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Frequency-dependent inhibition of neuronal activity by lappaconitine in normal and epileptic hippocampal slices

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1 Extracellular recording of the stimulus-evoked population spike in the CA1 region of rat hippocampal slices *in vitro* was performed in order to investigate whether lappaconitine affects neuronal excitability. Lappaconitine is a diterpene alkaloid of plants of the *Aconitum* genus and has analgesic properties.

2 The results reveal an inhibitory action of lappaconitine (10 μM) manifested in a slow attenuation of the orthodromic and antidromic population spike.

3 The lappaconitine-induced inhibitory action was activity-dependent, that is, it was potentiated when frequency of electrical stimulation was increased. In contrast, washout of the neurotoxin was accelerated when stimulation frequency was decreased.

4 The activity-dependent action of lappaconitine raised the question of whether the drug is effective in suppressing the aberrant neuronal activity that occurs during an epileptic seizure. The results obtained from experiments on epileptic hippocampal slices demonstrated a selective reduction of the later spikes in the bursts with less effect on normal neuronal activity.

5 These data support the conclusion that lappaconitine, in addition to its antinociceptive effect, also has antiepileptic potency due to its highly activity-dependent mode of action.

Keywords: hippocampus; extracellular recordings; epilepsy; lappaconitine

Introduction

Several structurally related aconitine derivatives, alkaloids of the plant *Aconitum* spec., have been shown to possess analgesic potential (Liu *et al.*, 1987; Ono & Satoh, 1988; 1989; 1990; 1991; Suzuki *et al.*, 1994). Aconitine is known to share a common binding site (site II) with veratridine and batrachotoxin on the sodium channel (Catterall, 1980), to induce persistent activation of the channel by blocking channel inactivation, to shift activation of the channel to more hyperpolarized potentials, and to decrease the maximal inward current (Ulbricht & Flacke, 1965; Schmidt & Schmitt, 1974; Mozhayeva *et al.*, 1977; Warashina, 1985). Recent work using electrophysiological techniques has demonstrated that aconitine exerts inhibitory effects on neuronal activity in rat hippocampal slices in an activity-dependent manner (Ameri *et al.*, 1996).

Lappaconitine has been shown to be a centrally-acting analgesic drug (Ono & Satoh, 1988) without affinity for opioid receptors (Ono & Satoh 1989; 1990). Furthermore, lappaconitine was found to exert an inhibitory effect on inward tetrodotoxin-sensitive sodium currents in neurones isolated from rat trigeminal ganglion and cardiomyocytes (Valeev *et al.*, 1990). Lappaconitine does not alter the voltage-dependence of the sodium current activation and inactivation, and it does not affect currents activated by various neurotransmitters such as glycine, taurine, γ-aminobutyric acid (GABA), glutamate, and adenosine 5'-triphosphate (ATP) in trigeminal ganglia neurones (Valeev *et al.*, 1990).

The present study was designed to investigate the effects of lappaconitine on neuronal activity in rat hippocampal slices. In addition, experiments were performed to determine if experimentally induced epileptiform activity is suppressed by lappaconitine. In order to examine neuronal activity we took advantage of synchronously discharging action potentials by a large population of pyramidal cells which are recorded extracellularly as distinct 'population spikes' in rat hippocampal slices.

Methods

Slice preparation

The experiments were performed on hippocampal slices of male Wistar rats (150-180 g), as has been previously described (Ameri & Jurna, 1991). In brief, the rats were decapitated under deep ether anaesthesia. The brain was removed and transferred into chilled artificial cerebrospinal fluid (ACSF) at 0-4°C. After 1 min, the hippocampus was isolated and transverse slices of 400 μm thickness were cut 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 by a flattened U-shaped platinum wire. The chamber temperature was slowly increased from room temperature to 32°C during a period of 30 min. The slices were continuously perfused with ACSF (flow rate 2 ml min^{-1}). Recordings were begun 1 h after slice preparation. The standard ACSF was 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, and glucose 15 (pH of 7.4). In some experiments a modified ACSF was used, i.e. either a low Ca^{2+}/high Mg^{2+} (omission of $CaCl_2$ and 4 mM $MgSO_4$ in order to suppress synaptic activity) or a nominal Mg^{2+}-free solution (omission of $MgSO_4$ in order to induce epileptiform activity).

Electrical stimulation and recording

Extracellular recordings were carried out with borosilicate glass microelectrodes filled with 3 M NaCl (resistance 5-10 MΩ). The recording electrode was connected via a chloride silver wire to the probe of the amplifier. Recordings of sti-

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mulus-evoked population spikes were performed in stratum pyramidale of area CA1, recordings of field excitatory potentials (f-e.p.s.ps) were performed in stratum radiatum. For electrical stimulation, a concentric bipolar electrode (Rhodes Medical Instruments) was positioned either into the Schaffer collateral commissural pathway (near the junction of CA1 and CA2 stratum radiatum) for orthodromic activation of CA1 pyramidal cells or in the alveus containing the axonal projections of the pyramidal neurones for antidromic activation. Extracellular stimuli were rectangular current pulses of 200 μs in duration delivered every 15 s (in particular experiments every 5 or every 60 s) through a digitally controlled stimulus isolation unit (Axon Instruments, U.S.A.). For each slice the amplitude of stimulation was adjusted to ensure that a submaximal response, approximately 50% of the maximum attainable, was used at the commencement of the experiment.

In one set of experiments epileptiform activity was elicited either by blockade of GABA receptors with bicuculline (Campbell & Holmes, 1984; Herron *et al.*, 1985; Ault & Wang, 1986; Chagnac-Amitai & Connors, 1989) or by omission of Mg^{2+} ions from the bath solution (low Mg^{2+}-ACSF) which leads to demasking of N-methyl-D-aspartate (NMDA) receptor-mediated responses (Coan & Collingridge, 1985; Anderson *et al.*, 1986; Mody *et al.*, 1987). In the control state, electrical stimulation produces a single population spike in CA1 stratum pyramidale. After treatment of hippocampal slices with convulsants, however, the same stimulus produced synchronized population bursts, each consisting of multiple spike discharges. Significant components of these bursts include the presynaptic fibre spike due to the Schaffer collaterals, the first population spike, and succeeding spikes which define epileptiform activity. The experimental protocol comprised 5 periods: Period 1: control; perfusion with ACSF. Period 2: induction of epileptiform activity; perfusion of the epileptogenic ACSF. Period 3: test of the anticonvulsant efficacy of lappaconitine; addition of lappaconitine (10 μM) to the solution of period 2. Period 4: washout of lappaconitine; perfusion with solution of period 2. Period 5: washout of the epileptogenic ACSF by standard ACSF.

The extracellular signals were recorded and amplified by means of a DP 301 amplifier (Warner Instruments, USA). Analogue data were digitized and analyzed by use of the data aquisition system TIDA (HEKA electronic, Germany).

Statistical analysis

Quantitative data are expressed as means ± standard deviation (s.d.). Statistical evaluation was performed by use of Student's t test. Significance was assumed when $P \leqslant 0.05$. The amplitude of the population spike was determined from the negative peak to a tangent drawn between the preceding and following maximum waveforms positivities.

Drugs

Lappaconitine hydrobromide (Latoxan, France) was dissolved in dimethyl sulphoxide (DMSO) to give stock solutions of 10 mM. Control experiments have revealed that the final concentration of DMSO (0.1%) did not affect any of the measured parameters. Bicuculline (Sigma, Germany) was dissolved in distilled water. These solutions were then diluted in ACSF to the desired concentration and gassed before being perfused into the bathing medium. All drugs were perfused for at least 30 min to reach equilibrium.

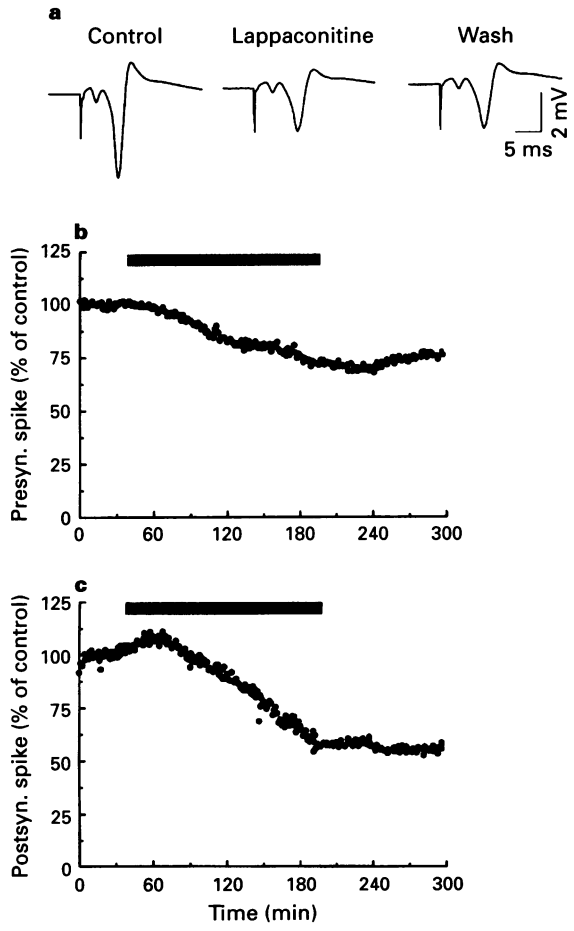

Figure 1 Inhibitory action of lappaconitine (10 μM) on the orthodromic population spike recorded extracellularly in hippocampal CA1 pyramidal cell layer. (a) Population spikes were elicited by electrical stimulation of the Schaffer collaterals every 15 s. The stimulus artefact is followed by the presynaptic fibre spike and the postsynaptic population spike. Each trace represents the average of 5 subsequent responses. (b) Time-course of the action of lappaconitine on the presynaptic fibre spike. Each point represents the average of the amplitudes of 5 subsequent measurements. The bar above the graph indicates when lappaconitine was applied. (c) Time-course of the action of lappaconitine on the postsynaptic population spike. Recordings in (a) and results in (b) were obtained from the same slice.

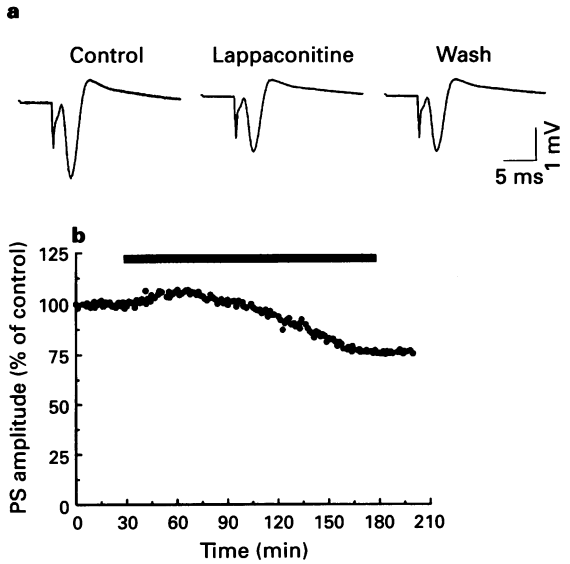

Figure 2 Inhibitory action of lappaconitine (10 μM) on the antidromic population spike. (a) Population spikes (PS) were elicited by electrical stimulation of alvear fibres every 15 s. Each trace is the average of 5 subsequent events. (b) Time-course of the action of lappaconitine on the antidromic population spike. Each point on the graph represents the average of 5 subsequent measurements. The time of application of lappaconitine is indicated by the bar. Recordings in (a) and results in (b) were obtained from the same slice.

Results

The effects of lappaconitine on the population spike in area CA1 of the rat hippocampus were investigated in 56 slices. Only those recordings were included into the data analysis where electrical stimulation of the Schaffer collaterals with maximal stimulus strength did not elicit a second population spike and where the amplitude of the population spike was stable for a control period of at least 30 min.

Effects of lappaconitine on the stimulus-evoked population spike

Lappaconitine (10 μM) slowly decreased the amplitude of the orthodromically evoked population spike. The inhibition affects both, the presynaptic fibre spike (afferent volley) which represents the compound action potential generated in presynaptic axons and the postsynaptic population spike (Figure 1). However, immediately after starting the application of lappaconitine there was a transient increase of $8.9 \pm 3.0\%$ ($n = 10$, $P \leqslant 0.02$) in the amplitude of the postsynaptic population spike in 10 of 14 slices tested. In contrast, the amplitude of the presynaptic fibre spike increased in only 1 of 14 slices. About 40 min after the start of perfusion with lappaconitine, the diminution of the presynaptic fibre spike and the postsynaptic population spike set in. The onset of the inhibitory action of lappaconitine on the presynaptic and the postsynaptic spike occurs simultaneously. After an application of lappaconitine of 90 min, the amplitude of the presynaptic fibre

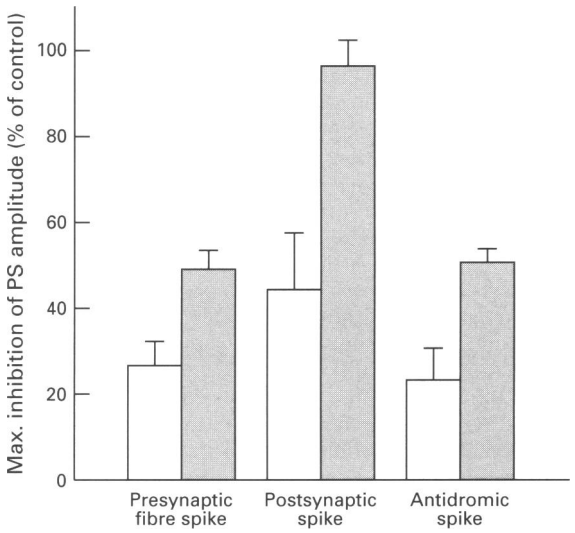

Figure 3 Frequency-dependence of the lappaconitine-induced inhibition of the presynaptic fibre spike, the postsynaptic spike and the antidromic spike, respectively. Lappaconitine (10 μM) was applied for 150 min. The columns represent the mean $\pm$ s.d. in % of the control response. Each column represents the data of at least 4 experiments. The stimulation frequency was increased from 1 pulse per 15 s (open columns) to 1 pulse per 5 s (hatched columns) and this enhanced significantly the extent of inhibition evoked by lappaconitine ($P \leqslant 0.002$ for the presynaptic fibre spike, $P \leqslant 0.001$ for the postsynaptic spike, $P \leqslant 0.002$ for the antidromic spike).

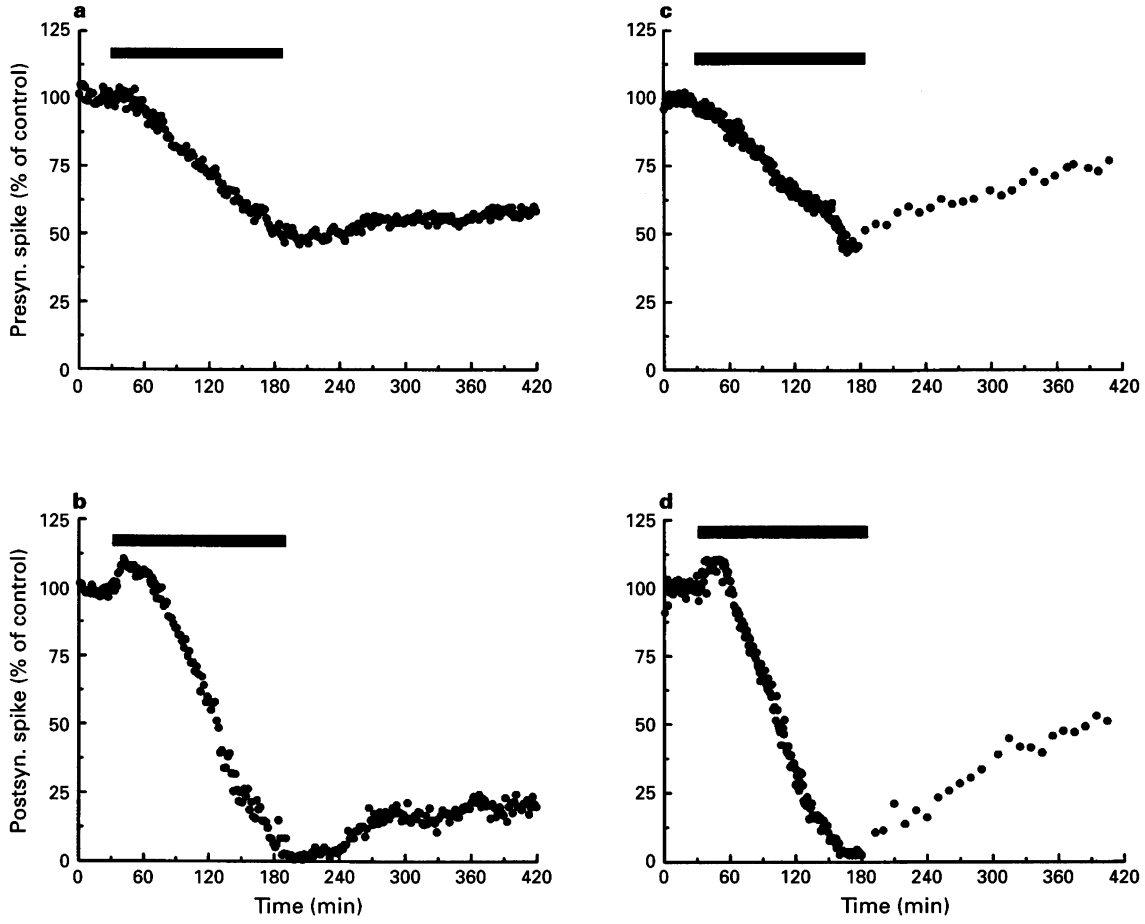

Figure 4 Inhibition of the orthodromic population spike by lappaconitine (10 μM) and dependence of its recovery on the stimulation frequency. Each data point represents the average of 5 subsequent measurements. The graphs in (a) and (b) were obtained from a slice which was stimulated every 5 s for the entire recording time. The graphs in (c) and (d) were obtained from a slice which was stimulated every 5 s until the end of the lappaconitine application. At the start of washout, the stimulation protocol was changed in so far that stimuli were applied only every 60 s. In both slices, the presynaptic fibre spike was reduced by about 50% (a, c), whereas the postsynaptic population spike was fully suppressed (b, d). Note that recovery of the drug-induced inhibition was enhanced by decreasing the stimulation frequency (c, d). The bar above each graph indicates when lappaconitine was applied.

spike was reduced by 17.7±3.9% ($n=6$, $P\leqslant0.001$) and the amplitude of the presynaptic spike by 34.1±7.6% ($n=6$, $P\leqslant0.001$). After an application period of 150 min, the inhibition was 26.4±5.6% ($n=5$, $P\leqslant0.001$) and 44.0±13.3% ($n=5$, $P\leqslant0.002$) for both components, respectively. The lappaconitine-induced inhibition was irreversible during washout of up to 5 h.

The antidromic population spike elicited by direct, alvear stimulation of CA1 pyramidal cells was also attenuated by lappaconitine (Figure 2). A transient increase in spike amplitude following onset of application was observed in only 2 of 13 slices stimulated antidromically. After an application of 90 min, the amplitude of the antidromic spike was reduced by 3.3±10.8% (not significant), after 150 min by 23.0±7.6% ($n=4$, $P\leqslant0.02$). 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 the possible contribution by presynaptic mechanisms to the lappaconitine-induced action, synaptic transmission was blocked by a low Ca^{2+}/high Mg^{2+} medium (Andersen *et al.*, 1978). One hundred and fifty min after the addition of lappaconitine (10 μM) to the low Ca^{2+}/high Mg^{2+}-ACSF, the amplitude of the antidromic population spike was attenuated by 24.5±6.7% ($n=4$, $P\leqslant0.01$) and did not differ from the inhibition caused by 10 μM lappaconitine added to standard ACSF.

Frequency-dependence of the lappaconitine-induced inhibition

Increasing the frequency of electrical stimulation from 1 pulse per 15 s to 1 pulse per 5 s potentiated the lappaconitine-induced inhibition such that a complete suppression of the postsynaptic population spike was achieved after an application of 140–160 min in every slice tested. In Figure 3, the mean inhibition of the presynaptic fibre spike, the postsynaptic population spike and the antidromic population spike obtained after 150 min of applying lappaconitine is compared for both stimulation frequencies used (1/15 s and 1/5 s). There is a significant increase in the inhibitory effect on stimulating the Schaffer collaterals or the alvear fibres, respectively, with the higher frequency. One hundred and fifty min after onset of the application of lappaconitine (10 μM), the amplitude of the presynaptic fibre spike was reduced by 49.9±3.9% ($P\leqslant0.001$) and the amplitude of the postsynaptic spike by 95.6±6.4% ($n=5$, $P\leqslant0.002$). Increasing the frequency of the antidromical stimulation from 1 pulse per 15 s to 1 pulse per 5 s exerted an attenuation of the antidromic population spike by lappaconitine by 50.3±3.1% ($n=4$, $P\leqslant0.001$).

While the inhibition induced by lappaconitine was accelerated by increasing the stimulation frequency, the washout of the drug was facilitated by decreasing the stimulation frequency. In order to determine the recovery from the lappaconitine-induced inhibition, the amplitude of the spike was determined after 4 h washout. Then this value was normalized to the maximum inhibition of the spike at the end of the lappaconitine-application. When the Schaffer collaterals were stimulated every 5 s (Figure 4a,b), the recovery of the presynaptic fibre spike was 8.9±3.3% ($n=4$, $P\leqslant0.05$) and the recovery of the postsynaptic spike 21.34±4.5% ($n=4$, $P\leqslant0.002$). In contrast, when the Schaffer collaterals were stimulated every 60 s during washout (Figure 4c,d), the recovery of the presynaptic spike amounted to 19.75±6.8% ($n=4$, $P\leqslant0.01$) and of the postsynaptic spike to 48.84±5.6% ($n=4$, $P\leqslant0.001$). The recovery obtained with the low stimulation frequency is significantly higher ($P\leqslant0.01$) than the recovery obtained during washout with the high frequency. A corresponding dependence of recovery on the stimulation frequency has also been observed for the amplitude of the antidromic population spike (Figure 5a,b). After 4 h washout the recovery of the antidromic spike amounted to 20.12±4.7% ($n=4$, $P\leqslant0.01$) when stimuli were applied every 5 s and to 52.38±8.9% ($n=4$, $P\leqslant0.001$) when stimuli were applied every 60 s.

Effects of lappaconitine on the f-e.p.s.p.

In order to investigate the effect of lappaconitine on synaptic transmission, recordings of f-e.p.s.ps were performed in the dendrite region of CA1 stratum radiatum ($n=7$). In four slices, lappaconitine (10 μM) failed to show an effect on the f-e.p.s.ps during the first 90 min of application. However, when stimulation-frequency was increased to 1 pulse per 5 s, these slices also showed a diminution of the f-e.p.s.ps which reflects the reduction of the presynaptic fibre spike. In the other three slices, there was an inhibition of the f-e.p.s.ps by 23.0±2.7% after 90 min of drug-application. This value does not differ significantly from the reduction of presynaptic fibre spike (17.7±3.9%) obtained after 90 min.

Diminution of epileptiform discharges during application of lappaconitine

The highly frequency-dependent mode of action of lappaconitine raises the question of whether or not it will selectively inhibit aberrant activity that occurs in pathophysiological states. Epileptiform activity can be induced in the hippocampal

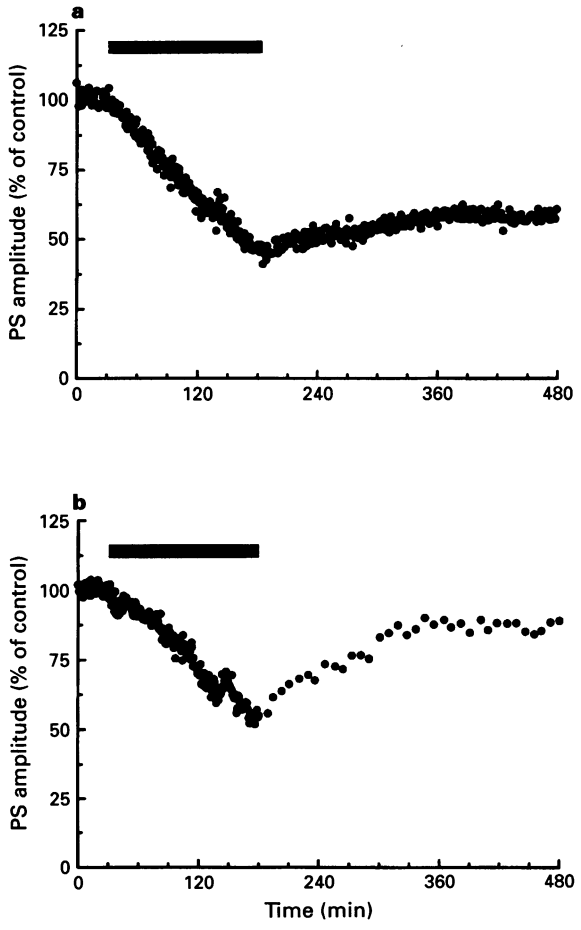

Figure 5 Inhibition of the antidromic population spike (PS) by lappaconitine and dependence of its recovery on the stimulation frequency. Each data point represents the average of 5 subsequent measurements. The graph in (a) was obtained from a slice which was stimulated every 5 s for the whole experiment. The graph in (b) was obtained from a slice which was stimulated every 5 s until the end of the lappaconitine application. At the start of washout, stimulation frequency was decreased to 1 pulse per 60 min. Note the enhancement of recovery in (b) compared with (a).

slice by the addition of convulsants which interfere with the inhibitory transmission such as bicuculline or by exposure to a nominally Mg^{2+}-free perfusate in order to activate N-methyl-D-aspartate (NMDA) receptors.

Control experiments ($n=4$) were performed in order to observe if the epileptic activity could be maintained for 6 h. After blockade of GABA receptors by bicuculline (10 μM), elicited epileptic activity manifested as 3–4 additional population spikes and persisted for the entire observation time.

In all slices tested so far ($n=11$), evoked epileptiform activity was reduced by lappaconitine (10 μM) in period 3. However, the extent of diminution of the additional population spikes occurring during perfusion with the epileptogenic ACSF differed markedly with the epilepsy model used. The amplitudes of the multiple population spikes evoked by application of bicuculline (10 μM) were attenuated continuously after addition of lappaconitine (Figure 6). When lappaconitine was applied for 150 min, the amplitude of the third population spike of the burst was reduced by $92.8 \pm 10.1\%$, whereas the first postsynaptic population spike was affected by only $49.1 \pm 5.3\%$ ($n=5$). Thus, the diminution of the third population spike is significantly ($P \leqslant 0.01$) higher than the diminution of the first postsynaptic population spike.

When epileptiform activity was induced by perfusing the slices with nominal Mg^{2+}-free ACSF (Figure 7), epileptic activity manifested in burst discharges of 4–6 additional population spikes. Lappaconitine (10 μM) applied for 150 min decreased the amplitude of the third population spike by $32.1 \pm 12.9\%$ ($n=6$). This value did not differ significantly from the inhibition of the first population spike ($26.7 \pm 4.5\%$, $n=6$). However, the fourth till sixth population spikes in the burst were blocked in every case.

After maximal inhibition, lappaconitine was omitted from the bathing solution and slices were perfused with the epileptogenic ACSF (period 4). During an observation period of up to 3 h, the pattern of epileptiform activity observed during period 2 (before application of lappaconitine) did not reappear suggesting a persistant antiepileptic effect of lappaconitine.

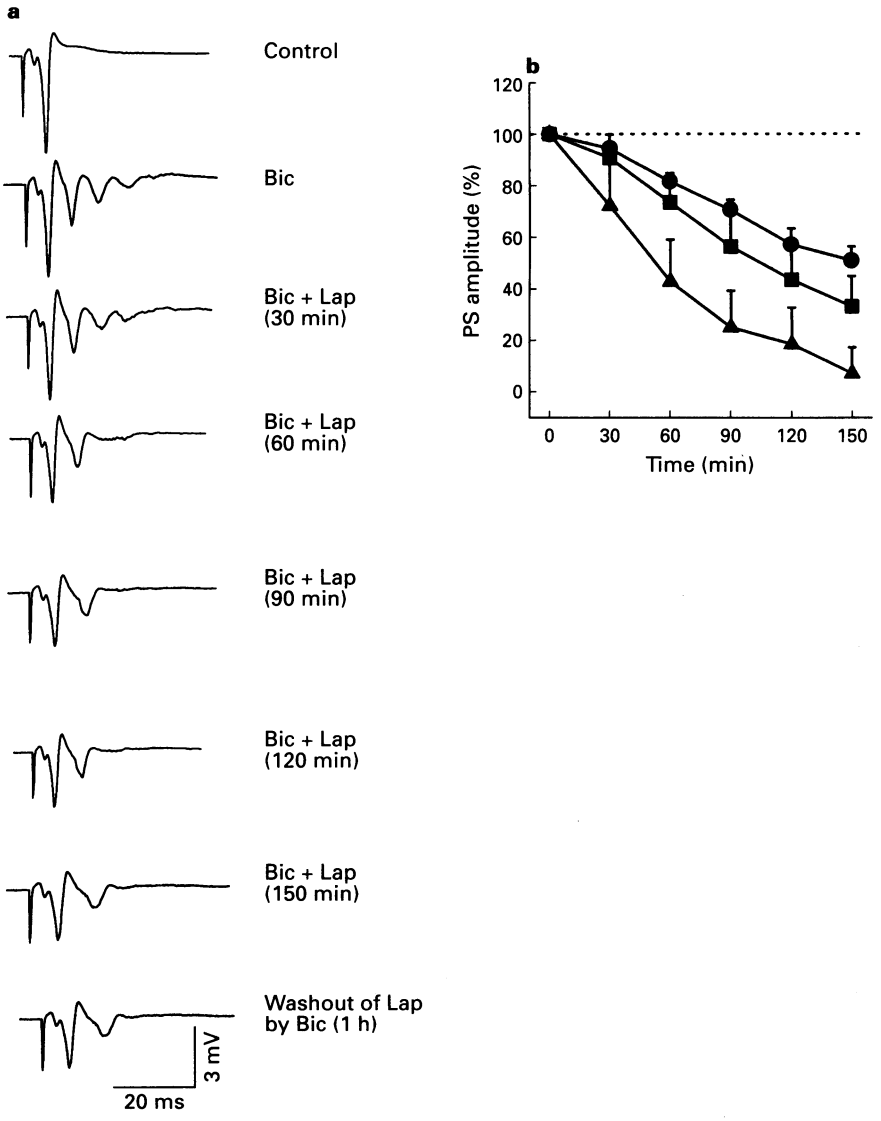

Figure 6 Effect of lappaconitine (Lap, 10 μM) on the orthodromically evoked population spike in the presence of 10 μM bicuculline (Bic). (a) Population spikes recorded in the CA1 region under control conditions, generation of epileptiform activity by bicuculline and its inhibition during application of lappaconitine. The calibration bars in the lower right corner apply to all records. (b) Time-course and sensitivity to lappaconitine of the primary (●), secondary (■) and tertiary (▲) population spike (PS) recorded in the presence of bicuculline. The amplitudes of the spikes were normalized with respect to the amplitudes achieved with bicuculline. Data points represent mean values $\pm$ s.d. ($n=5$). The differences between the effect on the first and the third population spike are significant ($P \leqslant 0.01$).

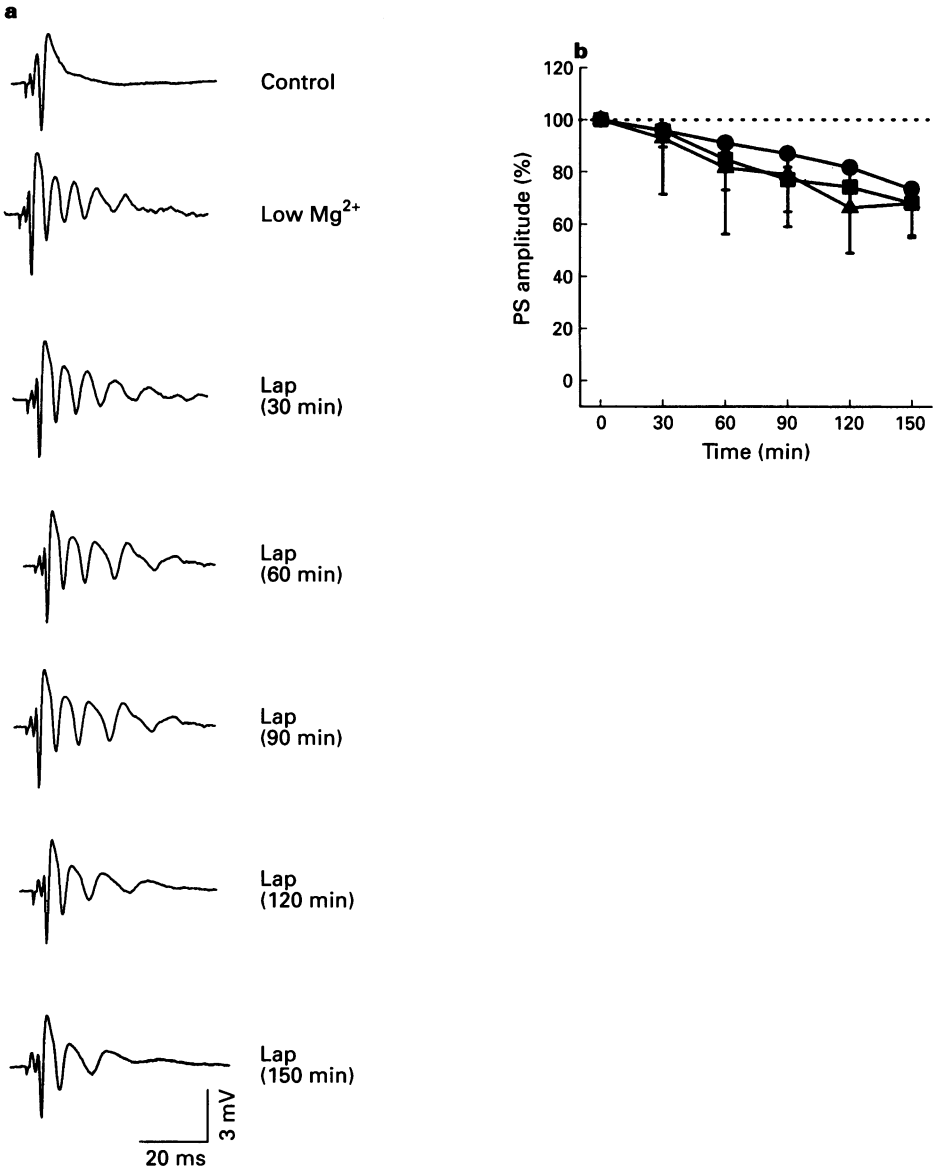

Figure 7 Effect of lappaconitine (Lap, 10 μM) on the orthodromically evoked population spike in nominal Mg^{2+}-free ACSF. (a) Extracellular responses from a representative experiment showing the effect of lappaconitine on an epileptiform burst induced by omission of Mg^{2+} from the bathing medium. The calibration bars in the lower right corner apply to all records. (b) Time-course and sensitivity to lappaconitine of the primary (●), secondary (■) and tertiary (▲) 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 low Mg^{2+}-ACSF. Data points represent mean values ± s.d. ($n = 6$).

Discussion

The main finding of the present study is an inhibitory effect of lappaconitine (10 μM) on neuronal activity in rat hippocampal slices which is highly activity-dependent. The inhibitory action of lappaconitine affects the orthodromic (Figure 1) and the antidromic (Figure 2) population spike. Time-course and extent of inhibition of the presynaptic fibre spike and of the antidromic spike, respectively, do not differ from each other. Both the presynaptic fibre spike and the antidromic spike are generated by synchronous discharges of axons in response to their electrical stimulation. The presynaptic fibre spike represents the compound action potential of the Schaffer collaterals, while the antidromic population spike reflects the response to electrical stimulation of the axons of the neurones being recorded from (Andersen *et al.*, 1978). Thus the present findings imply a direct action of lappaconitine on these fibres, which is in agreement with its previously described inhibitory action on inward tetrodotoxin-sensitive sodium currents (Valeev *et al.*, 1990). Despite the similar structure of aconitine and lappaconitine, the latter exerts a qualitatively and quantitatively different action from the former. Aconitine (1 μM) completely suppressed the postsynaptic population spike and the antidromic population spike within 30 min after starting the application, but failed to alter the presynaptic spike during this time (Ameri *et al.*, 1996). The similarity in action of lappaconitine concerning time-course and extent of inhibition of the presynaptic fibre spike and the antidromic spike stands thus in opposite to the effects observed with aconitine. A different mode of action of both alkaloids has been observed also in previous studies, although both drugs have been reported to decrease the peak amplitude of the sodium current. However, while aconitine shifts the threshold of sodium channel towards hyperpolarization and retards inactivation of sodium currents (Ulbricht & Flacke, 1965; Schmidt & Schmitt, 1974; Mozhayeva *et al.*, 1977; Warashina, 1985), lappaconitine decreases the peak amplitude of sodium current without changing its activation threshold (Valeev *et al.*, 1990).

Despite the pronounced inhibitory action of aconitine, a transient increase in the amplitude of the postsynaptic popu-

lation spike has been observed immediately after starting the application of lappaconitine in 10 of 14 slices. Since lappaconitine failed to increase transiently the amplitude of the presynaptic fibre spike, it seems unlikely that an increased afferent input is responsible for the enhancement of the postsynaptic spike. Further studies recording the membrane potential of the CA1 pyramidal cells during application of lappaconitine are required, in order to investigate if the early potentiation is due to changes in membrane potential.

It is obvious from the present results that lappaconitine is a less effective inhibitor of synaptic transmission at hippocampal CA1 pyramidal cells, since it is equally effective in reducing the orthodromic as well as the antidromic spike. Lappaconitine reduces the amplitude of the latter one also in a nominal Ca^{2+}-free medium, which abolishes all synaptic transmission in the slice. Although in some of the experiments a decrease in the amplitude of the f-e.p.s.p. has been observed, this is presumably the consequence of the simultaneously occurring decrease in the presynaptic fibre spike reflecting a lowered afferent input at the dendrites of CA1 pyramidal neurones. Moreover, it has been shown previously that lappaconitine has no effect on outward potassium, inward calcium, and neurotransmitter-activated currents as induced by, for example, glycine, taurine, GABA, glutamate and ATP (Valeev *et al.*, 1990). These findings are in accordance with the present results showing a lack of a direct effect on the f-e.p.s.p.

Thus, comparison of the lappaconitine-induced effects on the orthodromic spike, the antidromic spike and the f-e.p.s.p. imply that the main effect consists of an inhibition of axonal conductance, mediated probably by reduction of sodium currents.

Provided that lappaconitine inhibits sodium currents (Valeev *et al.*, 1990), one of the most striking features of its action described here is its affinity to the functional state of the sodium channel, i.e. to the open or to the inactivated state. This activity-dependent action of lappaconitine could result from drug binding to open or inactivated sodium channels and from the maintenance of the modified state of drug-bound channels during the interpulse intervals, as demonstrated for several anticonvulsive and antiarrhythmic drugs (Ragsdale *et al.*, 1991). Indeed, the present findings show that lappaconitine inhibits neuronal activity in an activity-dependent manner resulting in an increased extent of inhibition. Both the onset of the lappaconitine-induced inhibition and its recovery during washout with standard ACSF are dependent on the frequency of electrical stimulation (Figures 3, 4 and 5). This is in line with recently obtained effects of aconitine (Ameri *et al.*, 1996). An increase in stimulation frequency accelerates the drug-induced inhibition, whereas a decrease in stimulation frequency enhanced the extent of recovery during washout.

The activity-dependent mode of action of lappaconitine has raised the question of whether the drug would be effective in suppressing aberrant electrical activity of CA1 pyramidal cells which occurs during epileptic seizures. Epileptiform activity was induced in the present study, either by blockade of GABAergic inhibition with bicuculline (Campbell & Holmes, 1984; Herron *et al.*, 1985; Ault & Wang, 1986; Chagnac-Amitai & Connors, 1989) or by omission of Mg^{2+} in order to unmask NMDA receptor-mediated responses (Coan & Collingridge, 1985; Anderson *et al.*, 1986; Mody *et al.*, 1987). The present findings demonstrate that lappaconitine (10 μM) selectively blocks the later spikes in an epileptiform burst. The results are consistent with the interpretation that lappaconitine inhibits selectively excessive neuronal excitability, thus blocking the generation and spread of aberrant activity by sparing normal neuronal activity. The activity-dependent effects of lappaconitine may be important for filtering the high frequency bursts of action potentials characteristic of epileptiform activity. It is concluded, that the activity-dependent action reflects the accumulation of lappaconitine at activated sodium channels during an epileptic burst of action potentials as described already for conventional anticonvulsants, e.g. phenytoin and carbamazepine (Ragsdale *et al.*, 1991). However, the present study reveals differences in the anticonvulsive action of lappaconitine dependent on the epilepsy model involved. Obviously, lappaconitine is more potent in suppressing epileptiform activity evoked by the $GABA_A$ antagonist bicuculline. Thus, by reducing sodium currents (Valeev *et al.*, 1990) lappaconitine could be a selective blocker of the spread of epileptic activity which is mainly due to $GABA_A$-mediated disinhibition (Chagnac-Amatai & Connors, 1989).

Moreover, it is obvious from the present study, that the pattern of the multiple, epileptiform population spikes elicited in period 2 by perfusion with the epileptogenic ACSF did not reappear during washout of lappaconitine with the epileptogenic ACSF in period 4. In view of the control experiments performed, in which epileptiform activity was shown to persist in all cases during an observation period of 6 h, the inhibition is unlikely to be caused by rundown, but can be considered to be an antiepileptic effect of lappaconitine. Since the washout of lappaconitine has been shown in the present study to be accelerated by low stimulation frequencies (Figures 4 and 5), the maintenance of inhibition observed in epileptic slices is due to the activity-dependent unbinding of the drug from its binding-site.

In conclusion, the present study provides evidence that lappaconitine has, in addition to its antinociceptive effects, anticonvulsive properties due to its highly activity-dependent mode of action. Further experiments are required to clarify the mechanism of action of lappaconitine and to investigate the relation to membrane potential.

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