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Frequency- and structure-dependent inhibition of normal and epileptiform activity by 6-benzoyldeltamine in rat hippocampal slices

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Abstract

The present study investigated the effects of the *Aconitum* alkaloids 6-benzoyldeltamine and the structurally related eldeline on neuronal activity in rat hippocampal slices. 6-Benzoyldeltamine (1–30 μM) decreased the orthodromic field potentials recorded in area CA1 in a concentration-dependent manner. The inhibitory effect of eldeline (3-100 μM) was lower. The attenuation of the postsynaptic population spike was accompanied by a simultaneous decrease in the presynaptic fibre spike evoked by electrical stimulation of the Schaffer collaterals. The input-output relationship of the presynaptic fibre spike as function of the stimulation intensity, and for the postsynaptic population spike as function of the presynaptic fibre spike was shifted to the right. Thus, electrophysiologically, these alkaloids seem 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. The inhibitory action of 6-benzoyldeltamine revealed use-dependence as obvious by an enhanced attenuation of the antidromic spike when stimulation frequency was increased. 6-Benzoyldeltamine inhibited stimulus-triggered epileptiform population bursts in area CA1 elicited by omission of Mg^{2+}, as well as spontaneously occurring epileptiform discharges in area CA3 elicited by omission of Mg^{2+} and elevation of K^{+}. Complete suppression of spontaneous activity was observed at 1 μM 6-benzoyldeltamine, which reduced the population spike only by about 20% of control. It is concluded that the inhibitory and antiepileptiform effect of 6-benzoyldeltamine is mediated by a frequency-dependent decrease in excitability, which might be important for filtering high frequency bursts of action potentials characteristic for epileptiform activity in the hippocampus.

Keywords: Hippocampus; *Aconitum* alkaloid; Population spike; Epileptiform activity

1. Introduction

It has recently been reported that different alkaloids of the plants of *Aconitum* species have depressant effect in rat hippocampal slices (Ameri, 1997a,b). Interest in this group of compounds arises from use of *Aconitum* roots in Chinese and Japanese medicine. Different alkaloids are widely used as analgesics, anesthetics, and in the treatment of various neurological disorders (for review, see Ameri, 1998). At present, only heat-processed roots are used clinically, because the aconitine-like alkaloids are converted thereby into much less poisonous alkaloids by removal of the ester functions (Tang and Eisenbrand, 1992; Isono et al., 1994). The benzoylester side chain at position C14 and the acetyl group at C8 of the aconitine molecule are mainly responsible for the high toxicity, and it has been shown that structurally related alkaloids bearing also a benzoyl group in this position are activators of the voltage-dependent Na^{+} channel (Nilius et al., 1986; Catterall, 1992; Ameri, 1997b), due to an interaction with a specific neurotoxin binding site (site 2) on the channel protein (Catterall, 1992; Wann, 1993). In contrast, alkaloids lacking this group at C14 have been reported to block this channel (Valeev et al., 1990; Seitz and Ameri, 1998).

The diterpenoid alkaloids, 6-benzoyldeltamine and eldeline, which occur in several *Aconitum* species, have very closely related chemical structures (Fig. 1). The aim of the present study was to investigate the effect of 6-benzoyldeltamine, which possesses a benzoylester side chain at C6 position, and of eldeline, which bears no benzyolester side chain at the diterpene skeleton, in order to obtain further insight into the pharmacological action of this group of alkaloids in the central nervous system. We have investigated the actions of both alkaloids on electrically evoked synaptic potentials and cell excitability in the CA1

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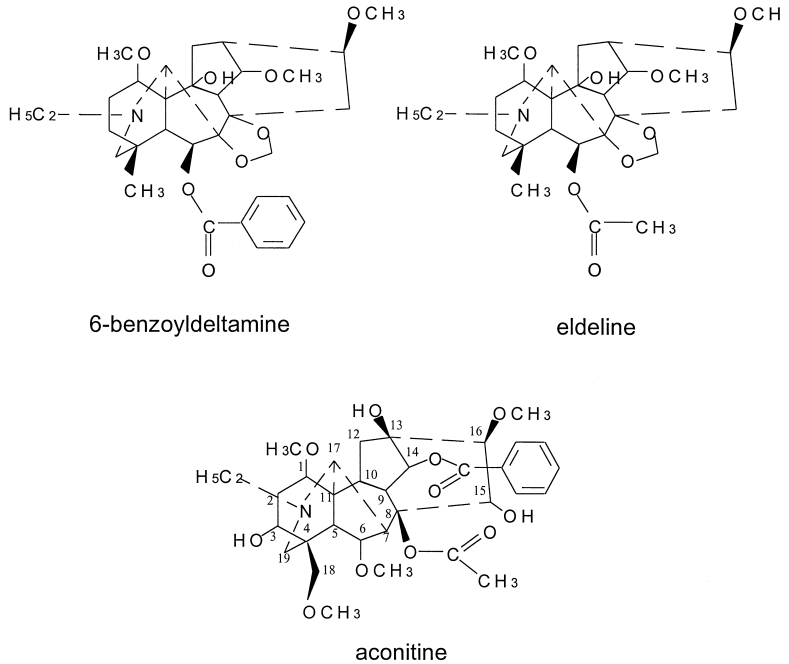

Fig. 1. Chemical structures of the *Aconitum* alkaloids, 6-benzoyldeltamine, eldeline and aconitine.

region of rat hippocampal slices by using extracellular recordings. In addition, we studied the effects of 6-benzoyldeltamine on epileptiform activity induced by activation of NMDA receptor-mediated responses in area CA1 and CA3.

2. Materials and methods

2.1. Slice preparation

Experiments were performed on hippocampal slices of male Wistar rats (150–180 g). The preparation of the hippocampus was performed as described previously (Ameri, 1997a). In brief, the rats were deeply anesthetized with diethyl ether and decapitated. The brain was quickly removed, 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 the recording chamber where it was kept submerged and held down on a nylon net with an U-shaped piece of flattened platinum wire. Chamber temperature was slowly increased from room temperature to 32°C during a period of 30 min. The other slices were maintained at room temperature in an incubation chamber. The standard artificial cerebrospinal fluid (ACSF) was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and contained (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.5, $MgSO_4$ 2, glucose 10 at a pH of 7.4. The ACSF was perfused with a flow rate of 3–4 ml min^{-1}. In some experiments, a modified ACSF was used in which no $MgSO_4$ was added (low Mg^{2+}-ACSF) in order to evoke epileptiform activity. For recording of spontaneously occurring epileptiform activity, a low Mg^{2+}/high K^+-ACSF was perfused. This solution was nominally Mg^{2+}-free, while the concentration of KCl was elevated to 5 or 8 mM.

2.2. Stimulation and recording

Recording began at least 1 h after slice preparation. Extracellular recordings of field excitatory postsynaptic potentials (field EPSPs) and population spikes were obtained from stratum pyramidale by use of microelectrodes filled with 3 M NaCl (resistance 5–10 MΩ). A concentric bipolar stainless steel electrode with 0.25 mm outer diameter (Rhodes Medical Instruments, USA) was positioned into the Schaffer collaterals for orthodromic and or in the alveus for antidromic activation of CA1 pyramidal neurons. Electrical stimuli were rectangular current pulses of 60 μs in duration delivered every 15 s (in some experiments every 5 s) through a digitally controlled stimulus isolation unit (Axon Instruments, USA). 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 isolate the presynaptic fibre spike, recordings were made in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), D-amino-5-phosphonovalerate (D-APV, 5 μM) and bicuculline (10 μM) to block postsynaptic potentials. The signal from the recording electrode was amplified by

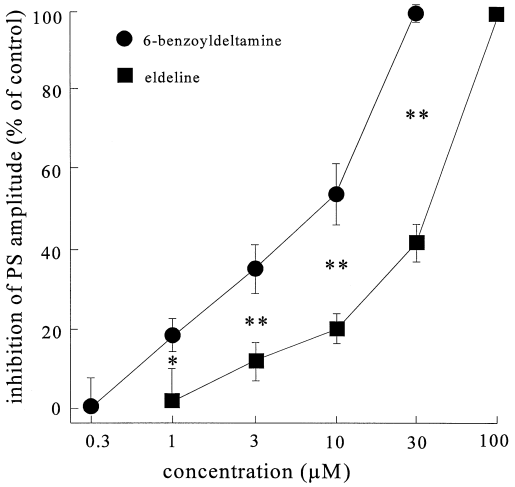

Fig. 2. Concentration–response relationship of 6-benzoyldeltamine and eldeline for the inhibition of the postsynaptic population spike (PS). The drugs were applied at each concentration to a single slice. The amplitude of the population spike was normalized with respect to control and plotted as a function of the logarithm of drug concentration. Each data point represent mean values ± S.D. from 7–10 slices. $^{*}P < 0.05$, $^{**}P < 0.001$.

means of a DP 301 amplifier (Warner Instruments, USA). Analog data were simultaneously digitized by use of the data acquisition system TIDA (HEKA Electronic, Germany).

Epileptiform activity in response to electrical stimulation was induced after a control period of 30 min in standard ACSF by omission of Mg^{2+} from the ACSF (low Mg^{2+}-ACSF) which leads to demasking of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses (Coan and Collingridge, 1985; Anderson et al., 1986; Mody et al., 1987; Tancredi et al., 1990). The experimental protocol consists of 4 periods characterized as follows: Period 1: superfusion with standard ACSF. Period 2: induction of epileptiform activity; superfusion of the low Mg^{2+}-ACSF. Period 3: test of the inhibitory effect of the alkaloids; addition of an alkaloid to the low Mg^{2+}-ACSF. Period 4: washout of the alkaloid by the low Mg^{2+}-ACSF. In all of the experiments, the spike amplitudes obtained at the end of period 3 were normalized with respect to the amplitude obtained at the end of period 2. The same experimental protocol was employed for investigation of spontaneously occurring epileptiform discharges which were elicited by superfusion of a low Mg^{2+}/high K^{+}-ACSF and recorded in the pyramidal cell layer of CA3 in absence of electrical stimulation.

Only the data of those hippocampal slices have been included into the present study which showed normal field potentials at control (i.e., no second population spike at maximal stimulation intensity) in response to electrical activation of Schaffer collaterals or alveolar fibres 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%.

2.3. Data analysis

Data are expressed as mean values ± standard deviation (S.D.). Statistical evaluation was performed by means of Student's *t*-test. Significance was assumed when $P \leq 0.05$. The amplitude of the population spike was determined from the negative peak to a tangent drawn between the preceding maximum and that following. The number of population bursts occurring during 1–5 min before as well as after 30 and 60 min of drug-application was counted, in

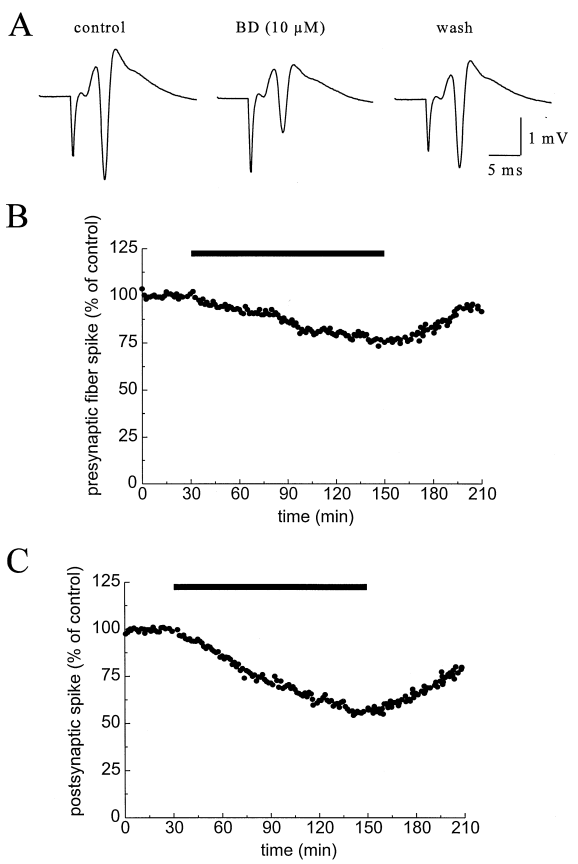

Fig. 3. Inhibitory effect of 6-benzoyldeltamine (BD, 10 μM) on the extracellularly recorded orthodromic population spike. (A) Population spikes were elicited by electrical stimulation of the Schaffer collaterals every 15 s. They represent the average of 5 subsequent responses recorded at the end of control, at the end of drug-application and at the end of the washout. (B) Time-course of the action of 6-benzoyldeltamine on the presynaptic fibre spike which preceds the postsynaptic population spike. Note that the presynaptic fibre spike partially overlaps with the postsynaptic potenial. (C) Time-course of the action of 6-benzoyldeltamine on the postsynaptic spike. Each point in (B) and (C) represents the average of the amplitudes of 5 subsequent measurements. The bar above the graphs indicates the time of drug application. The recordings in (A) and the graphs (B and C) were obtained from the same slice. One representative experiment out of 8 similar ones is shown.

order to determine the average firing rate which was used for the quantification of the depressions.

2.4. Drugs

6-Benzoyldeltamine and eldeline were both obtained from Latoxan (Rosans, France). For the present experiments, the alkaloids were dissolved in dimethylsulfoxide (DMSO) to give stock solutions of 10 or 100 mM. These solutions were diluted with ACSF to reach the desired concentrations and gassed before being perfused into the bathing medium. Control experiments have revealed that the highest final DMSO concentration (0.1%) did not affect any of the measured parameters. CNQX-HBC complex was purchased from RBI Biotrend Chemicals (Köln, Germany) and 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. When determining the input–output relationship, two concentrations were applied in a cumulative manner.

3. Results

3.1. Effects of the alkaloids on the orthodromic and antidromic response in standard ACSF

In a first series of experiments, we tested the effects of several concentrations of both 6-benzoyldeltamine and eldeline on evoked synaptic potentials recorded extracellularly from the CA1 region. As shown in Fig. 2, both alkaloids decreased the amplitude of the orthodromically evoked postsynaptic population spike in a concentration dependent manner. With a concentration of 1 μM of 6-benzoyldeltamine and 3 μM of eldeline, the effect became statistically significant. The inhibitory potency of eldeline was lower than that of 6-benzoyldeltamine. At a concentration of 10 μM, 6-benzoyldeltamine decreased the amplitude of the postsynaptic population spike by 54.84 ± 9.7% of control ($n = 8$, $P < 0.001$), eldeline by 20.10 ± 2.9% of control ($n = 5$, $P < 0.001$). Maximal effect, i.e., complete suppression of the postsynaptic spike, was observed by superfusion of 6-benzoyldeltamine and eldeline at a concentration of 30 and 100 μM, respectively. The

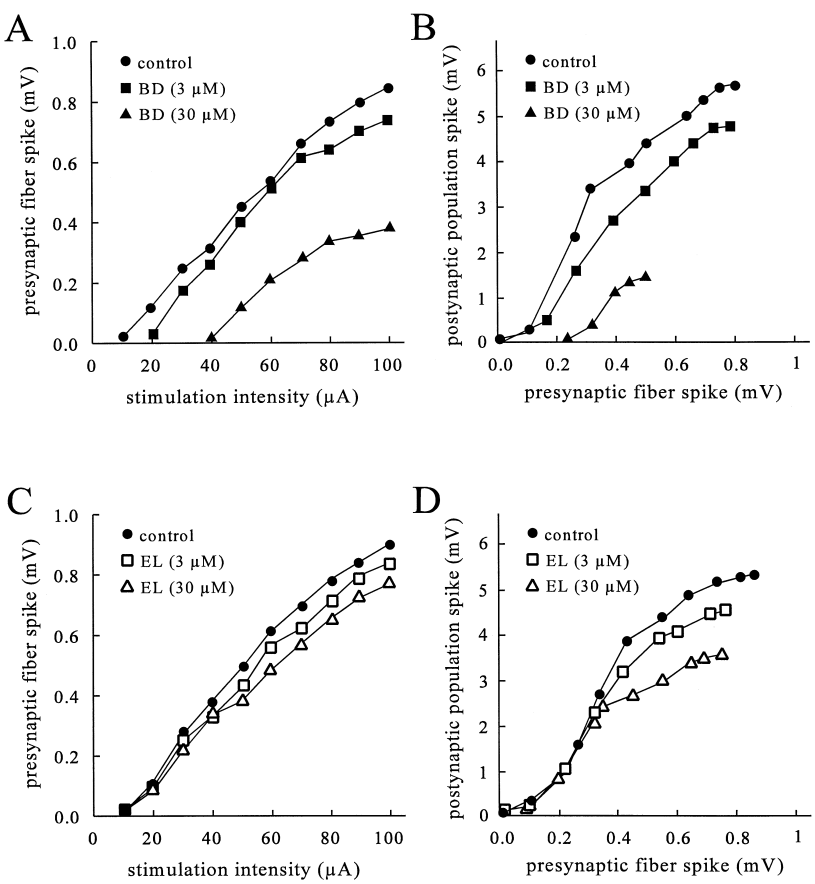

Fig. 4. Input–output relationship for the orthodromic response at control and in presence of two different concentration of (A, B) 6-benzoyldeltamine (BD) and (C, D) eldeline (EL). Each concentration was applied for 120 min. The hippocampal slices were stimulated with intensities ranging from subthreshold to maximal. The presynaptic fibre spike is related to the stimulus-intensity (A, C) and the postsynaptic population spike is related to the fibre spike (B, D). Due to the simultaneous recording of the presynaptic fibre spike and the field EPSP, there might be a partial overlap of the fibre spike with the synaptic potential. For each drug, a representative experiment out of five similar ones is shown.

decrease in population spike amplitude had a slow onset (about 10 min) and recovered within 60–90 min of drug washout at all concentration tested (Fig. 3). As obvious from Fig. 3B, the decrease in the postsynaptic population spike, which indicates the synchronous discharge of the pyramidal cells, was accompanied by a simultaneous decrease of the presynaptic fibre spike with the same onset. The presynaptic fibre spike which precedes the postsynaptic population spike represents the compound action potential and is generated in the stimulus-activated Schaffer collaterals. Under normal conditions, the presynaptic fibre spike partially overlaps with the synaptic field potentials. In order to quantify the effect of 6-benzoyldeltamine on the presynaptic excitability, we isolated the presynaptic fibre volley pharmacologically by recording the field potentials in the presence of the selective AMPA/kainate receptor antagonist CNQX (10 μM), the NMDA receptor antagonist D-APV (5 μM) and the GABA receptor antagonist bicuculline (10 μM), in order to block postsynaptic potentials. Under this condition, the presynaptic fibre spike was clearly visible without an overlapping postsynaptic potential. 6-Benzoyldeltamine (10 μM) reduced the amplitude of the isolated presynaptic fibre spike by 29.08 ± 9.3% of control ($n = 5$, $P < 0.01$, data not shown) indicating a direct depressant action on the excitability of the Schaffer collaterals.

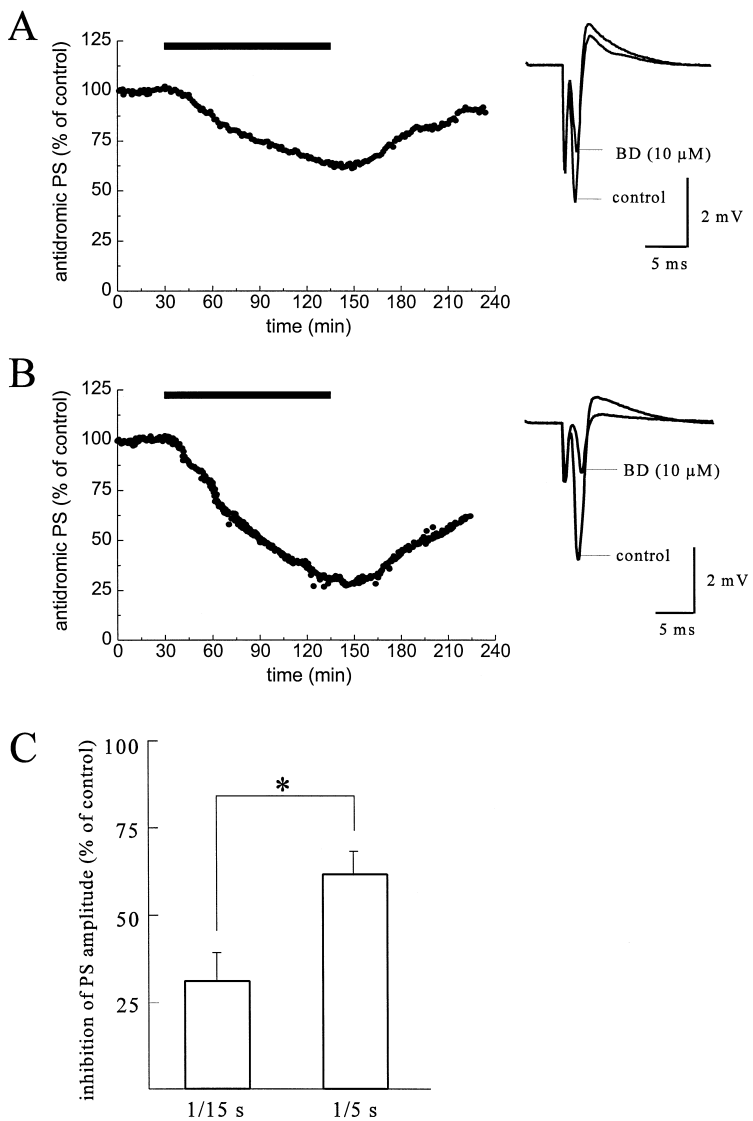

Fig. 5. Frequency dependence of the inhibitory action of 6-benzoyldeltamine (BD, 10 μM) on the amplitude of the antidromic population spike. The graphs show the time-course of the effect when the hippocampal slices were stimulated antidromically every 15 s (A) and every 5 s (B). For each stimulation frequency, one representative experiment out of 6 similar ones is shown. Each data point in the graphs represents the average of five subsequent measurements. The bar indicates the time of drug application. On the right of the graphs, antidromic population spikes recorded at the end of control and at the end of drug application of the according experiments are shown. (C) Comparison of the effects obtained with the two different stimulation frequencies. The increase in frequency significantly enhanced the inhibitory effect of 6-benzoyldeltamine (* $P < 0.001$). Each column represents the mean value ± S.D. of 7 experiments.

Since the postsynaptic population spike recorded in CA1 stratum pyramidale is driven partially by the stimulus-evoked excitability of the Schaffer collaterals, we wanted to determine the relationship of the presynaptic fibre spike to the stimulation intensity, as well as the relationship of the postsynaptic population spike to the fibre spike over a range of different stimulus intensities. For this purpose, input–output functions were measured before drug-application (control) and after application of 6-benzoyldeltamine and eldeline. Electrical stimuli of increasing intensity were applied to the Schaffer collaterals, and the amplitudes of the according fibre spikes and postsynaptic population spikes were measured. Fig. 4 shows a concentration-dependent shift of the input–output curve to the right. 6-Benzoyldeltamine decreased the amplitude of the presynaptic fibre spike at all stimulus intensities tested. This indicates that there was a clear attenuation in the excitability of the afferent fibres. Eldeline was less potent than 6-benzoyldeltamine in this respect and affected the fibre spike only at higher stimulation intensities. The input–output curve of the postsynaptic population spike as function of the presynaptic fibre spike was also shifted to right by 6-benzoyldeltamine and to a lesser extent also by eldeline. This indicates that, for identical fibre spikes, the cell is further from the firing threshold, so that the synchronous discharge of the same population of cells is reduced. In addition to the shift to the right of the input–output curves, their maximum was decreased. The maximum of the input–output curves depends mainly on two factors: (1) on the excitability of the afferents, and (2) on the properties of the postsynaptic neurons. The excitability of the afferents fibres innervating the CA1 pyramidal neurons is decreased obviously by recording the fibre spike in isolation of postsynaptic potentials. Therefore, the alkaloid-induced attenuation of the postsynaptic population spike seems at least in part to be due to a change in the excitability of the Schaffer collaterals.

However, the sensitivity of the postsynaptic population spike to the actions of 6-benzoyldeltamine and eldeline may suggest also a decrease in the excitability of pyramidal cells. We therefore investigated the effect on the antidromic population spike. The antidromic population spike was elicited by direct, alveolar stimulation of the CA1 pyramidal cells. The amplitude of the antidromic spike represents the compound action potential of the group of neurons from which recordings was performed. At a concentration of 10 μM, 6-benzoyldeltamine significantly reduced the amplitude of the antidromic population spike by $33.75 \pm 7.4\%$ of control ($n = 7$, $P < 0.001$). This means that 6-benzoyldeltamine had a significant lower inhibitory activity on the antidromic spike than on the orthodromic spike. However, as obvious from Fig. 5, the depressant action of the drug on the antidromic spike was significantly enhanced when stimulation frequency was increased from 4 min^{-1} (Fig. 5 A) to 12 min^{-1} (Fig. 5 B). Control experiments revealed that in untreated slices there was no frequency-dependent change in the amplitude of the antidromic spike during an observation period of up to 6 h.

To investigate whether the decrease in the amplitude of the postsynaptic population spike, which indicates the synchronous discharge of a population of cells in the vicinity of the recording electrode, is due to a reduced excitatory synaptic drive, extracellular recordings of the field EPSP were performed. The field EPSP reflects synaptic currents in the dendrites of the pyramidal cells as a result of the action of neurotransmitters. The major portion of the excitatory synaptic drive measured as the initial slope of the field EPSP is mediated by glutamate acting on AMPA receptors. 6-Benzoyldeltamine (10 μM) exerted a significant decrease in the slope of the field EPSP by $46.90 \pm 11.6\%$ of control ($n = 7$, $P < 0.001$). At the highest concentration tested, the drug completely suppressed the field EPSP.

3.2. Effects of 6-benzoyldeltamine on epileptiform activity in hippocampal area CA1 and CA3

Since 6-benzoyldeltamine exerted a frequency-dependent inhibitory action on hippocampal excitability, we

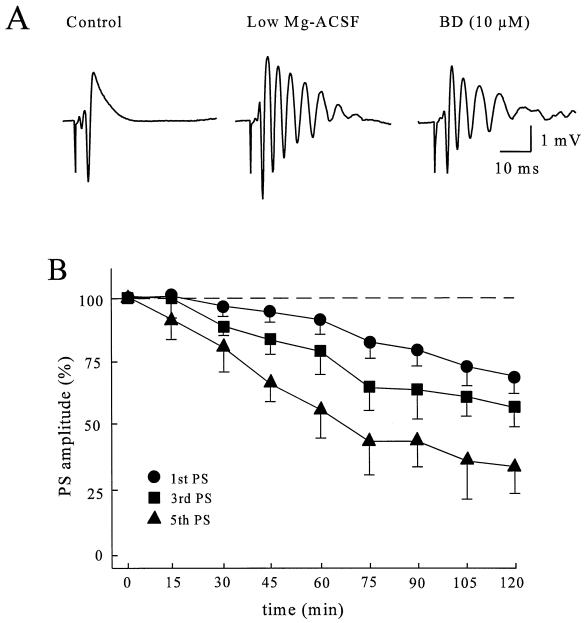

Fig. 6. Inhibitory effect of 6-benzoyldeltamine (BD, 10 μM) on the orthodromic response in a nominal Mg^{2+}-free ACSF recorded in CA1 stratum pyramidale. (A) Stimulus-triggered population spikes from a representative experiment out of nine similar ones showing the effect of 6-benzoyldeltamine on a epileptiform population discharge. (B) Time-course and sensitivity to 6-benzoyldeltamine of the first, the third and the fifth population spike (PS) in the epileptiform burst. The amplitudes of the spikes were normalized with respect to the amplitudes measured during application of the nominal Mg^{2+}-free ACSF. Data points represent mean value ± S.D. of 9 experiments. A significant decrease of the third and the fifth spike was observed after 30 and 15 min of drug-application, respectively, whereas the first postsynaptic population spike was significantly decreased after 45 min.

investigated the effects of this compound on (1) stimulus-triggered epileptiform activity in rat hippocampal area CA1 and (2) spontaneously occurring epileptiform activity in area CA3.

Epileptiform activity in response to electrical stimulation was induced after a control period of 30 min in standard ACSF by omission of Mg^{2+} from the ACSF (low Mg^{2+}-ACSF) which leads to an unmasking of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses (Coan and Collingridge, 1985; Anderson et al., 1986). After recording 15–20 min in absence of Mg^{2+}, the orthodromic response in CA1 stratum pyramidale was changed into an epileptiform bursting, made up by an increase in amplitude of the primary postsynaptic population spike and by the building up of additional multiple epileptiform population spike evoked by the electrical stimulation of the afferents. The epileptiform discharge of population spikes is the extracellular counterpart of an increase in the capability of the neuronal membrane to elicit an action potential. Significant components of the epileptiform burst discharges include the presynaptic fibre spike due to the stimulus-evoked activity of the Schaffer collaterals, the first population spike, and 7–10 succeeding spikes which define epileptiform activity. The amplitudes of these spikes became stable after another 15–20 min and were observed in control experiments to persist during the entire recording time of up to 6 h.

As shown in Fig. 6, 6-benzoyldeltamine (10 μM) exerted a depressant action on the stimulus-triggered population burst. The antiepileptiform effect of the alkaloid manifested itself as a decrease in the amplitudes of the additional population spikes, as well as a significant decrease in the number of the additional spikes. After 30 min of drug application, the additional spikes were significantly decreased, whereas the first postsynaptic population spike was not yet significantly changed. The number of spikes in the low Mg^{2+} burst was reduced from 8.44 ± 1.1 in absence to 6.13 ± 1.3 in presence of 6-benzoyldeltamine ($n = 9$, $P < 0.01$).

The experiments reported above indicate an inhibition of stimulus-triggered epileptiform activity in CA1 stratum pyramidale.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. The CA3 pyramidal neurons provide a synchronous excitatory drive for the downstream CA1 pyramidal cells. Accordingly, the antiepileptiform effect of 6-benzoyldeltamine observed in the CA1 subfield could represent a consequence of an inhibition of excitability occurring first in area CA3. This, in turn, would decrease the glutamatergic input in CA1. For this purpose, we performed recordings of spontaneous epileptiform activity elicited by omission of Mg^{2+} and elevation of K^+ in the pyramidal cell layer of the CA3 region. No electrical

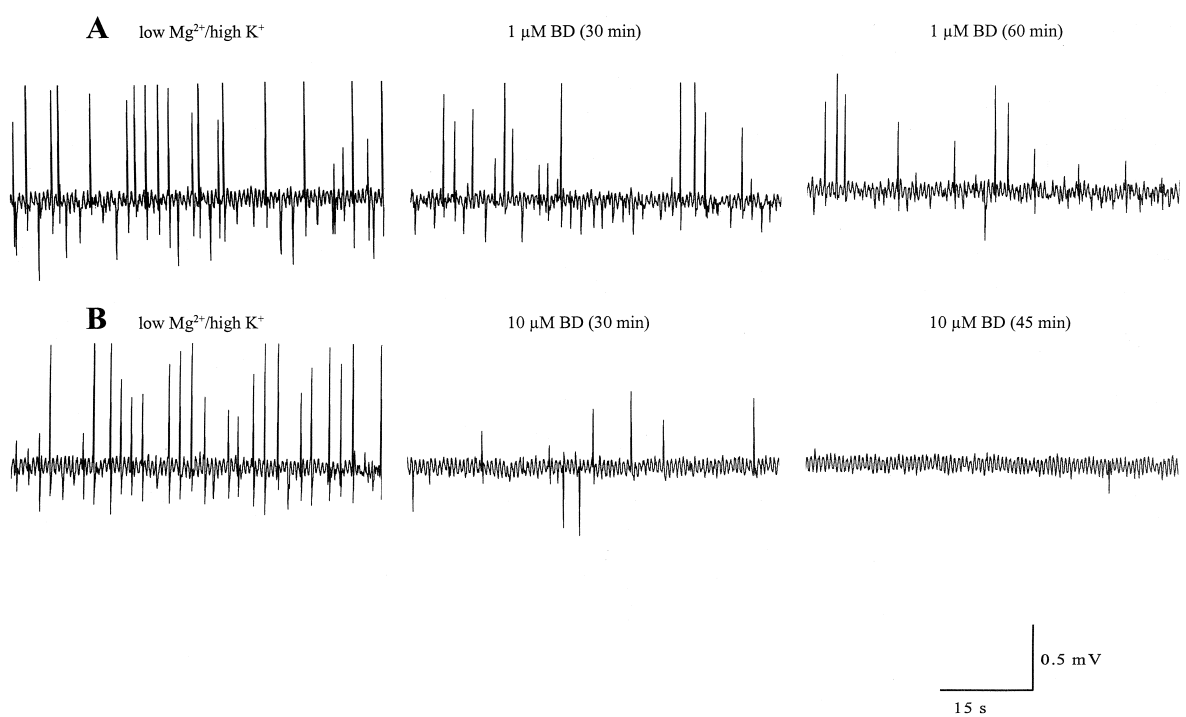

Fig. 7. Inhibitory effect of 6-benzoyldeltamine (BD, 1 and 10 μM) on epileptiform activity induced by omission of Mg^{2+} and elevation of K^+ to 8 mM. Spontaneously occurring epileptiform discharges were recorded in absence of electrical stimulation in stratum pyramidale of the CA3 region. Scale bars apply to the upper and lower traces.

stimuli were applied during these experiments. Since it is known that the appearance of spontaneous epileptiform discharges generated by hippocampal pyramidal cells is dependent upon the K^+ concentration (Tancredi and Avoli, 1987), we elevated $[K^+]_o$ either to 5 or to 8 mM. The augmentation of the $[K^+]_o$ caused recurrent epileptiform discharges with a regular repetition rate about 20–40 min after starting the superfusion of the low Mg^{2+}/high K^+-ACSF. In presence of 5 mM KCl, the epileptiform burst discharges occurred with a repetition rate of 19.57 ± 5.3 min^{-1} ($n = 7$), whereas at 8 mM KCl (Fig. 7), they occurred with a repetition rate of 32.47 ± 6.5 min^{-1} ($n = 16$). After stabilization of the activity (about 20–30 min after onset of the epileptiform discharges), 6-benzoyldeltaline was applied at a concentration of either 1 or 10 μM for a period of 60 min. As shown in Figs. 7 and 8, the alkaloid exerted a concentration-dependent decrease in the frequency of the spontaneously occurring recurrent discharges. Already 15 min after starting the application of 1 μM or 10 μM 6-benzoyldeltamine, the burst frequency elicited by 8 mM KCl was significantly reduced to $86.11 \pm 8.4\%$ ($n = 7$, $P < 0.001$) and to $56.01 \pm 12.8\%$ ($n = 9$, $P < 0.001$) of the repetition rate observed at the control prior to drug application. About 45 min after starting the superfusion with 10 μM 6-benzoyldeltamine, the bursting activity in area CA3 of the hippocampal slices was fully suppressed in every slice tested. When the K^+ concentration was elevated to 5 mM, there was no difference in the onset of antiepileptiform effect of 10 μM 6-benzoyldeltamine. After 15 min, burst frequency was reduced to $56.07 \pm 12.2\%$ ($n = 8$, $P < 0.001$). However, complete suppression of spontaneous bursting was achieved after an application time of 60 min.

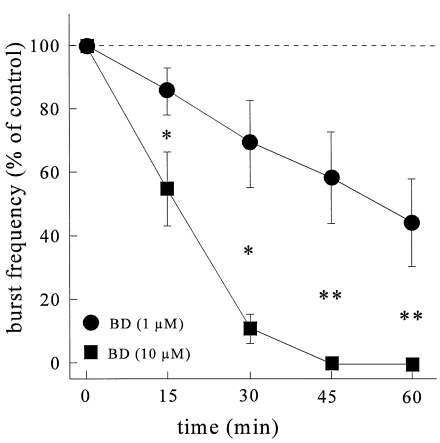

Fig. 8. Time-course of the effect of 6-benzoyldeltamine (BD, 1 and 10 μM) on the frequency of spontaneously occurring epileptiform burst discharges in stratum pyramidale of the CA3 region. Epileptiform activity was elicited by omission of Mg^{2+} and elevation of K^+ to 8 mM. Data points represent the mean value ± S.D. of 7 and 9 experiments with 1 and 10 μM 6-benzoyldeltamine, respectively. At a concentration of 10 μM, the antiepileptiform action of the alkaloid was significantly higher ($^*P < 0.01$, $^{**}P < 0.001$). Note that 45 min after starting the superfusion with 10 μM 6-benzoyldeltamine, burst activity was completely suppressed.

4. Discussion

In the present study, we investigated the effects of the *Aconitum* alkaloid 6-benzoyldeltamine on rat hippocampal excitability and compared them with the effects of the structurally related alkaloid eldeline. The primary goal of this investigation was twofold: first to examine the action of these alkaloids on neuronal excitability, and second, to get information about a possible relation between chemical structure and pharmacological effect of this group of alkaloids. The alkaloids 6-benzoyldeltamine and eldeline fulfill the requirement as a pharmacological tool for this purpose, because, as compared with aconitine, they lack 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. Changes of neuronal activity of pyramidal cells in the hippocampus can be 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).

The present findings demonstrated an inhibitory action of 6-benzoyldeltamine in a concentration range of 1 and 30 μM and of eldeline in a range of 3 and 100 μM. It is intriguing that although the molecular structures of both alkaloids differ only in the presence or absence of a benzoylester group at C6 position, the present results indicated a significant difference in their inhibitory potency. The major finding of the present study is that the inhibitory and antiepileptiform effect of 6-benzoyldeltamine is mediated by frequency-dependent changes in the threshold for both the presynaptic fibre spike and the postsynaptic population spike that indicate a possible effect on Na^+ channels. This is supported by the following results: First, the investigation of the input–output relationship indicated that the inhibitory effect on the postsynaptic potentials exerted by the alkaloids was mediated by a decreased excitability of the afferent fibres. This 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. Secondly, this conclusion is supported by the recordings of the presynaptic fibre spike after pharmacological isolation of postsynaptic potentials, which provide clear evidence for a decrease in fibre excitability by 6-benzoyldeltamine. Furthermore, the shift to the right of the input-output relationship indicates a change in firing threshold. In respect of a possible interaction with the neurotoxin binding site 2 on the voltage-dependent Na^+ channel, modification

of firing threshold for axonal discharges could be the result of a modification of the properties of Na^+ channels.

Furthermore, there is a large body of evidence for active Na^+ conductances in the dendrites of the pyramidal neurons which contribute to an amplification of the EPSPs at dendritic level and which have significant effects on the integration of synaptic inputs (Stuart and Sakmann, 1994, 1995; Andreasen and Nedergaard, 1996; Jung et al., 1997). If Na^+ channels are affected by the investigated alkaloids, they thereby could suppress amplification of the EPSPs at dendritic level and decrease the level of firing. Thus, this postsynaptic mechanism could contribute to the attenuation of both the field EPSP and the postsynaptic population spike reported in the present study.

It is obvious from the investigation of the antidromic population spike that the inhibitory action of 6-benzoyldeltamine was enhanced by an increase in stimulation frequency. This finding is in line with previously investigated alkaloids (Ameri, 1997a,b) and suggests an use-dependent mechanism of action. Moreover, control studies with untreated slices have shown that the spike amplitudes were stable when stimulation frequency was enhanced. Thus, due to the frequency-dependent attenuation of the antidromic spike, an involvement of long-term depression occurring at the higher stimulation frequency can be excluded. It is well known that several local anaesthetics, antiarrhytmics and anticonvulsants like phenytoin reduce the probability of Na^+ channels openings when membranes are depolarized while allowing channel opening when membranes are hyperpolarized (Catterall, 1987; Ragsdale et al., 1991; Wann, 1993; Taylor and Meldrum, 1995). Indeed, we have shown that 6-benzoyldeltamine depressed epileptiform activity in area CA1 and CA3. The multiple population spikes elicited by removal of Mg^{2+} from the superfusate were decreased in amplitude, albeit not completely suppressed.The antiepileptiform effect of 6-benzoyldeltamine was more pronounced in the CA3 pyramidal layer of the hippocampus, where it produced a complete suppression of spontaneously occurring recurrent burst discharges. Complete suppression of epileptiform activity was already observed at a concentration of 6-benzoyldeltamine of 1 μM, i.e., with a concentration which caused an attenuation of the postsynaptic population spike recorded in CA1 in standard ACSF by about 20%. Moreover, we have shown that the antiepileptiform effect of 6-benzoyldeltamine was further enhanced when increasing $[K^+]_o$. The enhanced depressant effect on spontaneously occurring epileptiform activity in presence of the higher KCl concentration, as well as the enhanced inhibition of the antidromic spike at the higher stimulation frequency might be explained by an accumulation of the alkaloid in Na^+ channels in highly active neurons. The use-dependent effect may be important for filtering high frequency bursts of action potentials characteristic for epileptiform activity in the hippocampus, and thereby sparing the normal neuronal activity.

5. Conclusion

6-Benzoyldeltamine exerts an antiepileptiform action in rat hippocampal slices by the use-dependent attenuation of afferent excitability, thereby decreasing excitatory neurotransmission at the CA3/CA1-synapse.

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