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Bidirectional Transfer of Intracerebrally Administered Pentylenetetrazol and Electrical Kindling[1]

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CAIN, D. P. *Bidirectional transfer of intracerebrally administered pentylenetetrazol and electrical kindling.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1111–1113, 1982.—Rats were kindled using electrical stimulation or the infusion of pentylenetetrazol (PTZ) in one amygdala, and subsequently rekindled using infusion of PTZ or electrical stimulation, respectively, in the contralateral amygdala. Control rats received infusions of saline into one amygdala, and were subsequently electrically kindled in the contralateral amygdala. The rats that previously had been kindled using one agent kindled significantly more rapidly and displayed significantly stronger generalized seizures when rekindled using the other agent. The results demonstrate that intracerebrally administered PTZ can effectively kindle seizures, and thus that a peripheral change in response to PTZ is not crucial for seizure development. The results also demonstrate that this form of kindling transfers bidirectionally to electrical kindling.

Kindling Seizures Pentylenetetrazol Transfer

THE repeated administration of a variety of convulsant agents in initially subconvulsant amounts results in the gradual kindling of seizures. Recent findings indicate that the increase in seizure susceptibility that occurs during kindling is not always limited to an increased susceptibility to other kindling agents—the transfer effect [10].

Although pharmacological kindling by repeated injection of pentylenetetrazol (PTZ) and bidirectional transfer to electrical kindling of the amygdala have been demonstrated [4,9], the mechanisms underlying these phenomena are not well understood. Because all previous kindling studies using PTZ utilized an intraperitoneal route of administration, there is some question whether the kindling of seizures that resulted was due to brain changes of the kind thought to underlie kindling [12], or whether a peripheral change in the organisms' response to or metabolism of the drug could account for the increased seizure susceptibility. A further question of the convulsive efficacy of direct intracranial application of PTZ was raised by the recent finding that a single intraventricular injection of a large dose of PTZ did not result in seizures [1]. The present study was an attempt to kindle seizures by repeated intracerebral infusion of PTZ, and to test for bidirectional transfer to electrical kindling of the amygdala.

METHOD

Thirty male hooded rats weighing 300 to 400 g served as subjects. They were anesthetized with pentobarbital and received implantation of a bipolar electrode into one basolateral amygdala and a 23 ga guide cannula into the contralateral hemisphere using standard stereotaxic techniques. The tip of the guide cannula was placed 1.0 mm above the basolateral amygdala and the 30 ga infusion cannula protruded 1.0 mm beyond the tip of the guide cannula. Electrodes were constructed of twisted Nichrome wire 127 μm in diameter, insulated except at the cut tips.

After a 14-day recovery period, the subjects were randomly separated into one control and two experimental groups. The subjects in one experimental group were initially kindled electrically through the amygdala electrode and subsequently rekindled in the other hemisphere using PTZ (E-PTZ group; n=9). The subjects in the other experimental group were initially kindled using PTZ, and subsequently rekindled in the other hemisphere through the amygdala electrode (PTZ-E group; n=10). In order to control for the possible effects of the repeated infusion of a fluid into one hemisphere upon the rate of electrical kindling in the other hemisphere, the control subjects (S-E group; n=10) received infusions of saline equal in number to the infusions of PTZ administered to the PTZ-E group. The control subjects first received the repeated infusion of saline into one amygdala, and subsequently were kindled electrically in the other amygdala.

For electrical kindling the subjects were connected to a polygraph and stimulator by means of a miniature plug, and the EEG was recorded before and after stimulation in order to obtain a prestimulation baseline and to record evoked

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afterdischarge (AD). The kindling stimulation consisted of 1 sec of 60 Hz biphasic constant current square waves, each 1 msec in duration. The AD threshold was determined in all of the subjects at the outset by applying an initial stimulation of 40 μA (base-to-peak), and increasing the intensity by 20 μA on each subsequent stimulation until an AD was evoked. The subjects in the E-PTZ group were then electrically kindled, beginning 24 hr later, using stimulations at an intensity of 200 μA, spaced at 24 hr intervals. The stimulations were repeated until a total of three generalized stage 5 seizures [11] had been evoked.

The subjects in the PTZ-E and S-E groups were rested for 48 hr after the AD threshold determination, and then infused with PTZ or saline once every 48 hr. The subjects in the PTZ-E group received 0.4 mg PTZ in a volume of 1.0 μl over 34 sec, and the subjects in the S-E group received an equivalent volume of isotonic saline over 34 sec. This dose of PTZ was determined to be effective for the kindling of seizures when infused repeatedly into a group of pilot subjects. All solutions were buffered. The infusions were delivered using a Sage infusion pump, and the infusion cannula was left in place for 15 sec, after which it was replaced by the obturator. The subjects were then connected to the polygraph and placed in a Faraday cage for the recording of EEG from the amygdaloid electrode during a 15-min observation period. Any seizures that occurred were closely monitored, and seizure latency and duration were recorded. The subjects in the PTZ-E group were infused until they had each displayed three generalized seizures.

After the completion of initial kindling or infusion of saline all subjects were rested for 1 wk. The AD threshold was then redetermined in the PTZ-E and S-E subjects, and they were kindled electrically beginning 48 hr later. The subjects in the E-PTZ group were kindled with PTZ using the methods described above.

At the end of testing all subjects were anesthetized and perfused with formol saline, and the brain was removed, frozen, and sectioned for verification of electrode and cannula placements. One subject in the E-PTZ group had a cannula placement outside of the amygdala and was discarded. All subjects described below had electrode and cannula placements within the amygdala.

The results were analyzed using a 2-way analysis of variance and post-hoc tests of simple main effects with an experiment-wise error rate of $p=0.05$ [8].

RESULTS

There was little or no response to the initial infusion of PTZ by most of the subjects in the PTZ-E group. However, most of these subjects displayed mild twitching or chewing (stage 1) in response to the second infusion of PTZ. The seizure signs became progressively more severe in response to each successive infusion, and the subjects in this group displayed a generalized seizure after a mean of 14.5 infusions. The seizure manifestations were very similar or identical to those typically displayed by a rat electrically kindled in the amygdala, and the subjects progressed through all of the seizure stages described by Racine [11]. Early in the development of PTZ-kindled seizures (during stage 1) epileptiform spiking was observed in the hemisphere contralateral to the infusion in approximately half of the subjects. Strong sustained spiking was invariably observed in subjects displaying a stage 2 through stage 5 seizure.

The main results appear in Fig. 1, where the kindling rates

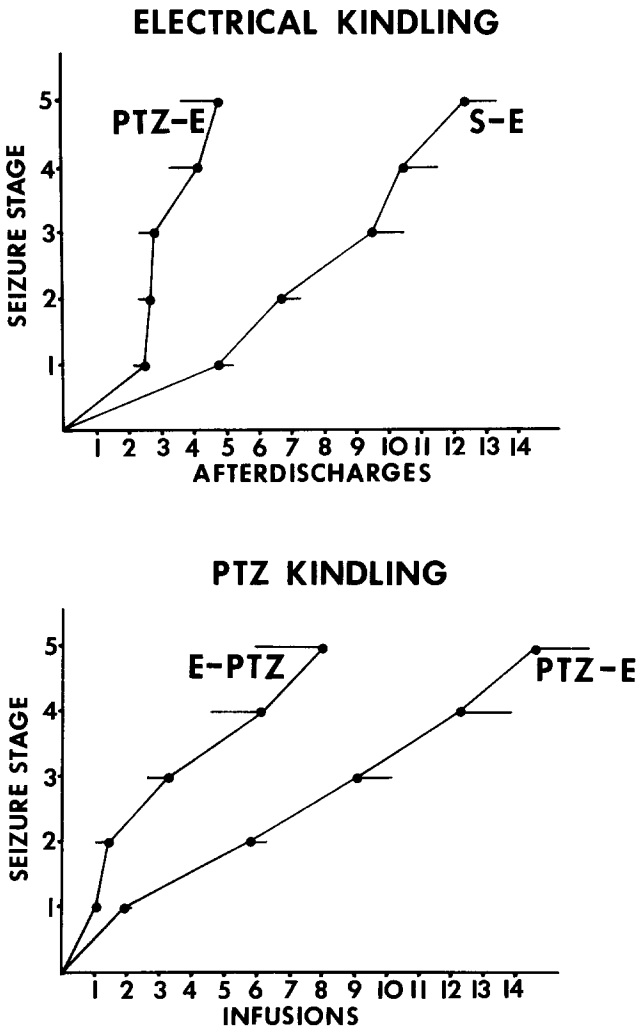

FIG. 1. Rate of electrical kindling (top) and pentylenetetrazol kindling (bottom). The values are means ±S.E.M.

of the groups are presented. The PTZ-E group required fewer than half as many ADs to develop generalized seizures during electrical kindling as the S-E control group required (Fig. 1, top). Analysis of variance indicated that there was a signifciant groups main effect ($F=22.91$; $p<0.0003$) and a significant groups × seizure stage interaction ($F=5.63$; $p<0.0007$). Tests of simple main effects indicated that the PTZ-E group kindled significantly more rapidly than the S-E group to all seizure stages (range of $Fs=141.9$ to 361.6; all p-values<0.0001).

The E-PTZ group required fewer infusions to develop generalized seizures during PTZ rekindling than the PTZ-E group required during initial PTZ kindling (Fig. 1, bottom). Analysis of variance indicated that there was a significant groups main effect ($F=16.27$; $p<0.0009$), and a significant groups × seizure stage interaction ($F=2.59$; $p<0.05$). Tests of simple main effects indicated that the E-PTZ group kindled significantly more rapidly than the PTZ-E group to stages 2 through 5 (range of $Fs=36.2$ to 58.3; all p-values<0.0001).

The generalized seizures that were displayed during rekindling were more severe than those evoked by the same

kindling agent during initial kindling. Thus, for electrical kindling the mean duration of the first generalized seizure in the PTZ-E group during rekindling (116.3 sec; range=88–180) was significantly longer ($p<0.025$, Mann-Whitney U) than that of the S-E group during initial kindling (64.5 sec; range=26–102). Similarly, for PTZ kindling the mean duration of the first generalized seizure in the E-PTZ group during rekindling (140.3 sec; range=45–295) was significantly longer ($p<0.05$) than that of the PTZ-E group during initial kindling (75.3 sec; range=40–122). The mean latency to the first generalized PTZ-induced seizure also was significantly shorter ($p<0.05$, Mann-Whitney U) in the E-PTZ group (75.7 sec; range=40–210) than in the PTZ-E group (173.2 sec; range=25–425).

The mean initial AD threshold of the PTZ-E group was 60.0 μA and of the E-PTZ group was 56.2 μA ($p>0.05$, Mann-Whitney U). The effect of initial PTZ kindling in the PTZ-E group was to reduce the AD threshold to 58.3 μA. This reduction was not significant ($p>0.05$, Sign test).

DISCUSSION

These results indicate that PTZ is capable of kindling generalized seizures when it is repeatedly infused directly into the amygdala. The fact that PTZ is effective for the kindling of seizures when it is repeatedly infused directly into the brain in constant amounts indicates that any peripheral mechanisms contributing to the occurrence of seizures that are brought into play as a result of its repeated peripheral administration are not crucial for the development of PTZ-kindled seizures. Such mechanisms, if they exist, might contribute to seizure development when PTZ is repeatedly administered in the periphery, however.

At the dose used in this study the initial infusion of PTZ nearly always failed to elicit a convulsive response, although the second and subsequent infusions elicited progressively stronger convulsions. It is not clear why Albala [1] failed to observe convulsions after a single intraventricular injection of a large dose of PTZ. It is possible that additional spaced injections might have evoked convulsions. It is also possible that the intraventricular route is not optimum for the elicitation of convulsions by PTZ.

These results also indicate that bidirectional transfer of kindling occurs when electrical stimulation of the amygdala and intracerebral infusion of PTZ are used as kindling agents. The amount of transfer observed in this study is similar to the amount reported to occur in transhemispheric transfer of electrical kindling of the amygdala and transfer of electrical and carbachol kindling of the amygdala [5,11]. The transfer was reflected in a variety of measures of seizure development. These included more rapid development of generalized seizures and longer seizures with both kindling agents during rekindling compared to initial kindling, and a shorter latency to seize during rekindling with PTZ compared to initial kindling with PTZ.

The mechanism by which each of these kindling agents facilitates kindling by the other is unknown. PTZ appears to exert its convulsant effect by a variety of neural mechanisms [14], any of which could conceivably interact with the brain changes associated with electrical kindling to produce transfer of kindling. A number of recent findings suggest that muscarinic cholinergic neurons are probably involved in the development of electrically kindled seizures [2, 3, 6, 13]. One of the potentially epileptogenic effects of PTZ on brain is the inhibition of acetylcholinesterase [7,14], and PTZ might rapidly rekindle electrically kindled subjects by inhibiting this enzyme [6].

Alternatively, any treatment that induces the repetitive and sustained discharge of amygdala neurons might transfer readily to electrical kindling of the amygdala. The recording of gross EEG activity during transfer testing with PTZ during the present study and with intracerebrally administered carbachol in a previous study [5] indicates that strong epileptiform discharge is evoked in the amygdala shortly after the infusion of these drugs. The epileptiform discharge and the resulting convulsive behaviors are very similar or identical in form to those observed during electrical kindling of the amygdala. These observations are at least consistent with the idea that the transfer of kindling by these three agents is based on their ability to induce the strong, repetitive and sustained discharge of amygdala neurons, thus driving a more caudal seizure generalizing mechanism. For a preliminary test of this hypothesis it would be useful to observe the response of single amygdala neurons to the administration of the different kindling agents discussed here.

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