tion. In the diabetic rats PAS-stained kidney sections revealed Armanni-Ebstein tubular lesions (fig. 1), collapsed or occluded glomerular capillary tufts, a marked reduction in the size of the glomeruli (fig. 2), and diffuse glomerular basement membrane thickening, as well as exudative deposits in the tubules and in glomerular subcapsular space. The Armanni-Ebstein lesions characteristically showed deposition of glycogen in distal tubules, especially the ascending limbs of the loop of Henle and those forming the juxta-glomerular apparatus. On the other hand the PAS-positive exudative deposits in tubules and glomeruli were α-amylase-resistant, which indicated their glycoprotein nature.

Treatment with MAG markedly reduced these tubular and glomerular lesions (fig. 3). The glomeruli were almost normal in appearance with open capillary tufts and were devoid of any noticeable PAS-positive thickening in the mesangial areas or the capillary walls. However, a mild form of tubular Armanni-Ebstein lesions was still evident but the tubules showed very little glycogen deposition (fig. 3). To our knowledge, this is the first definitive evidence that at least some of the diabetic kidney lesions can be prevented or reduced by MAG. This effect of monoaminoguanidine on renal pathology was, however, not associated with any significant reduction in the blood glucose levels, or with renal hypertrophy, which indicates that MAG-induced reversal of pathology is not via amelioration of hyperglycemia. We have recently observed that monoaminoguanidine inhibits nonenzymatic protein glycosylation and protein cross-linking in vitro [18]. Earlier, Brownlee et al. [6] also reported that MAG administration prevents the formation of fluorescent advanced nonenzymatic glycosylation products and the cross-linking of arterial wall connective tissue protein in diabetic rats. Similar changes in glomerular macromolecules are thought to be involved in diabetic nephropathy [5].

The results of the present study, therefore, suggest that the prevention of renal pathological lesions in the diabetic rat by monoaminoguanidine could be due to inhibition both of nonenzymatic protein glycosylation and of the accumulation of sorbitol.

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4-Aminopyridine and barium chloride attenuate the anti-epileptic effect of carbamazepine in hippocampal slices

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Summary. The exact mode of action of the anti-epileptic agent carbamazepine is unknown. In hippocampal slices in which epileptiform discharges were induced by addition of penicillin to the perfusion medium, the depressant effect of carbamazepine was attenuated by the potassium-channel blockers barium chloride (0.1 mM) and 4-aminopyridine (200 μM), which suggested that potassium fluxes might be involved in the mechanism of action of carbamazepine.
Key words. Carbamazepine; potassium; hippocampus; epilepsy; electrophysiology; 4-aminopyridine.

Although carbamazepine has ranked for more than twenty years as one of the major anti-epileptic agents and has recently also proved to be of value in the treatment of manic states[1], its primary mode of action is still unknown. None of its diverse pharmacological features is generally held to reflect its basic pharmacological mechanism[2-4]. In pilot experiments performed to evaluate different in vitro models of epilepsy for testing anticonvulsants, we found that carbamazepine inhibited penicillin-induced interictal discharges in hippocampal slices much more potently than it did similar discharges evoked by barium chloride. As $BaCl_2$ is known to block the potassium channels[5], this finding could indicate that potassium currents play a role in the mechanism of action of the drug. Other interpretations might be equally plausible, since Ba^{2+} ions can displace Ca^{2+} ions; however, in subsequent experiments we found that 4-aminopyridine (4-AP), a relatively selective blocker of K^+ currents, also potently blocked the effect of carbamazepine on penicillin-induced epileptiform discharges. Moreover, it has recently been reported that potassium-channel openers can prevent epilepsy in rats[6].

Materials and methods

Carbamazepine was synthesized in the chemistry laboratories of Ciba-Geigy Basel. Penicillin G was purchased from Sigma. 4-Aminopyridine and tetraethylammonium chloride were purchased from Fluka.

Hippocampal slices were prepared as previously described[7]. Briefly, male rats weighing 200–280 g were anesthetized and decapitated, and the brains quickly isolated and immersed in pregassed (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF). Transverse hippocampal slices (450 µm) were cut with a McIlwain tissue chopper and kept at 32 °C for 1 h in a perfusion chamber with the liquid level flush with the surface of the slices. In all experiments, the ACSF used up to this stage contained NaCl, 124 mM; KH_2PO_4, 1.25 mM; $MgSO_4$, 2.0 mM; $NaHCO_3$, 25.7 mM; $CaCl_2$, 2.5 mM; and glucose 10 mM. Unless otherwise stated, the KCl concentration was 2.5 mM. Superfusion was then started at a rate of 3 ml (1 chamber volume) per min. Epileptiform discharges were induced by adding penicillin 1.2 mM, 4-AP 200 µM, or $BaCl_2$ 0.1 mM to the perfusion medium. The activity was registered extracellularly in the CA3 area using a glass microelectrode filled with 4 M NaCl (2–5MΩ). The signals were amplified by a Grass P 16 amplifier. The epileptiform activity was separated from noise and single-unit acitity by a window discriminator, integrated over periods of 30 or 60 s, and plotted on a W + W 320 recorder.

Carbamazepine was dissolved in dimethylsulphoxide (DMSO) and diluted in ACSF to give a final DMSO concentration of 1.25%. It was added to the bath fluid in varying concentrations over periods of 10 min by means of an infusion pump connected to the main perfusion line. The maximal depression of the epileptiform discharges at each concentration was determined and calculated as percentage inhibition of the mean control activity. Results are expressed as means ± SEM. The results were analysed statistically using Student's t-test.

Results

Addition of penicillin or $BaCl_2$ to the perfusion medium was followed by spontaneous interictal discharges from CA3 pyramidal neurons with a latency of 10–30 min. Both substances led to the appearance of spontaneous multiple population spikes (fig. 1). These epileptiform events were recorded extracellularly as field potentials, as previously described[8]. The discharge frequency showed remarkably little variability. In the first series of experiments (6 rats), the depressant effects of carbamazepine were compared in 6 slices exposed to penicillin (1.2 mM) and 6 slices to which only 0.1 mM $BaCl_2$ was added throughout the experiment. The KCl concentration was 5 mM in the $BaCl_2$ experiments, but had to be raised to 10 mM in the penicillin experiments, because the frequency of epileptiform discharges at 5 mM was low. Infusion of carbamazepine for 10 min caused concentration-dependent suppression of the epileptiform discharge frequency in slices exposed to penicillin (fig. 2). At the highest concentration of 100 µM the mean inhibition was approximately 60%. DMSO alone did not affect the frequency of epileptiform discharges (fig. 1). Carbamazepine (60 µM) and DMSO (1.25%) had no effect on

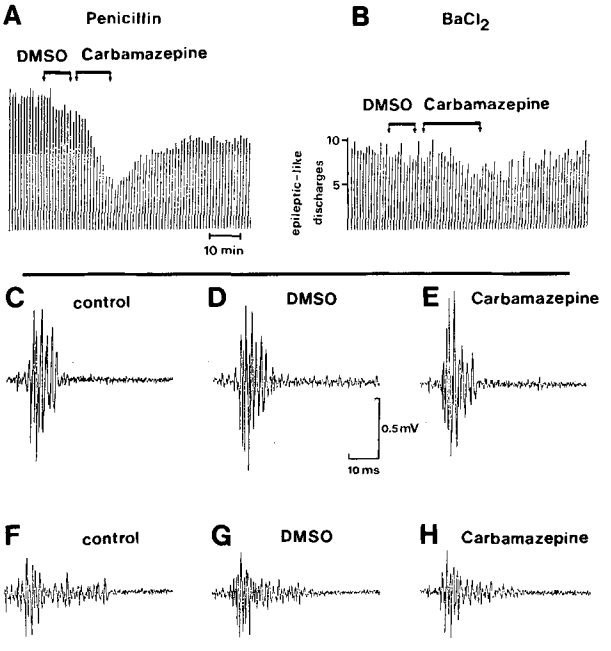

Figure 1. The inhibitory effects of carbamazepine (60 µM) on epileptiform discharges evoked by penicillin (1.2 mM) or $BaCl_2$ (0.1 mM) are compared. The upper panel shows that carbamazepine is more potent in the penicillin- than in the $BaCl_2$-model. The solvent DMSO (1.25%) has no effect in either model. The lower panel depicts individual epileptiform discharges recorded extracellularly in CA3 in slices exposed to penicillin (C, D, E) or $BaCl_2$ (F, G, H). DMSO and carbamazepine had no effects on the duration or amplitude of epileptiform field potentials.

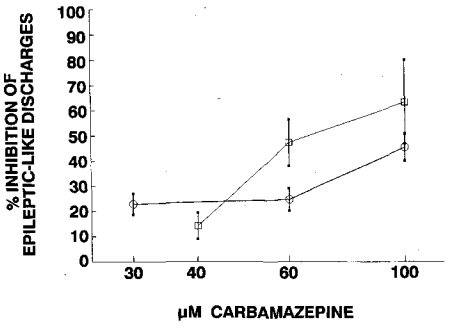

Figure 2. The depressant effect of carbamazepine on epileptiform discharges of CA3 pyramidal neurons are compared in 6 slices exposed to ACSF containing penicillin (1.2 mM) or $BaCl_2$ (0.1 mM). Each application of carbamazepine lasted 10 min. Results are expressed as the mean percent reduction (± SEM) of the maximal discharge, taking the mean predrug activity of each slice as its own control value. □ Penicillin; ○ $BaCl_2$.

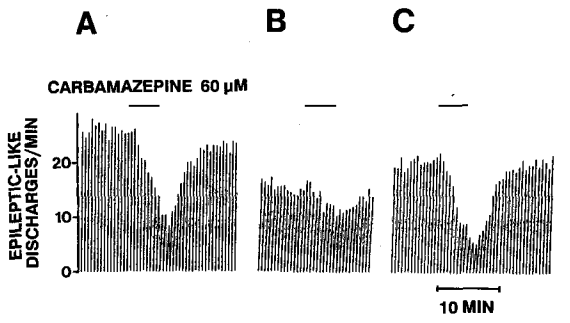

Figure 3. The depressant effect of carbamazepine on epileptiform discharges induced by penicillin is attenuated by 0.1 mM $BaCl_2$. Recordings A, B and C are from the same slice, taken at intervals of approximately 20 min. Penicillin (1.2 mM) was present in the perfusion medium throughout the experiment. Penicillin induced spontaneous interictal discharges of CA3 pyramidal neurons which are recorded extracellularly. *A* Carbamazepine depresses epileptiform activity; *B* upon addition of 0.1 mM $BaCl_2$, the action of carbamazepine is attenuated; *C* after termination of the $BaCl_2$ infusion, the depressant effect of carbamazepine is again observed.

either the shape or the duration of the individual discharges (fig. 1).

In separate experiments, the inhibitory effect of carbamazepine on epileptiform discharges in slices exposed to 0.1 mM $BaCl_2$ was less potent (fig. 2). The KCl concentration was 5 mM. The difference in sensitivity between the two models was particularly prominent at a concentration of 60 μM carbamazepine (fig. 2), at which the efficacy of the drug was lower by a factor of 2 ($p < 0.01$). The fact that different concentrations of KCl had to be used made it rather difficult to interpret the results of this first set of experiments. We therefore carried out two experiments to determine whether the depressant action of carbamazepine (60 and 100 μM) observed in slices exposed to penicillin is sensitive to $BaCl_2$. Penicillin (1.2 mM) was present in the medium throughout. The KCl concentration was 10 mM. Control responses were first recorded from all slices after exposure to carbamazepine for 3–10 min. $BaCl_2$ 0.1 mM was then infused for approximately 60 min. Carbamazepine was reinfused after 20 min exposure to $BaCl_2$, and again 20 min after termination of the $BaCl_2$ infusion to see whether the effect was reversible. In five out of six slices (KCl 10 mM), addition of 0.1 mM $BaCl_2$ led to a reversible reduction by 50–90% in the depressant effect of carbamazepine. A typical example is shown in figure 3. In one slice in which carbamazepine completely suppressed all epileptiform activity, $BaCl_2$ did not attenuate the effect. During infusion of $BaCl_2$, the spontaneous frequency of epileptiform discharges was reduced by 20–40% (fig. 3). Separate experiments were also performed to ascertain whether 4-AP or tetraethylammonium chloride (TEA) might interfere with the depressant action of carbamazepine on penicillin-induced epileptiform discharges. The KCl concentration was 2.5 mM. Penicillin (1.2 mM) was present in the ASCF throughout the experiment. Once spontaneous epileptiform activity was established, carbamazepine (100 and 200 μM) was added for 8–10 min. After recovery from the depressant action of

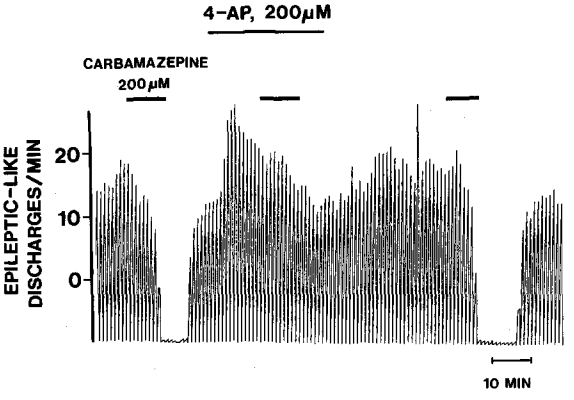

Figure 4. The blocking action of 4-aminopyridine on the action of carbamazepine is shown. Epileptiform activity induced by penicillin (1.2 mM) is recorded in CA3. The inhibitory effect of carbamazepine is strongly attenuated by 4-aminopyridine. All drugs were added to the perfusion medium.

carbamazepine, TEA (2 and 10 mM) or 4-AP (200 μM) was infused and exposure to carbamazepine repeated.

4-AP (200 μM) also strongly antagonized the action of carbamazepine (100 and 200 μM) in five slices exposed to penicillin (3 experiments) (fig. 4). It induced a brief initial increase in epileptiform activity, which during further perfusion then diminished to control levels or slightly below (fig. 4). In one experiment, epileptiform discharges were evoked by 4-AP at a KCl concentration of 2.5 mM. Spontaneous interictal discharges similar to those induced by penicillin developed in CA3 and were not depressed by 120 μM carbamazepine (4 slices).

The potentially antagonistic effect of TEA on the action of carbamazepine could not be tested, since it strongly suppressed the epileptiform activity in CA3 evoked by penicillin when applied at concentrations of 2 or 10 mM. TEA also lowered the amplitude of the penicillin-induced field potentials.

Discussion

Carbamazepine exerts a more potent inhibitory effect on the epileptiform activity induced by penicillin in the CA3 area than on that induced by $BaCl_2$. We showed that the effect of carbamazepine on penicillin-induced epileptiform discharges could be diminished by adding $BaCl_2$ to the perfusion medium. During exposure to $BaCl_2$, spontaneous epileptiform discharges induced by penicillin were moderately attenuated, indicating that the driving force of this activity may have been inhibited. The cause of this unexpected effect remains to be elucidated. Given the reduced intensity of epileptiform activity during the combined application of penicillin and $BaCl_2$, carbamazepine might have been expected to suppress these discharges more easily. On the contrary, however, its depressant effect was reduced, indicating that $BaCl_2$ antagonized the action of carbamazepine. Although these findings lend support to the potassium-flux hypothesis, they are not conclusive: Ba^{2+} ions penetrate the Ca^{2+} channels. For that reason we subsequently used the relatively selective potassium-channel blocker 4-AP. This agent strongly antagonized the action of carbamazepine and at the same time only transiently increased the epileptiform activity induced by penicillin. The inhibition of the action of carbamazepine by 4-AP is therefore unlikely to have been the result of changes in spontaneous epileptiform activity in the CA3 area. Moreover, carbamazepine did not diminish the epileptiform discharges induced by 4-AP alone.

In most of the experiments, rather high concentrations of carbamazepine were chosen. The fact that high concentrations of carbamazepine were strongly antagonized by 4-AP suggests that this is a potent blocker of the effects of carbamazepine. In patients receiving chronic treatment with carbamazepine the cerebrospinal fluid concentrations were reported to range from 4 to 14 μM[9].

It has been suggested that the anticonvulsant properties of carbamazepine could be due to its action on sodium channels[10]. Previous studies in 'epileptic' hippocampal slices demonstrated that carbamazepine has a postsynaptic depressant effect, and that it does not facilitate GABA-mediated paired-pulse inhibition[4,11]. Our present findings are not in conflict with these observations. An effect on the potassium channels tending to increase potassium fluxes would act in the same direction, namely to reduce cell excitability, and, perhaps even more specifically, to diminish repetitive firing.

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Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition

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Summary. Using flurbiprofen, a chiral anti-inflammatory and analgesic 2-arylpropionic acid derivative, the enantiomers of which are not converted to each other (less than 5%) in rats or man, we obtained evidence that prostaglandin synthesis inhibition is primarily mediating the anti-inflammatory activity but prostaglandin synthesis independent mechanisms contribute to the analgesic effects. Thus, the S-form inhibited prostaglandin synthesis, inflammation and nociception in rats. The R-form had much less effect on prostaglandin synthesis and did not affect inflammation. It did, however, block nociception in rats almost as potently as the S-form. S-flurbiprofen, in contrast to the R-form, was clearly ulcerogenic in the gastrointestinal mucosa. These results indicate additional molecular mechanisms of analgesia and suggest the use of R-arylpropionic acids as analgesics.
Key words. Aspirin-like drugs; flurbiprofen enantiomers; anti-inflammatory; analgesic; gastrointestinal toxicity; prostaglandin synthesis; rat.