APAMIN INCREASES EXCITABILITY OF CA1 HIPPOCAMPAL PYRAMIDAL NEURONS

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SUMMARY

The effects of bath application of apamin, a neurotoxin from bee venom, on CA1 hippocampal pyramidal neurons of young rabbits were examined *in vitro* using the intracellular sharp-electrode recording technique. Apamin dose-dependently reduced the AHP amplitude [$F(5,79) = 3.33$, $P < 0.009$]. The mean percent of AHP reduction at 250 - 1000 nM was ~ 20%. A small reduction of the spike-frequency adaptation was also observed. These significant, but small and dose-dependent, reductions of both the AHP amplitude and spike-frequency adaptation, reflecting increases in hippocampal pyramidal neuron excitability, may have been overlooked in previous reports. The results suggest that the apamin-sensitive AHP contributes little directly to the learning-induced reduction of the AHP and accommodation observed in hippocampal pyramidal neurons, but may contribute indirectly by enhancing cholinergic input to the hippocampus.

KEY WORDS: afterhyperpolarization, apamin, calcium-dependent potassium channel, I_{AHP}, sI_{AHP}, spike-frequency adaptation

INTRODUCTION

Previous studies from our laboratory have demonstrated that neuronal excitability of hippocampal pyramidal neurons are increased [via a reduction of the postburst afterhyperpolarization (AHP) and of spike-frequency adaptation (accommodation)] after hippocampal-dependent learning (20,35). The enhanced neuronal excitability was not observed in either pseudoconditioned controls

(which received the same stimuli, but without the paired association of the stimuli) or in animals that failed to acquire the trace eyeblink-conditioning task. The reduction of the AHP after associative learning is due to the reduction of a slow Ca^{2+}-dependent K^+ conductance (3,28). Thus, modulation of this current may be an important cellular mechanism contributing to associative learning (20,23).

Two slow Ca^{2+}-dependent K^+ conductances have been described in neurons which are distinguished by their sensitivity to apamin: the apamin-sensitive (I_{AHP}) and the apamin-insensitive (sI_{AHP}) (26). Apamin, a centrally acting neurotoxin from the bee *Apis mellifera* (7), has been shown to block a low conductance Ca^{2+}-dependent K^+ channel (SK_{Ca}: a source of the AHP) in various neurons, but was generally accepted to be absent in hippocampal pyramidal neurons (for reviews see: 26,33,36). However, apamin has been shown to bind to (5,9,18) and induce immediate early gene expression in (8) the hippocampus. In addition, apamin improved performance on various behavioral tasks (4,17). These paradoxes have prompted us (22) to reexamine the effect of apamin on the excitability of CA1 hippocampal pyramidal neurons.

The experiments reported here were undertaken to determine the likelihood that an apamin sensitive AHP contributes to the learning-induced reduction of the AHP and accommodation. A portion of these results has appeared in abstract form (22).

MATERIALS AND METHODS

Young (~3 mo) female New Zealand albino rabbits (*Oryctolagus cuniculus*) were used as subjects. Animal use procedures were approved by Northwestern University's Animal Care and Use Committee according to the standards of the US Department of Agriculture.

Details of the hippocampal slice (300 - 400 μm) preparation, intracellular current-clamp recordings and data collection have been described previously (23). The biophysical properties were recorded with the neuron held near -69 mV (using $< \pm 0.2$ nA) before and after the addition of either 0, 50, 250, 500, 750, or 1000 nM apamin into the perfusion solution [artificial cerebrospinal fluid (aCSF) in mM: 124 NaCl, 26 $NaHCO_3$, 10 D-glucose, 3 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.24 NaH_2PO_4, gassed with 95% O_2 - 5% CO_2, pH 7.4, heated to 31 °C]. Apamin was purchased from Sigma, and stock solutions were prepared and stored at -20 °C. The experimenter was blind to the identity of the perfusate until the end of data collection and analysis.

All data were digitized and analyzed on-line using Lab NB or PCI-MIO-16E-4 boards (National Instruments, Austin, TX) interfaced to computers using custom software routines written in LabView (National Instruments) (24). Complete analyses were performed off-line using procedures developed with LabView. Statistical analyses were performed using ANOVA, Kruskal-Wallis and Wilconxon's tests (StatView, SAS Institute Inc, Cary, NC). Significant main effects were evaluated using Fisher's Protected Least Significant Difference (PLSD) post hoc test. All data are reported as the mean ± SEM.

RESULTS

Apamin dose-dependently reduced the AHP amplitude in CA1 pyramidal neurons [$F(5,79) = 3.33$, $P < 0.009$]. The AHP amplitude was significantly reduced at 500, 750, and 1000 nM compared to 0 nM (Fisher's PLSD: p's < 0.006) nM (Figure 1C). The amount of the AHP amplitude reduction was similar (~20 %) at 250 – 1000 nM, indicating that a maximal block was produced by 250 nM apamin (Table 1). There was only a trend for a dose-dependent reduction of the AHP area [$F(5,79) = 2.01$, $P = 0.086$], even though the AHP area was reduced by ~ 30% (250 – 1000 nM).

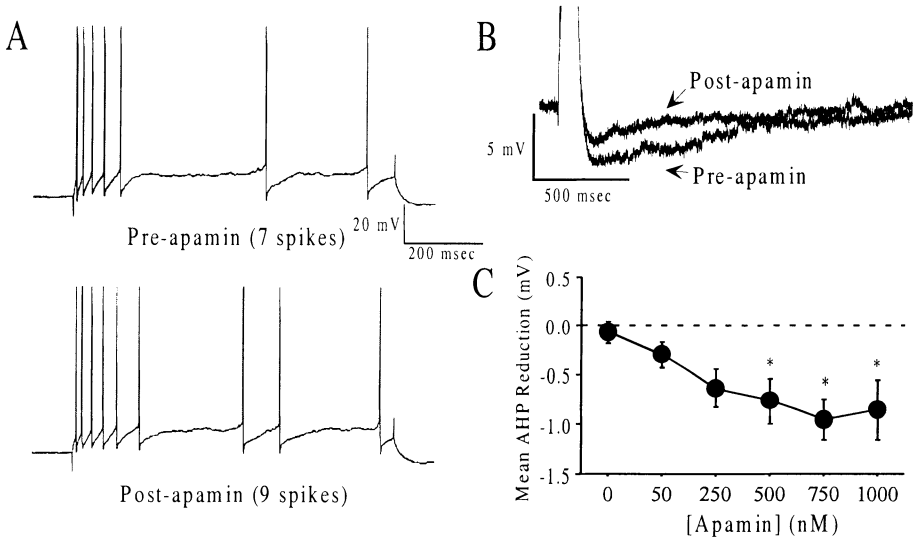

Figure 1. Bath application of apamin significantly decreased the accommodation and the postburst AHP in CA1 neurons. An example of apamin's (250 nM) effect on spike-frequency accommodation is illustrated in panel *A*. An example of AHP reduction observed after addition of 500 nM apamin is illustrated in *B*. Panel *C* illustrates the mean AHP peak amplitude reduction after the perfusion with apamin at various concentrations (mean ± SEM: * $p < 0.006$ for Fisher's PLSD post hoc test compared to 0 nM apamin). Illustrations A and B are from different cells.

The Kruskal-Wallis test revealed that apamin had a significant effect on the accommodation ($p < 0.05$). Wilcoxon's test revealed that the accommodation was significantly reduced by 50 ($p < 0.003$), 250 ($p < 0.02$), and 500 ($p < 0.02$) nM apamin (Table 1). The accommodation reduction caused an increase of 1 – 2 action potentials, a significant change from a 7 – 9 action potential baseline. In contrast, perfusion with normal aCSF (0 nM) was not associated with a significant change in any measure of cell excitability, indicating that the drug effects were not due to neuronal deterioration that may have occurred over time in the recording chamber.

Table 1. Biophysical properties of CA1 neurons before and after bath application of apamin *in vitro*.

			Postburst Afterhyperpolarization			
		n	Amplitude	Area	Duration	Accommodation
aCSF (0 nM)	Baseline		-3.57 ± 0.25	-3.08 ± 0.45	2.70 ± 0.29	9.14 ± 0.84
	Postdrug	24	-3.50 ± 0.23	-2.68 ± 0.44	2.35 ± 0.29	9.27 ± 0.84
	% Δ		-0.16 ± 3.28	-9.47 ± 7.89	-8.92 ± 6.04	2.64 ± 2.98
Apamin (50 nM)	Baseline		-3.59 ± 0.39	-2.71 ± 0.47	2.10 ± 0.26	7.90 ± 0.96
	Postdrug	12	-3.29 ± 0.39	-2.48 ± 0.55	2.21 ± 0.40	**9.21 ± 0.88**
	% Δ		-8.59 ± 4.13	-12.00 ± 10.95	1.22 ± 9.62	21.70 ± 6.00
Apamin (250 nM)	Baseline		-4.44 ± 0.68	-4.78 ± 1.39	3.24 ± 0.75	7.49 ± 0.85
	Postdrug	9	**-3.79 ± 0.67**	-3.44 ± 1.02	2.61 ± 0.64	**8.88 ± 1.11**
	% Δ		-16.53 ± 5.05	-26.89 ± 16.72	-9.17 ± 20.28	18.09 ± 6.73
Apamin (500 nM)	Baseline		-4.05 ± 0.27	-4.17 ± 0.66	2.97 ± 0.34	8.36 ± 0.60
	Postdrug	20	**-3.29 ± 0.22**	-2.63 ± 0.46	2.75 ± 0.36	**9.02 ± 0.66**
	% Δ		-16.42 ± 4.78	-31.56 ± 8.75	-4.71 ± 9.01	8.70 ± 3.61
Apamin (750 nM)	Baseline		-4.41 ± 0.49	-4.22 ± 0.70	2.87 ± 0.42	8.74 ± 1.27
	Postdrug	8	**-3.45 ± 0.35**	-2.51 ± 0.55	2.25 ± 0.55	9.33 ± 1.28
	% Δ		-20.48 ± 3.07	-38.67 ± 11.18	-14.04 ± 21.51	8.20 ± 4.54
Apamin (1000 nM)	Baseline		-4.20 ± 0.49	-4.10 ± 0.89	2.77 ± 0.39	7.66 ± 0.69
	Postdrug	12	**-3.34 ± 0.45**	-2.75 ± 0.68	2.20 ± 0.34	7.99 ± 0.84
	% Δ		-19.46 ± 6.16	-33.41 ± 9.81	-17.38 ± 9.82	5.70 ± 8.41

n indicates the number of cells recorded. The units of the measurements are: (mV) for AHP amplitude, (mVsec) for AHP area, (sec) for AHP duration, and (number of action potentials) for accommodation. The numbers in bold type indicate statistically significant difference compared to baseline measurements. The measurements are the mean ± SEM.

DISCUSSION

The results from the present experiment revealed that bath-application of apamin does reduce the AHP amplitude of CA1 hippocampal pyramidal neurons. This reduction was ~ 20%, similar to that found by Norris et al. (21), and plateaued at 250 – 1000 nM. This indicated that the apamin-sensitive I_{AHP} makes a small contribution to the total AHP, which is dominated by the apamin-insensitive sI_{AHP} in CA1 hippocampal pyramidal neurons (27). The reduction of the I_{AHP} was accompanied by only a modest decrease in the spike-frequency adaptation (< 10%).

We have previously demonstrated that reduction of the AHP and of accommodation in hippocampal pyramidal neurons are well correlated with learning an associative eyeblink conditioning task in rabbits (20,35). Both the AHP and accommodation are reduced in hippocampal pyramidal neurons from rabbits that acquire the task; but not in pseudoconditioned, control animals or in animals that are trained but fail to acquire the task (20,35). We have also demonstrated that the AHP and accommodation are greater in CA1 neurons from aging rabbits as compared to those from young animals (19,23), and that aging rabbits are impaired in acquiring the trace eyeblink

conditioning task (34). These observations led us to postulate that reduced hippocampal neuron excitability, as indicated by the enhanced AHP and accommodation, may contribute importantly to the learning deficits observed with aging.

Apamin administered *in vivo* has been demonstrated to reverse the water maze task deficit observed in mice with medial septal lesions (10), reverse the amnesia induced by potassium channel openers in a passive avoidance task in mice (6), facilitate the acquisition of bar pressing in mice (17), and improve object recognition learning in rats (4). It is possible that these learning enhancements are attributable to the reduced AHP and accommodation of the hippocampal pyramidal neurons due to apamin administration. However, this appears unlikely to be a full explanation because of the small contribution of the apamin-sensitive AHP to the total AHP. The magnitude of the reduction of the AHP and of accommodation reductions we observed here (~ 20% AHP and 9% accommodation) is considerably smaller than that we have observed in CA1 hippocampal neurons in young rabbits after learning the eyeblink conditioning task [~ 40% AHP and 60% accommodation: (20)]. In addition, we have demonstrated with voltage-clamp analysis that metrifonate [a cholinesterase inhibitor that facilitated acquisition of trace eyeblink conditioning (13) and reduced AHP and accommodation in hippocampal pyramidal neurons in aging rabbits (23)] reduced only the apamin-insensitive sI_{AHP} in CA1 neurons (25). Thus, a reduction in the apamin-sensitive AHP may contribute to the facilitation of learning observed following apamin treatment *in vivo*. However, it is unlikely that changes in apamin-sensitive channels are the only nor even a primary cause of the change in neuronal excitability observed following eyeblink conditioning.

An additional mechanism of apamin's facilitating effects on learning *in vivo* which should be considered is via the enhancement of cholinergic modulation of hippocampal pyramidal neuron excitability. Matthews and Lee (16) have demonstrated that bath application of apamin reduced the slow AHP and increased the firing frequency of cholinergic neurons in the medial septum/vertical diagonal band [a major source of cholinergic input to the hippocampus; (37)]. Numerous experiments have demonstrated that modulation of cholinergic transmission affects acquisition of various behavioral tasks. For example, pretreatment with scopolamine, a central cholinergic blocker, impaired acquisition of the eyeblink conditioning task in humans (30) and rabbits (11); whereas, pretreatment with a cholinesterase inhibitor (metrifonate) or muscarinic agonist (CI-1017) facilitated acquisition of the task in aging rabbits (13,38). Furthermore, numerous *in vitro* studies have illustrated that application of ACh, muscarinic agonists, or anticholinesterases increased neuronal excitability of hippocampal pyramidal neurons (1,2,15,23,38). Thus, it is plausible to hypothesize

that apamin facilitates learning by reducing the AHP and accommodation of hippocampal neurons directly and, also, indirectly by increasing the cholinergic input to the hippocampus.

Why did apamin's effect on hippocampal pyramidal neurons previously go unnoticed (for reviews see, 26,33)? As Lancaster and Adams (14) elegantly noted, their negative findings may have been due to problems with tissue penetration, toxin variability, and channel variability. Recently, there have been reports that methyl salts of bicuculline blocked the apamin-sensitive AHP in dopaminergic neurons (29); and more importantly, methyl salts of bicuculline blocked the cloned apamin-sensitive SK (12) and apamin-sensitive I_{AHP} in hippocampal CA1 neurons (31). The reports of bicuculline's effect are significant as it has been used in some of the previous reports examining the AHP in CA1 neurons (e.g., 32); and thus, the apamin-sensitive AHP may have been masked by the bicuculline.

In summary, the present findings illustrate that apamin does reduce the postburst AHP and spike-frequency accommodation in CA1 hippocampal pyramidal neurons. This small (~ 20%), but significant, reduction of the AHP and of spike-frequency adaptation may have been overlooked in earlier studies. The increase of hippocampal pyramidal neuron excitability reflected in these alterations may be a contributing factor in the facilitation of learning various behavioral tasks observed after apamin administration. We hypothesize that apamin may be effective directly, by enhancing the excitability of hippocampal pyramidal neurons, or indirectly, by increasing cholinergic drive onto the hippocampal system. Further experiments will be required to determine if learning causes either or both of these effects.

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