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Effect of Nootropic Solcoseryl® on Kainic Acid-Induced Excitotoxic Brain Injury

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MINTZ, M., B. KNOWLTON AND M. S. MYSLOBODSKY. *Effect of nootropic Solcoseryl® on kainic acid-induced excitotoxic brain injury.* PHARMACOL BIOCHEM BEHAV **45**(1) 55–58, 1993.—Solcoseryl® (S) has been shown to provide significant cytoprotection in a variety of models of cerebral hypoxia. In the present study, we quantified the epileptiform effects caused by kainic acid administered into the pontine reticular formation of rats and their response to S pretreatment. Compared to saline, the agent appeared to significantly reduce the mortality of rats in the course of status epilepticus. However, S-pretreated rats manifested an increased incidence of behavioral seizures. This untoward effect is attributed to the fact that S improves the functional potential of injured tissue and retards the period of metabolic exhaustion at a time when neuronal activity should be minimized.

Kainic acid Pontine reticular formation Status epilepticus Excitotoxic lesion Mortality Solcoseryl®

SOLCOSERYL® (S), a hemodialysate of calf blood that contains low-molecular-weight blood components such as amino acids, oligopeptides, nucleosides, and glycolipids, was shown to offer a significant cytoprotection in cerebral hypoxic episodes (24). Specifically, the agent was shown to increase cerebral blood flow (12), inhibit the development of cytotoxic (6-aminonicotinamid-induced) brain edema (9), and antagonize the decrement of glucose utilization in limbic areas (cingulate cortex, amygdala, and septal nuclei), the basal ganglia, and the thalamus following stroke in stroke-prone spontaneously hypertensive rats (24). Further, S appeared to reduce the formation of free radicals. It had a significant inhibitory effect on the activity of the archidonic cascade (22) and the production of lipid peroxides (16). A similar preparation, Actovegin®, was reported to increase cerebral metabolism depressed by alcohol (8) and barbiturates (1) and reduce the detrimental effects of an ischemic episode to cortical neurons (7). Actually, in the hippocampus the ischemia-related reduction of adenosine triphosphate (ATP) and CrP is counterbalanced by Actovegin (7).

In the present study, we examined the behavior of rats after kainic acid (KA) administration into the pontine reticular formation (PRF) and the response of KA-induced behavior to S pretreatment. KA is an excitotoxic analog of glutamate that affects cell bodies at the injection site but leaves fibers en passage relatively intact (5). Unlike other excitotoxins, KA injections also cause neuronal death in sites remote to the injection area. The main feature of such remote lesions is that they are limited to specific brain sites while the rest of the brain remains intact as though being immune to the neurotoxin-induced seizures (3). The excitotoxic and behavioral consequences of KA injection might be alleviated by S. Indeed, stroke-induced deficits in glucose utilization in the PRF were robustly alleviated by S (24).

METHOD

Subjects and Protocol

Male Wistar rats (n = 124) weighing 250–400 g were housed individually. Purina chow and tapwater were provided ad lib throughout the experiment. Rats were randomly allocated to either a group pretreated with S (2 ml/kg/day, IP, Solco Ltd., Basel, Switzerland) or to a control group that received an equal volume of physiological saline (IP) for 3 days. The last pretreatment injection took place 5–11 h before the intracerebral KA injection and was discontinued thereafter.

Intracerebral KA Injection

Rats were anesthetized with halothane (5% induction and then 2% maintenance dose). Using a Hamilton syringe (Hamilton Co., Reno, NV) with its opening directed caudally, KA (1.5 nmol in 1 μl 0.9% saline) or saline were injected (0.2 μl/min) into the PRF [coordinates: AP = 0.4, ML = 1.5, DV = 8.3 mm; (18)]. The cannula was left in place for another 1 min before removal.

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Behavioral Observations

Upon waking from anesthesia (approximately 30–60 min following KA injection), the rats were returned to their original cages and their behavior (mainly circular locomotion) and seizures were noted until cessation of epileptiform activity. In a group of 25 randomly selected rats, both locomotion and seizures were quantified in activity bowls (6). Each bowl was positioned on six microswitches along its circumference that responded to the animal's gyration, and the depressions were stored as counts by a CED 1401 (Cambridge) interface to an Olivetti PC. The system was calibrated to count only gross movements and/or locomotion and was not sensitive to stereotyped movements and localized myoclonus not associated with locomotion. Off-line time-domain analysis of the count pattern was used to extract the movements associated with gross seizures. A seizure event was operationally defined as a fast movement resulting in a burst of at least three counts appearing within a 3-s interval and followed by at least 10 s of postictal immobility. A pilot study confirmed that the number of visually observed behavioral seizures faithfully corresponded to the calculated number of seizure events.

Histology

Three weeks after surgery, rats were transcardially perfused with 0.9% saline followed by 10% formalin. Brains were removed and postfixed in 30% sucrose and 10% formalin solution. Postfixed brains were sectioned coronally through the entire anterior–posterior extent of the lesion site in the pons. The thionine-stained sections were examined microscopically for placement of the injection cannula track and the extent of the lesion.

RESULTS

PRF Lesion

Upon microscopic inspection, KA injections could be easily identified due to unilateral neuronal loss and perifocal gliosis (Fig. 1). Complete loss of neuronal somata was confined to the posterior part of the PRF pars oralis. Tissue surrounding this area was characterized by an extensive reduction in neuronal somata as compared to the contralateral spared structures. Lateral parts of the PRF (oral and caudal segments) were spared. There was a visible medial diffusion of KA that never encroached upon the nucleus of raphe or the most medial 0.5 mm of the PRF. Dorsal diffusion of KA was evident along the cannula track that passed through the colliculi. Ventrally, the lesion terminated above the pontine nucleus or superior olive. Because the cannula opening was directed posteriorly, diffusion of KA toward the anterior structures was limited so that the substantia nigra (SN) and retrorubral nucleus were spared in all animals. In its posterior extent, the lesion included the PRF caudalis but did not reach the level of the facial nerve. Microscopic observation did not reveal any major differences in the extent of the anatomic lesion between S- vs. saline-pretreated animals.

Mortality Due to KA-induced Status Epilepticus

Of 124 animals administered KA to the PRF, 10 (8.1%) died in the course of status epilepticus within the first 12 h after surgery. Table 1 shows that mortality was higher in saline-pretreated rats as compared to rats pretreated with S. This difference in rate of mortality appeared significant (χ^2 = 4.14, $p < 0.05$).

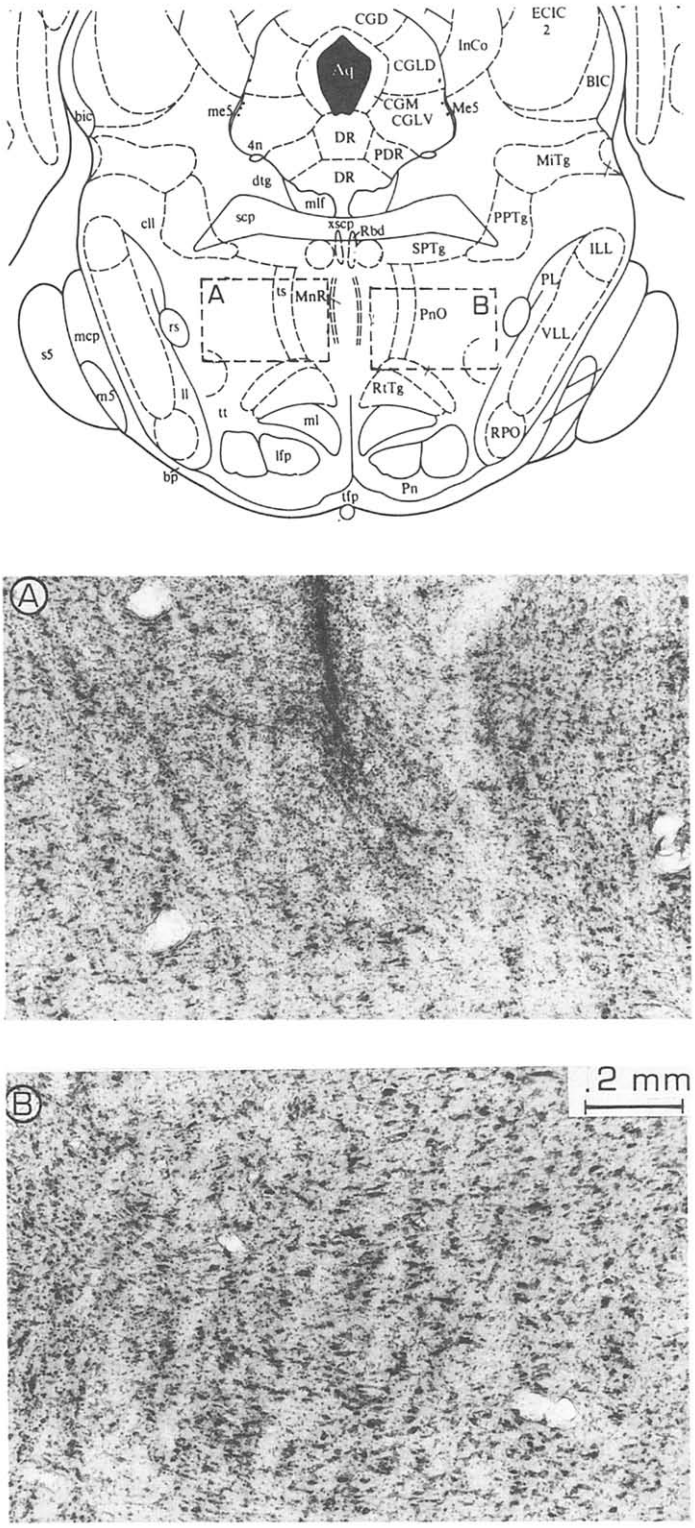

FIG. 1. Kainic acid (KA) lesion in rat pretreated with saline. Schematic drawing of a transversed section through the pontine reticular formation showing the location of the photomicrographs of thionine-stained brain sections at the side of KA ejection (A) and on the contralateral spared side (B).

TABLE 1

MORTALITY RATE, LOCOMOTION, AND SEIZURES SCORES (±SEM) AFTER KA-INDUCED LESION IN THE PONTINE RETICULAR FORMATION IN RATS PRETREATED WITH EITHER S OR SALINE (IP)

	Solocoseryl	Saline
Mortality	2/63*	8/61
	3.2%	13.1%
Locomotion	1,057	415
	(±293)	(±157)
	(n = 13)	(n = 12)
Seizures	15.8†	5.0
	(±6.8)	(±1.6)
	(n = 13)	(n = 12)

The seizure score was derived by isolation of bouts of fast locomotion (see the Method section).

*$\chi^2 = 4.14, p < 0.05$.

†$t(23) = 2.11, p < 0.05$, test was performed on log-transformed seizure scores.

Acute Behavioral Effects of KA Injection into the PRF

All animals showed a similar pattern of recovery from anesthesia consisting of a general state of quiescence and rigid head-trunk deviation toward the side of KA injection. Occasionally, rats performed short-lasting bouts of ataxic locomotion or climbing when they pushed themselves against the cage wall, climbed on it, and then froze in the climbing posture. The periods of quiescence were punctuated more frequently by episodes of ictal behavior comprised of pinna twitches, myoclonus of the oral and neck musculature, and tremor or clonus of forelimbs. On other occasions, rats exhibited "barrel rotation" or coordinated rotation (running gyratory fits) directed toward the side of KA injection, culminating in seizures and followed by postictal immobility. Although epileptiform events were spontaneous, they could have been associated with uncontrolled noise because sensory and auditory stimuli delivered to an immobile animal easily triggered seizures up to 4 h after KA injection. These seizures, as well, were terminated by postictal exhaustion as evidenced by immobility and a loss of the righting reflex. No seizure behavior was recorded 1 day after surgery while postural asymmetry returned to normal within 1–4 days after KA lesion. A tendency for spontaneous contralateral rotation remained for 1–6 days.

Despite the observers' inability to note qualitative differences in acute behavioral effects of KA between S- and saline-pretreated rats, the quantitative score of the activity of the two groups differed in the automatic activity bowls. Activity was scored until cessation of seizures but not less than for 3 h after KA injection. Following cessation of seizures, rats stayed basically motionless for several hours. Table 1 reveals that S-pretreated rats had enhanced overall motility, albeit it appeared nonsignificant when compared to the saline-pretreated group ($p = 0.28$). When locomotion associated with seizures was isolated from nonepileptiform locomotion, a significant increase of seizure events in S- compared to saline-pretreated rats was observed, $t(23) = 2.11, p < 0.05$.

DISCUSSION

The present findings indicate that rats with excitotoxic injury of the PRF responded to S pretreatment with enhanced locomotion and a reliably augmented seizure score. Given that the latter was derived from the pattern of locomotion, one cannot rule out the possibility that occasional cases of excessive or repeated hyperactivity were misclassified as convulsions. Notwithstanding this, arterial hypotension, hyperthermia, respiratory problems, etc. that must have been associated with enhanced locomotion did not seem to compromise the "central metabolic tone" to the extent that S would contribute to an increased mortality rate. If anything, there was a reliable reduction of mortality under S. This dissociation is consistent with the cytoprotective potency of the agent. It can be attributed to its beneficial action on peripheral circulation via reducing blood viscosity and increasing erythrocyte deformability (11) or other factors mentioned above (see the introductory section) regarding the life-protective effects of S in conditions associated with a drastic increase of metabolic demands. Therefore, the question posed by this study is this: What might be the mechanism via which a nontoxic agent, long known to improve functions of hypoxic tissue, can cause a facilitation of seizure behaviors?

Only a preliminary answer to this question is available. The cellular injury caused by excessive neuronal activity following KA is attributed to a cascade of pathobiochemical processes. The central one is a mismatch between excessive metabolic demands and insufficient blood supply that leads to hypoxia. Hypoxia sets in motion a chain of events involving the release of excitatory amino acids, elevation of free Ca^{2+}, depletion of cellular ATP stores, enhanced lactic acidosis, enhanced oxygen free radical formation, increased permeability of the blood–brain barrier, and edema, which all promote cell injury (2,21,23). In stressed animals, glucocorticoids may further exacerbate injury associated with transient ischemia (19), more likely to occur in waking animals exhibiting grand mal convulsions (14). It would be interesting to explore whether S is capable of the nonspecific potentiation of neuronal excitability by increasing membrane permeability to Ca^{2+}, similar to its effect in the canine vascular smooth muscle (10). Alternatively, because S is likely to be beneficial at each step of the foregoing cascade of injury-related events, one might inquire whether the effects observed in the present study are associated with the useful aspects of the agent. To put it boldly, the undesirable consequences of the agent are a result of giving too much of a good thing.

Assuming that the high energy demand of neurons overexcited with KA is upheld, seizures may continue for as long as the excitotoxic agent is not cleared from the brain. In the present study, rats did not convulse 1 day following surgery, consistent with the finding that 24 h after KA was administered into the striatum only 0.5% of total [^{3}H]KA was measured (25). Given that local seizures are the major determinant of neuronal loss (3), the risk of brain injury could have been reduced if rats had been kept sedated during that period. Pretreatment with diazepam prior to KA administration appears to protect against both seizures and cellular loss (3). The recurrent and lengthy seizures are more certain to compromise the oxygen (glucose) supply so that hypermetabolic regions exhibiting seizures typically sustain the maximal cell damage (13). A similar argument has been made elsewhere when γ-vinyl GABA, a potentially anticonvulsant agent, was found to prolong metrazol-induced convulsions in rats and even lead to *status epilepticus* (15). In contrast, reduced metabolic demand may conceivably reduce the size of neuronal damage. Although it seems counterintuitive, any maneuver directed at reducing metabolic reserves of convulsing tissue would probably also attenuate seizures. Bicuculline-induced seizures were

diminished in rats with increased GABAergic tone after transient reduction of cerebral blood flow (20). Bicuculline-induced convulsions are stopped under hypoxic conditions and the attenuation of epileptic discharges is achieved sooner in starved animals (4). Further, hypoxia superimposed on bicuculline injections reduced the histological evidence of lesion [microvacuolation and ischemic cell changes in the neocortex and hippocampus (4)]. It is of interest that in acute cerebral infarction where excitotoxic machinery seems to operate, reduced cerebral perfusion is, too, believed to be more damaging than complete metabolic lethargy with respect to the penumbral zone factors (17).

In summary, the reduced mortality under S is consistent with findings indicating that the agent improves the functional potential of injured tissue and retards the period of metabolic exhaustion. However, S seems to act at a time when excessive neuronal activity may lead to seizures. In other words, we posit that the increased incidence of seizures might be elicited by a significant cytoprotective effect of the tested agent. A corrolary of this suggestion is that salubrious effects of S could, too, be masked in some studies where ischemic episodes might have been accompanied by unrecognized seizure activity. It would therefore be of practical significance to examine S effects when the agent is coadministered with drugs controlling seizures in conditions where neuronal injury contributes to epileptogenicity.

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