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Effects of the *Aconitum* alkaloid mesaconitine in rat hippocampal slices and the involvement of α- and β-adrenoceptors

Angela Ameri

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

1 The effects of mesaconitine, the main alkaloid contained in *Aconiti* tuber, were investigated by use of extracellular recordings of stimulus-evoked population spikes and field excitatory postsynaptic potentials (e.p.s.ps) in the CA1 region of rat hippocampal slices.

2 At a concentration of 10 nM, mesaconitine evoked excitations, which were manifested as an increase in the amplitude of the orthodromic spike and the appearance of multiple spikes following the first postsynaptic spike, without affecting the magnitude of paired-pulse facilitation. The increase in spike amplitude was persistent and was not reversed by up to 90 min of washout. At concentrations of 30 and 100 nM, the alkaloid produced a biphasic effect, that is an excitation followed by an inhibition without having any effect upon the field e.p.s.p. At concentrations above 100 nM, mesaconitine suppressed the orthodromic population spike and the field e.p.s.p.

3 The excitatory effect was also observed when electrical stimulation was stopped completely during the application of mesaconitine (10 nM) and during the first 15 min of washout.

4 The enhancement of the population spike and the appearance of multiple spikes induced by mesaconitine (10–100 nM) were blocked by pretreatment with the β-adrenoceptor antagonists propranolol (1 μM) and timolol (1 μM), whereas the inhibitory effect was blocked by the α-adrenoceptor antagonists yohimbine (1 μM) and phentolamine (10 μM). However, when the β-adrenoceptor antagonist timolol was added 10 min after the application of mesaconitine, it failed to block the long-lasting enhancement of the spike amplitude and the appearance of multiple population spikes.

5 Application of the selective β-adrenoceptor agonist isoprenaline (500 nM) to the hippocampal slices induced an increase in the amplitude of the orthodromic population spike and elicited 2–3 additional spikes. Mesaconitine (10 nM) did not further potentiate this enhancement of the spike amplitude when added after a 15 min pretreatment with isoprenaline.

6 Perfusion of forskolin, which directly activates adenylate cyclase, enhanced the population spike. Mesaconitine had no additional effect when applied after pretreatment with forskolin.

7 It is concluded that the excitatory effects evoked by lower concentrations of the plant alkaloid mesaconitine are mediated by stimulation of β-adrenoceptors and the consequent activation of intracellular processes which lead to the long-lasting changes in excitability.

Keywords: Hippocampus; mesaconitine; timolol; propranolol; yohimbine; population spike

Introduction

It has been recently shown that the *Aconitum* alkaloids aconitine and 3-acetylaconitine exert a concentration-dependent inhibitory action on neuronal activity in rat hippocampal slices (Ameri *et al*., 1996; Ameri, 1997). This effect seems to be due to a sustained depolarization which appears as a consequence of the activation of the voltage-dependent sodium channel by the two structural related alkaloids (Schmidt & Schmitt, 1974; Mozahayeva *et al*., 1977; Catterall, 1980; Ameri, 1997).

Preparations of *Aconitum* roots are employed in Chinese and Japanese medicine for analgesic, antirheumatic and neurological indications (Hikino *et al*., 1982; Hikino & Murayama, 1985; Suzuki *et al*., 1994). The pharmacological effects are attributed to several diterpenoid alkaloids, some of which have been isolated in the past (Hikino *et al*., 1979; Han & Chen, 1988).

The C_{19} diterpenoid alkaloid mesaconitine is one of the main alkaloids contained in plants of the genera *Aconitum*. It occurs in a number of *Aconitum* species, often together with aconitine (Benn & Jacyno, 1983) and is the main alkaloid in the European subspecies *A. napellus*. The two alkaloids have very closely related chemical structures. Mesaconitine has a N-methyl function rather than the N-ethyl of aconitine. Mesaconitine has been shown to be a centrally-acting analgesic drug without affinity for opioid receptors (Isono *et al*., 1994). Recently it has been shown that mesaconitine activates inhibitory noradrenergic neurones of descending inhibitory pathways (Isono *et al*., 1994).

The present study was designed to investigate (1) if mesaconitine affects neuronal activity in rat hippocampal slices and (2) if the noradrenergic system contributes to this effect. The hippocampus is known to receive a diffuse projection of noradrenaline containing fibres which originate in the locus coeruleus (Loy *et al*., 1980) and activation of these noradrenergic afferents has a profound influence on neuronal activity in the hippocampus (Segal & Bloom, 1976; Olpe *et al*., 1986; Washburn & Moises, 1989). Noradrenaline, in turn, has been shown to interact with α-adrenoceptors to decrease pyramidal cell excitability, and with β-adrenoceptors to increase cell excitability (Mueller *et al*., 1981). Moreover, there is evidence that β-adrenoceptor activation is involved in long-term increases of neuronal excitability in hippocampal slices (Heginbotham & Dunwiddie, 1991; Dunwiddie *et al*., 1992; Thomas *et al*., 1996). Extracellular recordings of stimulus-evoked population spikes were studied as a physiological measure of the pharmacological response to mesaconitine.

Methods

Slice preparation

Experiments were performed on hippocampal slices from male Wistar rats (150–180 g). The preparation of the hippocampus was performed as described previously (Ameri *et al*., 1996). In brief, the rats were deeply anaesthetized with ether and killed by rapid decapitation. 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 a submerged brain slice recording chamber, where it was continuously perfused with warmed (32°C) artificial cerebrospinal fluid (ACSF) at 3–4 ml min^{-1} and held down on a nylon net by a U-shaped piece of flattened platinum wire. The other slices were maintained at room temperature in an incubation chamber. The standard ACSF was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and contained (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCI_2$ 2.5, $MgSO_4$ 2, glucose 15 at a pH of 7.4.

Stimulation and recording

The experimental protocol always included a recovery period of 1 h after slice preparation. For recordings of stimulus-evoked population spikes and field excitatory postsynaptic potentials (field e.p.s.ps), the recording electrodes were placed in stratum pyramidale and stratum radiatum of area CA1, respectively. The electrodes were pulled on a BB-CH-PC electrode puller (Mecanex S.A., Swiss) from 1.5 mm borosilicate glass and filled with 3 M NaCl (resistance 5–10 MΩ). A concentric bipolar stainless steel electrode with 0.25 mm outer diameter (Rhodes Medical Instruments, U.S.A.) was positioned into the Schaffer collaterals (i.e. near the junction of CA1 and CA2 stratum radiatum) for orthodromic activation of CA1 pyramidal neurones. Extracellular stimuli were rectangular current pulses of 200 μs in duration delivered every 15 s through a digitally controlled stimulus isolation unit (Axon Instruments, U.S.A.). At the beginning of each experiment, the stimulus intensity was adjusted until the responses to electrical stimulation were about 50% of the maximal response. In some experiments, stimulus pulses were delivered in pairs with an interstimulus interval of 40 ms and an interpair interval of 30 s. The response of the first stimulus of the pair was used to assess the effect of mesaconitine on synaptic transmission and the ratio of the second response to the first was used to assess its effect on paired facilitation. Responses evoked by each 10 consecutive stimulus pulses were averaged. The signal from the recording electrode by means of a DP 301 amplifer (Warner Instruments, U.S.A.). Analogue date were digitized and analysed with the date aquisition and analysis software TIDA (HEKA electronic, Germany).

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

Drugs

Mesaconitine (obtained from O. Krishtal, Bogomoletz Institute, Kiev, Ukraine) was dissolved in dimethylsulphoxide (DMSO) to give stock solutions of 1 or 10 mM. These solutions were diluted with ACSF to reach the desired concentrations and gassed before being perfused into the bathing medium. Staurosporine and forskolin (Sigma, Deisenhofen, Germany) and H-89 (N-[2-(f-bromocinnamylamino) ethyl)]-5-isoquinoline sulphonamide; Biomol, Hamburg, Germany) were dissolved in DMSO to give a stock solution of 10 mM. Control experiments had revealed that the highest final DMSO concentration (0.1%) did not affect any of the parameters measured. Propranolol-HCl, timolol-HCl, yohimbine-HCl, phentolamine-HCl, and isoprenaline-HCl were obtained from Sigma (Deisenhofen, Germany) and dissolved in distilled water. The drugs were delivered through the perfusion medium. In all experiments, each drug application was preceded by a control period of at least 30 min.

Data analysis

Mean data are presented as mean ± s.d. Comparisions of the effects of drug treatments (normalized as % of control) between groups of slices were performed by use of Student's *t* test for differences between two independent means. The statistical significance of the difference of the amplitude of the electrophysiological responses before and following the administration of a drug was assessed with paired Student's *t* test. In both cases, differences were considered statistically significant when $P \leqslant 0.05$. The amplitude of the population spike, which appeared as a large negative wave superimposed on a positive-going e.p.s.p., was determined as the length of a vertical line, drawn from the minimum of the population spike to the the line that joined the two positive peaks of the field response.

Results

Effects of mesaconitine on CA1 excitability

The effects of the *Aconitum* alkaloid mesaconitine were investigated in concentrations ranging from 1 nM–1 μM. Mesaconitine exerted variable effects on population spikes recorded in rat hippocampal pyramidal layer. At a concentration of 10 nM, mesaconitine elicited an enhancement of the orthodromic population spike. The enhancement began during the first 10 min of drug-application. Maximum increase amounted to 124.25 ± 4.5% of control (n = 7, $P \leqslant 0.001$). After 15 min of the superfusion with mesaconitine, a second population spike appeared in all slices. The amplitude of the presynaptic fibre spike was not affected by the drug. In 6 of 7 slices, the enhancement of population spike was persistent and was not reversed by up to 90 min of washout. Although the stimulation frequency (0.067 Hz) used in the present study to test the pyramidal cell response did not evoke an enhancement of the spike amplitude in control experiments, it seemed possible that the increase in the spike amplitude reflected an interaction between mesaconitine and repetitive stimulation. In order to exclude this possibility, stimulation was stopped completely during the application of mesaconitine (10 nM) and during the first 15 min of washout. As can be seen in Figure 1 and Table 1, increases in the amplitude of the postsynaptic population spike occurred also in absence of electrical stimulation.

A higher concentration of mesaconitine (30 nM) evoked biphasic effects (Figure 2a,b). First the drug increased the spike amplitude. The maximum increase was observed after 30–45 min of drug-application and amounted to 134.21 ± 7.2% of

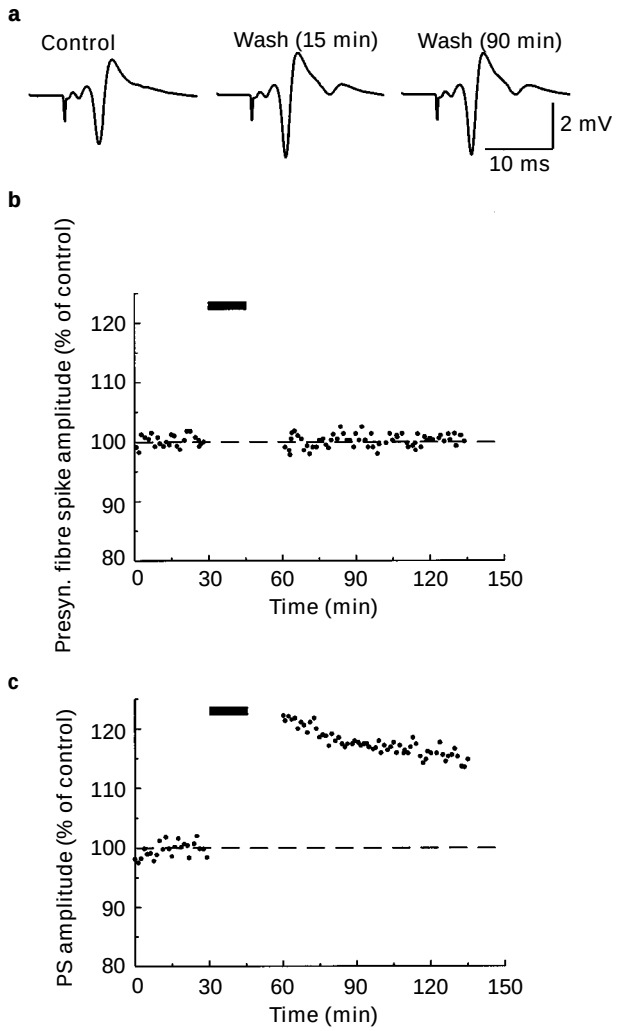

Figure 1 Effect of mesaconitine (10 nM) in absence of electrical stimulation. At control before the application of the alkaloid, the afferents were stimulated every 15 s. Stimulation was stopped during the perfusion of mesaconitine and for 15 min of washout. The traces in (a) show the averages of 5 subsequent responses at the end of the control, at 15 min after starting washout (start the stimulation) and 90 min after washout. The graphs in (b) and (c) show the time-course of the amplitudes of the presynaptic fibre spike and of the postsynaptic population spike, respectively. Note that mesaconitine failed to affect the presynaptic fibre spike. Each data point represents the average of responses evoked by each 5 consecutive stimulus pulses. The bar above the graphs indicates the time of drug application. One representative experiment out of 4 similar ones is shown.

Table 1 Effects of mesaconitine, isoprenaline and forskolin on the population spike (PS) amplitude

Treatment	*Pretreatment*	*PS amplitude* (as % of control)	
Mesaconitine (10 nM)	None	124.3±4	(8)**
	Isoprenaline (500 nM)	101.2±7	(4)
	Forskolin (1 μM)	108.6±8	(5)
	Staurosporine (1 μM)	96.8±8	(6)
	H-89	102.5±8	(4)
Mesaconitine (10 nM) (in absence of stimulation)	None	126.8±4	(5)**
Mesaconitine (30 nM)	None	134.2±7	(6)[a]**
	Propranolol (1 μM)	102.5±4	(6)[b]
	Timolol (1 μM)	105.1±7	(4)[b]
	Yohimbine (1 μM)	130.4±8	(5)*
Timolol (1 μM)	Phentolamine (10 μM)	127.6±7	(4)
	None	101.4±4	(4)
	Mesaconitine (10 nM)	125.8±6	(5)**
Isoprenaline (500 nM)	None	138.7±18	(8)*
	Staurosporine (1 μM)	102.1±11	(4)
Forskolin (10 nM)	None	127.6±9	(5)*

Data are expressed as mean±s.d. and are normalized with respect to the control before drug-application. Numbers in parentheses indicate the number of slices investigated. All comparisons were made by use of paired Student's t test, $*P \leqslant 0.01$, $**P \leqslant 0.001$. [a]Mesaconitine (30 nM) exerted a biphasic effect. Only the maximum increase of spike amplitude occurring 30 min after the start of the application was measured. [b]Determined after 30 min of mesaconitine-application.

control ($n=6$, $P \leqslant 0.001$). This enhancement was subsequently followed by a depression of the population spike. At a concentration of 100 nM ($n=7$), mesaconitine produced a very transient increase in spike amplitude in 4 slices which was followed by a decrease. In 3 slices there was only a depression of the orthodromic response without a preceding excitation. At higher concentrations of the alkaloid, the population spike was always inhibited without prior enhancement. Figure 2c shows the concentration response relationship for the effect of mesaconitine on the orthodromic population spike. No account was taken of the biphasic effect produced by 30 and 100 nM mesaconitine, because the spike amplitude was measured 30 min after the start of the application of the alkaloid.

Despite the excitatory effect of mesaconitine, at concentrations of 10 and 30 nM, on the population spike recorded in stratum pyramidale of area CA1, there was no effect on the field e.p.s.p. recorded in stratum radiatum of the CA1 region (Figure 2d). However, at concentrations above 100 nM, the field e.p.s.p. was diminished.

In order to determine whether the increase in the postsynaptic population spike observed during application of mesaconitine at low concentrations is localized presynaptically or postsynaptically, the effects of this alkaloid on paired-pulse facilitation were examined. When a synapse is activated twice with a short interval between each stimulus, the second response at most synapses, including excitatory synapses in the hippocampus, is facilitated. This phenomen is attributed to an increase in the amount of transmitter released in response to the second stimulus (Zucker, 1989) and, thus, represents a purely presynaptic mechanism. By use of paired stimuli with a 40-ms interstimulus interval, population spikes displayed facilitation when evoked in this manner (162.5±4.3 %, $n=6$). At a concentration of 10 nM, mesaconitine failed to alter the control value of paired pulse facilitation (158±6.2%, $n=6$). This lack of effect on paired pulse facilitation is consistent with a postsynaptic site of action of mesaconitine.

Effects of α- and β-adrenoceptor antagonists on the mesaconitine-evoked changes in excitability

The effects of mesaconitine on rat hippocampal excitability are similar to previously observed effects of noradrenaline. It has been shown that a low concentration of noradrenaline enhances the population spike recorded in the CA1 region by an interaction with β-adrenoceptors (Mueller *et al.*, 1981; 1982; Fowler & O'Donnell, 1988). At higher concentrations of noradrenaline, α-adrenoceptor-mediated attenuation of the spike is observed (Mueller *et al.*, 1982; Mynlieff & Dunwiddie, 1988). Noradrenaline does not affect the field e.p.s.p. recorded in area CA1 (Mueller *et al.*, 1981). On the other hand,

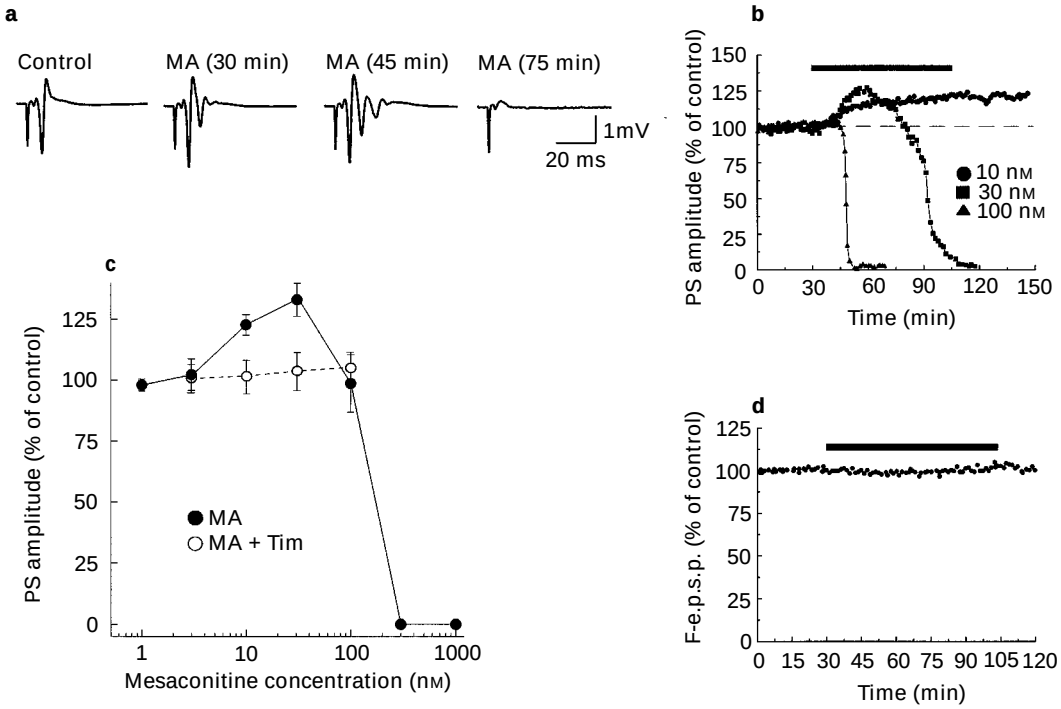

Figure 2 Effect of mesaconitine on the amplitude of the orthodromic responses recorded in area CA1. (a) Population spikes recorded extracellularly in CA1 stratum pyramidale. The excitatory effect of mesaconitine (MA, 30 nM) manifested itself as an increase in spike amplitude and an appearance of additional spikes. The excitation was followed by a complete suppression of the orthodromic response. (b) Time-course of the mesaconitine-induced effect. At a low concentration (10 nM), mesaconitine enhanced the population spike, whereas a high concentration (300 nM) suppressed the spike. An intermediate concentration (30 nM) evoked a biphasic effect, i.e. an increase followed by a depression of the orthodromic response. The solid bar indicates the time of drug-application. Each data point represents the average of responses evoked by each 5 consecutive stimulus pulses. For each concentration one representative experiments out of at least 4 similar ones is shown. (c) Concentration-response relationship for the effect of mesaconitine. The amplitude of the population spikes was measured at 30 min after the beginning of mesaconitine application so that the biphasic effects of 30 and 100 nM are not taken into account. Each data point represents the mean $\pm$ s.d. of at least 5 experiments. The excitatory effect of mesaconitine was blocked by pretreatment of the slices with the β-adrenoceptor antagonist timolol (Tim; 1 μM). (d) Time course of the field e.p.s.p. recorded in CA1 stratum radiatum. Mesaconitine (30 nM) completely failed to affect the synaptic response recorded at the dendrites of the pyramidal cells. Each data point represents the average of field e.p.s.p amplitudes evoked by each 5 consecutive stimulus pulses.

mesaconitine has been shown to activate noradrenergic neurones of descending inhibitory pathways (Isono *et al.*, 1994). Thus, it seems likely that mesaconitine might exert its inhibitory and excitatory effects via α-adrenoceptor and β-adrenoceptors, respectively. In order to test this possibility, experiments were performed with both α-adrenoceptor and β-adrenoceptor antagonists. Propranolol and timolol were employed as β-adrenoceptor antagonists, yohimbine and phentolamine as α-adrenoceptor antagonists. Control experiments revealed that none of the antagonists had any effects on the population spike amplitude. Mesaconitine was applied in a concentration of 30 nM, because this concentration exerted a biphasic effect, as described above. In the first set of experiments, slices were preincubated for 15 min with either propranolol (1 μM) or timolol (1 μM). When mesaconitine was added, the excitatory response was completely blocked, whereas the inhibitory effect on the population spike was not prevented by the β-receptor antagonist (Figure 3a, Table 1). In contrast, when mesaconitine was added to a ACSF containing either yohimbine (1 μM) or phentolamine (10 μM), a sustained enhancement of the spike amplitude, as well as additional spikes, became obvious, whereas the inhibitory action was effectively antagonized (Figure 3b, Table 1). However, both yohimbine and phentolamine failed to antagonize the inhibitory action evoked by mesaconitine at high concentrations (0.3 and 1 μM).

Due to the fact that both stimulation of the locus coeruleus (Olpe *et al.*, 1986; Washburn & Moises, 1989) and application of indirect-acting sympathomimetics to the hippocampal slice (Mueller *et al.*, 1982) produce increases in the population spike, it has been suggested that the β-receptor-mediated effect is the more physiological relevant of the two actions. Therefore, the excitatory effect elicited by mesaconitine was examined further. The sustained enhancement of the population spike produced by 10 nM of the drug, as well as the transient increase produced by 30 and 100 nM, were blocked by the β-receptor antagonist timolol (see also Figure 2c). However, when timolol (1 μM) was added 15 min after the application of mesaconitine (10 nM), it was not capable of blocking the excitation (Figure 4, Table 1). This suggests that the excitatory effect of mesaconitine, which seems to be β-receptor-mediated, is not just the result of continuous activation of β-adrenoceptors by the alkaloid, but rather that there might be a process occurring which, once initiated, is no longer affected by blockade of β-receptors with timolol.

Comparisons of the mesaconitine-evoked effect with those of isoprenaline and forskolin

As a further test of the hypothesis that the mesaconitine-evoked excitation is mediated by β-adrenoceptors, the effects of the selective β-receptor agonist isoprenaline (500 nM) was

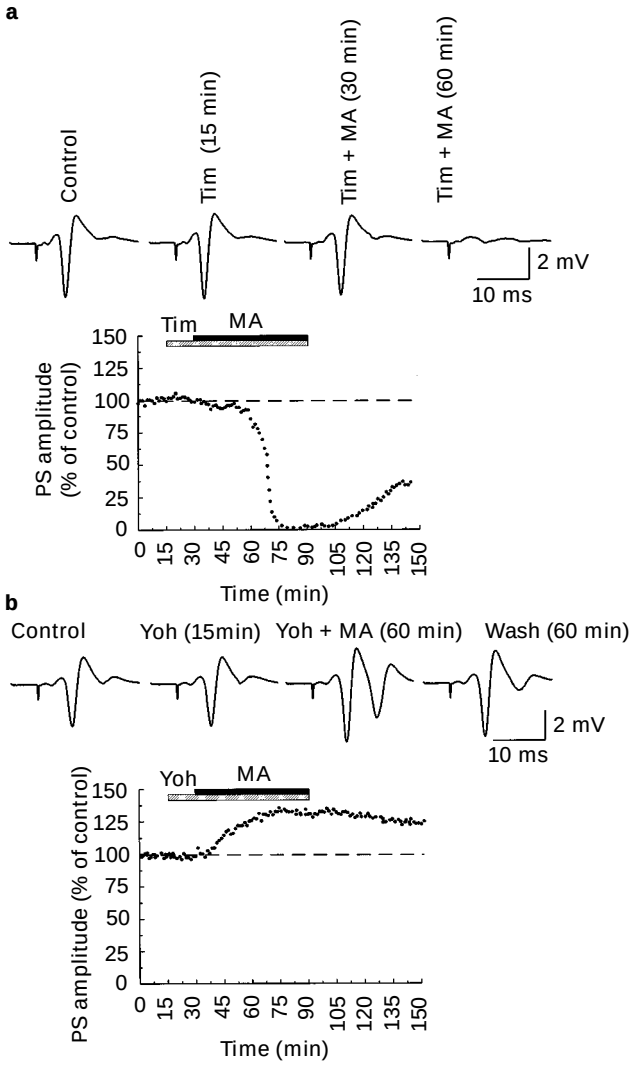

Figure 3 Interaction of mesaconitine and adrenoceptor antagonists on population spike amplitude. (a) Pretreatment with timolol (Tim; 1 μM), a β-antagonist, prevented the excitatory effect of mesaconitine (MA, 30 nM), but not its inhibitory effect. (b) Pretreatment with yohimbine (Yoh; 1 μM) caused mesaconitine (MA, 30 nM) to exert a persistent increase in population spike amplitude and to evoke an additional spike. The solid bars above the graphs indicate the application of mesaconitine, the hatched bars the application of the antagonists. Each data point represents the average of 5 consecutive measurements of orthodromic population spikes. One representative experiment for each antagonist is shown.

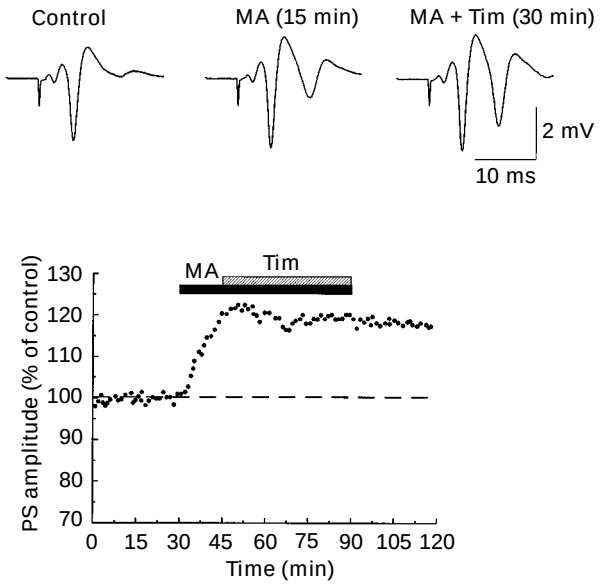

Figure 4 Pretreatment with mesaconitine prevents the effect of timolol. When 10 nM mesaconitine (MA) was applied 15 min before the β-receptor antagonist timolol (Tim, 1 μM), the excitatory response to the alkaloid was not blocked. The solid bar above the graph indicates the application of mesaconitine, the hatched bar the application of the timolol. Each population spike and each data point in the graph represents the average of responses evoked by each 5 consecutive stimulus pulses. One representative experiment out of 4 similar ones is shown.

investigated. According to previous studies (Heginbotham & Dunwiddie, 1991; Dunwiddie *et al.*, 1992), isoprenaline enhances the population spike (Table 1) and exerts 2–3 additional population spikes. In the next series of experiments, the effect of increasing the population spike amplitude by isoprenaline on the subsequent responses to mesaconitine was determined to see if they shared a common mechanism of action. If mesaconitine increased the population spike via different mechanisms of action, its effect should be additive with that of isoprenaline. In these experiments, slices were pretreated with 500 nM isoprenaline. After the increase in population spike had occurred, stimulation intensity was reduced, so that the spike amplitude was reduced to the control value obtained before the addition of isoprenaline. Then 10 nM mesaconitine was added. As shown in Figure 5a, pretreatment with isoprenaline completely prevented the response to mesaconitine.

The present results imply that mesaconitine, via activation of β-adrenoceptors, initiates intracellular processes leading to sustained excitability. Since the electrophysiological responses to β-receptor stimulation occur via activation of adenylate cyclase, the effects of mesaconitine (10 nM) were compared with those of forskolin which is known to activate adenylate cyclase directly (Seamon *et al.*, 1981; Daly *et al.*, 1982; Onodera & Kogure, 1989). Ten minutes after the start of the application of forskolin (10 μM) the amplitude of the population spike was significantly increased (Table 1). The effect of mesaconitine after a preincubation with forskolin was studied. As described above, the stimulation intensity was decreased so that the spike amplitude was reduced to the control value measured before application of forskolin, and mesaconitine (10 nM) was added. In all slices ($n=5$), mesaconitine failed to enhance the population spike in forskolin pretreated preparations (Figure 5b, Table 1).

Furthermore, the effects of the protein kinase inhibitor staurosporine on the excitatory effects of mesaconitine as well as those of isoprenaline were examined. Staurosporine (500 nM) does not affect the population spike, but has been shown to be effective at inhibiting adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-dependent protein kinase (Davis, 1989). When either 10 nM mesaconitine ($n=6$) or 500 nM isoprenaline ($n=3$) was applied after a 15 min preincubation with staurosporine, the increase in population spike amplitude and the appearance of additional spikes were blocked. In addition, the more selective inhibitor of cyclic AMP-dependent protein kinase, H89 (1 μM), completely prevented the enhancement of the population spike by 10 nM mesaconitine ($n=4$, Table 1).

The effects of membrane-permeable analogues of cyclic AMP were not studied because they depress synaptic transmission and inhibit cyclic AMP accumulation via activation of extracellular adenosine A_1 receptors (Dunwiddie

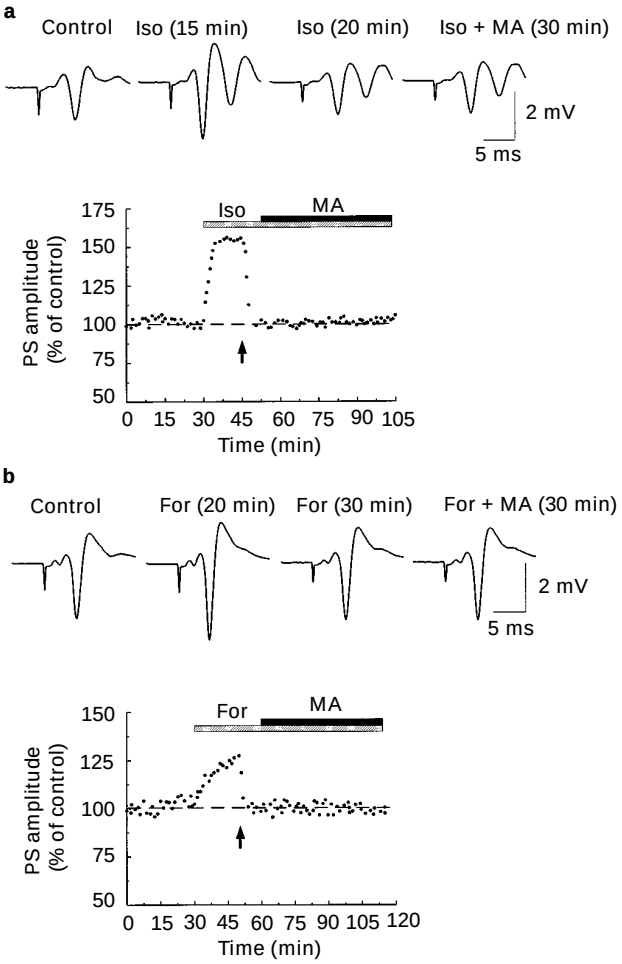

Figure 5 Effects of isoprenaline and forskolin on the population spike. (a) The β-receptor agonist isoprenaline (Iso; 500 nM) increased the spike amplitude and evoked additional spikes. After a 15 min application of isoprenaline (hatched bar), the stimulation intensity was reduced to return the spike amplitude to its control value as marked by the arrow-heads. Then mesaconitine (MA, 10 nM) was added (solid bar). (b) Forskolin (For; hatched bar, 10 μM) increased the spike amplitude without eliciting additional spikes. After 20 min, stimulation intensity was reduced and mesaconitine (10 nM, solid bar) was added. Note that mesaconitine was not capable of enhancing excitability after pretreatment with either isoprenaline or forskolin. Each population spike and each data point in the graph represent the average of responses evoked by each 5 consecutive stimulus pulses. One representative experiment out of 3 others is shown.

& Hoffer, 1980; Dunwiddie & Fredholm, 1989), complicating the interpretation of the effects of these compounds.

Discussion

The aim of the present study was the investigation of the electrophysiological effect of the *Aconitum* alkaloid mesaconitine and a possible contribution of the noradrenergic system. The preceding results demonstrated that mesaconitine was capable of evoking a long-lasting excitatory action at a low concentration, a depressant action at high concentrations and biphasic effects in an intermediate concentration range, probably via the involvement of the noradrenergic system. Exposure of the slices to low concentrations of mesaconitine failed to alter the magnitude of paired-pulse facilitation. At Schaffer collateral synapses, a result of this type may be taken to mean that the test substance does not alter the probability of transmitter release (Manabe *et al*., 1993; Debanne *et al*., 1996). Several lines of evidence support an involvement of the noradrenergic system. The biphasic effect observed during application of 30 and 100 nM mesaconitine is similar to the action of noradrenaline in the rat hippocampal CA1 region. The hippocampus receives a major noradenergic projection from the locus coeruleus (Loy *et al*., 1980) and activation of these fibres has variant effects (Segal & Bloom, 1976; Olpe *et al*., 1986; Washburn & Moises, 1989). In rat hippocampal slices, it has been shown that noradrenaline can either potentiate or attenuate the population spike in a concentration-dependent manner. Low concentrations of noradrenaline increase the excitability of neurones in the CA1 region (Mueller *et al*., 1981; 1982; Fowler & O'Donnell, 1988) and in the dentate gyrus (Stanton & Sarvey, 1985a; 1987), mediated by interaction with β-receptors which results in a long-lasting potentiation. At a higher concentration of noradrenaline, α-receptor-mediated inhibition has been observed (Mueller *et al*., 1981; 1982; Mynlieff & Dunwiddie, 1988). The fact that the two different kinds of actions evoked by 30 and 100 nM mesaconitine in a biphasic manner are selectively prevented by pretreatment with α- and β-adrenoceptor antagonists provides evidence that the effects involve activation of α- and β-receptors.

The persistant enhancement of the population spike evoked by 10 nM mesaconitine seems to be mediated via β-adrenoceptors, because it was blocked by both timolol and propranolol, and mimicked by the selective β-receptor agonist isoprenaline. The long-lasting increase in spike amplitude without any effect on the field e.p.s.p. elicited by isoprenaline observed in the present study is in accordance with previous findings (Heginbotham & Dunwiddie, 1991; Dunwiddie *et al*., 1992; Thomas *et al*., 1996). The finding that mesaconitine was without an effect when applied after pretreatment with isoprenaline is consistent with a common mechanism of action. Indeed, the long-lasting effect of iosprenaline (500 nM) as well as of mesaconitine (10 nM) consisted of an increase in population spike that was accompanied by an appearance of additional population spikes. Coupled with the fact that no changes in the magnitude of paired-pulse facilitation were observed, these results imply a postsynaptic locus of action. Thus, with respect to the low concentration employed, it appears that the main effect of mesaconitine in the CA1 region consisted of an enhancement of pyramidal cell excitability. In this respect the mesaconitine-evoked potentiation is clearly distinct from long-term potentiation (LTP) occurring in the hippocampal formation (Bliss & Lomo, 1973). With LTP in the CA1 region of the hippocampus an increase in the field e.p.s.p. has been demonstrated, that is concurrent with the increase in the population spike, and is similarly long lasting (Bliss & Collingridge, 1993). It has been shown that the induction of LTP in area CA1 (in contrast to the dentate gyrus) does not require the interaction of noradrenaline with β-adrenoceptors (Sarvey *et al*., 1989), and it has been suggested that the β-receptor-evoked potentiation of the population spike observed in CA1 is not identical with LTP (Heginbotham & Dunwiddie, 1992). However, in the dentate gyrus, both LTP and noradrenaline-induced potentiation involve activation of β-adrenoceptors (Stanton & Sarvey, 1985a,b), and it has been proposed that noradrenergic modulation of long-term neuronal plasticity in this region involves an interaction with N-methyl-D-aspartate (NMDA) receptors leading to enhanced Ca^{2+}-influx (Stanton *et al*., 1989). The physiological relevance of an activation of β-adrenoceptors in area CA1 has been demonstrated as a facilatory effect on the induction of LTP by

low frequency stimulation (Thomas *et al*., 1996). This is in accordance with the present findings demonstrating a pronounced excitatory effect of mesaconitine also in the absence of electrical stimulation. The β-receptor-mediated long-lasting enhancement of the population of spike occurring in CA1 which is not accompanied by an increase in the field e.p.s.p. was referred to as β-adrenoceptor-mediated potentiation by Heginbotham & Dunwiddie (1991). The present study provides evidence that a similar process may occur in CA1 stratum pyramidale which is initiated by the naturally occurring alkaloid mesaconitine.

The assumption that the excitatory effect of a low concentration of mesaconitine is mediated by activation of β-adrenoceptors is further supported by the finding that the production of the long-lasting effect was not dependent on the duration of exposure to mesaconitine. As shown in the present study, a 15 min application was sufficient. The finding that timolol failed to block the excitation after pretreatment with mesaconitine implies that the alkaloid had initiated an intracellular process leading to sustained excitability which was no longer antagonized by blockade of β-adrenoceptors receptors with timolol. It is in accordance with this assumption that mesaconitine had no effect after pretreatment of the slices with the selective inhibitor of cyclic AMP-dependent protein kinase, H-89. Moreover, mesaconitine had no additional effect on excitability after stimulation of adenylate cyclase by forskolin. It is concluded that it is the increase in cyclic AMP and protein kinase A activation, rather than the activation of β-adrenoceptors *per se*, that results in the long-term increase of population spike amplitude.

At higher concentrations (30 and 100 nM) of mesaconitine, the excitatory effect was only transient and followed by a depression of the population spike, which was antagonized by the α-receptor antagonists yohimibine and phentolamine. However, at the highest concentrations tested in the present study (0.3 and 1 μM), both antagonists failed to block the inhibitory effect of mesaconitine. Considering the structural relationship with aconitine, it seems that the inhibition caused by high concentrations of mesaconitine is mediated by an aconitine-like interaction with site 2 of the voltage-dependent sodium channel (Catterall, 1992), leading to sustained depolarization (Schmidt & Schmitt, 1974; Mozahayeva *et al*., 1977) and complete block of excitability (Ameri *et al*., 1996).

Although the present study provides evidence that the mesaconitine-evoked changes in neuronal excitability involve activation of adrenoceptors, the question remains in which manner the alkaloid interacts with these receptors. Assuming that mesaconitine, like aconitine and veratridine, is capable of activating voltage-dependent sodium channels (Catterall, 1980; 1992), it is possible that it can induce exocytotic release of noradrenaline and outward transport of noradrenaline by the noradrenaline transporter as shown for veratridine (Bönisch *et al*., 1983; Bönisch & Trendelenburg, 1987). In fact, it has been demonstrated that mesaconitine-induced contractions of the guinea-pig vas deferens is due to neuronal release of noradrenaline (Sato *et al*., 1979). The present study demonstrates that mesaconitine at low concentrations elicited an excitatory effect in rat hippocampal CA1 region which seems to be mediated by β-adrenoceptors. Further studies are required, in order to differentiate β_1 from β_2 actions of mesaconitine.

Mesaconitine has been shown to possess non-opioid antinociceptive properties *in vivo* (Hikino *et al*., 1982; Hikino & Murayama, 1985; Suzuki *et al*., 1994), which are at least in part mediated by activation of the descending noradrenergic fibres (Isono *et al*., 1994). In the present study it was shown that, in rat hippocampal slices, mesaconitine produced variant effects via α- and β-adrenoceptors in a concentration-dependent manner. Thus, it is of interest to compare the analgesic active doses with the concentrations used in the present experiments. The brain concentrations of mesaconitine after *in vivo* administration are not known. A dose of 1 mg kg^{-1}, p.o., in rat has been shown to induce to analgesia (Isono *et al*., 1994). Assuming that, based on the lipophilic nature of this substance, mesaconitine distributes easily from plasma to tissues and reaches about the same concentrations in tissue as in plasma, brain concentrations after a doses of 1 mg kg^{-1}, p.o., would be 2.64 μM. This is far above the concentrations which were found to exert a long-lasting increase in excitability, but this is in the concentration-range where complete suppression of neuronal activity occurred.

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