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Effects of Cold-Restraint and Swim Stress on Convulsions Induced by Pentylenetetrazol and Electroshock: Influence of Naloxone Pretreatment

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DE LIMA, T. C. M. AND G. A. RAE. *Effects of cold-restraint and swim stress on convulsions induced by pentylenetetrazol and electroshock: Influence of naloxone pretreatment.* PHARMACOL BIOCHEM BEHAV **40**(2) 297–300, 1991.—The influence of two stressogenic conditions, restraint at 4°C for 30 min (cold-restraint stress; CRS) or swimming at 20°C for 3 min (swim stress; SS), on nociception and on convulsions triggered by different agents was assessed in mice. In saline-pretreated mice CRS and SS caused analgesia (hot-plate test, 56°C), delayed the onset of convulsions induced by pentylenetetrazol (PTZ, 100 mg/kg, IP) and aggravated convulsions elicited by maximal transcorneal electroshock (150 mA pulses at 60 Hz for 0.2 s). Pretreatment with naloxone (10 mg/kg, SC, 30 min prior to testing), which did not affect the responsiveness of nonstressed mice to the hot plate or to the convulsant treatments, attenuated the development of analgesia following CRS, but not SS, and further prolonged the latency to onset of PTZ-induced convulsions in both stressed groups. Thus the extent to which CRS and SS can each delay the onset of PTZ-triggered convulsion appears to be limited by activation of a proconvulsant opioid system. In contrast, naloxone pretreatment did not modify the effects of CRS or SS on the severity of electroshock-induced seizures. In conclusion, CRS and SS can each, simultaneously, exert anticonvulsant and proconvulsant influences on responsiveness to PTZ and electroshock, respectively. Also, both forms of stress can activate an opioid system modulating the onset of PTZ-induced seizures, which is distinct from that controlling nociception. These findings, together with those of other studies, reveal a complex relationship between stress, convulsions and opioid systems, which depends on the characteristics of the stressogenic condition, species, convulsant agent and parameter considered.

Stress Analgesia Convulsions Opioids Naloxone Pentylenetetrazol Electroshock

STUDIES analysing the influences of stress on convulsions have yielded controversial results (19). Some authors found that stressogenic conditions increase the susceptibility and/or the incidence of convulsions in animals and in humans (5, 13, 27, 32, 36). Others, however, reported that stress can protect animals against convulsions (2, 17, 18, 20, 26, 34). One of the reasons which may account for these discrepant findings is that virtually all studies conducted, thus far, have each focused on the effects of a single stressogenic stimulus on a restricted number of convulsive parameters in a single convulsant model.

On the other hand, it is widely recognized that several forms of stress can cause analgesia by activating both opioid and/or nonopioid mechanisms, depending on the nature and the intensity of the stressogenic stimulus (3,31). Besides causing analgesia, opioids can affect seizures triggered by different agents (16,32). Thus opiates and opioid peptides elicit behavioral and/or electrographical seizures when injected intracerebrally (12,28) and aggravate amygdaloid-kindled seizures (29). In most studies, these effects involved activation of specific opioid receptors, as they were blocked by pretreatment with the opiate antagonist naloxone. However, others have detected a naloxone-sensitive protective action of opioids against seizures elicited audiogenically (8), spontaneously (2,16) or by fluorothyl (1), pentylenetetrazol (1) and electroshock (21). Furthermore, there are also reports showing both proconvulsant and anticonvulsant properties of opioids (6). For example, in rats, morphine delays the onset of PTZ-induced convulsions and shortens their duration, but also increases the number of seizures (33). Although the CNS sites mediating the analgesic, epileptogenic and anticonvulsant actions of opioids are probably distinct (12), stressogenic stimuli may activate not only opioid systems mediating analgesia, but also those capable of modulating seizure susceptibility and/or severity.

In light of the above considerations the aim of the present study was three-fold: 1) to analyse the influence of two distinct stressogenic procedures, cold-restraint (CRS) and swim stress (SS), on convulsions in mice; 2) to assess if this influence is similar in two different convulsant models, seizures induced by PTZ and by maximal transcorneal electroshock; and 3) to evaluate indirectly, by observing the influence of naloxone pretreatment, the participation of opioid systems in bringing about the effects of stress on convulsions. The two forms of stress em-

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ployed were chosen because they both cause significant analgesia, but only that induced by CRS is attenuated by naloxone pretreatment.

METHOD

Animals

Experiments were conducted with male Swiss mice (20–25 g), housed in groups of 20 per cage in a room of controlled temperature (22 ± 2°C) and lighting (lights on from 0600 to 1800 h), which had free access to food chow and water until submission to stress.

Experimental Protocol

The animals were divided into three groups: 1) a control nonstressed group, which remained in their home cages; 2) a group submitted to cold/restraint stress (CRS), i.e., restrained in perforated plastic tubes (2.5 cm wide and 10 cm long) and placed in a ventilated refrigerator at 4°C for 30 min; and 3) a swim-stressed group (SS), submitted to 3 min of forced swimming in water (22 ± 2°C, 20 cm deep) and allowed to dry on soft tissue paper in a cage for 5 min before receiving convulsant treatment. Mice in all 3 groups were pretreated SC with either saline (0.9% NaCl, 10 ml/kg) or naloxone (1 or 10 mg/kg).

Hot-Plate Test

Mice were placed on a hot plate (56°C) 30 min after pretreatment and immediately following exposure to stress. The time elapsed until the animal licked a hind paw was taken as the index of nociceptive threshold and the maximal time allowed on the apparatus (cut-off time) was 30 s.

Convulsions

Also 30 min after pretreatment and at the end of the stressogenic stimuli, but in a separate set of animals from those tested on the hot plate, mice were either treated with pentylenetetrazol (PTZ, 100 mg/kg, IP) or submitted to maximal transcorneal electroshock (rectangular pulses of 150 mA delivered at 60 Hz for 200 ms through stainless steel electrodes). The time elapsed until manifestation of the first myoclonic jerk (latency), as well as the duration, incidence and severity (10) of the convulsion induced by PTZ was recorded up to 30 min after treatment. When convulsions were triggered by electroshock, the durations of the hindlimb flexor and extensor components of the seizure were recorded and the extension time/flexion time ratio was used as an index of convulsion severity (7).

Drugs

The drugs used were: pentylenetetrazol (Knoll Pharmaceutical Co.) and naloxone hydrochloride (a kind gift from Du Pont Chemical Co.). Solutions were made up in double-distilled water.

Statistical Analysis

Most results are presented as the mean ± s.e. mean and were analysed by two-way ANOVA followed by Newman-Keuls' test. The severity indices of PTZ-induced convulsions are presented as the medians and were analysed by two-way ANOVA followed by Duncan's test. The incidence of convulsions and lethality were analysed by Fisher's test. Differences with $p<0.05$ were considered statistically significant.

RESULTS

Hot-Plate Test

Nonstressed saline-pretreated mice responded to the nociceptive stimulus within 4.2 ± 0.4 s. Prior exposure to CRS or to SS significantly ($p<0.05$) increased the nociceptive threshold to 23.4 ± 1.9 s and to 8.3 ± 1.0 s, respectively. Pretreatment with naloxone (10 mg/kg), which did not affect nociception in nonstressed animals (4.9 ± 0.7 s), attenuated the analgesic effect of CRS (14.3 ± 2.7 s, $p<0.05$), but not that of SS (7.3 ± 0.6 s; N = 10 in each group).

Pentylenetetrazol

Submission of saline-pretreated mice to either form of stress resulted in similar significant prolongations of the latency to convulsion induced by PTZ ($p<0.05$; Fig. 1A), without changing the incidence, duration or severity of such seizures (Table 1). Pretreatment with naloxone, which did not modify the convulsive latency of nonstressed animals, caused a dose-dependent potentiation of the effect of CRS and SS ($p<0.05$, at 10 mg/kg only). In addition, a greater incidence of deaths within the 24 h following PTZ-induced convulsions was detected in naloxone-pretreated (10 mg/kg) mice exposed to CRS.

Transcorneal Electroshock

Animals pretreated with saline and submitted to CRS or SS displayed more severe maximal electroshock-induced seizures than did nonstressed mice ($p<0.05$; Fig. 1B). The magnitude of this effect was similar in both stressed groups. Figure 1B also shows that the convulsive indices of stressed or nonstressed mice were not modified significantly by pretreatment with naloxone (1 or 10 mg/kg).

DISCUSSION

It is widely recognized that stress causes analgesia, through activation of opioid and/or nonopioid neural mechanisms (3, 20, 31). The participation of nonopioid mechanisms in the mediation of stress-induced analgesia appears to be directly related to the intensity of the stressogenic stimulus (31). In this regard it appears that CRS and SS were effective stressogenic conditions as they both enhanced response latencies in the hot-plate test. Moreover, as naloxone pretreatment attenuated analgesia induced by CRS, but not that following SS, SS may represent a greater stressogenic stimulus than CRS. It should be noted, though, that the CRS-induced analgesia was significantly greater than that detected after SS.

The main contribution of the current study is that, unlike all previous studies on the possible relationships between stress and convulsions, we analysed the influence of more than one stressogenic condition on convulsions elicited by two different agents, PTZ and electroshock. We found that both CRS and SS increased the latency to convulsions caused by PTZ and aggravated electroshock-induced seizures. Thus two distinct stressogenic conditions can each determine both anticonvulsant and proconvulsant influences, depending on the convulsive parameter considered and the type of convulsant agent used.

Stress decreases the incidence of electrographic and/or behavioral seizures induced by amygdaloid kindling in rats (26) or spontaneously in genetically prone epileptic gerbils (2), but enhances the incidence of convulsions in photosensitive baboons

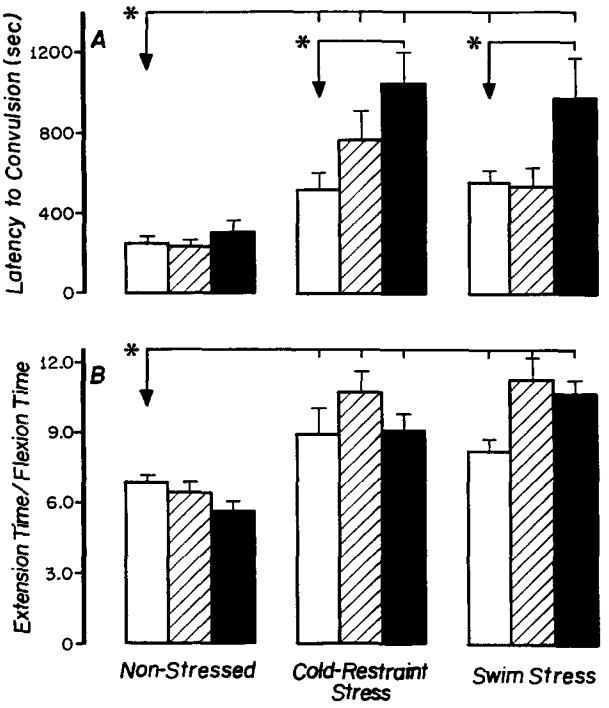

FIG. 1. Effects of cold/restraint and swim stress on (A) the latency to convulsion triggered by pentylenetetrazol (100 mg/kg, IP) and on (B) the severity of convulsion elicited by maximal transcorneal electroshock (rectangular pulses of 150 mA at 60 Hz for 200 ms). Mice were pretreated SC, 30 min before receiving each convulsant, with either saline (open columns) or naloxone at 1 (hatched columns) or 10 mg/kg (closed columns). The severity of electroshock-induced convulsions is shown as the ratio between hindlimb extension and flexion times (ET/FT). Columns represent the means of 10 observations and vertical lines indicate the s.e. means. Asterisks denote $p<0.05$ when compared to column marked with arrow (ANOVA + Newman-Keuls' test).

(13) and in humans (30). Such discrepancies could suggest that the effects of stress on convulsions are species-dependent. However, considerable variation can occur even within a same species. Thus mice inflicted with intermittent footshocks are less sensitive to electroshock-induced convulsions (18,20), whereas those submitted in the present study to CRS and SS showed no change in the incidence of convulsions triggered by submaximal currents of electroshock (50 or 100 mA, results not shown), yet presented more severe seizures in response to maximal electroshock. Furthermore, we have observed that mice submitted to the more natural stress of conspecific aggression show a reduced latency to convulsions triggered by PTZ (as opposed to the greater latency seen following CRS and SS) and no change in the severity of electroshock-induced convulsions (23). Also, Vale and Leite (34) found that mice submitted to the stress of REM sleep deprivation were more susceptible to PTZ-induced convulsions. Therefore, although stress-induced alterations in convulsive parameters may be species-dependent, different stressogenic conditions can clearly promote diverse effects in a single species.

The mechanisms underlying the variable influences of stress on convulsive parameters remain to be elucidated. Nevertheless, the fact that both CRS and SS enhanced the latency to PTZ-induced convulsion without modifying its severity may reflect alterations in the pharmacokinetics of this convulsant, due to altered regional blood flow at the injection site and/or to slower penetration of PTZ across the blood-brain barrier (34). Indeed we have found that CRS and SS both cause marked hypothermia in mice (De Lima, unpublished observations), which could possibly delay the absorption and/or distribution of PTZ. On the other hand, though inhibitory effects of hypothermia on seizures have been reported (4), we did not detect significant correlations between the intensity of the hypothermic responses to CRS and SS and the prolongation of the latencies to convulsions triggered by PTZ or pilocarpine, or the increases in severity of electroshock-induced seizures (De Lima, unpublished observations).

TABLE 1

EFFECT OF COLD-RESTRAINT AND SWIM STRESS ON PARAMETERS OF CONVULSIONS INDUCED BY PENTYLENETETRAZOL IN MICE

Condition	Naloxone (mg/kg)	Convulsive Parameter Incidence[a]	Duration[b]	Severity[c]	Lethality[a]
Nonstressed	—	100	23.7 ± 1.7	5	30
	1	100	22.0 ± 3.5	5	40
	10	100	17.3 ± 1.2	5	0
Cold/Restraint	—	100	25.0 ± 6.5	5	20
Stress	1	90	29.1 ± 5.2	5	30
	10	100	17.0 ± 2.9	6	80*
Swim Stress	—	100	25.8 ± 3.0	5	30
	1	100	28.4 ± 4.1	5	20
	10	100	19.2 ± 5.1	5	30

Each group was comprised of 10 mice.
[a]Percentage values.
[b]Mean ± s.e.m. (in seconds).
[c]Median score according to severity index proposed by Czuczwar and Frey (10).
*$p<0.05$ (Fisher's test).
The animals were pretreated with either saline or naloxone (1 or 10 mg/kg, SC) 30 min prior to injection of the convulsant (100 mg/kg, IP).

Naloxone aggravates audiogenic seizures in mice (25), sensitizes rabbits to convulsions caused by penicillin and strychnine (15) and prevents hyperthermia-derived seizures in immature rats (22). In the present study naloxone pretreatment did not modify PTZ- and electroshock-induced convulsions in nonstressed mice, but further prolonged the latencies to convulsions induced by PTZ after CRS and SS. If naloxone-induced changes can be taken as indirect evidence of ongoing opioid system activity, as the partial reversal of CRS-induced analgesia by the drug indeed suggests, it would appear that both CRS and SS can activate a proconvulsant opioid mechanism which selectively limits the increase in latency to PTZ-triggered convulsions. This view is strengthened by a report showing that morphine exerts proconvulsant effects when given prior to PTZ in mice (15). In contrast, another study found that morphine delays the onset and decreases the duration of PTZ-induced convulsions in rats (34). Treatment with naltrexone, 30 min after morphine and 5 min prior to PTZ, blocked the increase in latency induced by morphine, unveiled a proconvulsant effect of the opiate on seizure duration and protected all animals from ensuing death. On the other hand, we observed that naloxone enhanced the lethality of PTZ-induced seizures in CRS, but not in nonstressed or SS, mice. Altogether, these opposing findings in mice and rats illustrate the marked interspecies variation concerning the modulation of convulsions by opioids.

Caution must be exercised when interpreting results obtained using naloxone in light of reports showing that, in high doses or concentrations, it can synergize with GABA antagonists to cause

convulsions and displace radiolabelled ligands from neuronal membrane GABA receptors (11,24). However, the fact that the highest dose of naloxone we used did not modify the responses of nonstressed mice to either convulsant argues against this possibility.

Another important finding of the present study was that pretreatment with naloxone further prolonged the latency to PTZ-induced convulsions in both CRS and SS mice, but only attenuated the analgesia promoted by CRS. This fact strongly suggests that the opioid systems modulating nociception and responsiveness to convulsants are distinct. In this regard, it has been shown that the analgesic and proconvulsant effects of methionine-enkephalin in rats are each elicited by injection into different brain areas (12).

In conclusion, the results of the current study, which to our knowledge is the first to compare the effects of two distinct stressogenic stimuli on convulsive parameters, reveal a complex relationship between stress and convulsions. Both forms of stress elicited a proconvulsant or an anticonvulsant effect, depending on the convulsant model used or parameter analysed. The mechanisms involved in these stress-induced alterations remain to be determined, though it appears that opioid systems can play a significant modulatory role in some, but not all, of these changes. Finally, a comparison of these results with those of other studies reveals the inadequacy of generalising conclusions based on data obtained using a single stressogenic procedure, convulsant model and/or convulsive parameter.

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