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α_2-Adrenergic inhibition prevents the accompanied anticonvulsant effect of swim stress on behavioral convulsions induced by lithium and pilocarpine

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Abstract

There has been much debate regarding the potential influence of stress on epilepsy. Many studies have reported that stress can affect seizure susceptibility through eliciting either proconvulsant or anticonvulsant effects within the nervous system. In this study, we investigated the potential anticonvulsant effect of a 10-min swim stress on convulsions induced by a single systemic injection of lithium chloride followed 4 h later with pilocarpine. Rats pretreated with lithium chloride and exposed to a 10-min swim stressor prior to pilocarpine injection displayed a significant delay to seizure onset compared to unstressed rats or rats exposed to swim stress 10 min after lithium chloride, 2 h after lithium chloride, or immediately after pilocarpine injection. We then determined whether administration of a glucocorticoid antagonist (mifepristone; 10 or 50 mg/kg), an α_2-adrenergic antagonist (yohimbine; 2 or 5 mg/kg), or a nonspecific opioid blocker (naloxone; 0.2 or 1 mg/kg) could prevent the anticonvulsant effect of swim stress. Only the high dose of yohimbine was capable of inhibiting the anticonvulsant effect of swim stress on lithium–pilocarpine seizures. Our findings highlight the importance of an endogenous noradrenergic-dependent anticonvulsant system in mediating the effects of swim stress on seizures. Further studies exploring the benefits of treatments with noradrenergic acting drugs in epilepsy is well warranted.

Keywords: Swim stress; Lithium–pilocarpine; Seizure onset time (SOT); α_2-Adrenergic receptors; Noradrenaline; Yohimbine; Glucocorticoids; Opiates; Epilepsy

1. Introduction

The relationship between stress and epilepsy has long been recognized (Mostofsky and Balashak, 1977). Stress is commonly believed to precipitate and even exacerbate seizures in some patients with epilepsy (Schmid-Schonbein, 1998). Prolonged periods of stress can profoundly reduce the efficacy of various anticonvulsant treatments (Iancu et al., 2002; Denicoff et al., 1994; Mastropaolo et al., 1992; Lipka and Lathers, 1987). Although a multitude of both physical and psychological stresses can influence the number of convulsions reported by patients (Neufeld et al., 1994; Temkin and Davis, 1984; Rajna and Veres, 1989; Webster and Mawer, 1989), the precise mechanism underlying the relationship between stress and epilepsy remains unclear.

The major pathway implemented in coordinating the consequences of stress in most mammalian species is the hypothalamic–pituitary–adrenal (HPA) axis. Corticotropin-releasing factor (CRF)-expressing neurons within the parvocellular component of the paraventricular nucleus of the hypothalamus have been identified as one of the key elements in the stress response. The typical biochemical cascade in response to physiological and psychological stresses involves the release of CRF from paraventricular neurons into the hypophysial portal system that in turn stimulates the proopiomelanocortin-producing cells within the anterior lobe of the pituitary to release adrenocortico-

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trophic hormone (ACTH) into general systemic circulation (Herman and Cullinan, 1997; Herman et al., 1996). Circulating ACTH can then interact with adrenal cortex receptors to stimulate the synthesis of steroids (steroidogenesis) as well as cause a marked elevation in plasma glucocorticoids. Shunting of the stress response is provided by the negative-feedback inhibition that results from centrally circulating glucocorticoids acting within the initial hypothalamic and limbic sites responsible for eliciting the initial stress cascade (Vazquez, 1998; Sapolsky, 1994). Neuroplastic changes to any of the key structures within the HPA circuitry can quickly occur after the initial exposure to a variety of stressors (Mercier et al., 2003; Sapolsky, 1994). These changes can be both adaptive and beneficial in nature; however, chronic exposures to stressful stimuli could lead to pathological and maladaptive alterations within this circuit and result in prolonged or even exaggerated HPA activity. Dysfunctions to the HPA axis have been reported to occur in a variety of neurological conditions, including epilepsy (Nemeroff et al., 1984).

In various experimental models of epilepsy (see Fisher, 1989), many stressogenic factors can influence an animal's responsiveness to various convulsant compounds (Rae et al., 1990). In support of these findings is that exposure to a variety of acute stressors (such as 24-h food deprivation or exposure to nociceptive stimulation) can significantly lower the threshold required to evoke limbic motor seizures in lithium–pilocarpine-treated rats (see Persinger et al., 2002). Taken together, profound changes in the electrical characteristics of neurons may result from exposures to various stressful conditions which in turn can increase the susceptibility of neuronal ensembles to evoke epileptiform discharges and alter the vulnerability of hippocampal neurons to a variety of neurological insults.

Chronic and elevated levels of circulating stress hormones, such as cortisol in humans and corticosterone in rats, have been shown to produce long-lasting excitatory effects on the activity of hippocampal neurons and dentate granule cells (Karst and Joels, 2003; Kole et al., 2001). Both acute and chronic stresses can differentially affect susceptibility to pentylenetetrazole-induced convulsions (De Lima and Rae, 1991). Moreover, sex differences in the circulation of corticosteroids can affect the mortality associated with convulsions induced through picrotoxin (Pericic et al., 1996). Although many studies commonly highlight the proconvulsant effects of stress, there have been a variety of studies that have also shown the importance of certain acute stressors in significantly attenuating the actions of various chemoconvulsant drugs.

One such example is that exposure to a swim stressor can exert a significant anticonvulsant action on a wide variety of chemoconvulsant models of epilepsy (Pericic and Svob 2002). Many convulsant parameters have been shown to change in response to swim stress exposure (Pericic and Svob, 2002; Pericic et al., 2000, Rae et al., 1990). There are evident changes in the sensitivity to bicuculline- (Pericic et al., 2001; Drugan et al., 1985), picrotoxin-, and pentylenetetrazole-induced convulsions (Soubrie et al., 1980; Abel and Berman, 1993) following a 10-min exposure to a swim stressor.

In this study, we determined if exposure to a 10-min swim stressor could significantly influence the latency to seizure onset in rats subjected to the lithium–pilocarpine model of epilepsy. To our knowledge, there have been no studies to date that have investigated the effects of swim stress in rodents tested in this epilepsy model. First, we were interested in characterizing the anticonvulsant action from a swim stress by determining the length of time this effect would remain before rats were injected with pilocarpine and assessment of seizure onset began. Following this we assessed potential neurochemical mechanisms that might underlie the anticonvulsant effect of swim stress. Our findings suggest that exposure to a 10-min swim stressor immediately before pilocarpine injection can significantly delay the time to motor convulsion and that antagonism of the α_2-adrenergic system can dose-dependently block the anticonvulsant effect of swim stress in this epilepsy model.

2. Methods

2.1. Subjects

A total of 72 male Wistar albino rats, approximately 90 to 150 days of age and weighing between 530 and 760 g, were used in this study. All subjects were housed three per cage in standard metal grid cages during the entire duration of the study. They were housed at a constant temperature (~20 °C) with food and water available ad libitum. The light/dark cycle was 12-h light/12-h darkness with photophase onset at 0730 h. All testing procedures were conducted during the midphotophase period. The procedures used in this study were approved by the local Animal Care Committee and in compliance with the Canadian Council for Animal Care (CCAC) guidelines.

2.2. Experiment 1

Rats were injected subcutaneously with lithium chloride (3 mEq/kg) followed 4 h later by a single systemic injection of pilocarpine (30 mg/kg). This pretreatment with lithium chloride has been shown to reduce the convulsant dose of pilocarpine by a factor of 10 and also reduce the variability in seizure onset to approximately 30 min (±10 min; Fournier and Persinger, 2004; Persinger et al., 1988, 2002). Following lithium chloride injection, rats were removed from their home cages and subjected to a 10-min swim stressor at various time points either before or after injection of pilocarpine. We chose to administer our swim stress at a 10-min duration because of its common use for investigating the anticonvulsant effect of this stressogenic condition in mice (Pericic et al., 2000).

The swimming apparatus (135 cm diameter, 28 cm deep) was filled with water to a height of 20 cm. The temperature of the swimming apparatus was maintained between 18 and 19 °C. The different swim stress exposures were the following: (1) 10 min following lithium chloride injection (n=4), (2) 2 h after lithium chloride injection (n=4), (3) 10 min prior to injection of pilocarpine (n=4), or (4) immediately after injection of pilocarpine (n=4). The total duration of the swim stress was always 10 min. After swimming, animals were quickly returned to their home cages until pilocarpine injection was required. Approximately 5 min before injections were to be administered, the subject was taken to a separate room, injected with pilocarpine, and placed in a seizure observational cage until seizure onset had occurred. The subjects that received swim stress 10 min before pilocarpine injection were not returned to their home cages after exposure; instead, these animals were quickly transported to the seizure inducing room, injected with pilocarpine, and placed in separate observational cages. Another group of rats (n=4) that did not receive the swim stress served as a nonstressed control group.

The latency to seizure onset [SOT; defined as a conspicuous forepaw clonus, rearing, and falling motor sequelae (stage V motor seizures, see Racine, 1972)] was recorded for each animal. Electroencephalographic recordings at the time of forelimb clonus, rearing, and falling convulsive behavior have shown the presence of clear paroxysmal ("epileptiform") discharges (Cavalheiro et al., 1987; Turksi et al., 1989). After each rat was injected with pilocarpine, we set the maximum allotted time for the observation of seizure onset to be 60 min. Rats that did not exhibit an observable behavioral seizure during this period were assigned a value of 60 min (the maximum time that could be allotted for seizure onset). This time frame was chosen based upon previous published work (Persinger et al., 1988) and unpublished observations (N.M. Fournier and M.A. Galic) that have shown that rats which do not convulse within 60 min after pilocarpine injection do not exhibit indicators of status epilepticus (stages do not normally progress past stage II seizures on the Racine seizure classification scale), nor do they exhibit any observable histopathological evidence indicative of neuronal damage or gliosis following treatment (Persinger et al., 1988).

2.3. Experiment 2

For the second part of the study, a total of 52 male Wistar rats (three to four animals per group) were used. These subjects were of the same age and were housed under identical conditions as those described above. For this experiment, rats were injected with lithium chloride (3 mEq/kg s.c.) and 4 h later injected with pilocarpine (30 mg/kg). In order to assess the involvement of specific neurochemical systems that might mediate the anticonvulsant effect from swim stress, subjects were randomly assigned to either swim stress or nonswim stress groups and then treated with either naloxone (at 0.2 or 1 mg/kg s.c), yohimbine (at 2 or 5 mg/kg s.c.), mifepristone (RU 486: at 10 or 50 mg/kg s.c.), or physiological saline (0.9% s.c.) 10 min before exposure to their respective swim stress condition. Our rationale to administer the drugs at these dosages came from previous pharmacological studies that had revealed differential effects from these drugs on various chemoconvulsant and/or electrical stimulation models of epilepsy at these dosages (see Shan et al., 2004; Pericic et al., 2001; Homayoun et al., 2002; Jackson and Nutt, 1993; Velisek and Mares, 1992; Mariani, 1989; Loscher and Czuczwar, 1987; Baran et al., 1985). Rats in the nonswim stress groups were given one of the above drug injections 20 min prior to pilocarpine. All drugs used in this study were obtained from Sigma (St. Louis, MO, USA) and administered at a volume of 1 ml/kg. The latency to seizure onset (SOT) was recorded in a manner similar to that described above.

2.4. Statistical analyses

All analyses were completed using SPSS software loaded on a VAX 4000 computer. Between group differences in SOT were examined using a one-way analysis of variance (one-way ANOVA) with appropriate post hoc tests (Tukey). The criterion for statistical significance was set at $P<0.05$.

3. Results

3.1. Experiment 1

The results from a one-way ANOVA demonstrated a robust effect for SOT and swim stress [$F(4,15)=26.66$; $P<0.001$; $\eta^2=0.88$]. Post hoc tests revealed that rats exposed to the swim stress 10 min before pilocarpine injection exhibited a greater latency to convulse when compared to all other groups (Table 1). The SOTs in rats that were exposed to the stressogenic swim stimulus 10 min or 2 h after lithium chloride application were not significantly different from the SOTs of nonstressed control rats. Interestingly, the rats exposed to the swim stress immediately after pilocarpine injection displayed a seizure onset that was comparable to the SOT observed in nonstressed controls or rats from

Table 1
The mean SOT (in minutes) and standard error of the mean (S.E.M.) for animals exposed to a 10-min swim stress for different time intervals with respect to the injection of pilocarpine (PILO)

Group	Mean	S.E.M.
Nonswim-stressed controls	30.05	3.95
3 h 50 min before PILO	23.47	2.18
2 h before PILO	20.23	3.39
10 min before PILO	57.08*	1.83
Immediately after PILO	32.56	2.05

* Significantly different ($P<0.001$) from all other groups.

specific swim stress conditions (i.e., 10 min or 2 h after lithium chloride swim stress conditions).

3.2. Experiment 2

Fig. 1 illustrates the mean seizure onset time (SOT) for all swim stress/drug conditions. A highly significant difference was found for SOT and swim stress/drug conditions [$F(13,38)=9.31$; $P<0.0001$; $\eta^2=0.76$]. Because the assumption of homogeneity of variance was violated, a nonparametric Kruskal–Wallis test was performed to ensure that the significant differences observed were valid. The results confirmed the statistical finding of differences in SOTs for animals exposed to the various swim stress and drug conditions [$H=37.38$; $df=13$; $P<0.001$].

In nonstressed animals, post hoc tests revealed that there were no significant differences in SOT for rats pretreated with yohimbine or naloxone compared to saline-treated (nonstressed) controls. This indicates that pretreatment with any of these compounds approximately 20 min before administration of pilocarpine has no effect on the subsequent emergence of convulsions induced in this model of epilepsy. Rats treated with mifepristone at 10 mg/kg before swim stress showed no difference in SOT compared to nonstressed controls. A marginally significant difference ($P=0.07$) in SOT was reported for nonstressed rats treated with the high dose of mifepristone compared to nonstressed controls. There was a trend for these rats to exhibit a faster onset to convulsions compared to nonstressed controls.

In swim-stressed animals, post hoc tests revealed that rats pretreated with naloxone displayed SOT latencies that were significantly higher than nonstressed controls (Fig 1). There were also no differences in SOT for swim-stressed animals treated with naloxone at either 0.2 or 1 mg/kg. Mifepristone treatment before swim stress at either doses displayed SOT latencies that were significantly higher than nonstressed controls. Rats that were either pretreated with naloxone or mifepristone and exposed to the swim stress all displayed SOTs that were not significantly different from saline-treated swim-stressed animals (Fig. 1). These findings suggest that neither opioid nor glucocorticoid antagonism were sufficient in suppressing the accompanied increase in SOT elicited by swim stress.

For rats pretreated with yohimbine at 2 mg/kg prior to swim stress, the seizure onset latency was not significantly different from swim-stressed rats or nonswim-stressed controls (Fig. 1). However, when yohimbine was administered at 5 mg/kg prior to swim stress, there was a significant reduction in time to the onset of seizures compared to swim-stressed controls. These animals were not significantly different from nonswim-stressed controls. Together, these findings suggest that the anticonvulsant effect that normally accompanies exposure to a 10-min swim stressor prior to seizure induction can be prevented through antagonism of the α_2-adrenergic system in a dose-dependent fashion.

4. Discussion

An injection of lithium chloride (3 mEq/kg) followed 4 h later by a single systemic injection of pilocarpine (30 mg/kg) can reliably evoke limbic motor convulsions which produce an expression of brain damage and impairment similar to that found in human patients affected with temporal lobe epilepsy (Honchar et al., 1983). In the present study, we found that swim stress 10 min before seizures induced by the lithium–pilocarpine method exerted a profound delay to the onset of behavioral convulsions compared to animals that were in unstressed conditions. We also found that antagonism of the α_2-adrenergic receptor from a high dose of yohimbine was able to block the anticonvulsant effect mediated by swim stress.

Exposure to a 10-min swim stress revealed a time-dependent anticonvulsant action against lithium–pilocarpine-induced convulsions. The potent anticonvulsant effect from a swim stress occurred only when swim exposures were limited to the 10 min prior to seizure induction. A swim stress exposure at either 10 min or 2 h following lithium chloride injection had no substantial effect on SOT. This suggests that

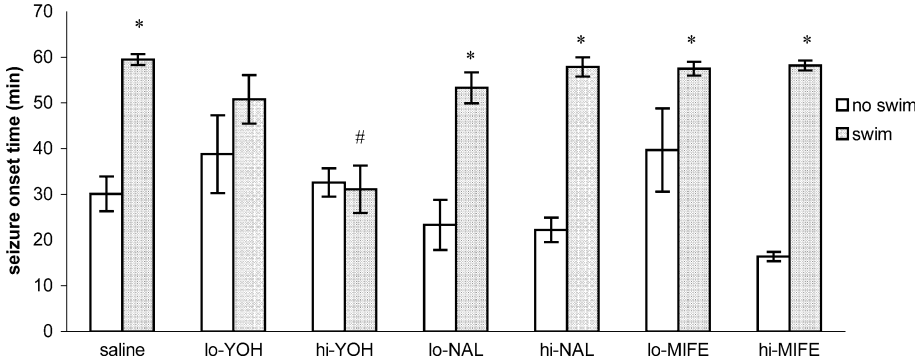

Fig. 1. The mean seizure onset time (±S.E.M.) for rats pretreated with saline, low-dose yohimbine (lo-YOH: 2 mg/kg), high-dose yohimbine (hi-YOH: 5 mg/kg), low-dose naloxone (lo-NAL: 0.2 mg/kg), high-dose naloxone (hi-NAL; 1 mg/kg), low-dose mifepristone (lo-MIFE; 10 mg/kg), or high-dose mifepristone (hi-MIFE; 50 mg/kg) prior to swim stress or non swim stress conditions. *Significant difference from non swim-stressed control rats ($P<0.001$). #Significant difference from swim-stressed rats ($P<0.05$).

whatever the action that mediates the anticonvulsant effect of swim stress may be, the effect is relatively short lasting and limited to when the exposure to the stress occurred in the time frame associated with seizure induction.

That activation in the neurocircuitry underlying stress could lead to the precipitation of seizures in certain forms of human epilepsy has been well documented (Hrachovy, 1997). An elevation in serum cortisol has been reported in epileptic patients (Wang et al., 2001; Pritchard, 1991). Despite the apparent widespread use of ACTH and corticosteroid treatments for regulating convulsions within the epileptic population (Hrachovy, 1997), strong proconvulsant effects following administration of ACTH have also been reported (Tartara et al., 1983). Interestingly, the pro- or anticonvulsant effects mediated by circulating stress hormones might be related to the type of model employed to elicit convulsions, as well as from the type of stressful stimulus used to activate endogenous stressogenic circuits (Persinger et al., 2002; Hrachovy, 1997).

The molecular consequences of stress have been shown to be dependent on the modulation from a variety of different neurochemical systems, including glucocorticoid, noradrenergic, and opiate systems (Rittenhouse et al., 2002; Schramm et al., 2001; Molina et al., 1994; Conner-Kerr et al., 1993). Moreover, the involvement of these specific neurochemical systems in modulating the threshold to evoke epileptiform activity in the limbic system has also been well established for a variety of animal models of epilepsy (see Rhodes et al., 2004; Liu et al., 2003; Homayoun et al., 2002; McEwen, 2001; Hong et al., 1993; Siggins et al., 1986). Our results suggest that the accompanied anticonvulsant action from exposure to swim stress is highly dependent upon the activation of the α_2-adrenergic system, because antagonism of this receptor by high doses of yohimbine produced a significant drop in the time required to display behavioral convulsions. None of the other systems tested (glucocorticoid- or opioid-based) were able to produce a similar change in SOT for swim-stressed animals. The SOT in high-dose yohimbine-treated animals exposed to the swim stress was virtually identical to that of nonstressed controls, further strengthening our conclusion that the anticonvulsant mechanism underlying swim stress is presumably through a noradrenergic-based system. These findings further support the earlier report from Pericic et al. (2000) showing that the elevation in threshold to evoke convulsions by picrotoxin following swim stress in mice can be suppressed by preadministration of α_2-adrenergic antagonists.

It was interesting to find that when mifepristone was administered at a high dose to unstressed animals, a significant shortening to the onset of motor convulsions occurred, whereas lower doses tended to delay the time to seizure onset. Mifepristone is known to have strong inhibitory effects at progesterone/glucocorticoid receptors. An elevation in CRF levels can also be found following exposure to mifepristone treatment (Hardwick et al., 1989). Pronounced excitatory and epileptogenic effects may also result from elevations in centrally circulating CRF (Piekut and Phipps, 1998). Because endogenous progesterone and the progesterone metabolite, 3α,5α-THP, can possess antiepileptic effects in some patients affected with epilepsy (Rhodes et al., 2004) and in a variety of animal models of epilepsy (Frye, 1995), it was not surprising to find that at high doses, mifepristone tended to lower the time to seizure onset in unstressed animals. Moreover, strong support for the involvement of progesterone's metabolite in eliciting pronounced anticonvulsant actions is supported by the observation that administration of finasteride, a 5α-reductase inhibitor (which can block the metabolism of progesterone to 3α,5α-THP) can cause an increase in the number of seizures reported in both human and animal epileptics (Rhodes et al., 2004).

A variety of stressful conditions have been shown to affect noradrenergic neurons of the locus ceruleus (Valentino et al., 1993). Stress can influence the firing rate of locus ceruleus neurons and cause a release of noradrenaline in brain regions that correspond with rich noradrenergic innervation (Bremner et al., 1996; Mabry et al., 1995). An increase in both central and plasma noradrenaline have been found in humans and rats following seizures (Shouse et al., 2001; Meierkord et al., 1994). After damage to noradrenergic terminals from the locus ceruleus, a microinfusion of bicuculline into the anterior piriform can elicit status epilepticus (Giorgi et al., 2003). Ferencz et al. (2001) also supported a role of the noradrenergic system in epilepsy by showing that direct lesions to the ascending noradrenergic pathway facilitated ventral hippocampal kindling. Magdaleno-Madrigal et al. (2002) and Fernandez-Guardiola et al. (1999) have both suggested that the delay in amygdala-kindled convulsions observed after prestimulation of the vagal complex or nucleus of the solitary tract might be related to the subsequent noradrenergic-driven increase in rapid eye movement sleep that occurs following stimulation. Administration of clonidine, a noradrenergic alpha (2) agonist, can delay amygdala kindling (Yoshioka et al., 2000; Shouse et al., 1996). And finally, the efficacy of the ketogenic (high fat, low carbohydrate) diet in abating convulsions has recently been shown to be dependent upon the possession of an intact and functional noradrenergic system (Szot et al., 2001). These studies strongly link a modulatory influence from the central noradrenergic pathway on limbic motor seizures and epilepsy.

Many of the brain regions implemented in the circuitry that underlies swim stress are also the same areas that receive dense noradrenergic innervation from the locus ceruleus (Aston-Jones and Shipley, 1995). Furthermore, the piriform cortex, a region of high noradrenergic innervation (Aston-Jones and Shipley, 1995), shows increased FOS immunoreactivity following swim stress (Duncan et al., 1993). It is interesting to note that the piriform cortex has also been considered a critical structure for the initiation and spread of seizures to other limbic and cortical areas (Peredery et al., 2000). Considering the strong innervation

from noradrenergic terminals to this structure, significant modulatory actions from the noradrenergic system at this site after exposure to a swim stress is likely.

It was extremely puzzling to observe that for rats exposed to the 10 min of swim stress immediately after pilocarpine injection, no effect on seizure onset was present; whereas, exposing an animal to the same swim stress 10 min before pilocarpine injection exerted a powerful and potent delay to convulsion. There may be numerous possibilities for why such an effect was found. Swim stress can cause an increase in the release of endogenous noradrenaline in brain regions that normally receive noradrenergic innervation (Mabry et al., 1995). A peak elevation in plasma noradrenaline can be observed approximately 10 to 15 min following exposure to swimming at 20 °C (Mabry et al., 1995). An increase in both the frequency of spontaneous postsynaptic currents and firing rate of cultured hippocampal neurons within 3 to 5 min following application of pilocarpine has also been reported (Priel and Albuquerque, 2002). This effect is presumably the result of increased activity at glutamatergic synapses that in turn produces an imbalance between excitatory and inhibitory (GABAergic) transmission (Priel and Albuquerque, 2002). Therefore, the antiepileptogenic effect from a swim stress 10 min before pilocarpine injection would correspond to a time frame when the increase release in endogenous noradrenaline resulting from swim stress occurs and the period when the initial imbalance between excitatory and inhibitory transmission produced by pilocarpine begins. Obviously, in rats that received the swim stress immediately following pilocarpine injection, the peak noradrenaline response would have occurred at a time significantly past the period when the initial imbalance between excitatory and inhibitory transmission appeared. Therefore, no anticonvulsant effect from swim stress would be found in this condition.

Many studies have shown that a definitive relationship between stress and the frequency (and severity) of convulsions in epileptic patients exists (Mostofsky and Balashak, 1977; Schmid-Schonbein, 1998). Animal studies have found that exposure to an acute stressor (such as 24-h food deprivation or thermal-induced nociception) prior to the induction of convulsions by the lithium–pilocarpine method or through other methods, can cause a significant lowering in the threshold required to elicit seizures and behavioral convulsions (Persinger et al., 2002; Reddy and Rogawski, 2002; Post and Weiss, 1998; Donzanti et al., 1985). Generally, the time of observation of forelimb clonus and its electrical characteristics can occur faster in stressed rats compared to unstressed rats (Persinger, 2002). However, in this study, we report that exposure to a 10-min swim stressor can exhibit a powerful anticonvulsant action on the induction of seizures in the lithium–pilocarpine model of epilepsy. Taken together, the type of stressor and how it impacts upon the neurocircuitry and neurochemistry of the animal may be one of the most important factors regarding whether stress will express an anticonvulsant or proconvulsant effect.

Our findings also extended the previous report from Pericic et al. (2000) in two important ways. First, by evaluating if this swim stress procedure would also yield a significant anticonvulsant effect in a different species of animals (i.e., rats) tested. And second, by determining if a swim stress exposure that has been found to previously elicit strong anticonvulsant effects against picrotoxin-induced convulsions would also produce similar actions against lithium–pilocarpine-induced convulsions. Although potent interactions between cholinergic and GABAergic systems following seizures have been described previously (Hamani and Mello, 2002), seizures that are induced by picrotoxin (a GABA-related convulsant) or pilocarpine (a cholinergic-related convulsant) can differ quite substantially in their mechanism of epileptogenesis and in their histopathological consequences. The anticonvulsant effect mediated from a swim stress, approximately 10 min prior to convulsions induced through lithium–pilocarpine, requires activation of an *endogenous anticonvulsant circuit* that involves the α_2-adrenergic system. These findings suggest a critical involvement of noradrenergic transmission in the modulation of epilepsy. Further work investigating the use of noradrenergic drugs as a treatment option against limbic epilepsy is needed.

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References

Abel AL, Berman RF. Effects of water immersion stress on convulsions induced by pentylenetetrazole. Pharmacol Biochem Behav 1993;45:823–5.

Aston-Jones G, Shipley MT, Grzanna R. The locus coeruleus, A5 and A7 noradrenergic cell groups. In: Paxinos G, editor. The rat nervous system, Second Edition. San Diego: Academic Press; 1995. p. 183–206.

Baran H, Sperk G, Hortnagl H, Sapetschnig G, Hornykiewicz G. α_2-Adrenoceptors modulate kainic acid-induced limbic seizures. Eur J Pharmacol 1985;113:263–9.

Bremner JD, Krystral JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: I Preclinical studies. Synapse 1996;3:28–38.

Cavalheiro EA, Silva DF, Turksi WA, Calderazzo-Filho LS, Bortolotto ZA, Turksi L. The susceptibility of rats to pilocarpine-induced seizures is age-dependent. Brain Res Dev Brain Res 1987;37:43–58.

Conner-Kerr TA, Simmons DR, Peterson GM, Terrian DM. Evidence for the corelease of dynorphin and glutamate from rat hippocampal mossy fiber terminals. J Neurochem 1993;61(2):627–36.

De Lima TC, Rae GA. Effects of cold-restraint and swim stress on convulsions induced by pentylenetetrazol and electroshock: influence of naloxone pretreatment. Pharmacol Biochem Behav 1991;40(2):297–300.

Denicoff KD, Meglathery SB, Post RM, Tandeciarz SI. Efficacy of carbamazepine compared with other agents: a clinical practice survey. J Clin Psychiatry 1994;55(2):70–6.

Drugan RC, McIntyre TD, Alpern HP, Maier SF. Coping and seizure susceptibility: control over shock protects against bicuculline-induced seizures. Brain Res 1985;342:9–17.

Donzanti BA, Green MD, Shores EI. Atropine sulfate and 2-pyridine aldoxime methylchloride elicit stress-induced convulsions and lethality in mice and guinea pigs. Drug Chem Toxicol 1985;8(6):431–49.

Duncan GE, Johnson KB, Breese GR. Topographic patterns of brain activity in response to swim stress: assessment by 2-deoxyglucose uptake and expression of fos-like immunoreactivity. J Neurosci 1993; 13(9):3932–43.

Ferencz I, Leanza G, Nanobashvili A, Kokaia Z, Kokaia M, Lindvall O. Septal cholinergic neurons suppress seizure development in hippocampal kindling in rats: comparison with noradrenergic neurons. Neuroscience 2001;102(4):819–32.

Fernandez-Guardiola A, Martinez A, Valdes-Cruz A, Magdaleno-Madrigal VM, Martinez D, Fernandez-Mas R. Vagus nerve prolonged stimulation in cats: effects on epileptogenesis (amygdala electrical kindling): behavioral and electrographic changes. Epilepsia 1999;40(7):822–9.

Fisher RS. Animal models of the epilepsies. Brain Res Rev 1989;14: 245–78.

Fournier NM, Persinger MA. The neuromatrix and the epileptic brain Behavioral and learning preservation in limbic epileptic rats treated with ketamine but not acepromazine. Epilepsy Behav 2004;5(1):119–27.

Frye CA. The neurosteroid 3α,5α-THP has anti-seizure and possible neuroprotective effects in an animal model of epilepsy. Brain Res 1995;696:113–20.

Giorgi FS, Ferrucci M, Lazzeri G, Pizzanelli C, Lenzi P, Alessandrl MG, et al. A damage to locus coeruleus neurons converts sporadic seizures into self-sustaining limbic status epilepticus. Eur J Neurosci 2003; 17(12):2593–601.

Hamani C, Mello LE. Spontaneous recurrent seizures and neuropathology in the chronic phase of the pilocarpine and picrotoxin model epilepsy. Neurol Res 2002;24(2):199–209.

Hardwick AJ, Linton EA, Rothwell NJ. Thermogenic effects of the antiglucocorticoid RU-486 in the rat: involvement of corticotropin-releasing factor and sympathetic activation of brown adipose tissue. Endocrinology 1989;124(4):1684–8.

Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis. Trends Neurosci 1997; 20(2):78–84.

Herman JP, Prewitt CM, Cullinan WE. Neuronal circuit regulation of the hypothalamo–pituitary–adrenocortical stress axis. Crit Rev Neurobiol 1996;10(3–4):371–94.

Hrachovy RA. ACTH and steroids. In: Engel Jr J, Pedley TA, editors. Epilepsy: a comprehensive textbook. Philadelphia: Lippincott-Raven; 1997. p. 1463–73.

Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium treated rats. Science 1983; 220:323–5.

Homayoun H, Khavandgar S, Dehpour AR. The involvement of endogenous opioids and nitricoxidergic pathway in the anticonvulsant effects of foot-shock stress in mice. Epilepsy Res 2002;49(2):131–42.

Hong JS, McGinty JF, Lee PH, Xie CW, Mitchell CL. Relationship between hippocampal opioid peptides and seizures. Prog Neurobiol 1993;40(4): 507–28.

Iancu I, Rosen Y, Moshe K. Antiepileptic drugs in posttraumatic stress disorder. Clin Neuropharmacol 2002;25(4):225–9.

Jackson HC, Nutt DJ. Investigation of the involvement of opioid receptors in the action of anticonvulsants. Psychopharmacology 1993;111(4): 486–90.

Karst H, Joels M. Effect of chronic stress on synaptic currents in rat hippocampal dentate gyrus neurons. J Neurophysiol 2003;89(1):625–33.

Kole MH, Koolhaas JM, Luiten PG, Fuchs E. High-voltage-activated Ca2+ currents and the excitability of pyramidal neurons in the hippocampal CA3 subfield in rats depend on corticosterone and time of day. Neurosci Lett 2001;307(1):53–6.

Lipka LJ, Lathers CM. Psychoactive agents, seizure production, and sudden death in epilepsy. J Clin Pharmacol 1987;27(3):169–83.

Liu H, Kaur J, Dashtipour K, Kinyamu R, Ribak CE, Friedman LK. Suppression of hippocampal neurogenesis is associated with developmental stage, number of perinatal seizure episodes, and glucocorticosteroid level. Exp Neurol 2003;184(1):196–213.

Loscher W, Czuczwar SJ. Comparison of drugs with different selectivity for central α_1- and α_2-adrenoceptors in animals models of epilepsy. Epilepsy Res 1987;1:165–72.

Mabry TR, Gold PE, McCarty R. Age-related changes in plasma catecholamine responses to chronic intermittent stress. Physiol Behav 1995; 58(1):49–56.

Magdaleno-Madrigal VM, Valdes-Cruz A, Martinez-Vargas D, Martinez A, Almazan S, Fernandez-Mas R, et al. Effect of electrical stimulation of the nucleus of the solitary tract on the development of electrical amygdaloid kindling in the cat. Epilepsia 2002;43(9):964–9.

Mariani PJ. Seizure associated with low-dose naloxone. Am J Emerg Med 1989;7(1):127–9.

Mastropaolo J, Park CH, Norris DO, O'Connor DA, Lukacs LG, Deutsch SI. Reduction of flurazepam's antiseizure efficacy persists after stress. Pharmacol Biochem Behav 1992;42(4):681–4.

McEwen BS. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Ann NY Acad Sci 2001;933:265–77.

Meierkord H, Shorvon S, Lightman SL. Plasma concentrations of prolactin, noradrenaline, vasopressin and oxytocin during and after a prolonged epileptic seizure. Acta Neurol Scand 1994;90(2):73–7.

Mercier S, Frederic C, Buguet A, Cespuglio R, Martin S, Bourdon L. Behavioural changes after an acute stress: stressor and test types influences. Behav Brain Res 2003;39(1–2):167–75.

Molina VA, Heyser CJ, Spear LP. Chronic variable stress or chronic morphine facilitates immobility in a forced swim test: reversal by naloxone. Psychopharmacology 1994;114(3):433–40.

Mostofsky D, Balashak R. Psychobiological control of seizures. Psychol Bull 1977;84:723–59.

Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, et al. Elevated concentrations of CSF corticotrophin-releasing factor-like immunoreactivity in depressed patients. Science 1984;226:1342–4.

Neufeld MY, Sadeh M, Cohn DF, Korczyn AD. Stress and epilepsy: the Gulf war experience. Seizure 1994;3(2):135–9.

Peredery O, Persinger MA, Parker G, Mastrosov L. Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. Brain Res 2000;881:9–17.

Pericic D, Svob D. Interaction of stress and noradrenergic drugs in the control of picrotoxin-induced seizures. Epilepsy Res 2002;51:179–87.

Pericic D, Manev H, Bujas M. Gonadal hormones and picrotoxin-induced convulsions in male and female rats. Brain Res 1996;736:174–9.

Pericic D, Dubravka S, Jazcinscak M, Mirkovic K. Anticonvulsant effect of swim stress in mice. Pharmacol Biochem Behav 2000;66(4):870–86.

Pericic D, Jazvinscak M, Svob D, Mirkovic K. Swim stress alters the behavioural response of mice to GABA-related and some GABA-unrelated convulsants. Epilepsy Res 2001;43:145–52.

Persinger MA, Makarec K, Bradley JC. Characteristics of limbic seizures evoked by peripheral injections of lithium and pilocarpine. Physiol Behav 1988;44:22–37.

Persinger MA, Stewart LS, Richards PM, Harrigan T, O'Connor RP, Bureau YRJ. Seizure onset times for rats receiving lithium and pilocarpine: sources of variability. Pharmacol Biochem Behav 2002;71:7–17.

Piekut DT, Phipps B. Increased corticotropin-releasing factor immunoreactivity in select brain sites following kainate elicited seizures. Brain Res 1998;781(1–2):100–13.

Priel MR, Albuquerque EX. Short-term effects of pilocarpine on rat hippocampal neurons in culture. Epilepsia 2002;43(5):40–6.

Pritchard III PB. The effect of seizures on hormones. Epilepsia 1991; 32(6):46–50.

Post RM, Weiss SR. Sensitization and kindling phenomena in mood, anxiety, and obsessive–compulsive disorders: the role of serotonergic mechanisms in illness progression. Biol Psychiatry 1998;44(3): 193–206.

Racine R. Modification of seizure activity by electrical stimulation: II. Motor seizures. Electroencephalogr Clin Neurophysiol 1972;56:225–35.

Rae GA, Pereira GH, De-Lima TC. The influence of stress on convulsive parameters in the mouse. Neurosci Biobehav Rev 1990;14(4):491–4.

Rajna P, Veres J. Life events and seizure frequency in epileptics: a follow-up study. Acta Med Hung 1989;46(2–3):169–87.

Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J Neurosci 2002;22(9):3795–805.

Rhodes ME, Harney JP, Frye CA. Gonadal, adrenal, and neuroactive steroids' role in ictal activity. Brain Res 2004;1000:8–18.

Rittenhouse PA, Lopez-Rubalcava C, Stanwood GD, Lucki I. Amplified behavioral and endocrine responses to forced swim stress in the Wistar–Kyoto rat. Psychoneuroendocrinology 2002;27(3):303–18.

Sapolsky RM. Individual differences in the stress response. Semin Neurosci 1994;6:261–9.

Schmid-Schonbein C. Improvement of seizure control by psychological methods in patients with intractable epilepsies. Seizure 1998;7(4): 261–70.

Schramm NL, McDonald MP, Limbird LE. The α_{2A}-adrenergic receptor plays a protective role in mouse behavioral models of depression and anxiety. J Neurosci 2001;21(13):4875–82.

Shan Y, Qin J, Chang XZ, Yang ZX. Effect of naloxone on remote seizure susceptibility. Beijing Daxue Xuebao 2004;36(1):57–60.

Shouse MN, Langer J, Bier M, Farber PR, Alcalde O, Moghimi R, et al. The alpha 2 adrenoreceptor agonist clonidine suppresses seizures, whereas the alpha 2 adrenoreceptor antagonist idazoxan promotes seizures: pontine microinfusion studies of amygdala-kindled kittens. Brain Res 1996;731(1–2):203–7.

Shouse MN, Staba RJ, Ko PY, Saquib SF, Farber PR. Monoamines and seizures: microdialysis findings in locus ceruleus and amygdala before and during amygdala kindling. Brain Res 2001;892(1): 176–192.

Siggins GR, Henriksen SJ, Chavkin C, Gruol D. Opioid peptides and epileptogenesis in the limbic system: cellular mechanism. In: Delgado-Escueta AV, Ward AA, Woodbury AM, Porter RJ, editors. Advances in Neurology, vol. 44. New York: Raven Press; 1986. p. 501–12.

Soubrie P, Thiebot MH, Jobert A, Montastrue JL, Hery F, Hamon M. Decreased convulsant potency of picrotoxin and pentetrazol and enhanced [^{3}H]flunitrazepam cortical binding following stressful manipulation in rats. Brain Res 1980;189:505–17.

Szot P, Weinshenker D, Rho JM, Storey TW, Schwartzkroin PA. Norepinephrine is required for the anticonvulsant effect of the ketogenic diet. Brain Res Dev Brain Res 2001;129(2):211–4.

Tartara A, Bo P, Maurelli M, Savoldi F. Centrally administered N-terminal fragments of ACTH (1–10, 4–10, 4–9) display convulsant properties in rabbits. Peptides 1983;4(3):315–8.

Temkin N, Davis G. Stress as a risk factor amongst adults with epilepsy. Epilepsia 1984;25:450–6.

Turksi L, Ikonomidou C, Turski WA, Bortolloto ZA, Cavalheiro EA. Cholinergeric mechanisms and epileptogenesis The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. Synapse 1989;3:154–71.

Valentino RJ, Foote SL, Page ME. The locus coeruleus as a site for integrating corticotrophin-releasing factor and noradrenergic mediation of stress responses. Ann NY Acad Sci 1993;697:173–88.

Vazquez DM. Stress and the developing limbic–hypothalamic–pituitary–adrenal axis. Psychoneuroendocrinology 1998;23(7):663–700.

Velisek L, Mares P. Differential effects of naloxone on postictal depression. Epilepsy Res 1992;12(1):37–43.

Wang W, Dow KE, Fraser DD. Elevated corticotropin releasing hormone/corticotropin releasing hormone-R1 expression in postmortem brain obtained from children with generalized epilepsy. Ann Neurol 2001; 50(3):404–9.

Webster A, Mawer G. Seizure frequency and major life events in epilepsy. Epilepsia 1989;30:162–7.

Yoshioka S, Mitani H, Maeda K, Takeo S, Matsuda K, Katayama S, et al. Age-specific effects of noradrenergic alpha-2 agonist clonidine on the development of amygdaloid kindling in developing rats. Brain Res Dev Brain Res 2000;119(2):283–8.