Differential Control of Spontaneous and Evoked GABA Release by Presynaptic L-Type Ca\(^{2+}\) Channels in the Rat Medial Preoptic Nucleus

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Submitted 2 July 2009; accepted in final form 6 May 2010

May 12, 2010; doi:10.1152/jn.00570.2009. To clarify the role of presynaptic L-type Ca\(^{2+}\) channels in GABA-mediated transmission in the medial preoptic nucleus (MPN), spontaneous, miniature, and impulse-evoked inhibitory postsynaptic currents (sIPSCs, mIPSCs, and eIPSCs, respectively) were recorded from MPN neurons in a slice preparation from rat brain. The effects of different stimulus protocols and pharmacological tools to detect contributions of L-type Ca\(^{2+}\) channels and of Ca\(^{2+}\)/activated K\(^{+}\) (K\(_{Ca}\)) channels were analyzed. Block of L-type channels did not affect the sIPSC and mIPSC properties (frequency, amplitude, decay time course) in the absence of external stimulation but unexpectedly potentiated the eIPSCs evoked at low stimulus frequency (0.1–2.0 Hz). This effect was similar to and overlapping with the effect of K\(_{Ca}\)-channel blockers. High-frequency stimulation (50 Hz for 10 s) induced a substantial posttetanic potentiation (PTP) of the eIPSC amplitude and of the sIPSC frequency. Block of L-type channels still potentiated the eIPSC during PTP, but in contrast, reduced the sIPSC frequency during PTP. It was concluded that L-type channels provide a means for differential control of spontaneous and impulse-evoked GABA release and that this differential control is prominent during short-term synaptic plasticity. Functional coupling of the presynaptic L-type channels to K\(_{Ca}\) channels explains the observed effects on eIPSCs.

INTRODUCTION

Transmitter release in central mammalian synapses is triggered by Ca\(^{2+}\) influx through P/Q-type (Ca\(_{V2.1}\)) channels or, in some cases, N-type (Ca\(_{V2.2}\)) channels into the presynaptic nerve terminal (Catterall and Few 2008; Evans and Zamponi 2006). In contrast, L-type (Ca\(_{V1} \)) channels, which in similarity with P/Q- and N-types belong to the high-voltage-gated group, do not seem crucial for triggering transmitter release in fast conventional synapses. In agreement with these general rules, GABA release from nerve terminals on neurons of the median preoptic nucleus (MPN) of rat is initiated by Ca\(^{2+}\) influx through N-, P-, and Q-type, but not L-type, channels (Haage et al. 1998). Nevertheless, L-type channels are also present in the synaptic terminals on MPN neurons. Blockage of these channels may, paradoxically, lead to a transient increase in spontaneous transmitter release, an effect that was ascribed to coupling to Ca\(^{2+}\)/activated K\(^{+}\) (K\(_{Ca}\)) channels and membrane potential and indirectly to altered activity in N- and P/Q-type channels (Druzin et al. 2002). A role of presynaptic L-type Ca\(^{2+}\) channels has also been suggested for a few other central synapses. Thus in Aplysia synapses and in rat hippocampal GABAergic synapses, L-type channels, although not detected anatomically, seem important for short-term plasticity. Although the L-type channels in these synapses do not contribute to low-frequency transmission, they do play a crucial role in the posttetanic potentiation (PTP) after high-frequency stimulation (Holmgaard et al. 2009; Jensen and Mody 2001; Murakami et al. 2002; Storozhuk et al. 2007). Similarly, L-type channels may contribute to slow excitatory transmission in the midbrain only after high-frequency stimulation (Bonci et al. 1998). This apparently different role of L-type channels in hippocampal and midbrain synapses compared with MPN synapses raises the question how the contribution of L-type channels depends on the stimulus conditions, i.e., the presynaptic impulse frequency. Does the suggested coupling to K\(_{Ca}\) channels in MPN neurons play a role during presynaptic impulse activity and does L-type channel activation facilitate or depress transmitter release?

Functional coupling of other presynaptic Ca\(^{2+}\)-channel types to large-conductance (BK) K\(_{Ca}\) channels has been shown for glutamatergic hippocampal synapses. Although first described as enigmatically silent and therefore ascribed a role as “emergency breaks” protecting against excessive detrimental activity under pathological conditions (Hu et al. 2001), BK channels do affect transmission between hippocampal CA3 neurons in slice cultures also under “basal conditions” (Rafaeili et al. 2004), in similarity with their role in the frog neuromuscular junction (Robitaille and Charlton 1992). In these cases, however, the K\(_{Ca}\) channels are thought to be coupled to the same Ca\(^{2+}\) channels that trigger transmitter release (Hu et al. 2001), as suggested by the tight anatomical co-localization (Robitaille et al. 1993). The proposed coupling to presynaptic L-type channels in MPN synapses (Druzin et al. 2002), on the contrary, implies a separate system, where activity of the K\(_{Ca}\) channels is controlled by Ca\(^{2+}\) influx through channels different from those directly triggering GABA release.

To clarify the role of L-type Ca\(^{2+}\) channels in GABA-mediated transmission in the MPN and to find out whether a possible role could be explained by coupling to K\(_{Ca}\) channels, we recorded spontaneous, miniature, and impulse-evoked inhibitory postsynaptic currents (sIPSCs, mIPSCs, and eIPSCs, respectively) from MPN neurons in a slice preparation from rat brain. The effects of different stimulus protocols and pharmacological tools to detect contributions of L-type Ca\(^{2+}\) channels and of K\(_{Ca}\) channels were analyzed. Our results suggest that L-type channels do contribute to spontaneous and impulse-evoked GABA-mediated transmission but in completely different ways. Whereas L-type channels seem to influence “spontaneous” GABA release only during PTP, after high-
frequency stimulation, evoked GABA release is influenced also at low-frequencies of presynaptic impulse activity. Furthermore, whereas spontaneous GABA release is facilitated by Ca\(^{2+}\) influx through L-type channels, evoked release is inhibited. The latter effect is explained by functional coupling of the presynaptic L-type channels to K\(_{Ca}\) channels of BK type.

**Methods**

Ethical approval of the procedures described was given by the local ethics committee for animal research.

**Slice preparation**

Young (50–120 g), male Sprague-Dawley rats were killed by decapitation, the brain was removed, and ~150-μm-thick coronal slices containing the preoptic area were prepared with the use of a vibroslicer (752M Vibroslice. Campden Instruments, Pembrokeshire, UK). During slicing, the tissue was submerged in preoxygenated (100% O\(_2\)) ice-cold incubation solution. Thereafter, the slices were incubated at a temperature of 25–27°C for ≥1 h in oxygenated incubation solution. Before recording, the slices were transferred to an experimental chamber perfused with extracellular solution. The MPN was localized on basis of comparison with the atlas by Swanson (1991) and simultaneous identification of a corresponding area rich in cell bodies as seen in the microscope used for recording (Malinina et al. 2006). All parts of the MPN were used for electrical recording. Neurons of the MPN were prepared for recording by cleaning with a microscope equipped with water-immersion objective (Nikon Microphot-FXA). Stimulation pipettes, filled with extracellular solution, were prepared from borosilicate glass (GC150-10, Harvard Apparatus, Edenbridge, UK). The resistance of patch pipettes was 3.5–4.5 MΩ when filled with extracellular solution. Stimulation pipettes, filled with extracellular solution, had a resistance of 0.5–1.0 MΩ. A gravity-fed fast perfusion (version 5.3.1, Synaptosoft, Decatur, GA), and Origin (version 6.0, Microcal Software, Northampton, MA) software. The spontaneous current in response to a 5-mV voltage step was repeatedly monitored during the experiments. Slow changes in series resistance were less than about 10% were accepted. Although the absolute series resistance was not determined for all of the studied cells, previous estimation with the same recording techniques yielded values of 18 ± 3 (SE) MΩ (n = 16) (Johansson et al. 2001). No series resistance compensation was used because of its introduction of extra noise and the small amplitude of the recorded signals under steady voltage conditions. The estimated mean voltage error during the peak eIPSC in control solution was <2 mV. The liquid-junction potential between the different solutions has been measured, as described by Neher (1992), to −14 mV, and subtracted in the data presented. The majority of experiments were performed at room temperature (21–23°C), where long-term stability of recordings is much better and noise level lower than at higher temperatures in the used preparation. A series of control experiments were, however, made at 30–31°C and with the HCO\(_3^-\)/CO\(_2^-\)-containing extracellular solution described below. Although current kinetics were faster with the latter solution (see results), neither the effects of stimulation nor the effects of the L-type Ca\(^{2+}\)-channel blocker calcepine on sIPSCs and eIPSCs differed significantly from the effects observed at 21–23°C with the standard extracellular solution.

**Solutions and chemicals**

The standard incubation solution contained (mM) 150 NaCl, 5.0 KCl, 2.0 CaCl\(_2\), 10 HEPES, 10 glucose, and 4.94 Tris-base. The standard extracellular solution used as control contained (mM) 137 NaCl, 5.0 KCl, 1.0 CaCl\(_2\), 1.2 MgCl\(_2\), 10 HEPES, and 10 glucose. Glycine (3.0 μM) was routinely added, and pH was adjusted to 7.4 with NaOH. An alternative extracellular solution, used for control experiments at 30–31°C, contained (mM) 125 NaCl, 26 NaHCO\(_3\), 3.1 KCl, 1.0 CaCl\(_2\), 1.3 MgSO\(_4\), 1.25 NaH\(_2\)PO\(_4\), and 10 glucose. This solution was saturated with 95% O\(_2\)-5% CO\(_2\), and the pH was 7.4. The standard intracellular solution, used for filling the patch pipettes, contained (mM) 140 Cs-acetate, 3.0 NaCl, 1.2 MgCl\(_2\), 1.0 EGTA, and 10 HEPES; pH was adjusted to 7.2 with CsOH. As an alternative to K\(^+\), Cs\(^+\) was used to reduce the background noise. Amphotericin B (Sigma), prepared from a stock solution (6 mg amphotericin B dissolved in 100 μl dimethylsulphoxide), was added to a final concentration of 120 μg amphotericin B per milliliter intracellular solution. To block large-conductance Ca\(^{2+}\)-dependent K\(^+\) (BK) channels, charybdotoxin or a mixture of iberiotoxin and paxilline was used. The peptide toxins apamin, calciseptine, and charybdotoxin were purchased from Alomone Labs (Jerusalem, Israel) or alternatively, as TTX, from Latoxan (Valence, France), and iberiotoxin and paxilline from Tocris (Bristol, UK). Paxilline was prepared from a stock solution (10 mM in dimethylsulphoxide). In all experiments, dimethylsulphoxide was routinely added to the control solution to achieve the same concentration as in the paxilline-containing test solutions. The other peptide toxins were dissolved in extracellular solution.

**Analysis**

All analysis and production of graphic presentations were performed using Clampfit (version 9.0, Axon Instruments), Mini Analysis (version 5.3.1, Synaptosoft, Decatur, GA), and Origin (version 6.0, Microcal Software, Northampton, MA) software. The spontaneous currents (sIPSCs and mIPSCs) were detected and counted semiautomatically, under visual inspection, using Clampfit and Mini Analysis software. The amplitude threshold for detection of sIPSCs and mIPSCs was set above the noise level (~5 pA) and kept constant in each experiment. Evoked postynaptic currents were elicited by stimulation at a steady rate of 0.1 or 2.0 Hz, and posttetanic potentiation (PTP) was elicited by 10-s intervals with 50-Hz stimulation, as specified in results. The peak current within a 20-ms window (starting 2.5 ms after stimulus onset) was detected for each trace, and the amplitude relative to the mean baseline current during the 0.5-ms interval preceding the inflection point at the start of the eIPSCs was measured. The average amplitude from 20–500 stimuli was calculated.

The different types of stimulation protocol (sequences of 0.1-, 2.0-, or 50-Hz stimulation and sequences without stimulation) were applied successively to each cell, first in control solution and thereafter in the presence of tested drugs. Between each series of stimuli at a given
frequency, there was a recovery period of >3 min after low-frequency (0.1 or 2.0 Hz) stimulation and of >6 min after high-frequency (50 Hz) stimulation, unless stated otherwise. This was sufficient for full recovery from the most long-lasting observed effects of stimulation (seen after 50-Hz stimulation; see RESULTS). The effects of channel blockers were analyzed after a minimum of 2.5-min exposure to these blockers. This time interval was sufficient for obtaining maximum effects of the used drugs on the eIPSC as verified by repeated application of 2.0-Hz stimulation after >15 min of drug exposure in a few cases (n ≥ 3 for each drug). The used protocols had the important advantage of enabling comparison of effects of poorly reversible substances on the results of several types of stimulation protocol within an individual cell but implied that the detailed time course of the onset of drug action was not obtained.

The data are presented as mean ± SE, unless stated otherwise. The Wilcoxon matched-pairs signed-ranks test was used for statistical evaluation, with effects considered as significant when P ≤ 0.05.

RESULTS

L-type Ca2+ channels do not contribute to spontaneous GABA release in the absence of external stimulation

To clarify the role of presynaptic L-type Ca2+ channels in GABA-mediated transmission in the MPN, we first analyzed the steady-state effect of the L-type Ca2+–channel blocker calciseptine on sIPSCs in the absence of external stimulation. In previous studies, we have shown that the sIPSCs and the eIPSCs recorded under the conditions described here are mediated by GABA_A receptors (Haage et al. 1998; Malinina et al. 2005). The sIPSCs to a large extent represent GABA release onto MPN neurons. Although these findings are consistent with earlier reports that L-type Ca2+ channels mediate suppression of impulse-evoked GABA release at low stimulation frequency (Jensen and Mody 2001; Murakami et al. 2002) and opposite to the finding that activa-
tion of presynaptic L-type Ca2+ channels reduces impulse-evoked GABA release onto MPN neurons. Although these findings are both in contrast to earlier reports that L-type Ca2+ channels do not contribute to “baseline” transmitter release (Bonci et al. 1998; Jensen and Mody 2001; Murakami et al. 2002) and opposite to the established triggering role of Ca2+ channels for transmitter release, we speculated that it may be caused by the relatively remote localization of L-type channels with respect to the presynaptic release site and to their functional coupling to presynaptic Ca2+-gated K+ (KCa) channels suggested in our earlier study (Druzin et al. 2002). The latter channels may affect the presynaptic membrane potential, e.g., the impulse time course, and indirectly the

Next, the effect of calciseptine on the eIPSCs at steady low-frequency (2.0 Hz) presynaptic stimulation was analyzed. In contrast to the lack of effect described above, calciseptine significantly increased the eIPSC amplitude by 50 ± 22% (Fig. 2, A and B; P = 0.042, n = 12; Wilcoxon matched-pairs signed-ranks test). The absence of changes in mIPSCs and sIPSC properties suggests that the effect on eIPSC amplitude was caused by a presynaptic mechanism. Thus the findings suggest that activation of presynaptic L-type Ca2+ channels reduces impulse-evoked GABA release onto MPN neurons. Although these findings are both in contrast to earlier reports that L-type Ca2+ channels do not contribute to “baseline” transmitter release (Bonci et al. 1998; Jensen and Mody 2001; Murakami et al. 2002) and opposite to the established triggering role of Ca2+ channels for transmitter release, we speculated that it may be caused by the relatively remote localization of L-type channels with respect to the presynaptic release site and to their functional coupling to presynaptic Ca2+-gated K+ (KCa) channels suggested in our earlier study (Druzin et al. 2002). The latter channels may affect the presynaptic membrane potential, e.g., the impulse time course, and indirectly the

FIG. 1. Spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs, respectively) in the absence of external stimulation are not affected by calciseptine. A: sIPSCs recorded at 0 mV in control solution (top) and after >3 min in the presence of 100 nM calciseptine (bottom; from same cell as top trace). B: averaged sIPSCs from 3-min intervals before (control) and >3 min after addition of 100 nM calciseptine, respectively, as indicated. (The 2 superimposed traces are not clearly separable.) From the same cell as used for A. C: mean amplitude, τ_decay, and frequency of sIPSCs recorded at 0 mV in control solution and in the presence of 100 nM calciseptine. Data from 12 cells recorded for 3 min in each condition. Normalization with respect to mean magnitude in control solution for each cell. D: mean amplitude, τ_decay, and frequency of mIPSCs recorded at 0 mV in control solution and in the presence of 100 nM calciseptine. Conditions as in C but with 2.0 µM TTX present throughout. Data from 7 cells. Normalization with respect to mean magnitude in TTX-containing solution without calciseptine, for each cell.
Ca\textsuperscript{2+} influx through channels close to the transmitter release site. To test this idea, we applied a set of KCa-channel blockers in two different combinations, both expected to block SK KCa channels and BK KCa channels: 1) a combination of the SK-channel blocker apamin (1.0 \textmu M) and the BK-channel blocker charybotoxin (200 nM) or 2) a combination of apamin (1.0 \textmu M) and the BK-channel blockers iberiotoxin (100 nM) and paxilline (1.0 \textmu M). (There was no significant difference between the effects of the two combinations and they are consequently treated as 1 group.) Consistent with our hypothesis and in similarity with the effect of calciseptine, application of the KCa-channel blockers significantly increased the amplitude of the eIPSC (Fig. 2C; by 35 \pm 14\%, \textit{P} \leq 0.013, \textit{n} = 17). This effect of KCa\textsubscript{Ca}-channel blockers was overlapping with the effect of calciseptine: no significant additional effect (\textit{P} \leq 0.75, \textit{n} = 17) of calciseptine on the eIPSC was detected when applied to slices already exposed to the KCa-channel blockers (Fig. 2C; potentiation 39 \pm 14\% with respect to control solution; \textit{P} \leq 0.015, \textit{n} = 17), suggesting a common underlying mechanism. Besides the effect on the eIPSC, the KCa-channel blockers also increased the sIPSC frequency by 53 \pm 18\% (\textit{P} \leq 0.017, \textit{n} = 13; cf. columns for pre-HFS periods in control solution and in KCa-channel blockers in Fig. 3E).

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The SK-channel blocker apamin (1.0 \textmu M) applied separately did not affect the eIPSC amplitude significantly (Fig. 2, D and E; change to 103 \pm 16\% of control; \textit{n} = 10), whereas a combination of the BK-channel blockers iberiotoxin (100

![Graph](image-url)
FIG. 3. Blocking L-type Ca\(^{2+}\) channel, but not blocking \(K_{Ca}\) channels, reduces posttetanic potentiation (PTP) of sIPSC frequency. A: raw data traces showing the PTP of sIPSC frequency induced by 50-Hz stimulation at 0 mV in control solution (top) and in 100 nM calciseptine (bottom) in the same cell. (Current during stimulation omitted for clarity.) B: frequency of sIPSCs recorded at 0 mV before and after a 10-s interval with 50-Hz stimulation in the absence and presence of 100 nM calciseptine. Data from 15 cells, plotted as mean frequency during 10-s intervals and normalized to the mean frequency during a 70-s interval immediately before 50-Hz stimulation. The smooth line is a fitted [to post-high-frequency stimulation (HFS) period in control solution] exponential with time constant 25 s. C: effects of 50-Hz stimulation and of 100 nM calciseptine on sIPSC frequency during the 1st 10 s after 50-Hz stimulation. Data from the same cells as in B. (The difference between control solution and calciseptine for the pre-HFS period was not significant; \(P < 0.80\).) D: frequency of sIPSCs recorded and plotted as in B but in the absence and presence of \(K_{Ca}\)-channel blockers and a combination of \(K_{Ca}\)-channel blockers and calciseptine. Data from 13 cells. E: effects of \(K_{Ca}\)-channel blockers and a combination of \(K_{Ca}\)-channel blockers and calciseptine on sIPSC frequency during the 1st 10 s after 50-Hz stimulation. Data from the same cells as in D. F: lack of effect of 50-Hz stimulation and of calciseptine on sIPSC amplitude and decay time constant. Data from 12 cells, normalized to the magnitudes in control solution before 50-Hz stimulation. Asterisks denote significant differences with \(P < 0.05\) (*), \(P < 0.01\) (**), or \(P < 0.001\) (***)
nM) and paxilline (1.0 μM) increased the eIPSC amplitude to 137 ± 16% (Fig. 2, F and G; P < 0.039, n = 9), an effect that was overlapping with that of 100 nM calciseptine (Fig. 2, F and G). Thus the results suggested that KCa channels of the BK type were involved in the control of GABA release and in the effect of calciseptine on such release.

**L-type Ca2+ channels contribute to the PTP of “spontaneous” GABA release**

The above results suggest an inhibitory role of L-type Ca2+ channels in evoked GABA release at low-frequency stimulation. This contrasts with the enhancing role reported for hippocampal synapses to take place only after high-frequency stimulation and being important for short-term synaptic plasticity (Jensen and Mody 2001; Murakami et al. 2002). To clarify whether L-type channels play different roles for GABA-mediated transmission in the MPN depending on the stimulus frequency, we therefore applied a protocol with 50-Hz extracellular, presynaptic stimulation for 10 s (see METHODS). This high-frequency stimulation (HFS) per se altered the “spontaneous” and evoked GABA-mediated transmission, in both cases giving rise to PTP: after the stimulus train, the frequency of sIPSCs was dramatically potentiated to 919 ± 79% of the basal frequency (as measured during the 1st 10 s after the HFS period; P < 0.002, n = 15; Fig. 3, A–C) and decayed to baseline with a time constant of 25 s (exponential fit in 15 cells). (For simplicity, we use the terminology spontaneous to describe all IPSCs that did not occur within 100 ms after external stimulation.)

After addition of calciseptine (100 nM) to the extracellular solution, the PTP of sIPSC frequency induced by HFS was reduced, now reaching only 595 ± 108% (n = 15) of the basal frequency in control solution (Fig. 3, A–C). The difference from the PTP in control solution was significant (P < 0.002, n = 15). sIPSC amplitude and decay time constant were neither affected by HFS in control solution nor in the presence of calciseptine (Fig. 3F; cf. Fig. 1). Similar results were obtained in control experiments at 30–31°C with the HCO3-/CO2-containing extracellular solution (see METHODS), with the PTP of sIPSC frequency reaching 833 ± 329% (n = 4) without channel blockers and 519 ± 170% (n = 4) in the presence of 100 nM calciseptine. The results suggest that L-type channels do contribute to PTP of sIPSC frequency but that a substantial PTP may occur also when these channels are blocked.

Thus L-type channels apparently affect GABA release evoked by low-frequency stimulation and “spontaneous” GABA release after HFS in opposite directions.

The results above suggest that the potentiating effect of calciseptine on eIPSC amplitude was indirect, mediated by BK-type KCa channels. It seems unlikely that KCa channels, which counteract membrane depolarization, should be essential also for the depressing effect of calciseptine on posttetanic sIPSC frequency. Consistent with this reasoning, but contrary to the effect on eIPSC, addition of KCa-channel blockers did not mimic the effect of calciseptine on posttetanic sIPSC frequency: in the presence of KCa-channel blockers, PTP of sIPSC frequency was not significantly different from that in control solution (P = 1, n = 13) and addition of calciseptine still reduced PTP (Fig. 3, D and E; P < = 7.3 × 10−4, n = 13).

**L-type Ca2+ channels mediate suppression of impulse-evoked GABA release also during PTP**

The results above suggest that L-type channels play different roles for GABA release evoked by low-frequency stimulation and for the “spontaneous” release during PTP of sIPSC frequency. To clarify whether the role of L-type channels for evoked GABA release also changes after HFS, we analyzed the effects of calciseptine on eIPSCs during PTP. In control solution, a 10-s interval with 50-Hz presynaptic stimulation was followed by PTP of the eIPSC amplitude to a peak level of 232 ± 43% of the control amplitude during a preceding 60-s interval with stimulation at low frequency (0.1 Hz) (Fig. 4A, ■). The peak amplitudes of eIPSCs evoked at 0.1 Hz after induction of PTP showed a relatively noisy time course and therefore effects of channel blockers were analyzed for mean eIPSC amplitude at six consecutive stimuli (at 0.1 Hz) after PTP induction. In control solution, this implied a PTP to 208 ± 31% of the pre-HFS eIPSC amplitude (Fig. 4B, control; P < 6.1 × 10−4, n = 14). A fitted exponential function with a time constant of 69 s roughly described the decay of the mean eIPSC amplitudes (in 14 neurons) to baseline, showing that PTP of eIPSC amplitude decayed slower than PTP of sIPSC frequency.

In the presence of calciseptine, the PTP of eIPSC amplitude was further increased to 283 ± 44% of the amplitude in control solution before HFS (Fig. 4, A and B). The difference from the PTP in control solution was significant (P < 1.7 × 10−3, n = 14). In similarity with the effect of calciseptine on eIPSCs evoked by low-frequency stimulation, the effect by calciseptine on eIPSC during PTP was mimicked by a combination of KCa-channel blockers (potentiation to 280 ± 33% of the amplitude in control solution before HFS; difference from PTP in control solution significant: P < 2.8 × 10−3, n = 18), and no further potentiation was induced when calciseptine was added in the presence of the KCa-channel blockers (Fig. 4, C–E). In the latter case, with KCa-channel blockers present, calciseptine reduced the mean eIPSC amplitude during PTP (to 231 ± 32% of the amplitude in control solution before HFS; P < 0.045, n = 17), which was opposite to the effect without KCa-channel blockers. These results show that the main effects of calciseptine and of the KCa-channel blockers on eIPSC amplitude are similar during low-frequency stimulation and during PTP. Apparently, the inhibitory effect of L-type channels on eIPSC amplitude is dependent on KCa channels for low-frequency “basal” transmission and during PTP evoked by high-frequency transmission.

Control experiments with the HCO3-/CO2-containing extracellular solution (see METHODS) and at 30–31°C gave qualitatively similar results, with 50-Hz stimulation evoking PTP of the eIPSC amplitude to 239 ± 71% (n = 4) in the absence of blocker and to 332 ± 140% (n = 3) in the presence of 100 nM calciseptine (mean of 6 consecutive responses after HFS normalized to control before HFS). In these conditions, calciseptine potentiated the eIPSC amplitude before HFS to 166 ± 47% (n = 3). Thus the main findings did not likely depend strongly on the temperature or composition of extracellular solution.
DISCUSSION

In this study, we showed that L-type Ca\(^{2+}\)/H11001 channels, as detected pharmacologically, do contribute to GABA-mediated transmission in the MPN. The lack of changes in amplitude and time course of mIPSCs and of sIPSCs on application of the L-type-channel blocker shows that these channels are presynaptically localized. The functional role of these presynaptic L-type channels for GABA release depends strongly on the stimulus conditions and, somewhat paradoxically, may involve facilitation of spontaneous GABA release after preceding high-frequency impulse activity but inhibition of synchronous impulse-evoked GABA release at low and high impulse frequency. We are not aware of previous studies showing such differential roles of presynaptic L-type channels. These differential roles may be explained by a model where L-type channels provide Ca\(^{2+}\)/H11001 that acts either locally, on BK-type KCa channels, or “globally,” with more direct effects on the presynaptic release machinery, depending on the presynaptic impulse frequency.

![Graphs and diagrams illustrating the effects of L-type Ca\(^{2+}\)/H11001 and KCa channel blockers on eIPSC amplitudes](http://jn.physiology.org/content/jn Supp 6/1/4103/F4.large.jpg)

**Fig. 4.** Blocking either L-type Ca\(^{2+}\) channels or KCa channels potentiates GABA-mediated eIPSCs during PTP. A: PTP of eIPSC amplitudes evoked by a 10-s interval of 50-Hz stimulation, preceded and followed by stimulation at 0.1 Hz, in the absence and presence of 100 nM calciseptine. Data from 14 cells normalized to the mean eIPSC amplitude at 7 consecutive stimuli immediately before 50-Hz stimulation. The smooth line is a fitted (to post-HFS period in control solution) exponential with time constant 69 s. B: effect of 100 nM calciseptine on mean eIPSC amplitude before (Pre-) and after (Post-HFS; mean amplitude from 1st 6 consecutive eIPSCs) 50-Hz stimulation. Data from the same cells as in A. C: raw data traces from 1 cell exemplifying PTP and the effects of KCa-channel blockers separately and in combination with 100 nM calciseptine on eIPSCs. Averages of last 10 eIPSCs before (Ca) and 1st 10 eIPSCs after (Cb) 10 s of 50-Hz stimulation. D: PTP of eIPSC amplitudes, as in C, in the absence and presence of KCa-channel blockers and a combination of KCa-channel blockers and 100 nM calciseptine. Data from 18 cells normalized as in A. E: effects of KCa-channel blockers and of calciseptine on mean eIPSC amplitude before (Pre-) and after (Post-HFS) 50-Hz stimulation, measured similarly as for B. Data from the same (17–18) cells as in D. Asterisks denote significant differences with \(P < 0.05\) (*), \(P < 0.01\) (**), or \(P < 0.001\) (***)
Mechanism for increased Ca\textsuperscript{2+}-dependent release on block of Ca\textsuperscript{2+} channels

The finding that block of L-type Ca\textsuperscript{2+} channels may lead to increase of GABA release, as was observed here in terms of eIPSC amplitudes, may at first seem surprising, especially because we previously showed that depolarization-induced GABA release onto MPN neurons depends on extracellular Ca\textsuperscript{2+} and voltage-gated Ca\textsuperscript{2+} channels (Haage et al. 1998). However, such an effect may arise as a consequence of functional coupling of L-type Ca\textsuperscript{2+} channels to Ca\textsuperscript{2+}-dependent K\textsuperscript{-} (K\textsubscript{Ca}) channels as hypothesized in this study. Ca\textsuperscript{2+} influx through L-type channels may activate K\textsubscript{Ca} channels that affect the membrane potential, e.g., shorten the impulse duration (Adams et al. 1982; Lancaster and Nicoll 1987; Raffaelli et al. 2004; Storm 1987), and thereby indirectly reduce the Ca\textsuperscript{2+} influx that triggers GABA release. K\textsubscript{Ca} channels are known to be present in several types of nerve terminals (Hu et al. 2001; Robitaille and Charlton 1992; Robitaille et al. 1993), including GABA-releasing nerve terminals on MPN neurons (Druzin et al. 2002). In excitatory central nerve terminals, they have been suggested to control transmitter release via effects on impulse time course (Raffaelli et al. 2004), although their contribution may be difficult to detect under basal conditions (Hu et al. 2001). This hypothesis was supported by the findings that the effect of L-type channel block on eIPSC amplitude was mimicked by, and overlapping with, the effect of K\textsubscript{Ca}-channel blockers. The proposed model is, however, dependent on the fact that Ca\textsuperscript{2+} entering through the L-type channels exerts an “indirect” inhibitory effect that dominates over any possible “direct” facilitating effect on the Ca\textsuperscript{2+}-dependent transmitter release machinery. How could such dominance be explained? The simplest explanation may be to assume a functional separation of the L-type channels from the transmitter release machinery. Such separation may arise as a consequence of physical distance. It is known that Ca\textsuperscript{2+} channels may be functionally coupled to some K\textsubscript{Ca}-channel types but not to others, even when present within the same patch of membrane (Marrion and Tavalin 1998). Our previous findings that L-type channels do not contribute to GABA release when terminals are depolarized by a high external K\textsuperscript{+} concentration (Haage et al. 1998) are consistent with the idea that, during low-frequency stimulation, these channels do not provide Ca\textsuperscript{2+} that directly triggers GABA release. (In the latter conditions with high external K\textsuperscript{+} concentration, K\textsubscript{Ca}-channel activation is not expected to significantly alter the presynaptic membrane potential.)

Role of L-type channels in GABA release during PTP

The hypothesis discussed above implies that L-type Ca\textsuperscript{2+} channels are functionally separated from the transmitter release machinery, possibly as a consequence of physical distance. It seems possible, however, that the situation may differ during PTP. The main function that has previously been ascribed presynaptic L-type channels is to contribute to PTP (Holmgaard et al. 2009; Jensen and Mody 2001; Murakami et al. 2002; Storozhuk et al. 2007). PTP is thought to arise as a consequence of presynaptic Ca\textsuperscript{2+} accumulation that takes place during HFS (Zucker and Regehr 2002). We should expect that the rise in presynaptic Ca\textsuperscript{2+} concentration mediated by L-type channels should be larger and affect more wide-spread regions of the presynaptic terminal during HFS than during stimulation at low frequency. The Ca\textsuperscript{2+} signal triggered by Ca\textsuperscript{2+} influx during the high-frequency impulse activity is in several cases thought to be amplified or prolonged by Ca\textsuperscript{2+} released from internal stores (endoplasmatic reticulum or mitochondria) and may act via several Ca\textsuperscript{2+}-sensing proteins that modulate transmitter release (Xu et al. 2007; for review, see Catterall and Few 2008; Zucker and Regehr 2002). Therefore it seems likely that, at HFS, L-type channel activation may contribute to a more global rise in terminal [Ca\textsuperscript{2+}], that increases GABA release probability during PTP, in a way consistent with our observations on the effect of calciseptine on siPSC frequency during PTP. Here we call this effect of Ca\textsuperscript{2+} influx through L-type channels on GABA release “direct” simply to denote that the effect is not mediated via K\textsubscript{Ca} channels.

Why then are eIPSCs potentiated by calciseptine also during PTP? Our interpretation is that the “indirect” effect via BK-type K\textsubscript{Ca} channels is still effective during PTP and simply dominating over the direct potentiating effect caused by L-type channel activation. This interpretation is supported by our finding that, when K\textsubscript{Ca} channels were blocked, calciseptine did reduce the eIPSC amplitude during PTP. Thus L-type channel activation may potentiate eIPSCs when not masked by the effect mediated by K\textsubscript{Ca} channels.

An intriguing possibility is that the recently described Ca\textsuperscript{2+}-dependent exocytosis pathway that is recruited only when Ca\textsuperscript{2+} floods the synaptic terminal (Déak et al. 2009) may contribute to the L-type channel–mediated PTP of siPSC frequency. However, this novel pathway is characterized as independent of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins synaptobrevin-2 and SNAP-25, whereas spontaneous transmitter release depends markedly on these proteins (Bronk et al. 2007; Schoo et al. 2001). Therefore this possibility seems unlikely, although we cannot rule out that the L-type channel–mediated component of PTP depends on a different exocytosis pathway than that contributing to the baseline siPSC frequency. Another possibility is that the Ca\textsuperscript{2+} influx through L-type channels contributes to PTP of siPSC frequency by increasing vesicular release probability via promotion of endocytosis, as suggested by Perissinotti et al. (2008).

Functional separation of Ca\textsuperscript{2+} channels coupled to transmitter release and Ca\textsuperscript{2+} channels coupled to K\textsubscript{Ca} channels

The proposed model, which provides a simple explanation of our results, implies that L-type Ca\textsuperscript{2+} channels are present in nerve terminals on MPN neurons but functionally separated from the Ca\textsuperscript{2+}-channel types (previously identified as N/P/Q-types; Haage et al. 1998) that more directly trigger GABA release. Thus there may be at least two different functional systems depending on Ca\textsuperscript{2+} channels in the nerve terminal: one that is adapted for direct triggering of GABA release (via the direct effect of incoming Ca\textsuperscript{2+} on the release machinery) and another that is adapted for controlling membrane potential via K\textsubscript{Ca} (BK) channels (and with indirect effects on GABA release) but that may also directly control GABA release (when incoming Ca\textsuperscript{2+} floods the terminal) after HFS. The Ca\textsuperscript{2+} ions that affect K\textsubscript{Ca} channels and thereby presynaptic membrane potential enter through different channel types than the Ca\textsuperscript{2+} channels.
ions that trigger transmitter release during low-frequency stimulation. This situation differs from earlier descriptions of the interaction between presynaptic Ca2+ channels and KCa channels. Thus, as verified by morphological [using charybdotoxin biotin–labeled KCa (BK) channels] and physiological (analyzing effects of EGTA and BAPTA on transmitter release) studies in the frog neuromuscular junction, the BK channels are thought to be tightly coupled to the same Ca2+ channels that also trigger acetylcholine release (Robitaille et al. 1993). The BK channels are activated by voltage and Ca2+ and are thought to be located within “nanodomains” of raised [Ca2+], (peak ~100 μM) that are generated within ~20–50 nm from the voltage-gated Ca2+ channels that constitute the source of Ca2+ (Fakler and Adelman 2008). The close coupling to the voltage-gated Ca2+ channels enable the BK channels to activate during an action potential and contribute to the repolarization (Lancaster and Nicoll 1987; Raffaelli et al. 2004; Storm 1987). The resulting shortening of the action potential curtails the opening of Ca2+ channels and thereby reduces Ca2+ influx and Ca2+-dependent transmitter release (Raffaelli et al. 2004; cf. Augustine 1990; Sabatini and Regehr 1997). In such cases, KCa channels may provide a negative-feedback mechanism that contributes to precise timing of synaptic transmission and prevents overexcitation (for review, see Fakler and Adelman 2008).

A functional separation between Ca2+ channels coupled to BK channels and Ca2+ channels coupled to the transmitter release machinery, as suggested on the basis of these findings, may be advantageous in some situations because it presents a possibility for separate control of presynaptic membrane potential (via Ca2+ acting on BK channels) and transmitter release (via Ca2+ acting on the release machinery). This in turn may allow for separate control of impulse-evoked transmitter release and release that is not tightly synchronized with impulse activity, such as sIPSC frequency in basal conditions and during PTP. Separate control mechanisms may provide a means for using spontaneous release for different functions, e.g., maintenance of synaptic stability and dendritic spines (McKinney et al. 1999) or regulation of postsynaptic sensitivity to transmitters (Sutton et al. 2007). It may also imply that pathological conditions may be associated with selective dysfunction of spontaneous, but not impulse-evoked, release (Hirsch et al. 1999).

To our knowledge, control of impulse-evoked release via functional coupling of presynaptic L-type and BK channels has not been described before. One reason may be that the L-type channels are not absolutely required for transmitter release but rather modulate release that requires contribution of other Ca2+-channel types. Furthermore, the presence of KCa channels and their coupling to L-type channels may vary in extent between different types of terminals. In synaptic terminals where such coupling is extensive, differential control of impulse-evoked and spontaneous transmitter release may be possible, whereas in terminals lacking such coupling, L-type channels may nevertheless be used to contribute to PTP (Jensen and Mody 2001).

Possible roles of L-type channels and PTP of GABAergic transmission for MPN function

The roles of L-type channels in GABAergic transmission as shown here are likely important for the information processing in the MPN. The interaction of L-type channels and BK channels during impulse-evoked release may be part of a negative-feedback mechanism that contributes to precise timing of synaptic transmission and prevents overexcitation, as suggested for BK channels in other nerve terminals (Fakler and Adelman 2008). In addition, synaptic plasticity of the type shown may contribute to the functional properties of the MPN during behavior. The firing frequency of medial preoptic neurons in male rats correlates well with the copulatory behavior that is regulated by the preoptic area. The frequency increases when a female is introduced, reaches a maximum (~50 Hz, similar to the frequency used to evoke PTP here) at copulatory behavior (pursuit mounting), but is suppressed during the postejaculatory refractory interval (several minutes) when the animals show no or very weak interest in sexual interactions (Horio et al. 1986; see also Shimura et al. 1994). Although glutamate contributes to copulatory behavior, GABA has the opposite effect: the concentration of GABA in cerebrospinal fluid increases to >1000% after ejaculation, and increased GABAergic transmission is thought to cause the postejaculatory refractory interval (Rodríguez-Manzo et al. 2000). It may be premature to speculate that PTP of GABAergic transmission as seen in this study plays a major role in this refractory interval, but it clearly will contribute to the dynamics of MPN neuronal excitability after high-frequency impulse activity such as seen during copulatory behavior.

Conclusion

In conclusion, L-type Ca2+ channels are present on GABA-containing nerve terminals on MPN neurons where they provide a mechanism for differential regulation of impulse-evoked, synchronous GABA release, and spontaneous/asynchronous GABA release. The former regulation is mediated via BK-type KCa channels. By taking part in the control of impulse-evoked release and contributing to synaptic plasticity, presynaptic L-type channels are likely to play a role in the behavior controlled by the MPN.

GRANTS

This work was supported by the Swedish Research Council (Project 21401), Gunvor och Josef Anés Stiftelse, the Royal Swedish Academy of Sciences, Stiftelsen J C Kempes Minnes Stipendiefond, and Umeå University.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


