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Effects of ω-toxins on noradrenergic neurotransmission in beating guinea pig atria

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Abstract

The effects of four ω-toxins, known to block various subtypes of neuronal voltage-activated Ca^{2+} channels, on the beating guinea pig left atrium have been analyzed. Atria were suspended in oxygenated Krebs-bicarbonate solution at 32°C and driven with electrical pulses delivered by a stimulator at 1 Hz, 1 ms, 4 V. A 10-fold increase of voltage caused a potent and rapid enhancement of the size of contractions (about 3- to 4-fold above basal), which reflects the release of endogenous noradrenaline from sympathetic nerve terminals. ω-Conotoxin MVIIC, ω-conotoxin MVIIA and ω-conotoxin GVIA inhibited the inotropic responses to $10 \times V$ stimulation with IC_{50} values of 191, 44 and 20.4 nM, respectively. ω-Agatoxin IVA did not affect the contractile responses. The inotropic responses to exogenous noradrenaline were unaffected by the toxins. The potent blocking effects of ω-conotoxin GVIA were present even in conditions in which the release of noradrenaline was strongly facilitated by presynaptic α_2-adrenoceptor blockade by phenoxybenzamine. These effects were not reversed upon repeated washing of the tissue with toxin-free medium. In contrast, the blockade induced by ω-conotoxin MVIIC and ω-conotoxin MVIIA were fully reversed, with $t_{1/2}$ of 13.5 and 31.2 min, respectively. ω-Conotoxin MVIIC (1 μM) protected against the irreversibility of the blockade induced by ω-conotoxin GVIA (100 nM). The results are compatible with the following conclusions: (i) at cardiac sympathetic neuromuscular junctions, the release of noradrenaline and the noradrenergic transmission is maintained and regulated by N-type Ca^{2+} channels; (ii) the modulation by presynaptic α_2-adrenoceptors of noradrenaline release is exerted via N-type Ca^{2+} channels; (iii) ω-conotoxin MVIIC and ω-conotoxin MVIIA reversibly block N channels and the noradrenergic neurotransmission; (iv) ω-conotoxin GVIA and ω-conotoxin MVIIC seem to recognize the same binding site on the N-type Ca^{2+} channels; however, their binding kinetics might considerably differ; (v) P-type Ca^{2+} channels are unlikely involved in the regulation of transmitter release at cardiac sympathetic nerve terminals.

Keywords: ω-Conotoxin GVIA; ω-Conotoxin MVIIC; ω-Conotoxin MVIIA; ω-Agatoxin IVA; Sympathetic neurotransmission; Ca^{2+} channel; Heart

1. Introduction

Two classes of polypeptide ligands, collectively referred to as ω-toxins, have been instrumental in defining various Ca^{2+} channel subtypes in neurones (Olivera et al., 1994). Thus, ω-conotoxin GVIA is considered as a selective, irreversible blocker of N-type Ca^{2+} channels (McCleskey et al., 1987). On the other hand, low concentrations of ω-agatoxin IVA irreversibly inhibit P-type Ca^{2+} channels (Mintz et al., 1992b). A new toxin from the marine snail *Conus magus*, ω-conotoxin MVIIC has been recently characterized (Hillyard et al., 1992). Though P- as well as N-type Ca^{2+} channels seem to recognize such toxin, a novel additional component of the whole-cell Ca^{2+} current is also blocked by ω-conotoxin MVIIC. This led to the belief that this toxin can identify a new subtype of neuronal Ca^{2+} channel named Q (Wheeler et al., 1994; López et al., 1994) or O (Adams et al., 1993).

It seems clear that different Ca^{2+} channel subtypes contribute differently to the influx of Ca^{2+} needed for the synaptic release of various neurotransmitters. Due

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to the small size of mammalian presynaptic nerve terminals, it is not possible to identify the channel subtypes contributing to transmitter release following conventional electrophysiological patch-clamp techniques. Therefore, the pharmacological sensitivity of the release process is used as a major criterion to determine the Ca^{2+} channel subtype involved in the regulation of transmitter release at a given synapse (Sher and Clementi, 1991).

N- as well as L-type Ca^{2+} channels have been identified in the soma of sympathetic neurones; however, P-type channels were not seen (Mintz et al., 1992a). In cultured rat superior cervical ganglion cells, release of noradrenaline is completeley blocked by ω-conotoxin GVIA, but not by dihydropyridines (Hirning et al., 1988). These experiments suggested that the control of secretion in sympathetic neurones was dominated by N-type Ca^{2+} channels. However, the question remained to whether this conclusion could be extrapolated to the situation of sympathetic nerve terminals innervating effector cells in intact organs. Thus, in rat vas deferens, electrically evoked muscle responses were inhibited by ω-conotoxin GVIA when stimulated at low frequencies, but not at higher frequencies (Keith et al., 1990). Similar findings have been obtained in rat and guinea pig vas deferens and bladder (Maggi et al., 1988; Maggi, 1991) and in the rabbit urethra and detrusor muscles (Zygmunt et al., 1993). Also, ω-conotoxin GVIA has been shown to block noradrenaline release induced by nicotine in the perfused guinea pig heart (Haass et al., 1991). On the other hand, ω-agatoxin IVA did not affect the noradrenergic responses induced by nerve stimulation of rabbit pulmonary artery, rat vas deferens or anococcygeus muscles (Lundy and Frew, 1994).

The present study was planned taking into account three facts: (1) the recent availability of a novel toxin, ω-conotoxin MVIIC which seems to recognize a non-N-non-P Ca^{2+} channel; (2) the lack of studies concerning the definition of the subtype of Ca^{2+} channel controlling noradrenergic neurotransmission in intact beating cardiac tissues; (3) the degree of selectivity of ω-toxins for different Ca^{2+} channel subtypes. Concerning the first, we expected to define resemblances and differences between the characteristics of the effects of ω-conotoxin MVIIC, ω-conotoxin MVIIA, ω-conotoxin GVIA and ω-agatoxin IVA on cardiac noradrenergic neurotransmission. Concerning the second, we expected to clearly establish whether the electrophysiological and neurochemical data obtained in the soma of sympathetic ganglion cells are valid for the intact nerve terminals innervating cardiac cells. Concerning the third, we wished to know the selectivity and reversibility of these toxins for blocking the cardiac sympathetic drive, and to find out whether they have common sites of action on nerve terminals. This is particularly relevant because ω-conotoxin GVIA has been shown to block N channels irreversibly while ω-conotoxin MVIIA has been suggested to block those channels reversibly (Yoshikami et al., 1989). To achieve these three goals, we have exploited the electrically driven guinea pig left atrium as a model. Electrical stimulation of sympathetic nerve terminals innervating its contractile cells produces a pronounced inotropic effect which adequately reflects the amount of noradrenaline being released from such nerve terminals.

2. Materials and methods

Male guinea pigs weighing 300–400 g were killed by a blow on the head. The heart was quickly removed and placed in oxygenated Krebs-bicarbonate solution. The left atrium was carefully dissected and placed between bipolar platinum electrodes. The base of the atrium was tied to one of the electrodes and the tip to an isometric transducer connected to an amplifier and recorder (Cibertec, Madrid, Spain). The preparation was placed in a 10-ml glass bath at 32°C, in Krebs-bicarbonate solution of the following composition (in mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.2, KPO_4H_2 1.2, $CaCl_2$ 1.8, $NaHCO_3$ 25, glucose 11, pH 7.4, at 32°C.

An initial 30-min period of electrical drive (4 V, 1 ms, 1 Hz) allowed the stabilization of the basal contraction. Thereafter, at 15-min intervals, the voltage was increased 10-fold (from 4 to 40 V) for 2 min, to allow the activation of sympathetic nerve terminals, the release of endogenous noradrenaline and the increase of the contraction. This increase in contraction is a quantitative index of the amount of noradrenaline being released, and will be termed $10 \times V$ stimulation (see Results). For comparative purposes, contractions were also elicited by addition to the bath of exogenous noradrenaline (3 μM). Initially, two to three $10 \times V$ stimuli were applied to each preparation to test the reproducibility of the inotropic response. Usually, the third stimulus was taken as control. After these stimuli, toxins were added to test their effects on $10 \times V$ stimulation and then they were washed out and the stimulation repeated to test the recovery.

Results are presented as means ± S.E.M. of the number (n) of individual animals. Statistical differences between means were calculated by using the Student t-test. P values smaller than 0.05 were taken as the level of significance. The IC_{50} to block heart contraction was calculated through the logarithmic transformation of the concentration-response curve. The increase of contraction in millinewtons (mN) above the basal resting contraction was normalized to 100% and expressed as $\log [Y/100 - Y]$ (ordinate) versus the log concentration of each toxin used. Straight lines were determined by least-squares fitting, and the inter-

cept with the abscissa ($Y = 0$) gave the IC_{50} to block contractions.

3. Results

3.1. The inotropic effects of voltage increase are due to the release of noradrenaline from sympathetic nerves

The threshold voltage for the contraction of left atrium by direct field electrical stimulation of myocardial cells was around 3–4 V. Under these conditions, the atrium was beating uniformly for hours when stimulated at 1 Hz with pulses of 1 ms duration. Atria which developed arrhythmias were discarded. The initial basal force contractions amounted to 3.8 ± 0.3 mN ($n = 35$). The size of these contractions decreased along the experiment, when repeated $10 \times V$ stimulations were applied to the tissues.

Upon 10-fold increase in voltage during 2 min (from 4 V producing basal contractions, to 40 V), a rapid increase of contraction was produced. The return to low voltage caused the gradual decline of contractions which reached baseline in about 2–4 min (Fig. 1A). The increase in contraction induced by high voltage varied from preparation to preparation (compare the two initial contractile responses in panels A, B and C of Fig. 1). On average, the increase of contraction was 9.4 ± 1.9 mN ($n = 6$), about 3- to 4-fold above the basal contraction.

The experiments in Fig. 1 were performed to demonstrate that $10 \times V$ stimulation augmented the myocardial contractions by releasing endogenous noradrenaline. If so, propranolol (a β-adrenoceptor antagonist) and tetrodotoxin (a Na^+ channel blocker) should block the response, and phenoxybenzamine (an α-adrenoceptor antagonist) should enhance it.

In Fig. 1A, inotropic responses were elicited by stimulating with $10 \times V$ for 2 min (to stimulate β-adrenoceptors by endogenously released noradrenaline) and with exogenous noradrenaline (3 μM for 2 min), to directly stimulate cardiac β-adrenoceptors. The responses to both stimuli were approximately similar (first two traces). In the presence of propranolol (3 μM) both inotropic responses were blocked by over 90%. Such blockade was partially reversed upon washing out propranolol (not shown).

If $10 \times V$ stimulation causes Na^+-dependent action potentials to induce Ca^{2+} entry through voltage-dependent Ca^{2+} channels, and noradrenaline release from sympathetic nerve terminals, tetrodotoxin should block the inotropic effects of $10 \times V$, but not of exogenous noradrenaline. This indeed occurred, as shown in Fig. 1B. The first two traces show control responses to $10 \times V$ and to noradrenaline and the second two traces in the presence of 1 μM tetrodotoxin. Tetrodotoxin

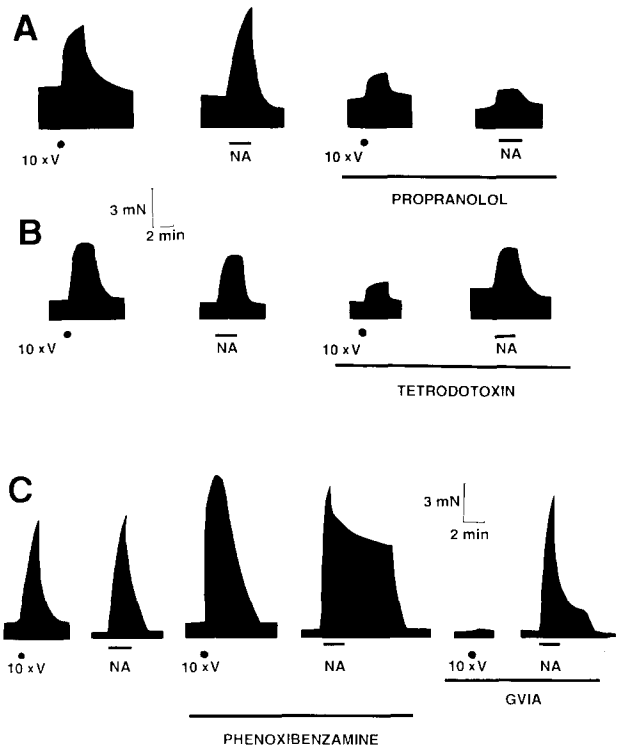

Fig. 1. Traces showing the beating pattern of guinea-pig left atrium in response to electrical field stimulation (1 Hz, 4 V, 1 ms). The basal contractions were quickly increased upon 10-fold enhancement of the voltage for 2 min (dots, $10 \times V$). Electrical stimulation ($10 \times V$ for 2 min) was alternated with 2-min application of exogenous noradrenaline (NA, 3 μM, horizontal bar below the trace). In A, the stimuli were repeated in the presence of 3 μM propranolol (A), 1 μM tetrodotoxin (B), 1 μM phenoxybenzamine or 100 nM ω-conotoxin GVIA (C, GVIA), as shown on horizontal bars. Calibration bars are in millinewtons (mN) and minutes. Traces are copies from the original records.

inhibited over 80% the inotropic response to $10 \times V$, but did not affect the response to exogenous noradrenaline.

The experiment using phenoxybenzamine is shown in Fig. 1C. As a blocker of presynaptic α_2-adrenoceptors, phenoxybenzamine increases the release of noradrenaline at low-frequency stimulation of sympathetic nerves; this potentiation effect is long-lasting and can be considered to be irreversible (Kirpekar and Puig, 1971). Therefore, it was expected that the compound might increase the inotropic response to $10 \times V$. Fig. 1C shows that this was the case. In addition, phenoxybenzamine also increased the response to noradrenaline, likely due to its ability to block the neuronal uptake carrier for this amine (Kirpekar and Puig, 1971). Addition of 100 nM ω-conotoxin GVIA fully blocked the contraction evoked by $10 \times V$ stimulation but did not affect the responses to exogenous noradrenaline (two last traces of panel C in Fig. 1). This suggests that ω-conotoxin GVIA strongly blocked the facilitated re-

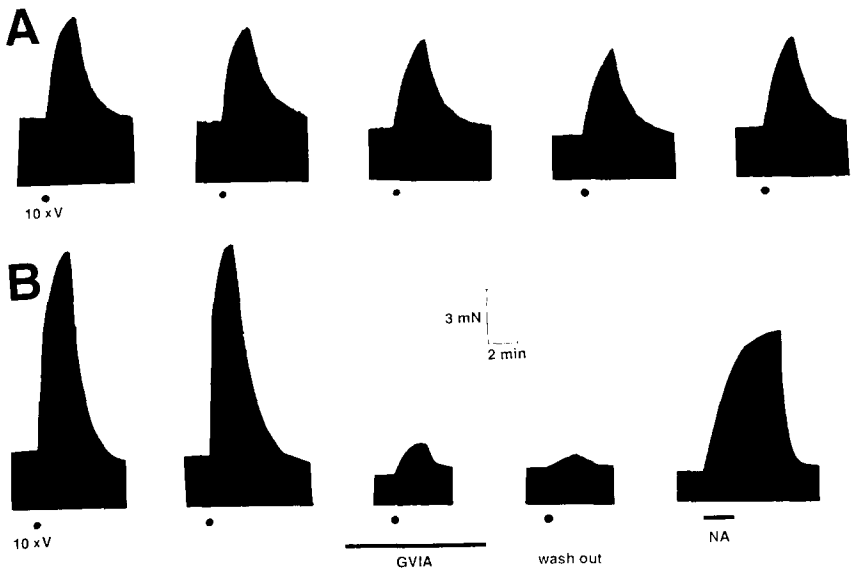

Fig. 2. In A, the reproducibility of the inotropic responses to electrical field stimulation is shown (1 Hz, 40 V, 1 ms for 2 min, dots). In B, the effect of ω-conotoxin GVIA (GVIA, 100 nM) and its irreversibility are illustrated (third and fourth traces respectively). The fith trace corresponds to the application of noradrenaline (NA), 3 μM for 2 min.

lease of noradrenaline in the presence of phenoxybenzamine.

3.2. The effects of ω-conotoxin GVIA, ω-agatoxin IVA, ω-conotoxin MVIIC and ω-conotoxin MVIIA on the inotropic responses to 10 × V stimulation

Fig. 2A shows that repeated stimulations with 10 × V of a beating atrium produced increases in contractions above basal levels which were very reproducible. Using this protocol, the intercalation of increasing concentrations of a toxin between two given pulses could provide information on its effects on the contractile response and, indirectly, on the release of noradrenaline from sympathetic nerve terminals.

Fig. 2B shows two similar initial control contractions evoked by two 10 × V stimuli applied 15 min apart. Ten minutes after addition of 100 nM ω-conotoxin

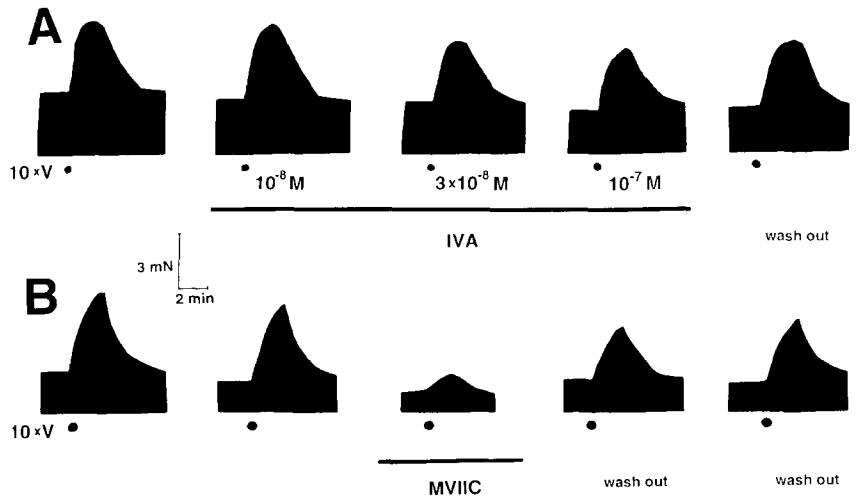

Fig. 3. Effects of ω-agatoxin IVA (IVA, A) and ω-conotoxin MVIIC (MVIIC, B) on the contractile responses to 10 × V stimulation (dots). In A, cumulative concentrations of ω-agatoxin IVA were present 10 min before and during each electrical stimulation of the atrium. The fith trace corresponds to a contraction obtained 15 min after washing out the highest concentration of ω-agatoxin IVA used. In B, after two initial control contractions, the blocking effect of ω-conotoxin MVIIC (1 μM) was tested, as well as its recovery upon washing out the tissue with toxin-free solution.

GVIA, the contractile response was inhibited over 90%. Forty minutes after washing out the toxin (four washouts of the tissue with fresh Krebs solution were applied) the contractile response remained depressed. The presynaptic location of the toxin target is strongly suggested on the basis of the fact that the contractile capability of the tissue was fully preserved. Thus, addition of exogenous noradrenaline (3 μM for 2 min) caused a strong inotropic effect (last trace in Fig. 2B).

ω-Agatoxin IVA did not affect the increased contraction induced by $10 \times V$. The experiment shown in Fig. 3A shows that increased additions of ω-agatoxin IVA to the bath (10–100 nM) did not affect the inotropic effects of $10 \times V$. After washing out the toxin, the contraction obtained was very similar to that seen before and in the presence of ω-agatoxin IVA. In one experiment, 300 nM ω-agatoxin IVA was used; the contraction induced by $10 \times V$ stimulation remained unmodified.

Fig. 3B shows the results obtained with ω-conotoxin MVIIC. After two initial similar control contractions, ω-conotoxin MVIIC (1 μM) was added and 10 min later, the response to $10 \times V$ tested again. A 90% blockade of such response was observed. In contrast to ω-conotoxin GVIA, the washout of ω-conotoxin MVIIC allowed the recovery of the contractile responses to almost the initial control level (last two traces in Fig. 3B).

Fig. 4 shows averaged results of experiments showing the concentration-response curves for various toxins on the inotropic response to $10 \times V$ stimulation. In the absence of toxins, the atria gave reproducible inotropic responses to repeated $10 \times V$ stimulation (open circles). ω-Agatoxin IVA did not affect the contractions at all. However, ω-conotoxin GVIA and ω-conotoxin MVIIC suppressed the inotropic responses in a concentration-dependent manner. The IC_{50} for ω-conotoxin GVIA was 20.4 nM and for ω-conotoxin MVIIC, 191 nM. It is worth noting that the concentration-response curve for ω-conotoxin GVIA was considerably steeper than for ω-conotoxin MVIIC. Thus at 10 nM, ω-conotoxin GVIA blocked by about 35% the inotropic response and at 30 nM the blockade reached 80%. At 30 nM, ω-conotoxin MVIIC inhibited the contraction by 45%, at 100 nM by 55% and at 300 nM by 80%. Thus, the concentration-response curve for MVIIC comprised more than 2-log units while that of ω-conotoxin GVIA developed in practically a 1-log unit. ω-Conotoxin MVIIA inhibited the atrial contractions to $10 \times V$ also in a concentration-dependent manner but apparently, the maximum blockade achieved at 100 and 300 nM stabilized at the 80% value. Thus, contractions were not fully suppressed by this toxin. Otherwise, its concentration-response curve was parallel to ω-conotoxin GVIA but shifted to the right ($IC_{50} = 44$ nM).

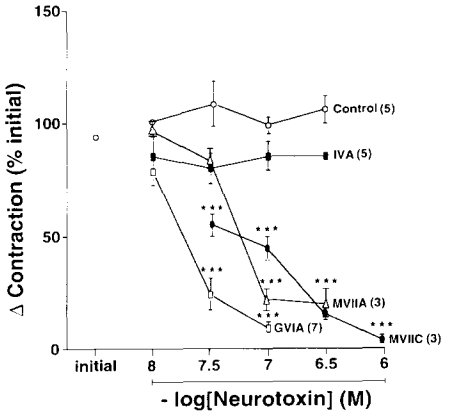

Fig. 4. Averaged data on the effects of various toxins on the contractions of guinea-pig left atria induced by electrical field stimulation. Cumulative concentrations of each toxin were used in every individual tissue, following the protocol shown in Fig. 3A. Data are means ± S.E.M. of the number of atria shown in parentheses. *** $P < 0.01$, compared to the initial contraction. IVA, ω-agatoxin IVA; MVIIA, ω-conotoxin MVIIA; GVIA, ω-conotoxin GVIIA; MVIIC, ω-conotoxin MVIIC.

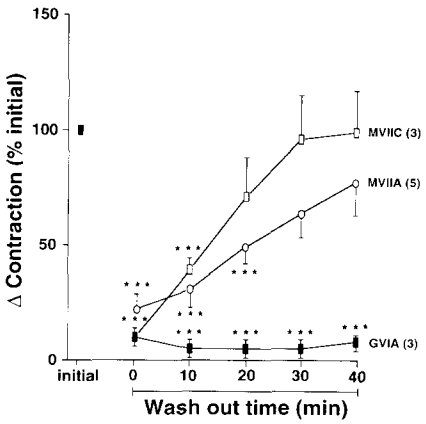

Fig. 5. Reversibility of the blocking effects of ω-conotoxin MVIIC (MVIIC) and ω-conotoxin GVIA (GVIA) on the increase in contraction induced by electrical field stimulation of guinea-pig left atria. Each atrium was tested for its ability to contract in response to $10 \times V$ stimulation (Δ contraction). Once stabilized, the initial contraction was normalized to 100% (ordinate). Then, the toxins were added (1 μM ω-conotoxin MVIIC or 100 nM ω-conotoxin GVIA) and their effects on contraction tested (0-min point on the abscissa). Then, the preparations were washed with toxin-free Krebs solution and their contractile responses tested at 10-min intervals (abscissa). Data are means ± S.E.M. of the number of atria shown in parentheses. *** $P < 0.01$, compared to the initial contraction.

3.3. Reversibility of the blocking effects of conotoxins and interactions between ω-conotoxin MVIIC and ω-conotoxin GVIA

Full blockade of contractile responses by ω-conotoxin GVIA as well as by ω-conotoxin MVIIC raised the question concerning whether these two toxins were

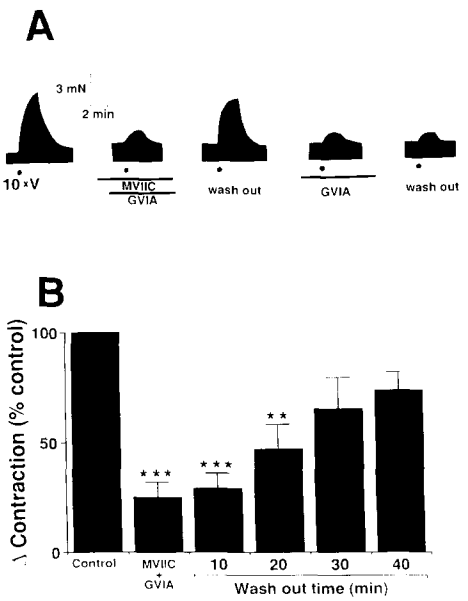

Fig. 6. Protection by ω-conotoxin MVIIC (MVIIC) against the irreversibility of the blocking effects of ω-conotoxin GVIA (GVIA) on atrial contractions induced by 10×V stimulation. In A, the protocol used is shown. After the initial test contraction, ω-conotoxin MVIIC (1 μM) was added and 10 min later, ω-conotoxin GVIA was added and maintained during an additional 10-min period before the 10×V stimulation was applied (dots). The third trace was obtained 30 min after washing out the two toxins. The fourth trace was obtained after giving GVIA alone for 10 min. The last trace was obtained 30 min after washing out the toxin. In B, average results from four atria are plotted, using the protocol shown in A. The preparations were stimulated at 15-min intervals. ** $P < 0.05$, *** $P < 0.01$, compared to control.

acting at the same site on the Ca^{2+} channel. This hypothesis could be tested if one toxin was reversibly blocking the contractions, and the other was irreversible. Fig. 5 shows the averaged results of various experiments designed to test this point. In a given atrium, a concentration of toxin was selected capable of blocking over 90% the inotropic response to 10 × V stimulation. Once blocked, the tissue was washed out at 10-min intervals with toxin-free Krebs solution. Fig. 5 shows that the blocking effects of ω-conotoxin GVIA were fully preserved after a 40-min washing period. On the contrary, the tissue treated with ω-conotoxin MVIIC started to recover its contractile ability 10 min after the toxin washout, and was fully reversed 30 min thereafter. Reversibility from the blockade induced by ω-conotoxin MVIIA was slower and smaller than with ω-conotoxin MVIIC.

In the light of these results, it was interesting to test whether ω-conotoxin MVIIC (which was reversibly blocking the heart inotropism) would protect against the irreversible blockade of ω-conotoxin GVIA. In the experiments shown in Fig. 6A, an atrium was treated with ω-conotoxin MVIIC first (1 μM) and 10 min later ω-conotoxin GVIA (100 nM) was added in the presence of ω-conotoxin MVIIC. The contractile response to 10 × V stimulation was thus blocked 90% (second trace). After a 30-min washout period with toxin-free Krebs solution, the contraction recovered almost fully (third trace); blockade was not fully reversible probably because the affinity of both toxins for a common binding site considerably differed (see Discussion). The subsequent treatment with ω-conotoxin GVIA alone (100 nM for 10 min) re-established the blockade. This time, however, a 30-min washout period did not allow any recovery from blockade. Fig. 6B shows averaged data from four different preparations with a similar protocol. In the presence of ω-conotoxin MVIIC and ω-conotoxin GVIA, contraction was inhibited 80%. Upon washing out the toxins, the contraction recovered in a time-dependent manner, being complete 30 min thereafter.

4. Discussion

Of the four ω-toxins tested, we have found that ω-conotoxin MVIIC, ω-conotoxin MVIIA and ω-conotoxin GVIA blocked the inotropic actions of 10 × V stimulation of guinea pig left atrium, in a concentration-dependent manner; ω-agatoxin IVA was devoid of blocking effects. None of the toxins affected the atrial basal contractions, nor the inotropic response to exogenously added noradrenaline. Considering that these two functional components of the heart beat are mediated by L-type Ca^{2+} channels located on the sarcolemma of cardiac cells (Fleckenstein et al., 1983), and that these toxins do not block L-channels (Olivera et al., 1994), the lack of direct effects of the toxins on the myocardium is not surprising. Rather, the suppression of the inotropic effects to 10 × V electrical field stimulation are correctly explained by the toxins' interference with the release of endogenous noradrenaline from sympathetic nerve terminals innervating cardiac myocytes. This is strengthened by the results obtained in control experiments in which propranolol and tetrodotoxin inhibited the inotropic effects, while phenoxybenzamine potentiated such response (see comments in the Results section). Of special concern was the drastic potentiation by phenoxybenzamine of the response to 10 × V which likely reflects its well-established ability to break-down the inhibitory α_2-adrenoceptor-mediated control of noradrenaline release at sympathetic nerve terminals (Kirpekar and Puig, 1971). The full blockade by 100 nM ω-conotoxin GVIA of such facilitated response strongly suggests that the modulation by α_2-adrenoceptors of transmitter release at sympathetic synapses is exerted via N-type Ca^{2+} channels.

If we accept the selectivity of ω-toxins for specific Ca^{2+} channel subtypes (see Introduction), it seems

clear that P-type Ca^{2+} channels are not involved in the regulation of noradrenergic neurotransmission in the guinea pig left atrium. ω-Agatoxin IVA irreversibly blocks P-type Ca^{2+} channels in Purkinje cell bodies with high affinity ($K_D = 2$ nM) (Mintz et al., 1992a). However, higher concentrations of ω-agatoxin IVA (up to 1 μM) reversibly block a component of the whole-cell current through Ca^{2+} channels in bovine chromaffin cells (Albillos et al., 1993). Also, ω-agatoxin IVA blocks with low potency α_{1A} channels expressed in oocytes, where they generate a Ca^{2+} channel phenotype quite different from P-type currents (Sather et al., 1993). The fact that ω-agatoxin IVA did not affect the inotropic effects of atrial $10 \times V$ stimulation at concentrations up to 300 nM suggests that neither P-type nor α_{1A} channels are responsible for regulating noradrenaline release in cardiac sympathetic nerve terminals. Conversely, the results strongly suggest that ω-agatoxin IVA does not recognize N channels regulating secretion from cardiac sympathetic nerves; there, its selectivity for non-N channels seems to be quite high.

The IC_{50} for ω-conotoxin GVIA to irreversibly block the inotropic responses was 20 nM. These two properties agree with electrophysiological findings that this toxin irreversibly blocks N-type Ca^{2+} channels in sympathetic neuronal cell bodies, with affinities ranging from picomolar to micromolar concentrations (Olivera et al., 1994). High sensitivity to ω-conotoxin GVIA is considered to be a definitive proof for the involvement of N-type Ca^{2+} channels in a given function. On the other hand, ω-conotoxin MVIIA is a high-affinity but reversible blocker of N-type Ca^{2+} channels (Yoshikami et al., 1989). Thus, irreversible blockade by ω-conotoxin GVIA and reversible blockade by ω-conotoxin MVIIA of the inotropic effects of $10 \times V$ stimulation strongly agree with the conclusion that N-type Ca^{2+} channels located on sympathetic nerve terminals are certainly involved in the regulation of noradrenergic transmission in the guinea pig left atrium.

Particularly interesting were the blocking effects of ω-conotoxin MVIIC. This is so because this *Conus magus* toxin seems to recognize an entirely new family of Ca^{2+} channels named 'OPQ' (Olivera et al., 1994). Peripherally, ω-conotoxin MVIIC seems to mimic ω-agatoxin IVA to interfere with mouse neuromuscular transmission (Bowersox et al., 1993). In contrast, ω-conotoxin GVIA has little or no effect on evoked acetylcholine release at the mammalian neuromuscular junction (Sano et al., 1987; Wessler et al., 1990; Uchitel et al., 1992). Thus, it seems that at the mammalian neuromuscular junction, a Ca^{2+} channel with similar affinity for ω-agatoxin IVA and ω-conotoxin MVIIC belongs to the 'OPQ' family and controls the release of acetylcholine. On the other hand, K^+-induced glutamate release in the hippocampus is potently blocked by ω-conotoxin MVIIC, whereas the N-type blocker ω-conotoxin MVIIA was three orders of magnitude less potent (Valentino et al., 1993). This implies a predominant role for non-N-type, non-L-type Ca^{2+} channels in controlling glutamate release.

At the cardiac sympathetic neuromuscular juction we have found only a 10-fold higher potency of ω-conotoxin GVIA ($IC_{50} = 20.4$ nM) and MVIIA ($IC_{50} = 44$ nM) than ω-conotoxin MVIIC ($IC_{50} = 191$ nM) to block neurotransmission. The binding of ω-conotoxin MVIIC to a class of sites in rat brain is in a range of affinities between 40 pM and 1 nM (Hillyard et al., 1992; Adams et al., 1993). Thus, these affinities are considerably higher than both the binding affinity of ω-conotoxin MVIIC for N-type channels (Adams et al., 1993) and its affinity to block noradrenergic neurotransmission in the guinea pig left atrium ($IC_{50} = 191$ nM; this study). It seems, therefore, that these effects are related more to the low-affinity reversible blocking effects of ω-conotoxin MVIIC on N-type Ca^{2+} channels, than to its effects on a channel of the 'OPQ' family.

In conclusion, noradrenergic neurotransmission at the sympathetic neuromuscular junction in the guinea pig left atrium seems to be fully controlled by N-type Ca^{2+} channels present in noradrenergic nerve terminals. Those channels are reversibly blocked by ω-conotoxin MVIIA and ω-conotoxin MVIIC, and irreversibly blocked by ω-conotoxin GVIA. The affinities of ω-conotoxin MVIIA and ω-conotoxin GVIA for N channels are 3- and 10-fold higher than the affinity of ω-conotoxin MVIIC. Though the binding site for ω-conotoxin GVIA and ω-conotoxin MVIIC on the N channel seems to be the same, judging by the shape of their dose-response curves the kinetics of binding might considerably differ for both conotoxins. ω-Agatoxin IVA, even at concentrations 100-fold higher than its K_D to block P-type Ca^{2+} channels did not affect cardiac noradrenergic neurotransmission.

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