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Effect of sodium-bromide on CA1-pyramidal cells, GABAergic transmission, synaptic plasticity in rat pilocarpine-induced seizures

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Introduction

Bromide was the first effective anticonvulsant, but became less conventional due to the development of more modern compounds. Nevertheless, it is still a valuable drug for the treatment of refractory epilepsy in pediatric patients.

Since $GABA_A$ receptor related chloride channels also conduct bromide-ions the antiepileptic effect of bromide is likely due to an increase in inhibition. Moreover, different affinity of chloride-ions and bromide-ions for Na^+-K^+-$2Cl^-$ cotransporter (NKCC) and K^+-Cl^- cotransporter (KCC2) respectively may improve the anticonvulsant effect of bromide.

Since the mechanism of bromide acting as antiepileptic drug is still under debate we tested the impact of chronic sodium bromide treatment in chronically epileptic rats following a pilocarpine-induced status epilepticus. Additionally in consequence of the age-dependent use of bromide we compared the effect of acute bromide application on GABAergic transmission between juvenile pilocarpine treated rats and adult pilocarpine treated rats.

Materials & Methods

Animals and slice preparation: All experiments were performed with male Wistar rats purchased from Charles River (Sulzfeld, Germany). Animals received pilocarpine treatment either at age P9, P11 and P15 (juvenile group), or at age P30 (adult group). Four weeks after pilocarpine treatment animals were prepared for electrophysiological recordings. After deep anesthesia with diethyl ether, animals were decapitated and the brain was rapidly removed and submerged into oxygenated ice-cold sucrose solution. Subsequently, horizontal brain slices of the hippocampus were prepared in this sucrose solution with a vibratome. The hippocampal formation on both hemispheres were carefully excised, and then transferred into a holding chamber at room temperature filled with ACSF solution.

Chronic and acute bromide treatment: For chronic bromide treatment animals were implanted subcutaneously with osmotic pumps filled with NaBr. In ASCF used for acute treatment 20 mM NaCl was replaced by 20 mM NaBr. Slice storage and electrophysiological recordings were conducted in NaBr containing ACSF.

Field potential recordings: For electrophysiological recordings, hippocampal slices were transferred into an interface chamber maintained at 32°C and superfused with standard ACSF and NaBr containing ACSF respectively. Field excitatory postsynaptic potentials (fEPSPs) were recorded using borosilicate glass pipettes filled with ACSF. Borosilicate glass pipettes were also used for stimulation. Stimulation was delivered using a Master-8 stimulator and a stimulus isolator at a baseline rate of 0.033 Hz. After input-output measurements the baseline stimulation strength was adjusted to 50 % of the maximal fEPSP. Stimulating and recording electrode were placed into stratum radiatum of CA1. Long-term potentiation (LTP) was induced by a high frequency stimulation (HFS) consisting of 100 pulses at 100 Hz (doubled baseline stimulation strength). LTP values (average of fEPSP at 55-60 minutes after HFS) are given as fEPSP slope expressed as the percentage of the baseline response.

Intracellular recordings: Sharp micropipettes were pulled from quartz capillaries (60-120MΩ; O.D. 1.2mm I.D. 0,9mm; Science Products) and filled with pipette solution composed of 3 mM potassium acetate and 0.3 M KCl. Intracellular recordings were performed in CA1 pyramidal cells. For all recordings the membrane potential was adjusted to -70 mV by current injection. Current injections from -1.3 nA to + 1.0 nA (0.1 nA steps) and the corresponding I/V-relation was used to determine membrane resistance. Hyperpolarizing voltage steps were used for analyzing the membrane time constant. The voltage sag was calculated as the difference between maximal hyperpolarization and the steady state potential at the end of stimulation in consequence of a current injection of -1 nA. Current injections of +1 nA were used to determine the number of actionpotentials and the subsequent afterhyperpolarizing potential. The afterhyperpolarizing potential was calculated as the amplitude between the maximal hyperpolarization after depolarization-induced actionpotentials and the resting membrane potential. For recordings of $GABA_AR$-mediated inhibitory postsynaptic potentials (IPSP) glutamatergic transmission was blocked by bath application of D-AP5 and CNQX. The input-output relation was analyzed by extracellular stimulation of the Schaffer collaterals with stimulation intensities ranged from 0.02mA to 0.3 mA (0.02mA increment). Reversal potentials of $GABA_AR$-mediated IPSP were determined by intracellular current injections ranged from -0.5 nA to +0.3 nA and extracellular stimulation at 50 % of maximal IPSP amplitude.

Statistical analysis: All data are expressed as means ± SEM. Statistical comparisons were performed using the Mann–Whitney U test and two way-analysis of variance (ANOVA). Significant differences are indicated by asterisks (*p<0.05).

Results

Acute bromide treatment (*in vitro*)

Table 1 Active and passive membrane properties of hippocampal CA1 pyramidal neurons in pilocarpine treated rats at P9, P11 and P15

P11 pilocarpine treatment	Control		Pilocarpine	
	w/o bromide (n=11)	Bromide (n=12)	w/o bromide (n=19)	Bromide (n=7)
Membranresistance (MΩ)	47.4 ± 7.1	53.2 ± 5.7	40.2 ± 4.1	31.8 ± 4.4
Membrane time constante (ms)	9.1 ± 0.6	8.6 ± 1.1	8.1 ± 0.7	10.7 ± 0.6*
Voltage sag (mV)	2.5 ± 0.9	3.9 ± 1.0	3.2 ± 0.8	3.0 ± 0.4
Number of actionpotentials	11.8 ± 2.2	8.8 ± 2.1	9.2 ± 1.4	6.5 ± 0.5
Afterhyperpolarizing potential (mV)	-7.0 ± 0.8	-9.6 ± 1.0	-9.0 ± 1.0	-8.3 ± 0.9

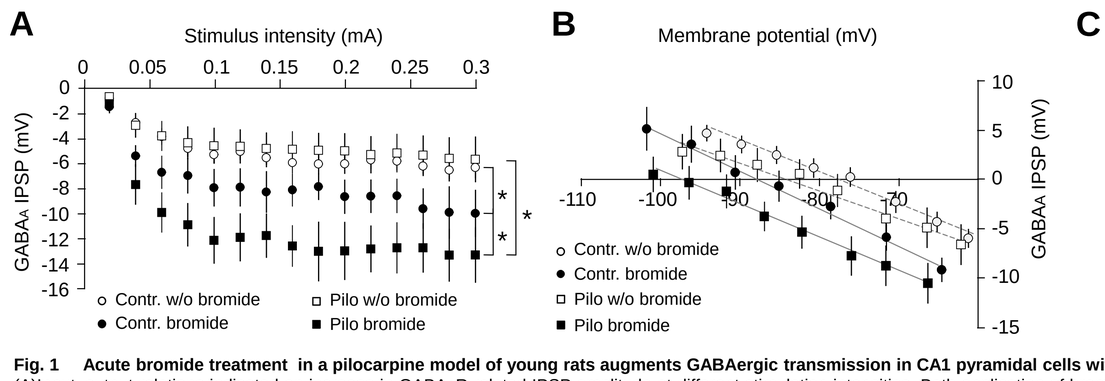

Fig. 1 Acute bromide treatment in a pilocarpine model of young rats augments GABAergic transmission in CA1 pyramidal cells without affecting long-term potentiation (LTP).
(A)Input–output relations indicated an increase in $GABA_AR$ related IPSP amplitude at different stimulation intensities. Bath application of bromide significantly enhanced evoked IPSP in both controls (closed circles, n=9) and pilocarpine treated rats (closed squares, n=7). The effect of bromide was significantly stronger in pilocarpine treated rats compared to controls. There was no significant difference in Input–output relation between control rats (open circles, n=7) and pilocarpine treated rats (open squares, n=7) under control conditions.
(B) Reversal potentials of electrically evoked $GABA_AR$ related IPSP show no significant effect of bromide. Membrane potentials were systematically varied by current injections from -0.5 nA to +0.3 nA.
(C)The bar graph summarizes LTP levels of fEPSP slope after HFS of Schaffer collaterals. The number of slices is given in parentheses.

Table 2 Active and passive membrane properties of hippocampal CA1 pyramidal neurons in pilocarpine treated rats at ~P30

P30 pilocarpine treatment	Control		Pilocarpine	
	w/o bromide (n=19)	Bromide (n=10)	w/o bromide (n=9)	Bromide (n=7)
Membranresistance (MΩ)	49.8 ± 4.7	47.7 ± 9.4	49.3 ± 8.4	43.4 ± 4.4
Membrane time constante (ms)	10.5 ± 1.1	9.2 ± 0.9	9.9 ± 1.5	10.6 ± 1.3
Voltage sag (mV)	6.6 ± 0.8	3.9 ± 0.4*	4.5 ± 1.0	5.3 ± 0.7
Number of actionpotentials	10.7 ± 1.2	8.5 ± 1.9	11.0 ± 2.1	12.7 ± 2.4
Afterhyperpolarizing potential (mV)	-8.5 ± 1.1	-8.1 ± 1.1	-5.2 ± 1.5	-7.1 ± 1.1

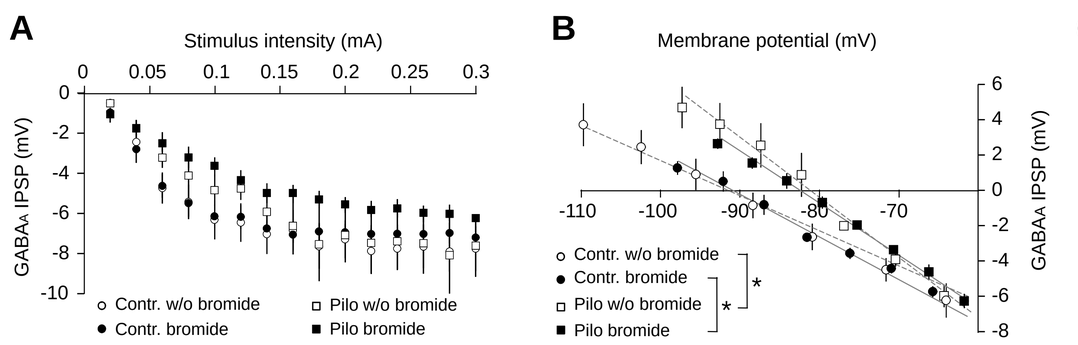

Fig. 2 Acute bromide treatment in a pilocarpine model of adult rats had no effect on GABAergic transmission in CA1 pyramidal cells and long-term potentiation (LTP).
•Input–output relations indicated an increase in $GABA_AR$-mediated IPSP amplitude at different stimulation intensities. In control rats bath application of bromide (closed circles, n=9) had no effect on GABAergic transmission compared to control experiments w/o bromide (open circles, n=7). The input-output relation was not different between control rats (open circles) and pilocarpine treated rats (open squares, n=8). Moreover, $GABA_AR$-mediated IPSP in epileptic rats were unaffected by bromide treatment (closed squares, n=7).
• Reversal potentials of electrically evoked $GABA_AR$-mediated IPSP show no significant effect of bromide. Membrane potentials were systematically varied by current injections from -0.5 nA to +0.3 nA. Note the shift of reversal potential in positive direction in pilocarpine treated rats (open squares) compared to control rats (open circles).
•The bar graph summarizes LTP levels of fEPSP slope after HFS of Schaffer collaterals. The number of slices is given in parentheses.

Chronic treatment (*in vivo*)

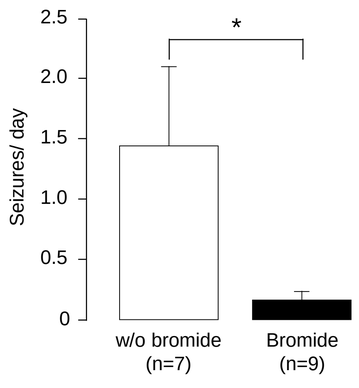

Fig. 3 Chronic bromide treatment reduces seizure frequency in pilocarpine treated rats.
The bar graph summarizes seizures/day in bromide treated (closed symbol) and saline treated (open symbol) epileptic rats

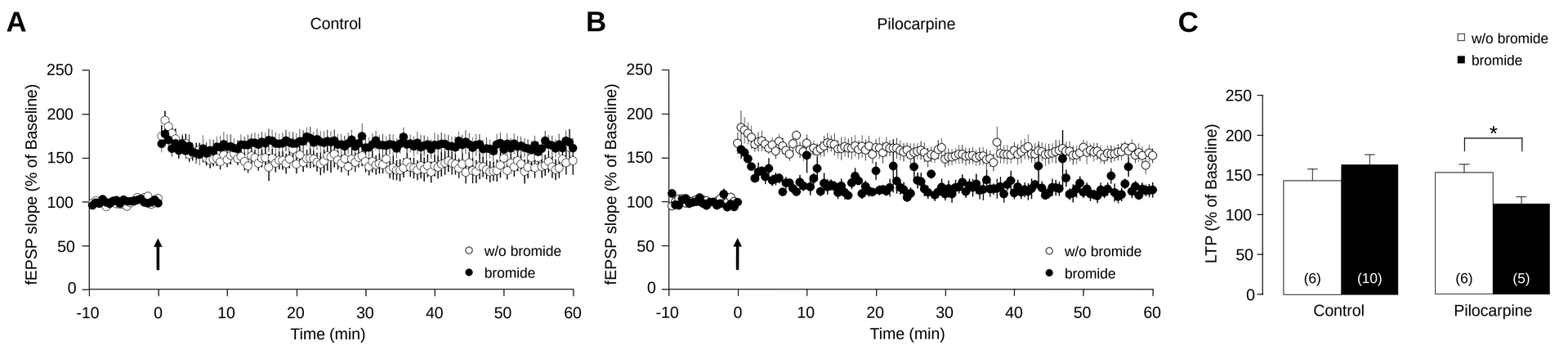

Fig. 4 Chronic bromide treatment reduces long-term potentiation exclusively in hippocampal slices from epileptic rats.
•Time course of excitatory postsynaptic field potentials (fEPSPs) following stimulation of Schaffer collaterals. High frequency stimulation (arrow) in control rats displays LTP to a similar level in saline treated (open symbols) and bromide treated rats (closed symbols).
•Time course of excitatory postsynaptic field potentials (fEPSPs) in pilocarpine treated rats. LTP following high frequency stimulation (arrow) was reduced in bromide treated rats (closed symbols) compared to saline treated rats (open symbols).
•The bar graph summarizes LTP levels in control rats and epileptic rats under both conditions, with chronic bromide treatment (closed bars) and without bromide treatment (open bars). The number of slices is given in parentheses.

Conclusions

The results provide evidence that chronic treatment with bromide is an effective anticonvulsant in the pilocarpine model of epilepsy in adult rats. However, the therapeutic effect of sodium bromide cannot be explained by acute effects on GABAergic transmission or synaptic plasticity. Rather, chronic effects seem to be required for antiseizure efficacy, but the underlying mechanisms during chronic treatment await further investigation. Furthermore an age-dependent effect of bromide can not be excluded since acute bromide treatment affected basal GABAergic transmission only in juvenile, pilocarpine treated rats.