Involvement of N-Type Voltage-Activated Ca^{2+} Channels in the Release of Endogenous Noradrenaline from the Isolated Vascularly Perfused Rat Stomach

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ABSTRACT—We characterized the voltage-activated Ca^{2+} channels involved in noradrenaline (NA) release from gastric sympathetic neurons using isolated, vascularly perfused rat stomach. The evoked NA release by electrical stimulation of periarterial nerves was abolished by calcium removal from the perfusion medium and by cadmium. ω-Conotoxin GVIA (N-type Ca^{2+}-channel blocker) effectively and ω-conotoxin MVIIC (N/P/Q-type blocker) slightly inhibited the evoked NA, while ω-agatoxin IVA (P-type blocker) had no effect. These results suggest that ω-conotoxin GVIA and ω-conotoxin MVIIC-sensitive N-type Ca^{2+} channels are involved in NA release from the rat gastric sympathetic nerve terminals.

Keywords: Noradrenaline release, Rat stomach, N-type Ca^{2+} channel

Calcium (Ca^{2+}) influx essential for neurotransmitter release is governed by a number of high voltage-activated channels. These channels have been subdivided into the L-, N-, P- and Q-type. Various polypeptide toxins have subtype selectivities for neuronal Ca^{2+} channels (1). ω-Conotoxin GVIA (ω-CTX GVIA) is considered to be a selective blocker of N-type channels (2, 3), while ω-agatoxin IVA (ω-AGA IVA) is considered to be a blocker of P-type channels (4). ω-Conotoxin MVIIC (ω-CTX MVIIC) is able to identify a new type of channel named "Q" in addition to N- and P-type channels (5, 6). These toxins have been shown to attenuate the release of several kinds of neurotransmitters.

In the sympathetic nervous system such as the rat superior cervical ganglia (7), rat isolated kidney (8), rat tail artery (9) and rat mesenteric arteries (10), ω-CTX GVIA-sensitive N-type Ca^{2+} channels have been shown to be involved in noradrenaline (NA) release. More recently, however, it has been reported that the ω-CTX MVIIC-sensitive Q type Ca^{2+} channel is also involved in NA release from mouse vas deferens (11). The high K^+-evoked dopamine release from rat retina is also mediated by ω-AGA IVA- and ω-CTX MVIIC-sensitive Q-type Ca^{2+} channels (12).

In the present study, therefore, we attempted to characterize the Ca^{2+} channels involved in NA release from gastric sympathetic nerve terminals, using an isolated, vascularly perfused rat stomach.

Male Wistar rats weighing about 350 g were fasted overnight before the experiments. Isolated stomach preparations were made as described previously (13, 14). The preparations were equilibrated for 60 min by perfusion with modified Krebs-Ringer solution (pH 7.4, 37℃) bubbled with a mixture of 95% O_2 – 5% CO_2 via the celiac artery with a flow rate of 4 ml per min. Modified Krebs-Ringer solution was composed of 117.5 mM NaCl, 4.7 mM KCl, 2.4 mM $CaCl_2$, 1.1 mM $MgCl_2$, 1.1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose, 0.1% bovine serum albumin, 10 μM pargyline and 1 μM phentolamine. Each 2-min effluent from the portal vein was collected in chilled tubes. The 1st electrical stimulation of periarterial nerves (ESP) around the left gastric artery, which contain the postganglionic sympathetic nerves, was applied using bipolar electrodes, and the 2nd stimulation was carried out 26 min after the 1st ESP. ESP consisted of square-wave pulses of 2.5 Hz, 10 mA, 2-msec duration for 1 min. Perfusion with test substances was started 14 min before the 2nd ESP and continued throughout the experiments (Fig. 1) or for 20 min (Figs. 2 and 3). The amounts of NA released above the basal levels by the 1st and 2nd ESP were expressed as S_1 and S_2, and the effects of test substances were evaluated as S_2/S_1 ratios. Drugs used were cadmium chloride and diltiazem hydrochloride (Sigma, Chemical Co., St. Louis, MO, USA) and ω-

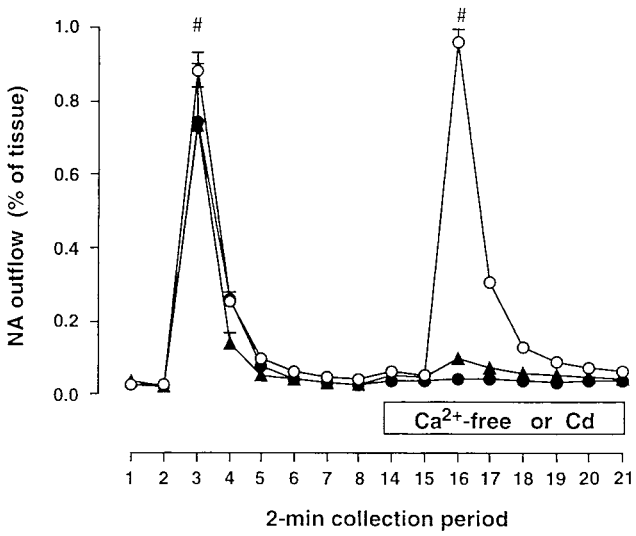


Fig. 1. Effects of Ca^{2+} removal and cadmium on the evoked noradrenaline (NA) release by electrical stimulation of periarterial nerves. NA release was expressed as % of total NA contained in the stomach. ○, normal medium (n=5); ●, Ca^{2+}-free medium containing 2 mM EGTA (n=4); ▲, medium containing 10^{-4} M cadmium (n=5). Application of these media was started 14 min before the 2nd stimulation and continued until the end of the experiments. #Electrical stimulation of periarterial nerves at 2.5 Hz for 1 min. Values are each a mean±S.E.M.

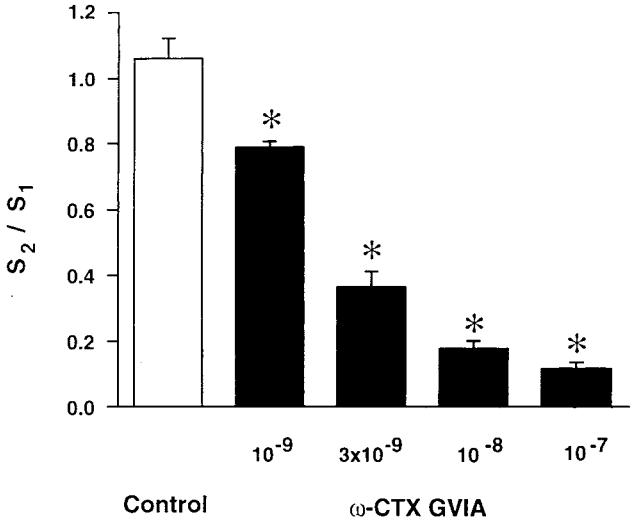
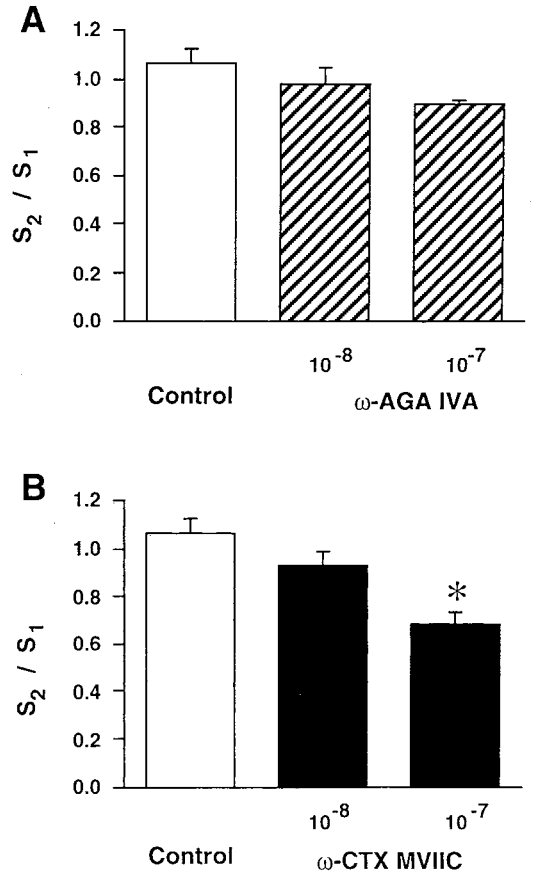


Fig. 3. Effects of ω-conotoxin GVIA (ω-CTX GVIA) on the evoked NA release by electrical stimulation of periarterial nerves. Treatments with ω-CTX GVIA (10^{-9}–10^{-7} M) were started 14 min before the 2nd stimulation and continued for 20 min. Control (cited from Fig. 2A); 10^{-9} M ω-CTX GVIA, n=4; 3×10^{-9} M ω-CTX GVIA, n=4; 10^{-8} M ω-CTX GVIA, n=4; 10^{-7} M ω-CTX GVIA, n=5. Other conditions were the same as those for Fig. 2.



AGA IVA, ω-CTX GVIA and ω-CTX MVIIC (Peptide Institute, Inc., Osaka). Catecholamines in the perfused medium and the supernatant of tissue homogenate were electrochemically assayed by high-performance liquid chromatography as described elsewhere (13, 14).

The content of NA remaining in the stomach was 655±14 ng (n=49). The amount of NA spontaneously released per 2 min was about 0.03% of the total NA contained in the stomach. In a previous study (14), ESP induced frequency-dependent (1, 2.5, 5 and 10 Hz) increases in NA release, and the evoked NA release was abolished by 3×10^{-7} M tetrodotoxin. In the present study, therefore, periarterial nerves were stimulated at 2.5 Hz. Repetitive stimulations of periarterial nerves produced almost the same responses (Fig. 1). The evoked

Fig. 2. Effects of ω-agatoxin IVA (ω-AGA IVA) and ω-conotoxin MVIIC (ω-CTX MVIIC) on the evoked NA release by electrical stimulation of periarterial nerves. Treatments with ω-AGA IVA (10^{-8} and 10^{-7} M) and ω-CTX MVIIC (10^{-8} and 10^{-7} M) were started 14 min before the 2nd stimulation and continued for 20 min. The amounts of NA released above the basal levels by the 1st and 2nd electrical stimulations were expressed as S_1 and S_2, and the effects of toxins were evaluated as S_2/S_1 ratios. A: control, n=5; 10^{-8} M ω-AGA IVA, n=4; 10^{-7} M ω-AGA IVA, n=5. B: control (cited from Fig. 2A); 10^{-8} M ω-CTX MVIIC, n=4; 10^{-7} M ω-CTX MVIIC, n=5. *Significantly different from the control ($P<0.05$) (analyzed by one-way ANOVA followed by *post hoc* analysis with Bonferroni's method for comparing a control with all other means). Other conditions were the same as those for Fig. 1.

NA release was abolished in the Ca^{2+}-free medium containing 2 mM EDTA and in the medium containing 10^{-4} M cadmium (Fig. 1), but not affected by diltiazem (10^{-6} and 10^{-5} M) (data not shown). These results indicate that voltage-activated Ca^{2+} channels other than the L-type are involved in the release of gastric NA.

In the next series of experiments, we used three kinds of polypeptide toxins. ω-AGA IVA (10^{-8} and 10^{-7} M) had no effect on the evoked NA release, while ω-CTX MVIIC slightly, but significantly, inhibited the evoked NA release only at a higher concentration of 10^{-7} M (Fig. 2). On the other hand, ω-CTX GVIA markedly inhibited the evoked NA release in a concentration-dependent manner ($10^{-9}-10^{-7}$ M) (Fig. 3). These polypeptide toxins had no effect on the basal NA release. The present results clearly indicate that N-type voltage-activated Ca^{2+} channels, but not P or Q-type Ca^{2+} channels, mediate the release of NA from the rat stomach as shown in other sympathetically innervated organs (7–10).

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