ELSEVIER

European Journal of Pharmacology 335 (1997) 145–152

ejp

Inhibition of rat hippocampal excitability by the *Aconitum* alkaloid, 1-benzoylnapelline, but not by napelline

Angela Ameri *

Department of Pharmacy and Pharmacology of Natural Compounds, University of Ulm, Helmholtzstraße 20, D-89081 Ulm, Germany

Received 17 February 1997; revised 21 July 1997; accepted 25 July 1997

Abstract

The effects of the two structurally related *Aconitum* alkaloids, 1-benzoylnapelline and napelline, were investigated by extracellular recording of the stimulus-evoked population spike in the CA1 region of rat hippocampal slices in vitro. 1-Benzoylnapelline (1–100 μM) exerted a depressant action on the orthodromic as well as on the antidromic population spike. Napelline failed to evoke a significant effect at concentrations up to 100 μM. The inhibitory action induced by 1-benzoylnapelline was enhanced when the frequency of electrical stimulation was increased. In contrast, reversal of the inhibitory effect was accelerated when stimulation frequency was decreased. The activity-dependent mode of action of 1-benzoylnapelline raised the question of whether the drug is effective to suppress epileptiform activity. The results obtained from experiments on epileptiform hippocampal slices revealed a reduction of the burst duration and of the number of spikes in the burst as well as attenuation of the amplitude of the population spikes. These data support the conclusion that 1-benzoylnapelline, in contrast to the structurally related compound, napelline, has an activity-dependent inhibitory action on central neurons. © 1997 Elsevier Science B.V.

Keywords: Hippocampus; Epileptiform activity; *Aconitum* alkaloid

1. Introduction

It has recently been reported that different alkaloids of the plants, *Aconitum* spec., have depressant effects in rat hippocampal slices (Ameri et al., 1996a,b). Interest in this group of compounds arises from the use of *Aconitum* roots in Chinese traditional medicine and folk medicine. Different *Aconitum* alkaloids are widely used as antirheumatics, analgesics, anesthetics, and in the treatment of various neurological disorders (Bisset, 1981; Han et al., 1988). Aconitine, the main alkaloid of *Aconitum* spec., binds to site 2 of the voltage-dependent Na^+ channel, activates the Na^+ channel at resting membrane potential and inhibits inactivation (Catterall, 1980). In contrast, lappaconitine, which has been shown to inhibit epileptiform activity in rat hippocampal slices (Ameri et al., 1996a), is a blocker of the voltage-dependent Na^+ channel (Valeev et al., 1990). The plant alkaloids, 1-benzoylnapelline and napelline, occur in several *Aconitum* species, including *A. napellus* from which their name derives (Benn and Jacyno, 1983). These two alkaloids have very closely related chemical structures (Fig. 1). The effects of both compounds in the central nervous system have not been investigated.

The aim of the present study was to examine the effects of 1-benzoylnapelline and napelline on rat hippocampal excitability in order to obtain further insight into the action of this class of alkaloids. Moreover, it was of interest to investigate if the pharmacological effects of these alkaloids correlate with their chemical structure. In the present study, the effects of both compounds on stimulus-evoked population spikes in normal and epileptiform rat hippocampal slices were investigated by means of extracellular recordings.

2. Materials and methods

2.1. Slice preparation

Experiments were performed on 108 hippocampal slices from 150 to 180 g male Wistar rats. The preparation of the

* Tel.: (49-731) 502-4286; Fax: (49-731) 502-4299.

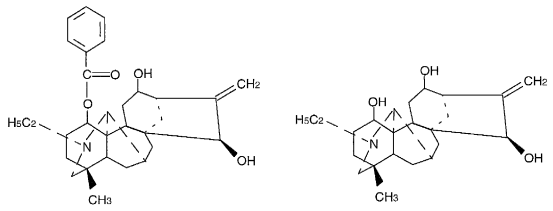

Fig. 1. Chemical structure of the *Aconitum* alkaloids 1-benzoylnapelline and napelline.

hippocampus was performed as described previously (Ameri and Jurna, 1991). Slices (400 μm thick) were cut transversely to the longitudinal axis of the hippocampus with a McIlwain tissue chopper. In the recording chamber, the slices were kept submerged and held down on a nylon net by a U-shaped piece of flattened platinum wire. The standard artificial cerebrospinal fluid (ACSF) was gassed with 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 15 at a pH of 7.4. It was superfused at a flow rate of 2 ml min^{-1} and had a temperature of 32°C. After 2 min, the perfusion fluid reached the recording chamber which had a volume of 1.5 ml. In some experiments, a modified ACSF was used in which either no $CaCl_2$ was added and $MgSO_4$ was increased to 4 mM (low Ca^{2+}/high Mg^{2+}-ACSF) in order to block synaptic transmission, or in which no $MgSO_4$ was added (nominally Mg^{2+}-free ACSF) in order to evoke epileptiform activity. For recording of spontaneous epileptiform activity a low Mg^{2+}/high K^+-ACSF was perfused. This solution was nominally Mg^{2+}-free and contained 8 mM KCl.

2.2. Extracellular recording and electrical stimulation

Extracellular recording electrodes (3 M NaCl, 5–10 MΩ) were placed in CA1 stratum pyramidale for recording of stimulus-evoked population spikes. Field excitatory postsynaptic potentials (field e.p.s.ps) were recorded from the stratum radiatum. A bipolar concentric stimulation electrode (Rhodes Medical Instruments, USA) was positioned in the Schaffer collateral commissural pathway or in the alveus for orthodromic or antidromic stimulation, respectively. The distance between the recording electrode and the stimulation electrode was approximately 0.5 mm. Extracellular stimuli (200 μs in duration) were delivered every 15 s (in some experiments every 5 s). Drug effects were investigated on population spikes elicited by using half-maximal stimulus strength which was adjusted for each slice at the beginning of the experiment. The signal from the recording electrode was amplified by means of a DP 301 amplifier (Warner Instruments, USA). Analog data were digitized by use of the TIDA data acquisition system (HEKA electronic, Germany).

2.3. Induction of epileptiform activity

Epileptiform activity was induced after a control period of 30 min in standard ACSF by means of two different experimental protocols. In one set of experiments, epileptiform activity was elicited with bicuculline in order to block GABA receptors (Campbell and Holmes, 1984; Herron et al., 1985; Ault and Wang, 1986; Chagnac-Amitai and Connors, 1989), in another set of experiments, $MgSO_4$ was omitted from the bathing medium, leading to activation of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses (Coan and Collingridge, 1985; Anderson et al., 1986; Mody et al., 1987). In standard ACSF, electrical stimulation elicits a single population spike superimposed on a field excitatory postsynaptic potential. During perfusion of the slices with the epileptogenic ACSF, however, the same stimulus evokes synchronized population bursts, each consisting of multiple spike discharges. Significant components of the epileptiform burst discharges include the presynaptic fiber spike, the first postsynaptic population spike and succeeding spikes which define epileptiform activity. The experimental protocol consisted of 4 periods characterized as follows: Period 1: perfusion with standard ACSF (control). Period 2: induction of epileptiform activity; perfusion of the epileptogenic ACSF. Period 3: test of the effect of the alkaloid; addition of the alkaloid to the epileptogenic ACSF used in period 2. Period 4: washout of the alkaloid with the epileptogenic ACSF. In all experiments, the individual amplitudes of the multiple population spikes were quantified as mean percentages of the change in response amplitude when compared to responses obtained during perfusion of the epileptogenic ACSF in period 2.

2.4. Data analysis

Data are expressed as means ± 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.

2.5. Drugs

1-Benzoylnapelline and napelline (obtained from O. Krishtal, Kiev, Ukraine) were dissolved in dimethylsulfoxide (DMSO) to give stock solutions of 100 mM. These solutions were diluted with ACSF to yield final concentrations varying between 1 and 100 μM. The final concentration of DMSO never exceeded 0.1%, a concentration which did not affect any of the parameters measured as shown in control experiments. Bicuculline (Sigma, Germany) was dissolved in distilled water and applied at a concentration of 10 μM to the ACSF.

3. Results

Only data from the hippocampal slices which showed normal field potentials (i.e., no second population spike by maximal stimulation intensity) in response to electrical activation of Schaffer collaterals or alvear fibers in standard ACSF were used for calculations. Furthermore, population spikes had to be stable during a control period of at least 30 min prior to application of drugs.

3.1. Effects of 1-benzoylnapelline and napelline on the orthodromic and antidromic population spike

1-Benzoylnapelline (1–100 μM) exerted a depressant action on the amplitude of the orthodromic and antidromic population spike recorded in CA1 stratum pyramidale in a concentration-dependent manner (Fig. 2). At concentrations from 10–100 μM, the inhibition of the orthodromic spike was significantly stronger than the inhibition of the antidromic spike. The attenuation of the orthodromic response affected both the presynaptic fiber spike (afferent volley) and the postsynaptic population spike which is generated by synchronous activity of the CA1 pyramidal neurons. There was no visible effect on the onset of the presynaptic fiber spike and of the antidromic population spike, indicating that conduction velocity was not affected by the alkaloid. The onset of the inhibitory action of 1-benzoylnapelline on the presynaptic fiber spike occurred simultaneously with the inhibition of the postsynaptic population spike. The amplitude of the presynaptic fiber spike, which represents the compound action potential generated in the stimulus-activated Schaffer collaterals, was reduced by 1-benzyolnapelline (10 μM) by 18.36 ± 4.8% ($n = 7$, $P \leq 0.01$). This decrease did not differ from the corresponding decrease of the antidromic spike (20.87 ± 2.1%, $n = 7$).

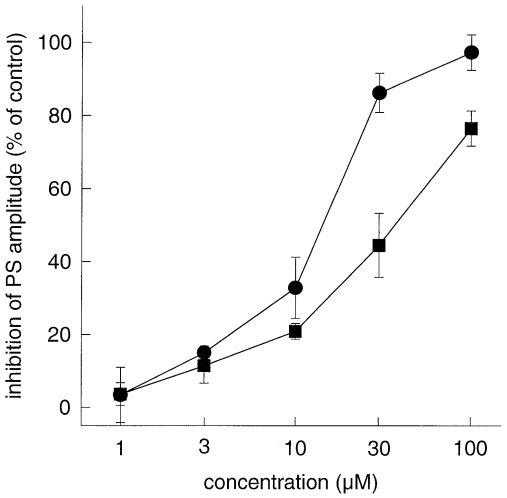

Fig. 2. Concentration–response curve for 1-benzoylnapelline in rat hippocampal slices. 1-Benzoylnapelline (1 μM to 100 μM) exerted an inhibitory action on both the orthodromic (●) and the antidromic (■) population spike (PS). Each slice was treated with a single concentration of the drug. At concentrations from 10–100 μM the differences between the curves were significant (at least $P \leq 0.05$) The amplitude of the population spike was normalized with respect to the control and was plotted as a function of the logarithm of drug concentration. Each point represents the mean value ± S.D. recorded from 7 or 8 slices.

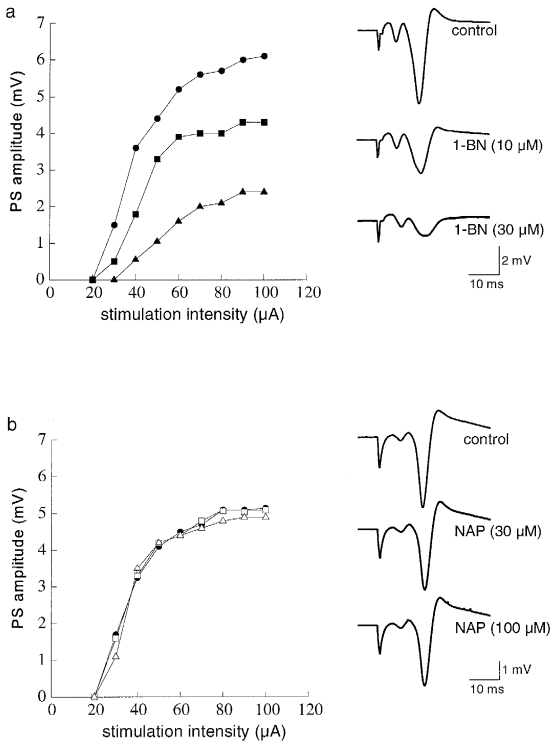

Fig. 3. Effect of 1-benzoylnapelline and napelline on the input–output relationship. The amplitude of the postsynaptic population spike was measured as a function of stimulus intensity in the control (●) and in the presence of various concentrations of the alkaloids. 1-Benzoylnapelline (a) was applied at a concentration of 10 μM (■) and 30 μM (▲), napelline (b) at a concentration of 30 μM (□) and 100 μM (△), respectively. Each graph shows a representative experiment out of 7 similar ones. On the right of the graphs, orthodromic population spikes for the relevant experiment are shown. The spikes were evoked by half-maximal stimulation intensity. Each trace is the average of 5 subsequent events measured at the end of drug application.

The antidromic population spike is elicited by direct, alvear stimulation of the CA1 pyramidal cells recorded. In response to antidromic stimulation, synaptic activation of CA1 pyramidal cells via recurrent loops or perhaps by orthodromic stimulation of basal dendritic afferents might occur and could contribute to the generation of the evoked potentials observed in these experiments. In order to eliminate a possible contribution of presynaptic mechanisms to the inhibitory action induced by 1-benzoylnapelline, synaptic transmission was blocked by a low Ca^{2+}/high Mg^{2+} medium (Andersen et al., 1978). There was no significant difference in the effect of 1-benzoylnapelline (10 μM) on the antidromic population spike, when a low Ca^{2+}/high Mg^{2+}-ACSF was used in order to eliminate a possible contribution of presynaptic processes to the action of the drug ($n = 6$). The inhibition of the antidromic spike in-

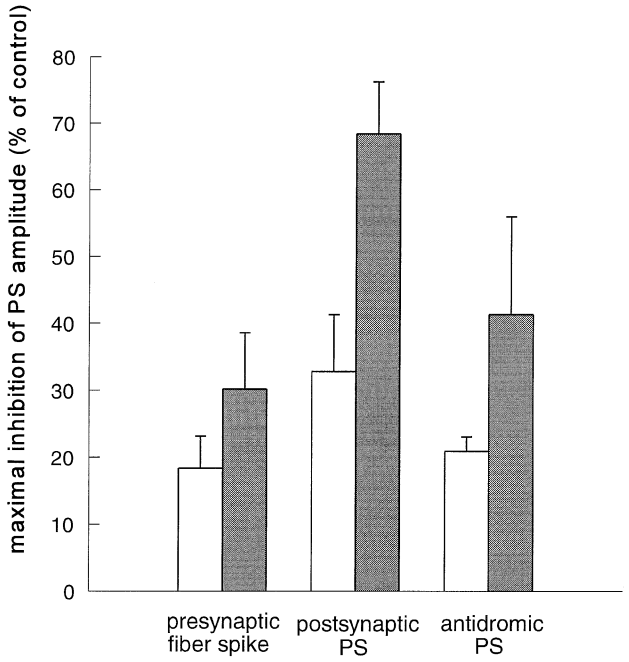

Fig. 4. Potentiation of the inhibitory action of 1-benzoylnapelline (10 μM) by an increase in stimulation frequency. The columns represent the means ± S.D. in % of the control amplitude. Each column represents the data from 7–8 experiments. An increase in stimulation frequency from 1 pulse per 15 s (white columns) to 1 pulse per 5 s (grey columns) enhanced significantly (at least $P \leq 0.01$) the drug-induced inhibition of the population spikes (PS).

duced by this alkaloid even in the absence of synaptic transmission suggests that it is mediated by a change in axonal excitability.

The structurally related alkaloid, napelline, however, did not affect neuronal excitability at concentrations of 3–100 μM. Neither the antidromic nor the orthodromic population spike was changed in the experiments with napelline. Due to the low solubility of this compound it was not possible to test concentrations higher than 100 μM.

In a subsequent series of experiments, the influence of the two alkaloids on the input–output relationship of the synaptic response was investigated. For this purpose, electrical stimuli of increasing intensity were applied to the Schaffer collaterals and the amplitudes of the corresponding population spikes were measured in the absence and presence of different concentrations of 1-benzoylnapelline and napelline, respectively (Fig. 3). The input–output curves of the control show that the population spike amplitude increased with stimulus intensity until a maximum was attained. In all slices tested, 1-benzoylnapelline (10 and 30 μM) produced a concentration-dependent shift to the right of the input–output curve and decreased its maximum, whereas napelline (30 and 100 μM) was without effect.

In order to investigate if the inhibitory action of 1-benzoylnapelline was use-dependent, the frequency of electrical stimulation was increased from 1 stimulus pulse per 15

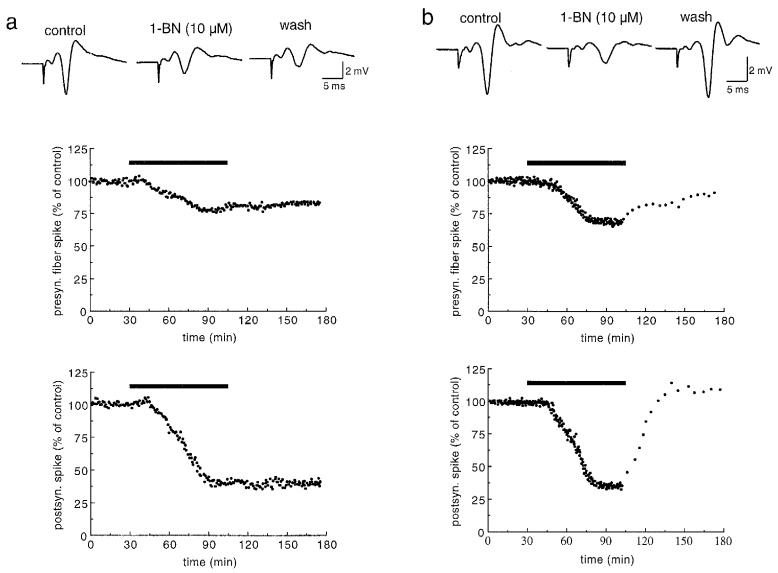

Fig. 5. Frequency dependence of recovery from the inhibition induced by 1-benzoylnapelline (10 μM). Each data point represents the average of 5 subsequent measurements. The graphs in (a) were obtained from a slice which was stimulated orthodromically every 5 s during the entire recording time. The graphs in (b) were obtained from a slice which was stimulated every 5 s until the end of drug application. When washout was started, the stimulation protocol was changed in so far that stimuli were applied only every 60 s. Note the full recovery of the drug-induced inhibition when stimulation was at 60 s interval (b). The bar above each graph indicates when 1-benzoylnapelline was applied. The upper graphs show the time-course of the presynaptic fiber spikes and the lower graphs show the time-course of the corresponding postsynaptic population spikes. The insets above the graphs represent sample spikes recorded prior to drug application, and at the end of washout.

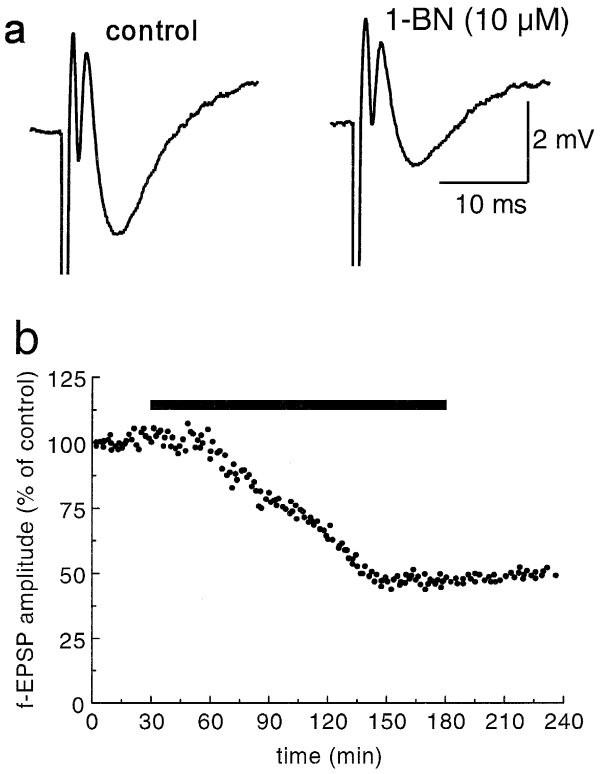

Fig. 6. Inhibitory action of 1-benzoylnapelline (1-BN, 10 μM) on the field excitatory potential (f-e.p.s.p.). (a) Field e.p.s.ps recorded extracellularly in the apical dendritic region of area CA1. Schaffer collaterals were stimulated every 15 s. Each curve is the average of 5 subsequent responses. (b) Time-course of the inhibitory action of 1-benzoylnapelline on the field e.p.s.p. The filled bar in the graphs indicates the time of drug application. One representative experiment out of 8 similar ones is shown.

s to 1 pulse per 5 s. In Fig. 4, the mean amplitude of the presynaptic fiber spike, the postsynaptic population spike, and the antidromic population spike measured at the end of the application of 1-benzoylnapelline is compared for both stimulation frequencies employed. Stimulating the Schaffer collaterals or the alvear fibers with a higher frequency caused a significant increase in the inhibitory effect induced by this alkaloid.

When the higher stimulation frequency was also applied during washout of 1-benzoylnapelline, the inhibitory effect of the drug was irreversible (Fig. 5a, b). In contrast, when stimulation frequency during washout of the drug was decreased from 1 pulse per 5 s to 1 pulse per 60 s (Fig. 5c,d), the inhibitory effect on the postsynaptic population spike was reversible in each slice tested ($n = 8$).

3.2. Effect of 1-benzoylnapelline on the field e.p.s.p.

In order to further investigate whether the decrease in the amplitude of the postsynaptic population spike was due to attenuated transmitter release at the dendrites of the CA1 pyramidal cells, extracellular recordings of field e.p.s.ps were performed in the dendrite region of the CA1 pyramidal cells (Fig. 6). The field e.p.s.p. reflects synaptic currents in the dendrites as a result of the action of neurotransmitters. 1-Benzoylnapelline (10 μM) reduced the amplitude of the field e.p.s.p. by $42.33 \pm 6.7\%$ of the control ($n = 8$, $P \leq 0.001$). There was no significant difference in the 1-benzoylnapelline-mediated decrease of the field e.p.s.p. and in the decrease of the postsynaptic population spike.

3.3. Effect of 1-benzoylnapelline on epileptiform population spikes

Considering the highly frequency-dependent inhibitory action of 1-benzoylnapelline the question arises of whether or not this drug will be capable to suppress experimentally induced epileptiform activity. To further examine this question, epileptiform activity was induced either by bicuculline (10 μM) which interferes with the inhibitory transmission or by perfusion of the slices with a nominally Mg^{2+}-free ACSF in order to activate NMDA receptor-mediated responses.

Control experiments ($n = 4$) were performed with 10 μM bicuculline in order to observe if epileptiform activity could be maintained for at least 6 h. About 5 min after the addition of bicuculline, bursts of population spikes, which reflect synchronous discharges of the neurons in the vicinity of the recording electrode (Anderson et al., 1986), were induced in area CA1. The elicited epileptiform burst activity became manifest as 4–6 negative population spikes of declining amplitude on top of a positive postsynaptic field potential of 40–50 ms in duration. The amplitudes of these spikes became stable after 10–15 min and persisted during the entire observation time of up to 6 h.

Table 1
Inhibitory effect of 1-benzoylnapelline (10 μM) on epileptiform field bursts induced either by bicuculline or by a nominally Mg^{2+}-free bathing solution

	BIC	1-BN	Mg^{2+}-free	1-BN
Number of spikes	4.78 ± 0.4	3.05 ± 0.8	7.25 ± 1.4	4.25 ± 1.4
Burst duration (ms)	45.28 ± 6.1	32.15 ± 4.2	58.23 ± 4.5	32.52 ± 6.1
Amplitude of the first spike (%)	100	67.09 ± 6.0	100	63.16 ± 8.9
Amplitude of the third spike (%)	100	52.64 ± 10.2	100	44.62 ± 3.3

Data represent the means ± S.D. of 8–9 experiments. 1-Benzoylnapelline (1-BN, 10 μM) was applied for a duration of 150 min. The amplitudes of the spikes were normalized with respect to the corresponding spikes obtained by perfusion with bicuculline (BIC, 10 μM) or nominally Mg^{2+}-free ACSF, respectively. Each parameter measured was significantly decreased by 1-benzoylnapelline (at least $P \leq 0.05$).

In all slices tested, 1-benzoylnapelline (10 μM) inhibited the epileptiform population spikes. This effect was similar when epileptiform activity was elicited by bicuculline ($n = 9$) and by the nominally Mg^{2+}-free ACSF ($n = 8$). In order to quantify the inhibitory action of 1-benzoylnapelline, the number of spikes in the burst, the duration of the burst and the size of the first and third population spike were determined at the end of period 2 and at the end of the drug-application (period 3). As shown in Table 1 and Fig. 7, all parameters were significantly diminished by 1-benzoylnapelline. It is obvious (Fig. 7a) that the late spikes in the bursts were completely suppressed by the drug.

After 150 min of application, 1-benzoylnapelline was omitted from the bathing medium and slices were perfused with the epileptogenic ACSF (period 4). During the entire observation period of at least 2 h, the pattern of epileptiform activity observed during period 2 (i.e., before application of 1-benzoylnapelline) did not reappear. In view of the control experiments, where epileptiform activity persisted in all slices for at least 6 h, the inhibition was unlikely to be due to fatigue, but can be considered to be an inhibitory effect of 1-benzoylnapelline. This result is consistent with the failure to recover observed during washout, when electrical stimuli were applied at high frequency. In contrast, the experiments performed with low stimulation frequency during washout showed that the inhibition induced by the drug was completely reversible (Fig. 5).

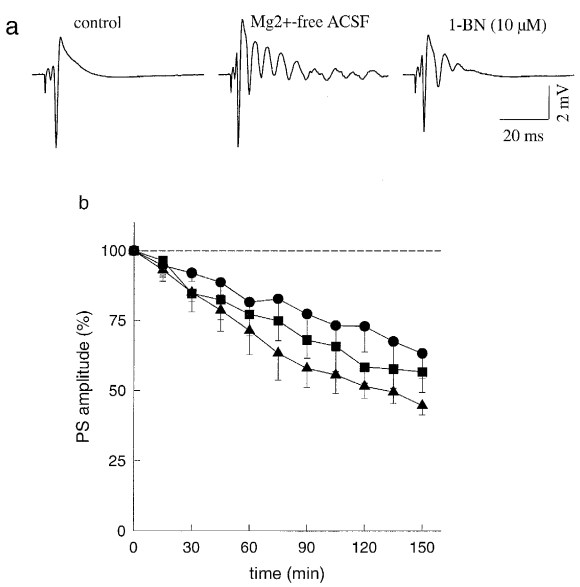

Fig. 7. Effect of 1-benzoylnapelline (1-BN, 10 yM) on the orthodromic response in nominally Mg^{2+}-free ACSF. (A) Extracellularly recorded population spikes from a representative experiment out of 8 similar ones, showing the effect of 1-benzoylnapelline on an epileptiform burst induced by omission of Mg^{2+} from the bathing medium. The calibration bars in the lower right corner apply to all records. (B) Time-course and sensitivity to 6-benzoylnapelline of the first (●), second (■) and third (▲) population spike (PS) recorded in the absence of Mg^{2+}. The amplitudes of the spikes were normalized with respect to the amplitudes achieved with perfusion of Mg^{2+}-free ACSF. Data points represent mean values ± S.D. ($n = 8$).

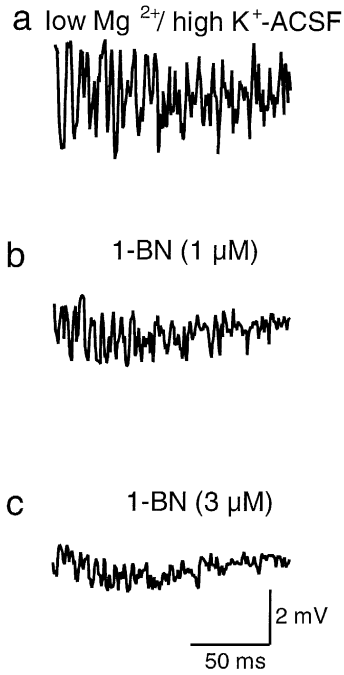

Fig. 8. Effect of 1-benzoylnapelline (1 and 3 μM) on spontaneous epileptiform activity which developed after omission of Mg^{2+} and increase of the K^+ concentration from 3 to 8 mM (low Mg^{2+}/high K^+-ACSF). Spontaneous activity was attenuated after the addition of 1-benzoylnapelline.

In order to examine if 1-benzoylnapelline is capable of inhibiting spontaneous epileptiform activity, slices were perfused with a low Mg^{2+}/high K^+-ACSF ($n = 4$). Spontaneous epileptiform activity occurred within 90–120 min of perfusing with the modified ACSF. At a concentration of 1 and 3 μM, 1-benzoylnappelline diminished both the frequency and the duration of the bursts within 60–90 min (Fig. 8).

4. Discussion

The aim of the present study was to investigate the action of the plant alkaloid, 1-benzoylnapelline, on hippocampal excitability and to compare its effect with that of its structural analog, napelline. The present results demonstrated an inhibitory effect of 1-benzoylnapelline at A concentration of 1–100 μM, whereas the closely related compound napelline lacked any effect at concentrations up to 100 μM. 1-Benzoylnapelline attenuated the amplitude of the orthodromically evoked presynaptic fiber spike and the postsynaptic population spike as well as the antidromically evoked population spike. Electrophysiologically, this drug seems to inhibit predominantly axonal excitability of the afferents and, in consequence, neurotransmission between Schaffer collaterals and the CA1 pyramidal neurons, thus suppressing the firing of the latter. This conclusion is

supported by three experimental findings: First, the onset of the diminution of the presynaptic fiber spike and the onset of the postsynaptic population spike occurred simultaneously. Second, the decrease in size of the antidromic spike did not differ significantly from the decrease in size of the presynaptic fiber spike. Third, 1-benzoylnapelline had a very similar inhibitory potency both in the presence and in the absence of synaptic transmission. 1-Benzoylnapelline thus seems to possess local anaesthetic activity. Local anesthetics like lidocaine are reported to decrease the amplitude of the presynaptic fiber spike. Due to the decreased afferent input they decrease the EPSP and the postsynaptic population spike (Weber and Taylor, 1994). Both local anesthetics such as lidocaine and anticonvulsants such as phenytoin are known to cause a frequency-dependent block of the voltage-dependent Na^+ channel (Catterall, 1987; Ragsdale et al., 1991; Wann, 1993; Weber and Taylor, 1994). In contrast to the Na^+ channel blockers, tetrodotoxin and saxitoxin, which block the channel independently of its conformational state, lidocaine and phenytoin bind to the channel in its open state. When stimulation is at a higher frequency a cumulative block of the channel is achieved with these drugs, that is, more molecules become bound with each opening of the channel. The more frequently the Na^+ channels are opened, the greater the degree of block. Lidocaine and phenytoin reduce the probability of Na^+ channel opening when membranes are depolarized, while allowing channel opening when membranes are hyperpolarized (Catterall, 1987; Ragsdale et al., 1991). It is obvious from the present data that the alkaloid, 1-benzoylnapelline, has a frequency-dependent mode of action. 1-Benzyolnapelline shares this mode of action with local anesthetics as well as with aconitine and lappaconitine which also have been reported to evoke a stronger inhibition when stimulation frequency is increased (Ameri et al., 1996a,b). It has been proposed that the molecular site of local anaesthetic block is within the ion conduction pathway, near the inactivation gate and the selectivity filter and near the receptor site 2 for batrachotoxin and aconitine (Wann, 1993; Fozzard and Hanck, 1996). Aconitine is well known to block the inactivation of the voltage-dependent Na^+ channel (Catterall, 1980), and there is a report that lappaconitine blocks this channel (Valeev et al., 1990).

An important implication of the frequency-dependent mode of action of Na^+ channel-active drugs is suppression of aberrant neuronal activity that occurs in the pathophysiological state of epileptic seizures (Catterall, 1987). Indeed, 1-benzoylnapelline diminished epileptiform burst activity in both models of epileptiform activity employed in the present study. The first model (bicuculline model) exploits the fact that seizures are associated with a disturbed inhibition (Campbell and Holmes, 1984; Herron et al., 1985; Ault and Wang, 1986; Chagnac-Amitai and Connors, 1989), whereas in the second model (low-Mg^{2+} model) epileptiform activity has been described as the consequence of activation of NMDA receptor-mediated responses due to the release of the Mg^{2+} block (Coan and Collingridge, 1985; Anderson et al., 1986; Mody et al., 1987). The present data show that the duration of the bursts, the number of spikes in the burst, and the amplitudes of the population spikes were equivalently inhibited by 1-benzoylnapelline, indicating a powerful blockade of all Na^+ channel-dependent activity. 1-Benzoylnapelline attenuated spontaneous epileptiform activity even at a concentration of 1 μM, which failed to significantly affect the population spike in control slices. However, since the neurones are depolarized by the increased extracellular K^+ concentration, it cannot be excluded that 1-benzoylnapelline reduces the probability of Na^+ channel openings at this depolarized potential.

Together, the results showed that the predominant effect of the plant alkaloid, 1-benzoylnapelline, is a frequency-dependent inhibition of axonal excitability which may distort the spread of epileptiform events. The action is concentration-dependent and, since napelline lacks any effect, is specific for 1-benzoylnapelline. Further electrophysiological experiments are required to investigate the interaction of this compound with the voltage-dependent Na^+ channel.

References

Ameri, A., Jurna, I., 1991. Adenosine A1 and non-A1 receptors: Intracellular analysis of the actions of adenosine agonists and antagonists in rat hippocampal neurones. Brain Res. 546, 69–78.

Ameri, A., Metzmeier, P., Peters, T., 1996a. Frequency-dependent inhibition of neuronal activity by lappaconitine in normal and epileptic hippocampal slices. Br. J. Pharmacol. 118, 577–584.

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

Andersen, P., Silvenius, H., Sundberg, S.H., Sveen, O., Wigström, H., 1978. Functional characteristic of myelinated fibers in the hippocampal cortex. Brain Res. 144, 11–18.

Anderson, W.W., Lewis, D.V., Swartzwelder, H.S., Wilson, W.A., 1986. Magnesium-free medium activates seizure-like events in rat hippocampal slice. Brain Res. 398, 215–219.

Ault, B., Wang, C.M., 1986. Adenosine inhibits epileptiform activity arising in hippocampal area CA3. Br. J. Pharmacol. 87, 695–703.

Benn, M.H., Jacyno, J.M., 1983. The toxicology and pharmacology of diterpen alkaloids. In: Pelletier, S.W. (Ed.), Alkaloids – Chemical and Biological Perspectives. John Wiley and Sons, New York, NY, pp. 153–210.

Bisset, N.G., 1981. Arrow poisons in China. Part II. Aconitum – Botany, chemistry, and pharmacology. J. Ethnopharmacol. 4, 247–336.

Campbell, A., Holmes, O., 1984. Bicuculline epileptogenesis in the rat. Brain Res. 323, 239–246.

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

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

Chagnac-Amitai, Y., Connors, B.W., 1989. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. J. Neurophysiol. 61, 747–758.

Coan, E.J., Collingridge, G.L., 1985. Magnesium ions block a *N*-methyl-D-aspartate receptor-mediated component of synaptic transmission in rat hippocampus. Neurosci. Lett. 53, 21–26.

Fozzard, H.A., Hanck, D.A., 1996. Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. Physiol. Rev. 76, 887–926.

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

Herron, C.E., Williamson, R., Collingridge, G.L., 1985. A selective *N*-methyl-D-aspartate antagonist depresses epileptiform activity in rat hippocampal slices. Neurosci. Lett. 61, 255–260.

Mody, I., Lambert, J.D.C., Heinemann, U., 1987. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J. Neurophysiol. 57, 869–888.

Ragsdale, D.S., Scheuer, T., Catterall, W.A., 1991. Frequency and voltage-dependent inhibition of type IIA Na^+ channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs. Mol. Pharmacol. 40, 756–765.

Valeev, A.E., Verkhratskii, A.N., Dzhakhangirov, F.N., 1990. Effects of allapinine on sodium currents in neurons isolated from the rat trigeminal ganglion and cardiomyocytes. Neirofiziologiya 22, 201–206.

Wann, K.T., 1993. Neuronal sodium and potassium channels: Structure and function. Br. J. Anaesth. 71, 2–14.

Weber, M.L., Taylor, C.P., 1994. Damage from oxygen and glucose deprivation in hippocampal slices is prevented by tetrodotoxin, lidocaine and phenytoin without blockade of action potentials. Brain Res. 664, 167–177.