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ORIGINAL ARTICLE

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Sodium bromide: effects on different patterns of epileptiform activity, extracellular pH changes and GABAergic inhibition

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Abstract Results regarding the anticonvulsant potency of bromide have been questioned, and the mechanisms of its action are unclear. Using combined rat hippocampus-entorhinal cortex slices we analyzed the effects of NaBr on four types of epileptiform discharges in two different models of epilepsy, the low-Ca^{2+} and the low-Mg^{2+} model. NaBr concentration-dependently reduced the frequency and finally blocked the low Ca^{2+}-induced discharges. Low Mg^{2+}-induced short recurrent discharges were also reduced in a concentration-dependent manner. In the entorhinal cortex the frequency of seizure-like events was reduced by 3 and 5 mM and the discharges were blocked by 7 mM NaBr. Also, the late recurrent discharges in the entorhinal cortex which do not respond to most clinically employed anticonvulsants were reduced by concentrations of 10 and 15 mM and completely blocked by 30 mM NaBr.

Using pH-sensitive microelectrodes different effects of NaBr were seen than those of acetazolamide on extracellular pH under control conditions and after stimulation. Acetazolamide at 1 mM caused a reversible acidification of ΔpH: 0.2±0.14 at rest whereas no change on extracellular pH was seen with 5 mM NaBr. Acetazolamide increased the transient alkalosis induced by repetitive stimulation of the stratum radiatum in area CA1 and reduced the subsequent acidosis. NaBr also increased the alkalosis but had no effect on the subsequent acidosis.

A significant increase in paired-pulse inhibition was seen in a paired-pulse stimulation protocol used to monitor the efficacy of GABAergic inhibition at concentrations of 5 mM NaBr. This finding was confirmed in whole-cell patch clamp recordings from cultured hippocampal neurons showing an increase in inhibitory postsynaptic current amplitude.

In summary, our results suggest a broad-spectrum anticonvulsant activity which is likely to be caused by its effects on membrane excitability, by an increase in GABAergic inhibition and is less likely caused by its effects on extracellular pH.

Key words Bromide · Epileptiform discharges · Carboanhydrase · pH · Membrane excitability · GABAergic inhibition

Abbreviations *ACSF* artificial cerebrospinal fluid · *GABA* γ-aminobutyric acid · *IPSC* inhibitory postsynaptic current · *LRDs* late recurrent discharges · *SLEs* seizure-like events · *SRDs* short recurrent discharges

Introduction

Bromides were the first effective anticonvulsants, but their use in the treatment of epilepsy dropped dramatically after the introduction of phenobarbital and phenytoin. Since then the drug gained a reputation of having a very narrow target range and being less effective than other anticonvulsant drugs (Joynt 1974). In contrast, there are studies which have shown that bromides are more effective than other drugs in the treatment of special syndromes such as refractory tonic-clonic seizures of childhood (Boenick et al. 1985; Ernst et al. 1988; Steinhoff and Kruse 1992) and of severe myoclonic epilepsy in infants (Oguni et al. 1994). In addition, bromides are also used for the treatment of various seizure types in epileptic dogs (Trepanier et al. 1998).

To re-evaluate the anticonvulsant potency of bromide and thus to clarify contradictory views reported in the lit-

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erature we tested the effects of the substance on four types of epileptiform discharges in two different in vitro models of epilepsy, the low-Ca^{2+} and the low-Mg^{2+} model. The discharges in these models result from different physiological mechanisms. The epileptiform discharges in the low Ca^{2+} model of epilepsy are largely mediated by non-synaptic mechanisms (Jefferys and Haas 1982; Yaari et al. 1983). Epileptiform discharges induced by washout of Mg^{2+} ions are caused by enhanced excitatory transmission due to unblocking of the *N*-methyl-D-aspartate (NMDA) receptor (Anderson et al. 1986). In this model, prolonged treatment of the slice with Mg^{2+}-free artificial cerebrospinal fluid (ACSF) results in the induction of late recurrent discharges (LRDs) in the entorhinal cortex which have been shown to be resistant to currently clinically used anticonvulsant drugs (Zhang et al. 1995). Such refractory late activity is preceded by seizure-like events (SLEs) which display a longer duration but reduced frequency and a different pharmacological profile than that of the late activity (Dreier and Heinemann 1990). In addition, a third type of activity is seen in the hippocampus proper consisting of short recurrent discharges (SRDs) (Mody et al. 1987).

The mechanisms by which bromide produces anticonvulsant effects are still unknown. According to Balcar et al. (1987) bromide does not affect the GABAergic inhibitory system. This was indicated by a lack of changes in the metabolism or transport of γ-aminobutyric acid (GABA) and also by unchanged characteristics of the receptor-associated GABA-binding sites under acute or chronic bromide exposure. In contrast, Suzuki et al. (1994) have shown that bromide potentiates GABA-activated currents in cultured cerebral neurons. To clarify such contradictory results we chose different approaches by testing the effect of the substance on GABAergic inhibition in a paired-pulse protocol and on inhibitory postsynaptic currents.

It has also been suggested that bromide may interact with the enzyme carboanhydrase resulting in extracellular acidosis and consequent inhibition of epileptiform activity (Woodbury and Pippenger 1982). This prompted us to compare the effect of both the carboanhydrase blocker acetazolamide and NaBr on extracellular pH changes at rest and following electrical stimulation.

Methods

Slice preparation. The experiments were performed as described previously (Dreier and Heinemann 1990). In brief, brain slices (400 μm) containing the temporal cortex area 3, the perirhinal cortex, the entorhinal cortex, the subiculum, the dentate gyrus and the ventral hippocampus were prepared in a nearly horizontal plane from male or female Wistar rats (150–200 g) after decapitation under deep ether anesthesia (Fig. 1). The slices were placed on lens cleaning tissue and continuously perfused (1.5–2 ml/min) in an interface chamber with prewarmed (35°C) and oxygenated (95% O_2, 5% CO_2) ACSF which contained (in mM): NaCl 124, KCl 3, $MgSO_2$ 2, $CaCl_2$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 26 and glucose 10 (pH 7.4). Mg^{2+} was omitted in the Mg^{2+}-free ACSF, Ca^{2+} in the Ca^{2+}-free ACSF. NaBr was bath applied at concentrations of 1–30 mM in the Ca^{2+}-free or Mg^{2+}-free ACSF after induction of the various types of epileptiform activity. The concentrations of Na^+ and Cl^- were replaced by the same amount of NaBr to ensure that the total amount of ions remained constant in each experiment.

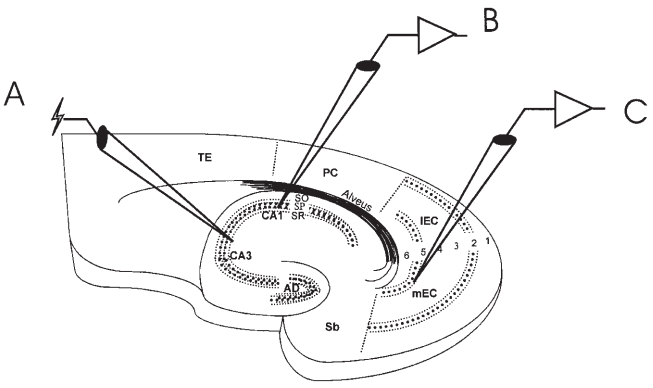

Fig. 1 Hippocampal-entorhinal cortex slice preparation. Schematic drawing of the hippocampal-entorhinal cortex slice preparation containing temporal cortex area 3 (*TE*), perirhinal cortex (*PC*), lateral entorhinal cortex (*lEC*), medial entorhinal cortex (*mEC*), subiculum (*Sb*), area dentata (*AD*), and the hippocampal regions CA1 and CA3 with stratum oriens (*SO*), stratum pyramidale (*SP*), stratum radiatum (*SR*) and the alveus. Recordings were made in deep layers of the mEC (*C*), in stratum pyramidale of hippocampal region CA1 (*B*). Orthodromic activation of CA1 pyramidal cells was achieved by positioning the stimulus electrode in SR of the hippocampus (*A*)

Extracellular field potential recordings. The recordings were started after more than 1 h of equilibration of the slices in the ACSF. The viability of slices was tested by stimulation of the stratum radiatum with a bipolar stimulation electrode. Only those slices were accepted for further experiments in which stimulus induced population-spikes recorded in stratum pyramidale of area CA1 yielded amplitudes between 3.5 and 4 mV at 80% of maximal stimulus intensity and when no evidence of multiple spike responses was present. Stimulus pulses were 0.1 ms square pulses of 1–12 V applied through a bipolar platinum wire glass insulated electrode. Repetitive stimulation was applied at 20 Hz for 10 s. Extracellular potential recordings were obtained with 150 mM NaCl-filled microelectrodes in area CA1 of the hippocampus and in the deep layers of the medial entorhinal cortex. Due to interaction with the AgCl pellet used as ground electrode the addition of NaBr caused a considerable offset voltage. Therefore, we grounded the bath through an agar bridge in most experiments which prevented the offset voltage.

Ion-sensitive microelectrodes. Ion-sensitive microelectrodes (ISMs) were used to measure both the equilibration time course of NaBr within the slice (Cl^--sensitive microelectrode) and stimulus-induced pH changes with and without pharmacological manipulation. The ISMs were produced using methods previously described (Heinemann and Arens 1992). In short, the electrodes were pulled from double-barreled theta glass. One barrel was used as reference electrode and was filled with 154 mM NaCl, whereas the other (ion sensitive) barrel was filled with 50 or 100 mM solutions of the ion to be measured. The ion sensitive barrel was silanized with 5% trimethyl-1-chlorosilane in 95% CCl_4 and subsequently the ion-sensitive resin was sucked into the tip of the silanized barrel to a height of about 200–300 μm. The following resins were used: chloride ionophore 1–cocktail A (Fluka 24902), pH–hydrogen ionophore I, cocktail A (Fluka 95291). Cl^-sensitive microelectrodes for bromide measurements were calibrated in solutions of NaCl 123 mM plus 1 mM NaBr, NaCl 119 plus 5 mM NaBr, NaCl 114 mM plus 10 mM NaBr, and NaCl 104 mM plus 20 mM NaBr. They were accepted for experiments when their sensitivity to a tenfold increase in Br^- concentration was 58±3 mV. ISMs for pH

measurements were calibrated using solutions with a pH ranging from 6.5 to 8.0, the electrodes had to react with a slope of 50±5 mV to a change of 1 pH unit.

Repetitive stimulation. In order to mimic changes in ion concentrations as observed during epileptiform discharges, trains of stimuli (20 Hz for 10 s with 0.1 ms pulse duration) were applied through a bipolar stimulus electrode placed in stratum radiatum of area CA1. In a first step the intensity of stimulation which evoked maximal changes in extracellular pH was determined. This intensity ranged from 4–12 V and the particular intensity in a given slice was kept constant in the following experiments. After five to ten control stimuli, acetazolamide or NaBr was applied for 60–80 min and washout was awaited for 60–80 min. Effects of acetazolamide and NaBr on stimulus train induced pH changes were compared with those during control and washout periods. The stimulus train induced changes in extracellular pH were recorded on chart recorder and simultaneously with an IBM-compatible PC using SignalAverager 5.0.1 (Cambridge Electronic Design, Cambridge, UK) or pClamp 6.0.3 (Axon Instruments, Foster City, Calif., USA) data acquisition software and were analyzed off-line.

Patch clamp recordings. Inhibitory postsynaptic currents (IPSCs) were recorded from cultured hippocampal neurons using whole-cell patch-clamp techniques. Cultures were prepared from rat embryos at day 18 of pregnancy after decapitation of the deeply anesthetized mother. The hippocampus was removed, cells were mechanically dissociated by trituration and cultured in MEM (minimal essential medium) with 5% horse serum at 35°C and 5% CO_2 (for details, see Banker and Cowan 1977; Draguhn et al. 1997). Whole-cell currents (Hamill and Sakmann 1981) were recorded in voltage clamp at –60 mV using an EPC 7 amplifier (HEKA Instruments, Lambrecht, Germany) and symmetrical Cl^- (extracellular solution (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 25; HEPES, 10; pH 7.4; intracellular pipette solution: KCl, 120; $MgCl_2$, 2; $CaCl_2$, 1; HEPES, 10; EGTA, 11; glucose, 25). To isolate spontaneous GABA-mediated IPSCs we added 10 µM NBQX (1,2,3,4,-tetrahydro-6-nitro-2,3-di-oxo-benzo-quinoxaline-7-sulfonamide) and 30 µM±-APV (DL-2-amino-5-phosphonovaleric acid). Data were filtered at 3 kHz and stored on video-tape for off-line analysis.

Data analysis. Spontaneous epileptiform activity was recorded on chart recorder (AstroMed DASH IV, West Warwick, R.I., USA). The change in frequency of the various epileptiform activities was compared by counting the number of epileptiform events during an interval of 4 min (and for 12 min in the case of seizurelike events) before perfusion with the drug, the same time interval following equilibration and after wash of the substance. All results are expressed as mean value±standard error of the mean. For statistical evaluation we only accepted slices which showed at least partial recovery from drug effects. We employed the Wilcoxon matched-pairs signed-rank test (significance level $P<0.05$). To translate the potentials recorded with ISMs into concentration values (mM) we used a modified Nernst equation:

$$\log[Ion]_s = \frac{E_M}{s * v} + \log[Ion]_c$$

where E_M: recorded potential, s: electrode slope obtained at calibration, v: valence of the specific ion, $[Ion]_c$: ion concentration at rest, and $[Ion]_s$: ion concentration during stimulation. Inhibitory postsynaptic currents from patch clamp recordings were replayed from video tape, filtered at 1 kHz and redigitized at 3 kHz (CED 1401 interface, Cambridge Electronic Design, Cambridge, UK). Current amplitudes and frequencies were analyzed using a personal computer and the CDR and SCAN program by Dr. J. Dempster, University of Strathclyde, Scotland, UK. Frequency of events as well as mean IPSC amplitudes in 60 to 90-s-long traces before, during, and after addition of bromide were calculated and compared using Student's *t* test (data expressed as mean±SEM; significance level at $P<0.05$).

Drugs. NaBr and acetazolamide were obtained from Sigma (Deisenhofen, Germany). All experiments were carried out in compliance with the current laws in Germany.

Results

Equilibration time course

Bath application of NaBr resulted in rapid equilibration of the tissue (as measured with Cl^--sensitive electrodes, see above) approaching the new steady-state concentration after about 15 min. Similarly, washout to baseline levels took approximately 15 min. Accordingly, all measurements were carried out after at least 15–20 min equilibration with the respective drug concentration.

Effects of sodium bromide on spontaneous epileptiform activity

Low Ca^{2+}-induced activity

Washout of Ca^{2+} resulted after 45±21 min in seizure-like events consisting of several population spikes superimposed on slow negative field potential shifts of 1.6–4 mV lasting 3–15 s and recurring at a frequency of 3±1 per min (Fig. 2A). The activity was restricted to the hippocampal area CA1 and did not spread to the entorhinal cortex and neighboring temporal cortex. NaBr applied at 1 and 2 mM (n=4) had no visible effect on the low Ca^{2+}-induced discharges. At a concentration of 5 mM the frequency of the low Ca^{2+}-induced discharges was reduced by 25% (n=9). At a concentration of 7 mM there was a reduction of 50% and a block was seen in one slice. At a concentrations of 9 mM a frequency reduction of 80% (n=4; Fig. 2A) was seen and a complete block in one slice and a concentrations of 11 mM blocked the discharges completely in all slices.

Low Mg^{2+}-induced short recurrent discharges

Lowering the Mg^{2+} concentration resulted after 66±25 min in the appearance of short recurrent discharges in area CA1 and CA3, sometimes after some initial seizure-like events. The discharges consisted of 40–80 ms positive-going field potential bursts recurring at a frequency of about 20–30 per minute (Fig. 2B). NaBr (3 mM) had no visible effect on this activity (n=4). At 9 mM a suppression by 40% was seen and at 10 mM NaBr the frequency of discharges was reduced by 62% (n=3). When NaBr was applied at 15 mM there was a reduction of 80% and a block in 1 slice (Fig. 2B). With 20 mM a block was seen in all five slices.

Low Mg^{2+}-induced seizure-like events

Lowering the Mg^{2+} concentration induced seizure-like events in the entorhinal cortex after 35±20 min character-

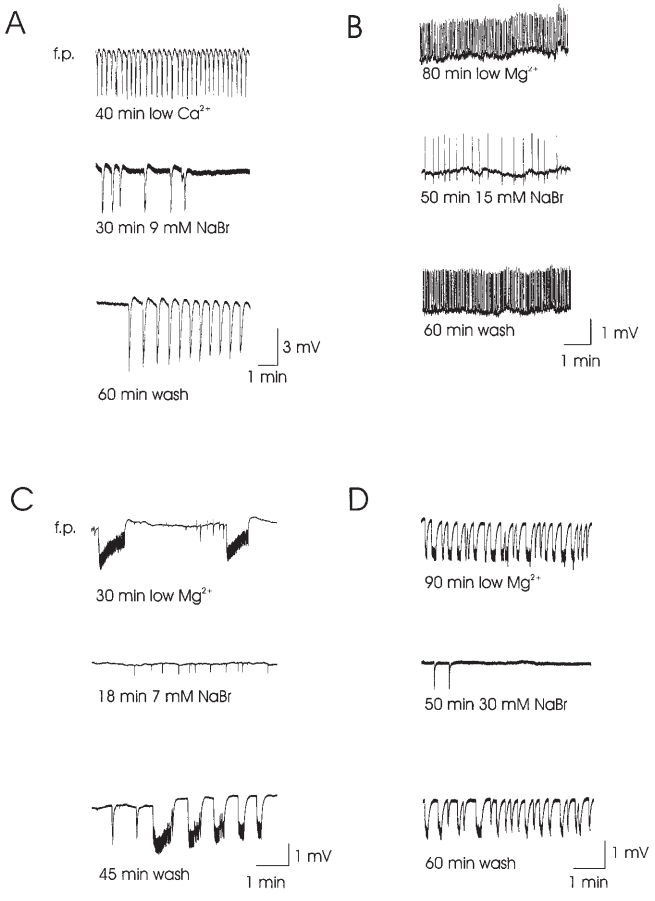

Fig. 2A–D Effects of NaBr on various types of spontaneous epileptiform activities. **A** Effects on low Ca^{2+}-induced epileptiform activity. Epileptiform discharges resulting from washout of Ca^{2+} with negative-going potentials superimposed by series of population spikes (*upper trace*) after 40 min of application of low Ca^{2+} artificial cerebrospinal fluid. Application of NaBr (9 mM) resulted in frequency reduction followed by blockade of the epileptiform discharges (*middle trace*). After washout of NaBr reappearance of the discharges is represented in the lowest trace. **B** Effects on low Mg^{2+}-induced short recurrent discharges (SRDs). Low Mg^{2+}-induced activity in hippocampal area CA1 is characterized by short recurrent discharges associated with positive field potentials superimposed by repetitively bursting spikes following 80 min of washout of Mg^{2+} (*upper trace*). SRDs in hippocampal region CA1 are reduced under 15 mM NaBr (*middle trace*) in frequency but not in amplitude while under 20 mM the SRDs were completely blocked (not shown, see text). *Lower trace* shows reversibility of the anticonvulsant effects. **C** Low Mg^{2+}-induced seizure-like events (SLEs). SLEs by tonic- and clonic-like electrographic activity superimposed on slow negative field potentials appear in the entorhinal cortex but not in hippocampus proper after 30 min of washout of Mg^{2+} (*upper trace*). NaBr in concentrations of 7 mM blocked these discharges (*middle trace*). Reversibility of the effects (lower trace). **D** Effects on low Mg^{2+}-induced late recurrent discharges (LRDs). Following prolonged washout of Mg^{2+} (90 min) the SLEs in the entorhinal cortex change to late recurrent discharges. NaBr in a concentration of 30 mM blocked this activity (*middle trace*). *Lower trace* shows reversibility of the effects

ized by slow negative field potential shifts superimposed by an initial toniclike and then cloniclike electrographic activity lasting for 30–180 s and recurring at a frequency of 6±2 events in an observation period of 12 min (Fig. 2C). Between "seizures" interictal discharges were frequently noted. NaBr at 1 mM had no significant effect (n=5), whereas 3 mM reduced SLEs by 20% (n=6). With 5 mM a reduction in SLEs by 80% was seen (n=5), and 7 mM blocked this activity in seven slices but interictal discharges still were present (Fig. 2C).

Low Mg^{2+}-induced late recurrent discharges

After 60±20 min of ongoing activity seizure-like events in the entorhinal cortex changed to late recurrent discharges (Fig. 2D). These were not affected by concentrations of 1 and 5 mM NaBr (n=6). At 10 mM, a reduction in frequency of the discharges by 20% was seen (n=4). With 15 mM a reduction of 50% occurred and at 30 mM there was a complete block in five slices (Fig. 2D). Figure 3 summarizes the concentration-response relationship for all four types of spontaneous epileptiform discharges.

Effects of acetazolamide and NaBr on extracellular pH

Baseline pH changes

To test the hypothesis that bromide action may be mediated by an acidification through inhibition of the enzyme carboanhydrase we compared the effects of 1 mM acetazolamide and 5 mM NaBr on both baseline pH and on stimulus induced alkaline and acidic transients. Lowering the microelectrode to a recording depth of approximately 100 µm into the slice showed a baseline value of 0.16±0.04 pH units more acidic than at the surface (n=8). The pH at the surface of the slice was equal to the pH of the perfusate (7.40±0.02). The pH of the perfusate was not altered by the addition of 1 mM acetazolamide or of 5 mM NaBr. Following bath application of acetazolamide an acidification of the extracellular pH inside the tissue was observed

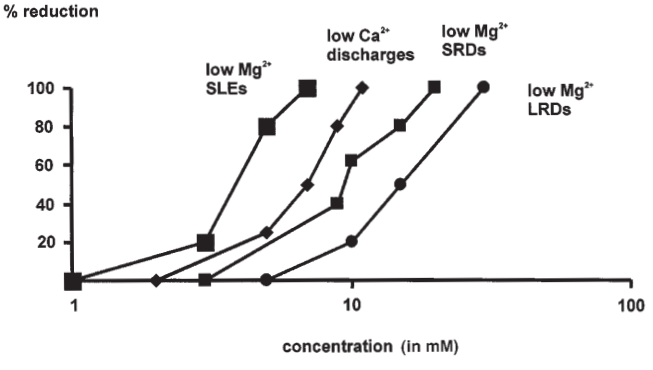

Fig. 3 Concentration-response relationship for the four types of discharges. The effect of NaBr in reducing and suppressing various types of discharges was concentration-dependent. Lowest concentrations were required to reduce and block the low Mg^{2+}-induced seizure-like events (*SLEs*) and highest concentrations were needed to suppress the late recurrent discharges (*LRDs*) in the entorhinal cortex. *SRDs* short recurrent discharges

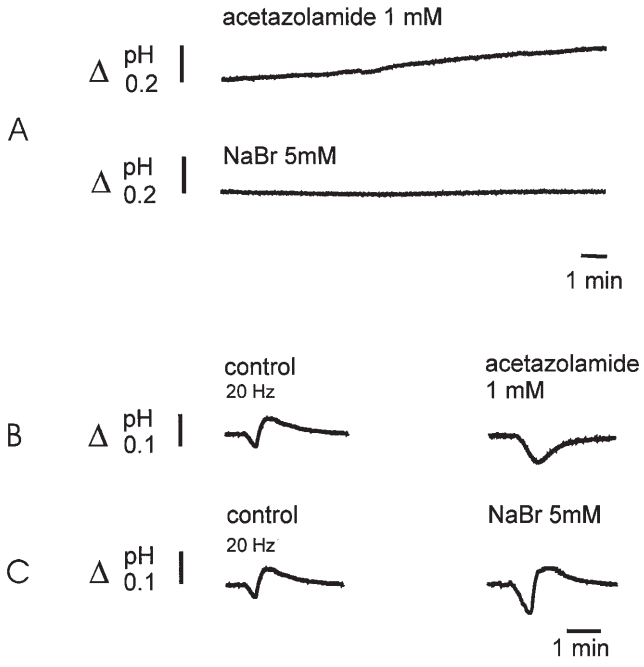

Fig. 4A–C Comparison of the effects of acetazolamide and NaBr on extracellular pH changes under control conditions and after stimulus trains. **A** At rest the extracellular pH under acetazolamide shifted in the acidotic direction but no such effect was seen following NaBr application. **B** Stimulus-induced pH transients changed from a biphasic alkalotic-acidotic sequence under control conditions to a monophasic increased alkalotic one under acetazolamide. **C** Under NaBr stimulus-induced pH transients changed to an enhanced alkalotic one while the acidotic component was not altered

which reached a maximum after 18±4 min (n=8; ΔpH: 0.2±0.14, P<0.01; n=6; Fig. 5A). After washout of acetazolamide complete recovery of baseline pH value was seen in all slices after 20±4 min. In contrast, NaBr did not change the baseline extracellular pH (n=6; Fig. 4A).

Stimulus train-induced pH transients

Under control conditions the stimulus train-induced pH transient consisted of an alkalotic shift (ΔpH: 0.04±0.01) followed by an acidosis (ΔpH: 0.06±0.02) with a time course of 1–2 min (n=13; Fig. 4B). Following 20±5 min of addition of acetazolamide (1 mM) the initial alkalosis after a stimulus train increased by a factor of two to three (ΔpH: 0.10±0.07; P<0.05) with no subsequent acidosis (Fig. 4B). The changes in the stimulation-evoked pH-signal were completely reversible after 40±10 min washout of acetazolamide. NaBr (5 mM) after 20±6 min increased the initial alkalosis by a factor of two (ΔpH 0.08±0.02, P<0.05) with no subsequent inhibition of acidosis (Fig. 4C).

Effects of NaBr on paired pulse stimulus induced field potentials

NaBr was tested at concentrations of 1 and 5 mM on field potential responses recorded in stratum pyramidale (SP) induced by stimulation of stratum radiatum. Stratum radiatum stimulation elicited positive field excitatory postsynaptic potentials superimposed by population spikes. We looked at paired pulse stimulus induced responses evoked with stimulus intensities of 30, 50 and 80% of maximal responses and each of the intensities at stimulus intervals of 20 and 120 ms. During paired pulse stimulation the second response to an stratum radiatum stimulus was always larger than the first response. In a concentration of 1 mM NaBr did not alter paired pulse responses (n=5; not shown). At 5 mM the second population spike in a 20 ms stimulus pair was reduced by 33±15% at 30% of maximum stimulation intensity (n=7; Fig. 5), by 9±5% at 50% (n=7), and by 6±4% at 80% (n=6). At intervals of 120 ms the second population spike was only slightly reduced at all stimulation intervals.

Effects of NaBr on spontaneous IPSCs

Spontaneous IPSCs were recorded from cultured hippocampal neurons in whole-cell patch clamp configuration. All cells tested expressed such spontaneous events at varying frequency and amplitude (mean amplitude of cur-

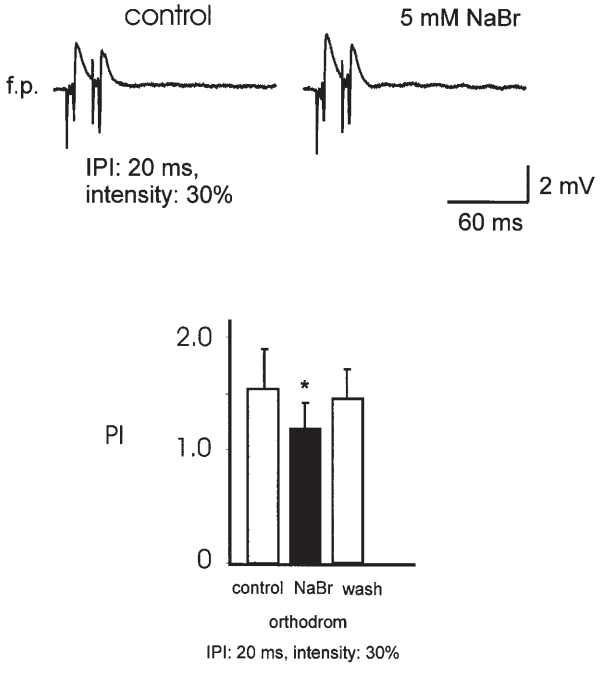

Fig. 5 Effects of 5 mM NaBr on stimulus-induced paired-pulse field potentials. The amplitude of the second population spike was reduced under 5 mM NaBr in normal ACSF following orthodromic stimulation at an interpulse interval (*IPI*) of 20 ms and an intensity of 30% (*above*). To determine the effects of the drug on synaptic behavior we calculated the paired pulse index (PI) according to the formula: $PI=P_2/P_1$, with P2 being the average of ten responses to the second stimulus in a paired-pulse paradigm. The figure shows the effects of 5 mM NaBr on the PI calculated from responses to paired pulse stimulation with an interval of 20 ms and intensity of 30% (n=7) in area CA1 of the hippocampus. The orthodromic paired-pulse inhibition was significant

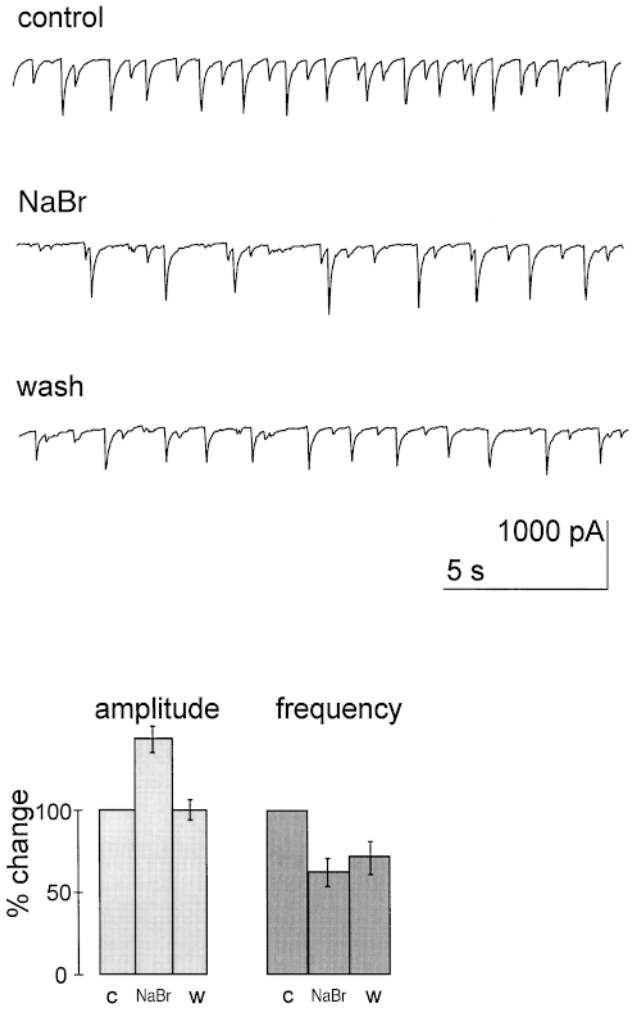

Fig. 6 Effects of NaBr on spontaneous inhibitory currents in cultured hippocampal neurons. *Above* inhibitory postsynaptic currents were pharmacologically isolated and recorded in whole-cell patch-clamp technique. Bromide (5 mM) reversibly enhanced the mean current amplitude and decreased the frequency of events. *Below* summary of the effects of 5 mM NaBr on mean amplitude and frequency of inhibitory postsynaptic currents (11 cells). Amplitude was enhanced ($P<0.01$) and frequency lowered ($P<0.05$). *c* control, *w* wash

rents: 1526±370 pA; mean frequency: 46±14 events/minute, $n=11$). Addition of NaBr (5 mM) to the bath solution changed the pattern of synaptic currents within a few seconds. Frequency was reduced to 61±10% of control ($n=11$; $P<0.05$) and the mean amplitude of events was potentiated to 144±18% of control (Fig. 6; $n=11$; $P<0.01$). The changes were completely (amplitude) or partially (frequency) reversible upon washout of the drug.

Discussion

The current experiments were designed to test the anticonvulsant properties of NaBr in two in vitro epilepsy models with different epileptiform activities resulting from different physiological mechanisms. Previous studies have shown that the various types of discharges used in the current experiments display a different sensitivity towards anticonvulsant drugs.

The low Ca^{2+}-induced epileptiform activity has been shown to be sensitive to substances altering membrane excitability such as valproic acid, phenytoin, carbamazepine and barbiturates but not to glutamate antagonists or to benzodiazepines (Heinemann et al. 1985) in accordance with the assumption that synaptic transmission is of minor importance in this model. NaBr concentration-dependently reduced the frequency or blocked these discharges which suggests that it affects membrane excitability of nerve cells. The different patterns of low Mg^{2+}-induced epileptiform activity depend predominantly on the activation of glutamate receptors of the NMDA type and are therefore all sensitive to NMDA receptor antagonists (Stanton et al. 1987). Regarding clinically employed drugs the SRDs in CA1 have been shown to be sensitive to valproic acid only, but not to benzodiazepines and barbiturates at clinically relevant concentrations. NaBr significantly reduced or blocked these discharges at concentrations of 10, 15, and 20 mM. The seizure-like events originating in the entorhinal cortex are sensitive to most anticonvulsant substances and were significantly reduced in frequency or blocked at the lowest concentration of NaBr (Fig. 3). In contrast, the entorhinal LRDs are not blocked by any of the currently clinically employed anticonvulsant drugs including carbamazepine, phenytoin, valproic acid, benzodiazepines and barbiturates (Zhang et al. 1995). Therefore, this activity represents a model system to test new anticonvulsant substances (Dreier and Heinemann 1990). Again, NaBr at concentrations of 10, 15, and 30 mM reduced and blocked the discharges. To date, no single clinically employed substance has been demonstrated to suppress all these different forms of epileptiform activities. Our results are broadly in line with the study of Grewal et al. (1956) who demonstrated the efficacy of NaBr in different electroshock procedures and pentylenetetrazol-induced convulsions in vivo.

In humans, therapeutic Br^- serum ranges of 750–1250 mg/l (Dreifuss 1989) or more recently 2000–3000 mg/l (Steinhoff and Kruse 1992) have been found. In the current experiments the majority of epileptiform discharges were reduced or blocked at concentrations ranging between 5 and 20 mM in the slice preparation corresponding to serum levels of 500–2000 mg/l. Since almost complete equilibration between serum and brain levels can be assumed (Pierson et al. 1978), concentrations used in the current experiments may well correspond to therapeutic brain concentrations in humans. Toxic concentrations are individually variable but with levels over 2000 mg/l patients should be monitored very closely.

The fact that bromide did suppress all patterns of epileptiform activity suggests that the drug has a direct membrane action. This is particularly supported by the observation that NaBr reduced the low Ca^{2+} induced activity which is so far only sensitive to anticonvulsants with direct membrane effects.

To gain some further insight into the mechanisms of action of bromide we investigated a hypothesis put forward by Woodbury and Pippenger (1982) postulating that, similar to acetazolamide, bromide may act by inhibition of the enzyme carbonic anhydrase resulting in extracellular pH changes. Respiratory systemic alkalosis has long been known to promote ictogenesis (Foerster 1924). In brain slices too, it has been demonstrated that extracellular alkalosis may induce epileptiform discharges (Aram and Lodge 1987), while extracellular acidosis reduces excitability and may even block spontaneous epileptiform discharges (Velisêk et al. 1994).

The effects of the carbonic anhydrase inhibitor acetazolamide on baseline and stimulation-induced pH-shifts were clearly different from those of bromide. Whereas an acidic baseline shift resulted from acetazolamide application no effect was seen with bromide. In addition, upon strong synaptic activation acetazolamide increased the alkaline shift and reduced the acidic shift. Bromide, instead, increased the alkalotic shift but did not alter the acidic shift. Therefore the current experiments do not support a direct action of Br^- on carboanhydrase. Moreover, the activity-induced alkalotic shift was potentiated by Br^- which is rather pro- than anticonvulsant. Thus, it is unlikely that the anticonvulsant potency of bromide is mediated via extracellular pH effects.

It has well been established that a decrease in GABAergic inhibition is a crucial step in the development of epileptiform activity. Indeed, blocking 20% of $GABA_A$ inhibition is sufficient to initiate synchronous discharges in neocortex (Chagnac-Amitai and Connors 1989). Furthermore, during status epilepticus there is severe reduction in GABA-mediated inhibition (Kapur and Coulter 1995) indicating that loss of GABAergic control could be a major factor in the transition from treatable to refractory forms of epilepsy. Therefore we assessed the effects of NaBr on the inhibitory system by employing a paired-pulse protocol. The decrease in amplitude of the second of a pair of paired-pulse evoked responses can be attributed to the activation of feed-forward and recurrent inhibitory circuits in the case of orthodromic stimulation (Andersen et al. 1964; Buzsaki 1984). A significantly increased paired-pulse inhibition was seen with 5 mM NaBr upon orthodromic stimulation suggesting increased GABAergic inhibition. This is in line with the previous suggestion that the action of GABA may be potentiated by the presence of bromide (Takeuchi and Takeuchi 1967).

To directly test for this hypothesis we performed patch-clamp recordings from cultured hippocampal neurons. The data revealed two effects: an increase in IPSC amplitude as well as a decrease in the frequency of spontaneous synaptic events. Br^- passes the $GABA_A$ receptor ionophore more easily than chloride (Bormann et al. 1987). An increase in extracellular bromide should, therefore, slightly reduce the driving force for the outward current observed in symmetrical Cl^- at –60 mV. However, we found increased mean amplitudes of the currents, indicating either potentiation of the $GABA_A$ receptors or increased release of GABA from presynaptic terminals under bromide. While the mechanism of action of bromide remains unclear, the resulting potentiation of GABAergic inhibition is consistent with the anticonvulsant effect of the drug and fits the enhanced paired-pulse inhibition of evoked field potentials. The decrease in frequency of spontaneous synaptic events can be explained by the increased ratio of IPSCs to inhibitory postsynaptic potentials at inhibitory synapses in the network which will result in a decreased discharge probability of all cells.

The results of this study show that the activity profile of bromide in controlling various epileptiform discharges is broader than that of other standard anticonvulsant drugs. This is in keeping with a recent clinical study (Oguni et al. 1994) in which bromide was shown to be effective in controlling a number of different seizure types including focal, generalized, convulsive and non-convulsive seizures all of which may occur in the malignant syndrome of severe myoclonic epilepsy.

Given the fact that bromides are cheap and widely available anticonvulsants with powerful anticonvulsant properties as shown in the current study their use should be considered more frequently in severe forms of epilepsy.

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