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Effects of repeated systemic penicillin injections on nonconvulsive and convulsive epileptic seizures in the rat

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Abstract. Changes in the spontaneous and induced epileptic activity in the course of repetitive systemic i.p. administration of crystalline penicillin (Pc) were examined in imp-DAK rats. In all the rats used, nonconvulsive seizures characterized by bursts of spike and wave discharges (SWD) in the neocortex occurred spontaneously. A single i.p. injection of Pc at doses of 1,000,000, 1,500,000 or 2,000,000 IU/kg resulted in a transient increase in SWD activity. For the latter two doses, Pc injections also induced convulsive activity, i.e. single spikes and trains of spikes accompanied by myoclonies. When 1,500,000 IU/kg of Pc was administered repeatedly (six injections, one every 48 h), the amount of the convulsive activity induced by successive injections decreased but the increase in the number of SWD bursts became more pronounced. This result gives rise to some questions about the development of tolerance to the epileptogenic Pc effects in the course of repeated administration of this antibiotic.

Short communication

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Repeated, systemic injections of a convulsant drug, e.g. pentylenetetrazol, may result in kindling, i.e. a permanent lowering of the seizure threshold (Mason and Cooper 1972), and spontaneous recurrent seizures may develop after induction of a seizure state by an electrical (Bertram and Cornett 1993) or chemical (Leite et al. 1990) stimulus. Penicillin (Pc) is the most commonly used antibiotic, but it is also a known convulsant (see De Deyn et al. 1992). The risk of epileptic seizures arising from its repeated administration, however, is not well recognized. There are clinical reports of epileptic fits in patients receiving Pc intravenously, usually in large doses. In some of the reported cases convulsive symptoms were absent after the first injection, but appeared after the successive ones (see Keskin and Konkol 1993). Data from animals, concerning the Pc epileptogenicity, are abundant. In the cat, a single systemic injection of crystalline Pc may induce nonconvulsive seizures with bursts of spike and wave discharges (SWD) in ECoG (Fisher and Prince 1977), and aggravate the occurrence of SWD bursts in rats in which this form of epileptic activity appears spontaneously (Marescaux et al. 1992). In both species, convulsive (myoclonic and clonic) seizures accompanied by single spikes and trains of spikes in ECoG may follow i.p. administration of Pc at large doses (Fisher and Prince 1977, Chen et al. 1986). Reports concerning the effects of repeated Pc administration are scarce. In a series of experiments on rats, Bo et al. observed that the Pc induced convulsive symptoms were markedly reduced after successive i.p. Pc injections, which suggested that tolerance to the proconvulsive effect of Pc might have developed (Bo et al. 1984, 1986). No effects of Pc on the SWDs were observed in these studies, since in the animals used by Bo et al. this form of epileptic activity neither occurred spontaneously nor was induced by Pc. The purpose of the present work was to find out whether, in the course of repeated Pc administration, the changes in the spontaneous nonconvulsive SWD activity and those in the induced convulsive activity proceeded similarly. It is known that these two types of epileptic activity differ by their pharmacological profiles (Coenen et al. 1992, Marescaux et al. 1992) as well as by their neuronal mechanism (Bleck and Klawans 1990, Kostopoulos and Antoniadis 1993). Hence, there is a possibility that in the course of repeated Pc administration the changes in SWD activity need not necessarily proceed in the same direction as those seen in the convulsive activity.

Twenty-three male Wistar rats, (IMP/DaK stock, outbreds), weighing 380-600 g, were used. The animals were housed in single cages under standard laboratory conditions. All had chronic unipolar, gold plated electrodes (diam. about 1.5 mm) implanted epidurally over fronto-parietal cortex, bilaterally, under general (pentobarbital, 50 mg/kg) and local (polocainum) anaesthesia. The reference and the ground electrodes were placed on the surface of dura mater over the cerebellum and on the nasal bone, respectively. The outer ends of the electrodes were soldered to a miniature socket and the whole assembly was fastened to the skull with dental acrylic (Duracryl, Spofa). In all the rats, the spontaneous occurrence of SWD bursts had been verified on the basis of preliminary EEG recordings made two weeks after the surgery.

The ECoGs were recorded on an 8-channel electroencephalograph with time constant set at 0.3 s, and high frequency filter at 35 Hz. At the time of the recording, the rats were put into plastic opaque open-top boxes (30 x 30 x 40 cm), and shielded low-weight flexible cables, connecting the electrodes to the inputs of the electroencephalograph amplifiers, were attached. The recording equipment was located in a room adjacent to the recording compartment. A TV system enabled observation of the rat's behaviour during the recording.

Crystalline penicillin G (potassium salt, Polfa, Tarchomin) was dissolved in water (aqua pro injectione, Polfa) to the concentration of 375,000IU/ml. This solution was administered i.p. in a volume containing the appropriate dose per kg body weight. Control animals were given i.p. injections of water in volumes corresponding with the volumes of the Pc solution in the experimental rats.

The rats were recorded in squads of four. Three--hour ECoG recordings, one hour before and two

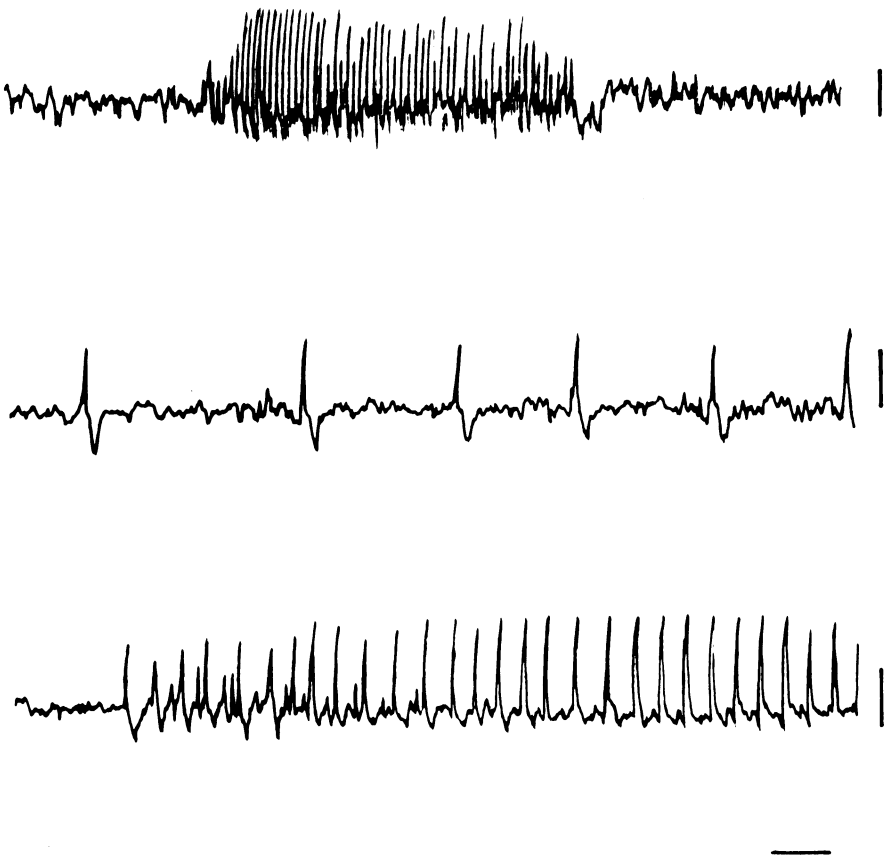

Fig. 1. Fragments of ECoGS showing the three forms of epileptic activity influenced or induced by intraperitoneal injections of 1,500,000 IU/kg of crystalline penicillin. Upper section, a spontaneous 7-9 Hz burst of spike and wave discharges with no overt motor manifestations. Middle section, single spikes accompanied by twitches of neck muscles or whole body jerks. Lower section, a train of spikes accompanied by clonic motor convulsions. Calibration: horizontal, 1.0 s; vertical, 500 V.

hours after injection, were performed. For each rat, the ECoG from one derivation (out of two), which yielded the smallest number of artifacts, was analysed. The number and duration of SWD bursts as well as single spikes and trains of spikes (see Fig 1.) were counted in successive one hour sections of the recordings. At first, in order to establish the Pc dose for repeated injections, the effects of single i.p. injections of water (n=2) and single injections of Pc at doses: 1,000,000 IU/kg (n=2), 1,500,000 IU/kg (n=4) and 2,000,000 IU/kg (n=3) were tested. A transient increase in SWD activity was observed after each Pc dose, but not after injection of water. The 1,500,000 IU/kg and 2,000,000 IU/kg doses of Pc also induced single spikes accompanied by body jerks, and trains of spikes with clonic seizures. After 2,000,000 IU/kg of Pc, the convulsions were severe. Therefore, the 1,500,000 IU dose of Pc was selected for repeated injections. The remaining twelve rats were divided into two groups: A (n=6) and B (n=6). The rats from Group B were given six i.p. injections of Pc at the selected dose. The interval between successive injections was 48 h, similarly as in Bo et al. (1986). Six water injections were given to the animals from Group A in the same way. ECoGs were recorded on the day of the first (recording 1), the third (recording 2) and the sixth (recording 3) injections.

During the first hour of recording 1, the mean number of SWD bursts in group A was 21.7 and the mean cumulative burst duration was 536 s. The corresponding values for group B were 39 and 531 s.

A two-way parametric ANOVA (groups x recordings) and Tukey's test (Winer 1961) was applied in order to assess the changes in the number of SWD episodes in the course of the experiment (Fig. 2, A and B, diagrams to the left). No differences in this respect were found for the first (preinjection) hour of recording. As for the second hour, i.e. the first hour after the injection, a significant effect was noted of the recordings factor ($F_{1,10}$=9.89, P<0.02) and the groups x recordings interaction was signi-

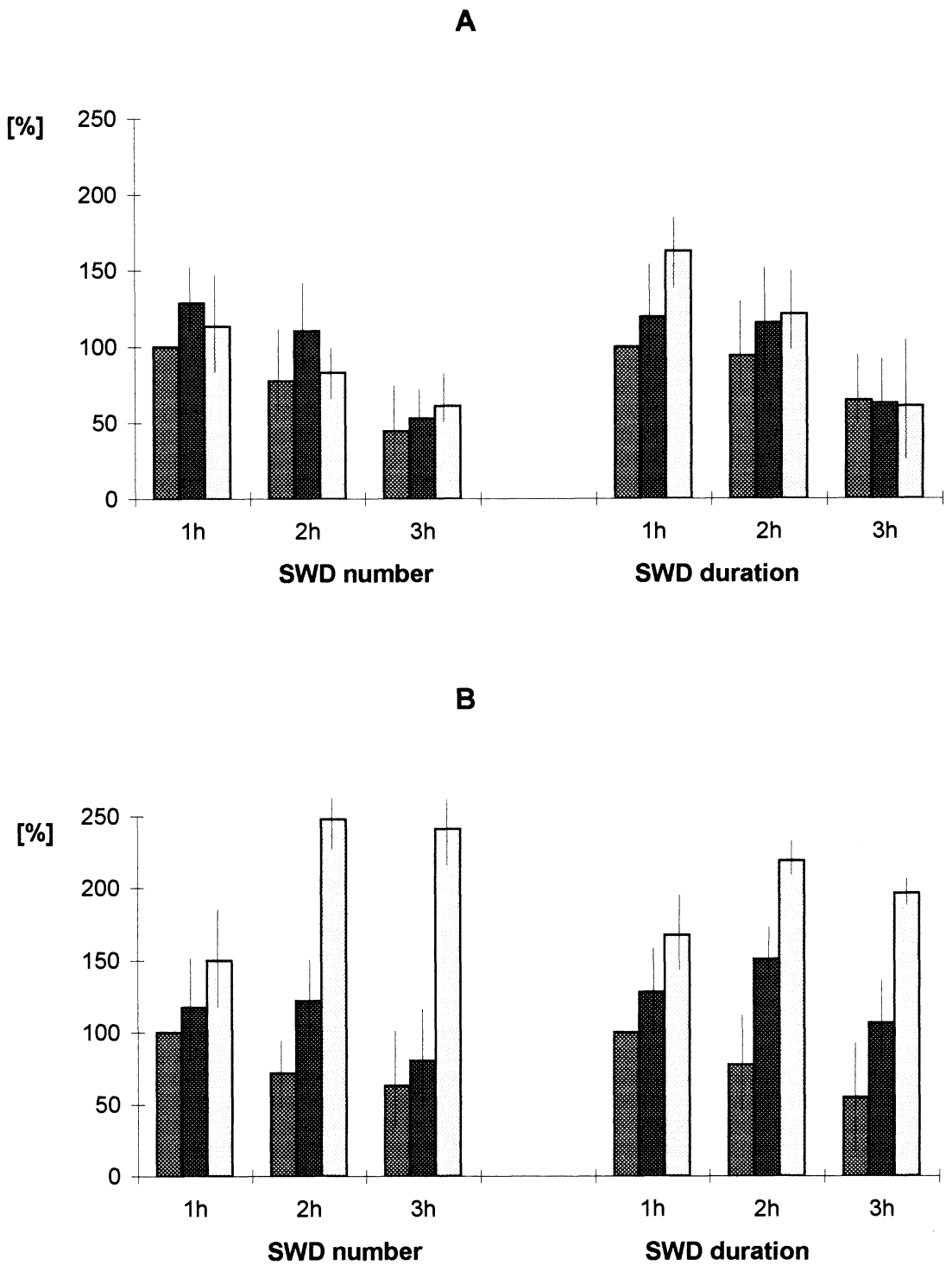

Fig. 2. Diagrams (means and SEs) illustrating changes in the number and duration of SWD episodes during successive 3 h of three ECoG recordings in rats receiving repeated i.p. injections of water (A), and rats receiving repeated i.p injections of 1,500,000 IU/kg of crystalline penicillin (B). Shadowed bars, recording 1 (first injection day); black bars, recording 2 (third injection day); open bars, recording 3 (the last injection day). The values from the first injection day were considered 100%.

ficant ($F_{1,10}$=5.36, P<0.05). The subsequent within-group comparisons revealed that successive recordings differed significantly with respect to the number of SWD bursts only in Group B ($F_{2,20}$=14.77, P<0.001): in recording 3 the number of SWD bursts was significantly higher than in recording 1 and 2. Comparisons between groups revealed differences only in recording 3 ($F_{1,30}$=5.34, P<0.03); in Group B the number of SWD bursts was significantly higher than in Group A. In the case of the third hour, a significant effect only of the groups factor was found: ($F_{1,10}$=6.60, P<0.03); there were significantly more SWD bursts in Group B than in Group A.

A similarly performed analysis of the total duration of SWD activity (Fig. 2, A and B, diagrams to the right) revealed no significant differences, although in group B the mean values of this parameter were clearly increased in successive recordings.

Single spikes (i.e. spikes with usually irregular occurrence at less than 1.0 Hz frequency) were observed after Pc injections in all rats of group B, and trains of spikes with clonic convulsions were noted in five of these rats. For statistical purposes, the total number of single spikes encountered in the post injection parts of all the three recordings was assumed to be 100%. The percentage distribution of the number of spikes in consecutive records is presented in Fig.3. In all but one rat the highest number of spikes occurred after the first Pc injection (recording 1) and in all animals the smallest number of single spikes occurred after the last injection (recording 3). Statistical analysis by the Friedmann non parametric two-way ANOVA and Wilcoxon test (Siegel 1956) showed that these differences were significant (X^2= 6.33, P<0.05). The proportion of single spikes in recording 3 was significantly smaller than in recording 1 and 2 (P<0.05 in both instances).

The observed trains of spikes lasted from 5 to 20 s and the frequency of spikes in the trains varied from 2.0 to 9.0 Hz. After the first Pc injection (recording 1), they were noted only in one rat, and after the third injection (recording 2) in four animals. The sixth injection (recording 3) produced no trains of spikes and clonic convulsions in any of the rats. In all cases, the convulsive activity started within the first post injection hour. In three rats it continued to appear intermittently until the end of recording, and in two convulsive activity was limited to the first post injection hour. Owing or due to the uneven distribution, statistical evaluation of this form of activity was impossible. However, a very prominent

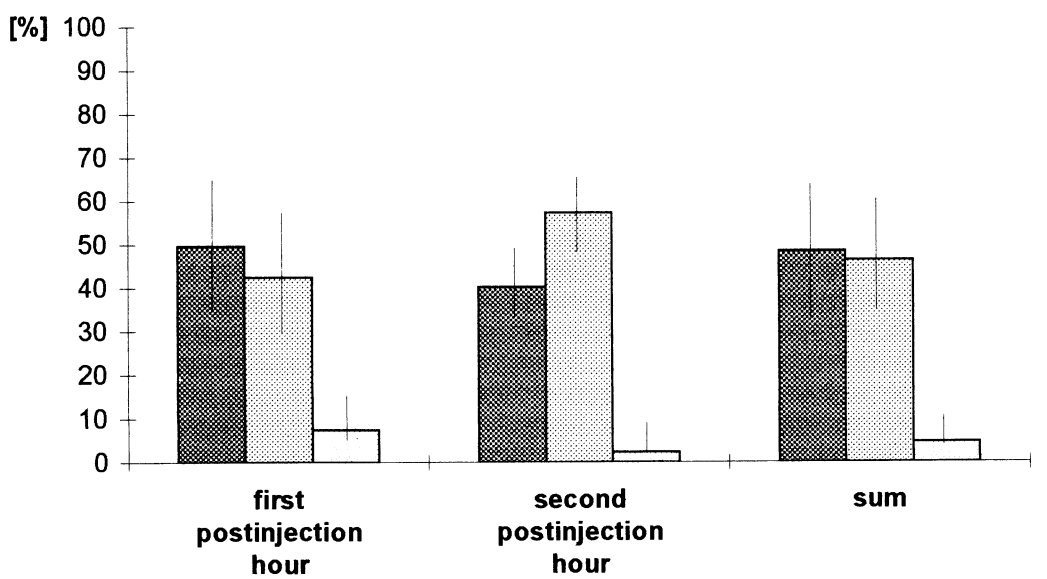

Fig. 3. Diagrams (means and SEs) illustrating the percentage distribution of the single spikes in the post injection hours of three ECoG recordings in rats receiving repeated i.p. injections of 1,500,000 IU/kg of crystalline penicillin. Strongly shadowed bars, recording 1; moderately shadowed bars, recording 2; lightly shadowed bars, recording 3.

relationship between the occurrence of SWD bursts and the occurrence of the trains of spikes with clonic seizures was clearly seen; after each train of spikes the SWD occurrence was reduced for several minutes or longer.

The results described above have confirmed that Pc administered intraperitoneally may stimulate spontaneous nonconclusive SWD activity (Marescaux et al. 1992) and provoke convulsive seizures (Bo et al. 1984, 1986, Chen et al. 1986) in rats. They have also revealed, however, that when Pc is administered repeatedly, the SWD response to Pc increases in strength, whereas the convulsive response decreases.

According to Bo et al. (1986), the decrease in the convulsive effects of Pc after successive injections may suggest the development of tolerance. The authors excluded an increased Pc elimination, or reduction of the blood-brain barrier permeability to Pc, as the possible causes of the tolerance development, but proposed no alternative explanation.

It has been suggested that the action of Pc in CNS is based on its competition for GABA at the GABA receptor (McDonald and Barker 1977), and that the target area for Pc given systemically is the neocortex (Avoli and Gloor 1981). It has also been shown that a blockade of the GABA-mediated postsynaptic inhibition of cortical pyramidal neurones is the probable cause of the Pc-induced ECoG changes which are accompanied by motor convulsions (Quesney and Gloor 1978). It is known that when the normal interactions between transmitters and their receptors are impaired, compensatory alterations within a given neurotransmitter system may occur (Mandell 1976). It is then likely that when Pc is administered repeatedly, an adaptive compensatory increase in the GABA receptor sensitivity, or GABA availability, may occur in the neocortex. The question remains, however, whether the decrease in the convulsive ECoG and motor effects, and the increase in SWD activity after successive Pc injections might be due to the same adaptive changes. According to the existing evidence, SWD may be considered a response of hyperexcitable cortex to the thalamic volleys, which normally induce spindles (Kostopoulos and Antoniadis 1992, Steriade 1993). Unlike the Pc-induced convulsive seizures, this form of epileptic activity is not associated with an impairment of the GABAergic inhibition on the somatic level of cortical cells. It has therefore been proposed that the SWD-promoting action of Pc may be due to a reduction in remote dendritic inhibition which may result in an increased effectiveness of the afferent thalamic volleys in the cortex (Giaretta et al. 1987). As some *in vitro* data have shown, the dendritic GABA-ergic inhibition is more vulnerable to Pc than somatic inhibition (Avoli 1980, Alger and Nicoll 1982). It is then likely that when Pc is applied repeatedly, the presumed adaptive changes will prevent the effect of Pc on the somatic level earlier than that on the dendrites. This supposition may account for the reduced convulsive activity seen after successive Pc injections in our rats, but is not a sufficient explanation of the substantial parallel increase in SWD activity. Several observations (Brankack et al. 1993), including those described in this paper, suggest a depressing influence of convulsive seizures on SWD activity. It may thus be possible that the observed increase in the SWD response to Pc, seen after successive injections, might be secondary to the reduction of the convulsive response. If the above explanation is correct, then one may expect that in the case of repeated Pc injections at doses too small to induce convulsive seizures, but sufficient to increase SWD activity, the latter response would diminish progressively. If no diminution occurs, it may suggest that in the course of repeated Pc injections the CNS may develop tolerance only to the convulsive effects of this antibiotic, but not to that promoting the nonconvulsive SWD activity. Checking the validity of the above assumption will be the subject of our further research.

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