

Spike-Triggered Spreading Depression: Facilitation by Pyrrolopyrimidine

V. I. KOROLEVA* and J. BUREŠ

*Institute of Physiology, Czechoslovak Academy of Sciences,
Videňská 1083, 142 20 Prague, Czechoslovakia*

Abstract. Spike-triggered spreading depression (STSD) waves can be elicited from the penicillin focus in the cerebral cortex of rats by the first spike appearing after an interruption (>30 sec) of the interictal discharge, caused by postictal suppression or by spreading depression (SD). STSD incidence is markedly increased by administration of BW 58-271 (2 methyl 4 benzoyl-amino-pyrrolo/2, 3-d/pyrimidine). At doses (5—10 mg/kg) which do not elicit spontaneous SD waves in locally anaesthetized curarized rats, STSD waves appear not only in the penicillin focus but also in the thalamus and caudate nucleus. Pentobarbital anaesthesia blocks the projected STSD waves without interfering with their generation in the focus. BW 58-271 increased the success of STSD induction from 17% to 58 % in rats under pentobarbital anaesthesia and allowed thus systematic examination of the conditions for STSD continuation or termination. Sequences of up to 20 STSD waves were generated at 6—8 min intervals when the SD elicited suppression of the interictal discharge outlasted the period of SD refractoriness. The STSD sequence was terminated when repetitive penicillin spikes were triggered by electrical stimulation or by projected discharge during SD refractoriness. Frequent interictal activity prevented STSD generation by increasing the potassium clearance in the activated tissue. Repetitive STSD waves are an example of a slow periodic process generated by complex interaction of synaptic, neurohumoral and metabolic influences.

Key words: Spreading depression — Potassium clearance — Penicillin focus — Thalamus — Caudate nucleus

Introduction

Interaction between epileptic activity and Leão's (1944) spreading depression (SD) of EEG is not limited to their mutual facilitation and/or inhibition (Bureš et al. 1974) but may generate complex interactive phenomena, including reverberation

* Visiting scientist from the Institute of Higher Nervous Activity and Neurophysiology, Academy of Sciences of the USSR, Moscow, USSR

of SD around an electrically or chemically stimulated cortical area (Koroleva and Bureš 1979) and repetitive SD waves triggered by single discharges of an epileptic focus (Koroleva and Bureš 1983). The latter phenomenon appears when interictal activity of the penicillin focus is interrupted for a few minutes by changes in the focus (e.g. after an SD wave) and/or by extrafocal inhibitory influences (e.g. during postictal depression). The reduced activity of the focus decreases demands on the metabolically maintained electrolyte transport. As a consequence, the potassium clearing capacity of the focal area is so reduced that the first penicillin spike appearing after the interruption increases the focal $[K^+]_e$ to the SD-threshold level and triggers the avalanche increase of $[K^+]_e$. The SD wave inhibits the focus for a few minutes again and the sequence repeats for several cycles. Although series of spike-triggered SDs (STSDs) could be elicited in 42% of experiments, their reliable generation depends on a number of poorly controlled variables including the level of anesthesia, metabolic state and SD susceptibility. Especially the latter factor is critical because it determines the probability that an isolated epileptic spike will trigger an SD wave. SD susceptibility is considerably increased by a pyrrolopyrimidine drug BW 58-271 (2-methyl-4-benzoyl-amino-pyrrole/2,3-d-/pyrimidine), the systemic application of which elicits repeated waves of SD in cats (Norton and Jewett 1966) and rats (Norton and Jewett 1967) and a low dosage facilitates SD reverberation (Shibata and Bureš 1975) and transition of cortical SD into the caudate nucleus (DeLuca et al. 1975). The purpose of the present paper was to assess the effect of BW 58-271 on the STSD and related processes.

Method

Sixteen male hooded rats (Druckray strain), aged 2–3 months were used throughout. The animals were anesthetized with pentobarbital (45 mg/kg) and trephine openings were made according to the scheme shown in Fig. 1. The animals were then fixed in the head holder of a stereotaxic apparatus. In some experiments trephine openings were made under ether anesthesia, the animal was paralysed by d-tubocurarine, fixed in the stereotaxic instrument equipped with a face mask for application of artificial respiration (1 Hz, open system), while all pressure points and wounds were infiltrated with 2% procaine. Epileptic foci were elicited by application of a few μ g of powdered Na penicillin salt directly on the exposed cerebral cortex. EEG activity, epileptic spikes and slow potentials were picked up with capillary electrodes connected to calomel half-cells. The microelectrodes were stereotaxically inserted into the cortex (0.5 mm), into the head of the caudate nucleus and anterior thalamic nuclei (stereotaxic coordinates AP-2, L 2, V 4 and AP 2, L 1, V 5, respectively, according to the atlas by Fifiková and Maršala 1967). The electrodes were connected through high impedance input DC preamplifiers to a 4-channel polygraph. All recordings were monopolar against a wick calomel cell electrode contacting the exposed neck tissue. SD waves were evoked by intracortical microinjections of 0.5 μ l of 5% KCl.

Results

Fig. 1 illustrates the BW 58-271 effect in a curarized rat. Shortly after i.p. injection of 15 mg/kg of the drug, spontaneous SD waves appeared in both hemispheres.

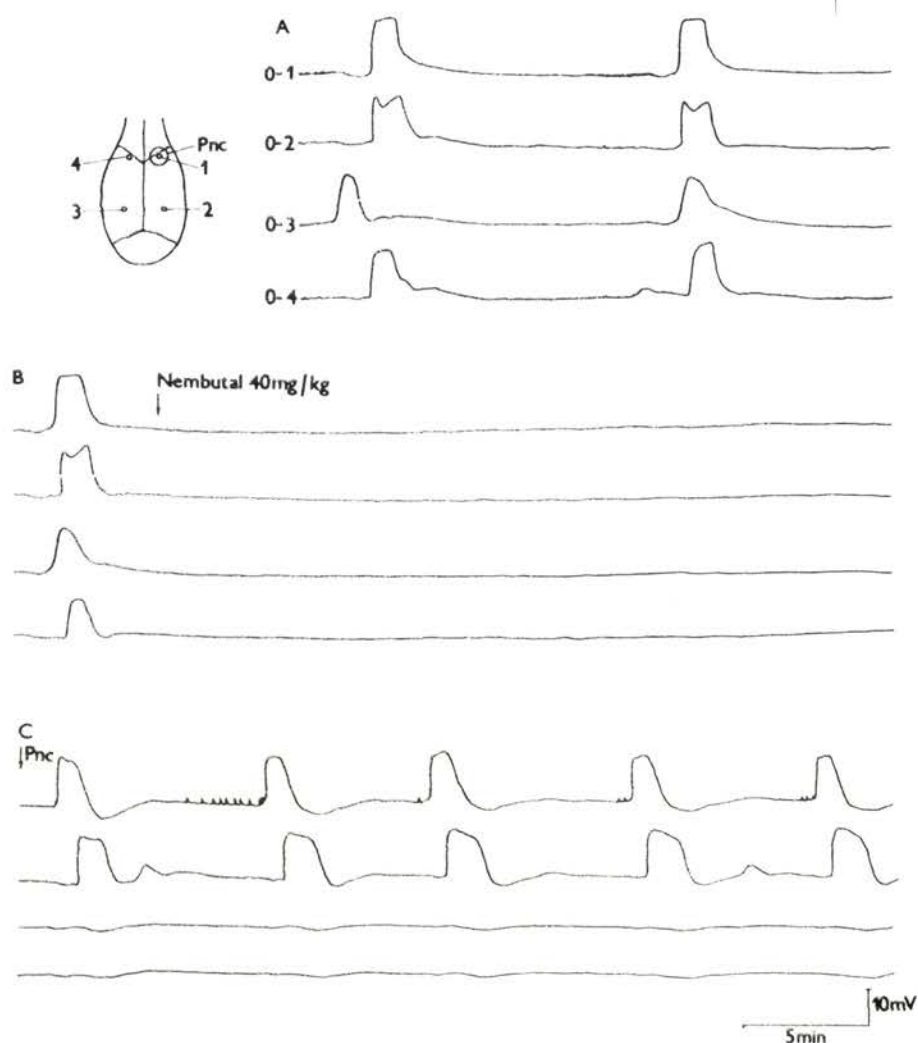


Fig. 1. Spontaneous and spike triggered SD waves in a BW 58-271 treated curarized rat. Brain diagram shows the position of the capillary electrodes (1 to 4), inserted 0.5 mm below cortical surface, and of the penicillin focus (Pnc). 0-1 to 0-4: polygraph recordings. Reference electrode on the cut skin. Calibration: 5 min, 10 mV. **A:** Spontaneous SD waves 30 min after application of BW 58-271 (15 mg/kg). Note absence of regular conduction delay between SD waves recorded from electrodes 1, 2, and 3, 4. **B:** Injection of pentobarbital (40 mg/kg) stops generation of spontaneous SD waves. **C:** Application of Na-penicillin to the right hemisphere elicits a sequence of STSDs. Note that the STSD waves appear only in the right hemisphere and spread from the focus (electrode 1) to the remote electrode 2.

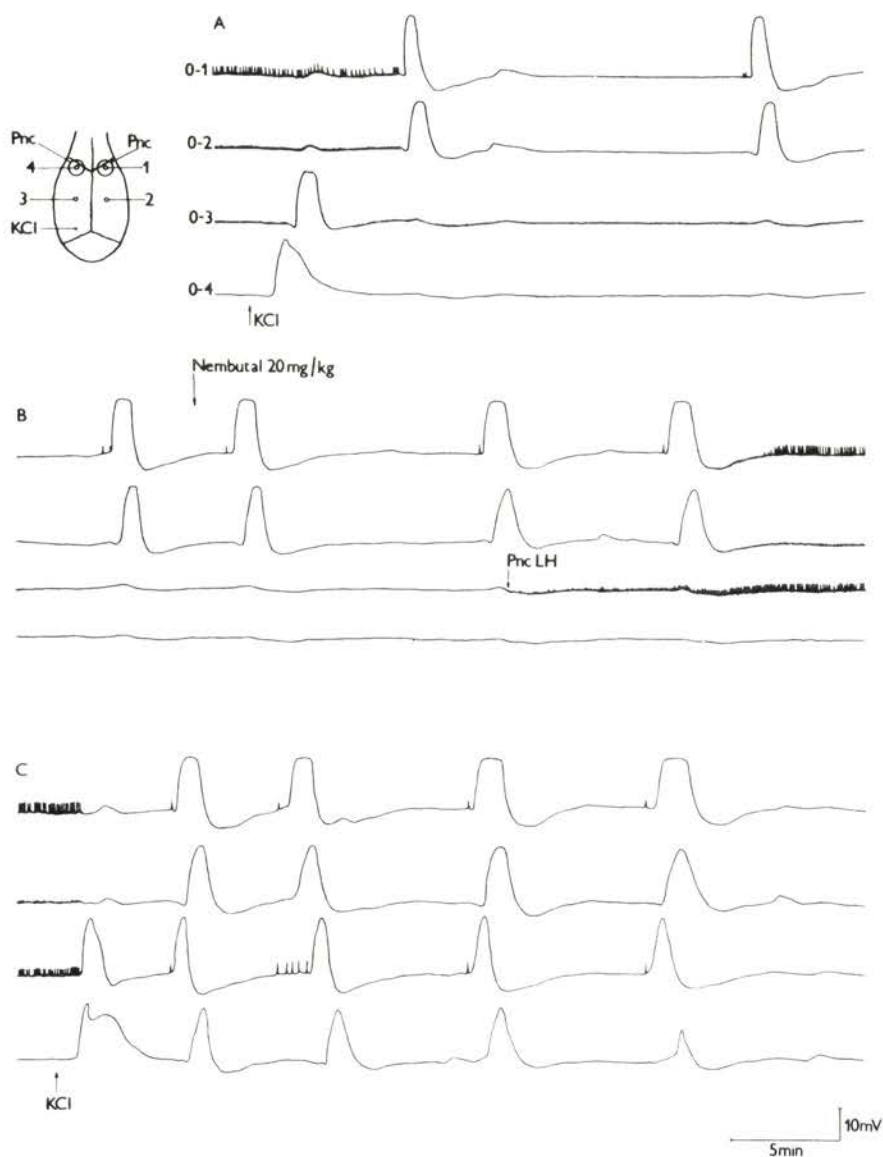


Fig. 2. Eliciting and stopping STSD waves in a rat anaesthetized with pentobarbital and treated with BW 58-271. KCl: site of intracortical injection of 5% KCl. Other description as in Fig. 1. *A*: SD wave elicited by KCl injection into the left hemicortex slows down interictal discharge of a penicillin focus in the right hemisphere and starts thus an STSD sequence. *B*: An on-going STSD sequence in the right hemisphere is interrupted when penicillin focus established in the left hemisphere starts triggering the right focus in the relative refractory period of SD. *C*: Blockade of the left focus by KCl-elicited SD induces synchronous STSD generation in both hemispheres.

They were initially asynchronous and reached the recording electrodes in the same hemisphere almost simultaneously. After the spontaneous SD generation had been stopped by pentobarbital (40 mg/kg), an epileptic focus was established by application of sodium penicillin on the left hemisphere.

The first spike appearing 7 min after penicillin application triggered an SD wave which delayed further development of the focus. The focal activity in the interval following the first STSD wave was so slow and irregular that a sequence of 3 spikes elicited a second STSD wave followed by regular STSD generation.

In most experiments (Fig. 2), BW 58-271 (10 to 15 mg/kg) was administered to rats anesthetized with pentobarbital (40 mg/kg). No spontaneous SD waves were observed in this case. Spikes elicited by topical application of penicillin on the right hemicortex gradually developed and regular interictal activity stabilised. An SD wave elicited by KCl microinjection into the occipital area of the left hemisphere slowed down the discharge rate of the focus. A group of spikes triggered an SD wave which started in the focus. The interictal activity was suppressed again and the STSD continued for 13 cycles in the right hemisphere. After completion of the 12th cycle, a symmetrical penicillin focus was established in the left hemisphere. The interictal spikes started to trigger the depressed focus in the right hemisphere soon after the passage of the 13th STSD wave. This gradual development of interictal activity prevented triggering of the next STSD and disrupted the ongoing STSD sequence. However, the right penicillin focus was still inhibited and displayed only projected spikes triggered by the discharge of left focus. When at this stage an SD wave was elicited in the left hemisphere by microinjection of KCl into the occipital region, SD blocked not only the left focus, but also the driven discharge of the right focus. The first spikes appearing after 4 min synchronously in both foci triggered SD waves in both hemispheres and STSD generation continued with bilaterally synchronous waves for 4 cycles completed within 30 min.

The synchronous discharges of the penicillin focus can trigger SD waves in unanesthetized BW 58-271 treated animals not only in the focus, but also in remote cortical or subcortical areas. This is illustrated in Fig. 3 showing a curarized rat, the SD susceptibility of which has been increased by BW 58-271. Application of penicillin to the right hemisphere started repetitive STSDs. The spike triggered the SD wave not only in the penicillin treated cortex but also in the projection areas, i.e. in the head of the caudate nucleus and in thalamus. After i.p. injection of pentobarbital (40 mg/kg) the STSD cycle continued in the cortex, but the projected STSD waves in thalamus and in the head of the caudate nucleus stopped. Due to facilitation of neocortico-caudatal propagation of SD by BW 58-271, all cortical SD waves reached the caudate electrode after a 4-min delay. STSDs of this type continued for 3 hours. After the pentobarbital effect dissipated, the projected penicillin spikes started to trigger caudate and thalamic SD waves again.

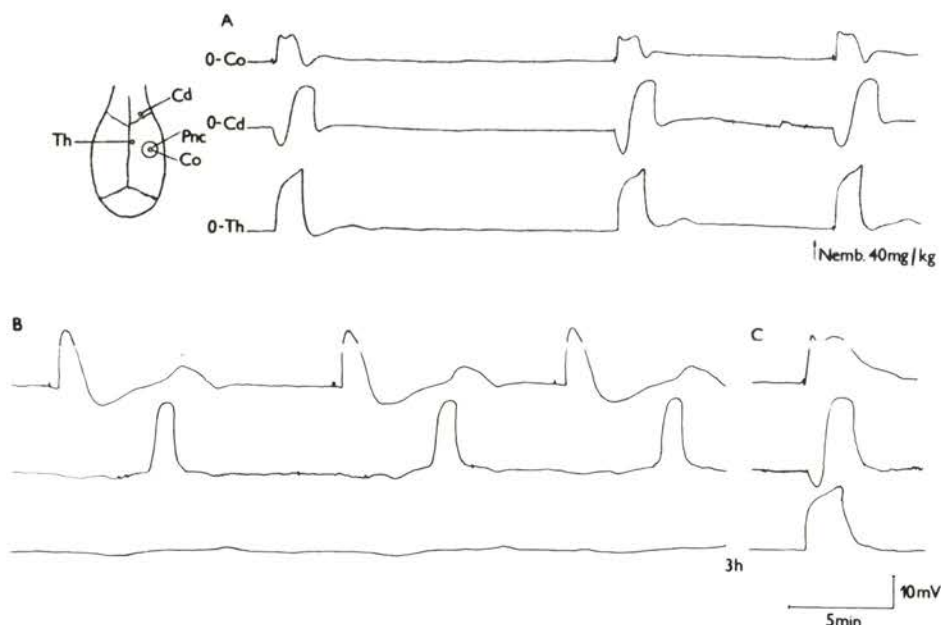


Fig. 3. Blockade of subcortical generation of STSD waves in a curarized rat by pentobarbital. Brain diagram shows position of the capillary microelectrodes in parietal cortex (Co), caudate nucleus (Cd, AP-2, R2, V5) and anterior thalamus (Th, AP + 2, L 0.5, V4). Other description as in Figs. 1 and 2. **A:** STSDs appear in the curarized animal simultaneously in the cortical focus and in the caudate nucleus and thalamus. **B:** 10 min after 50 mg/kg pentobarbital STSD generation continues in cortex but stops in caudate nucleus and thalamus. Cortical STSD waves reach the caudate nucleus with the usual conduction delay of 4 min. **C:** Three hours after pentobarbital injection simultaneous STSD waves reappear in both caudate nucleus and thalamus.

The overall incidence of STSD in pentobarbital anesthetized rats was increased by application of BW 58-271 from 17.3% to 59% when the same technique of STSD initiation was used (penicillin foci of similar discharge rate, suppression of focal discharges by microinjections of KCl into a distant cortical area or by electrical stimulation of the focus or of the symmetrical cortical area).

The increased reliability of STSD generation is also indicated by higher incidence of long sequences of STSD cycles. The maximum duration of the STSD sequences was not systematically examined, because the cycle was usually deliberately interrupted, but occasionally more than 20 STSD cycles were generated within an interval of 3 to 5 h. Probability of continued generation of STSD waves decreased as a linear function of time (Fig. 4). The slope of the descent was considerably steeper in the untreated than in the BW 58-271 treated rats. On the other hand, the drug prolonged the duration of the STSD cycle from 6 to 9 min and shifted the distribution of STSD cycle durations to the right (Fig. 5).

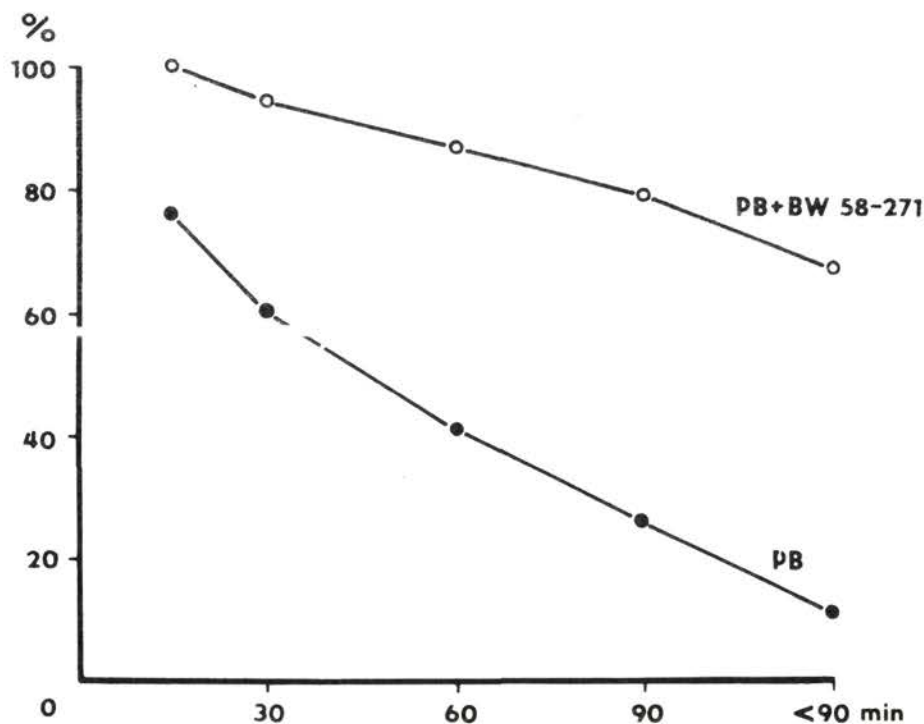


Fig. 4. Percentage of STSD sequences (*ordinate*) continuing at the various time intervals after sequence onset (*abscissa*). Full line — rats anaesthetized with pentobarbital ($n=45$). Interrupted line — rats treated with pentobarbital and BW 58-271 ($n=16$).

High reliability of STSD generation in the BW 58-271 treated rats made it possible to examine conditions of continued STSD generation and STSD termination. Interventions increasing the activity of the focus during the period of relative SD refractoriness stabilized the cortical $[K^+]_e$ at a new equilibrium protecting the focus against SD generation. Discharges of the inhibited focus could be triggered either by another focus established in the contralateral cortical area (see Fig. 2) or more reliably by electrical stimulation of the focus or of the afferents to the focal region. This is shown in Fig. 6 where low frequency stimulation (0.16 Hz, 100 μ s, 20 V) of the focus was applied during a stable STSD sequence. Amplitude of the triggered penicillin spikes gradually increased during post-SD recovery and further generation of STSDs was prevented during 30 min of stimulation. The triggered focal activity was not strong enough to cause SD blockade, however, since SD waves elicited from the occipital cortex could enter the penicillin focus and block the triggered spikes. With continued stimulation,

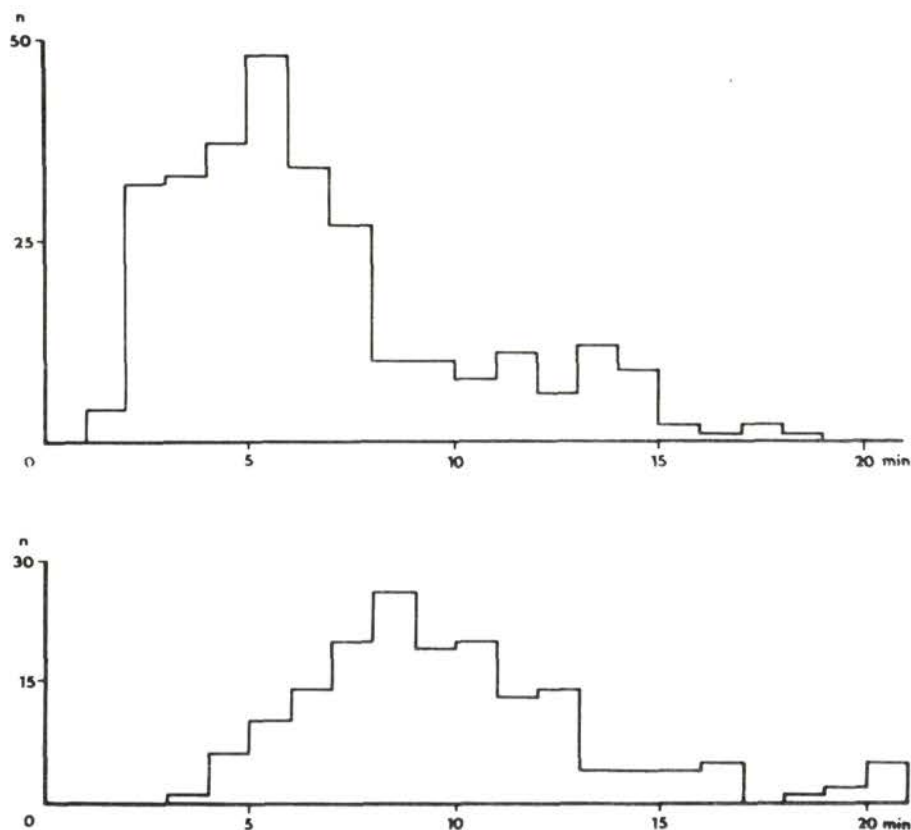


Fig. 5. Distribution of intervals between successive STSD waves in rats under pentobarbital anaesthesia with (*above*) and without (*below*) additional BW 58-271 treatment. *Abscissa*: Interval between two successive STSD waves in min. *Ordinate*: Incidence of intervals of the given duration.

spike amplitude recovered during one minute and no SD wave was evoked. A pause following cessation of stimulation reinstated conditions for STSD generation. After a 50 s-long spike-free interval a spontaneously generated spike elicited STSD again. STSD could also be elicited by single pulse stimulation applied to the focus at least 2–3 min after the previous SD wave.

Discussion

The main result of the present study is the finding that application of BW 58-271 greatly increases the reproducibility of STSD and makes thus the phenomenon amenable to more detailed analysis. The effect is probably due to decreased SD threshold and increased K^+ release during SD waves (DeLuca et al. 1975).

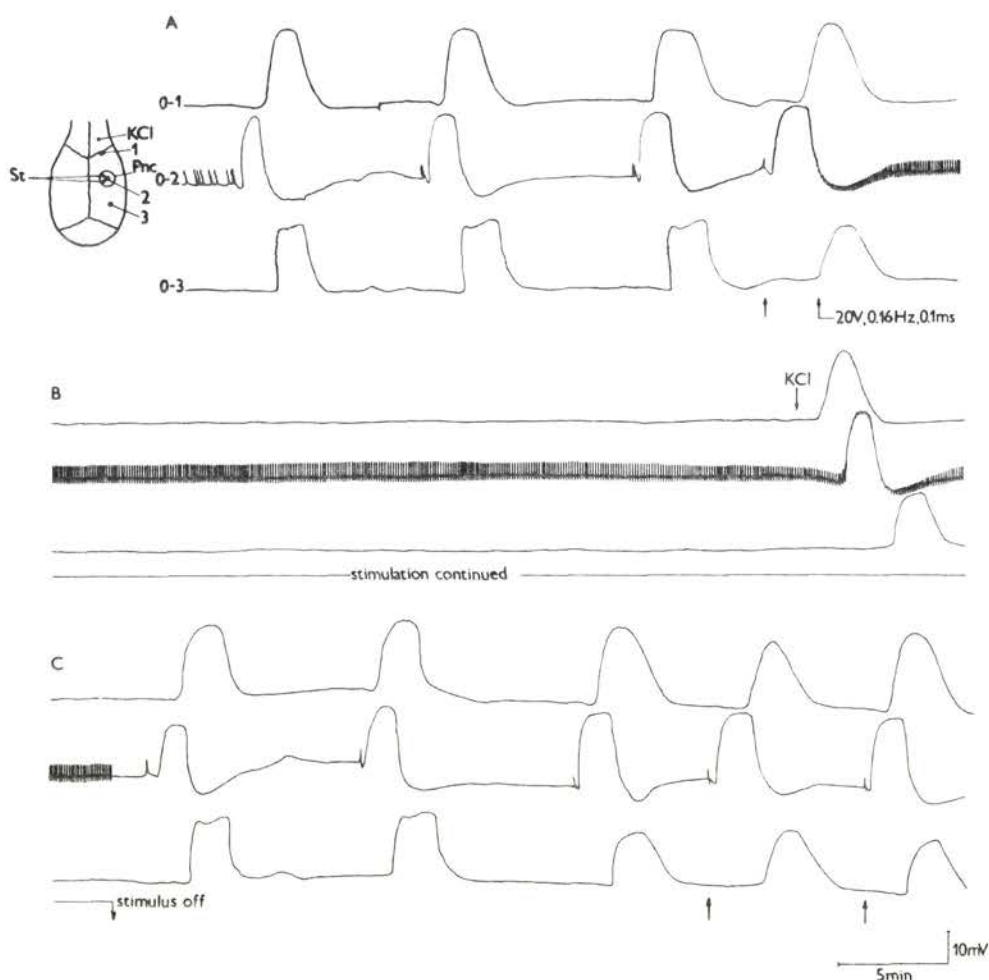


Fig. 6. Suppressing and eliciting STSD waves by electrical stimulation of the penicillin focus. Rat under pentobarbital anaesthesia, 15 mg/kg of BW 58-271. St — stimulation electrode. Other description as in Figs. 1 and 2. *A, B* spontaneously appearing STSD cycle is blocked by low frequency (1/6 s) electrical stimulation of the focus. *C*: Spontaneous STSD waves reappear after cessation of stimulation. STSD waves can be evoked before recovery of the interictal discharge by isolated penicillin spikes elicited by electrical stimulation of the focus (arrows).

On the other hand, BW 58-271 interferes neither with the effect of penicillin nor with its SD-elicited changes. The drug does not decrease the efficiency of the metabolic pump and probably facilitates synaptic transmission. In fact, spontaneous generation of SD waves in unanesthetized curarized rats is probably due to combined effect of synaptic facilitation and decreased SD threshold which accounts

for simultaneous appearance of multiple SD foci. In unanesthetized BW 58-271 treated animals an epileptic spike triggers SD waves not only in the focus, where synaptic efficacy is increased under the influence of penicillin but also in remote areas receiving direct synaptic projections from the focus: in the thalamus, caudate nucleus and symmetrical cortical region. The blockade of the plurisynaptic transmission by barbiturate anesthesia stops spontaneous SD generation and SD-triggering by the projected focal discharge, but does not prevent STSD generation in the focus. The STSD cycle duration can be regulated by the depth of anesthesia which slows down the rate of recovery of the interictal discharge. Surprisingly, the STSD cycle is longer in the BW 58-271 treated animals than in animals under pentobarbital anesthesia alone. This may indicate that BW 58-271 potentiates the inhibitory effect of barbiturates on interictal activity in the focus or at some subcortical level (e.g. reticular formation).

The BW 58-271 induced increase of STSD susceptibility does not interfere with the basic mechanisms of STSD. This is clearly demonstrated by experiments in which external triggering of the focus by electrical stimulation applied during the absolute refractory period of STSD stopped further STSD generation. This observation is in good agreement with the hypothesis that STSD appears only when the potassium clearing capacity of the focus is low due to prolonged activity reduction. During the absolute refractory period of SD, potassium clearance is decreased by persisting activation of the Na^+ , K^+ -ATPase (Křivánek and Reddy 1980). Increased activity of the metabolic pump induces electrogenic hyperpolarization of neural membranes and blocks spontaneous generation of interictal spikes in the penicillin focus. The spikes can be triggered during this interval by direct electrical stimulation of the focus or by synchronous afferent volleys. The subsiding activity of the metabolic pump during SD repolarisation is increased by the stimulation and maintained at the level, which prevents STSD generation, but not the invasion of the focus by SD waves (Fig. 6). At high stimulus rates the potassium clearance is so much enhanced, that SD waves generated in extrafocal cortical areas stop at the boundary of the focus, the activity of which continues unimpaired (Koroleva and Bureš 1980). Since SD blockade induced by high rate interictal spikes is preserved under BW 58-272, the drug induced increase of SD susceptibility does not overcome the blocking effect of enhanced potassium transport.

The electrically evoked penicillin spikes make it also possible to assess the shortest interval at which an STSD can appear after a preceding SD wave. Although this problem was not systematically examined in the present study, scattered experimental evidence indicates that single pulse stimulation of the focus can trigger STSD considerably before appearance of the first spontaneous interictal spike. It seems that at certain phase of post-STSD recovery, the slowly subsiding activity of the metabolic pump still counteracts spontaneous generation of interictal spikes, but does not prevent the autoregenerative increase of $[\text{K}^+]_i$. The events

induced by an SD wave in a penicillin focus stimulated by single electrical pulses proceed through the following stages: 1. Electrical stimuli fail to elicit penicillin spikes during SD negativity. 2. Low amplitude spikes are elicited during the repolarization phase of SD. 3. Full amplitude penicillin spikes, evoked during the positive phase of SD slow potential are not followed by an STSD. 4. Electrical pulses applied during a later phase of SD recovery elicit spikes followed by STSD. 5. Still later, STSD is elicited by spontaneous interictal spikes. Further experiments are needed to establish the exact duration of the above phases.

High SD-susceptibility of the BW 58-271 treated rats increases the incidence of STSDs after short lasting irregularities of the interictal discharge. SD elicited in the hemisphere contralateral to the penicillin focus may slow down or even briefly interrupt the interictal discharge and decrease the potassium clearance below the STSD preventing level. Such SD-induced inhibition of the contralateral penicillin focus is probably mediated by remote effects on the non-specific thalamus and reticular formation (Bureš et al. 1974). The minimum interruption of the interictal discharge causing subsequent appearance of STSD waves probably depends on the preinterruption discharge rate. The quantitative aspects of these relationships can be established more precisely in the electrically stimulated penicillin focus.

Acknowledgement. The authors wish to thank Burroughs-Wellcome and Co., 3030 Cornwallis Road, Research Triangle Park, N.C. 27709, for supplying the BW 58-271.

References

- Bureš J., Burešová O., Krivánek J. (1974): The Mechanism and Applications of Leão's Spreading Depression of Electroencephalographic Activity. Academic Press, New York—London
- DeLuca B., Shibata M., Brožek G., Bureš J. (1975): Facilitation of Leão's spreading depression by a pyrrolopyrimidine derivative. *Neuropharmacol.* **14**, 537—545
- Fifková E., Maršala J. (1967): Stereotaxic atlases for the cat, rabbit, and rat. In: *Electrophysiological Methods in Biological Research* (Eds. J. Bureš, M. Petráň, J. Zachar) pp. 653—731, Publ. House Czech. Acad. Sci., Prague
- Koroleva V. I., Bureš J. (1979): Circulation of cortical spreading depression around electrically stimulated areas and epileptic foci on the neocortex of rats. *Brain Res.* **173**, 209—215
- Koroleva V. I., Bureš J. (1980): Blockade of cortical spreading depression in electrically and chemically stimulated areas of cerebral cortex in rats. *EEG Clin. Neurophysiol.* **48**, 1—15
- Koroleva V. I., Bureš J. (1983): Cortical penicillin focus as a generator of repetitive spike-triggered waves of spreading depression in rats. *Exp. Brain Res.* (in press)
- Křivánek J., Reddy M. M. (1980): Does vanadate participate in the response of cerebral (Na^+ , K^+)-ATPase to electrical stimulation? *Brain Res.* **195**, 506—510
- Leão A. A. P. (1944): Spreading depression of activity in the cerebral cortex. *J. Neurophysiol.* **7**, 359—390
- Norton S., Jewett R. E. (1966): A pyrrolopyrimidine with depressant action on the CNS. *J. Pharmacol. Exp. Ther.* **154**, 152—160

- Norton S., Jewett R. E. (1967): Effect of prolonged EEG suppression by a pyrrolopyrimidine on learned behavior. *Physiol. Behav.* **2**, 317—322
- Shibata M., Bureš J. (1975): Techniques for termination of reverberating spreading depression in rats. *J. Neurophysiol.* **38**, 158—166

Received November 19, 1982/ Accepted November 25, 1982