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INHIBITION IN PENICILLIN-INDUCED EPILEPTIC FOCI [1]

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As an acute model of focal epilepsy, the penicillin focus has been widely utilized since the initial report by Walker and Johnson (1945). However, the cellular mechanisms responsible for the epileptogenic effect of penicillin remain poorly understood. A characteristic feature of penicillin-induced epileptic foci is the paroxysmal depolarizing shift (PDS), which may be observed in many of the neurons in the focus (Matsumoto and Ajmone Marsan 1964). The PDS is well correlated temporally with the interictal spike seen in the electrocorticogram and appears to be a giant excitatory postsynaptic potential (EPSP) (Matsumoto et al. 1969; Prince 1969). Attempts to explain the basis for the PDS have led to investigation of the effect of penicillin on inhibitory processes in the cortex. Curtis et al. (1972) demonstrated that penicillin increased the firing rate of unidentified neurons in cat postcruciate cortex and antagonized the inhibitory action of gamma-aminobutyric acid (GABA) on the discharge of those neurons.

Still, the effect of penicillin upon inhibitory processes in mammalian cortex remains an open question. While inhibitory postsynaptic potentials (IPSPs) are still present in epileptic foci (Dichter and Spencer 1969; Matsumoto et al. 1969; Prince 1969), their functional effectiveness may be impaired. To attack more directly the question of functional inhibition in the cortex, Van Duijn et al. (1973) used the surface negative wave evoked by peduncular stimulation as an indicator of effectiveness of recurrent inhibition in the pericruciate cortex of cats. Humphrey (1968) demonstrated that the surface negative (SN-wave) component of the complex potential recorded at the surface of pericruciate cortex was a reflection of postsynaptic potentials (PSPs) generated through recurrent inhibitory pathways. Van Duijn et al. (1973) showed that in barbiturate anesthetized cats the amplitude of the SN-wave diminished over time subsequent to topical application of penicillin. The time course for maximal decay of amplitude was comparable to that for development of an epileptic focus, and the amplitude would slowly recover after the penicillin was removed from the cortex. Penicillin which had been deactivated by pre-incubation with penicillinase had no effect on SN-wave amplitude. The lemniscal response was unaffected, as were the late component evoked by peduncular stimulation (thought to represent excitatory events) and the contralateral SN-wave. The implication was that effectiveness of recurrent inhibition was selectively reduced by penicillin and that this might be one of the bases for penicillin's epileptogenicity. However, important questions remained which could only be answered through analysis of the phenomenon at the neuronal level. Was recurrent inhibition functionally reduced at the pyramidal cell body subsequent to penicillin application or was

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the reduced amplitude of the SN-wave the result of some other effect of penicillin? If recurrent inhibition was reduced by penicillin, what was the most likely mechanism? The goal of the present research was to determine the most likely of 4 alternative explanations for the results of Van Duijn et al. (1973). The first mechanism which may be postulated is that penicillin acts on the deep pyramidal cells axons, their recurrent collaterals or the synapses of these axons onto inhibitory interneurons. This is rather unlikely, since it has been determined that penicillin diffuses passively and that 95% of the labeled drug is confined to layers I—III of the cortex at the onset of interictal spiking (Pedley and Noebels 1976). A second alternative is that the responsiveness of the inhibitory interneurons is reduced specifically through an effect of penicillin on the neuronal membrane. Third, penicillin may block transmission at the inhibitory synapses onto pyramidal tract cells (and possibly other neurons also). Finally, inhibitory pathways may not be specifically blocked by penicillin at all, but rather the drug may act to enhance excitatory input. An experimental design was chosen which could distinguish between these alternatives.

Materials and Methods

Experiments were performed on adult cats initially anesthetized with ether. After tracheostomy and femoral vein cannulation, alpha-chloralose (in propylene glycol, 35 mg/kg i.v.) was administered. Ether was withdrawn as soon as chloralose anesthesia was effective. Cisternal drainage was performed to help minimize cortical pulsations. After a wide unilateral craniotomy, exposing the entire precruciate cortex, the dura was reflected and the exposed cortex covered with warm mineral oil, maintained at 38.5° C. Animals were immobilized with gallamine triethiodide and artificially respirated to maintain expired CO_2 at 3—4%. A solution of 5% dextrose in saline was administered at a rate of 5 ml/h. Body core temperature was maintained at 37—38° C.

A bipolar stimulating electrode, insulated except for 0.5 mm at the tip and with tip separation of 2 mm, was placed in the ipsilateral cerebral peduncle at coordinates F 5.5—6, L 4—5 and H —4 to —6, according to the atlas of Jasper and Ajmone Marsan (1954) Position of the electrode was verified electrophysiologically by appearance of a and b waves at the cortical surface following single stimulus pulses to the peduncle (Stefanis and Jasper 1964; Humphrey 1968). Stimulus parameters were of 0.05 msec duration and 0.8 mA intensity and clearly did not activate lemniscal fibers with the electrode positioned in the peduncle. The SN-wave was evoked by stimulation of the peduncle with a brief train of pulses (3 pulses, intraburst frequency 300 c/sec, 0.05 msec duration, 0.8 mA intensity) and the site of maximal SN-wave amplitude was determined using a platinum ball electrode. Neuronal activity was sampled in this area using tungsten microelectrodes which allowed simultaneous recording of 2—4 neurons.

A monopolar platinum ball stimulating electrode was placed on the surface of the cortex 2—3 mm anterior to the site of microelectrode penetration, and a pair of needle electrodes inserted into the contralateral forepaw footpad. Epicortical stimulus was of 0.8 mA intensity, 0.2 msec duration, while intensity for forepaw stimulation was determined prior to immobilization as slightly suprathreshold for just discernible foot twitch maintaining duration at 0.2 msec.

Spontaneous activity and evoked responses of neurons to peduncular stimulation (to test recurrent inhibition at pyramidal tract cells, Humphrey 1968), epicortical stimulation (to test local inhibitory circuits, Krnjević 1966a, b) and forepaw footpad stimulation (to test afferent pathways) were displayed on an oscilloscope and recorded on film for later analysis. Only neurons exhibiting no signs of injury discharge were studied.

A neuron was categorized as a pyramidal

tract (PT) neuron if it responded with a short (less than 1.2 msec) and constant latency to single shock stimulation of the peduncle and would follow stimulation to at least 100 c/sec. Non-PT cells were further categorized by spontaneous and forepaw stimulation evoked firing behavior as bursting (B-NPT) or not (NPT) and the latency of an excitatory response to peduncular stimulation was compared to the SN-wave peak latency for possible involvement of the neuron in recurrent inhibitory pathways.

A 1 mm cube of gelfoam was soaked in a freshly prepared solution of potassium-penicillin in saline, 80,000 U/ml. In no instance was the edge of the gelfoam pledget placed more than 1 mm distant from the site of microelectrode insertion, and the distance was typically 0.5 mm. Spontaneous activity and response to the 3 stimuli were recorded on film immediately subsequent to penicillin application and at 5 min intervals therafter for at least 20 min after interictal spiking appeared in the slow wave record.

In most cases, spontaneously active neurons were selected so inhibition could be assessed against a background level of activity. However, to help guard against sampling bias, some non-spontaneously active PT cells were analyzed also. Antidromic invasion probability was used as a test for inhibition (Stefanis and Jasper 1964). Stimulus intensity was adjusted so a single pulse would result in antidromic invasion of the neuron studied 100% of the trials. This stimulus was then used as a test stimulus, considering the 100 msec poststimulus interval.

For spontaneously active neurons, data were analyzed from film records. A stimulus artifact suppressor enabled observation of very short latency effects by switching the microelectrode out of the recording circuit during stimulation and supplying a constant voltage level to the preamplifier during that period; the voltage was determined by the baseline voltage level recorded from the microelectrode immediately preceding stimulation and automatically adjusted. From the film records histograms were generated for each neuron's spontaneous and evoked responses by discrimination and counting of individual action potentials (requiring constancy within 5% for a single spike amplitude and differentiation from other spikes in the record by at least 20%). Inhibition or excitation was defined as a deviation from the mean spontaneous count per 10 msec by more than 1 S.E.M. (or change in antidromic invasion probability of more than 20%), and the 100 msec poststimulus interval was the time interval analyzed. If the maximal deviation of the histogram from the mean spontaneous count did not exceed these criteria, then the stimulus was determined to have no effect on the spontaneous discharge of the neuron.

Results

Acute epileptic foci were successfully induced in 23 cats and the activity of 84 neurons was recorded extracellularly. These neurons consisted of 22 PT cells, 44 B-NPT cells and 18 NPT cells. Since the penicillin was applied so close to the site of microelectrode penetration all neurons were assuredly located in the active focus. The responses of the neurons to the stimuli stabilized within 5 min after interictal spiking first appeared, and it is this stabilized pattern of responses which is reported here under the post-penicillin category. Neurons were monitored for at least 20 min and usually 1 h or more after interictal spiking was observed. Depth of the neurons recorded was determined from microdrive readings, and ranged from 1080 to 1860 μm, with a mean of 1588 μm. Although an attempt was made to penetrate perpendicular to the surface of the cortex for the microelectrode tracks, curvature of the cortex near the cruciate sulcus confounds the correlation of depth with cortical layer structure in the absence of histological marking and identification of recording sites. Therefore, no such correlation attempts were made.

PT neurons

The PT neurons had slow spontaneous rates (1—5/sec) and 11 of the PT cells studied were not spontaneously active. Ten PT cells were followed through the development of a focus with responses of an additional 8 neurons recorded from normal cortex and of 4 from epileptic cortex without the benefit of continuous recording of the neuronal activity through the transition from normal to epileptic cortex. Since the PT cells were almost all inhibited by peduncular stimulation prior to penicillin application and have been shown to exhibit predominantly inhibitory effects in response to pyramidal tract stimulation in other studies as well (Stefanis and Jasper 1964; Renaud and Kelly 1974a, b), observations regarding the 4 neurons whose responses were recorded only post-penicillin have been included.

The responses from one of the 10 spontaneously active PT cells are given in Fig. 1, and histograms calculated from these data are shown in Fig. 2. Histograms were calculated by averaging 2—3 film frames (for this neuron, three) of the response to each of the stimuli pre- and post-penicillin. Thus, the apparent inhibition seen in the film records of response to peduncular and epicortical stimulation prior to application of penicillin is seen to be a deviation from the mean spontaneous activity which is 2.5 times greater than the standard error, clearly satisfying the criterion for an inhibitory effect of the stimulation. The response to forepaw stimulation is similarly seen to satisfy the criterion for excitation followed by inhibition. Post-stimulus effects are more obvious in histograms than in single frames due to the random distribution of spontaneous activity compared to constant latency post-stimulus effects. While the 10—20 superimposed sweeps comprising a single frame in Fig. 1 are sufficient to demonstrate an effect of stimulation, additional averaging is necessary to satisfy the statistical criteria used to define post-stimulus effects.

Similarly, post-penicillin, peduncular and epicortical stimulation did not cause significant deviation of neuronal firing from mean spontaneous activity, while forepaw stimulation results in an initial excitatory deviation from mean spontaneous activity 3 times the standard error.

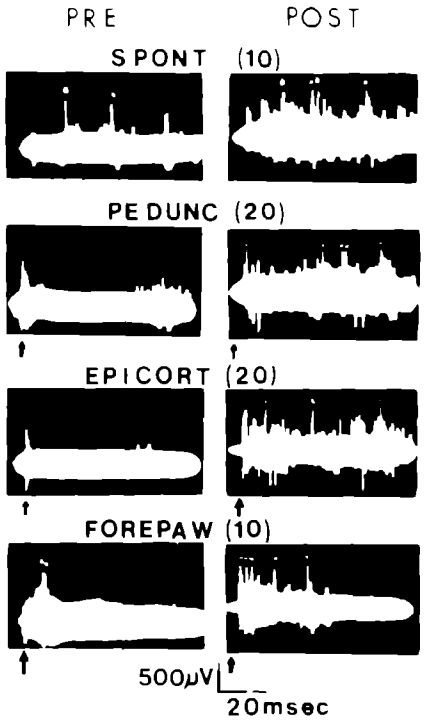

Fig 1 Post-stimulus responses of a sample PT neuron (discharge marked by dots) pre- and post-penicillin. The number of superimposed sweeps per frame is indicated in parentheses. Pedunc., peduncular stimulation, Epicort, epicortical stimulation. Stimulus delivery is indicated by the arrow

The effects of the stimuli on non-spontaneously active PT cells were determined by assessing the antidromic invasion probability of a single peduncular shock capable to invading the neuron soma 100% of the trials in the absence of a prior conditioning stimulus. (Since these neurons were inactive, collision of antidromic and orthodromic action potentials was not a complication.) Utilizing the test pulse, invasion probability was measured as a function of time subsequent to the conditioning stimulus, either peduncular, epicortical or forepaw footpad stimulation. Prior to penicillin all 6 neurons were inhibited by

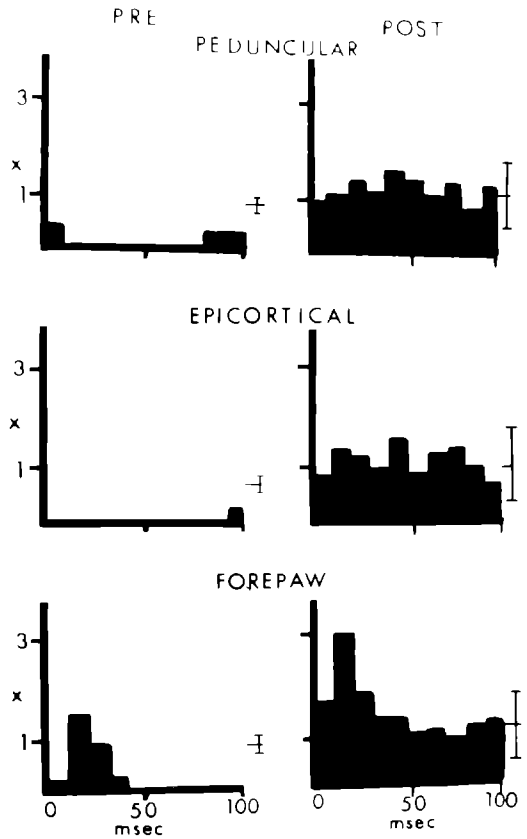

Fig. 2 Post-stimulus histograms pre- and post-penicillin derived for the PT cell shown in Fig. 1. The spontaneous rate and standard error of the mean are indicated at the right (spontaneous rate = 0 6 pre-, 0 8 post-penicillin). Note that inhibition of PT-cell discharge, clearly evident pre-penicillin, is absent following development of the penicillin focus.

peduncular stimulation, while epicortical stimulation had a less potent inhibitory effect with two neurons inhibited and three unaffected. In the penicillin focus, neither stimulus had an effect on antidromic invasion probability. Pre-penicillin, forepaw stimulation evoked action potentials in addition to the antidromically evoked spikes, raising the invasion probability above 1.2, the criterion defining an excitatory effect, and the initial excitation was followed by a period of invasion probability less than 0.8, satisfying the criterion for inhibition. Post-penicillin, only the increased invasion probability was observed. Interpretations of the PT cell data are summarized in Table I.

The averaged responses for the 10 PT cells followed pre- through post-penicillin are shown in Fig. 3. Note the sequence of excitation followed by inhibition, pre-penicillin, in response to forepaw stimulation, while later in the focus, excitation alone was observed. The time courses are consistent with interaction of excitation and inhibition in cortex pre-penicillin, with initial excitation damped by longer latency inhibition. Loss of this later inhibition post-penicillin could result in greater excitatory deviation from mean spontaneous activity and increased duration of excitation.

B-NPT cells

Thirty of these neurons (representing 68%

TABLE I

Response characteristics of PT cells Data are tabulated as the number of cells in each category, both pre- and post-penicillin. Excitation, inhibition and no effect are as defined in the text. Excit. → inhib indicates an initial excitation followed by inhibition in response to the stimulus. Response characteristics of neurons recorded only pre- or post-penicillin are given in parentheses.

Stimulation		Excit	Excit. → inhib	Inhib.	No effect
Peduncular	Pre	0 (1)	(1)	10 (6)	0
	Post	3	0	0	7 (4)
Epicortical	Pre	0	0	9 (2)	1 (3)
	Post	4	0	0	6 (4)
Forepaw	Pre	1 (2)	9 (3)	0	(1)
	Post	10 (4)	0	0	0

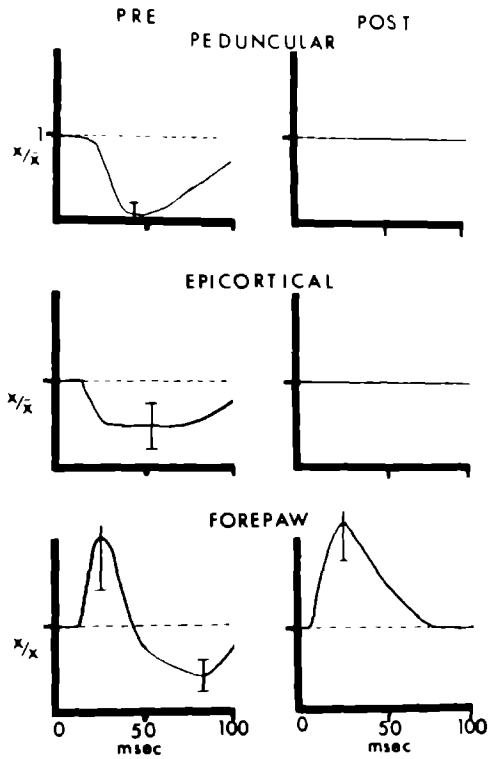

Fig 3 Post-stimulus average responses of 10 PT neurons pre- and post-penicillin The ordinate gives the averaged response, calculated by first normalizing the spike count per 10 msec time bin by dividing by the average spontaneous spike count per 10 msec time bin, for that neuron. These normalized values were then averaged to generate the plots shown, where no effect on spontaneous discharge is thus 1 The standard errors of the mean are vertical bars, indicated only at the point of maximal deviation from no effect for clarity

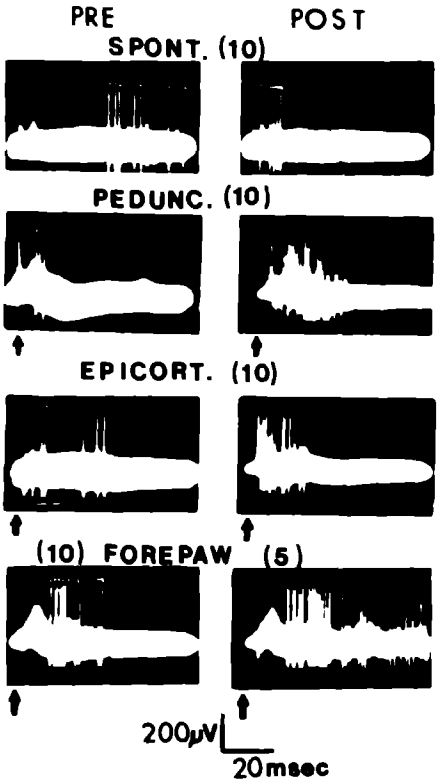

Fig. 4 Post-stimulus responses of a sample B-NPT neuron (discharge marked by dots) pre- and post-penicillin. The number of superimposed sweeps per frame is indicated in parentheses. Abbreviations are as in Fig 1 Stimulus delivery is indicated by the arrow.

of the sample) responded to peduncular stimulation with a short latency burst of spikes. The latency to this excitation (determined from histograms calculated as described for the PT cell above) was less than the latency to the SN-wave peak amplitude, indicating possible involvement of those neurons in the generation of the SN-wave, and hence, possible involvement in recurrent inhibitory pathways.

The responses from one of these B-NPT cells with short latency excitation in response to peduncular stimulation are given in Fig. 4, and histograms calculated for this neuron are shown in Fig. 5. Using the same criteria as for PT cells, it can be seen from Fig. 4 (and more clearly from the histograms in Fig. 5) that this neuron had a strong excitatory response to peduncular, epicortical and forepaw stimulation pre-penicillin, and these excitatory responses were also observed post-penicillin. In fact, excitatory deviations from mean spontaneous activity were even greater post-penicillin (when assessed as 'multiples of the standard error'). In spite of possible involvement of these 30 neurons in inhibitory pathways, a maintained or enhanced excitability (as illustrated in the example) was typical (Fig. 6). The remaining 14 B-NPT cells were also more likely to exhibit an excitatory

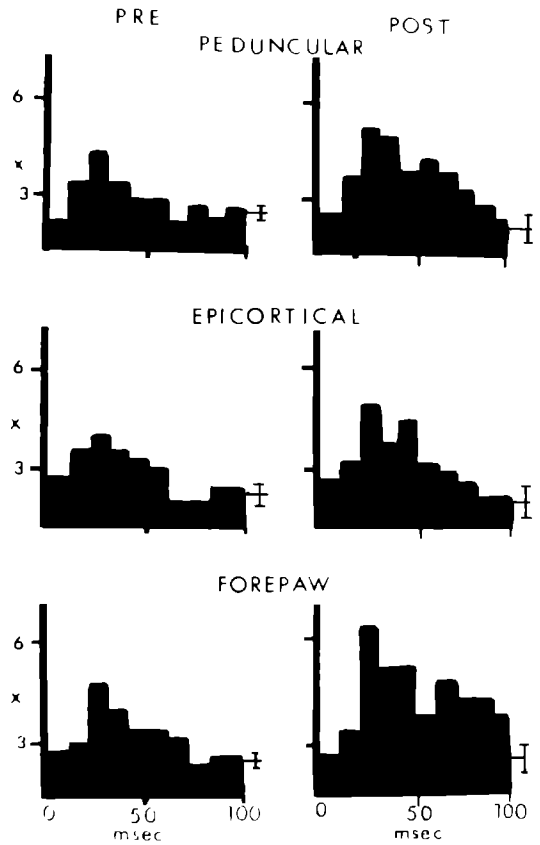

Fig 5 Post-stimulus histograms pre- and post-penicillin derived for the B-NPT cell shown in Fig 4 The spontaneous rate and standard error of the mean are indicated at the right (spontaneous rate = 1.4 pre-, 1 2 post-penicillin) Note that excitatory responses of the neuron are maintained following development of the penicillin focus

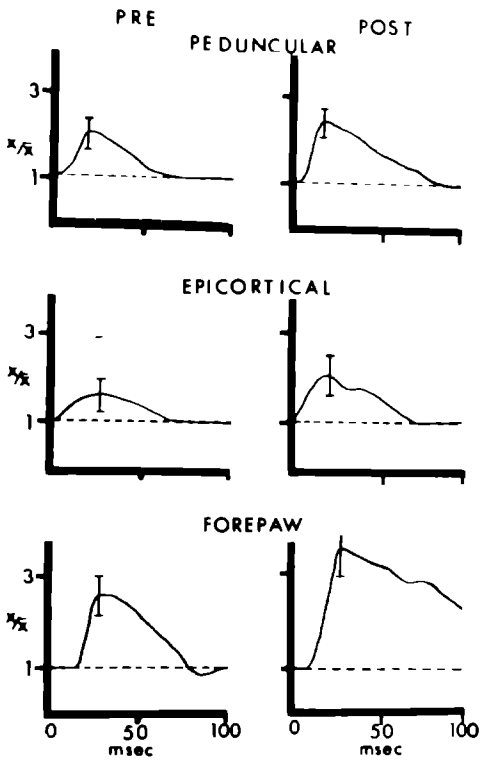

Fig 6. Post-stimulus average responses of 30 B-NPT neurons which exhibited short-latency excitatory response to peduncular stimulation. Calculations were as for Fig. 3, and standard errors are again indicated by vertical bars

response to any of the 3 stimuli post-penicillin, as can be seen from the summary given in Table II. Thus, excitatory responses of B-NPT cells observed pre-penicillin were maintained and often enhanced post-penicillin, and excitatory responses were more commonly observed post-penicillin than pre-penicillin.

NPT cells

While PT cells and B-NPT cells were fairly consistent in their response patterns to the various stimuli, no such uniformity was observed in the responses of the NPT cells. This is not altogether surprising, since the NPT cells are probably a heterogeneous group which may include long and short association neurons, commissural neurons and some PT cells whose axons were simply too distant from the peduncular stimulating electrode to be antidromically activated. For these cells also, inhibition was the most commonly observed effect of peduncular or epicortical stimulation, and they all exhibited an initial excitation in response to forepaw stimulation. Subsequent to penicillin application, excitatory effects of the stimuli were more commonly observed. Excitation in response to forepaw stimulation was enhanced post-penicillin, both in respect to the number of spikes generated by the stimulus and in the duration of the enhanced firing period. However, the

TABLE II

Response characteristics of B-NPT cells. Data are tabulated as number of cells in each category. Definitions of categories are as in Table I

Stimulation		Excit	Excit. → inhib	Inhib	No effect
Peduncular	Pre	36	3	5	0
	Post	44	0	0	0
Epicortical	Pre	20	0	11	13
	Post	41	0	0	3
Forepaw	Pre	39	4	0	1
	Post	44	0	0	0

TABLE III

Response characteristics of NPT cells. Data are tabulated as the number of cells in each category Definitions of the categories are as in Table I

Stimulation		Excit.	Excit → inhib.	Inhib	No effect
Peduncular	Pre	2	2	14	0
	Post	9	0	1	8
Epicortical	Pre	5	3	10	0
	Post	10	0	0	8
Forepaw	Pre	11	7	0	0
	Post	18	0	0	0

fact that the identity of the NPT cells is uncertain precluded their usefulness in drawing conclusions regarding the probable mechanisms underlying penicillin epileptogenesis. The response characteristics of the NPT cells are summarized in Table III.

Discussion

The purposes of this study were 2-fold. The first goal was to verify or refute the hypothesis advanced by Van Duijn et al. (1973) that inhibition is functionally less effective at PT cells in mature epileptic foci when compared with PT cells in normal cortex. The present observations clearly confirm this hypothesis. Since almost all PT cells are inhibited by peduncular stimulation prior to penicillin application, yet none are inhibited in the penicillin focus, strong conclusions may be drawn regarding the population of PT cells from which the sample was taken. The population of PT cells is large compared to the sample size (10) and we can view these data as a binomial distribution sampled with replacement, where the two alternatives are (1) inhibition is preserved in the penicillin focus, and (2) inhibition is not preserved in the focus. From a standard table giving 95% confidence levels for such a sampling, we see that the observed effect, loss of the inhibitory effect of peduncular stimulation on PT cell discharge, can be expected to occur in at least 69% of all PT neurons, the population sampled. If the similar effects upon responses of neurons only recorded post-penicillin are included, increasing the sample size to 14, the inhibition resulting from peduncular stimulation would be expected to be lost post-penicillin in at least 76% of the general population of PT cells. Loss of inhibition on PT cells in a penicillin focus can now be regarded as an established fact.

A schematic diagram of circuitry in the precruciate cortex is given in Fig. 7, with sug-

gested mechanisms leading to reduced inhibition at PT neurons. Alternative 1 is that penicillin acts on PT axons, their recurrent collaterals or the synapses of those collaterals onto inhibitory interneurons. The observation that excitatory responses of B-NPT cells, likely interneurons, to peduncular stimulation persist in the penicillin focus argues against this alternative. Also, inhibition in response to epicortical stimulation was decreased in the penicillin focus, a finding which would not be expected if effects on pyramidal cell processes were the major effect of penicillin. The second alternative is that the responsiveness of inhibitory interneurons is decreased in

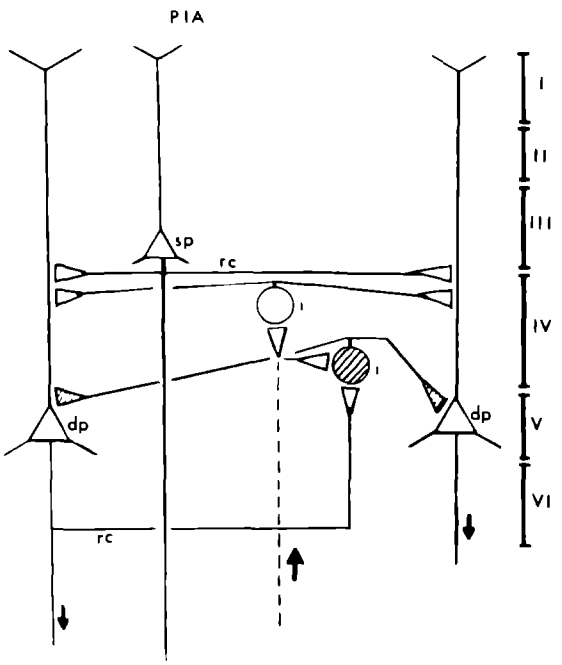

Fig. 7. Schematic diagram of neuronal circuitry in layers of pericruciate cortex. dp, deep pyramidal tract cell; sp, superficial pyramidal tract cell; i, interneuron, rc, axon recurrent collaterals. The deep lying pyramidal neuron has a recurrent excitatory contact on an inhibitory interneuron, thereby mediating recurrent inhibition. Inhibitory interneurons and synapses are shaded. The dashed line indicates afferent input. Note the recurrent excitatory contacts of superficial pyramidal neurons. Cells whose discharges were recorded were probably located primarily in layers IV and V

penicillin foci, and that this results in decreased effectiveness of recurrent inhibition. However, the class of neurons most likely to represent inhibitory interneurons, the B-NPT cells with short latency excitatory responses to peduncular stimulation (Stefanis 1969), show maintained excitatory response to peduncular stimulation in the penicillin focus. In fact, the excitatory response often shows greater deviation from mean spontaneous activity (measured as a multiple of the standard error) in the penicillin focus than in normal cortex, as illustrated by the example in Fig. 4 and also the averaged responses in Fig. 6. Thus, the second alternative is similarly unlikely to hold.

The third alternative is that penicillin acts at the inhibitory synapses onto PT cells, blocking or reducing effectiveness of transmission. The data are consistent with this alternative, as inhibitory effects were diminished in the penicillin focus regardless of the stimulus which initially generated the inhibition. This would be expected if the deficit in the inhibitory pathway was at a final common point, the inhibitory synapse. In support of this, it has been demonstrated that iontophoretically applied penicillin will reversibly reduce the inhibitory effect of GABA upon discharge of neurons in both spinal cord and pericruciate cortex of cats (Curtis et al. 1972).

The fourth alternative is that rather than acting specifically on inhibitory pathways, penicillin acts to enhance excitatory input, thereby reducing the effectiveness of inhibitory input to the PT cells. This could be accomplished through a direct effect of penicillin on presynaptic terminal membrane, as has been demonstrated to occur in both invertebrate preparations (Futamachi and Prince 1975) and isolated mammalian nerve-muscle preparations (Noebels and Prince 1977). Further, antidromic spiking, elicitable by an orthodromic action potential, has been observed in mammalian nerves subsequent to application of penicillin in the terminal region of the neuron, both in isolated preparations

(Noebels and Prince 1977) and in thalamocortical projection neurons (Gutnick and Prince 1972). Indeed, excitatory effects of forepaw stimulation often did result in greater deviation from spontaneous activity of PT cells in penicillin foci when compared to normal cortex, as is apparent in Fig. 3. However, this appeared to be a result of a delayed peak of excitation rather than a faster rise in the deviation away from spontaneous activity. This would be expected if longer latency inhibitory input normally served to damp excitatory responses, and the inhibitory control feature was lost or greatly impaired in the penicillin focus.

The most likely mechanism for the epileptogenicity of penicillin is a combination of alternatives 3 and 4. That excitatory pathways in the uppermost 3 layers of cortex are crucial to epileptogenic effects of penicillin was verified by Reichenthal and Hocherman (1977) in an attempt to determine the minimum area of rat cortex which could sustain epileptiform activity. They found that subpial incisions dividing an active focus into two or more regions which were each less than the critical area (0.5 mm^2) rendered each subfocus inactive, even when the cuts were restricted to layers I, II and III of the cortex with no damage discernible histologically in deeper layers. Thus, recurrent and feed-forward excitatory pathways appear to be crucial to development of epileptiform activity.

In conclusion, a functional decrease in recurrent inhibition has been demonstrated at the PT cell level subsequent to penicillin application to the surface of precruciate cortex. Some degree of caution should be maintained in the interpretation of these observations as they relate to the mechanisms underlying the epileptogenicity of penicillin, and more generally, the mechanisms underlying epileptiform activity in cortex. Since chloralose anesthesia as used in these experiments does alter neuronal responsiveness, these observations should now be confirmed in the unanesthetized preparation. However, in no case in the present experiments did interictal spiking develop except in the localized area surrounding the site of penicillin application. Furthermore, since the level of chloralose anesthesia probably remained essentially constant throughout the course of the experiment, comparisons of neuronal response properties pre- and post-penicillin remain valid, even if the baseline responsiveness of the neuron differs somewhat from the unanesthetized case.

The observed decrease in recurrent inhibition is not due to general reduced responsiveness of PT cells to synaptic input, since forepaw stimulation elicited excitatory responses are maintained in the penicillin focus. The decreased inhibition is most likely due to a direct effect of penicillin upon the inhibitory synapses onto PT cells, possibly through interference with a GABA mediated inhibition. This decreased inhibition, however, probably requires intact recurrent excitatory pathways before epileptiform activity can be sustained.

Summary

A previous study indicated that the early surface negative component (associated with recurrent inhibition) of the evoked potential recorded from cat pericruciate cortex, subsequent to pyramidal tract stimulation, was altered after application of penicillin to the cortical surface (Van Duijn et al. 1973). This suggested that decreased effectiveness of recurrent inhibition might be the basis for epileptogenicity of penicillin.

To verify that recurrent inhibition is functionally decreased in the penicillin epileptic focus and to assess alternative sites for penicillin action, this phenomenon was investigated at the cellular level. Neurons were recorded extracellularly and response to stimuli monitored throughout the transition from normal cortex to epileptogenic cortex. Stimuli employed were peduncular stimulation (to test recurrent inhibitory pathways), epicortical stimulation (to test inhibitory pathways,

bypassing the recurrent collateral system), and forepaw footpad shock (to test the responsiveness of neurons to afferent input).

In normal cortex, PT cells were inhibited by peduncular or epicortical stimulation and excited by forepaw stimulation, with the excitation followed by a period of inhibition. In the penicillin focus, inhibition was not observed in response to any of the 3 stimuli, and the excitatory response to forepaw stimulation was maintained.

The bursting non-PT cells, most likely candidates for interneurons, exhibited excitation in response to peduncular and epicortical stimulation, consistent with involvement in inhibitory pathways. Nonetheless, in the penicillin focus, excitatory response to peduncular and epicortical stimulation was maintained. Excitatory response to forepaw stimulation was also maintained in the penicillin focus.

The results demonstrate a loss of effectiveness of recurrent inhibition measured at the PT cell body in the penicillin focus. Further, the reduction in inhibitory feedback occurs in conjuction with maintained or enhanced excitability of the neurons which are most likely candidates for inhibitory interneurons. Thus, penicillin is most likely exerting its effect at the inhibitory synapses onto PT cells in the cortex, thereby allowing excitatory input to have greater influence on neuronal firing.

Résumé

Inhibition dans les foyers épileptiques induit par pénicilline

D'après une étude antérieure, la composante précoce surface négative (associée à l'inhibition récurrente) des potentiels évoqués enregistrés au niveau du cortex péricrucié du chat, à la suite d'une stimulation du tractus pyramidal, est altérée après application de penicilline à la surface du cortex (Van Duijn et al. 1973). Ceci suggère que la diminution d'efficacité de l'inhibition récurrente pourrait être à la base du caractère épileptogène de la penicilline.

Pour vérifier que l'inhibition récurrente est fonctionnellement diminuée au niveau des foyers épileptiques à la pénicilline et pour établir d'autres points d'action de la pénicilline, ce phénomène a été étudié au niveau cellulaire. Des neurones ont été enregistrés de façon extra-cellulaire et la réponse à des stimuli a été contrôlée au cours de la transition du cortex normal au cortex épileptogène. Les stimuli utilisés ont été la stimulation pédonculaire (pour tester les voies inhibitrices récurrentes), la stimulation épicorticale (pour tester les voies inhibitrices, dérivant du système collatéral récurrent), et le choc à la plante du pied (pour tester la réactivité des neurones aux afférences). Dans le cortex normal les cellules PT ont été inhibées par la stimulation pédonculaire ou épicorticale et excitées par la stimulation de la plante du pied, cette excitation étant suivie par une période d'inhibition. Dans le foyer épileptogène, l'inhibition n'est observée en réponse à aucune des trois stimulations, et la réponse excitatrice à la stimulation de la plante du pied se maintient. Les décharges des cellules non-PT, plus vraisemblablement des interneurones, montrent une excitation en réponse à la stimulation pédonculaire et épicorticale, confirmant que les voies inhibitrices sont impliquées. Néanmoins, dans le foyer épileptogène, la réponse excitatrice à la stimulation pédonculaire et épicorticale se maintient. La réponse excitatrice à la stimulation de la plante du pied se maintient également dans le foyer à la pénicilline.

Ces résultats montrent la perte d'efficacité de l'inhibition récurrente mesurée au niveau du corps des cellules PT du foyer à la pénicilline. De plus, la réduction du feedback inhibiteur survient en même temps que le maintien ou l'augmentation de l'excitabilité de neurones qui sont plus vraisemblablement des interneurones inhibiteurs. Ainsi, la pénicilline exerce très probablement ses effets au niveau des synapses inhibitrices qui vont aux cellules PT du cortex, permettant alors à l'afférence

excitatrice d'avoir une influence plus grande sur la décharge neuronique.

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