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Interaction of the structurally related *Aconitum* alkaloids, aconitine and 6-benzyolheteratisine, in the rat hippocampus

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Abstract

Aconitine is a highly toxic diterpenoid alkaloid occurring in plants of the *Aconitum* genus. Aconitine is known to shift the voltage-dependence of the voltage-dependent Na^+ channel towards hyperpolarized direction, thereby leading to a permanent activation of the channel. 6-Benzoylheteratisine is a plant alkaloid which is structurally related with aconitine. The aim of the present study was to investigate the interaction of aconitine and 6-benzoylheteratisine in the rat hippocampus. The experiments were carried out as extracellular recordings of stimulus evoked population spikes and field excitatory postsynaptic potential (EPSP) in rat hippocampal slices. Aconitine (10–100 nM) exerted a concentration-dependent decrease in the amplitude of the orthodromic population spike. When aconitine was applied in presence of 6-benzoylheteratisine (3 μM), the concentration–response curve was shifted to the right. Furthermore, the complete suppression of the population spike evoked by 100 nM aconitine was reversed by 10 μM 6-benzoylheteratisine. The closely related alkaloid heteratisine (3 and 30 μM), however, was not capable to antagonize the aconitine action. 6-Benzoylheteratisine shifted the input–output relationship of the presynaptic fiber spike as function of the stimulation intensity and the input–output relationship of the field EPSP as function of the presynaptic fiber spike to the right. Thus, electrophysiologically this alkaloid seems to inhibit predominantly the excitability of the afferent fibres and, in consequence, neurotransmission between Schaffer collaterals and the CA1 neurons, thereby suppressing the firing of the latter. Spontaneously occurring epileptiform activity in area CA3 elicited by omission of Mg^{2+} and elevation of K^+ was attenuated by 6-benzoylheteratisine (1 and 10 μM). Patch clamp studies performed on cultured rat hippocampal pyramidal cells revealed an inhibitory action of 6-benzoylheteratisine on whole cell Na^+ currents. It is concluded that the inhibitory and antiepileptiform effect of ajacine and lappaconitine is mediated by an inhibition of the voltage-dependent Na^+ channel which might be important for filtering high frequency bursts of action potentials characteristic for epileptiform activity in the hippocampus. Thus, 6-benzoylheteratisine seems to be a naturally occurring antagonist of the Na^+ channel activator aconitine.

Keywords: Hippocampus; Electrophysiology; Epileptiform activity; Na^+ current; *Aconitum* alkaloid

1. Introduction

Plant extracts of different *Aconitum* species are employed in traditional Chinese and Japanese medicine as analgesics, antirheumatics and treatment of neurological disorders. For this purpose, the highly toxic diester diterpenoid alkaloids are transformed to less toxic monoesters by heat-processing [for review see Ameri, 1998]. Aconitine, the main alkaloid in plants of the *Aconitum* genus, binds to site 2 of the Na^+ channel, thereby producing a persistent activation of Na^+ channels at resting membrane potentials by blocking channel inactivation and shifting the voltage-dependence of channel activation to a more hyperpolarized state (Catterall, 1980, 1987, 1992). Previously, aconitine has been shown to completely suppress neuronal activity in rat hippocampal slices at concentrations of 0.1 μM and 1 μM (Ameri et al., 1996)

6-Benzoylheteratisine, the main alkaloid of *Aconitum tanguticum*, is a monoester diterpene alkaloid, structurally related to aconitine (Fig. 1). This alkaloid is less toxic than aconitine and known to possess antinociceptive properties (Bisset, 1981; Han et al., 1988). Previously, it has been shown that 6-benzoylheteratisine exerts an inhibitory action on hippocampal excitability in a frequency-dependent

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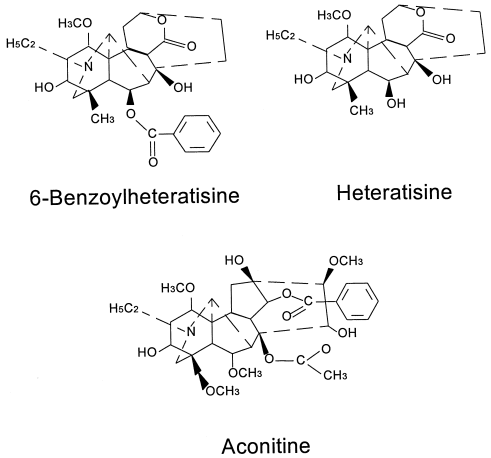

Fig. 1. Chemical structures of the *Aconitum* alkaloids 6-benzoylheteratisine, heteratisine and aconitine.

manner (Ameri, 1997a,b). The inhibitory effect of 6-benzoylheteratisine was shown to depend on the presence of a benzoylester side chain at position C4 of the molecule (Ameri, 1997b), since the alkaloid heteratisine which is closely related to 6-benzoylheteratisine lacks pharmacological activity.

The aim of the present study was to examine the interaction of 6-benzoylheteratisine with aconitine on rat hippocampal excitability, in order to obtain further insight into this class of alkaloids. In the present study, the effects of the alkaloids on stimulus evoked population spikes in area CA1 of normal and epileptiform rat hippocampal slices as well as spontaneously occurring epileptiform activity in area CA3 were investigated by means of extracellular recordings. To examine the effect of 6-benzoylheteratisine on the voltage-dependent Na^+ current, whole-cell patch clamp experiments were performed at cultured hippocampal neurons.

2. Materials and methods

2.1. Brain slice preparation

Experiments were performed on hippocampal slices from male Wistar rats (150–180 g). The rats were deeply anaesthetized with diethyl ether and killed by rapid decapitation. The brains were quickly removed from the skulls and the hippocampus of one hemisphere was isolated. Slices of 400 μm thickness were cut transversely to the longitudinal axis of the hippocampus by use of a McIlwain tissue chopper. Immediately after cutting, one slice was transferred into a submerged brain slice recording chamber, where it was continuously superfused with warmed (32°C) artificial cerebrospinal fluid (ACSF) at a flow rate of 3–4 ml min^{-1} and held down on a nylon net by a U-shaped piece of flattened platinum wire. The other slices were maintained at room temperature in an incubation chamber. The standard ACSF was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and contained (in mM): NaCl 124, KCl 3, NaH_2PO4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.5, $MgSO_4$ 2, glucose 10 at a pH of 7.4. To elicite spontaneously occurring epileptiform activity, a low Mg^{2+}/high K^+-ACSF was superfused. This solution was nominally Mg^{2+}-free, while the concentration of KCl was levated to 8 mM.

2.2. Stimulation and recording

The experimental protocol always included a recovery period of 1 h after slice preparation. For recordings of stimulus-evoked population spikes and field EPSPs, the recording electrode was placed in stratum pyramidale and stratum radiatum of area CA1, respectively. The electrodes were pulled on a BB-CH-PC electrode puller (Mecanex, Switzerland) from 1.5 mm borosilicate glass and filled with 3 M NaCl (resistance 5–10 M). A concentric bipolar stainless steel electrode with 0.25 mm outer diameter (Rhodes Medical Instruments, U.S.A.) was positioned into the Schaffer collaterals (i.e., near the junction of CA1 and CA2 stratum radiatum) for orthodromic activation of CA1 pyramidal neurons. Extracellular stimuli were rectangular current pulses of 60 μs in duration delivered every 15 s through a digitally controlled stimulus isolation unit (Axon Instruments, Foster City, U.S.A.). Drug effects were investigated on population spikes elicited by stimulus strength which was adjusted for each slice to the half-maximal amplitude at the beginning of the experiment. For recording of field EPSPs stimulus intensities were adjusted to subthreshold for spike initiation.

To construct stimulus–response curves, electrical stimuli of increasing intensity were applied to the Schaffer collaterals and the amplitude of the presynaptic fiber spike and the maximum rate of change (i.e., slope) of the field EPSP was measured before (control) and at the end of a 90-min application of drug, and plotted as a function of stimulus intensity.

The signal from the recording electrode was amplified by means of a DP 301 amplifier (Warner Instruments, U.S.A.). Analog data were digitized and analyzed using the data acquisition and analysis software TIDA (HEKA electronic, Germany).

2.3. Patch clamp experiments

For patch clamp recordings of whole-cell Na^+ currents, rat hippocampal pyramidal cells which had been held in culture for 10–14 days (obtained from S. Himmelseher, Experimental Anesthesiology, University of Ulm) were employed. The external solution consisted of (in mM): NaCl 150, KCl 3, $CaCl_2$ 2, $MgCl_2$ 2 2, HEPSES-Na 5, HEPES 5, and glucose 10, pH 7.4. Experiments were performed using an Axopatch 200 A amplifier (Axon

Instruments) and an ITM phase contrast microscope (Olympus, Hamburg, Germany). Patch pipettes (2–4 M) were filled with (in mM) CsF 125, TRIS–Cl 10, HEPES 10, and EGTA 10, pH 7.2. Internal Cs^+ (109.4 mM) was necessary for suppression of voltage-dependent K^+ currents in order to allow stable recordings of the Na^+ current. To avoid space clamp, only medium-sized cells with processes shorter than the diameter of the cell body were employed for patch clamp recordings, because they are particularly advantageous for space clamp conditions (Huguenard et al., 1988; Ogata and Tatebayashi, 1990). Voltage commands and data acquisition were performed with a TIDA system (HEKA Electronic, Lamprecht, Germany). Whole-cell currents were recorded at room temperature (20–22°C), filtered at 5 kHz with a four-pole Bessel filter, and sampled at 10 kHz. The seal resistances ranged from 10 to 20 G . The series resistance of the whole-cell patches ranged between 5 and 15 M and 0.3 and 0.8 M when compensated by 75–85%. The voltage drop caused by the compensated series resistance never exceeded 4 mV in these experiments. Drugs were added to the external solution. The flow rate of the perfusion medium was adjusted to 1.5 ml/min.

2.4. Drugs

Aconitine (Sigma, Deisenhofen, Germany), 6-benzoylheteratisine and heteratisine (both Latoxan, Rosans, France) were dissolved in DMSO (Dimethyl sulfoxide) to give stock solutions of 10 or 100 mM and diluted with ACSF to the final concentration. Control experiments have revealed that the highest final DMSO concentration (0.1%) did not affect any of the measured parameters. All drugs were delivered through the superfusion medium. In all experiments, each drug application was preceded by a control period of at least 30 min. Lidocaine hydrochloride (Sigma) was dissolved in distilled water. The drugs were applied to the superfusion medium. To determine the concentration–response relationship only one concentration of each drug was added to a single slice.

2.5. Data analysis

All data are expressed as mean ± standard deviation (S.D.). Comparisons of the effects of drug treatments (normalized as percent of control) between groups of slices or cells were performed using Student's *t* test for differences between two independent means. The statistical significance of the difference of the amplitude of the electrophysiological responses prior to and following the administration of a drug was assessed with the paired Student's *t* test. In both cases, differences were considered statistically significant when $P = 0.05$. The amplitude of the population spike which appears as a large negative wave superimposed on a positive-going was determined as the length of a vertical line, drawn from the minimum of the population spike to the line that joined the two positive peaks of the field response. Drug effects on the field EPSP were determined as changes in the slope of the field EPSP to avoid contamination by the population spike. The slope was measured as the ascending gradient between 20 and 80% of the maximum field EPSP.

Spontaneously occurring epileptiform discharges were counted for periods of 1 min during superfusion with high K^+/low Mg^{2+}-ACSF until the burst frequency was stable. Then the bathing medium was switched to the drug-containing high K^+/low Mg^{2+}-ACSF and frequency was determined after 15, 30, 45 and 60 min. Additionally, spontaneously occurring epileptiform discharges were quantified by coastline measurement which is implemented in the TIDA software system. The coastline index was determined according to Apland and Cann (1995). In brief, the length of the line representing the epileptiform activity was measured at control (CI_{con}), after superperfusion of the slices by the Mg^{2+}-free ACSF (CI_{epi}) and after addition of the test compounds to the Mg^{2+}-free ACSF ($CI_{epi+test}$). The coastline index was determined by using the following formula:

$$\text{anticonvulsant index} = (CI_{epi+test} - CI_{con})/(CI_{epi} - CI_{con}) \times 100$$

An index $< 100\%$ indicates inhibition of burst activity, and index > 100 indicates an enhancement of epileptiform activity.

3. Results

Only the data of those hippocampal slices have been included into the present study which showed normal field potentials (i.e., no second population spike at maximal stimulation intensity) in response to electrical activation of Schaffer collaterals during the control in standard ACSF. Furthermore, the amplitudes of the population spikes had to be stable during a control period of at least 30 min prior to the application of drugs. During this control period differences in spike amplitude had to be below 5%.

3.1. Effect of the alkaloids on normal hippocampal excitability

When aconitine was applied in concentrations of 10 to 100 nM to the hippocampal slices it exerted a concentration-dependent decrease in the amplitude of the orthodromic population spike. At a concentration of 100 nM, aconitine caused a complete suppression of the population spike within 30 to 45 min. During the first 10 to 15 min of application, the alkaloid evoked an enhancement of neuronal excitability, which became evident as a transient increase in the amplitude of the postsynaptic population spike (Fig. 2A). This increase was accompanied by the

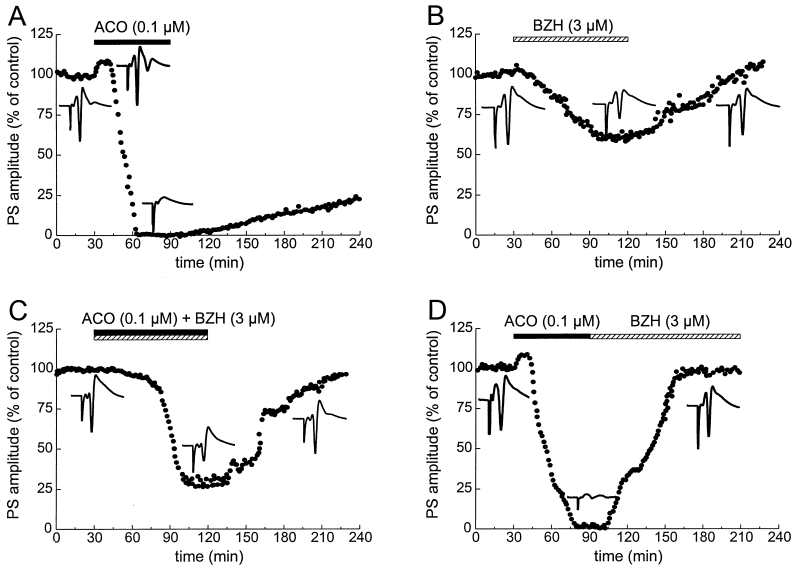

Fig. 2. Time-course of the effects of aconitine and 6-benzoylheteratisine, alone and in combination, on the amplitude of the orthodromic population spike. Population spikes were elicited by electrical stimulation of the Schaffer collaterals every 15 s and recorded extracellularly in the CA1 pyramidal layer. (A) Aconitine (ACO) completely suppressed the postsynaptic population spike (PS). The decrease in neuronal excitability is preceded by a transient phase of hyperexcitability which became evident by an increase in spike amplitude and by occurrence of a second population spike. (B) Time-course of the inhibitory action of 6-benzoylheteratine (BZH). (C) When aconitine was applied together with 6-benzoylheteratisine, the depressant effect of the former was reduced and reversible. (D) Reversion of the aconitine-induced suppression by 6-benzoylheteratisine. Each point in the graphs represents the average of the amplitudes of five subsequent measurements. The bar above the graphs indicates the time of drug-application. The insets show population spikes which represent the average of five subsequent recordings. Each graph shows one representative experiment out of 6–9 similar ones.

occurrence of a second population spike. The final inhibition of the population spike was only partially reversible during washout by ACSF. At a concentration of 3 μM, 6-benzoylheteratisine caused a reversible decrease in the spike amplitude by 28 ± 4% of control ($n = 7$, $P \leq 0.001$, Fig. 2B). To investigate whether the blocking effect of aconitine on neuronal excitability was antagonized by 6-benzoylheteratisine, experiments were performed with a combination of 100 nM aconitine and 3 μM 6-benzoylheteratisine. When both alkaloids were applied together to the hippocampal slices (Fig. 2C), the inhibitory action of aconitine was significantly attenuated and reversible during washout by ACSF. After coapplication of aconitine (100 nM) with 6-benzoylheteratisine (3 μM), the population spike amplitude was decreased only by 58 ± 6% of control ($n = 8$). Moreover, when 6-benzoylheteratisine was added subsequent to the aconitine application, i.e., after the suppression of the population spike had occurred, complete reversion of the aconitine-induced suppression was achieved within 60 to 90 min in all slices tested ($n = 6$, Fig. 2D).

The antagonism of aconitine by 6-benzoylheteratisine was mimicked by the local anesthetic lidocaine. When applied alone, lidocaine (100 μM) reversibly decreased the amplitude of the postsynaptic population spike by 40.3 ± 4% of control ($n = 6$). In combination with 100 nM aconitine, lidocaine (100 μM) applied for 60 min decreased the amplitude of the postsynaptic population spike by 17 ± 4% of control ($n = 7$).

To further characterize the relationship between aconitine and 6-benzoylheteratisine the concentration–response relationship was examined. As shown in Fig. 3, 6-benzoylheteratisine caused a shift to the right of the concentration–response curve of aconitine. Despite its structural

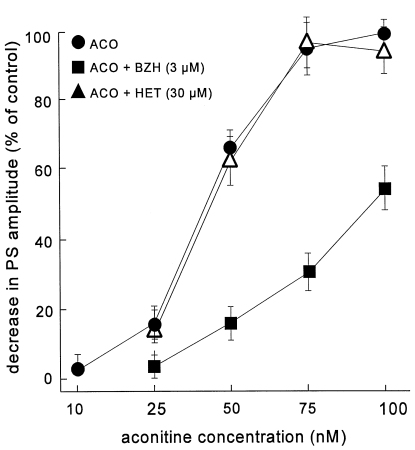

Fig. 3. Concentration–response curve of aconitine (ACO) alone and in combination with either 6-benzoylheteratisine (BZH) or heteratisine (HET) for the inhibition of the postsynaptic population spike (PS). The drugs were applied at each concentration to a single slice. The amplitude of the population spike was normalized with respect to control and plotted as a function of drug-concentration. Each data point represents mean values ± S.D. from 5–8 slices. 6-Benzoylheteratisine shifted the concentration–response curve of aconitine to the right and significantly (at least $p = 0.05$) reduced the depressant effect of all aconitine concentrations. In contrast, heteratisine failed to affect the concentration–response curve of aconitine.

similarity the alkaloid heteratisine (30 μM) failed to affect the concentration–response curve of aconitine.

To determine the stimulus–response relationship, electrical stimuli of increasing intensity were applied to the Schaffer collaterals, and the amplitudes of the according fiber spikes as well as the slope of the field EPSPs were measured. Fig. 4 shows the relationship of the presynaptic fiber spike to the stimulation intensity (A and C) as well as the relationship of the field EPSP to the fiber spike (B and D) over a range of different stimulus intensities. The input–output functions were measured before drug-application (control) and after application of 6-benzoylheteratisine or heteratisine. 6-Benzoylheteratisine (3 μM) decreased the amplitude of the presynaptic fiber spike at all stimulus intensities tested and shifted the curve to the right (Fig. 4A), indicative for a decrease in the excitability of the afferent fibers. The input–output curve of the field EPSP as function of the presynaptic fiber spike was also shifted to the right by this alkaloid (Fig. 4B). This implies that, for identical fiber spikes, the cell is further from the firing threshold, so that the synchronous discharge of the same population of cells is reduced. In contrast to 6-benzoylheteratisine, the alkaloid heteratisine (30 μM) did not affect the stimulus–response relationship of the presynaptic fiber spike and the field EPSP (Fig. 4C,D).

To further investigate the mechanisms of action of 6-benzoylheteratisine, patch clamp recordings in whole cell configuration were performed at cultured hippocampal

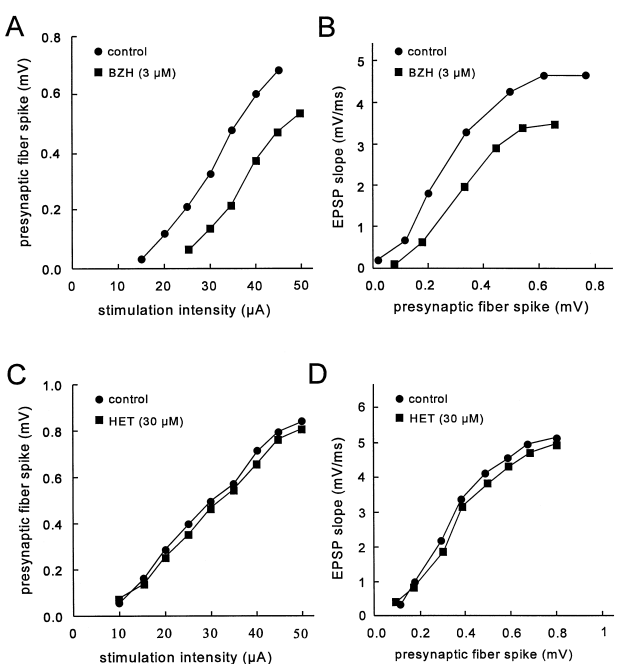

Fig. 4. Effects of 6-benzoylheteratisine (BZH) and heteratsine (HET) on the stimulus–response relationship. The slices were stimulated with intensities ranging from subthreshold to maximal. For each response, the amplitude of the presynaptic fiber spike (A,C) and the slope of the field EPSP (B,D) were measured and plotted as function of stimulus-intensity. One representative experiment out of 5–7 similar ones is shown.

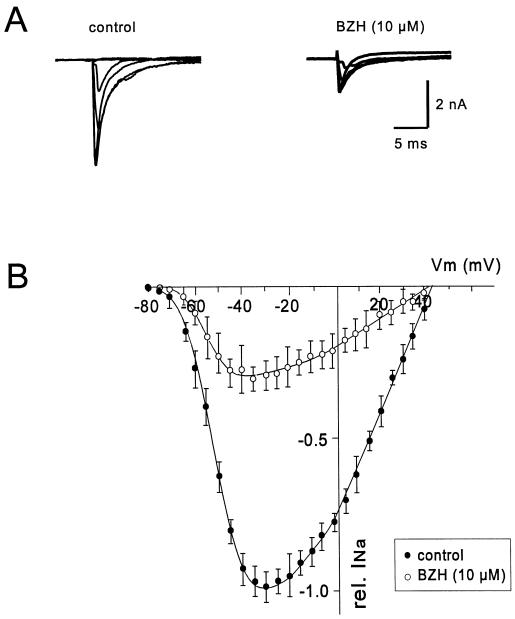

Fig. 5. Effects of 6-benzoylheteratisine (BZH) on voltage-dependent Na^+ currents in hippocampal neurons. (A) Original traces of currents evoked by voltage steps from −80 mV at control and in presence of 6-benzoylheteratisine. (B) Current–voltage relationship of peak Na^+ currents in the absence and presence of 6-benzoylheteratisine. Peak Na^+ currents are plotted as a function of membrane potential (V_m). Data points represent the means ± SD of five experiments. Cells were maintained at a holding potential of −90 mV.

pyramidal cells. Inward currents were elicited by depolarizing voltage steps from a holding potential of −90 mV to potentials between −80 and +40 mV. The currents had a peak amplitude of 3.14 ± 0.7 nA ($n = 7$) and were blocked by 0.5 μM tetrodotoxin. At a concentration of 10 μM, 6-benzoylheteratisine reduced the peak amplitude of the Na^+ current (Fig. 5).

3.2. Effects of 6-benzoylheteratisine on spontaneous epileptiform activity in hippocampal area CA3

6-Benzoylheteratisine has previously been shown to attenuate stimulus-evoked epileptiform activity in the hippocampal area CA1 (Ameri, 1997a,b). Since the CA1 subfield of the hippocampus received powerful excitatory projections from the CA3 region via Schaffer collaterals, we performed in the present study recordings of spontaneously occurring epileptiform activity in the CA3 pyramidal cell layer. For this purpose, the slices were perfused by a low Mg^{2+}/high K^+. During these experiments no electrical stimuli were given. The increase in the K^+ concentration of the ACSF caused recurrent epileptiform discharges with a regular repetition rate of about 20–40. The epileptiform burst potentials were monophasic or biphasic with a duration of 177 ± 10 ms and occurred with a regular repetition rate of 32 ± 6 min^{-1} ($n = 23$). After stabilization of the epileptiform activity (about 20–30 min after onset of the epileptiform discharges), either 6-benzo-

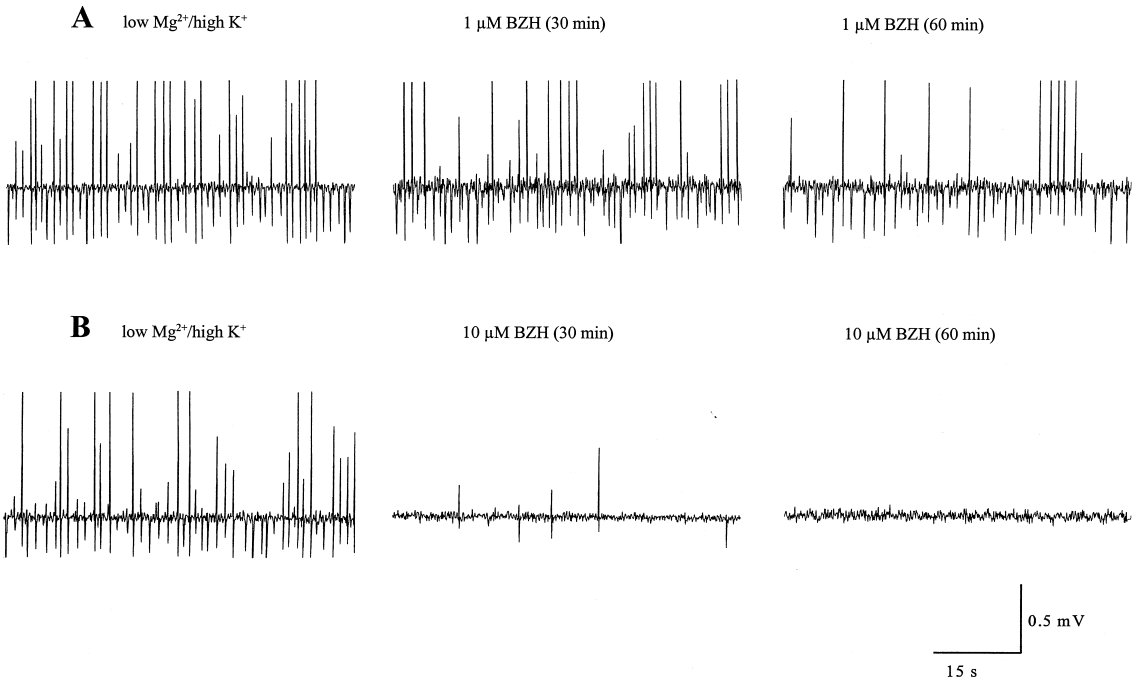

Fig. 6. Inhibitory effect of 6-benzoylheteratisine (BZH) on epileptiform activity induced by omission of Mg^{2+} and elevation of KCl to 8 mM. Spontaneously occurring epileptiform discharges were recorded in absence of electrical stimulation in stratum pyramidale of the CA3 region. 6-Benzoylheteratisine was applied at 1 μM (A) and 10 μM (B). Scale bars apply to the upper and lower traces.

ylheteratisine (1 and 10 μM) or heteratisine (10 and 100 μM) were applied for a period of 60 min. As shown in Figs. 6 and 7, 6-benzoylheteratisine exerted a concentration-dependent decrease in the frequency of the spontaneously occurring recurrent discharges. Thirty minutes after starting the application of 1 μM 6-benzoylheteratisine (Fig. 6A) and 15 min after starting the application of 10 μM (Fig. 6B), the burst frequency elicited by the low Mg^{2+}/high K^+-ACSF was significantly reduced. The anticonvulsant index, determined as described in Methods

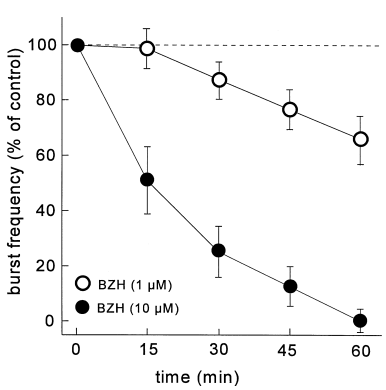

Fig. 7. Time-course of the effect of 6-benzoylheteratisine on the frequency of spontaneously occurring epileptiform burst discharges in the stratum pyramidale of the CA3 region. Epileptiform activity was elicited by omission of Mg^{2+} and elevation of K^+ to 8 mM. Data points represent the mean ± S.D. of 8–10 experiments. After 30 min, both 1 and 10 μM 6-benzoylheteratisine decreased the burst frequency significantly (at least p 0.01).

was reduced to $57.8 \pm 9\%$ ($n = 9$, $P \leq 0.001$) and to $18.5 \pm 6\%$ ($n = 9$, $P \leq 0.001$) after a 60-min application of 1 and 10 μM 6-benzoylheteratisine, respectively. At both concentration tested, heteratisine failed to attenuate the frequency of the spontaneously occurring bursts (data not shown).

4. Discussion

The *Aconitum* alkaloid 6-benzoylheteratisine has been reported previously to inhibit neuronal excitability in rat hippocampal slices (Ameri, 1997a). The aim of the present study was to investigate the mechanisms of this inhibitory effect as well as the interaction of this alkaloid with the main alkaloid of the *Aconitum* species, aconitine. Changes of neuronal activity of pyramidal cells in the hippocampus were recorded by monitoring the excitatory synaptic drive (initial slope of the field EPSP) and the amplitude of the synchronous discharge of a population of cells (amplitude of the population spike).

Aconitine is known to bind at the neurotoxin binding site 2 of the voltage-dependent Na^+ channel and to cause the channel to remain open at rest (Catterall, 1980,1987). In consequence, aconitine depolarizes neuronal membranes and leads to a final inexcitability. This is in line with the result of this study which has shown a complete suppression of the population spike following an initial period of hyperexcitability. 6-Benzoylheteratisine has in common

with aconitine an inhibitory action on the stimulus-evoked population spike in hippocampal area CA1. Compared with aconitine, 6-benzoylhetertisine lacks the benzoylester side chain at position C14 as well as the acetyl group at C8 (Fig. 1), which are mainly responsible for the toxicity of the former (Ameri, 1998).

The present study provides evidence that despite their structural similarity and their common inhibitory action on neuronal excitability both alkaloids seem to antagonize each other. This is supported by three findings: First, 6-benzoylheteratisine diminished the suppressing action of aconitine on the population spike and shifted the concentration–response curve of aconitine to the right. Second, the suppression of the spike by aconitine, which is irreversible during washout, did recover during the subsequent application of 6-benzoylheteratisine. Third, as shown by the whole cell patch clamp recordings, 6-benzoylheteratisine blocks the voltage-dependent Na^+ channel. Furthermore, the antagonizing effect appears to be specific for 6-benzoylheteratisine, because the closely related alkaloid heteratisine did not affect the aconitine action. Recently, it has been reported that lappaconitine, an *Aconitum* alkaloid which bears like 6-benzoylheteratisine only one benzoylester side chain at the diterpene skeleton, also blocks the voltage-dependent Na^+ current (Seitz and Ameri, 1998) whereas 3-acetylaconitine, an alkaloid which similar to aconitine is bearing two benzoylester side chains, activates this current at resting membrane potential (Ameri, 1997c).

The different modifications of the voltage-dependent Na^+ channel by aconitine and 6-benzoylheteratisine which activate or block this current, respectively, explain the present finding that the aconitine-induced depression of neuronal activity is antagonized by 6-benzoylheteratisine. Due to the structural relationship of the *Aconitum* alkaloids investigated in the present study, the blockage of the aconitine-induced suppression of neuronal activity by 6-benzoylheteratisine might reflect a competitive antagonism at the same binding site at the Na^+ channel protein. However, as shown in the present study, the effect of aconitine is also blocked by the local anesthetic lidocaine. This finding suggests that the aconitine-modified Na^+ channel can be blocked by local anesthetics, probably by an allosteric inhibition due to binding at a separate site on the Na^+ channel protein (Ulbricht, 1998).

Furthermore, the present results imply that the inhibitory effect of 6-benzoylheteratisine is mediated by a decrease in afferent excitability. This is supported by the investigation of the input–output relationship, which indicated that the inhibitory effect on the postsynaptic potentials exerted by the alkaloids was mediated at least in part by a decreased excitability of the afferent fibers. The decreased afferent input, in consequence, led to an inhibition of neurotransmission between the Schaffer collaterals and CA1 neurons, thus suppressing the firing of the latter. Therefore, the alkaloid-induced attenuation of the postsynaptic population spike seems at least partially to be due to a change in the excitability of the Schaffer collaterals. However, the input–output curve of the field EPSP as function of the presynaptic fiber spike shows a rightward shift. This indicates that a fiber spike of a given size induced a decrease in the efficiency of synaptic transmission after the application of the alkaloids. Given the presence of Na^+ channels at the dendritic level (Stuart and Sakmann, 1994, 1995; Andreasen and Nedergaard, 1996; Jung et al., 1997) as well as the Na^+ channel blocking properties of 6-benzoylheteratisine reported in the present study, this alkaloid can suppress the amplification of the EPSPs at dendritic level and thereby decrease the firing level. Therefore, it is likely that this postsynaptic mechanism could contribute to the attenuation of both the field EPSP and the postsynaptic population spike reported in this study.

Previously, it has been reported that 6-benzoylheteratisine is capable of inhibiting stimulus-triggered epileptiform activity recorded in CA1 stratum pyramidale (Ameri, 1997a). In this study, we have further investigated if this compound affects spontaneously occurring epileptiform discharges in the CA3 subfield of the hippocampus. The excitatory synaptic input driving the glutamatergic excitation of CA1 pyramidal cells involved in bursting includes the powerful synaptic input of the area CA3 via Schaffer collaterals. Therefore, the CA3 pyramidal neurons provide a synchronous excitatory drive for the downstream CA1 pyramidal cells. The recordings of spontaneous epileptiform burst discharges in area CA3 revealed that 6-benzoylheteratisine diminished these spontaneous epileptiform discharges in a concentration-dependent manner. These findings suggest that the antiepileptiform effect of 6-benzoylhetertisine observed in the CA1 subfield (Ameri, 1997a) might be a consequence of an inhibition of excitability occurring first in the CA3 subfield. This, in turn, would decrease the glutamatergic input in the pyramidal cell layer of area CA3 and might be important for filtering high frequency bursts of action potentials characteristic for epileptiform activity in the hippocampus.

The antiepileptiform activity is in line with the Na^+ channel blocking properties of 6-benzoylheteratisine, since Na^+ channels are known to be involved in the genesis of abnormal activities in epilepsy. Several anticonvulsants (e.g. phenytoin and lamotrigine), antiarrhythmic agents (class I) and local anesthetics have been shown to inhibit neuronal excitability by interacting with the neurotoxin receptor site 2 of the voltage-dependent Na^+ channel (Rogawski and Porter, 1990), an effect that is considered to contribute to the therapeutic actions of these agents.

In conclusion, the *Aconitum* alkaloid 6-benzoylheteratisine exerted an inhibitory and antiepileptiform effect in the hippocampal slices which antagonizes the activation of Na^+ currents by aconitine. This effect is likely to be mediated by a direct or indirect interaction at the neurotoxin binding site 2 of the voltage-dependent Na^+ channel.

References

Ameri, A., 1997a. Electrophysiological actions of the plant alkaloid 6-benzoylheteratisine in rat hippocampal slices. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 538–544.

Ameri, A., 1997b. Effects of the alkaloids 6-benzoylheteratisine and heteratisine on neuronal activity in rat hippocampal slices. Neuropharmacology 36, 1039–1046.

Ameri, A., 1997c. Inhibition of rat hippocampal excitability by the plant alkaloid 3-acetylaconitine mediated by an interaction with voltage-dependent sodium channels. Naunyn-Schmiedeberg's Arch. 355, 273–280.

Ameri, A., 1998. The effects of Aconitum alkaloids on the central nervous system. Prog. Neurobiol. 56, 211–235.

Ameri, A., Shi, Q., Aschhoff, J., Peters, T., 1996. Electrophysiological effects of aconitine in rat hippocampal slices. Neuropharmacology 35, 13–22.

Andreasen, M., Nedergaard, S., 1996. Dendritic electrogenesis in rat hippocampal CA1 pyramidal neurons: functional aspects of Na^+ and Ca^{2+} currents in apical dendrites. Hippocampus 6, 79–95.

Apland, J.P., Cann, F., 1995. Anticonvulsant effects of memantine and MK-801 in guinea pig hippocampal slices. Brain Res. Bull. 37, 311–316.

Bisset, N.G., 1981. Arrow poissons in China: Part II. Aconitum — botany, chemistry, and pharmacology. J. Ethnopharmacol. 4, 247–336.

Catterall, W.A., 1980. Neurotoxins that act on the voltage-sensitive sodium channels in excitable membranes. Annu. Rev. Pharmacol. Toxicol. 20, 15–43.

Catterall, W.A., 1987. Common modes of drug action on Na^+ channels: local anesthetics, antiarrhythmics and anticonvulsants. Trends Pharmacol. Sci. 8, 57–65.

Catterall, W.A., 1992. Cellular and molecular biology of voltage-gated sodium channels. Physiol. Rev. 72, 15–48.

Han, G.-Q., Chen, Y.Y., Liang, X.-T., 1988. Distribution of alkaloids in traditional Chinese medicine plants. In: Brossi, A. (Ed.), The Alkaloids 32 Academic Press, New York, pp. 241–270.

Huguenard, J.A., Hamill, O.P., Prince, D.A., 1988. Developmental changes in Na^+ conductances in neocortical neurons: appearance of a slowly inactivating component. J. Neurophysiol. 59, 778–779.

Jung, H.Y., Mickus, T., Spruston, N., 1997. Prolonged sodium channel inactivation contributes to dendritic action potential attenuation in hippocampal pyramidal neurons. J. Neurosci. 17, 6639–6646.

Ogata, M., Tatebayashi, H., 1990. Sodium current kinetics in freshly isolated neostriatal neurones of adult guinea pig. Pflugers Arch. 416, 594–603.

Rogawski, M.A., Porter, R.J., 1990. Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol. Rev. 42, 223–286.

Seitz, U., Ameri, A., 1998. Different effects on [^{3}H] noradrenaline uptake of the *Aconitum* alkaloids 3-acetylaconitine, lappaconitine, and *N*-deacetyllappaconitine in rat hippocampus. Biochem. Pharmacol. 55, 883–888.

Stuart, G.J., Sakmann, B., 1994. Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. Nature 367, 69–72.

Stuart, G.J., Sakmann, B., 1995. Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. Neuron 15, 1065–1076.

Ulbricht, W., 1998. Effects of veratridine on sodium currents and fluxes. Rev. Physiol. Biochem. Pharmacol. 133, 1–54.