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EFFECTS OF STIMULUS INTENSITY ON THE INHIBITION BY ω-CONOTOXIN GVIA AND NEOMYCIN OF K^+-EVOKED [^{3}H]NOREPINEPHRINE RELEASE FROM HIPPOCAMPAL BRAIN SLICES AND SYNAPTOSOMAL CALCIUM INFLUX

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Abstract—The effects of various K^+ concentrations on the inhibition of [^{3}H]norepinephrine release from rat hippocampal brain slices and evoked synaptosomal $^{45}Ca^{2+}$ influx by ω-conotoxin GVIA (ω-CgTx) and neomycin were examined. K^+ (15–75 mM) caused a concentration-dependent release of [^{3}H]-norepinephrine that was greater than 90% dependent on extracellular calcium. The ability of ω-CgTx to inhibit [^{3}H]norepinephrine release was optimal at 25 mM K^+ and was reduced substantially at higher concentrations of K^+. ω-CgTx maximally inhibited [^{3}H]norepinephrine release by 49% (15 mM K^+), 58% (25 mM K^+), 22% (50 mM K^+), and 12% (75 mM K^+). In contrast, neomycin caused a concentration-dependent and virtually complete inhibition of [^{3}H]norepinephrine release at all concentrations of K^+, with IC_{50} values of 210 μM (15 mM K^+), 150 μM (25 mM K^+), 450 μM (50 mM K^+), and 1500 μM (75 mM K^+). ω-CgTx (1 μM) had little effect (10% or less inhibition) on hippocampal synaptosomal $^{45}Ca^{2+}$ influx at any concentration of K^+, whereas 3 mM neomycin caused at least 75% inhibition of $^{45}Ca^{2+}$ influx, with the largest inhibition (96%) occurring at 25 mM K^+. The results suggest that increasing stimulus intensity decreases the contribution of N-type voltage-sensitive calcium channels (VSCC) in mediating K^+-evoked release of [^{3}H]norepinephrine. The comparative absence of ω-CgTx-sensitive synaptosomal $^{45}Ca^{2+}$-influx sites suggests that N-type calcium channels are a small subset of channels in rat hippocampal synaptosomes. The demonstration that neomycin can inhibit ω-CgTx-sensitive and -insensitive neurotransmitter release and calcium influx suggests that neomycin may block N-type VSCC as well as non-N-type VSCC.

ω-Conotoxin GVIA (ω-CgTx)† is thought to be a selective and irreversible inhibitor of N-type voltage-sensitive calcium channels (VSCC) [1, 2]. Although it has been suggested that N-type VSCC play a dominant role in mediating neurotransmitter release [3], ω-CgTx has been shown to cause an incomplete inhibition of evoked neurotransmitter release in some studies [4–6]. In one instance, ω-CgTx had no effect on evoked neurotransmitter release [7]. Recent studies, however, have suggested that variations in stimulus conditions, as well as species differences may account for reported differences in the effectiveness of ω-CgTx to inhibit neurotransmitter release. For example, ω-CgTx has been reported to be more effective under conditions of electrical field stimulation than K^+ stimulation of neurotransmitter release [8–10]. ω-CgTx inhibition of neurotransmitter release was also shown to be more effective at low frequency than at high frequency stimulation [11, 12]. ω-CgTx also inhibited neurotransmitter release more effectively from chicken than from rat brain preparations [13, 14].

The aminoglycoside antibiotic neomycin was shown to inhibit [^{125}I]ω-CgTx binding [15, 16], suggesting that it interacts with the N-type VSCC. In contrast to ω-CgTx, neomycin causes a complete inhibition of evoked neurotransmitter release under conditions in which ω-CgTx and L-type dihydropyridine VSCC antagonists cause incomplete or no inhibition of neurotransmitter release [14]. Based on these observations, we have suggested that in addition to blocking N-type VSCC, neomycin may also inhibit neuronal L- and non-L/non-N VSCC associated with evoked neurotransmitter release.

In an attempt to characterize further the role of VSCC in mediating evoked neurotransmitter release under varying stimulus conditions, the inhibitory effects of ω-CgTx and neomycin on the release of [^{3}H]norepinephrine from rat hippocampal brain slices were evaluated at different concentrations of K^+. The inhibition of K^+-evoked hippocampal synaptosomal $^{45}Ca^{2+}$ influx by ω-CgTx and neomycin at different K^+ concentrations was also investigated. In this way it may be determined whether variations

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† Abbreviations: ω-CgTx, ω-conotoxin GVIA; VSCC, voltage-sensitive calcium channels; and KRB, Krebs–Ringer buffer.

in stimulus intensity alter the relative importance of ω-CgTx-sensitive VSCC in mediating neurotransmitter release and calcium influx.

MATERIALS AND METHODS

Materials. ω-Conotoxin GVIA was purchased from Peninsula Laboratories Inc., Belmont, CA. Nitrendipine was a gift from Miles Pharmaceuticals, West Haven, CT. Neomycin, pargyline, ascorbic acid and desipramine were purchased from the Sigma Chemical Co., St. Louis, MO. [^{3}H]Norepinephrine and $^{45}Ca^{2+}$ were purchased from NEN Research Products, Boston, MA.

K^+-Evoked release of [^{3}H]norepinephrine from hippocampal brain slice preparations. Male Sprague–Dawley rats (250–300 g) were killed by decapitation and brains were removed rapidly. The method for measuring evoked neurotransmitter release was similar to that described previously [6, 7]. Hippocampal brain slices (0.25 × 0.25 mm) were prepared using a McIlwain tissue slicer (Brinkmann Instruments, Westbury, NY). The tissue slices were suspended in a Krebs–Ringer bicarbonate buffer, pH 7.4, of the following composition (mM): NaCl, 134; KCl, 5; $MgSO_4$, 1.2; KH_2PO_4, 1.25; $CaCl_2$, 2; $NaHCO_3$, 25; glucose, 10; ascorbic acid, 0.17; and NaEDTA, 0.03 that had been equilibrated with a mixture of 95% O_2 and 5% CO_2. Rat hippocampal brain slices were incubated for 20 min with 0.1 μM [^{3}H]norepinephrine (11.3 Ci/mmol) in Krebs–Ringer solution (37°) that contained 1 μM pargyline to prevent metabolic degradation of [^{3}H]norepinephrine. After the incubations, slices were washed three times with approximately 50 mL of buffer. Aliquots (200 μL) of slices (equivalent to 7 mg wet weight of rat brain slices) were transferred into chambers of a superfusion apparatus and were superfused at a rate of 1 mL/6 min with Krebs–Ringer solution (37°). The Krebs–Ringer superfusion solution contained 1 μM pargyline to block monoamine oxidase and 1 μM desipramine to prevent neuronal uptake. Following an initial 20-min washout period, eleven successive 6-min fractions were collected (i.e. t = 20 min to t = 86 min). Brain slices were exposed to a single 6-min stimulation of buffer containing 15, 25, 50 or 75 mM K^+ at t = 56 min (equimolar replacement of NaCl by KCl). Where indicated, compounds were added 18 min prior to K^+ exposure at t = 38 min, and remained in the medium throughout the K^+ stimulus (t = 62). In calcium-free studies, 20 μM ethyleneglycolbis-(aminoethylether)tetra-acetate (EGTA) replaced Ca^{2+} 18 min prior to K^+ exposure. At the conclusion of each experiment, slices from each chamber were sonicated. The radioactivity present in the collected fractions and the tissue slices was estimated by liquid scintillation spectroscopy.

The percent fractional efflux of tritium was estimated as the fraction of the amount of radioactivity in the superfusate fraction relative to the total amount of radioactivity in the slice at that particular point in time, multiplied by 100. The total amount of evoked tritium release by a K^+ stimulus was estimated by summing the percent fractional release values over baseline for the four fractions following exposure to K^+. The percent fractional release of tritium in the fraction before superfusion with K^+ was used as an estimate of baseline release. Results were expressed as the percent of the control total evoked release values obtained from paired brain slice preparations not exposed to drugs.

Synaptosomal calcium influx. Rat synaptosomes (P2) were prepared as described by Reynolds *et al.* [4]. Male Sprague–Dawley rats (225–325 g) were killed by decapitation. All preparative steps were performed at 4°. Hippocampal preparations were minced and homogenized in 9 vol. of 0.32 M sucrose, using nine up-and-down strokes of a loose-fitting motor-driven Teflon–glass homogenizer. The homogenate was centrifuged at 1000 *g* for 10 min. The supernatant was decanted, diluted 1:1 with a low-Na^+ resuspension Krebs–Ringer buffer (KRB; composition in mM: choline Cl, 140; KCl, 5; $MgCl_2$, 1.3; glucose, 10; Na-HEPES, 10; pH 7.45; aerated with O_2 for 60 min), and centrifuged at 10,000 *g* for 15 min. The pellet was resuspended in 3 mL of resuspension KRB per g wet weight of tissue (approximately 5 mg protein/mL), with five strokes of the homogenizer. Synaptosomes were stored on ice for no more than 30 min prior to beginning the experiment. Drugs were added to synaptosomes during this incubation period on ice, and were allowed to equilibrate for at least 30 min prior to initiating $^{45}Ca^{2+}$ influx. $^{45}Ca^{2+}$ influx was initiated by the rapid addition of 450 μL of either low- or high-K^+, low-Na^+ KRB, which contained approximately 2 μCi/mL $^{45}Ca^{2+}$, to 50 μL of synaptosomal suspension (approximately 250 μg protein, prewarmed for 12 min at 34°). The composition of the low-K^+ KRB was (mM): choline Cl, 140; KCl, 5; $MgCl_2$, 1.3; $CaCl_2$, 1; glucose, 10; Na-HEPES, 10; pH 7.45. The high-K^+ KRB was equivalent to the low-K^+ KRB except that it contained appropriate amounts of KCl (isosmotically substituted with choline Cl) to provide final K^+ concentrations of 15, 25, 50 and 75 mM in the reaction mixture. After a 1-sec incubation period (timed with an electronic metronome), $^{45}Ca^{2+}$ influx was stopped by the addition of 3 mL of quench KRB (composition in mM: choline Cl, 130; KCl, 5; $MgCl_2$, 1.3; glucose, 10; Na-EGTA, 10; Na-HEPES, 10; pH 7.45; 4°). The samples were filtered immediately through Whatman GF/C glass fiber filters using a Hoefer FH225V filtration instrument. The filters were washed with 3 × 4 mL of wash KRB (composition in mM: choline Cl, 139; KCl, 5; $MgCl_2$, 1.3; $LaCl_3$, 2; glucose, 10; Na-HEPES, 10; pH 7.45; 4°) and dried at 24° for 16 hr; the retained radioactivity was determined by liquid scintillation spectroscopy. Net voltage-dependent $^{45}Ca^{2+}$ uptake was determined as the difference between uptake in high-K^+ and low-K^+ KRB. Protein concentration was determined by the method of Bradford [17], using reagents purchased from Bio-Rad (Richmond, CA), and bovine serum albumin as the standard.

Statistical analyses. Data are expressed as means ± SEM. Statistically significant differences between two means were determined by Student's *t*-test for unpaired observations. Significant differences between three or more means were determined by one-way analysis of variance and the Bonferroni

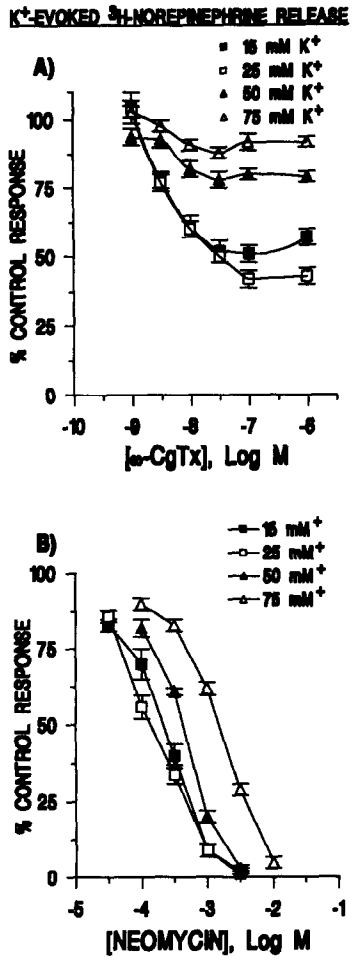

Fig. 1. Inhibition of K^+-evoked release of [^{3}H]norepinephrine from rat hippocampal slices by (A) ω-CgTx and (B) neomycin at various concentrations of K^+. Percent total evoked release values for control preparations in this series of experiments were: 13 ± 1.6 (15 mM K^+), 42 ± 3.0 (25 mM K^+), 101 ± 4.9 (50 mM K^+) and 126 ± 9.2 (75 mM K^+). Values represent means ± SEM; N = 6–12.

modified *t*-test as described by Wallenstein *et al.* [18]. The IC_{50} values were determined from regression lines of probit transformed log concentration–response curves.

RESULTS

The inhibitory effects of ω-CgTx on K^+-evoked release of [^{3}H]norepinephrine were tested at several concentrations of K^+ (Fig. 1A). The maximal inhibition by ω-CgTx for each concentration of K^+ occurred at either 30 or 100 nM ω-CgTx. The largest inhibition induced by ω-CgTx occurred at 25 mM K^+, causing a 58 ± 3% inhibition at 100 nM ω-CgTx. The maximal inhibition at 15 mM K^+ was somewhat less (49 ± 3% inhibition at 100 nM ω-CgTx; statistically nonsignificant compared to 25 mM K^+ value, $P > 0.05$). At higher concentrations of K^+ the maximal extent of inhibition induced by ω-CgTx was reduced dramatically, causing only 22 ± 3% (50 mM K^+; $P < 0.05$ compared to 25 mM K^+ value) and 12 ± 2% (75 mM K^+; $P < 0.05$ compared to 25 mM K^+ value) inhibition at 30 nM ω-CgTx. Thus, on a percentage basis, the ability of ω-CgTx to maximally inhibit K^+-evoked [^{3}H]norepinephrine release was optimal at 25 mM K^+ and was decreased substantially at higher concentrations of K^+.

In contrast to ω-CgTx, increasing the concentration of K^+ altered the potency with which neomycin inhibited release, but did not prevent neomycin from causing essentially a complete inhibition of release (Fig. 1B). The IC_{50} values for neomycin were: 210 μM (15 mM K^+); 150 μM (25 mM K^+); 450 μM (50 mM K^+); and 1500 μM (75 mM K^+). Thus, like ω-CgTx, the inhibition induced by neomycin was optimal at 25 mM K^+ and slightly reduced at 15 mM K^+. Although concentrations of K^+ greater than 25 mM did not prevent neomycin from causing a complete inhibition of release, the potency of neomycin at 50 and 75 mM K^+ was decreased.

Increasing the concentration of K^+ caused a greater amount of total [^{3}H]norepinephrine release in absolute terms (Fig. 2A; note that total evoked release can be greater than 100% because this represents the sum of four fractional release values expressed as percent values as described in Materials and Methods). At all concentrations of K^+, release was inhibited by greater than 90% by removal of extracellular calcium (Fig. 2A), suggesting that calcium influx was the predominant effector of release at all concentrations of K^+. We wondered whether the ω-CgTx-sensitive pool decreased in absolute terms or whether the decrease in maximal inhibitory effect of ω-CgTx at higher concentrations of K^+ was merely due to a relative increase of ω-CgTx-insensitive [^{3}H]norepinephrine release with no change in the absolute size of the ω-CgTx-sensitive pool *per se*. To address this question the total evoked release values of [^{3}H]norepinephrine in both the presence and absence of 100 nM ω-CgTx were evaluated at the different concentrations of K^+ (Fig. 2A). The difference between total evoked release values in the absence and presence of ω-CgTx represents, in absolute terms, the ω-CgTx-sensitive pool of [^{3}H]norepinephrine release (Fig. 2B). The total amount of [^{3}H]norepinephrine release in the presence of ω-CgTx represents in absolute terms the ω-CgTx-insensitive pool of [^{3}H]norepinephrine release (Fig. 2B). The absolute values of the ω-CgTx-sensitive pools of [^{3}H]norepinephrine release were 8.1 ± 2% (15 mM K^+), 28.4 ± 3% (25 mM K^+), 21.6 ± 2% (50 mM K^+), and 9.8 ± 2% (75 mM K^+). The absolute values of the ω-CgTx-insensitive pools of [^{3}H]norepinephrine release were 8.8 ± 2% (15 mM K^+), 20.5 ± 4% (25 mM K^+), 87.1 ± 3% (50 mM K^+), and 113 ± 3% (75 mM K^+). Thus, in absolute terms, the maximal extent of inhibition induced by ω-CgTx was greatest at 25 mM K^+. As K^+ concentrations were increased above this value, the ω-CgTx-sensitive pool decreased and the ω-CgTx-insensitive pool of [^{3}H]-norepinephrine release increased. The dihydropyridine VSCC antagonist nitrendipine (1 μM) caused a small but significant inhibition of K^+-evoked [^{3}H]norepinephrine release only at 15 mM K^+

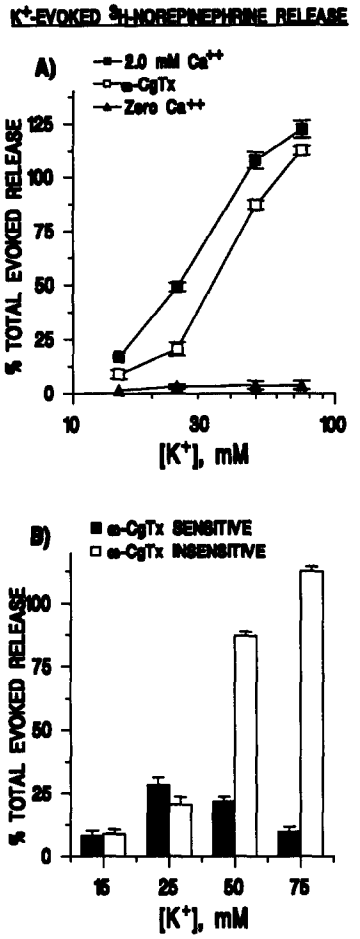

Fig. 2. Effects of ω-CgTx on the total amount of K^+-evoked [^{3}H]norepinephrine release at various concentrations of K^+. (A) Total evoked release values of K^+-evoked [^{3}H]-norepinephrine release in the absence and presence of 100 nM ω-CgTx. Release of stimulated [^{3}H]norepinephrine in the absence of extracellular calcium is also shown. Note that total evoked release can be greater than 100% because this represents the sum of four fractional release values expressed as percent fractional release values as described in Materials and Methods. Values represent means ± SEM; N = 6–12. (B) ω-CgTx-sensitive and -insensitive pools of K^+-evoked release of [^{3}H]norepinephrine at various concentrations of K^+. Values are derived from data in Fig. 2A.

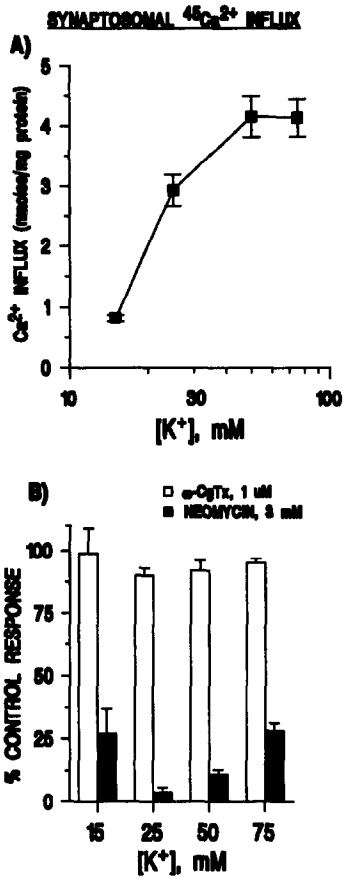

Fig. 3. (A) Dependence of K^+ concentration on evoked $^{45}Ca^{2+}$ influx into rat hippocampal synaptosomes. Values represent means ± SEM; N = 5 experiments, each performed in triplicate. (B) Effects of 1 μM ω-CgTx and 3 mM neomycin on synaptosomal $^{45}Ca^{2+}$ influx evoked by various concentrations of K^+. The net stimulated $^{45}Ca^{2+}$ influx values (nmol/mg protein) were: 0.82 ± 0.06 (15 mM K^+), 2.93 ± 0.26 (25 mM K^+), 4.15 ± 0.35 (50 mM K^+), and 4.13 ± 0.31 (75 mM K^+). Values represent means ± SEM; N = 5 experiments, each performed in triplicate.

(17 ± 6% inhibition) and 25 mM K^+ (12 ± 4% inhibition), but had no significant effect at either 50 or 75 mM K^+.

K^+ caused a concentration-dependent stimulation of hippocampal synaptosomal $^{45}Ca^{2+}$ influx that was maximal at 50 mM K^+ (Fig. 3A). The net stimulated $^{45}Ca^{2+}$ influx values (nmol/mg protein) were: 0.82 ± 0.06 (15 mM K^+); 2.93 ± 0.26 (25 mM K^+); 4.15 ± 0.35 (50 mM K^+); and 4.13 ± 0.31 (75 mM K^+; data represent means ± SEM values from five experiments, each performed in triplicate). At 1 μM, ω-CgTx caused no greater than a 10% inhibition of evoked $^{45}Ca^{2+}$ influx (Fig. 3B). Only the inhibition induced by ω-CgTx at 25 mM K^+ was marginally statistically significant ($0.02 < P < 0.05$). In contrast, 3 mM neomycin substantially inhibited evoked $^{45}Ca^{2+}$ influx at all concentrations of K^+. The largest inhibition (96 ± 2%) induced by neomycin occurred at 25 mM K^+. Nitrendipine (1 μM) had no significant effect on evoked $^{45}Ca^{2+}$ influx at any concentration of K^+ (data not shown).

DISCUSSION

Recent studies suggest that differences in the effectiveness of ω-CgTx to inhibit neurotransmitter release may be due, at least in part, to the use of different stimulation conditions [8, 10–12]. The present report demonstrates that variation in the concentration of K^+ used to evoke neurotransmitter release altered the effectiveness of ω-CgTx to maximally inhibit [^{3}H]norepinephrine release from rat hippocampal brain slices. ω-CgTx caused a

maximal inhibition of release at 25 mM K^+. Increasing the concentration of K^+ resulted in a depressed maximal inhibitory effect induced by ω-CgTx. In absolute terms, the ω-CgTx-sensitive pool of [^{3}H]-norepinephrine release was largest at 25 mM K^+ and was decreased substantially at higher concentrations of K^+.

ω-CgTx has been shown to inhibit both N- and L-type VSCC in some studies [19] or only N-type VSCC in other studies [1, 2]. We have shown that the inhibitory effects of ω-CgTx and nitrendipine are additive on K^+-evoked release of [^{3}H]-norepinephrine [6], suggesting that ω-CgTx had no effect on the functional responses of L-type VSCC. On the basis of this observation, we propose that the inhibitory effects of ω-CgTx on K^+-evoked release of [^{3}H]norepinephrine from brain slice preparations are due to inhibition of N-type VSCC only. Thus, the observation that the maximal inhibition of neurotransmitter release induced by ω-CgTx declined with increasing concentrations of K^+ suggests that the contribution of N-type VSCC to neurotransmitter release is reduced as stimulus intensity is increased. This is consistent with the demonstration that ω-CgTx inhibition of neurotransmitter release can be overcome by high frequency electrical field stimulation [11, 12]. Thus, for both electrical field stimulation or K^+ stimulation of neurotransmitter release, increasing stimulus intensity decreases the contribution of ω-CgTx-sensitive N-type VSCC to neurotransmitter release.

The results of the present study suggest that variations in stimulus intensity alter the gating characteristics of N-type VSCC that are involved in mediating [^{3}H]norepinephrine release. Suszkiw *et al.* [20] have estimated the membrane depolarization values of step changes in K^+ concentrations. These calculations suggest that a step from 5 to 15 mM K^+ causes a 25–30 mV depolarization, 5 to 25 mM K^+ a 40–45 mV depolarization, 5 to 50 mM K^+ a 60–65 mV depolarization, and 5 to 75 mM K^+ a 70–75 mV depolarization. Thus, our studies would suggest that membrane depolarizations greater than 65 mV are sufficient to trigger the alteration in gating characteristics. One may speculate that either a voltage-dependent or a calcium-dependent increase in the rate of inactivation could account for the decreased contribution of N-type VSCC to mediate neurotransmitter release at higher levels of stimulus intensity. Further studies, however, would be required to discriminate between these or other possibilities.

In contrast to N-type VSCC, the contribution of non-N-type VSCC to evoked release increases with greater membrane depolarization, in both relative and absolute terms. The relative ineffectiveness of nitrendipine suggests that L-type VSCC, at best, play only a minor role. While the nature of the non-L/non-N-type VSCC cannot be determined from the present study, recent studies suggest that P-type and possibly other types of VSCC may be involved. ω-Aga-IVA has been described recently as a putative selective inhibitor of P-type VSCC [21], calcium channels that are resistant to inhibition by dihydropyridines and ω-CgTx [22, 23]. We have shown recently that ω-Aga-IVA inhibits K^+-evoked (25 mM K^+) [^{3}H]norepinephrine release from rat hippocampal brain slices that is insensitive to inhibition by ω-CgTx [24]. In addition, Turner *et al.* [25] have shown that increasing stimulus intensity results in the apparent activation of non-L/non-N/non-P-type VSCC in the mediation of K^+-evoked synaptosomal [^{3}H]glutamate release. Thus, the non-L/non-N-type VSCC involved in mediating [^{3}H]-norepinephrine release may at least be partially due to the activity of P-type VSCC, but other VSCC subtypes may be involved as well.

Although ω-CgTx has been shown to inhibit neurotransmitter release from rat brain slice preparations [6, 14, 26, 27], ω-CgTx is essentially ineffective in inhibiting rat synaptosomal $^{45}Ca^{2+}$ influx at concentrations that cause a maximal inhibition of neurotransmitter release [20, 28]. We wondered whether variations in stimulus intensity might reveal optimal conditions to measure ω-CgTx-sensitive synaptosomal $^{45}Ca^{2+}$ influx. A 1 μM concentration of ω-CgTx (ten times higher than required to cause maximal inhibition of neurotransmitter release) was generally ineffective in inhibiting rat hippocampal synaptosomal $^{45}Ca^{2+}$ influx at all concentrations of K^+ tested. ω-CgTx caused no greater than a 10% inhibition of $^{45}Ca^{2+}$ influx. In contrast to evoked [^{3}H]norepinephrine release, varying stimulus intensity had only a marginal effect on the ω-CgTx sensitivity of K^+-evoked rat synaptosomal $^{45}Ca^{2+}$ influx. These observations are consistent with the hypothesis proposed by Lundy *et al.* [28] that ω-CgTx blocks only a small proportion of total calcium influx in synaptosomal preparations, but does so at "hot spots" that may be intimately associated with neurotransmitter-relevant calcium influx. Alternatively, interaction with proposed subtypes of N channels [29] by ω-CgTx may be the underlying mechanism that could explain the relative ineffective inhibition of rat synaptosomal $^{45}Ca^{2+}$ influx.

Neomycin has been shown to inhibit [^{125}I]ω-CgTx binding [15, 16], and prevent irreversible inhibition induced by ω-CgTx [12]. Although neomycin interacts with the ω-CgTx binding site, and therefore, presumably N-type VSCC, the profile of inhibition with increasing concentrations of K^+ is different from ω-CgTx. Although the potency of neomycin to inhibit the release of [^{3}H]norepinephrine was changed by varying K^+ concentrations, neomycin could essentially abolish [^{3}H]norepinephrine release at all concentrations of K^+ tested. This suggests that neomycin can inhibit ω-CgTx-sensitive and -insensitive calcium influx sites that mediate K^+-evoked [^{3}H]norepinephrine release. Neomycin has also been shown to cause a concentration-dependent and virtually complete inhibition of K^+-evoked rat synaptosomal $^{45}Ca^{2+}$ influx [14, 30, 31], and K^+-evoked neurotransmitter release processes that are both sensitive and resistant to inhibition by ω-CgTx and dihydropyridine VSCC antagonists [14]. Based on these observations we have proposed that neomycin can inhibit neuronal L- and non-L/non-N VSCC *in addition* to its ability to inhibit N-type VSCC. Observations from the present report provide additional evidence to support this notion.

Nitrendipine, a selective inhibitor of L-type VSCC,

caused a small or no inhibition of either K^+-evoked [^{3}H]norepinephrine release or synaptosomal $^{45}Ca^{2+}$ influx. This suggests that L-type VSCC exert only a minor role in synaptic transmission under the conditions of the present study. However, inhibition of L-type VSCC by dihydropyridine antagonists is voltage-dependent, implying that the lack of effectiveness of dihydropyridines is not necessarily evidence that L-type VSCC are not involved [32].

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