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Long-Lasting Changes in Morphine Sensitivity Following Amygdaloid Kindling in Mice

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MANSOUR, A., R. DOYLE, R. KATZ AND E. S. VALENSTEIN. *Long-lasting changes in morphine sensitivity following amygdaloid kindling in mice.* PHYSIOL. BEHAV. 27(6) 1117–1120, 1981.—Mice tested at either 3, 27 or 90 days following their last amygdala kindled convulsion exhibited a marked increase in response to morphine compared to controls. Kindled animals showed a higher incidence of clonic convulsions and an exaggerated Straub tail response, both of which could be blocked by naloxone pretreatment. The changes in response to morphine produced by kindling may provide a model for studying the long-lasting effects of convulsions.

Kindling Amygdala Morphine Seizures Mice Convulsion Straub tail response

KINDLING refers to the gradual development of electrical discharges and behavioral convulsions in response to a regimen of brief, intermittent electrical stimulation of the amygdala and other brain structures [19]. The demonstration that kindling can induce a permanent seizure propensity in animals has raised the possibility that this technique may provide a tool for studying the brain mechanisms that underlie the long-lasting behavioral changes claimed to be produced by temporal lobe seizures. In addition to increasing seizure propensity, amygdala kindling has also been reported to produce persistent changes in predatory behavior [1], emotionality [32], and some types of learning [28].

In pursuing our interest in the mechanism that may underlie interictal changes in behavior, we have been influenced by several lines of evidence suggesting a relationship between opiate systems in the brain and seizure activity. Intraventricular injections of endogenous opiates have been shown, for example, to produce epileptiform activity [16,17] and electroconvulsive shock (ECS) in rats produces "opiate-like" effects such as elevation of pain thresholds, catalepsy, and hypothermia [4] and all of these effects can be blocked by the opiate antagonist, naloxone. Repeated ECS has also been shown to increase sensitivity to opiates [3] and to elevate met-enkephalin levels in the hippocampus [22]. These reports of increased endogenous opiates following a regimen of convulsions may be relevant to the finding that kindling reduces morphine withdrawal symptoms in rats [25].

In addition, the amygdala, which is most sensitive to kindling [19], has one of the highest concentrations of opiate receptors in the brain [38]. Micro-injections of morphine into the amygdala produce seizures [39] while systemic morphine has been reported to induce electroencephalographic spiking in this structure [8]. The precise role of opiates in kindling, however, remains unclear. Morphine potentiates amygdala kindled seizures and increases the frequency of interictal spiking in kindled rats [24], but naloxone has been reported not to inhibit amygdala or caudate kindling [11,34] and may even potentiate the rate of kindling [20].

To date, most studies have only examined the direct effects of opiates and opiate antagonists on kindling or the short-term changes in opiate systems following convulsions in rats. We report here that C57BL/6J mice exhibit a marked increase in morphine sensitivity following kindling that is detectable up to three months following an animal's last kindled convulsion.

METHOD

Adult C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were implanted with bipolar electrodes in the amygdala. With the skull level, the stereotaxic coordinates were 1.7 mm posterior to bregma, 3.6 mm lateral, and 5.2 mm below the dorsal surface of the skull. The elctrodes consisted of two twisted teflon coated stainless steel wires (0.006 in dia.) soldered to microminiature pins (Winchester Co. 8456). An additional pin attached to a skull screw was placed anterior to the coronal suture and served as a ground for EEG recording.

Following recovery from surgery, the animals were divided into three experimental and three control groups. Twenty-five experimental animals were stimulated once

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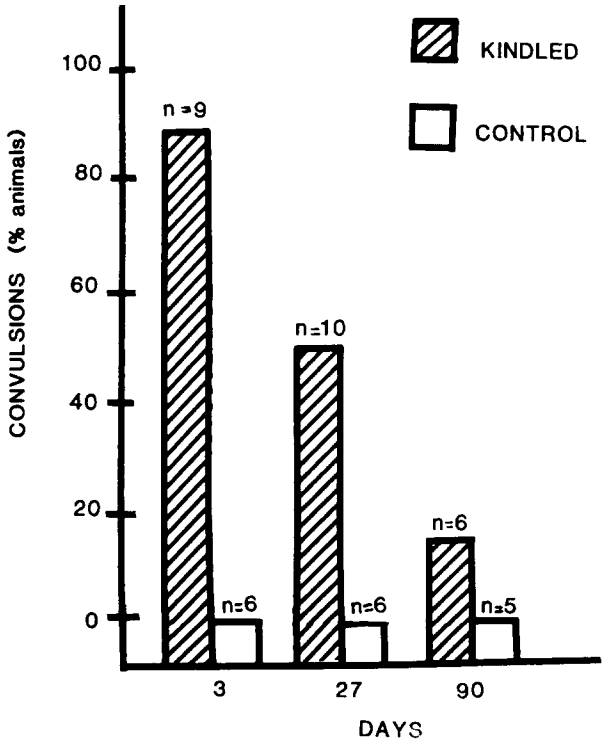

FIG. 1. Percentage of mice exhibiting clonic convulsions in response to morphine (25 mg/kg IP) at 3, 27, and 90 days after termination of kindling. Control mice were not kindled.

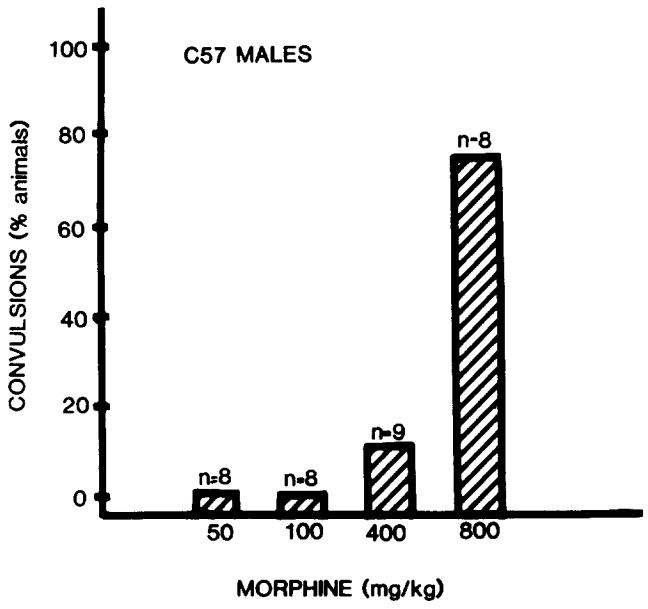

FIG. 2. Percentage of naive C57 mice showing tonic convulsions following different doses of morphine.

daily (4–7 hrs into the 12 hr day cycle) with a 1 sec duration, 50 μA, 60 Hz sine wave current. Kindled convulsions were rated on a modified Racine scale [36] as follows: 1=mouth movements, 2=head bobbing, 3=clonus, 4=generalized convulsions (GC) plus rearing, 5=GC plus rearing and falling, 6=GC plus repeated rearing and falling, 7=GC plus repeated rearing and falling followed by running.

The mice received daily stimulation until they reached a criterion of seven consecutive generalized convulsions (stages 5–7). Typically, mice had the first generalized convulsion after 13–14 stimulations and reached the criterion on approximately the 21st stimulation day. After the animals had reached the criterion, they were injected with morphine (25 mg/kg IP) at either 3 days (N=9), 27 days (N=10) or 90 days (N=6) following their last kindled convulsion. Seventeen additional mice made up the three groups of unstimulated controls. These animals were implanted with amygdala electrodes, handled identically to the kindled animals (including time in the test chamber connected to the electrode cables), and tested with morphine at the 3, 27, and 90 day intervals.

The occurrence of convulsions was recorded during the morphine test session. Animals were placed in Plexiglas boxes (23×23×30 cm) and observed continuously for the occurrence of convulsions over a 105 min period following the injection of morphine. An additional group of 7 mice were kindled to the same criterion and 3 days after their last convulsion they were pretreated with naloxone (5 mg/kg IP) 5 mintes prior to the administration of morphine (25 mg/kg IP).

Following the termination of the experiment all the animals were anesthetized and perfused through the heart with saline and 10% Formalin. The brains were sectioned (40 μm) and stained with cresyl violet to verify the electrode placements. The data presented here represent animals whose electrode tips were localized in the amygdaloid complex.

RESULTS

The kindled animals showed a high incidence of clonic convulsions following morphine (Fig. 1). The convulsions were behaviorally similar to those produced by electrical kindling and generally first occurred 25–35 min after the drug injection. Many of the animals had several convulsions. The incidence of morphine induced convulsions was highest in the 3 day group (89%) and progressively declined in the 27 (50%) and 90 day (16%) groups. In marked contrast, none of the control animals (N=17) had a clonic convulsion following morphine.

In order to determine the extent to which amygdala kindling had increased morphine sensitivity, we treated separate groups of naive male C57 mice with 50, 100, 400, and 800 mg/kg doses of morphine and recorded the incidence of clonic convulsions. None of the naive mice convulsed at doses of 100 mg/kg or lower and only 11% (1 of 9) convulsed following a 400 mg/kg dose of morphine. Although the majority (88%) of the animals did convulse and die at a dose of 800 mg/kg (Fig. 2), none of the animals displayed the clonic convulsion typical of kindled animals injected with much lower doses of morphine. The convulsions of the naive animals were tonic in nature and occurred at the time the animals expired from respiratory failure.

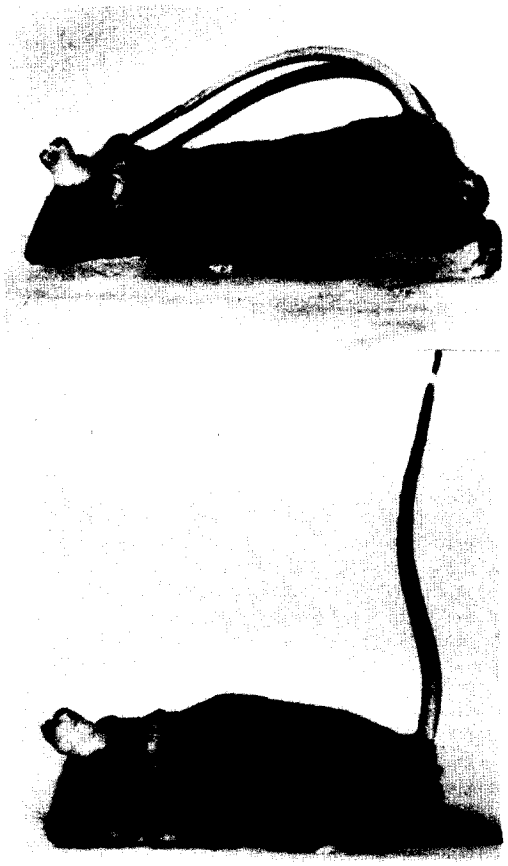

FIG. 3. Straub tail response of kindled (top) and control (bottom) mice given morphine (25 mg/kg IP) 3 days following the termination of kindling.

In addition to a high incidence of convulsion, kindled animals showed a very exaggerated Straub tail response compared to controls. When injected with morphine, the stiffened tails of the kindled mice typically extended forward in an arch to the top of the animal's heads. In contrast, the tails of control animals given morphine rarely extended beyond a vertical position (Fig. 3).

Naloxone pretreatment blocked the morphine induced convulsions in 6 of the 7 kindled mice and attenuated the Straub-tail response. These results are clearly different from those obtained with the 3-day group given morphine alone where 8 of 9 animals had convulsions.

DISCUSSION

The results clearly indicate that kindling produces a long-lasting increase in morphine sensitivity. The increase in morphine sensitivity in kindled mice is reflected in the significant increase in clonic convulsions and Straub tail response induced by this drug. The magnitude of this effect is demonstrated by the fact that normal C57 mice do not display clonic convulsions in response to morphine even in lethal doses, and also by recent work in our laboratory demonstrating that doses as low as 10 mg/kg elicit clonic convulsions in 40% of kindled C57 mice. It should be noted in this context, that a dose of 10 mg/kg morphine produces only a mild analgesia in normal C57 mice [6].The ability of naloxone to block the heightened response to morphine seen in kindled mice raises the possibility that kindling induces changes in the endogenous opiate systems of these animals.

The convulsions induced by morphine may be dependent on the establishment of an epileptic focus by kindling. Evidence in support of this position include the observation that morphine induced convulsions are very similar to those displayed during kindling and the report that morphine produced electroencephalographic spiking in the amygdala [8]. It might be argued that kindled animals have already been shown to have an increased sensitivity to many different convulsant agents such as lidocaine [35] and metrazol [32]. The significant point here is that morphine is not normally a convulsant for C57 mice as we have shown that it generally requires lethal doses to produce convulsions in these animals and even then the convulsions are qualitatively very different than those produced following kindling.

The time dependent decline in the morphine effects observed in our experiment may seem to be inconsistent with the generally accepted idea that kindled rats exhibit clonic convulsions upon the first stimulation even months after the termination of the kindling regimen. In actuality, however, kindled rats left unstimulated for 12 weeks required an average of 4 stimulations before observing a generalized (stage 5) convulsion [19]. We tested, therefore, the permanence of kindling in those mice in the 90 days group that had not exhibited convulsions when tested with morphine. These animals required an average of 4.3 stimulations before showing the first stage 5 convulsion. The results, therefore, are in close agreement with the earlier data from rats and suggest that some aspect of kindling delines with time. This decline appears to be due to local changes at the electrode tip, because most of the animals progressed immediately to a stage 5 convulsion after showing little or no afterdischarge following several preceding stimulations. Two additional daily stimulations produced generalized (stage 5–7) convulsions. When tested a second time with morphine 3 days later, all animals had clonic convulsions. These additional results suggest that the increase in morphine sensitivity parallels the persistence of the kindling effect.

Although the electrodes in the present study were localized in the amygdala, apparently convulsions do not have to be triggered from a temporal lobe structure, as mice undergoing motor cortex kindling also show a heightened sensitivity to morphine when tested 3 days after their last convulsion. Recent work in our laboratory has shown that mice kindled in the anterior cortex also showed a higher incidence of clonic convulsions and an exaggerated Straub tail response when tested with morphine (25 mg/kg).

While the long-lasting effects produced by kindling have been demonstrated in tests with morphine and can be blocked by pretreatment with naloxone, it is certainly possible that other neural systems are actually undergoing the changes. The biogenic amines, for example, have been shown to suppress seizures [2, 9, 10, 13, 27, 37, 40] and kindling in turn has been shown to alter all of the amines as well as other neurotransmitters [5, 14, 15, 18, 30, 31]. Moreover, kindling has been shown recently to produce long-lasting decreases in response to such catecholamine agonists as amphetamine [12] and cocaine [35]. Without ad-

ditional work, it is not possible to decide among alternative hypotheses as the endogenous opiate systems activated by morphine are believed to modulate activity of the biogenic amines [7, 21, 23, 26].

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