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Arrest of Seizure Series Induced by an Intracortical Injection of Penicillin in the Awake Rat

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HORN, E. AND K. ESSELING. *Arrest of seizure series induced by an intracortical injection of penicillin in the awake rat.* PHARMACOL BIOCHEM BEHAV 45(4) 857–863, 1993.—Experiments were performed to answer the question, whether series of generalized tonic-clonic seizures, induced in the awake rat by a local injection of Na-penicillin (PCN) solution into the motor cortex, terminates at the same critical concentration C_t of PCN within the focal area independently of the concentration C_0 of PCN injected. Using the PCN diffusion coefficient $D = 3.52 \times 10^{-4}$ mm^2/s and the tortuosity factor $\lambda = 1.62$, the concentration C_t at the onset of the last generalized seizure was calculated. The median duration of seizure series increased from 32 to 190 min, when the dose of injected PCN was raised from 32 to 1000 IU. At the onset of the last seizure, the median concentration C_t within the artificial focus ranged from 2.1 to 4.0 IU/0.5 μl saline in rats treated with 32 to 125 IU PCN. After induction of convulsive behaviour with C_0 = 250, 500, or 1000 IU PCN/0.5 μl saline, however, C_t was at a higher level between 6.1 and 7.4 IU PCN/0.5 μl saline. The difference between the cumulated data from the low-dose vs. the high-dose range was significant ($p < 0.01$). It is concluded that during long-lasting series of generalized seizures, the brain takes advantage of its plastic properties. By forming a counteracting mechanism, it protects itself from extreme epileptiform activity. This autoprotection may be due to the activation of neuronal networks which probably needs a certain frequency of seizures to become operatively.

Diffusion Dose relationship Epilepsy Generalized seizures Protective mechanisms Rat

GENERALIZED seizures can be elicited by GABA antagonists. Their local intracerebral or epicortical application [cf., (12)], as well as a systemic injection [cf., (1,3)] leads to disinhibition of brain activity. The convulsant effect of Na-penicillin (PCN) detected by chance (28) is probably due to its antagonizing action to GABA (5,9,18,26). After a single application of PCN into the motor cortex, a series of regularly occurring generalized seizures is initiated lasting some time (10). The most likely reason for their disappearence is the decrease of PCN concentration in the application area below a critical threshold due to diffusion. However, also physiological factors may be effective as, for example, the stability of neuronal activity shown by the aggravating effect of cortical lesions (11). Other physiological mechanisms of seizure arrest may include ionic changes in the extra- and intracellular space (2), the activation of endogeneous substances, or other autogenic factors with anticonvulsive properties (6), or even the recruitment of neurons during the convulsive behaviour (25). Similar processes may also be involved in the arrest of a series of seizures induced by a single injection of PCN into the motor cortex.

Investigations about the regularities of diffusion in brain tissue (15,20,21) have opened the possibility to determine the contribution of this physical process on the termination of seizure series induced by a minute intracortical volume of a PCN solution. Therefore, different doses of PCN were injected into the cortex and the duration of the induced seizure series was determined. These experimental values were compared with those obtained from calculations on the basis of the diffusion equation (20). In this way, the question could be answered whether there is a critical threshold concentration C_t of PCN at which seizure activity stops.

METHOD

Experiments were performed with 81 awake female and male Wistar rats from the stock of the Centre for Experimental Animals of the University of Ulm (Germany). Animals were kept under a light–dark rhythm of 12L : 12D h for their whole life. They were prepared for the experiments when they were 5 to 7 months old. Under Na-pentobarbital anaesthesia (75 mg/kg body weight IP), a guiding tube for the local PCN

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treatment was fastened to the skull. Furthermore, six AgAgCl-electrodes, three on each hemisphere, were attached to the skull (cf., inset of Fig. 2), to record seizure activity. For recovery, animals were housed individually and handled carefully every day for at least 2 weeks, until the critical seizure test started.

Convulsive behaviour was elicited in the awake rat by injecting 0.5 μl of a Na-penicillin (PCN) solution at a depth of about 1.5 mm into the foreleg field of the right motor cortex near the stereotaxic coordinates A2 (bregma) and R2. PCN was diluted in a sterile 0.9%-NaCl solution. Seven doses were used, the lowest being 16 IU, the highest 1,000 IU. Controls received an equal volume of the solvent. The application was performed without anaesthesia, while the carefully handled rats were kept in the hand of the experimenter. Animals were sacrified 1–3 days after the experiment. Inspection of the brain sections [preparation according to (22)] stained with cresylviolett revealed that all injections were located within the cerebral motor cortex between the stereotaxic coordinates A2.8 to A1.4 (with respect to bregma), R0.8 to R2.7 and H −1.4 to H −1.6 (sub dura).

Generalized seizures were recorded by means of the electroencephalogram (EEG). The AgAgCl-electrodes had a tip diameter of 0.3 mm and were positioned 0.4 mm above the dura at the stereotaxic coordinates A4.5 and R/L1.8, P1.0 and R/L2.3, P5.8, and R/L2.7 (with respect to bregma). Bipolar recordings were taken between adjacent electrodes on both sides of the brain. During the recordings, the rats could move freely in a chamber of 25 × 25 × 35 cm^3 standing in a large Faraday box. Experiments started with a control session lasting 1 h. After the PCN injection, experiments were followed until the last generalized seizure was observed. We considered a seizure as the last if it was followed by a period of at least 3 h completely seizure free.

The time course of PCN concentration after the injection was calculated by using the diffusion equation (15,20)

$$C_t = 1/2\, C_0 \left\{ \operatorname{erf} \frac{\lambda(r+b)}{2\sqrt{Dt}} - \operatorname{erf} \frac{\lambda(r-b)}{2\sqrt{Dt}} - \frac{2\sqrt{Dt}}{\lambda r \sqrt{\pi}} \left[\exp \frac{-\lambda^2 (r-b)^2}{4Dt} - \exp \frac{-\lambda^2 (r+b)^2}{4Dt} \right] \right\}$$

In this equation, C_t is the concentration of PCN at the time t after the injection, and C_0 the concentration of PCN injected at time zero into the brain; b is the radius of the injected volume, and r the distance from the center of the volume to the site at which C_t was calculated. The diffusion coefficient $D = 3.52 \times 10^{-4}$ mm^2/s for PCN and the tortuosity factor $\lambda = 1.62$ for brain tissue (15,20) were used. C_t was calculated

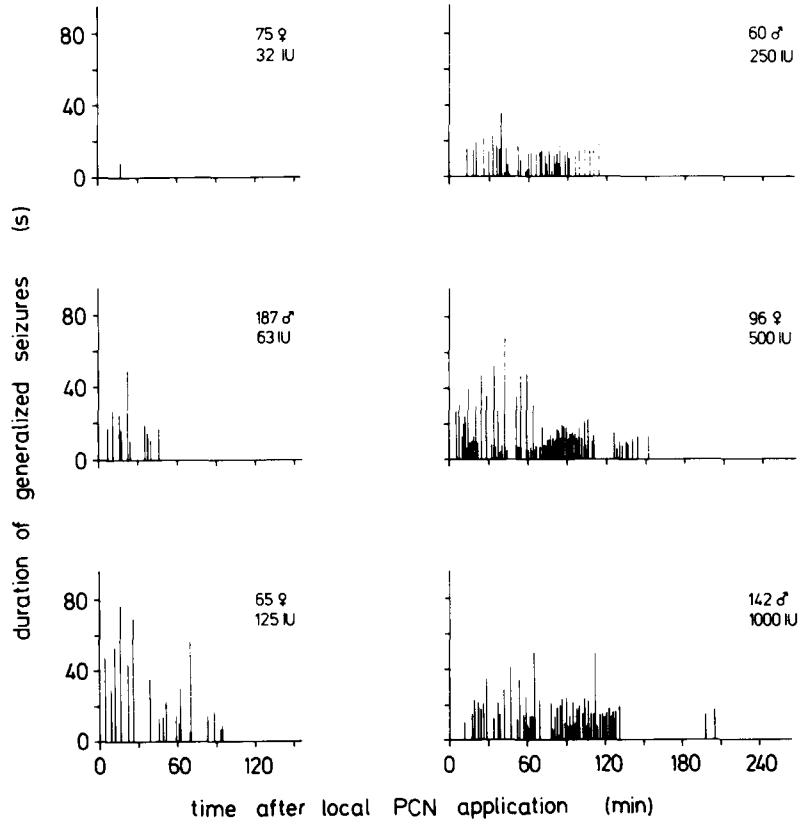

FIG. 1. Time course of series of generalized tonic-clonic seizures induced by an intracortical injection of PCN into the motor cortex. Vertical bars indicate the onset and duration of seizures. If more than one generalized seizure occurred during a minute, the vertical bar represents the sum of them. For each dose, one example was chosen. Identification numbers and sex of the rat as well as the doses of PCN are given at each plot. Time zero indicates the injection time.

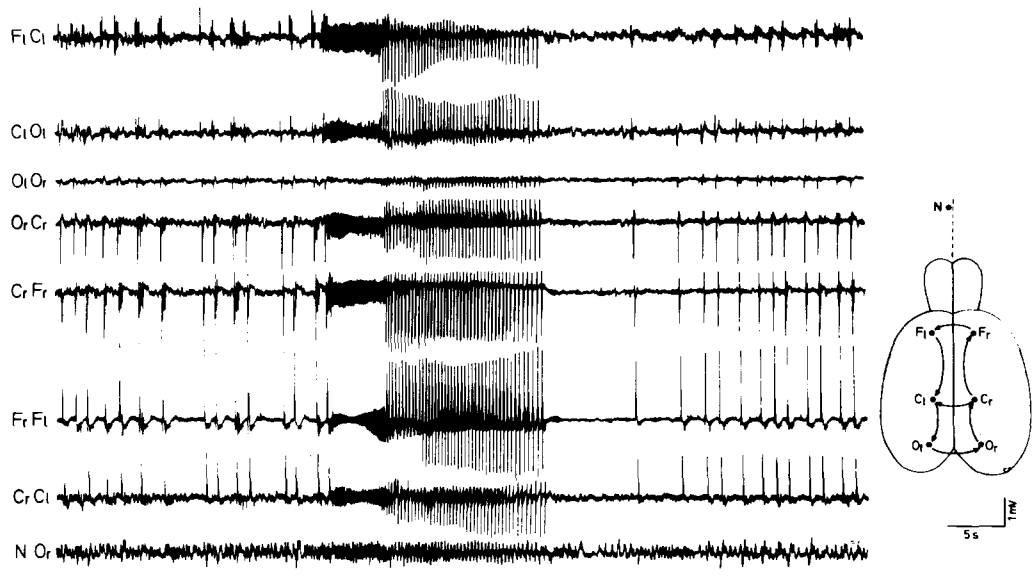

FIG. 2. Typical EEG pattern of a generalized tonic-clonic seizure induced by a local injection of PCN into the foreleg field of the motor cortex of the right hemisphere. Note the high cortical activation near the application area. The tonic component at the beginning of the seizure is followed by the clonic period characterized by the large potentials. The picture shows seizure number 13 out of 73 recorded for this animal. F, C, O, frontal, central, and occipital electrodes, respectively; l, r, left and right side, respectively.

in a distance $r = 0.2$ mm from the center of the injection area, which was a volume with a radius of $b = 0.5$ mm.

The nonparametric *U*-test from Wilcoxon, Mann and Whitney (29) was used. Additionally, calculations were performed to look for correlations between the different seizure-related values and the onset of the first or last seizure. In figures, individual values and median values are presented; if not given in the figures, levels of significances are mentioned in the text.

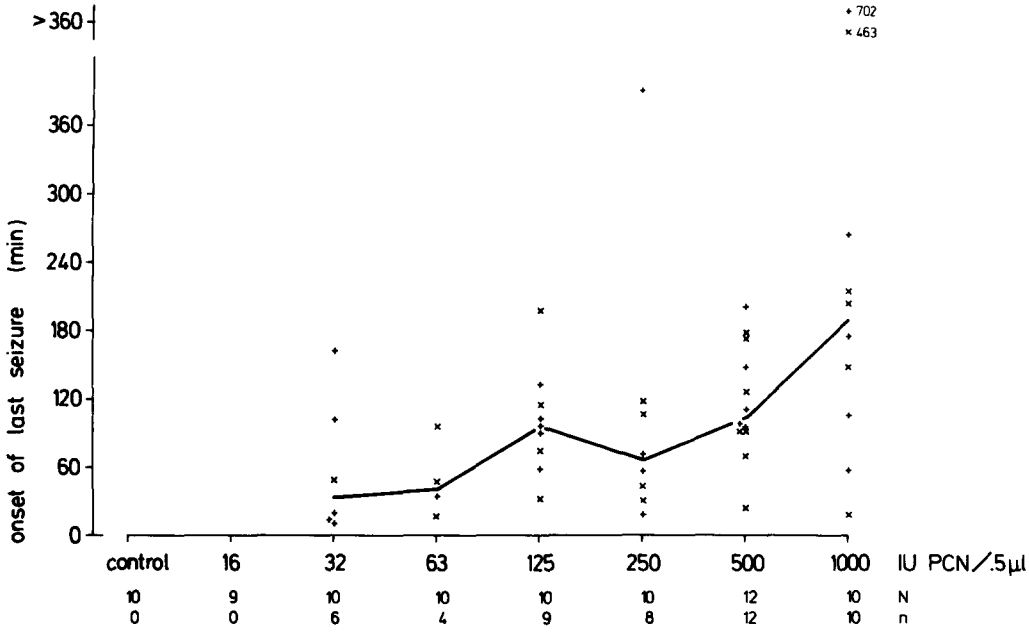

FIG. 3. Onset of the last seizure in relation to the dose of PCN injected into the motor cortex of the right hemisphere. Time zero marks the injection time of PCN. The heavy line interconnects the median values obtained from the male (x) and female (+) rats for the corresponding dose. N, number of animals tested for each dose; n, number of rats in which seizures could be recorded.

TABLE 1

MEDIAN VALUES FOR THE TOTAL NUMBER OF SEIZURES, THEIR NUMBER PER HOUR, AND THEIR LENGTH IN RELATION TO THE DOSE OF PCN INJECTED INTO THE FORELEG FIELD OF THE RIGHT MOTOR CORTEX

Dose (IU)	Number (n_s)	Number/Hour (n_s/h)	Length (s)	n	N
1000	87 (3-327)	24 (6-74)	11 (8-17)	10	10
500	37 (2-213)	19 (1-64)	15 (8-28)	12	12
250	16 (1-156)	15 (6-44)	17 (11-13)	8	10
125	20 (3-100)	13 (2-40)	15 (10-29)	9	10
63	7 (1- 17)	8 (5-14)	18 (9-26)	4	10
32	5 (1- 41)	9 (4-24)	14 (8-29)	6	10
16	–	–	–	0	9
solvent	–	–	–	0	10

PCN was always solved in 0.5 μl of 0.9% NaCl solution. Each median value is followed by the lowest and largest value within the respective dosis-group (in parentheses).

N = number of animals used for the corresponding dose. n = number of animals from which the corresponding value could be determined.

n_s = number of seizures of a rat.

RESULTS

Data Obtained From the Experiments

A local application of a suprathreshold amount of PCN into the superficial layer of the right foreleg field of the motor cortex of awake rats induced a series of tonic-clonic, tonic, or clonic generalized seizures (cf., Fig. 1). Tonic phases of a seizure were characterized by potentials of high repetition rates up to 12/s. During clonic phases, large potentials could be recorded which correlated with jerks up to 4/s. Mostly, each of these large potentials was followed by a short-lasting high-frequent EEG-baseline fluctuation up to 15/s. For tonic-clonic seizures, the mean duration of the tonic phase was about 4 s (Fig. 2). For low PCN concentrations C_0, seizures were initiated at regular intervals, while for high concentrations the repetition rate increased especially between the 60th and 120th min after PCN injection (cf., Fig. 1). No seizures were obtained from rats treated with the solvent or a PCN solution containing only 16 IU in 0.5 μl sterile saline. Threshold doses ranged from 32 to 63 IU PCN, because after this stimulus, 10 out of 20 (= 50%) rats developed seizures (cf., Fig. 3). Male and female rats did not differ, so that all the following calculations included both sex (cf., Figs 1, 3, 5).

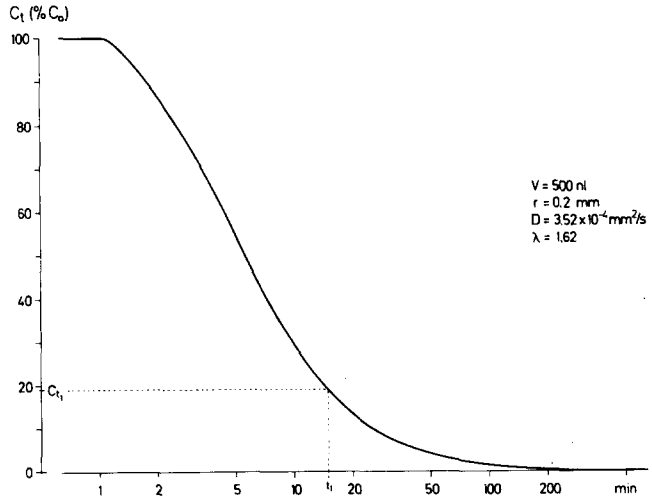

FIG. 4. Time course of PCN concentration C_t as a percentage of the injected concentration C_0. Injection time of PCN: t = 0 min. Parameters for calculation of this function: V, injected volume; r, distance from the center of the injected volume; D, diffusion coefficient for PCN; λ, tortuosity factor for brain tissue.

The time of onset of the first seizure ranged from 123 to 5400 s after PCN injection if all experiments were considered. The median values for the six dose groups varied irregularly between 359 and 615 s. These differences were not significant ($p > 0.2$). The median values for the onset of the last generalized seizure increased with increasing dose. They ranged from 32 min for the 32 IU-group to 190 min for the 1,000 IU group (Fig. 3). To overcome the large interindividual variation of the values and the low number of animals (Table 1), p levels of significances were calculated after cumulation of two dose groups. In particular, the cumulated values of the groups treated with 32 or 63 IU PCN differed significantly ($p < 0.05$) from the cumulated values obtained from the 500 and 1,000 IU-groups.

The median number of seizures (n_s) increased from 5, obtained from the 32 IU-group, to 87 seizures for the 1,000 IU-group (Table 1). The median of the cumulated values from the 32 and 63 IU group differed significantly ($p < 0.05$) from that obtained from the cumulated 500 and 1,000 IU group. Also, the number of seizures per hour (n_s/h) increased with increasing PCN dose (Table 1). Despite of the large interindividual variability of these values, the increase of the median from 9/h for the 32 IU group to 24/h for the 1,000 IU-group was significant ($p < 0.05$). In contrast, the length of individual seizures was only slightly affected by the PCN dose (Table 1). The medians were between 11 and 18 s, the interindividual variation ranged from 8 to 28 s for all dose groups. The differences were not significant ($p > 0.2$) even if data cumulation for the low-dose and high-dose range was used for statistics.

All seizure-related parameters like the time of onset of the last seizure, the number and frequency of all seizures, as well as the mean seizure-duration were independent of the time of onset of the first seizure. The corresponding correlation coefficients were $r = -0.08$, $r = 0.21$, $r = -0.38$, and $r =$

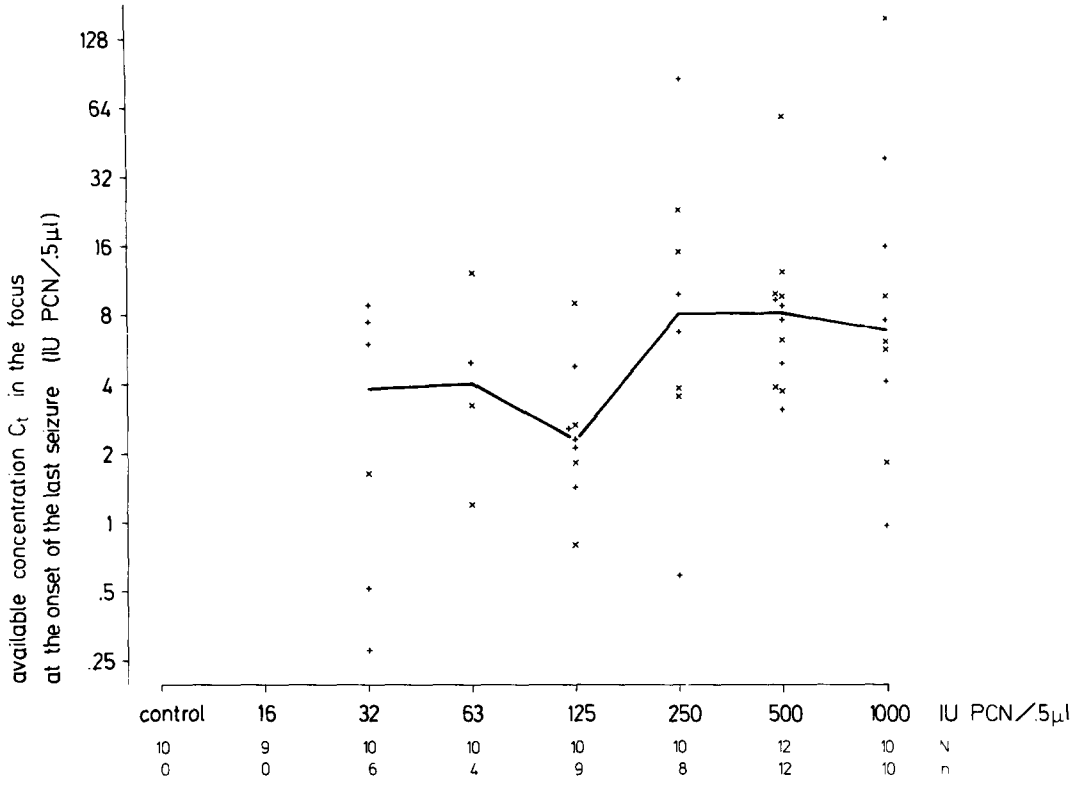

FIG. 5. The concentration C_t of PCN in the focus available at the onset of the last seizure in relation to the concentration C_0 of PCN applied into the motor cortex. Calculation of C_t was based on the diffusion curve, shown in Fig. 4 and on the data presented in Fig. 3. +, females; x, males. The fat line connects the median values obtained for each concentration.

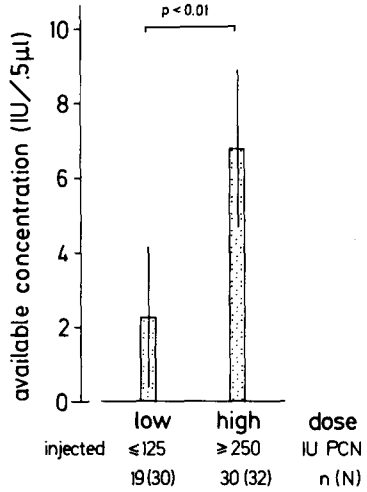

FIG. 6. The concentration C_t of PCN within the injection area available at the onset of the last PCN induced seizure. The cumulated data for the three groups treated with 32, 63, and 125 IU PCN were compared with the cumulated data obtained from the three groups treated with 250, 500, and 1000 IU. Levels of significances between these cumulated groups are indicated below the columns. N, number of rats used; n, number of rats from which generalized seizures could be recorded.

0.22, respectively ($p > 0.2$; $n = 6$). In contrast, number and frequency of seizures were highly correlated to the time of onset of the last seizure. Correlation coefficients obtained from the median values were $r = 0.98$ and $r = 0.93$ ($p < 0.01$; $n = 6$). No significant correlation existed between the onset time of the last seizure and the mean duration of the seizures from individual series ($r = -0.76$, $p > 0.05$; $n = 6$).

Data Calculated on the Basis of the Diffusion Equation

Calculations on the basis of the diffusion equation (20) assume a spherical shape of the injected volume. Obviously, this condition is never fulfilled in an experimental situation. Nevertheless, it leads to an approximation of the spatial distribution of a substance within the brain tissue. With these limitations in mind, the calculation showed a dramatic decrease of the PCN concentration C_t for distances r from the application center larger than 1 mm. For $r = 0.6$ to 1.0 mm, the peak concentration C_t decreased from 20% to 4% with respect to C_0, and was reached after 8 and 50 min, respectively. Therefore, the PCN injections used obviously caused local effects, originating in the foreleg field of the motor cortex. With this assumption in mind, the theoretical time relationship of the diffusion characteristic was calculated within the application area ($r = 0.2$ mm) (Fig. 4).

Based on the figured diffusion characteristic (cf., Fig. 4), the concentration C_t within the application area at the time t of the onset of the last seizure (cf., Fig. 3) was calculated.

The result showed that C_t depended on the concentration C_0 of PCN applied into the cortex (Fig. 5). In particular, for the rats of the 32 IU group, C_t ranged from 0.3 to 8.0 IU PCN/0.5 μl (median: 3.4 IU/0.5 μl), for the 63 IU group from 1.1 to 10.9 IU/0.5 μl (median 4.0 IU/0.5 μl), and for the 125 IU group from 0.7 to 8.0 IU/0.5 μl (median 2.1 IU/0.5 μl). For larger doses, the individual values for C_t ranged from 0.5 to 75.4 IU/0.5 μ (median 7.4 IU/0.5 μl) in the 250 IU group, from 2.8 to 51.9 IU/0.5 μl (median 7.3 IU/0.5 μl) for the 500 IU group, and from 0.9 to 137.5 IU/0.5 μl (median 6.1 IU/0.5 μl) for the 1,000 IU group (Fig. 5). For statistical calculations, the cumulated values obtained from the 32 to 125 IU group were compared with the combined values from the rats treated with doses of 250 IU PCN or more. Both collectives differed significantly ($p < 0.01$) (Fig. 6). This means that the PCN concentration C_t in the focus at the arrest of a long-lasting seizure series was higher compared to C_t obtained after a short-lasting series.

DISCUSSION

The performed experiments should clarify whether the duration of a series of seizures induced by an intracortical injection of the GABA antagonist penicillin (PCN) is only determined by physical factors like the spread of PCN due to diffusion, or whether physiological mechanisms are involved. The model of PCN-induced epilepsy was used for this analysis because the diffusion of PCN within the brain is well investigated (15). If only regularities of diffusion are responsible, seizure activity would arrest at a critical PCN concentration C_t within the application area which is independent of the concentration C_0 used to elicit the seizures. Each deviation of this rule would imply that physiological mechanisms are involved. If C_t decreases with increasing C_0, an augmentation of brain sensitivity is likely; on the other hand, a reduction of brain sensitivity can be postulated, if C_y increases with increasing C_0.

Our results clearly demonstrate that for high PCN concentrations C_0, the critical threshold value C_t, characteristic for the onset of the last seizure is significantly higher than for low C_0 levels (Figs. 5, 6). This means that seizure activity arrests earlier than from the regularities of diffusion can be expected. It is unlikely that metabolic exhaustion due to the increased metabolic demand (4,13,23) or motor fatique during seizure activity are responsible for the observed effects because the period of seizures series can be prolonged by cortical lesions, even in cases of severe epileptiform activity (11). It is also unlikely that changes of the ionic distribution or the extracellular space (ES) are responsible for the observed effects. In general, a rise of K^+ and a decline of Ca^{2+} concentration within the ES (8,14), as well as a shrinkage of the ES (16), occur during seizure activity. These factors, however, rather support the development of seizure activity than reduce it.

We, therefore, postulate protective processes which develop in response to long-lasting epileptiform activity. In fact, epileptiform activity can inhibit mechanisms of seizure initiation. An effective anticonvulsant feedback system was described in cats in which interictal activity induced by cortical cobalt application could be reduced if each burst triggered a stimulation of the caudate nucleus (24). There are also reports that it is just the occurrence of seizures that changes the susceptibility of the nervous system for seizure initiation. In amygdala-kindled rats [(cf., (27)], a stimulation administered 90 min after a generalized seizure elicits normal seizures, while a series of 19 kindled motor seizures elicited at 1.5-h intervals suppresses seizure initiation for several days (19). Similar results have been obtained by seizures elicited by electroconvulsive shock on the subsequent elicitation of electroshock seizures (7). Finally, the performance of a large number of kindled seizures increased the anticonvulsant effect of phenobarbital (17). These results are important for the understanding of our experiments. We have shown that the repetition rate of seizures increased with increasing concentration C_0 of injected PCN (Table 1). It is likely that due to the decreasing interval between seizures the threshold for seizure initiation is shifted to higher levels due to postseizure inhibition. In this way, the period of seizure initiation compared to that calculated on the basis of PCN diffusion is shortened.

In conclusion, it is likely that during long-lasting periods of penicillin-induced seizures the brain takes advantage of its plastic properties. By forming a counteracting mechanism, it protects itself from extreme disturbances. This autoprotection may be due to the activation of neuronal networks which needs some time to suppress the mechanisms responsible for seizure initiation.

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