Spermine modulates neuronal excitability and NMDA receptors in juvenile gerbil auditory thalamus

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Abstract

Medial geniculate body (MGB) neurons process synaptic inputs from auditory cortex. Corticothalamic stimulation evokes glutamatergic excitatory postsynaptic potentials (EPSPs) that vary markedly in amplitude and duration during development. The EPSP decay phase is prolonged during second postnatal week but then shortens, significantly, until adulthood. The EPSP prolongation depends on spermine interactions with a polyamine-sensitive site on receptors for N-methyl-D-aspartate (NMDA). We examined effects of spermine application on EPSPs, firing modes, and membrane properties in gerbil MGB neurons during the P14 period of highest polyamine sensitivity. Spermine slowed EPSP decay and promoted firing on EPSPs, without changing passive membrane properties. Spermine increased membrane rectification on depolarization, which is mediated by tetrodotoxin (TTX)-sensitive, persistent Na + conductance. As a result, spermine lowered threshold and increased tonic firing evoked with current injection by up to 150%. These effects were concentration-dependent (ED 50 = 100 μM), reversible, and eliminated by NMDA receptor antagonist, 2-amino-5-phosphonovalerate (APV). In contrast, spermine increased dV/dt of the low threshold Ca 2+ spike (LTS) and burst firing, evoked from hyperpolarized potentials. LTS enhancement was greater at −55 mV than at hyperpolarized potentials and did not result from persistent Na + conductance or glutamate receptor mechanisms. In summary, spermine increased excitability by modulating NMDA receptors in juvenile gerbil neurons.

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Key words: Spermine; Auditory thalamic neuron; Medial geniculate body; N-methyl-D-aspartate; NR2B

1. Introduction

Neurons of the medial geniculate body (MGB) integrate synaptic information from glutamatergic projections of the auditory cortex and inferior colliculus (Gonzalez-Lima and Scheich, 1984; Morel and Imig, 1987; Hu et al., 1994; Metherate and Cruikshank, 1999). Stimulation of the corticothalamic pathway activates postsynaptic N-methyl-D-aspartate (NMDA) and non-NMDA receptors on MGB neurons (Bartlett and Smith, 1999). The receptors for NMDA, robustly expressed in the MGB (Sucher et al., 1995; Khan et al., 2000), become functional at an early age. By the end of the second postnatal week in the rat, NMDA receptors on thalamocortical neurons mediate the transformation of sound-related synaptic inputs to output firing patterns (Heierli et al., 1987). The glutamatergic responses of neurons in visual cortex depend on the subunit composition of the NMDA receptor, including the NR2B subunit (Roberts and Ramoa, 1999). The NR2B subunit, which MGB neurons express in high levels at postnatal day 14 (P14; Hsieh et al., 2002), confers an NMDA receptor sensitivity to spermine and other polyamines which enhance the response to glutamatergic stimulation (Williams et al., 1994).

Abnormal polyamine modulation of glutamatergic systems may occur in pathological states of auditory function (reviewed by McCann and Pegg, 1992). Pharmacological inhibition of polyamine synthesis decreases...
polyamine concentrations in the cochlea (Schweitzer et al., 1986) and induces a temporary hearing loss in humans and rats (McCann and Pegg, 1992). On the other hand, excessive NMDA-mediated excitation contributes to absence epilepsy in an animal model (Koerner et al., 1996). Audiogenic seizure activity, initiated in brainstem nuclei (Faingold et al., 1989), enhances MGB responsiveness to acoustic stimuli (N’Gouemo and Faingold, 1997). Hence, high concentrations of polyamines may induce excessive NMDA receptor activation in MGB neurons which can impair auditory information processing.

Despite the potential significance, there is little information about the effects of polyamines on the firing modes as well as other functional aspects of thalamocortical neurons, especially during postnatal stages of development. The present study on MGB neurons examines, for the first time, the effects of spermine and receptor antagonists on the tonic and burst firing modes, excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs), and the electrical membrane properties. For these experiments, we used gerbils which have a hearing capacity similar to humans (Kraus et al., 1987). At P14, the gerbil’s thalamus has abundant NR2B subunits, implying a neuronal sensitivity to polyamines (Hsieh et al., 2002).

2. Materials and methods

The procedures and conditions for thalamic slice preparation were similar to those described previously for rats (Tennigkeit et al., 1996). In brief, young gerbils (10–14-day-old) were decapitated while under deep isoflurane anesthesia. For the preparation of slices, the cerebral hemispheres were removed rapidly (~1 min) from the cranial vault and immersed for ~1 min in ice-cold (0–2°C) artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM): NaCl, 124; NaHCO3, 26; glucose, 10; KCl, 4; CaCl2, 2; MgCl2, 2; and KH2PO4, 1.25. The ACSF, on saturation with 95% O2 and 5% CO2, had a pH of 7.3. The brain tissue was trimmed into a cube (~0.125 cm3) that contained the cortex and thalamus. We used a Vibroslicer (Campden Instruments, London, UK) to cut slices (thickness, 300–500 μm) in a horizontal orientation. The slices included portions of the medial geniculate, inferior collicular, and reticularis thalamic nuclei. The slices were incubated for 2–3 h in ACSF at room temperature (21–25°C), until required for recording, which was carried out at 21–25°C.

Whole-cell patch-clamp techniques were used to record from thalamocortical neurons of a slice situated on a nylon mesh, and perfused with oxygenated ACSF (Tennigkeit et al., 1996). The electrode pipettes were pulled from borosilicate glass tubing (WP-Instruments, Sarasota, FL, USA), using a Narishige puller (Narishige Instruments, Tokyo, Japan, Model PP83). The pipette solution contained (in mM): K-gluconate, 140; N-2-hydroxyethylpiperazine-N-2-ethanesulfonate (HEPES), 10; KCl, 5; NaCl, 4; adenosine 5-triphosphate (disodium salt), 3; guanosine 5-triphosphate (trisodium salt), 0.3; ethylene glycol-bis-(β-aminoethyl-ether)-N,N,N’,N’-tetraacetic acid (EGTA), 10; and CaCl2, 1. This combination of EGTA and Ca2+ yielded a final [Ca2+] of 10 nM (calculated with Max Chelator software). In experiments with the Ca2+ chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetate (BAPTA), we substituted EGTA with an equimolar concentration of BAPTA, which yielded a final [Ca2+] of 1 nM. The calculated Ec1 was ~55 mV. The pipette solution had a pH of 7.3. The tip resistances of the pipettes were ~5–10 MΩ and access resistances were below 25 MΩ.

Guided by differential interference contrast microscopy (100 × objective) and using a micromanipulator, we positioned the electrode tip in the ventral partition of the medial geniculate nucleus. The electrical recordings were performed in the current-clamp mode with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA, USA). We used Digidata 1320A hardware with pClamp 8 software (Axon Instruments), running on a Pentium computer for data acquisition, storage, and analysis. The input resistance (Ri) of the neuron was computed from the voltage displacements (range, 5–8 mV) to intracellular injection of hyperpolarizing current pulses or from the linear slope of current–voltage (I–V) relationships. The membrane time constant was estimated from a single exponential fit to a voltage response of 5–10 mV to a hyperpolarizing current pulse. The voltage values have been corrected for a measured junction potential of ~10 mV.

We evoked tonic firing of action potentials from neurons at DC-held potentials near ~–60 mV by injecting depolarizing current pulses with an amplitude of 1.5 times the amplitude of ‘just-threshold’ pulses, which was estimated from the voltage deflection that resulted in action potentials in 50% of the trials. We evoked EPSPs by electrical stimulation, using a bipolar tungsten electrode (tip diameter ~100 μm), placed in the slice at 0.2–0.3 mm mediadorsal to the MGB and near corticothalamic axons. Stimulation at this position resulted only in EPSPs. The stimuli consisted of single pulses of approximately 30 V in amplitude (range, 10–100 V) and 100–200 μs in duration. The stimulation rate was 0.5 Hz. Using these stimulus parameters, we evoked inhibitory postsynaptic potentials (IPSPs) when the electrode was placed in the brachium, midway between the inferior colliculus and MGB. The postsynaptic potentials were averaged and fitted with an α func-
tion (pClamp 8 software), yielding the rise and decay time constants for the EPSPs.

The drugs were prepared in distilled water as stock solutions. The stock solutions were frozen for storage and thawed just before the experiment. Spermine, tetrodotoxin (TTX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and d-2-amino-5-phosphono-valerate (APV) were diluted ~1000 times with ACSF to the concentrations required for the particular experiment. These substances, ATP, EGTA, GTP, HEPES, and the inorganic chloride salts were obtained from Sigma (St. Louis, MO, USA). As with the ACSF, the drugs were applied with a roller-type pump at a rate of 2 ml/min through a submersion-type chamber that had a volume of ~1.0 cc.

In most cases, we analyzed data from neurons of 14-day-old gerbils; however, some exceptions were made due to the availability of the age group. We incorporated four neurons from P10, P12 and P13 in the data analysis, including: (1) one out of 19 neurons from a 13-day-old gerbil in the spermine group in studies of tonic firing; (2) two out of eight neurons from a 12-day-old gerbil in the APV group; and (3) one out of four neurons from a 13-day-old gerbil in the BAPTA group. All figures are from neurons of 14-day-old gerbils. The data are presented as means ± S.E.M. A Student’s paired t-test was used for comparison of responses to drug application between two groups. For comparison of more than two groups, we used analysis of variance (ANOVA). P < 0.05 was considered significant.

3. Results

3.1. Spermine application increases tonic firing

Spermine application reversibly increased the number of action potentials in all neurons depolarized from rest by current pulse injection. Spermine (100 μM) applied for 3–6 min induced tonic firing of action potentials on top of subthreshold responses. When action potentials were present in the control, spermine application increased the rate of firing (Fig. 1A). Long recovery times of 35–45 min characterized spermine’s effects on thalamic firing modes after 6 min applications. In the neuron of Fig. 1A, substantial recovery occurred at ~32 min after discontinuing the spermine application.

The spermine-induced increase in the firing frequency was concentration-dependent over the range between 50 and 500 μM (n = 19, Fig. 1B). In addition, application of a spermine concentration of 1 μM did not affect firing (n = 2); however, at 1 mM, there was a marked increase in the firing rate (n = 2), without any apparent recovery (data not shown). Spermine, applied at an

![Graph showing the effect of spermine on firing rate](image)

Fig. 1. Spermine enhanced tonic firing in a concentration-dependent manner in MGB neurons. (A) Spermine application (100 μM, 3 min) enhanced action potential firing evoked by current pulses (25 and 50 pA, 500 ms, 1.5×threshold). Holding potential, −65 mV. Vertical upper bar, 30 mV and lower bar, 60 pA; horizontal bar, 150 ms. (B) Increase in number of action potentials per pulse was concentration dependent. The control firing was 1.8±0.3 action potentials/pulse (n = 9). The ED₉₀ was ~100 μM for spermine-enhanced firing which approached saturation at 200 μM.

ED₉₀ of 100 μM (cf. Fig. 1B), reversibly increased the number of action potentials per pulse by an average of ~80% in nine neurons (control, 1.8 ± 0.3 action potentials/pulse and spermine application, 3.3 ± 0.4 action potentials/pulse, paired t-test, P < 0.01).

The increased firing due to spermine did not likely result from changes in the passive membrane properties which did not greatly change during 3–6 min spermine applications (cf. subthreshold responses in Figs. 1A and 3A). The average resting potentials were −67 ± 4 mV during the control period, and −66 ± 5 mV during applications of spermine at 50–500 μM (n = 19). Spermine application did not significantly change the mean membrane time constant (τ_m = 64 ± 6 ms in control, and 76 ± 6 ms during spermine application, paired t-test) and mean input resistance (R_i = 772 ± 38 MΩ in control, and 756 ± 61 MΩ during spermine application, paired t-test), computed from the responses to hyperpolarizing current pulse injections in 19 neurons held at −65 mV. Hence, the spermine-induced effects on the passive properties could not account for the changes in firing threshold.

Spermine (100 μM) decreased the latency to tonic firing by decreasing the threshold (Fig. 2). Spermine decreased the firing threshold from −50.9 ± 0.6 mV to
Significant changes in action potential amplitude did not accompany the decreased threshold. Fig. 2B summarizes the effects of spermine on firing threshold for six neurons.

3.2. NMDA receptors mediate the spermine-induced increase in firing

We examined the possibility that NMDA receptors mediated the effects on the firing threshold and tonic firing rate by determining the interactions of spermine and the competitive antagonist, APV (50 μM). As shown in Fig. 2B, spermine reduced the threshold voltage for an action potential evoked with a 500 ms current pulse, by an average of 6.2 ± 1.1 mV. On recovery from spermine (washout, Fig. 2B), application of APV alone, or in combination with spermine, did not significantly change the firing threshold (during APV, −52.1 ± 1.9 mV and APV+spermine, −53.0 ± 1.7 mV; n = 6) or changes in membrane properties that could account for the blockade of the spermine-induced reduction in firing threshold. This signified that APV acted on NMDA receptors to completely block spermine action. Since these neurons had received a spermine application prior to APV, we also applied APV to five neurons that had not previously received a drug application in order to assess the possibility of constitutive release of glutamate in the slice. Here, APV produced an increase in threshold, which remained largely unaltered by a subsequent, combined application with spermine (Fig. 2C). All neurons showed substantial recovery at 15 min after discontinuing the application. These data implicated an NMDA receptor mechanism in the spermine-induced decrease in the threshold.

NMDA receptors also mediated the spermine-induced increase in firing rate. In the neuron of Fig. 3A, spermine application (100 μM, 3 min) reversibly increased the number of action potentials during a 500 ms current pulse injection from one action potential in the initial control, to three action potentials. In the neurons that had not previously received a spermine application, an increase in the current pulse amplitude during action potential blockade due to APV application, produced a return of the action potential (Fig. 3B). The APV blockade of spermine-induced increase in firing also was overcome by an increase in the current amplitude. The APV-induced blockades of action potentials and spermine enhancement of firing were not attributable to an increased input conductance and were completely reversible. The graph of Fig. 3C summarizes the data that implicate NMDA receptor mediation.

We assessed the possibility that non-NMDA receptors for glutamate may have contributed to the increased firing during spermine application. In these
studies, we determined the interactions of spermine with an AMPA receptor antagonist, CNQX. Application of CNQX (30 μM) for 6 min did not result in significant changes in evoked action potential firing, configuration, or membrane electrical properties. A subsequent, combined CNQX and spermine application did not greatly alter the reduction in threshold and a 200% increase in firing rate evoked by current pulses (amplitude ~1.5 × threshold), as observed with prior spermine application in all five neurons (CNQX, 1.4 ± 0.3 action potentials/pulse, and CNQX+spermine, 4.2 ± 0.4 action potentials/pulse; data not shown). Hence, the increase in tonic firing rate due to spermine application did not likely involve AMPA receptors.

3.3. Effects on membrane rectification

We examined the possibility that the spermine-induced increase in tonic firing involved voltage-dependent membrane properties. For example, thalamocortical neurons exhibit larger responses to depolarizing, compared to hyperpolarizing current pulses (Tennigkeit et al., 1996; Parri and Crunelli, 1998; cf. Fig. 4B). Spermine (100 μM) application for 3 min increased this rectification in a range between the rest and firing threshold but did not appreciably change the responses at hyperpolarized potentials, down to −100 mV (n = 19; Fig. 4A). Quantification of the increase in rectification on depolarization was difficult because spermine application shortened the latency to firing (cf. arrows in Figs. 2A and 4A). Application of APV (50 μM, 6 min) completely blocked the rectification in the upper right quadrant of the current–voltage (I–V) relationship. A subsequent co-application with spermine (100 μM) did not greatly change this curve. The graph of Fig. 4A (right) summarizes these findings for six neurons.

There was little or no involvement of AMPA receptors in the spermine-induced (100 μM, 3 min) enhancement of rectification produced by depolarizing current pulses. The application of CNQX (30 μM, 6 min) did not affect spermine’s effects on the rectification in five neurons. The average voltage response during co-application of CNQX and spermine (15.9 ± 0.6 mV) was significantly different from control (11.5 ± 0.5 mV) or CNQX application (11.3 ± 0.4 mV; ANOVA, P < 0.05). Hence, the spermine-induced increase in the depolarizing responses involved NMDA receptors, but not likely AMPA receptors.

We investigated the possibility that spermine increased the rectification in the upper right quadrant of the I–V relationship by interacting with a persistent Na+ conductance. The rectification observed on depolarization from ~ −70 mV to threshold involves a persistent Na+ conductance, sensitive to TTX blockade (Tennigkeit et al., 1996; Parri and Crunelli, 1998).

Fig. 3. Spermine increased tonic firing by interacting with NMDA receptors. (A) Spermine application (100 μM, 3 min) reversibly induced firing. After a 15 min washout from spermine, APV (50 μM, 6 min), an NMDA receptor antagonist, blocked the evoked action potential. Firing was not observed during co-application of APV and spermine (3 min). Washout shows recovery at 10 min after discontinuing the co-application. Lower traces show hyperpolarizing tests for input resistance. (B) Application of APV (50 μM, 6 min) abolished firing induced by just-threshold current pulse (40 pA). A subsequent 3 min co-application of spermine and APV did not alter this suppression (lower superimposed traces in middle panel). A two-fold increase in current amplitude overcame the blockade during APV application, alone, or during co-application with spermine (upper superimposed traces in middle panel). Recovery was observed after 10 min washout. (C) Summary of spermine effects on firing in six neurons. ANOVA; *P < 0.01, **P < 0.05. Vertical bar, 30 mV; horizontal bar, 200 ms.
Blockade of voltage-dependent Na\(^+\) channels with TTX (0.6 \(\mu\)M, 6 min) decreased the slope of the \(I-V\) relationship, more in the depolarizing quadrant than in the hyperpolarizing quadrant. The response on depolarization increased from 12.5 \(\pm\) 0.5 mV (control) to 17.5 \(\pm\) 0.8 mV during spermine application, and after 15 min washout, recovered to 12.9 \(\pm\) 0.7 mV. Subsequent APV application reduced the response on depolarization from 12.9 \(\pm\) 0.7 mV (first washout) to 9.2 \(\pm\) 0.4 mV (APV). Co-applied APV and spermine did not greatly alter rectification (8.7 \(\pm\) 0.3 mV). Recovery from APV and spermine occurred after 15 min (12.7 \(\pm\) 0.5 mV). Holding potential, \(-70\) mV. (B) \(I-V\) relationship for a neuron shows that TTX application (0.6 \(\mu\)M, 6 min) decreased rectification on depolarization over a \(>10\) mV range. Co-applied with TTX, spermine did not alter rectification in upper right quadrant. A 20 pA pulse was sufficient to observe rectification on depolarization, whereas a \(-50\) pA pulse produced little or no rectification on hyperpolarization. Graph at right summarizes effects of TTX and spermine on rectification. Rectification on depolarization decreased from 13.1 \(\pm\) 0.8 mV (control) to 10.2 \(\pm\) 0.8 mV during TTX application. A subsequent co-application with spermine did not greatly alter depolarizing responses (10.8 \(\pm\) 0.7 mV). Inserts in upper left quadrants of (A) and (B) show superimposed responses (7 mV) to depolarizing and hyperpolarizing current pulses (duration 500 ms) of 60 and \(-60\) pA, during control (C), spermine (S), and at 3 min of co-application of TTX and spermine (TTX+S). Bar graph values are mean \(\pm\) S.E.M. ANOVA; * and ** \(P<0.05\).

The spermine-induced enhancement of rectification on depolarization of the neuron also may depend on extra- or intracellular Ca\(^{2+}\), as demonstrated for neocortical neurons (Crill, 1996). Hence, we measured the spermine-induced enhancement of voltage responses to depolarizing current injections during intracellular application of BAPTA (10 mM) and extracellular perfusion with Ca\(^{2+}\)-free ACSF. In the neuron of Fig. 5A, perfusion of Ca\(^{2+}\)-free ACSF did not greatly alter these depolarizing responses. In four neurons, a 50 pA current pulse evoked average responses of 9.8 \(\pm\) 0.6 mV in control ACSF and 10.0 \(\pm\) 0.5 mV in 0 mM [Ca\(^{2+}\)]; the response increased to 15.2 \(\pm\) 1 mV during spermine application (in 2 mM Ca\(^{2+}\) ACSF; ANOVA, \(P<0.01\))

Fig. 4. Effects of spermine (100 \(\mu\)M, 3 min) on membrane rectification. (A) Current–voltage (\(I-V\)) relationship of a neuron shows that spermine increased depolarizing response which was abolished during combined application (3 min) with APV. APV (50 \(\mu\)M, 6 min), alone, reduced rectification in upper right quadrant. \(I-V\) curve after 15 min washout shows substantial recovery. Graph at right summarizes effects of spermine, APV, and their co-application on rectification. The response on depolarization increased from 12.5 \(\pm\) 0.5 mV (control) to 17.5 \(\pm\) 0.8 mV during spermine application, and after 15 min washout, recovered to 12.9 \(\pm\) 0.7 mV. Subsequent APV application reduced the response on depolarization from 12.9 \(\pm\) 0.7 mV (first washout) to 9.2 \(\pm\) 0.4 mV (APV). Co-applied APV and spermine did not greatly alter rectification (8.7 \(\pm\) 0.3 mV). Recovery from APV and spermine occurred after 15 min (12.7 \(\pm\) 0.5 mV). Holding potential, \(-70\) mV. (B) \(I-V\) relationship for a neuron shows that TTX application (0.6 \(\mu\)M, 6 min) decreased rectification on depolarization over a \(>10\) mV range. Co-applied with TTX, spermine did not alter rectification in upper right quadrant. A 20 pA pulse was sufficient to observe rectification on depolarization, whereas a \(-50\) pA pulse produced little or no rectification on hyperpolarization. Graph at right summarizes effects of TTX and spermine on rectification. Rectification on depolarization decreased from 13.1 \(\pm\) 0.8 mV (control) to 10.2 \(\pm\) 0.8 mV during TTX application. A subsequent co-application with spermine did not greatly alter depolarizing responses (10.8 \(\pm\) 0.7 mV). Inserts in upper left quadrants of (A) and (B) show superimposed responses (7 mV) to depolarizing and hyperpolarizing current pulses (duration 500 ms) of 60 and \(-60\) pA, during control (C), spermine (S), and at 3 min of co-application of TTX and spermine (TTX+S). Bar graph values are mean \(\pm\) S.E.M. ANOVA; * and ** \(P<0.05\).
which did not significantly change during combined application of spermine and 0 mM [Ca\(^{2+}\)] (ANOVA; 10.2 ± 0.3 mV, n = 4).

In contrast, the intracellular application of BAPTA, a more rapid Ca\(^{2+}\) chelator than EGTA, eliminated the spermine-induced enhancement of voltage responses, observed on depolarization (Fig. 5B). In neurons recorded with BAPTA-containing pipettes, spermine application did not alter the responses to depolarizing currents (average of 12.4 ± 1 mV in control and 12.4 ± 0.7 during spermine; n = 4). This implied that spermine induced Ca\(^{2+}\) entry into the neuron which enhanced the subthreshold depolarizing responses and promoted rectification in the upper right quadrant of the I–V relationship.

The spermine-induced increase in rectification on depolarization would reduce firing threshold. We determined if the spermine-induced reduction of the action potential threshold also depended on extra- and intracellular [Ca\(^{2+}\)]. The omission of Ca\(^{2+}\) from the ACSF, which normally contained 2 mM Ca\(^{2+}\), decreased the threshold from −53.7 ± 2.9 mV to −60.6 ± 3.1 mV (n = 3, ANOVA, P < 0.05). On application of spermine in ACSF that was nominally Ca\(^{2+}\)-free, we observed no change in the threshold (control, −60.6 ± 3.1 mV, and spermine, −60.2 ± 3.2 mV; n = 3). Recovery of the threshold from the effects of the Ca\(^{2+}\)-free solution occurred at 10 min after returning to normal Ca\(^{2+}\) perfusion. A subsequent application of spermine for 3 min in normal solution (2 mM Ca\(^{2+}\)) reversibly reduced the firing threshold to −60.0 ± 2.6 mV (n = 3, ANOVA, P < 0.05). These effects, observed when the pipette solution contained 10 mM EGTA, were largely reversible (recovery, −55.3 ± 2.3 mV). We then examined the effects of the fast Ca\(^{2+}\) chelator, BAPTA (10 mM), applied internally, on the spermine-induced reduction in action potential threshold. A 3 min application of spermine did not significantly change the threshold in four neurons recorded with BAPTA-containing pipettes (control, −49.3 ± 2.1 mV, and spermine, −49.3 ± 2.3 mV). These experiments demonstrated that the effects of spermine on action potential threshold depended on Ca\(^{2+}\) entry.

3.4. Effects of spermine on low threshold Ca\(^{2+}\) spike (LTS) firing

Application of Ca\(^{2+}\)-free ACSF abolished the transient, low threshold spike (LTS), evoked at the offset of hyperpolarizing current pulses or on step depolarization in neurons held at hyperpolarized potentials. This blockade confirmed the Ca\(^{2+}\) mediation of the LTS. Spermine application increased action potential firing on top of a LTS in only 10 out of 19 neurons, in contrast to the increased tonic firing rate on spermine ap-

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Fig. 5. Alterations in extra- and intracellular Ca\(^{2+}\) influence spermine effects on depolarizing current-voltage (I–V) relationships in MGB neurons. (A) Voltage responses to current pulses (80 pA, 500 ms in upper traces) and I–V diagram show that removal of extracellular Ca\(^{2+}\) from ACSF abolished the increase in voltage responses induced by spermine during internal application of EGTA (10 mM). Perfusion of Ca\(^{2+}\)-free media for 6 min (0 Ca\(^{2+}\)), alone, and with spermine (100 μM, 3 min) did not alter voltage response. After a 10 min washout, and perfusion with control media containing 2 mM Ca\(^{2+}\) (10 min), spermine application (100 μM, 3 min) increased the voltage response. I–V diagram in same neuron shows that spermine did not change the slope of the voltage responses during Ca\(^{2+}\)-free perfusion. When applied during extracellular perfusion with normal [Ca\(^{2+}\)], spermine increased the voltage responses to current pulses that were > 25 pA. (B) Voltage responses to current pulses (80 pA, 500 ms in upper traces) and I–V diagram show that spermine did not increase the voltage responses in a neuron recorded during internal BAPTA (10 mM). Vertical bar, 10 mV; horizontal bar, 100 ms.
plication, observed in all neurons. As shown in Fig. 6A, spermine induced an action potential on the rebound depolarizing response at the termination of hyperpolarizing current pulses. In five out of the 10 neurons hyperpolarized with DC to \(-80\) mV, spermine application induced one or two action potentials on a subthreshold rebound response to hyperpolarizing current pulses. Spermine application increased the amplitude of the rebound LTS that did not reach action potential threshold in the remaining five neurons. The effects of spermine were reversible, requiring 20–40 min for recovery.

Blockade of voltage-dependent Na\(^+\) channels with TTX did not significantly alter the ability of spermine to enhance the LTS in six neurons (cf. Fig. 6A and B). During TTX blockade, the spermine enhancement of the LTS depended on the holding potential. In these experiments, we evoked the LTS by injecting hyperpolarizing currents of different amplitude into neurons held at different holding potentials (Fig. 6C). Co-application of spermine (100 \(\mu\)M) and TTX induced an LTS in neurons at potentials that caused marked inactivation of the LTS. At potentials where an LTS was present, a spermine application increased its amplitude and rate of rise (d\(V/dt\)). There was a greater increase in the d\(V/dt\) of the LTS when the neuron was held at \(-55\) mV than at \(-85\) mV (Fig. 6C). After spermine application, the LTS evoked in a neuron held at \(-55\) mV had an average d\(V/dt\) of 3.1 ± 0.2 mV/ms, compared to 1.5 ± 0.3 mV/ms in the control during TTX application. The average rate of decay was −1.7 ± 0.3 mV/ms (\(n = 6\)) with fast (26 ± 5 ms, \(n = 5\)) and slow (146 ± 12 ms, \(n = 5\)) components (trace 2, Fig. 6B). Fig. 6C summarizes the effects of spermine on the d\(V/dt\) of the LTS, showing a maximal effect at a holding potential \((V_h) = -55\) mV.

![Fig. 6](image)

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**Fig. 6.** Effects of spermine (100 \(\mu\)M, 3 min) on the low threshold Ca\(^{2+}\) spike (LTS) firing. (A) Superimposed voltage responses (control, spermine, and recovery) show that spermine induced an LTS on termination of a hyperpolarizing current pulse (−40 pA, 500 ms). (B) Spermine increased the rate of rise and amplitude of the LTS at the end of a hyperpolarizing current pulse (−80 pA) just before (1), during (2), and after (3) spermine application during TTX blockade (0.6 \(\mu\)M) of voltage-dependent Na\(^+\) conductances. Holding potential, −55 mV. (C) Bar graph summarizes spermine effects on d\(V/dt\) of the LTS at the end of hyperpolarizing responses and during TTX blockade. Spermine increased d\(V/dt\) in neurons held at −85, −75, −65, and, maximally, at −55 mV (\(n = 6\), paired t-test, *\(P < 0.01\), **\(P < 0.005\)). (D) APV (50 \(\mu\)M) did not block the effects of spermine on the LTS, as shown by sub- and suprathreshold responses to current pulses (60, 120 pA) during application of APV, alone, and co-application with spermine. Application of APV reduced the subthreshold response, LTS rate of rise, and number of action potentials. Co-application (3 min) of APV and spermine transformed a subthreshold response to an LTS, increased LTS rate of rise, and shortened the latency to the action potential. Recovery (in APV) was observed after a 10 min washout. Vertical bar, 15 mV in (A) and (B); 30 mV in (D). Horizontal bar, 150 ms. Insert in (B) (right): Vertical bar, 3 mV; horizontal bar, 30 ms.
and a minimal increase at $V_h = -85$ mV in six neurons (paired $t$-test, $P < 0.01$).

Since the hyperpolarization-activated current influences the rate of rise of the LTS, we examined whether spermine affected the voltage sag, mediated by this current (Tennigkeit et al., 1996). The voltage sag was not prominent in the majority of neurons. Spermine application (100 μM) produced no change in the voltage sag induced by a hyperpolarizing current pulse in three neurons. Hence, the increase in the rate of rise of the LTS did not likely involve interactions with this hyperpolarization-activated conductance.

We determined if the spermine potentiation of the LTS involved NMDA receptors in eight neurons. In six of these neurons, APV application decreased the amplitude of the subthreshold responses to current pulses (cf. Fig. 6D, lower traces). As shown for the neuron of Fig. 6D, APV application also decreased an LTS burst to a single action potential and LTS rate of rise, increasing the latency to the first action potential on top of the LTS. Despite APV antagonism of NMDA receptors, spermine application transformed the subthreshold response into an LTS, as well as decreased the latency to an action potential on top of the LTS (cf. APV and spermine traces in Fig. 6D). In eight out of eight neurons, APV application (50 μM, 6 min) reduced the average rate of rise of the LTS from $1.6 \pm 0.3$ mV/ms in naive controls to $1.2 \pm 0.2$ mV/ms. A subsequent co-application of APV and spermine caused a significant increase in the rate of rise of the LTS to $2.2 \pm 0.1$ mV/ms (ANOVA, $P < 0.05$).

We determined if the spermine potentiation of the LTS involved interactions with AMPA receptors. During blockade of AMPA receptors with CNQX (30 μM, 6 min), spermine significantly increased the $dV/dt$ of the LTS (control, $1.8 \pm 0.1$ mV/ms and spermine, $3.6 \pm 0.2$ mV/ms; $n = 5$, ANOVA, $P < 0.05$). Hence, the effects of spermine on the LTS did not likely involve NMDA or AMPA receptors.

3.5. Effects of spermine on excitatory and inhibitory postsynaptic potentials

Spermine application (100 μM) to 18 neurons resulted in bursts of action potentials on EPSPs evoked by electrical stimulation of corticothalamic projections (Fig. 7A). Spermine had little or no effects on the rate of rise of the EPSP, but always enhanced the late decay phase amplitude. The EPSP amplitude increased slightly (3–5 mV) during spermine application to five neurons, but this was not a consistent finding in the 18 neurons. The spermine-induced action potentials on the EPSPs were reversible in all neurons. Complete recovery was observed in 13 of 18 neurons at 35 min after terminating the application.

Spermine prolonged the EPSP decay time constant ($\tau_{\text{decay}}$), as estimated with an $\alpha$ function fit of the EPSPs (Fig. 7B). This promoted the occurrence of action po-

Fig. 7. Spermine (100 μM, 3 min) prolonged late component of corticothalamic EPSPs mediated by NMDA receptors. (A) Spermine increased EPSP amplitude and duration, resulting in three action potentials. (B) Spermine delayed the late component (2) of the EPSPs. The bar graph summarizes the spermine-induced prolongation of EPSP decay time constant ($\% \tau_{\text{decay}}$), expressed as % of the control. Control $\tau_{\text{decay}}$ was $142 \pm 8.5$ ms ($n = 15$, paired $t$-test, $^*P < 0.01$). (C) Spermine did not affect EPSPs during NMDA receptor blockade by 50 μM APV or significantly change remaining EPSP components. Bar graph summarizes the reduction in EPSP $\tau_{\text{decay}}$ by APV and lack of spermine effect during APV blockade, expressed as % of control. Control $\tau_{\text{decay}}$ was $143.6 \pm 14$ ms ($n = 8$, ANOVA, $^*P < 0.01$). Vertical bar, 5 mV; horizontal bar, 200 ms.
tentials on top of the EPSPs (Fig. 7A). The ED$_{50}$ for the spermine-induced increase in $\tau_{\text{decay}}$ of EPSPs was $\sim 100$ $\mu$M which was approximately the same for the spermine-induced increase in firing (cf. Fig. 1B). Recovery to the control value occurred after 30 min (148 ± 15 ms). Fig. 7B summarizes these results for 15 neurons. Table 1 summarizes the effects of single or cumulative applications of spermine on the 90–10% decay time in 18 neurons.

Spermine application (100 $\mu$M) did not significantly affect the amplitude or time course of depolarizing potentials evoked by stimulation of the brachium colliculi inferioris ($n$ = 6; data not shown). These potentials, 100–200 ms in duration, were likely IPSPs mediated by GABA$_A$ receptors because they reversed at potentials near $E_{Cl}$ ($\sim 55$ mV) and were sensitive to blockade by bicuculline (50 $\mu$M, $n$ = 6).

Spermine prolonged the decay of the late EPSP component mediated by an NMDA-type receptor. The application of APV (50 $\mu$M), blocked the late component, resulting in shorter rise (10–90%) and decay (90–10%) times of the EPSPs (Table 1). During NMDA receptor blockade, EPSP mean $\tau_{\text{decay}}$ was 100 ± 14 ms, compared to 143 ± 15 ms in control (Fig. 7C; $n$ = 8, ANOVA, $P < 0.01$). This mean value did not change significantly during combined spermine and APV application (Fig. 7B).

By applying spermine in combination with CNQX (30 $\mu$M, 6 min), we determined if the prolongation of EPSPs resulted from interactions with AMPA receptors. We found that spermine prolonged the EPSP 90–10% decay time during CNQX blockade to the same extent as in the absence of AMPA receptor blockade (Table 1). In three additional neurons, spermine was applied before the co-application with CNQX. The co-application resulted in a significant prolongation of the EPSP to the same extent as in the absence of CNQX blockade of AMPA receptors (Table 1). This confirmed that spermine affected only the NMDA-mediated component. Co-application of CNQX (30 $\mu$M) and APV (50 $\mu$M) then abolished the early and late components of the EPSP which remained absent despite a subsequent spermine application ($n$ = 4). From these results, we suggest that spermine increased the duration of the EPSP decay phase by interacting with NMDA receptors.

We considered the possibility that spermine prolonged the EPSPs by acting on an extracellular polyamine-sensitive site of the NMDA receptor (cf. Beneviste and Mayer, 1993). We studied the interactions of spermine and arcaine, an antagonist that acts at the polyamine-sensitive site on the NMDA receptor (Reynolds, 1990). In these studies, we sequentially applied arcaine (40 $\mu$M), spermine (100 $\mu$M), arcaine and spermine, each for 3 min ($n$ = 3). Arcaine, alone, did not greatly alter the configuration of the EPSP (Fig. 8A) or produce changes in the EPSP amplitude, 90–10% decay time, and half-width (Table 1). After a 15 min washout from arcaine application, spermine significantly prolonged the EPSP $\tau_{\text{decay}}$ to 180 ± 32 ms from 101 ± 16 ms in the control (Fig. 8A). A subsequent co-application of spermine and arcaine abolished the actions of spermine, resulting in $\tau_{\text{decay}}$ of 118 ± 16 ms (Fig. 8A). The graph of Fig. 8A summarizes the spermine-induced increases in EPSP $\tau_{\text{decay}}$ and arcaine blockade of spermine effects.

We also determined if spermine increased the NMDA-mediated component of the EPSP by potentiating the actions of glycine on the NMDA receptor. In the presence of a saturating concentration of glycine (40 $\mu$M), spermine still prolonged the EPSP by $\sim 49$% (Fig. 8B). In three neurons, spermine increased EPSP $\tau_{\text{decay}}$ from 255 ± 44 ms to 379 ± 53 ms (ANOVA, $P < 0.05$). In summary, spermine actions on the EPSP likely involved an extracellular polyamine-sensitive site, and not a glycine-sensitive site of the NMDA receptor.

We attempted to assess a contribution of extracellular Ca$^{2+}$ or Mg$^{2+}$ to the spermine-induced enhancement...
of EPSPs. Spermine application did not alter the amplitude or duration of the EPSPs during a 6 min perfusion of Ca$^{2+}$-free ACSF in three neurons (data not shown). Hence, spermine effects on NMDA-mediated EPSPs likely depended on Ca$^{2+}$ entry. In two neurons, the omission of Mg$^{2+}$ from ACSF perfusion resulted in subthreshold oscillations of the membrane potential and spontaneous firing of action potentials. These observations were consistent with previous studies on thalamocortical neurons (Jacobsen et al., 2001) which prevented critical assessment of an agonist role of Mg$^{2+}$ at the polyamine-sensitive site on the NR2B receptor subunit (Kew and Kemp, 1998).

4. Discussion

These studies demonstrate that extracellular spermine has distinctive actions on thalamocortical neurons, consistent with a neuromodulator role in the medial geniculate body. Spermine actions on NMDA receptors produced a heightened state of excitability which we viewed as prolonged corticothalamic EPSPs, and increased EPSP bursting and tonic firing of action potentials. To a large extent, these effects resulted from increased membrane rectification on depolarization and reduction in threshold for action potential genesis. Spermine also modulated the burst firing mode by increasing the rate of rise and amplitude of low threshold Ca$^{2+}$ spikes (LTSs). This unusual effect did not involve interaction with glutamate receptors. The modulation of corticothalamic excitation and LTSs of MGB neurons may be critical in the transformation of auditory signals in gerbil thalamus at the P14 stage of development.

4.1. Enhancement of late component of NMDA-mediated EPSPs

Spermine application increased the decay time constant of corticothalamic EPSPs mediated by NMDA receptors. This finding is consistent with the increased amplitude of NMDA-evoked currents during spermine application to cultured hippocampal and spinal neurons (Lerma, 1992; Benveniste and Mayer, 1993). In the present studies, the actions of spermine were selective and required extracellular Ca$^{2+}$ because they were abolished in three neurons by brief Ca$^{2+}$-free perfusion.
Spermine also did not significantly alter the early EPSP component mediated by AMPA receptors, or appreciably affect IPSPs mediated by GABA receptors that were sensitive to bicuculline antagonism. Application of APV completely blocked the spermine-induced increase in the EPSP decay time constant. This implicated NMDA receptors in spermine actions.

The effects of spermine on MGB neurons involved a polyamine-sensitive site on the NR2B subtype of NMDA receptors, as we demonstrated with arcaine and glycine applications. Arcaine, itself, did not have significant effects on the passive and active membrane properties but reversed the spermine-induced decrease of the EPSP decay. Previous studies have shown that arcaine blocks spermine actions by inverse agonism, antagonism, and open-channel blockade of the polyamine-sensitive site on NMDA receptors (Reynolds, 1990; Pritchard et al., 1994). The actions of spermine at this site decreased the EPSP decay, despite saturating concentrations of glycine. These observations are consistent with the glycine-independent potentiation of NMDA currents by spermine at the NR2B receptor subunit in cultured hippocampal neurons (Benveniste and Mayer, 1993). In thalamocortical neurons, the persistence during high glycine concentrations and arcaine reversal imply that spermine acted independently of the glycine site at a specific polyamine-sensitive site on the NR2B receptor subunit of the NMDA receptor.

The NR2B subunit may modulate the decay time constant of the NMDA receptor-mediated EPSP during the development in MGB neurons. At the end of the second postnatal week, thalamocortical neurons express an abundance of the NR2B polyamine-sensitive receptor subtype in the MGB and lateral geniculate body (LGB) of the rat (Chen and Regehr, 2000). The duration of EPSPs mediated by NMDA receptors in LGB neurons of the rat is similar at P14 to that in gerbil MGB neurons. The decay time constant in LGB neurons is longer at P14 in rats than at earlier (P7-P13) or later (P16-P28) stages of development (Chen and Regehr, 2000; cf. also rat MGB at P21-P42, Bartlett and Smith, 1999). Hence, spermine modulation of the NR2B subunit may cause the longer EPSP duration in MGB neurons at P14.

4.2. Enhancement of membrane rectification on depolarization

Spermine enhanced excitability by increasing inward rectification on depolarization, without greatly affecting the passive properties of MGB neurons. It is not known if the passive and active membrane properties of MGB neurons mature by P14 in gerbil, as in the rat (Tennigkeit et al., 1998). Thalamocortical neurons of the adult guinea pig and P7-P28 rat inwardly rectify because the activation of persistent Na\(^{+}\) conductance on depolarization results in an amplification of the voltage response (Jahnsen and Llinas, 1984; Tennigkeit et al., 1996; Parrini and Crunelli, 1998). In the present studies, blockade of the TTX-sensitive rectification or NMDA receptors eliminated the spermine-induced enhancement of rectification on depolarization. These findings imply that spermine interactions with NMDA receptors led to activation of a persistent Na\(^{+}\) conductance in MGB neurons.

An elevation in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) due to NMDA receptor activation (Jahr, 1992) may have enhanced rectification on depolarization. We did not observe a spermine-induced increase in voltage responses on depolarization during Ca\(^{2+}\)-free perfusion or rapid chelation of Ca\(^{2+}\) with intracellular BAPTA. It seems likely that an elevation of [Ca\(^{2+}\)], initiated by spermine actions at NMDA receptors activated intracellular messengers and increased this rectifying behavior. In neocortical neurons, transmitter activation of dendritic NMDA receptors increases Ca\(^{2+}\) entry (Schwindt and Crill, 1995) that may increase channel phosphorylation (Siekevitz, 1991) and a persistent Na\(^{+}\) conductance (Schwindt et al., 1992). Hence, the spermine-induced enhancement of TTX-sensitive rectification on depolarization may result from NMDA-mediated Ca\(^{2+}\) entry in MGB neurons.

The effects of spermine on membrane rectification and firing threshold may involve the recruitment of a Ca\(^{2+}\)-dependent second messenger, subsequent to NMDA receptor activation. Activation of NMDA receptors increases Ca\(^{2+}\) entry, resulting in a Ca\(^{2+}\) gradient in the dendrites (Connor et al., 1988) and activation of a protein kinase C (PKC) pathway. A rise in intracellular [Ca\(^{2+}\)] also may activate calmodulin kinase II which enhances Na\(^{+}\) currents (Carlier et al., 2000). PKC activation increases membrane excitability by shifting the activation curve for the persistent Na\(^{+}\) current along the voltage axis to more hyperpolarized potentials (Astman et al., 1998; Franceschetti et al., 2000). This voltage range is consistent with the range of spermine enhancement of voltage rectification in our experiments.

The increased rectification on depolarization may have reduced the threshold for an action potential in MGB neurons (cf. neocortical neurons, Stafstrom et al., 1982). Antagonism of NMDA receptors, perfusion with Ca\(^{2+}\)-free ACSF or rapid chelation of Ca\(^{2+}\) with BAPTA, eliminated the reduction in threshold and increased tonic firing due to spermine application. Hence, the modulation of NMDA receptor-mediated Ca\(^{2+}\) entry likely increased membrane rectification on depolarization and reduced firing threshold. This mechanism explains the ability of spermine to increase postsynaptic excitability and tonic firing in MGB neurons.
4.3. Facilitation of low threshold Ca\(^{2+}\) spike (LTS) firing

Spermine facilitated LTS firing by a mechanism that did not involve interactions with NMDA receptors. Spermine increased the rate of rise and amplitude of the LTS, despite APV blockade of NMDA receptors. This was evident on depolarization to action potential threshold where there is a smaller gradient for Ca\(^{2+}\) as well as greater inactivation of T-type Ca\(^{2+}\) channels (Hernandez-Cruz and Pape, 1989). Spermine enhanced the LTS during blockade of voltage-dependent Na\(^{+}\) channels by TTX. Hence, a change in some parameter of the T-type Ca\(^{2+}\) current, e.g. voltage dependence of the inactivation–activation relationship, may have increased the LTS.

4.4. Significance

Spermine is widely distributed in rat and human brain (Harman and Shaw, 1981; Morrison et al., 1995). A membrane transporter appears to maintain low extracellular concentrations of \(<1\ \mu\text{M}\) (Dot et al., 2000). These concentrations may increase on NMDA stimulation to \(>50\ \mu\text{M}\) in striatal neurons of adult rat brain (Fage et al., 1992). The effects of spermine on NMDA receptors and low threshold Ca\(^{2+}\) spikes in juvenile MGB neurons (ED\(_{50}\approx100\ \mu\text{M}\) are consistent with neuromodulatory actions at high micromolar concentrations (Williams, 1997). Given the role of NMDA receptors during development, such modulation by spermine is likely important for learning processes (Chida et al., 1992).

The present results are relevant to the normal function of the central auditory system. The NMDA receptor-mediated effects of spermine would enhance the ability of MGB neurons to detect simultaneous inputs, as in coincidence detection. For example, an overexpression of the spermine-sensitive NR2B subunit (Williams et al., 1994) prolongs EPSPs and shortens the time window between two coincident signals in hippocampal neurons (Tang et al., 1999). In thalamic neurons, the generation of synchronous activity may involve coincidence detection (Roy and Alloway, 2001) as well as amplitude selectivity in the MGB neurons (Kuwabara and Suga, 1993).

The effects of spermine on the low threshold Ca\(^{2+}\) spikes of MGB neurons may have relevance for conscious or sleep states and disorders of consciousness. The LTS is essential in the generation of bursting and oscillatory activity in the auditory nuclei (Hu, 1995; Tennigkeit et al., 1996). By increasing the rate of rise and amplitude of the LTS and slowing its decay, spermine modulation may increase an MGB neuron’s responsiveness of neurons at hyperpolarized potentials (Hu et al., 1994) to inputs during these states (He and Hu, 2002). Modulation by spermine may have importance for bursting behavior during sleep states whereas excessive modulation may occur in absence epilepsy as in audiogenic seizures (Porta et al., 1981), sensitive to blockade by polyamine antagonists (Kotlinska and Liljequist, 1996).

The present study has shown that spermine enhanced the excitability of thalamocortical neurons in specific ways that were consistent with a neuromodulator role in the medial geniculate body at P13–P15 stage of development. Spermine acted on a polyamine site of NMDA receptors, to increase membrane rectification on depolarization, reduce firing threshold and to slow the decay of corticothalamic EPSPs in MGB neurons. The heightened excitability increased tonic firing evoked by depolarizing current pulses and EPSP bursts of action potentials. Spermine also increased the rates of rise and amplitudes of low threshold Ca\(^{2+}\) spikes by an unknown mechanism, not mediated by NMDA receptors. By increasing the efficacy of corticothalamic excitation, spermine actions have importance in the transformation of auditory signals to tonic and burst firing during the juvenile stage of development.

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