clonus; 4, clonus plus rearing; 5, clonus, rearing and falling. The duration of limb clonus, if present, was timed by stopwatch. Electroencephalographic activity was recorded through the implanted electrodes immediately before and after stimulation. Afterdischarge duration (ADD) was defined as the duration of poststimulation spike-trains of more than twice baseline amplitude and faster than 1 Hz. Sporadic interictal spikes, wet-dog shakes, and secondary afterdischarges were disregarded.

Drug Treatment

CHA (Sigma Chemical Co., St. Louis, MO) was dissolved in isotonic saline and injected bilaterally by means of a Harvard Apparatus dual infusion pump (Harvard Apparatus, Reno, NV) connected to the injection cannulae with flexible tubing. Each injection was 0.5 μl in volume, delivered at a rate of 0.25 μl/min. Injection cannulae were left in situ for a further 2 min after completion of injection. The dose of CHA injected (2.0 nmol/side) was selected after preliminary experiments had shown behavioral depression, hypotonia, and ataxia following a 10-nmol dose. The dose selected did not produce obvious behavioral effects, but systematic behavioral testing was not undertaken.

Procedure

Rats were divided arbitrarily into two equal groups. Kindling stimulation was administered to both groups at 24- or 48-h intervals six times per week. The first five kindling sessions were preceded according to group by intranigral injections of CHA (CHA group, $n = 7$) or saline (NaCl group, $n = 7$). Kindling stimulation was administered 5 min after injection. Daily kindling was continued until at least one stage 5 seizure had been elicited, but for not less than 12 sessions.

RESULTS

In the first five kindling sessions, both groups developed characteristic twitches of mouth or head (stages 1 and 2) (Fig. 1). Both groups also showed a closely parallel increase in mean afterdischarge durations (Fig. 2). Little clonic activity was evident in the first five sessions, apart from two control rats that developed incipient forepaw clonus at the end of this period (Fig. 3); at this stage, most rats gave zero scores for clonus, and group differences in the incidence or mean duration of clonic activity did not approach statistical significance [Fisher's exact probability $p = 0.23$; Mann-Whitney U (7, 7) = 17.5, $p > 0.38 < 0.46$]. There was thus no evidence of immediate antikindling activity by the intranigral CHA injections during the 5-day treatment period.

In the seven ensuing sessions, all rats went on to develop full stage 5 seizures (bilateral tonic-clonic convulsions with rearing and falling), and the number of kindling stimuli needed to reach or pass each of the five stages en route were similar in the two groups (Fig. 1). Afterdischarge durations were slightly shorter in the CHA-treated group in the 7 days following treatment (Fig. 2), but the difference was not significant [analysis of variance (ANOVA) treatment × session interaction, $F(6, 72) = 0.19, p > 0.90$].

The only feature that clearly distinguished the two groups was the duration of the clonic activity that developed in sessions subsequent to the last injection (Fig. 3). A split-plot repeated-measures ANOVA for all seven posttreatment sessions showed a highly significant treatment × session interaction, $F(6, 72) = 3.4, p < 0.01$. This was due to a significant reduction in clonus duration in the CHA-treated group in sessions 7 and 8 [$F(1, 84) = 8.2$ and 6.3, $p < 0.01$ and $p < 0.025$, respectively]. By session 9, the CHA group had caught up with the control group, and the drug treatment had no apparent effect on the later seizures of fully kindled rats. Thus, the effect of intranigral CHA was to shorten the clonic component in sessions 48–72 h after the last injection.

Histology

The tips of the stimulating electrodes in both groups were clustered between coronal planes A −0.4 and A +1.2 in the anterior, lateral, or basolateral nuclei of the amygdala or in an area within the amygdaloid complex between these nuclei (33). All rats included in the study showed the presence of injected dye bilaterally within the substance of the SNr. Dye was in general also seen extending up the cannula track.

DISCUSSION

Intranigral injections of CHA failed to affect kindled afterdischarges or seizure progression, unlike GABAergic agents injected intranigrally in previous studies (21,23). The SNr has

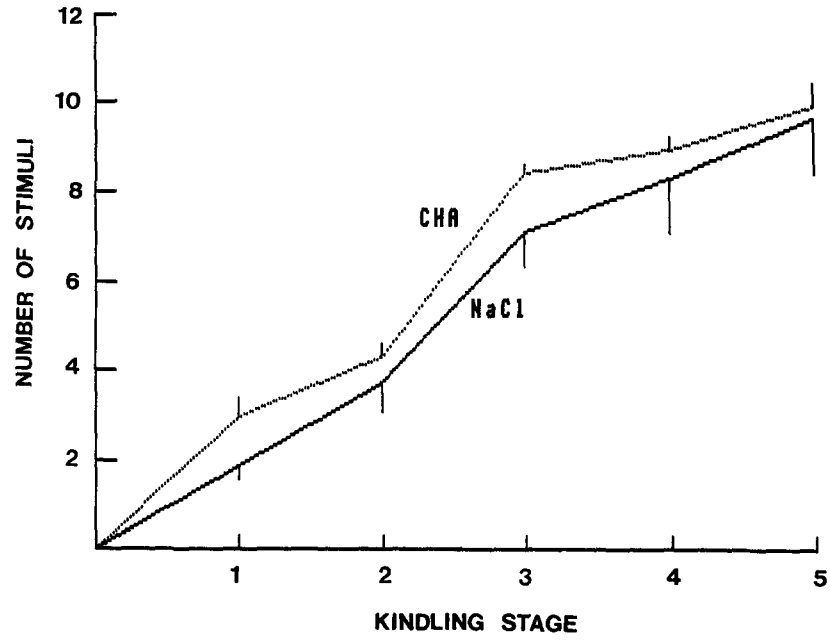

FIG. 1. Mean number of kindling sessions required to reach successive seizure stages in groups treated with intranigral injections of N^6-cyclohexyladenosine (CHA) (n = 7) or NaCl (n = 7). Vertical bars indicate SEs.

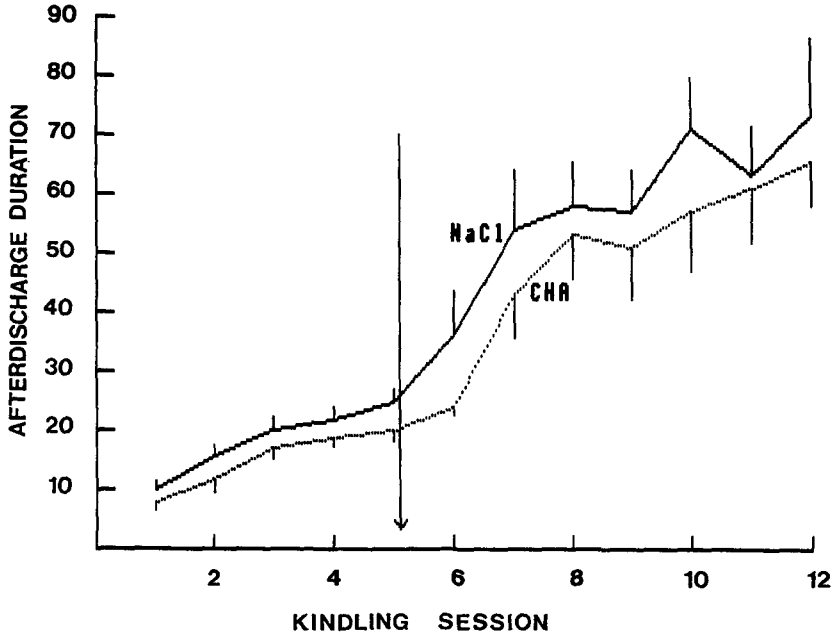

FIG. 2. Mean duration (±SE) of electroencephalograph (EEG) afterdischarges recorded from the stimulating electrodes in 12 consecutive kindling sessions. The first five sessions, shown to the left of the vertical line, were each preceded by intranigral injection of N^6-cyclohexyladenosine (CHA) or NaCl, according to group.

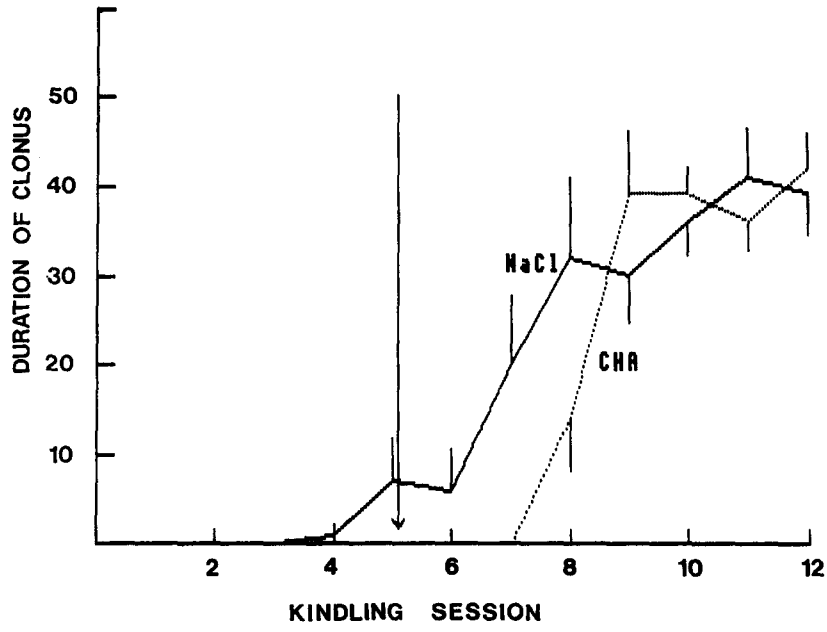

FIG. 3. Mean duration (±SE) of clonic movements (stages 3–5) in successive kindling sessions. Sessions with seizure activity less severe than stage 3 were assigned a zero score. Other details as in Fig. 2.

been reported to contain only a "low" concentration of A_1 receptors [cf. most other brainstem regions with "very few" (14)], but this is unlikely to have been the reason for our failure to affect kindling because intranigral injection of an A_1 agonist, 2-chloroadenosine, has previously been shown to be effective against electroconvulsive shock (8). Nor is tolerance to repeated injections of CHA likely to have played a significant role because repeated systemic or intraventricular injections of adenosine analogs appear to have retained their anticonvulsant properties in previous studies (6,11). Our 2.0-nmol doses of CHA are unlikely to have been too low because CHA is comparable in potency to 2-chloroadenosine (31), which has displayed significant anticonvulsant activity in the SNr even in picomole doses (8). Further evidence for the potential efficacy of intranigral CHA in the present study was the significant shortening of convulsions that occurred 48 and 72 h after the last injection [a more immediate anticonvulsant effect, as seen in previous studies (8), could not be seen here because our injections were restricted to the early, nonconvulsive sessions].

Thus, intranigral CHA injections appeared to affect subsequent convulsions but not the acquisition of kindling. A similar specificity in the effects of adenosine has been reported in previous studies: adenosine agonists or antagonists were found to affect the duration of convulsions or the postictal refractory period (3,4,36,42) rather than seizure threshold or the number of kindling sessions needed. These findings are consistent with the suggestion by Dragunow and Goddard (11) that each seizure itself releases adenosine that serves to end it. The necessary surge in extracellular adenosine is initiated within seconds of seizure onset (43) and may activate inhibitory pre-, post-, and extrasynaptic receptors (37) on cholinergic and glutamatergic neurons (7,18).

But, this short-term feedback process is over in only a few minutes (22) and cannot easily account for the delayed effects seen 48–72 h after CHA in the present study. Nor could it account for the long-term inhibitory state typically produced by massed kindling (2,30,38). Other changes, lasting longer than a few days, initiated or modulated by extracellular adenosine include long-term potentiation (5,9), neuronal differentiation with neurite outgrowth (1,17), and the induction of enzymes involved in neurotramission (17,19). The induction of long-term inhibitory processes by such means following intranigral injection of adenosine analogs would be consistent with the suggested role of the SNr as an endogenous mechanism inhibiting seizure expression (28,29).

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