ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 673-681

The influence of repeated systemic penicillin injections at subconvulsive doses on spontaneous Spike-Wave Discharges in the rat

Jolanta Stankiewicz and **Sławomir Gralewicz**[1]

Laboratory of Neurotoxicity Evaluation, Nofer Institute of Occupational Medicine, 8 Teresy St., 90-950 Łódź, Poland

Abstract. Changes in nonconvulsive spontaneous epileptic activity - Spike-Wave Discharges (SWD) - induced by repeated intraperitoneal (i.p.) administration of crystalline penicillin (PC) at subconvulsive doses were evaluated in imp-DAK rats. Three groups received ten daily i.p. injections of PC at doses of 750,000, 500,000 and 250,000 IU/kg b.w. For comparison, another classic convulsant, pentylenetrazol (PTZ) was applied in the same way to another group. PTZ was also given to all rats before and after injection series for better evaluation of changes in CNS excitability. Repeated PC injections resulted in a progressive increase in the basal level of the spontaneous SWD activity and in an increase in the SWD response to PC, which was statistically significant in the case of the dose 750,000 IU/kg. Moreover, in all rats given PC the response to PTZ (increase in SWD activity) was reduced. The results obtained in this and previous experiment suggest that in the course of repeated systemic Pc administration adaptive changes in the rat CNS develop which prevent the convulsive effects of Pc but promote the occurrence of the spontaneous nonconvulsive SWD activity.

[1] To whom correspondence should be addressed

Key words: penicillin, pentylenetetrazol, Spike Wave Discharges (SWD), neurotoxicity, rat

INTRODUCTION

Epileptogenic properties of penicillin (PC) applied either directly to the brain or systemically have been known for many years (see de Deyn et al. 1992). Considering that repeated, systemic injections of a convulsant drug may result in a durable change of the convulsive threshold (Mason and Cooper 1972) a question about the effects of prolonged PC therapy arises. Experimental data concerning this problem are scarce. In a series of experiments on rats, Bo et al. (1984, 1986) found a decrease in the epileptiform activity induced by crystalline PC after successive injections, which suggested the development of tolerance to PC. In these experiments PC induced single spikes and trains of spikes in ECoG, accompanied by muscle twitches and clonic convulsions. It is known, however, that systemic injections of PC may also induce generalized nonconvulsive petit mal-like seizures characterized by bursts of spike and wave discharges (SWD) in ECoG (Gloor and Testa 1974, Gloor 1979), or aggravate this type of epileptic activity in animals in which it occurs spontaneously (see Marescaux et al. 1992). The convulsive and nonconvulsive seizures differ with respect to their sensitivity to anticonvulsant drugs (Coenen et al. 1992, Marescaux et al. 1992), which suggests that different neural mechanisms and/or neurotransmitter systems are involved in their generation. It would therefore be of interest to find out whether in the course of repeated PC administration the effect of PC on the SWD activity will change in the same direction as that on the convulsive activity. In our earlier experiments on rats with spontaneous SWDs we observed that while the convulsive activity (single spikes and trains of spikes) induced by subsequent PC injections was markedly attenuated, similarly as in experiments of Bo et al. (1986), the SWD activity increased (Stankiewicz et al. 1995). Such effect may suggest that in the course of repeated PC injections CNS developed tolerance to the convulsive effect of this antibiotic, but not to that promoting SWD activity. Another possibility was that the increase in the SWD activity was secondary to the reduction in strength of the convulsive activity; according to some observations SWD activity is being suppressed during the latter (Brankack et al. 1993). Should this be true then one could expect that in the case of repeated PC injections at doses too small to induce convulsive seizures but sufficient to promote SWD activity, the latter response would also diminish progressively owing to the development of tolerance. Smaller doses should produce no change in SWD activity. The main purpose of the present experiment was to verify the above supposition. According to literature reports, the mechanism of PC action in CNS is similar to that of another classic convulsant, pentylenetetrazol (McDonald and Barker 1977). Therefore, for comparison, the effect of pentylenetetrazol (PTZ) applied repeatedly at a dose just sufficient to produce a transient increase in SWD activity was also examined. In order to reveal enduring changes in the CNS excitability which may be a consequence of the repeated PC or PTZ administration, the response (increase in SWD activity) to a higher dose of PTZ injected before and after the series of PC and PTZ injections was compared in all animals.

METHODS

Forty four Wistar rats from the IMP/Wist stock, weighing 370-520 g, were used. The animals were kept in single plexiglas cages, at constant temperature of 22° C, and maintained on standard diet. Under general (Nembutal 60 mg/kg b.w.) and local (0.5 ml of 4% xylocainum) anaesthesia, unipolar surface electrodes (gold-plated discs of 1.5 mm in diameter) were implanted over the fronto-parietal cortex bilaterally, without breaking meninges. The reference and ground electrodes were placed on the surface of dura mater over the cerebellum and on the nasal bone, respectively. The experiment began after a 14-day recovery period.

Apparatus

An 8-channel electroencephalograph (Beckman, Acutrace) was used to perform the ECoG recording. The time constant was set at 0.3 s and the high fre-

quency filter at 35 Hz. For the time of the recording the rats were placed into plastic opaque open-top boxes (30 x 30 x 40 cm) and the 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 animal compartment. A TV system enabled observation of the rat behaviour during the recording.

Drugs and drug administration

Crystalline penicillin G (potassium salt, Polfa, Tarchomin) was dissolved in water for injection (aqua pro injectione, Polfa) at the concentration of 300,000 IU/ml and given intraperitoneally (i.p.) in a volume containing an appropriate dose per kg b.w. Pentylenetrazol (Sigma) was also dissolved in water for injection, at the concentration of 20 mg/ml and administered i.p. Control animals were given i.p. injections of water.

EEG recording and evaluation of records

The rats were recorded in the squads of four. In all rats, spontaneous occurrence of SWD bursts had been verified on the basis of preliminary EEG records obtained two weeks after the surgery. Two-hour recordings (monopolar derivations), one hour before and one hour after injection, were performed. Each one-hour ECoG section was analysed visually with respect to the number and cumulative duration of SWD bursts. Each clearly discernible spike-wave series lasting at least one second was regarded as an SWD burst (Fig. 1).

Experimental procedure

The rats were divided into five groups, of eight animals each. Three groups received ten daily i.p. injections of PC at doses of 750,000 IU/kg (PC750), 500,000 IU/kg (PC500) and 250,000 IU/kg (PC250). The rats of the fourth group (PTZ) received ten daily i.p. injections of PTZ at a dose of 10.0 mg/kg. The rats of the fifth, control group (W) received ten daily injections of 2 ml/kg of water. Six days before the first and six days after the last of the injection series, PTZ at a dose of 20.0 mg/kg was injected (PTZ test) to each rat of every group. The highest PC dose and all doses of PTZ used for daily injections were determined on the basis of pilot experiments. In these experiments a single injection of 750,000 IU/kg of PC and 10.0 mg/kg of PTZ resulted in a moderate (by about 20-50%) increase in the number of SWD bursts during a one-hour postinjection period. The 20.0 mg/kg PTZ dose used for the PTZ test, resulted in about twofold increase in the number of SWD bursts in the postinjection one-hour ECoG section.

The ECoGs were performed on the days when PTZ at the dose of 20.0 mg/kg was injected and on the day of the first (record 1), fifth (record 2) and tenth (record 3) injection of the series of daily injections.

Statistical analysis

The data were analysed by a parametric two-way ANOVA and Tukey's test for multiple comparisons (Winer 1961). The differences were regarded as significant at $P<0.05$.

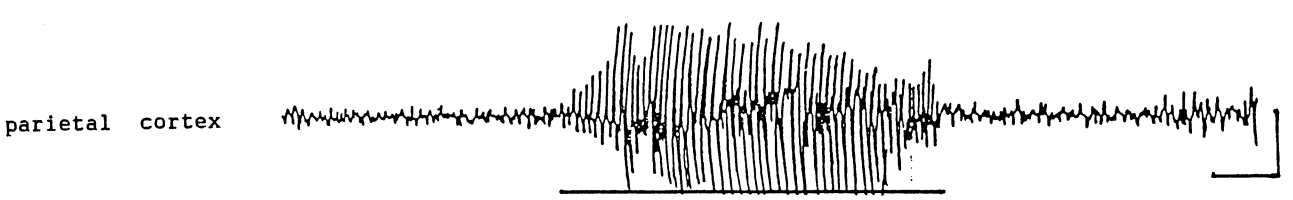

Fig. 1. Fragment of ECoG showing spontaneous 7-9 Hz burst of spike and wave discharges. Calibration: horizontal, 1.0 s, vertical, 500 V.

RESULTS

The number of SWD bursts in the preinjection and postinjection one-hour ECoG sections was denoted as n1 and n2, respectively. Consequently, the total duration of the SWD activity in the pre- and postiniection ECoG sections was denoted as t1 and t2. Direct n1, n2, t1 and t2 values as well as n2/n1 and t2/t1 ratios calculated on the basis of ECoGs obtained during the period of repeated daily injections (recordings 1, 2 and 3), and those from the PTZ tests, were analysed separately.

Repeated injections stage

The analysis of n1 by the two-way parametric ANOVA revealed no effect of group factor but the effect of recordings as well as the groups x recordings interaction was significant ($F_{1,35}$=6.88, P<0.02 for recordings, $F_{4,35}$=29.51, P=0.001 for interaction); generally, in the preinjection part of recording 3 the number of SWD episodes was significantly higher than in recordings 1 and 2 and this increase was most reliable in the PC750 group (Fig. 2A). Within-group comparisons however, revealed that this difference was not significant (0.05<P<0.07).

The results for the t1 value replicated in part those for n1. Generally, t1 in recording 3 was significantly higher than in recording 1 (effect of recordings: $F_{1,34}$=5.42, P<0.03). However, no significant differences between groups within consecutive recordings as well as between recordings within groups were found (Fig. 2B).

The analysis of the n2/n1 proportion revealed no significant effect of any of the main factors. However, the group x recordings interaction, was found to be significant ($F_{4,34}$=34,75, P<0.0001). Further comparisons revealed no differences between groups within the successive recordings. Comparisons between recordings revealed differences only in the PC500 group ($F_{2,68}$=5.37, P<0.01); the n2/n1 ratio in recording 3 was significantly higher than in recordings 1 and 2 (Fig. 3A). It should be noted, however, that in all groups receiving PC injection the n2/n1 ratio changed in the same direction, i.e. increased, across successive recordings, whereas in group W and PTZ10, the downward tendency was observed. Moreover, it needs mentioning, that contrary to our expectations based on the results of pilot experiments, only in three rats from the PC750 group the n2/n1 ratio in recording 1 was higher than 1.0, i.e. the PC injection resulted in an overt increase in SWD activity. There were two such animals in group PC500, one in group PC250 and six in the PTZ10 group. Even in the W group, in two rats an

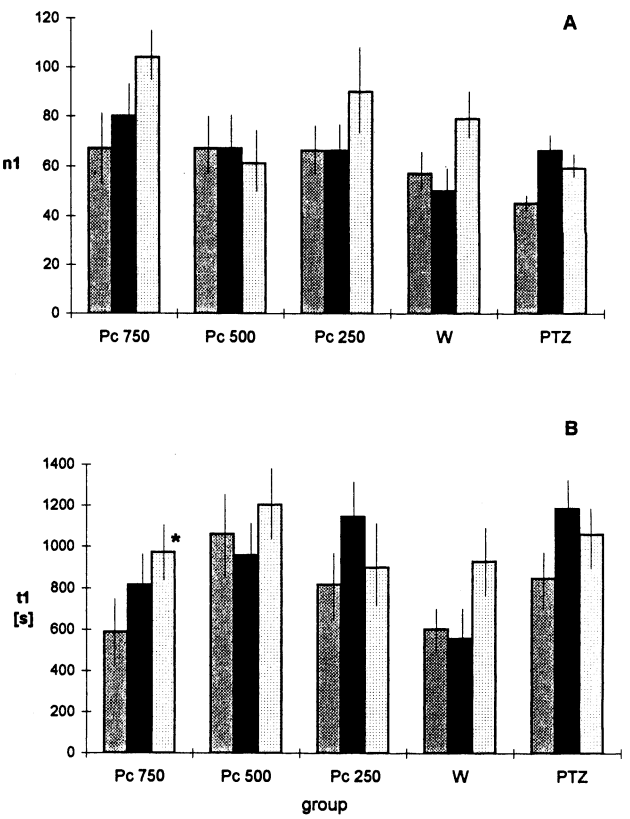

Fig. 2. Diagrams (means and SEs) illustrating the number (A) and duration (B) of SWD episodes in the preinjection hour of three ECoG recordings in rats receiving repeated i.p. injections of crystalline penicillin at the doses of 750,000 I.U./kg (Pc750), 500,000 I.U./kg (Pc500) and 250,000 I.U./kg (Pc 250) and in rats receiving repeated i.p. injections of water (W) and 10 mg/kg of pentylenetrazol (PTZ). Shadowed bars, recording 1 (before the first injection); black bars, recording 2 (before the fifth injection); open bars, recording 3 (before the tenth injection). *P<0.05 in comparison with recording 1.

increase in the SWD number after the first injection was observed. In recording 3, however, the n2/n1 ratio was higher than 1.0 in four rats of the PC750 group, in six of the PC500 group and in four of the PC250 group. In the PTZ10 group the number of animals showing an increase in the SWD number after the injection fell to two and in group W to zero. In other words, within the PC groups the proportion of rats showing postinjection increase in SWD number became higher in the course of repetitive PC treatment. In PTZ10 group, on the contrary, the response to successive PTZ injections seemed to become weaker. The latter was more clearly illustrated by the results of t2/t1 analysis. In this case, the two-way ANOVA revealed a significant group effect ($F_{4,34}$=3.35, P<0.03), and significant groups x recordings interaction ($F_{4,34}$=20.73, P<0.0001). Subsequent one-way ANOVAs revealed differences in recording 1 ($F_{4,102}$=3.93, P<0.01), and recording 2 ($F_{4,102}$=2,71, P<0.05). In recording 1 the t2/t1 ratio in PTZ10 group was significantly higher than in all the remaining groups except the PC750 group. In recording 2, only in group W the t2/t1 ratio was lower than in the PTZ10 group. In recording 3 no within-group differences with respect to the value of the t2/t1 proportion were found (Fig. 3B).

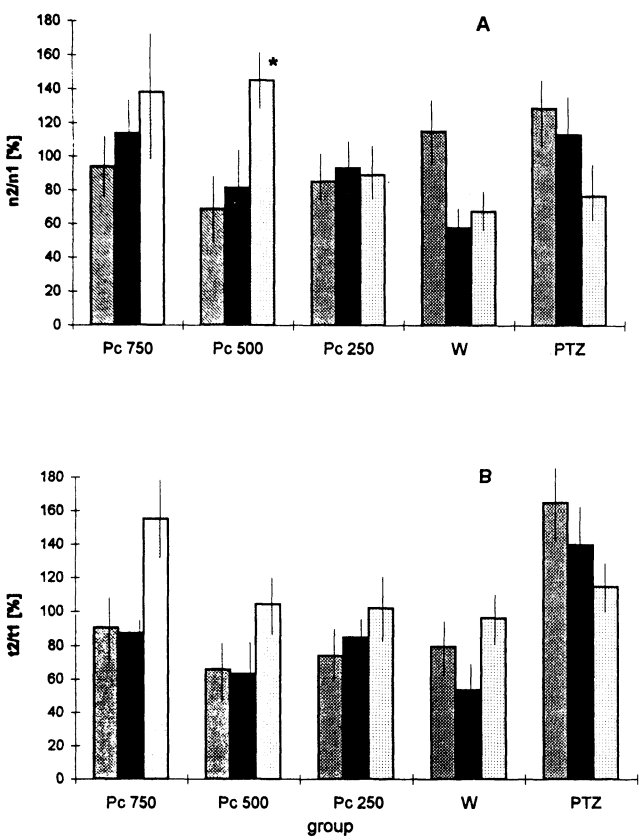

Fig. 3. Diagrams (means and SEs) illustrating the n2/n1 (A) and t2/t1 (B) ratios in rats receiving repeated i.p. injections of crystalline penicillin at the doses of 750,000 I.U./kg (Pc750), 500,000 I.U./kg (Pc500) and 250,000 I.U./kg (Pc 250) and in rats receiving repeated i.p. injections of water (W) and 10 mg/kg of pentylenetrazol (PTZ). The remaining description as in Fig. 2.

Results of PTZ tests

The comparison of the number of SWD episodes in the preinjection phase of the PTZ tests revealed no significant group effect but the effect of recordings was significant ($F_{1,35}$=4.32, P<0.05); on the day of the second test the n1 value was significantly increased. The group x recordings interaction was also significant ($F_{4,35}$=47.45, P<0.0001). Further comparisons revealed that in group PC750 on the day of the second PTZ test, i.e. six days after the last PC injection, the n1 value was significantly increased as compared with respective value for the first PTZ test ($F_{1,35}$=15.6, P<0.0005). In the remaining groups the n1 values for the day of the first and second PTZ tests did not differ. The analysis of the t1 value revealed no significant differences, although on the day of the second PTZ test it was evidently increased in the PC750 group (Fig.4).

As for the n2/n1 ratio the effects of the main factors were not significant but the interaction was ($F_{4,35}$=80.40, P<0.0001). Comparisons between successive tests revealed differences only in the PC750 group ($F_{1,35}$=21.14, P<0.0001); on the day of the second test the n2/n1 proportion was significantly decreased in comparison with the day of the first PTZ test.

The analysis of the t2/t1 proportion revealed no significant effect of the group factor but the effect of tests was significant ($F_{1,35}$=5.79, P<0.03); generally, the t2/t1 proportion on the day of the second PTZ test was significantly reduced. The groups

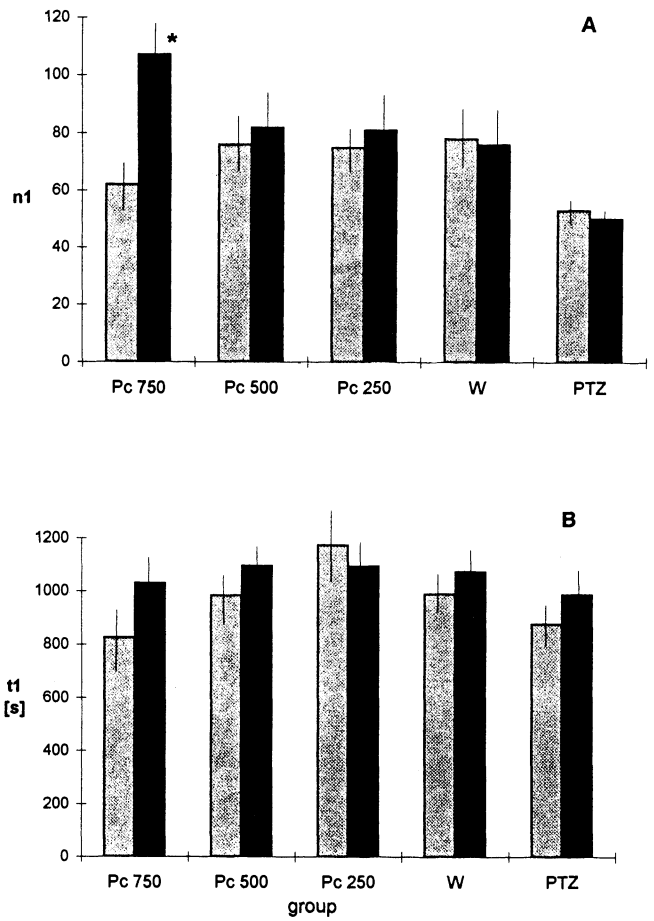

Fig. 4. Diagrams (means and SEs) illustrating the number (A) and duration (B) of SWD episodes in the preinjection hour of ECoG recordings on the days of pentylenetrazol administration at the dose of 20 mg/kg in rats receiving series of i.p. injections of crystalline penicillin at the doses of 750,000 I.U./kg (Pc750), 500,000 I.U./kg (Pc500) and 250,000 I.U./kg (Pc 250), water (W) and 10 mg/kg of pentylenetrazol (PTZ). Shadowed bars, recording I - six days before; black bars, recording II - six days after the injection series. *P<0.05 in comparison with recording 1.

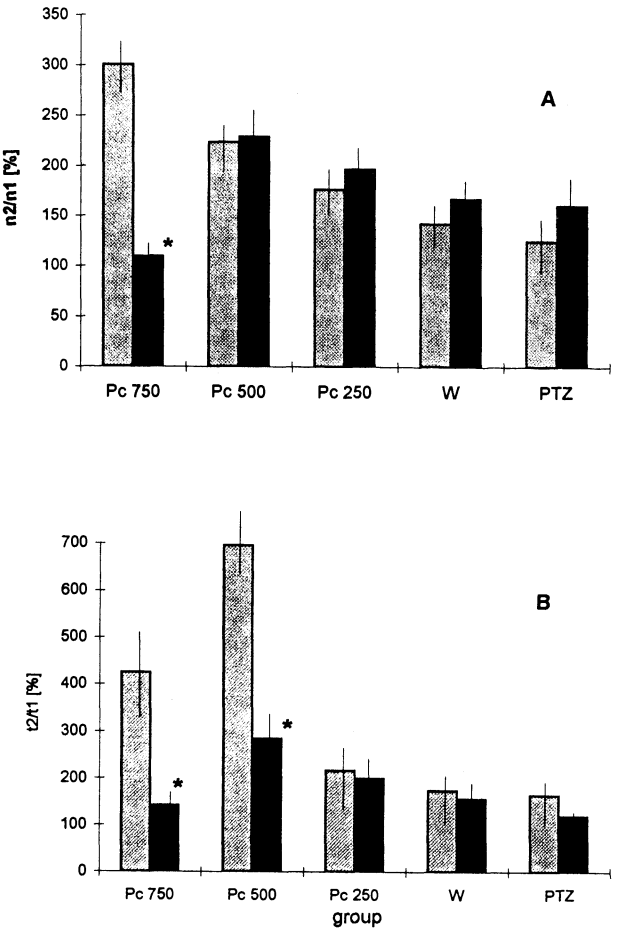

Fig. 5. Diagrams (means and SEs) illustrating the n2/n1 (A) and t2/t1 (B) ratios on the days of pentylenetrazol administration at the dose of 20 mg/kg in rats receiving series of i.p. injections of crystalline penicillin at the doses of 750,000 I.U./kg (Pc750), 500,000 I.U./kg (Pc500) and 250,000 I.U./kg (Pc 250), water (W) and 10 mg/kg of pentylenetrazol (PTZ). The remaining description as in Fig. 4.

x tests interaction was also significant ($F_{4,35}$=25.48, P<0.0001). In group PC750 as well as in group PC500, the value of the t2/t1 proportion was significantly reduced on the day of the second test in comparison with test I ($F_{1,35}$=4.88, P<0.05, and $F_{1,35}$=8.15, P<0.01, respectively) (Fig. 5).

DISCUSSION

The major objective of the present study was to find out whether the transient increase in SWD activity, which may follow systemic injection of PC at appropriate dose, diminishes when the antibiotic is given repeatedly. If such effect occurred, it might suggest, following the data of Bo et al. (1986) and the results of our previous studies (Stankiewicz et al. 1995), that in the course of repeated injections, changes in CNS develop, which compromise not only the convulsive but also the nonconvulsive (SWD) epileptic effects of PC. Unfortunately, the expected response to the first PC injection, even at the highest dose (750.000 IU/kg) i.e. the transient increase in the number of SWD episodes, appeared to be much less frequent than it was expected on the

basis of pilot studies. Nonetheless, detectable changes have been produced by the repeated PC injections. Firstly, in the PC-injected animals, unlike in the control and PTZ10 groups, the intensity of SWD activity (i.e. the number and/or duration of the SWD bursts) in the postinjection part of the recording period in general, as well as the percentage of animals with the n2/n1 or t2/t1 1, apparently increased. One may consider this increase controversial owing to the lack of overt dose-response relationship. In fact, only at the medium PC dose (PC500 group) the n2/n1 increased significantly. It should be noted, however, that at the highest PC dose (PC750 group), there was also a pronounced increase in the n1 value (see below) which might be responsible for the flattening of differences in the n2/n1 ratios between successive records. Different individual sensitivity to PC might be another factor responsible for the lack of overt dose-response relationship.

Secondly, repeated PC administration seemed to result in a general increase in the level of SWD activity persisting for at least several days after the PC injections had been discontinued. It has been evidenced by a significantly higher n1 value in group PC750 during the second PTZ test as compared with that seen during the first one. The above observations by no means suggest a diminution of a facilitatory effect of PC on the SWD activity, but they allow one to suspect its strengthening in the course of repeated PC injections. However, repeated i.p. administration of PC made the rat CNS apparently less susceptible to the SWD-promoting influence of PTZ given via the same route. This is suggested by the lower n2/n1 and t2/t1 proportions in the second PTZ test as compared with the first one. This effect appeared to be dose-related since it was evident only in the PC750 and PC500 groups.

The available data concerning the mechanism of PC activity on the CNS and generation of SWD do not allow simple, straightforward explanation of the results obtained in the present experiment. According to several reports, in the mammalian nervous system PC behaves as an antagonist of GABA (GABA-A) receptors (Curtis et al. 1972, McDonald and Barker 1977). A deficient GABA-mediated inhibition in the neocortex was assumed to be the most likely cause of the convulsive activity induced by PC in cats (Quesney and Gloor 1978, Giaretta et al. 1987). In rats, the cortical spiking activity induced by intraperitoneal PC injections, can be blocked by systemic GABA (Loeb et al. 1982, Benassi et al. 1992). Thus, it is possible that in both species the mechanism of PC-induced convulsive activity is similar, if not the same. One may assume that in case of repeated PC administration compensatory modifications of the GABA-ergic synapses in the cortex occur which make the GABA-ergic transmission less vulnerable to the action of an antagonist. Conceivably, the changes may consist, like in other neurotransmitter systems, in receptor up-regulation in the presence of an antagonist (Ben-Barak and Dudai 1980, McKinney and Coyle 1982). This could explain the decrease in the strength of the convulsive response to subsequent PC injections (Bo et al. 1986, Stankiewicz et al. 1995). It could also account for some effects of repeated PC injections on SWD activity observed in the present experiments. According to literature data, strengthening the GABA-ergic transmission by systemic injections of an agonist of GABA-A and GABA-B receptors, inhibitors of GABA reuptake, or an inhibitor of GABA-transaminase, results in a dose-related facilitation of SWD activity in rat genetic models of absence epilepsy, which suggests that this type of ECoG abnormality may be related to an augmented GABA-ergic transmission (see Marescaux et al. 1992). In view of the above, the persisting, increased basal level of the SWD activity noted in the present experiments could be regarded as an evidence of increased GABA-ergic functional tone, the presumed adaptive response to repeated PC injections. The above explanation, however, cannot be accepted in the context of the second effect of repeated PC administration found in the previous (Stankiewicz et al. 1995) and in the present experiment, i.e. the gradually increasing postinjection SWD response. If a PC- induced inactivation of GABA-A receptors were the direct cause of the transient increase in SWD activity following PC injection, and if this inactivation, if repetitive, led to

a compensatory increase in the functional GABA-ergic tone, then the effect of subsequent PC injections should be diminished rather than increased. There are also other reasons which make the presumed modification in the GABA-ergic transmission an unreliable explanation of the PC-induced changes in SWD activity. Firstly, no signs of deficient GABA-mediated postsynaptic inhibition were found in cat's pericruciate cortex during SWDs induced by systemic PC administration (Giaretta et al. 1987). Secondly, it has been shown that systemic injections of classic GABA-A agonists, bicuculline and picrotoxin, do not facilitate spontaneous SWD activity in the rat genetic models of absence epilepsy (see Coenen et al. 1992, Marescaux et al. 1992). The above data allow one to assume that the transient increase in SWD activity following systemic PC administration in our rats could not be related to a blockade of GABA-A receptors; consequently the lasting elevation in the basal level of the spontaneous SWD activity could not be attributed to the presumed compensatory increase in GABA-ergic functional tone. The locus and the character of the PC action responsible for the PC-induced changes in SWD activity remain to be demonstrated.

An intriguing observation from the present studies is the lack of any overt changes in SWD activity in rats of the PTZ10 group and the reduced effectiveness of PTZ (20 mg/kg) in facilitating SWD activity in the PC groups, noted on the 6-th day after the last PC injection. In cultured mammalian neurons, PTZ, like PC, behaves like a selective antagonists of GABA (GABA-A) receptors (McDonald and Barker 1977), and both PTZ and PC, administered systemically, aggravate spontaneous SWD activity in rats (Marescaux et al. 1992). What is more, neocortex is the primary locus of the epileptogenic action of PC (Gloor et al. 1977), and at least some epileptiform phenomena induced by PTZ are due to its direct action on the cortex (Stringer 1994). In the light of the above, one could expect that the effect of repeated PTZ administration on SWD activity would be similar as that of PC (i.e. stimulation), and that the effect of the second PTZ test (after a series of PC injections) would be rather stronger that that of the first one (preceding the PC injections). The diminished effectiveness of PTZ in the second test suggests that PC, administered repeatedly, induces changes which protect against SWD induction (or stimulation) by PTZ. It is likely that the changes are not identical to those responsible for the elevated basal level of the SWD activity and the increased SWD response to PC. The recently obtained evidence suggests that PTZ, administered systemically, induces SWD activity in rats through an action on mediodorsal and intralaminar thalamic nuclei; lesions located in these areas prevent the SWD induction by PTZ (Banerje and Snead III 1994). Such lesions, however, do not affect the SWD generation in rats in which, like in those used in the present studies, this form of activity occurs spontaneously (Vergnes and Marescaux 1992). One can thus conclude that PTZ may influence SWD through thalamocortical connections not engaged in spontaneous SWD generation. Then there is a possibility that the PC-induced changes responsible for lowering of the PTZ effectiveness concern the neocortical projections from intralaminar thalamic nuclei, whereas those facilitating SWD develop somewhere in the thalamo-cortical circuitry involved in spontaneous SWD generation (Avanzini et al. 1992, Kostopoulos and Antoniadis 1993, Steriade et al. 1993).

Summing up, the obtained results demonstrate the complexity of the spectrum of PC activity within the CNS. They also confirm the different pathophysiology of the convulsive epileptic phenomena and nonconvulsive SWD activity. The previous (Stankiewicz et al. 1995) and present data suggest that as a result of repeated PC administration CNS develops tolerance to the convulsive activity of this antibiotic. At the same time, it becomes more susceptible to the SWD-promoting action of PC but not to a similar action of another classic convulsant, PTZ. The obtained results should be of interest for clinicians. The neurotoxicity of beta-lactam antibiotics, even at therapeutic doses, especially when risk factors exist, is well known (Keskin and Konkol 1993). Unfortunately the results of treatment with these antibiotics in doses producing no clinically

evident toxicity, were not investigated. Therefore, experiments on animal models might provide evidence allowing better estimation of the possible consequences of PC therapy on CNS functions.

REFERENCES

Avanzini G., de Curtis M., Spreafico R. (1992) Physiological properties of GABAergic thalamic reticular neurons studied in vitro: relevance to thalamocortical synchronizing mechanisms. In: Neurotransmitters in epilepsy (Eds. G. Avanzini, J. Engel Jr, R. Fariello and U. Heinemann) (Epilepsy Res. suppl. 8). Elsevier, Amsterdam, p. 117-124.

Benassi E., Bessio G., Cupello A., Mainardi P., Patrone A., Rapallino M.V., Vignolo L.., Loeb C.W. (1992) Evaluation of the mechanisms by which gamma-amino-butyric acid in association with phosphatidylserine exerts an antiepileptic effect in the rat.. Neurochem. Res. 17: 1229-1233.

Ben-Barak J., Dudai Y. (1980) Scopolamine induces an increase in muscarinic receptor level in rat hippocampus. Brain Res. 185: 309-313.

Bo G.P., Fonzari M., Scotto P.A., Benassi E. (1984) Parenteral penicillin epilepsy: tolerance to successive treatments. Exp. Neurol. 85: 229-232.

Bo G.P., Mainardi P., Benassi E., Besio G., Faverio A., Scotto P.A., Loeb C. (1986) Parenteral penicillin model of epilepsy in the rat: a reappraisal. Meth. Find. Exptl. Clin. Pharmacol. 8: 491-496.

Brankack J., Lahtinen H., Koivisto E., Riekkinen P.J. (1993) Epileptogenic spikes and seizures but not high voltage spindles are induced by local frontal cortical application of gamma-hydroxybutyrate. Epilepsy Res. 15: 91-99.

Coenen A.M.L., van Luijtelaar E.L.J.M.I. (1992) Genetic models of absence epilepsy with emphasis on the WAG/Rij strain of rats. Epilepsy Res. 12: 75-86.

Curtis D.R., Game C.J.A., Johnston G.A.R., McCulloch R.M., MacLachlan R.M. (1972) Convulsive action of penicillin. Brain Res. 43: 242-245.

De Deyn P.P., D'Hooge R., Marescau B., Pei Y. (1992) Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsant. Epilepsy Res. 12: 87-110.

Giaretta D., Avoli M., Gloor P. (1987) Intracellular recordings in pericruciate neurons during spike and wave discharges of feline generalized penicillin epilepsy. Brain Res. 405: 68-79.

Gloor P. (1979) Generalized epilepsy with spike-and-wave discharge: A reinterpretation of its electrographic and clinical manifestations. Epilepsia 20: 571-588.

Gloor P., Quesney L.F., Zumstein H. (1977) Pathophysiology of generalized penicillin epilepsy in the cat: the role of cortical and subcortical structures. II. Topical application of penicillin to the cerebral cortex and to subcortical structures. Electroencephalogr. Clin. Neurophysiol. 43: 79-94.

Gloor P., Testa G. (1974) Generalized penicillin epilepsy in the cat: effects of intracarotid and intravertebral pentylenetetrazol and amobarbital injections. Electroencephalogr. Clin. Neurophysiol. 36: 499-515.

Keskin S., Konkol R.J. (1993) Seizure exacerbation related to betalactam antibiotics in a child with cerebral dysgenesis, Dev. Med. Child Neurol. 35: 258-274.

Kostopoulos G., Antoniadis G. (1992) Active role of cortical inhibition in the development of generalized epilepsy with spike-and-wave discharges: evidence from electrophysiological, microiontophoretic and simulation studies. In: Neurotransmitters in epilepsy (Eds.G. Avanzini, J. Engel Jr., R. Fariello and U. Heinemann), (Epilepsy Res., suppl. 8). Elsevier, Amsterdam, p. 125-133.

Loeb C., Benassi E., Besio G., Maffini L., Tanganelli P. (1982) Liposome entrapped GABA modifies behavioral and electrographic changes of penicillin-induced epileptic activity. Neurology 32: 1234-1238.

McKinney M., Coyle J.T. (1982) Regulation of neocortical muscarinic receptors: Effects of drug treatment and lesions. J. Neurosci. 2: 97-105.

Marescaux C., Vergnes M., Bernasconi R. (1992) $GABA_{(B)}$ receptor antagonists: potential new anti-absence drugs. J. Neural Transm. 35: 179-188.

Marescaux C., Vergnes M., Depaulis A. (1992) Genetic absence epilepsy in rats from Strasbourg - a review. J. Neural Transm. 35: 37-69.

Mason C.R., Cooper R.M. (1972) A permanent change in convulsive threshold in normal and brain damaged rats with repeated doses of pentylenetetrazol. Epilepsia 13: 663-674.

Stankiewicz J., Stankiewicz M., Wiaderna-Stępień D., Tomas T., Gralewicz S. (1995) Effects of repeated systemic penicillin injections on nonconvulsive and convulsive epileptic seizures in the rat. Acta Neurobiol. Exp. 55: 281-287.

Steriade M., McCormic D.A., Sejnowski T.J. (1993) Thalamocortical oscillations in the sleeping and aroused brain. Science 262: 679-685.

Stringer J.L. (1994) Pentylenetetrazol elicits epileptiform activity in the dentate gyrus of the urethane anesthetized rat by activation of the entorhinal cortex. Brain Res. 636: 221-226.

Quesney L.F., Gloor P. (1978) Generalized penicillin epilepsy in the cat: correlation between electrophysiological data and distribution of ^{14}C-penicillin in the brain. Epilepsia 19: 35-45.

Vergnes M., Marescaux C. (1992) Cortical and thalamic lesions in rats with genetic absence epilepsy. J. Neural Transm. 35 (suppl): 71-83.

Winer B.J. (1962) Statistical principles in experimental design. Mc Graw Hill Book Company, New York.

Received 22 September 1995, accepted 14 May 1996