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EFFECTS OF AN INDENE-DERIVATIVE, TN-871, ON SYNAPTIC TRANSMISSION IN A SYMPATHETIC GANGLION: PRESYNAPTIC ACTIONS ON NEUROTRANSMITTER RELEASE

BY

Ying-Lan SHEN, Keiji HIRAI and Yoshifumi KATAYAMA*

ABSTRACT

Intracellular recordings were made from bullfrog sympathetic ganglion cells to elucidate effects of 2-n-butyl-1-(4-methylpiperazinyl)-5,6-methylenedioxyindene·2HCl (TN-871) on synaptic transmission. TN-871 at 30 nM augmented cholinergic nicotinic fast excitatory postsynaptic potentials (fast EPSPs), whereas the drug at 3 μM reversibly depressed them, without affecting acetylcholine-induced depolarizations. TN-871 did not affect active and passive electrical properties of the ganglion cells. The quantal analysis method was applied to the fast EPSPs in a 0.54 mM Ca^{2+}/7.56 mM Mg^{2+} Ringer's solution. The mean quantal content was significantly increased by TN-871 at 30 nM but significantly decreased at 3 μM. TN-871 at 300 nM either increased or decreased the mean quantal content. The mean quantal size of the fast EPSPs was not changed by TN-871 at the concentrations examined. Fast EPSPs in a 0.99 mM Ca^{2+}/4.86 mM Mg^{2+} Ringer's solution were not affected by nicardipine, but were inhibited in amplitude by ω-conotoxin in a concentration-dependent manner. It is likely that TN-871, in high concentrations, might block ω-conotoxin-sensitive N-type calcium channels in the presynaptic terminals. These results indicate that TN-871 modulates transmitter release from preganglionic nerve terminals without changing the postsynaptic sensitivity of the ganglion cells to ACh.

Key words: Intracellular recording, Ganglionic transmission, Sympathetic ganglion, Fast EPSP, Acetylcholine, Quantal analysis, Transmitter release, Indene-derivative, TN-871.

INTRODUCTION

There is a wide variety of compounds designed for maintaining or improving cerebral activities; these compounds might be tentatively classified into cerebral vasodilators and metabolic enhancers according to their possible pharmacological mechanisms of action. An indene-derivative, indeloxazine, is reported to enhance the acquisition of learned behavior and to desynchronize the spontaneous EEG in rats; thus, it has been suggested that indeloxazine possesses "activating effects" on cerebral functions (Yamamoto and Shimizu [1]). Furthermore, this drug has been shown to augment long-term potentiation in guinea-pig hippocampal slices (Sugimura et al. [2]). Recently, a new indene-derivative, 2-n-butyl-1-(4-methylpiperazinyl)-5,6-methylenedioxyindene·2HCl (TN-871) was reported to have anti-anoxic effects in mice (Ikeda et al. [3]). Little is yet known, however, about the actions of these indene-derivatives on neuronal function.

Our preliminary studies (Katayama et al. [4]; Katayama and Morita [5]) have shown

* 申　英蘭，平井恵二，片山芳文：Department of Autonomic Physiology (Chief: Prof. Y. KATAYAMA), Medical Research Institute, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku)

that TN-871 modulated synaptic transmission in the myenteric plexus of the guinea-pig ileum; this compound augmented nicotinic, fast EPSPs at low concentrations, whereas it depressed them at high concentrations. Since TN-871 did not affect the ACh-induced depolarization, this drug was thought to act presynaptically to modulate ACh release without changing the sensitivity of postsynaptic neurons to ACh. A further analysis has been necessary to determine the sites and possible mechanisms of the action of this potentially interesting drug, TN-871, on synaptic transmission, and the statistical quantal analysis (variance or failure) method should be employed to examine whether TN-871 actually changes the amount of neurotransmitter released from presynaptic nerve terminals. The statistical methods could not be applied to the synaptic transmission in myenteric ganglia and other mammalian autonomic ganglia, because neurons in those ganglia have multiple preganglionic inputs and show fast EPSPs of graded amplitude (Purves and Hume [6]; Forehand [7]; Tabatabai et al. [8]; Hadley [9]).

However, using bullfrog sympathetic ganglion neurons of which electrophysiological properties have been well investigated (Nishi and Koketsu [10]; Kuba and Koketsu [11]), the statistical quantitative analysis of transmitter release has been successfully carried out (Hirai and Koketsu [12]; Kuba et al. [13]; Hirai and Katayama [14]). Release of the transmitter ACh is known to be quantal in nature, and the amplitude of nicotinic fast EPSPs is considered to be of a Poisson distribution in a low Ca^{2+}/high Mg^{2+} Ringer's solution (see Hirai and Koketsu [12]; Kuba et al. [13]). Thus, the bullfrog sympathetic ganglion could be employed in the present experiments using the quantal analysis method. This paper describes the modulation by TN-871 of the amount of the transmitter released from preganglionic nerve terminals, and possible mechanisms underlying these actions of TN-871 on transmitter release are discussed. A preliminary account of this work has been published (Shen et al. [15]).

Materials and Methods

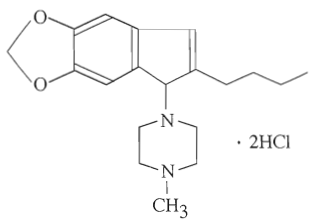
The ninth or tenth lumbar sympathetic ganglia were isolated from bullfrogs (*Rana catesbiana*) of either sex together with preganglionic nerves (Nishi and Koketsu [10]; Hirai and Katayama [14]). Each ganglion was mounted in a recording chamber and was superfused with Ringer's solution of the following composition (mM):NaCl 112.0, KCl 2.0, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4. The volume of the chamber was approximately 0.6 ml, and flow rate was 2–3 ml/min.

Intracellular recordings were made from ganglion cells using glass microelectrodes filled with 3M KCl. Microelectrodes with resistances from 20 to 50 MΩ were chosen. Transmembrane currents were passed through the recording electrodes by using a bridge circuit of a preamplifier. The resting membrane potential was determined by a sudden withdrawal of the recording electrode from the cell impaled. Somatic action potentials were evoked by depolarizing current pulses (duration, 3 ms), and neuronal input resistance was estimated from the amplitude of electrotonic potentials induced by hyperpolarizing current pulses (duration, 250 ms). Fast EPSPs were evoked by supramaximal electrical stimulation applied to preganglionic nerves. The fast EPSPs for quantal analysis were evoked in a low Ca^{2+}/high Mg^{2+} Ringer's solution of the following composition (mM): NaCl 102.8, KCl 2.0, $CaCl_2$ 0.54, $MgCl_2$ 7.56 and $NaHCO_3$ 2.4. Acetylcholine-depolarizations (ACh potentials) were induced by

brief ionophoresis of ACh (duration, 5 ms) from a micropipette positioned immediately above the ganglion cell examined; the micropipette was filled with 2M ACh, and constant backing-currents (10 nA) were continuously passed to prevent ACh leakage. Recorded signals were digitized, stored in a memory oscilloscope (VC-10: Nihon Kohden) and subsequently displayed on a three-channel pen chart recorder (RECTI-HORIZ-8K, San-ei). The mean quantal content and the mean quantal size were calculated for every 60 fast EPSPs evoked at 0.2Hz in the low Ca^{2+}/high Mg^{2+} Ringer's solution. Calculations were made by using both variance and failure methods (Castillo and Katz [16]; Martin [17]), and the values obtained by both methods were almost the same, as shown in Table 2.

All experiments were carried out at room temperature (22–26°C). Drugs were applied by superfusion or ionophoresis (see above). There was a time delay of about 15s before the first arrival of drug-containing solution at preparations in the chamber after changing the solution. Drugs used in the present study were acetylcholine chloride (ACh, Sigma), d-tubocurarine chloride (d-TC, Wako), TN-871 (see Fig. 1, gift of Taiyo Pharmaceutical Industry Co. Ltd.), ω-conotoxin GVIA (Peptide Institute, Osaka) and nicardipine hydrochloride (Sigma). Results were presented as the mean±S.E.M. with the number of cells in parentheses. Significant differences between means were determined by Student's *t*-test.

TN-871



· 2HCl

$C_{19}H_{26}N_2O_2 \cdot 2HCl$

Fig. 1 The structural formula and molecular formula of TN-871 (2-n-butyl-1-(-4-methylpiperazinyl)-5,6-methylenedioxyindene·2HCl).

Results

I. Actions of TN-871 on electrophysiological properties of ganglion cells

The present results are based on observations from sixty-eight B ganglion cells (Nishi et al. [18]). Their electrophysiological properties are shown in Table 1; they are essentially the same as those that appeared in recent publications (Hirai and Katayama [14]; Rafuse et al. [19]).

TN-871 (3nM~3μM) did not significantly change passive and active electrophysiological properties of the ganglion cells. The amplitude and the shape of electrotonic potentials induced by hyperpolarizing current pulses were not signi-

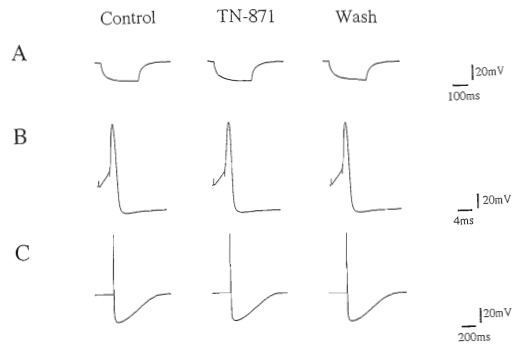


Fig. 2 Effect of TN-871 (3 μM) on sympathetic ganglion cells in the Ringer's solution. A, electrotonic potentials were generated by constant hyperpolarizing current pulses (duration, 250ms; intensity, 0.2nA). B, action potentials were elicited by depolarizing pulses (duration, 3ms), shown at fast sweep speed. C, an action potential was followed by a slow afterhyperpolarization, shown at a slow sweep speed. Control (left), during the application of TN-871 (3 μM) (middle), and 5 min after discontinuing exposure to the drug (right).

Table 1. Effect of TN-871 on passive and active electrophysiological membrane properties of bullfrog sympathetic ganglion cells.

	Control	TN-871 (30 nM)	TN-871 (3 μM)
Resting Membrane Potential (mV)	−64.6±3.7 (n=26)	−64.4±3.7 (n=10)	−64.9±3.7 (n=10)
Input Resistance (MΩ)	110±40 (n=6)	118±40 (n=5)	120±37 (n=5)
Spike Peak Amplitude (mV)	97.4±9.2 (n=10)	100±9.2 (n=6)	98.5±10 (n=8)
Spike Duration* (ms)	2.8±0.1 (n=10)	2.8±0.1 (n=6)	2.8±0.1 (n=8)
Amplitude of After-hyperpolarization (mV)	30.6±3.6 (n=10)	31.7±3.4 (n=6)	30.4±3.7 (n=8)

All data are given as the mean±S.E.M. with the number of cells in parentheses. *Action potential (spike) duration was measured at 1/2 of peak amplitude. The values were not statistically different in either drug-treatment group compared with controls.

ficantly changed in the presence of the drug (Fig. 2A), indicating that the input membrane resistance was not altered. Neither action potentials caused by outward current pulses (Fig. 2B) nor the afterhyperpolarizations following the action potentials (Fig. 2C) were affected by the drug at these concentrations. The resting membrane potential, the amplitude and duration (measured at half-maximum amplitude) of the action potential and the amplitude of the after-hyperpolarization, in the control and in the presence of the drug, are summarized in Table 1.

II. Effects of TN-871 on nicotinic ganglionic transmission

It is known that fast EPSPs in the bullfrog sympathetic ganglion are mediated by ACh and are nicotinic in nature (Nishi and Koketsu [10]). The effects of TN-871 both on fast EPSPs partially blocked by d-TC and on ACh potentials (see Methods) were examined in normal Ca^{2+} Ringer's solution (Fig. 3). TN-871 at 30nM reversibly augmented fast EPSPs (Fig. 3A-a), whereas the drug at 3 μM reversibly depressed them (Fig. 3A-b). On the other hand, the ACh potentials were not significantly affected by TN-871 at the concentrations tested (3nM to 3 μM, see Fig. 3B for 3 μM); indeed, the mean amplitude of ACh potentials (not less than 10 observa-

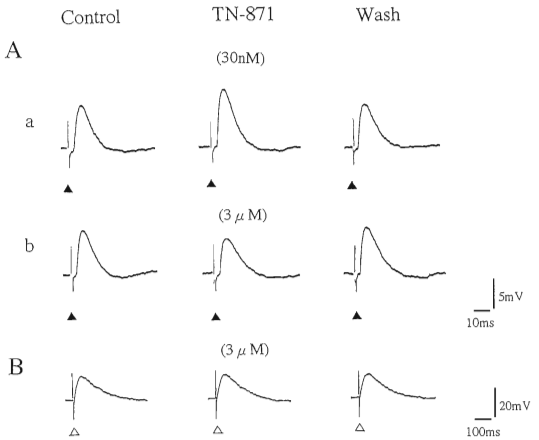


Fig. 3 Effects of low and high concentrations of TN-871 on fast EPSPs and ACh potentials in normal Ringer's solution. A, the fast EPSPs were evoked by supramaximal preganglionic stimulation indicated by filled triangles. The fast EPSPs were partially blocked with a low concentration of d-TC (4 μM). B, ACh potentials were induced by ionophoresis of ACh (duration, 5ms; intensity, 100nA; indicated by open triangles). Control (left), during the application of TN-871 (middle), and 5 min after cessation of exposure to TN-871 (right).

Table 2. Values of the mean quantal content calculated by the variance method and by the failure method in the presence of TN-871 (15 min after drug application).

		Concentration			
		3nM (n=5)	30nM (n=5)	300nM (n=7)	3 μM (n=5)
Variance	Control	1.7±0.1	1.7±0.2	1.7±0.1	1.7±0.2
	TN-871	1.9±0.1	**2.3±0.1	1.8±0.3	**1.1±0.2
Failure	Control	1.5±0.3	1.6±0.2	1.5±0.2	1.6±0.3
	TN-871	1.7±0.2	**2.1±0.2	1.7±0.3	**1.0±0.2

All data are given as the mean±S.E.M. with the number of cells in parentheses. **P<0.01, compared with the mean control value before drug application.

tions from each cell) was 11.3±2.8 mV in control (n=4 cells), 11.5±2.6 mV at 30nM (n=4 cells) and 11.5±2.5 mV at 3 μM (n=4 cells) at the resting membrane potential. The results indicate that TN-871 may facilitate or inhibit the nicotinic ganglionic transmission without affecting the post-synaptic sensitivity of ganglion cells to ACh.

III. Quantal analysis of fast EPSPs

The amount of ACh released from preganglionic nerve terminals was subjected to quantal analysis (Table 2). Fast EPSPs were recorded in the 0.54 mM Ca^{2+}/7.56 mM Mg^{2+} Ringer's solution in which the probability of transmitter release is lowered. The control values of the mean quantal content of the fast EPSPs calculated by using both variance and failure methods were 1.7±0.1 (n=17 cells) and 1.6±0.1 (n=17 cells), respectively.

TN-871 at 30nM increased the amplitude of the fast EPSPs and decreased the number of failures of synaptic transmission (Fig. 4A). During drug application, the mean amplitude and the mean quantal content of the fast EPSPs were significantly (P<0.01) increased, reversibly (Fig. 4B). As shown in Fig. 4B, the drug did not change the mean quantal size of the fast EPSPs (ratio of the mean amplitude to the mean quantal content of the fast EPSPs). This means that the sensitivity of ganglion cells to ACh was unchanged. The mean amplitude and the mean quantal content slowly returned to control values about 15 min after exposure to the drugs was discontinued. These results indicate that TN-871 at low concentration (30 nM) increased the amount of ACh released from presynaptic nerve terminals.

TN-871 at 3 μM depressed fast EPSPs; their amplitude decreased, and their frequency of failure increased (Fig. 5A). The mean amplitude and the mean quantal content of the fast EPSPs were both significantly (P<0.01) reduced in the presence of the drug without changing the mean quantal size (Fig. 5B). The mean amplitude and the mean quantal content slowly recovered to the control levels after 10 to 15 min after cessation of exposure to TN-871. Thus, it is concluded that TN-871 at high concentration (3 μM) inhibited the release of ACh without affecting the ACh-sensitivity of ganglion cells.

Results obtained at an intermediate concentration of TN-871 seemed complex. At 300nM, the drug either facilitated or inhibited fast EPSPs (Fig. 6A); e.g., the failure of the fast EPSPs became more frequent in three cells (Fig. 6A-a) but became less frequent in four cells (Fig. 6A-b) in the

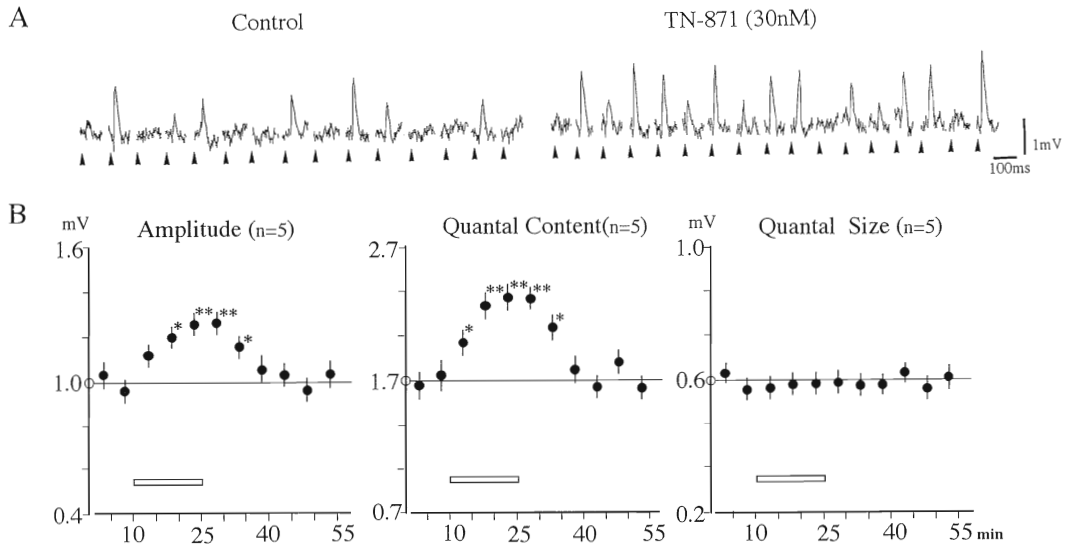


Fig. 4 Effects of TN-871 (30nM) on fast EPSPs recorded in 0.54 mM Ca^{2+}/7.56 mM Mg^{2+} Ringer's solution. A, the fast EPSPs were evoked by preganglionic stimulation applied at 0.2 Hz (arrow heads). Left: control recordings, and right: recordings in the presence of the drug. B, TN-871 (30nM) was applied by superfusion during the period indicated by the horizontal bar in each graph. The mean amplitude, quantal content and quantal size were calculated for sixty consecutive fast EPSPs for every 5 min (n=5 cells) using the variance method; these values were plotted (ordinate) against time (abscissa); the mean control values (open circles) were obtained by averaging two values before drug application. Each point indicates the mean value and vertical lines represent S.E.M. *: $P<0.05$, **: $P<0.01$, compared with the mean control value before drug application (open circle).

presence of the drug. When the effects of TN-871 at 300nM were statistically evaluated from all results of 7 cells, it was found that the mean values for the amplitude, quantal content and quantal size were not significantly ($P>0.1$) altered by the drug (Fig. 6B). However, it should be noted that the values of the S.E.M. for the amplitude and the quantal content were large during drug application (Fig. 6B) (compare Fig. 6 to Figs. 4 and 5). These results may reflect the opposing effects of TN-871 (300nM) on fast EPSPs.

IV. Relationship between actions of calcium blockers and TN-871 on the fast EPSPs

Fast EPSPs in a 0.99 mM Ca^{2+}/4.86 mM Mg^{2+} Ringer's solution were not affected by nicardipine at 10 μM (n=4), as shown in Fig. 7A. On the contrary, they were inhibited by ω-conotoxin in a concentration-dependent manner (Fig. 7B); the fast EPSPs were reduced in amplitude to 62.5±9.2% (n=4) of the control values at 200nM and to 27.0±6.3% (n=4) of controls at 300nM, being abolished at 1 μM (n=3). These inhibitory actions of ω-conotoxin were essentially irreversible.

After the fast EPSPs were partially inhibited with 200nM ω-conotoxin, they were further depressed when TN-871 (3μM) was added (Fig. 7C). Upon withdrawal of TN-871, the fast EPSPs started to recover in amplitude (see Fig. 7C far right); afterwards, they were totally abolished by treatment with a high concentration of ω-conotoxin (1μM) (not shown). These results suggest the possibility that a high concentration of TN-871 (3 μM) may inactivate ω-conotoxin-sensitive calcium

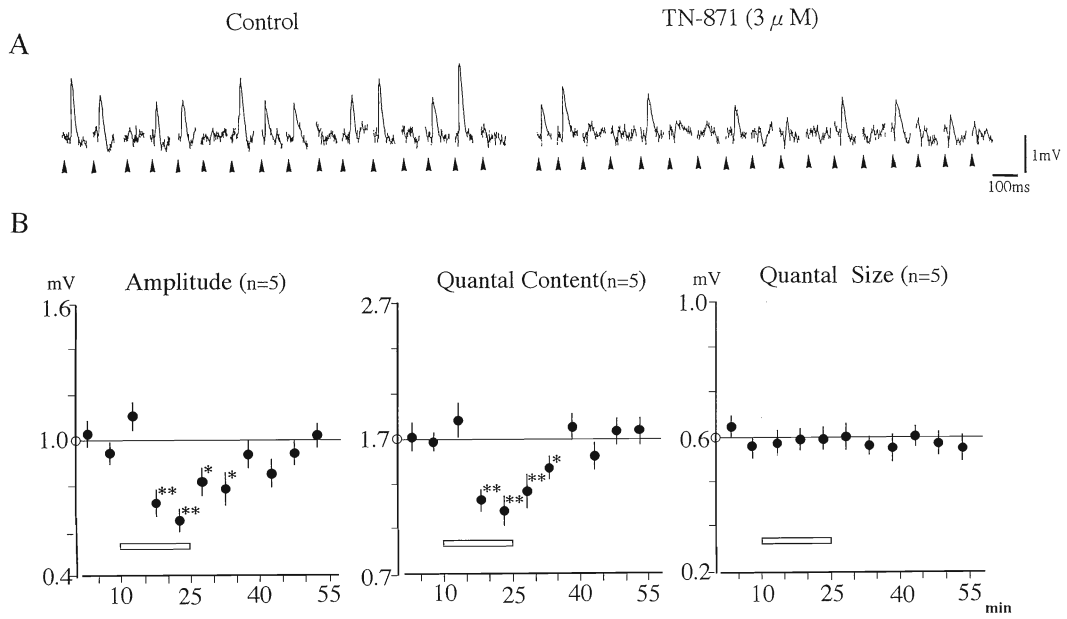


Fig. 5 Effects of TN-871 (3 μM) on fast EPSPs recorded in 0.54 mM Ca^{2+}/7.56 mM Mg^{2+} Ringer's solution. A, the fast EPSPs were evoked at 0.2 Hz (arrow heads). Left: control recordings, and right: recordings in the presence of the drug. B, TN-871 at 3 μM was applied by superfusion during the period indicated by the horizontal bar in each graph. The mean amplitude, quantal content and quantal size were calculated for sixty consecutive fast EPSPs for every 5 min (n=5 cells) using the variance method; these values were plotted (ordinate) against time (abscissa); the mean control values (open circles) were obtained by averaging two values before drug application. Each point indicates the mean value and vertical lines represent S.E.M. *: $P<0.05$, **: $P<0.01$, compared with the mean control value before drug application (open circle).

channels, which can be expected to be completely blocked by a high concentration of ω-conotoxin (1 μM). Lower concentrations of TN-871 (3 and 30 nM) failed to affect the fast EPSPs that were already inhibited by pretreatment with 200nM ω-conotoxin.

Discussion

The present results indicate that the new indene-derivative TN-871 may modulate cholinergic synaptic transmission in the bullfrog sympathetic ganglion; TN-871 at a low concentration augmented the fast EPSPs but at high concentrations inhibited them. In fact, the mean amplitude and the mean quantal content of the fast EPSPs were significantly changed at 30nM and 3 μM, whereas the mean quantal size was unchanged. The mean quantal size indicates the amplitude of a response to a quantum of transmitter, assuming a constant amount of ACh in each quantum. No change in the mean quantal size means that the postsynaptic sensitivity to the neurotransmitter ACh was not affected by TN-871. This conclusion can be supported by the experimental fact that the amplitude of the ACh potentials was not altered by TN-871. All of these results may suggest that TN-871 either increases or decreases the release of ACh from presynaptic terminals in the bullfrog sympathetic ganglion without affecting postsynaptic sensitivity to ACh. However, mechanisms underlying the concentration-dependent

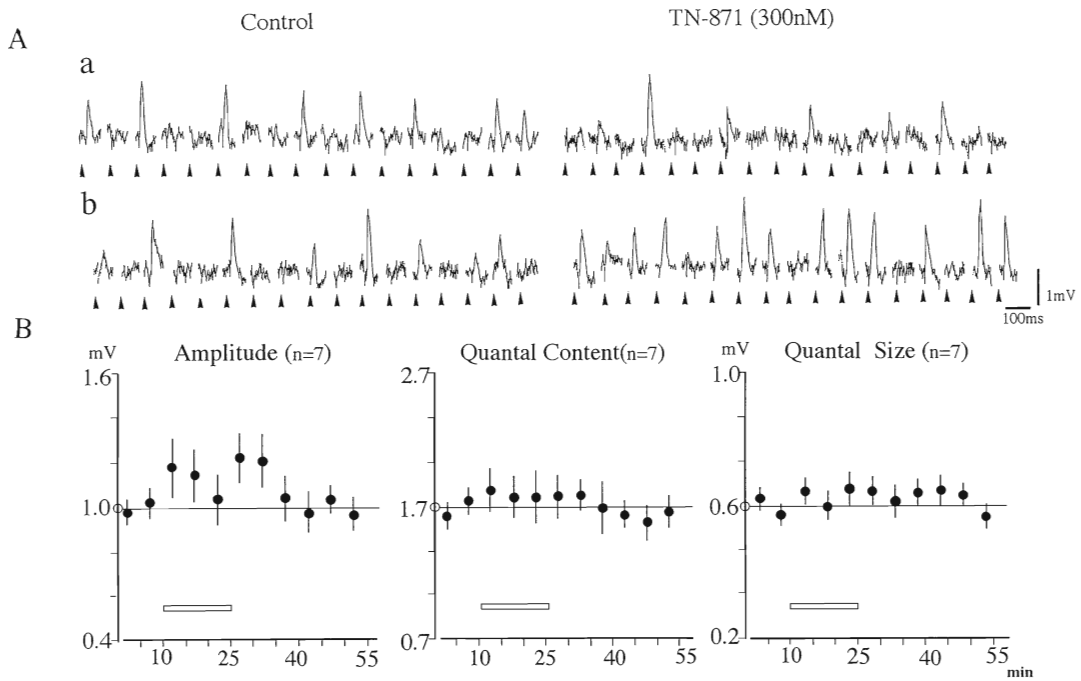


Fig. 6 Effects of TN-871 (300 nM) on fast EPSPs recorded in 0.54 mM Ca^{2+}/7.56 mM Mg^{2+} Ringer's solution. A, the fast EPSPs were evoked at 0.2 Hz (arrow heads). Left: control recordings, and right: recordings in the presence of the drug. The fast EPSPs were either inhibited (A-a) or facilitated (A-b) by TN-871. B, TN-871 at 300 nM was applied by superfusion during the period indicated by the horizontal bar in each graph. The mean amplitude, quantal content, and quantal size were calculated for sixty consecutive fast EPSPs for every 5 min (n=7 cells) using the variance method. These values were plotted (ordinate) against time (abscissa); the mean control values (open circles) were obtained by averaging two values before drug application. Each point indicates the mean value and vertical lines represent S.E.M. There are no statistically significant points.

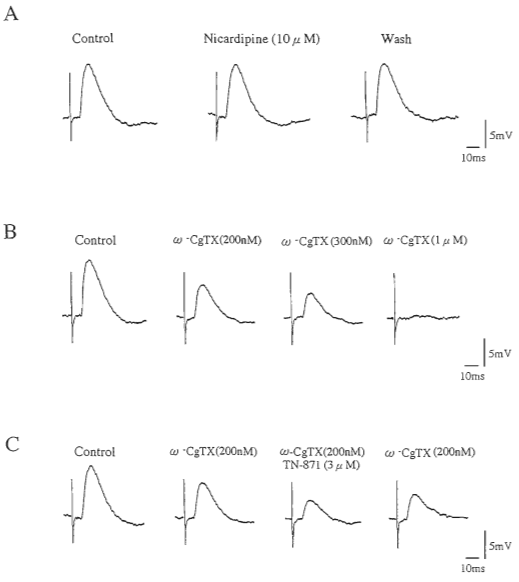


Fig. 7 Effect of calcium channel blockers on the fast EPSPs in 0.99 mM Ca^{2+}/4.86 mM Mg^{2+} Ringer's solution. A, effect of nicardipine on fast EPSPs. Control (left), 5 min after addition of nicardipine 10 μM (middle), and 5 min after cessation of exposure to nicardipine (right). B, effect of three different concentrations of ω-conotoxin (ω-CgTX) applied consecutively (200nM, 300nM and 1 μM). C, effect of ω-conotoxin and TN-871 on fast EPSPs. Control (left), ω-conotoxin 200nM alone (middle left), ω-conotoxin 200nM plus TN-871 3 μM (middle right) and ω-conotoxin 200nM alone, again, after withdrawal of TN-871 (right). Records in A, B, and C were obtained from three different cells.

opposite actions of the drug remain to be clarified (see below). It should be added that similar concentration-dependent dual effects were observed with methionine enkephalin in the same preparation (Hirai and Katayama [14]).

It is widely believed that release of neurotransmitter is associated with a transient elevation of internal calcium concentration in the presynaptic nerve terminals (Zucker and Lando [20]; Zuker and Haydon [21]). Therefore, it is speculated that TN-871 may modulate transmitter release by affecting intracellular calcium concentration at release sites. Since voltage-dependent calcium entry during presynaptic action potentials is known to be an important step for transmitter release (Stanley and Atrakchi [22]), TN-871 is supposed to regulate calcium channels in presynaptic nerve terminals as follows.

Synaptic transmission in a variety of preparations is reported to be inhibited by N-type calcium channel blockers such as ω-conotoxin (Kerr and Yoshikami [23]; Sano et al. [24]; Obaid et al. [25]; Horne and Kemp [26]; Takahashi and Momiyama [27]). It is also shown that the postsynaptic sensitivity to ACh is unaltered by ω-conotoxin in frog nerve-muscle preparations (Sano et al. [24]). The present experiments demonstrated that fast EPSPs of the bullfrog sympathetic ganglion were depressed by ω-conotoxin but not by an L-type calcium channel blocker, nicardipine (see Stanley and Atrakchi [22]; Obaid et al. [25]), suggesting the involvement of ω-conotoxin-sensitive N-type calcium channels in the transmitter release in the bullfrog sympathetic ganglion. TN-871 inhibited the fast EPSPs possibly by blocking N-type calcium channels.

It is reported that TN-871 at 1 μM reversibly depressed the slow afterhyperpolarization following the action potential of myenteric AH neurons (Katayama and Morita [5]). The slow afterhyperpolarization of the myenteric AH neurons is considered to be generated by an increase in potassium conductance which is activated by calcium ions entered through voltage-dependent calcium channels (Morita et al. [28]). Then, there is a possibility that TN-871 at high concentration might block the voltage-dependent calcium entry. However, the sensitivity of the calcium channel of the myenteric AH neurons to ω-conotoxin is not known. In the present study, the afterhyperpolarization of bullfrog sympathetic ganglion cells was not affected by the drug (see Fig. 2C). A possible mechanism underlying the afterhyperpolarization in the bullfrog sympathetic cells might not be the same as that of the slow afterhyperpolarization in myenteric AH neurons. That is, calcium ions released from internal store sites are thought to play a major role in causing the afterhyperpolarization of the bullfrog sympathetic ganglion cells (Hua et al. [29]).

In the presence of ω-conotoxin (200 nM), fast EPSPs were not affected by TN-871 at the same low concentration as that at which TN-871 alone augmented fast EPSPs. There are three possible interpretations. First, ω-conotoxin might interfere with TN-871 in blocking the voltage-sensitive calcium channels. This seems unlikely, because the facilitatory action at a low concentration cannot be induced by blocking calcium channels. Second, TN-871 could not reverse the ω-conotoxin-induced blockade of calcium channels. However, it is conceivable that the drug at low concentration could activate several types of calcium channels including N-type channels in the absence of ω-conotoxin. Finally, there may be different sites of action for TN-871 at the low concentrations. The second and the last possibilities prompted us to discuss mechanisms of the augmenting action by the low concentra-

tion of TN-871. For this, it might be helpful to observe cytosolic calcium concentration by using a fluorescent calcium indicator, fura-2. It is technically difficult, however, to measure the cytosolic calcium concentration in the nerve terminals in the bullfrog sympathetic ganglion. Our preliminary fura-2 study using enteric neurons of guinea-pigs (Katayama et al. [4]) demonstrated that the resting intrasomatic calcium concentration was elevated by TN-871 at a low concentration, probably mediated by increasing resting calcium influx (Tatsumi et al. [30]). In this context, it is also supposed that TN-871 might change internal calcium concentration by affecting the rate of cytosolic calcium sequestration and by controlling the amount of intracellular calcium binding proteins.

It is concluded that TN-871 modulates nicotinic synaptic transmission in the bullfrog sympathetic ganglion by controlling transmitter release from preganglionic nerve terminals without affecting postsynaptic sensitivity to ACh.

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