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Effects of the *Aconitum* alkaloid songorine on synaptic transmission and paired-pulse facilitation of CA1 pyramidal cells in rat hippocampal slices

[1]Angela Ameri

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

1 The present study investigated the electrophysiological effects of songorine (1 – 100 μM), an alkaloid occurring in plants of the *Aconitum* genus, in rat hippocampal slices.

2 Songorine (10 – 100 μM) evoked a concentration-dependent increase in the amplitude of the orthodromic population spike and in the slope of the field e.p.s.p. The enhancement was long-lasting and was not reversed by up to 90 min of washout. Songorine failed to affect size and shape of the presynaptic fiber spike which represents the compound action potential of the Schaffer collaterals. This indicates that enhancement of the synaptic response is no consequence of an increased afferent excitability.

3 The antidromically evoked population spike was not affected by songorine at concentrations up to 100 μM suggesting that the enhancement of the orthodromic population spike and of the field e.p.s.p. was not due to an increase in pyramidal cell excitability.

4 The input-output curve for the postsynaptic population spike was shifted to the left implying that a presynaptic fiber spike of the same size elicited a larger postsynaptic response, indicating a decrease in threshold for generation of the population spike.

5 The songorine-evoked increase in excitability was not affected by the NMDA receptor antagonist, D-AP5. However, the effect of songorine was completely abolished by the selective dopamine D_2 receptor antagonist sulpiride (0.1 μM) as well as by haloperidol (10 μM) and was mimicked by application of the dopamine releaser, amantadine (100 mM). In contrast, the selective D_1 receptor antagonist, SCH23390, did not block the action of songorine.

6 The results indicate that the plant alkaloid songorine enhances excitatory synaptic transmission which may be due to an agonistic action at D_2 receptors.

Keywords: *Aconitum* alkaloids; hippocampus; dopamine; paired-pulse facilitation

Introduction

Songorine is a C_{20} diterpenoid alkaloid from plants of the *Aconitum* genus. Preparations of *Aconitum* roots are employed in Chinese and Japanese medicine as antirheumatics, analgesics, anaesthetics, and in the treatment of various neurological disorders (Bisset, 1981, Han & Chen, 1988). 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). Songorine was first isolated from *Aconitum soongaricum*. It is the 12-keto analog of napelline, which fails to affect neuronal excitability, whereas its structural analog, 1-benzoylnapelline inhibits neuronal excitability in rat hippocampal slices (Ameri, 1997a). It has been reported from *in vivo* studies that the physiological effects of songorine differ from those of other diterpenoid alkaloids, for although cardiovascular activity is seen at high doses, the most noticeable effects are on CNS activity (Benn & Jacyno, 1983). Songorine has been shown to exhibit stimulant effects on the CNS (Tulyaganov *et al*., 1978). It has been reported that subcutaneous administration of 50 – 100 mg kg^{-1} of songorine to cats resulted in a stereotypic rocking motion of the head (Benn & Jacyno, 1983). Furthermore, it has been shown that the sedative effect of the dopamine receptor antagonist bulbocapnine is antagonized by songorine (Tulyaganov *et al*., 1978). With a LD_{50} of 142.5 mg kg^{-1} (mice, i.v.) the toxicity of songorine appears to be relatively low (Benn & Jacyno, 1983).

The aim of the present study was to investigate (1) if songorine affects neuronal activity in rat hippocampal slices and (2) if the dopaminergic system contributes to these effects. A further objective of the present study was to determine whether songorine acts at a presynaptic or postsynaptic site in the hippocampal slice.

Previous studies have shown that the hippocampus receives rich dopaminergic innervation from the mesocorticolimbic dopamine system (Meador-Woodruff *et al*., 1991, 1994), and receptor-binding, molecular cloning and *in situ* hybridization studies have ascertained the presence in the hippocampus of both D_1 and D_2 subtypes of dopaminergic receptors (Kohler *et al*., 1991; Meador-Woodruff *et al*., 1991; Mansour *et al*., 1992). Although the effects of dopamine in the hippocampus are predominantly inhibitory and result in elevation of spike threshold and slower firing rates (Pockett, 1985; Malenka & Nicoll, 1986; Beretta *et al*., 1990), dopamine can produce both inhibitory and excitatory effects on neuronal activity mediated by dopamine D_1 and D_2 receptors, respectively. At low concentrations, dopamine predominantly binds to the D_2 receptor to induce depolarization accompanied by an increase in the spontaneous firing rate and increase in the amplitude of population spike (Gribkoff & Ashe, 1984; Smialowski & Bijak, 1987; Hsu, 1996; Wang *et al*., 1997). At high concentrations, dopamine binds to both dopamine D_1 and D_2 receptors to evoke a dominant inhibition of spontaneous firing of action potential and hyperpolarization mediated by dopamine D_1 receptors.

In the present study, extracellular recordings of stimulus-evoked population spikes and field excitatory postsynaptic potentials (field e.p.s.ps.) were studied as a physiological measure of the pharmacological response to songorine. To distinguish between pre- and postsynaptic site of action of songorine the paired-pulse facilitation method was employed. 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 release in response to the second stimulus, and there is strong evidence that residual Ca^{2+} from the first presynaptic volley increases the probability of transmitter release to a second volley (Hess *et al*., 1987; Zucker, 1989; Debanne *et al*., 1996). Therefore, paired-pulse facilitation is considered to be an example of purely presynaptic plasticity. The magnitude of paired-pulse facilitation is decreased when transmitter release is enhanced. No change in the magnitude of paired-pulse facilitation is consistent with a postsynaptic site of action (Zucker 1989).

Methods

Brain slice preparation

Experiments were performed on hippocampal slices from male Wistar rats (150–180 g). The rats were deeply anaesthetized with ether and killed by rapid decapitation. The brains were quickly removed from the skulls and the hippocampus of one hemisphere was isolated. Slices of 400 μm thickness were cut transversely to the longitudinal axis of the hippocampus by use of a McIlwain tissue chopper. Immediately after cutting, one slice was transferred into a submerged brain slice recording chamber, where it was continuously perfused with warmed (32°C) ACSF at a flow rate of 3–4 ml min^{-1} and held down on a nylon net by a U-shaped piece of flattened platinum wire. The other slices were maintained at room temperature in an incubation chamber. The standard ACSF was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and contained (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.5, $MgSO_4$ 2, glucose 10 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 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) or in the alveus for orthodromic and antidromic activation of CA1 pyramidal neurons, respectively. 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 interval of 20, 40 and 80 ms between the stimuli and an interpair interval of 30 s. The response of the first stimulus of the pair was used to assess the effect of songorine on synaptic transmission and the ratio of the second response to the first was used to assess its effects on paired-pulse facilitation. Responses evoked by each 10 consecutive stimulus pulses were averaged. The signal from the recording electrode was amplified by means of a DP 301 amplifier (Warner Instruments, U.S.A.). Analog data were digitized and analysed using the data acquisition and analysis software TIDA (HEKA electronic, Germany).

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

Drugs

Songorine (Latoxan, Rosans, France) was dissolved in dimethylsulfoxide (DMSO) to give stock solutions of 1 mM. Haloperidol was purchased from Sigma (Deisenhofen, Germany) and D-AP5, SCH-2330- and sulpiride were purchased from RBI Biotrend Chemicals (Cologne, Germany) and were dissolved in DMSO to give stock solutions of 10 mM. Amantadine hydrochloride (Sigma, Deisenhofen, Germany) was dissolved in distilled water. Control experiments have revealed that the highest final DMSO concentration (0.1%) did not affect any of the measured parameters. These solutions were diluted with ACSF to reach the desired concentrations and gassed before being perfused into the bathing medium. All drugs were delivered through the perfusion medium. In all experiments, each drug application was preceded by a control period of at least 30 min. In some cases the application of songorine was preceded by a 30 min application of an antagonist.

Data analysis

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

Results

Effect of songorine on Schaffer collateral-commissural synaptic transmission

The effects of songorine on the orthodromic and antidromic population spike recorded in CA1 stratum pyramidale was investigated at concentrations between 1–100 μM. At concentrations ranging from 10–100 μM, songorine significantly

increased the orthodromic population spike during 30 min application in a concentration-dependent manner (Table 1, Figure 1). The effect of songorine developed gradually, reaching a steady-state value in about 20 min. The enhancement of the postsynaptic population spike was long-lasting and was not reversed by up to 90 min of washout. One mechanism which could account for the increase in the amplitude of the postsynaptic population spike is that more presynaptic fibers are activated in the presence of songorine than in control. The excitability of the afferent fibers (Schaffer collaterals) innervating the CA1 pyramidal neurons is expressed by the amplitude of the presynaptic fiber spike preceding the postsynaptic population spike. The presynaptic fiber spike (afferent volley) represents the compound action potential of the Schaffer collaterals and is elicited by their electrical stimulation (Dunwiddie, 1986). However, as shown in Figure 1B, the amplitude of the presynaptic fiber spike was not affected by the alkaloid. Moreover, the effect of songorine on the antidromically evoked population spike was investigated to determine whether the drug acts by increasing pyramidal cell excitability. As shown in Figure 2, songorine (10–100 μM) failed to alter the amplitude of the antidromically evoked population spike ($n=7$) indicating a lack of effect on cell excitability.

In order to examine if the effect of songorine is dependent on stimulation intensity, the input-output relationship for the orthodromic response was determined. For this purpose, electrical stimuli of increasing intensity were applied to the Schaffer collaterals and the amplitudes of the according presynaptic fiber spikes and the postsynaptic population spikes were measured at control and at the end of a 30 min application of two different concentrations of the alkaloid and plotted as function of the stimulus-intensity (Figure 3). The input-output curve (Figure 3A) indicate that there is no obvious change in fiber excitability after application of songorine as determined by recording the size of the presynaptic fiber spike. Figure 3B shows that the amplitude of the postsynaptic spike increased with stimulation intensity until a maximum was attained. In all slices tested ($n=7$), songorine (10 and 30 μM) exerted a concentration-dependent shift to the left of the curves. The shift to the left means that a presynaptic fiber spike of the same size elicited a larger postsynaptic response, indicating a decrease in threshold for generation of the population spike.

In order to further investigate whether the enhancement in the size of the postsynaptic population spike is due to an increase in transmitter release at the dendrites of the CA1 pyramidal cells, recordings of field e.p.s.ps were performed in the dendrite region of area CA1 (Figure 4). The field e.p.s.p. reflects synaptic currents in the dendrites of the pyramidal neurones as a result of the action of neurotransmitters. After 30 min of application, the slope of the field e.p.s.p. was increased by songorine (30 μM) to 121.2±4.1% of control ($n=7$, $P\leqslant 0.001$). The enhancement of the field e.p.s.p. did not differ significantly from the action of songorine on the postsynaptic population spike.

Table 1 Effect of the investigated drugs on the amplitude of the orthodromic population spike (PS)

Drug	*Pretreatment*	*PS amplitude* (% of control)
Songorine (1 μM)	None	103.6±4.5 (7)
Songorine (10 μM)	None	119.0±5.3 (6)*
Songorine (30 μM)	None	128.7±5.6 (8)*
	SCH-23390 (0.1 μM)	122.0±5.1 (6)*
	Sulpiride (0.1 μM)	101.3±7.2 (7)
	Haloperidol (10 μM)	97.3±8.1 (5)
	D-AP5 (10 μM)	128.4±4.4 (7)*
Songorine (100 μM)	None	143.2±5.2 (5)*
Amantadine (100 μM)	None	119.3±4.0 (7)*

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 performed by the paired Student's *t*-test. Significance is expressed by an asterix ($P\leqslant 0.01$).

Paired-pulse facilitation

The shift to the left of the input-output curve (Figure 3B) raises the question of whether the enhancement of the postsynaptic population spike evoked by songorine occurs presynaptically or postsynaptically. In order to determine if pre- or postsynaptic mechanisms underly the increase in the amplitude of the postsynaptic population spike observed during application of songorine, the effects of this compound on paired-pulse facilitation were investigated. Paired-pulse facilitation of neurotransmission has been attributed to a presynaptic process in response to a second stimulus which

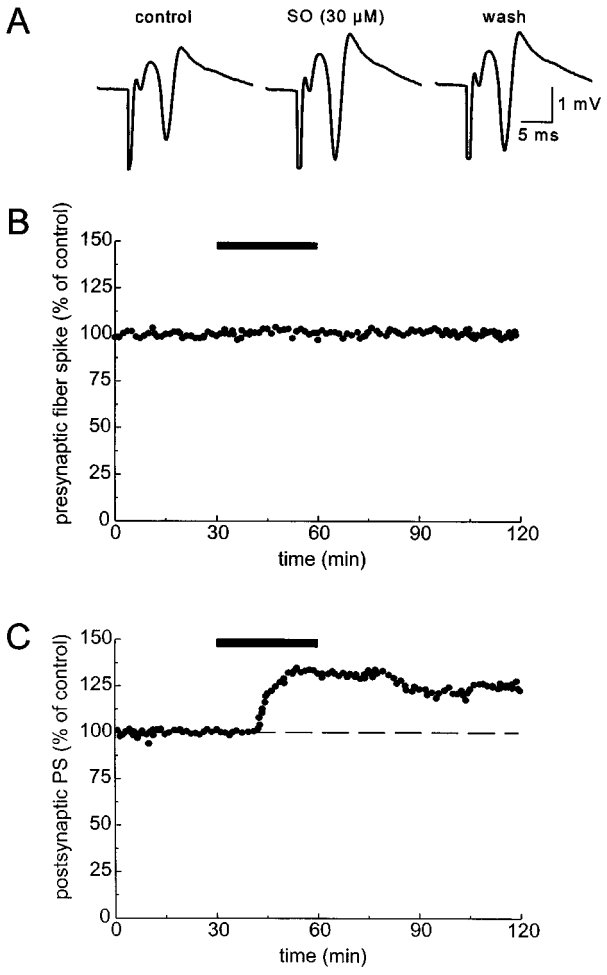

Figure 1 Excitatory effect of songorine (SO, 30 μM) on the orthodromic population spike. (A) Population spikes were elicited by half-maximal stimulation intensity. Each trace represents the average of five subsequent responses at the end of the control, 30 min after starting the application of songorine and at the end of the washout. (B,C) Time-course of the amplitude of the presynaptic fiber spike and of the postsynaptic population spike, respectively. Note that songorine failed to affect the presynaptic fiber spike. The bar indicates when songorine was applied. Each point represents the average of five consecutive measurements. A representative experiment out of eight similar ones is shown.

triggers a proportionally larger amount of transmitter release when it follows shortly after the first stimulus. It is generally assumed that a decrease in the ratio of the second pulse to the first pulse response (P_2/P_1) indicates an increase in release probability. On the other hand, manipulations that depress transmitter release usually increase the magnitude of P_2/P_1 (Hess *et al.*, 1987; Manabe *et al.*, 1993; Schulz *et al.*, 1994). If the songorine-induced enhancement of the orthodromic population spike involves a presynaptic mechanism of action, it will be associated with an alteration of the magnitude of P_2/P_1. Alternatively, if songorine enhances the orthodromic response by another type of mechanism (e.g. by increasing the sensitivity of postsynaptic receptors), then presynaptic events should be relatively unaffected. This hypothesis was tested by comparing the magnitude of P_2/P_1 before and after

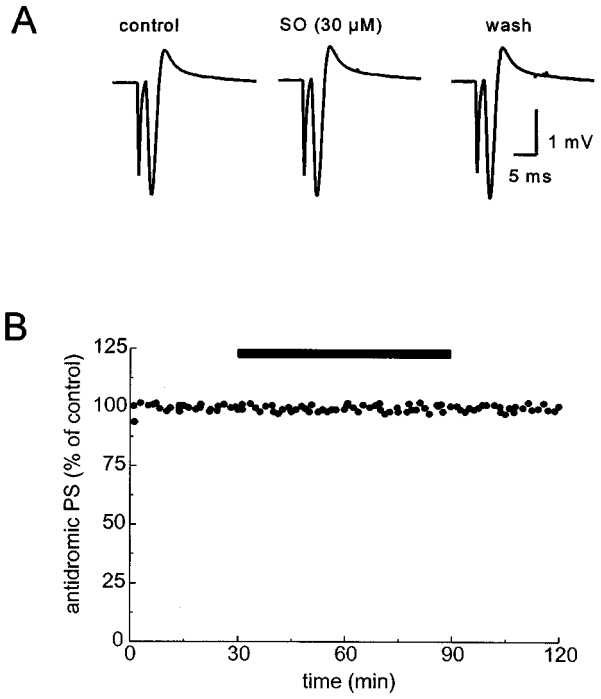

Figure 2 Lack of effect of songorine (SO, 30 μM) on the antidromic population spike. (A) Population spikes were elicited by half-maximal stimulation intensity. Each trace represents the average of five subsequent responses at the end of the control, 60 min after starting the application of songorine and at the end of the washout. (B) Time-course of the amplitude of the antidromic population spike. The bar indicates when songorine was applied. Each point represents the average of five consecutive measurements. A representative experiment out of seven similar ones is shown.

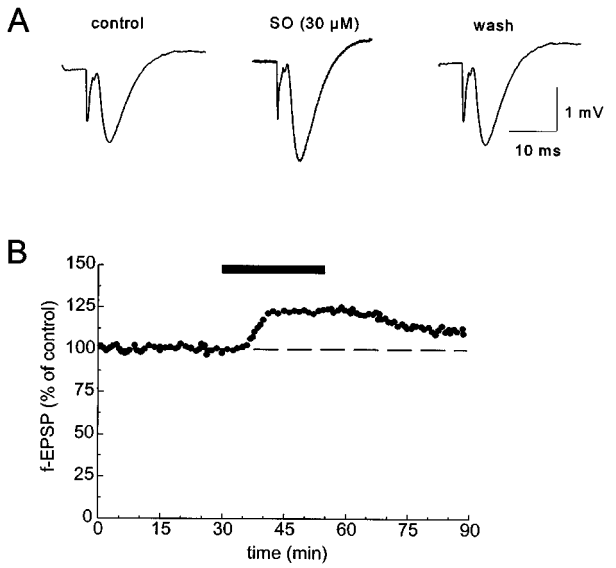

Figure 4 Effect of songorine (30 μM) on the field excitatory postsynaptic potential (e.p.s.p.). (A) Field e.p.s.p. recorded extracellularly in the apical dendritic region of area CA1. Schaffer collaterals were stimulated every 15 s. Each trace is the average of five consecutive recordings. (B) The graph shows the time-course of the action of songorine on the slope of the field e.p.s.p. Each data point in the graph represents the average of five consecutive measurements. The bar indicates the time of drug-application.

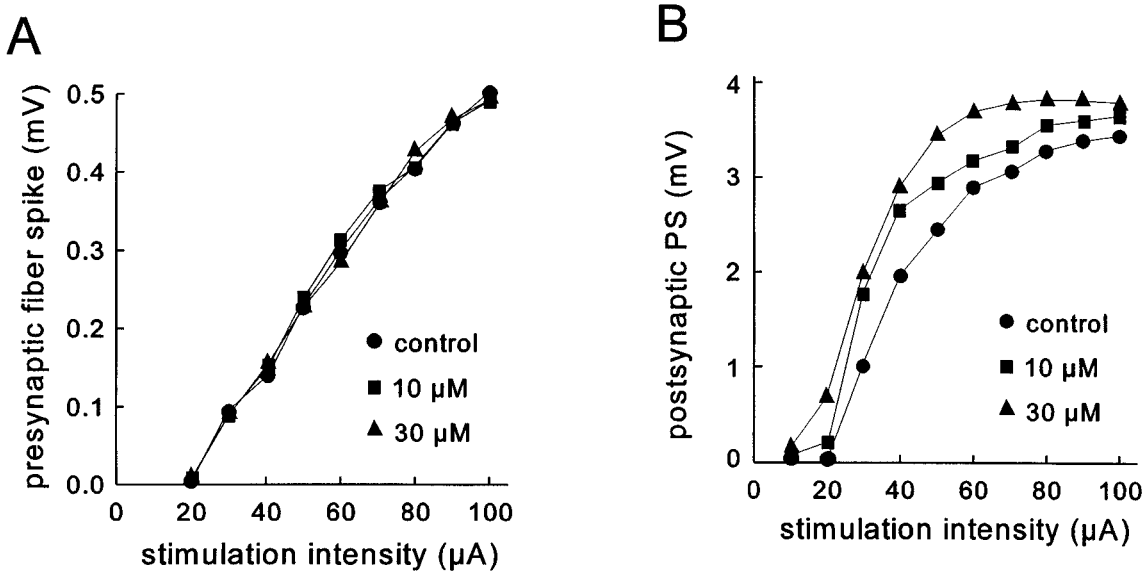

Figure 3 Effect of songorine (SO, 10 and 30 μM) on the input-output relationship of the orthodromic response. The amplitudes of the presynaptic fiber spike (A) and the postsynaptic population spike (B) were measured as a function of the stimulation intensity at control and in the presence of the alkaloid (10 μM and 30 μM). Note that the input-output relationship for the presynaptic fiber spike was not affected by songorine, whereas the input-output curve of the postsynaptic spike was shifted to the left. A representative experiment out of eight similar ones is shown.

treatment of the slices with songorine (30 μM). In the present experiments, paired-pulse stimulus induced population spikes were evoked with stimulus intervals of 20, 40 and 80 ms. At control, i.e. prior to the application of songorine, the second response was always larger than the response to the first stimulus. Maximum values of facilitation were achieved with a stimulus interval of 40 ms. Figure 5A shows population responses to a pair of stimuli with a stimulus interval of 40 ms. Using paired stimuli delivered with a 40 ms stimulus interval, population spikes displayed facilitation of $180.3 \pm 12\%$ of control ($n=8$) when evoked in this manner.

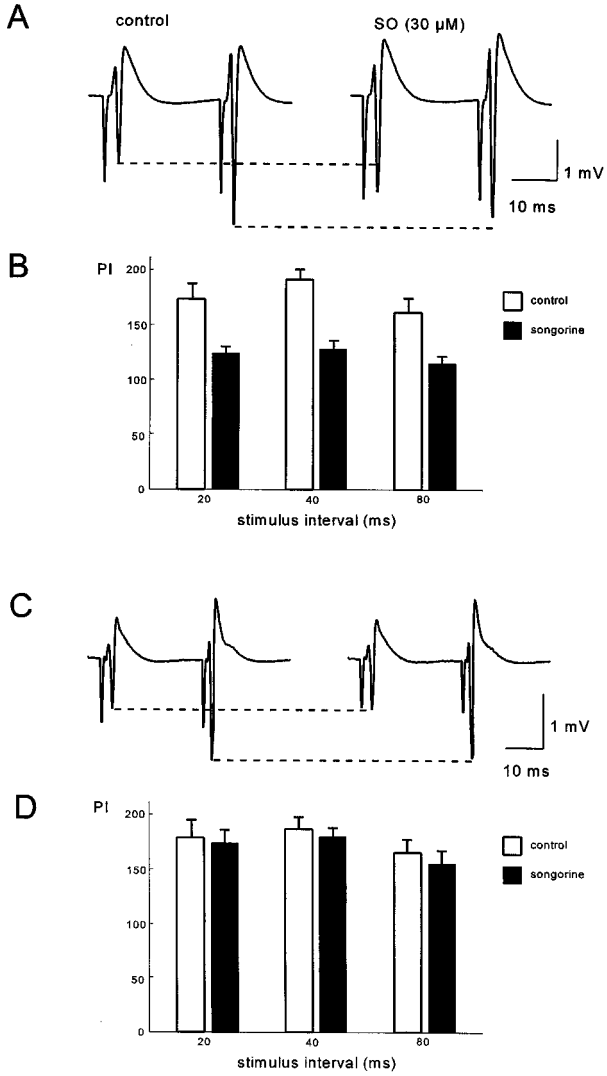

Figure 5 Effect of songorine (30 μM) on the orthodromic paired-pulse stimulus induced responses in CA1 pyramidal cell region. (A) Average of ten consecutive population spikes evoked by paired stimuli (40 ms interval) at control and 30 min after starting the application of songorine. Exposure to songorine enhanced the response to the test pulse, but the ratio was decreased. (B) Comparison of the effects of 30 μM songorine on the paired-pulse index (PI) calculated from responses to paired-pulse stimulation of different intervals (20 ms, 40 ms and 80 ms) in CA1 stratum pyramidale. Songorine decreased the PI values significantly at all intervals ($P<0.001$, $n=8$). (C) After 30 min of songorine application, the stimulation intensity was decreased to counteract a direct effect of songorine on the first postsynaptic population spike. (D) songorine (30 μM, $n=7$) failed to affect PI values at all intervals after the decrease in stimulation intensity. The PI was calculated according to the formula $PI = P_2/P_1 \times 100\%$ with P_1 being the average of ten responses to the first stimulus and P_2 being the average of ten responses to the second stimulus.

Bath application of 30 μM songorine increases the response to the first stimulus, but decreases the magnitude of this paired-pulse facilitation in all slices tested ($132.3 \pm 8.4\%$ of control, $n=8$). The results from eight experiments for all three stimulus intervals tested are summarized in Figure 5B. However, recently, it has been shown that the magnitude of paired-pulse facilitation decreased when the response to the first stimulus increased (Debanne *et al*., 1996). In order to investigate if the relative reduction in amplitude of the second spike is due to the effect of songorine on the response to the first stimulus, further experiments ($n=7$) were performed. At the end of the songorine application, the stimulation intensity was decreased to return the amplitude of the population spike to its control value in order to counteract the direct enhancing effect of songorine on the first spike. At these conditions, however, the paired-pulse facilitation observed after application of songorine (30 μM) was not significantly reduced (Figure 5C and D). This result suggests that songorine is not likely to act on the presynaptic site to modulate the transmitter release mechanism in the CA1 region of rat hippocampus.

Involvement of the dopaminergic system in the action of songorine

Since a previous *in vivo* study has reported that songorine antagonizes the effects of the dopamine D_2/D_1 receptor

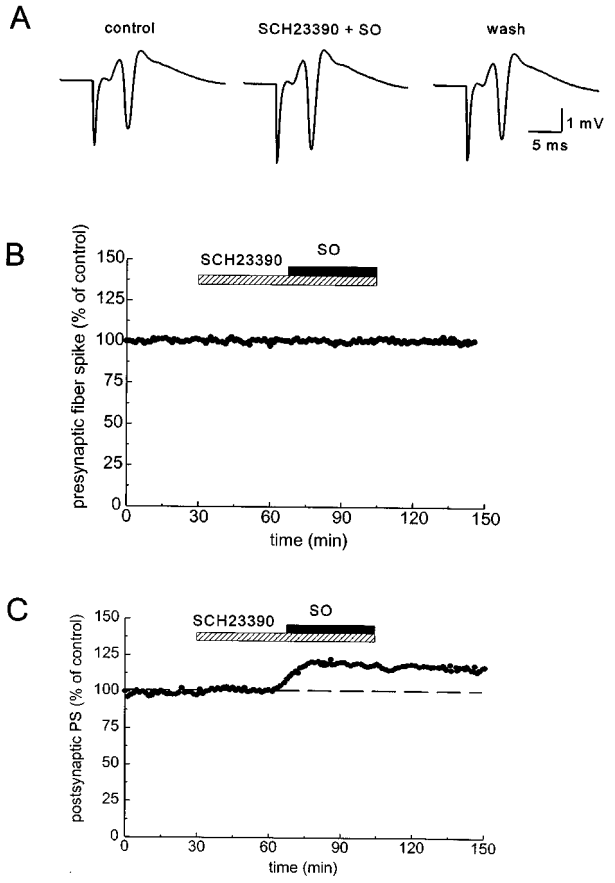

Figure 6 Effect of songorine (SO, 30 μM) after preapplication of the dopamine D_1 receptor antagonist SCH23390 (0.1 μM). (A) Each trace represents the average of five subsequent orthodromic population spikes evoked by half-maximal stimulation-intensity. (B, C) Time-course of the amplitude of the presynaptic fiber spike and of the postsynaptic population spike. Each point represents the average of five subsequent measurements. A representative experiment out of six similar ones is shown.

antagonist bulbocapnine (Tulyaganov *et al*., 1978), a next series of experiments was designed, in order to investigate whether the action of songorine in rat hippocampal CA1 region involves the dopaminergic system. For this purpose, selective dopaminergic receptor antagonists as well as amantadine, a dopamine releasing agent, were used to block or to mimic, respectively, the excitatory effect of songorine. SCH-23390 was used as a selective dopamine D_1 receptor antagonist. The results of these experiments are summarized in Table 1. When SCH-23390 (0.1 μM) was applied 30 min prior to the application of songorine (30 μM), the songorine-induced sustained enhancement of the postsynaptic population spike was not altered (Figure 6). However, as shown in Figure 7, the selective dopamine D_2 receptor antagonist sulpiride (0.1 μM) completely blocked the enhancement of the orthodromic population spike induced by 30 μM songorine. Moreover, the dopamine D_2/D_1 receptor antagonist haloperidol (10 μM) abolished the songorine induced effect. There was on effect of the dopamine receptor antagonists tested in the present study, when applied prior to songorine.

Amantadine, a dopamine releaser, was employed at concentrations from 1, 10 and 100 μM. Bath application of 10 μM amantadine resulted in an increase in spike amplitude to $108.5 \pm 2\%$ of control ($n=5$, $P \leqslant 0.01$), bath application of 100 μM enhanced the spike amplitude to $119.3 \pm 4\%$ of control ($n=7$, $P \leqslant 0.001$). Both magnitude of effect of amantadine and its time-course (Figure 8) resembled the action of 10 μM songorine.

Effect of the NMDA receptor antagonist D-AP5 on the songorine-induced effect

As shown in Figures 1 and 4, the songorine-induced enhancement of the orthodromic population spike and the field e.p.s.p. was sustained still after washout of the alkaloid. It is well known that most forms of long-lasting plasticity in hippocampal CA1 region require the activation of N-methyl-D-aspartate (NMDA) receptors for their induction (Malenka & Nicoll, 1993; Bear & Abraham, 1996). In order to examine a possible involvement of NMDA receptors in the action of songorine, a further set of experiments was performed with the NMDA receptor antagonist D-AP5. Coapplication of D-AP5 (10 μM) with 30 μM songorine failed to block the enhancement of the spike amplitude evoked by the alkaloid (Table 1).

Discussion

The aim of the present study was to investigate the effects of the plant alkaloid songorine on central neurons. Previously, it

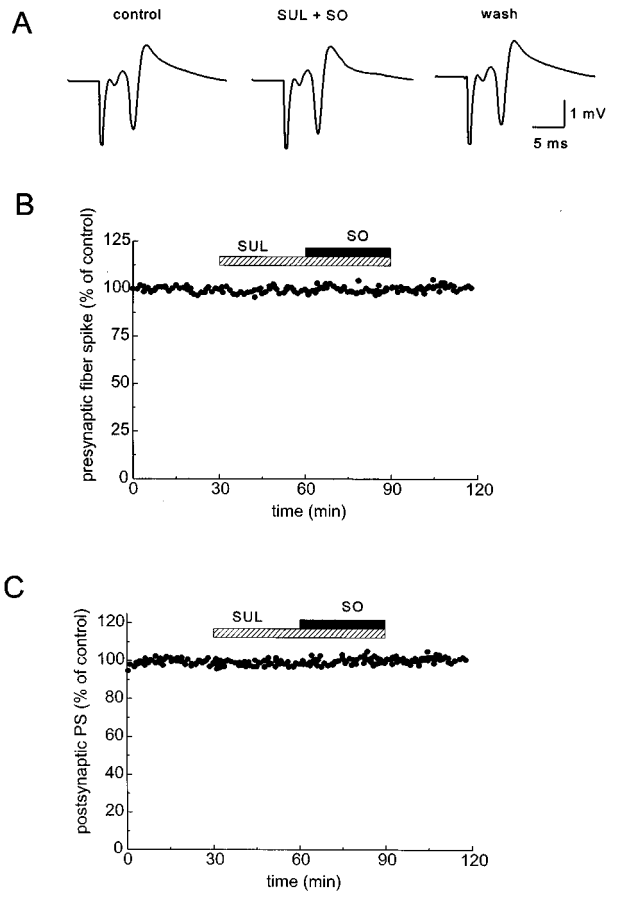

Figure 7 Blockade of the songorine-evoked excitation by sulpiride. The dopamine D_2 receptor antagonist sulpiride (SUL, 0.1 μM) was applied for a period of 30 min before songorine (SO, 30 μM) was added. Under this condition, songorine completely failed to increase the amplitude of the population spike. (A) Each trace represents the average of five subsequent orthodromic population spikes evoked by half-maximal stimulation-intensity. (B, C) Time-course of the amplitude of the presynaptic fiber spike and of the postsynaptic population spike. Each point represents the average of five subsequent measurements. A representative experiment out of seven similar ones is shown.

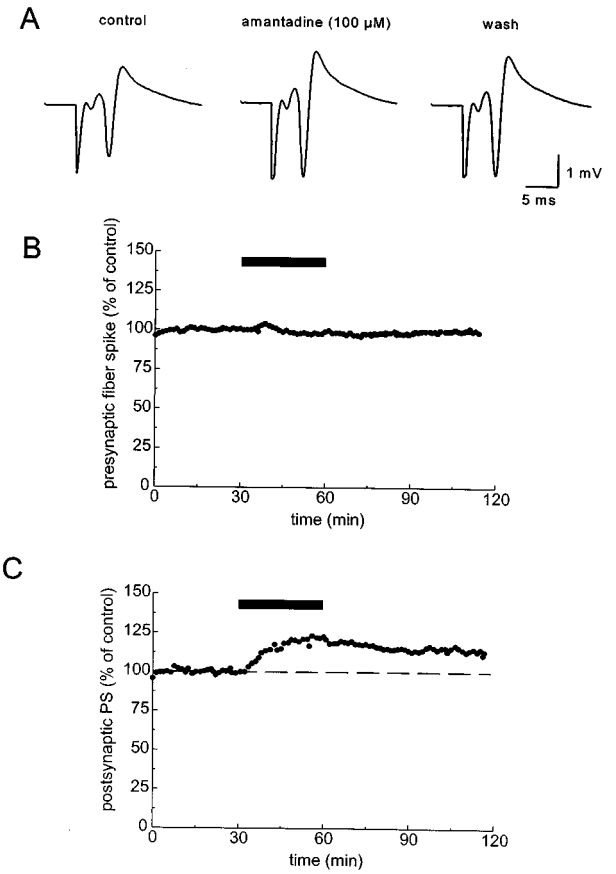

Figure 8 Effect of the dopamine releasing agent, amantadine (100 μM) on the orthodromic population spike. (A) Each trace represents the average of five subsequent orthodromic population spikes evoked by half-maximal stimulation-intensity at the end of control, 30 min after starting the application of amantadine and at the end of the washout. (B, C) Time-course of the amplitude of the presynaptic fiber spike and of the postsynaptic population spike. Each point represents the average of five subsequent measurements. The bar indicates when amantadine was applied. A representative experiment out of seven similar ones is shown.

has been reported that songorine has a stimulatory action *in vivo* which is likely to involve the dopaminergic system (Tulyaganov *et al*., 1978; Benn & Jacyno, 1983).

The present findings provide electrophysiological evidence that songorine produced a concentration-dependent increase in the amplitude of the orthodromic population spike and the field e.p.s.p. The enhancement was neither due to an increased afferent input nor to an enhancement of pyramidal cell excitability as indicated by the lack of effect on the presynaptic fiber spike and antidromic population spike, respectively. The songorine-induced potentiation far outlasted the period of songorine application and did not require the activation of NMDA receptors, because the enhancement of the spike amplitude was not abolished by coapplication of the NMDA receptor antagonist D-AP5. Due to its excitatory effect, the action of songorine differs markedly from the action of other *Aconitum* alkaloids which have been reported recently to inhibit CA1 excitability (Ameri *et al*., 1997a,b).

In accordance with the above mentioned *in vivo* studies, the present findings provide evidence for an involvement of the dopaminergic system in the effects of songorine. The effects of songorine were specifically antagonized by the selective dopamine D_2 receptor antagonist sulpiride as well as by the D_2/D_1 receptor antagonist haloperidol. In contrast, the selective D_1 receptor antagonist possessed no significant inhibitory effect on the sustained enhancement of the orthodromic population spike evoked by songorine. These results imply that the alkaloid songorine may act as a dopamine D_2 receptor agonist. Furthermore, the finding that the effects of songorine were mimicked by the dopamine releaser, amantadine, provides additional evidence that the dopaminergic system is involved in the action of this drug. These findings are in line with previous findings of Tulyaganov *et al*. (1978) who have reported that songorine has stimulant effects in the CNS and demonstrated that the sedative effect of the dopamine D_2/D_1 receptor antagonist bulbocapnine is antagonized by songorine (Tulyaganov *et al*., 1978).

Dopamine mediates its effects in mammalian brain through five subtypes of dopaminergic receptors, (D_1-D_2). Dopamine D_1-like receptors (D_1 and D_5) have been shown to couple to stimulation of adenylate cyclase activity, whereas D_2-like receptors (D_2-D_4) inhibit this enzyme activity (Stoof & Kebabian, 1984; Andersen *et al*., 1990; Silbey & Monsma, 1992; O'Dowd, 1993). All five of the known dopamine receptor subtypes are expressed in the hippocampus (Meador-Woodruff *et al*., 1994) which receives a strong dopaminergic innervation from the mesocorticolimbic dopamine system. Dopamine has both excitatory and inhibitory effects on neuronal excitability depending on the receptor subtype activated. Previously, it has been demonstrated that dopamine evokes an excitatory response in rat hippocampal CA1 region which is long-lasting and which is antagonized by the dopamine D_2 receptor antagonist sulpiride and haloperidol, but not by the D_1 receptor antagonist SCH-23390 (Smialowski & Bijak, 1987). It is still unknown if songorine binds to dopamine receptors, in particular the D_2 receptor.

In the present study, songorine was shown to have no discernible effect on the shape or size of the presynaptic fiber spike, indicating that changes in presynaptic axonal excitability cannot account for the action of songorine on the postsynaptic population spike. This is supported by the input-output curves which revealed that an increase in the number of presynaptic fibers activated do not account for the synaptic enhancement. It is suggested, therefore, that the central effect of songorine may be a result of either an enhancement of transmitter release or a result of a postsynaptic action, and it is unlikely to be due to an increase in axonal excitability.

As demonstrated in Figure 3B, songorine caused a shift to the left of the input-output curve, which means that a presynaptic fiber spike of the same size elicited a much larger postsynaptic response. These results indicate that there is an additional increase in synaptic efficiency during applications of songorine. Schaffer collateral-commissural synaptic transmission may have been enhanced by presynaptic or postsynaptic mechanisms. In order to investigate if songorine potentiates synaptic transmission at a presynaptic site paired-pulse stimulation experiments were performed. Paired-pulse facilitation, in which the second response to two closely spaced stimuli is enhanced, has been studied at a number of synapses and has been attributed to an increase in transmitter release (Hess *et al*., 1987; Zucker, 1989; Debanne *et al*., 1996), and manipulations which are known to have a presynaptic action affect the magnitude of the paired-pulse facilitation (Muller *et al*., 1988). However, Debanne *et al*. (1996) have shown that the magnitude of paired-pulse facilitation is influenced by the stimulation intensity. Although songorine apparently reduced the magnitude of paired-pulse facilitation, it has been shown in the present study that this effect was due to the enhancing effect of the alkaloid on the response to the first stimulus. With respect to the two main findings of the present study (1) the lack of effect on paired-pulse facilitation by songorine and (2) the block of action of songorine by the selective D_2 receptor antagonist sulpiride, it is concluded that this drug does not seem to evoke its excitatory action by stimulating presynaptic neurotransmitter release, but rather by activating dopamine D_2 receptors.

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