British Journal of Pharmacology (1995) 116, 1577-1582

Calcium channel subtypes for the sympathetic and parasympathetic nerves of guinea-pig atria

S.J. Hong & [1]C.C. Chang

Department of Pharmacology, College of Medicine, National Taiwan University, No. 1, Sec. 1, Jen-Ai Road, Taipei, Taiwan

1 The Ca^{2+} channel subtypes of the autonomic nerves of guinea-pig atria were elucidated by monitoring the effects of specific Ca^{2+} channel blockers on the negative and positive inotropic responses associated respectively, with stimulation of the parasympathetic and sympathetic nerves.

2 In left atria paced at 2-4 Hz, the negative inotropic effect induced by field stimulation of parasympathetic nerves (in the presence of propranolol) was abolished by ω-conotoxin MVIIC, a blocker of N-type and OPQ subfamily Ca^{2+} channels. ω-Conotoxin GVIA (an N-type blocker), ω-agatoxin IVA (a P-type blocker), nifedipine (an L-type blocker) and Ni^{2+} (a T- and R-type blocker) were much less effective.

3 The positive inotropic response resulting from field stimulation of the sympathetic nerves (in the presence of atropine) was abolished by both ω-conotoxins, while ω-agatoxin IVA, nifedipine and Ni^{2+} were ineffective.

4 In the spontaneously beating right atria, the early negative inotropic effect produced by 1,1-dimethyl-4-phenylpiperazinium was abolished by ω-conotoxin MVIIC, whereas the late positive inotropic effect was partially reduced, but not abolished, by a high concentration of ω-conotoxin GVIA.

5 None of the peptide toxins affected the chronotropic and the inotropic responses evoked by carbachol and isoprenaline.

6 These results suggested that, under physiological conditions, the release of acetylcholine from paraysmpathetic nerves is dominated by an OPQ subfamily Ca^{2+} channel while that of noradrenaline from sympathetic nerves is controlled by an N-type Ca^{2+} channel. Ligand-induced noradrenaline release appeared to recruit additional type(s) of Ca^{2+} channel.

Keywords: ω-Conotoxin GVIA; ω-conotoxin MVIIC; Ca^{2+} channel; inotropic effect; parasympathetic nerve; sympathetic nerve

Introduction

The release of neurotransmitter is triggered when action potentials depolarize nerve terminals and open voltage-gated Ca^{2+} channels, the principal mechanism for enabling rapid Ca^{2+} influx into the nerve terminal (Bertolino & Llinás, 1992; Llinás *et al.*, 1992b; Zucker, 1993). Electrophysiological and molecular cloning studies have revealed multiple types of neuronal Ca^{2+} channels, which have different distribution, voltage-dependence, kinetics of activation/inactivation and conductance (Nowycky *et al.*, 1985; Hess, 1990; Llinás *et al.*, 1992a; Hofmann *et al.*, 1994; Olivera *et al.*, 1994). Using specific channel blockers, e.g., 1,4-dihydropyridines, ω-conotoxin GVIA, ω-conotoxin MVIIC, ω-agatoxin IVA and low concentrations of Ni^{2+}/Cd^{2+}, the high voltage-activated Ca^{2+} channels have been subdivided into L-, N-, O-, P-, Q- and R-types (*cf.* Zhang *et al.*, 1993; Olivera *et al.*, 1994). It is apparent that different types of Ca^{2+} channels are operational for transmitter release in different neurones and species, though multiple Ca^{2+} channel types may coexist to regulate neurotransmitter release (Lemos & Nowycky, 1989; Turner *et al.*, 1993; Yawo & Chuhma, 1993; Momiyama & Takahashi, 1994; Regehr & Mintz, 1994; Wheeler *et al.*, 1994; Dunlap *et al.*, 1995).

In mammalian atria which are innervated with both sympathetic and parasympathetic nerves for regulation of heart rate and contractile strength, the subtypes of Ca^{2+} channels involved in neurotransmitter release have not yet been elucidated. Because of the tiny size/mass of nerve terminals/varicosities of the postganglionic nerves which are embedded within muscle fibres, the release of transmitter is difficult to assess by electrophysiological techniques or by direct measurement. We therefore studied the properties of the Ca^{2+} channels pharmacologically by monitoring the effects of specific Ca^{2+} channel blockers on the inotropic/chronotropic effects in response to excitation of the autonomic nerves. The release of neurotransmitters was evoked by means of electrical field stimulation to simulate the physiological process. A ganglionic stimulant was also used to evoke transmitter release.

[1] Author for correspondence.

Methods

Atrial preparations

Left and right atria were isolated from guinea-pigs (Hartley strain, 0.35-1.1 kg) of either sex. Preparations were bathed in Tyrode solution (composition in mM: NaCl 137, KCl 2.8, $MgCl_2$ 1.1, $CaCl_2$ 1.8, NaH_2PO_4 0.33, dextrose 11.2 and $NaHCO_3$ 11.9) maintained at 35-37°C with the pH adjusted to 7.2-7.4 by aeration with a gas mixture (95% O_2 + 5% CO_2). Depending on the body weight of the animal, the resting tension was adjusted between 0.2-0.5 g. Contractile forces were recorded isometrically by means of a strain gauge force transducer (HSE F30, Germany).

Excitation of autonomic nerves

Field stimulation Left atria were juxta-positioned to the centre of the stimulation assembly consisting of one pair of punctate electrodes for pacing cardiac muscle and one pair of bar electrodes for field stimulation of autonomic nerves similar to that described by Blinks (1966). Pulses were generated with digital stimulators having high voltage output (A-M Systems, U.S.A.). Left atria were driven at 2 or 4 Hz with rectangular pulses (10-20 V, 0.2-0.3 ms) unless otherwise indicated. The

nerves were excited by field stimulations, consisting of short trains of 1–4 pulses (40–60 V, 0.05–0.1 ms, pulse interval 5 ms). Field pulses were applied within 30 ms following pacing pulse (within the absolute refractory period of cardiac muscle) for up to 120 s. Since the field stimulation (train of pulses) was coupled to the pacing pulse, the frequency of field stimulation increased simultaneously as the pacing rate increased. Atropine (1 μM) and propranolol (3 μM) were applied to differentiate the effects resulting respectively, from stimulation of sympathetic and paraysmpathetic nerves.

Excitation by a ganglionic stimulant Spontaneously beating right atria were used. 1,1-Dimethyl-4-phenylpiperazinium (DMPP) was applied to stimulate the nicotinic receptor (Lindmar *et al.*, 1968; Westfall & Brasted, 1972). Changes in both contractile force and beating rate were monitored. The drug was washed out within 4 min in order not to cause deterioration of tissues.

Assessment of the effects of Ca^{2+} channel blockers

The effects of Ca^{2+} channel blockers on the inotropic responses were monitored up to 60 min. For every atrium, the magnitude of the maximal inhibition of the electrical or chemical stimulation-induced negative/positive effects was expressed as a percentage of the *respective* control values obtained before application of channel blockers. The curves relating the logarithmic concentrations of channel blockers *vs.* the magnitude of effects were constructed and the half inhibition concentrations (IC_{50}) of Ca^{2+} channel blockers were estimated by interpolation.

Chemicals

ω-Agatoxin IVA, ω-conotoxin GVIA and ω-conotoxin MVIIC were purchased from Peptide Institute (Japan). Atropine, carbachol, DMPP, tubocurarine, isoprenaline, propranolol, nifedipine and tetrodotoxin were purchased from Sigma Chem. Co. (U.S.A.). Toxins were applied cumulatively for the study of dose-response relationships.

Statistics

Data are expressed as means ± s.e. from at least 4 preparations. The results were analyzed with Student's paired *t* test with the level of significance taken as $P<0.05$.

Results

Effects of peptide toxins on atrial contractility

At the concentrations applied (0.1–3 μM), ω-conotoxin GVIA, ω-conotoxin MVIIC, and ω-agatoxin IVA did not inhibit the contractile strength and beating rate of right atria. These peptide toxins were also without effect on the muscarinic receptor agonist, carbachol (10–30 μM), induced negative chronotropic/inotropic effects nor the β-adrenoceptor agonist, isoprenaline (3–30 nM), induced positive responses (not shown).

Effects of field stimulation on contractile force

The contractile strength of isolated left atria varied with pacing frequency. It increased from 0.47 ± 0.08 g at 0.5 Hz to 1.53 ± 0.11 g at 4 Hz and then declined to 1.20 ± 0.13 g at 6 Hz ($n=13-17$). Pacing frequency was set at 2–4 Hz in the following experiments. Application of field stimulation to excite intra-cardiac nerves elicited biphasic changes of contractile strength: an initial rapid decline, followed in 10–15 s, by a progressive increase. Upon termination of stimulation, a prominent rebound increase of contractile strength occurred which gradually returned to the pre-stimulation level (Figures 1 and 2). The extent of the stimulation-induced negative response and the post-stimulation rebound positive inotropic response became more marked as the pulse number (1–4) in each train of field stimulation increased. Both the negative and positive inotropic responses were abolished by a low concentration of tetrodotoxin (0.3 μM) that had no effect on the contractility of regularly paced atria (not shown).

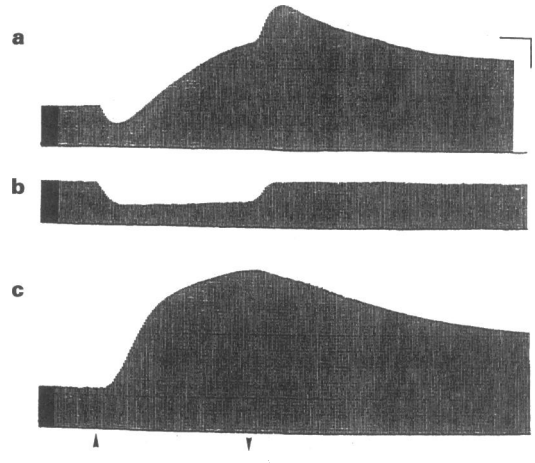

Figure 1 Responses to field stimulation of the contractile force of guinea-pig left atrium. Left atria were paced at 2 Hz. In each panel, field stimulations were delivered for 50 s in between the upward and downward arrow heads. Each field stimulation consisted of a short train of 2 pulses (0.05–0.1 ms duration, 5 ms apart) per pacing pulse. (a) Control; (b) the same atrium, 20 min after treatment with 3 μM propranolol; (c) the same atrium, after washout of propranolol and treatment with 1 μM atropine for 20 min. Note the rebound facilitation phase in (a). Similar responses were obtained from 7 preparations. Calibrations: 1 g and 10 s.

To see if the effects of field stimulation were mediated by release of acetylcholine and noradrenaline from autonomic nerves, we tested the antagonistic effects of atropine (a muscarinic receptor antagonist) and propranolol (a β-adrenoceptor antagonist). In the presence of propranolol (3 μM), the facilitatory phase was abolished and the field stimulation caused a monophasic sustained inhibition (Figures 1b and 2Ib). In contrast, atropine (1 μM) abolished the initial inhibitory phase but enhanced the facilitatory component (Figures 1c and 2IIb). The post-stimulation rebound facilitation was absent when either atropine or propranolol was present, indicating that the rebound phenomenon is due to a more rapid termination of the muscarinic component than the adrenergic one. In the following experiments, the effects of Ca^{2+} channel blockers on the field stimulation-induced negative and positive inotropic effects were analyzed quantitatively in preparations pretreated, respectively, with propranolol and atropine to differentiate cholinergic inhibitory and adrenergic facilitatory components.

Effects of ω-conotoxin GVIA

In the presence of ω-conotoxin GVIA, the profile of inotropic responses on field stimulation was changed. The adrenergic facilitation, but not the cholinergic inhibition, was depressed (Figure 2Ic *vs.* a). Figure 3 illustrates dose-dependent effects of the toxin on the cholinergic and adrenergic components. ω-Conotoxin GVIA abolished the adrenergic component with an IC_{50} value of 0.42 ± 0.05 μM ($n=6$). However, the toxin inhibited the cholinergic component by only 49 ± 7% at 10 μM. The effect on the adrenergic component was irreversible on washout.

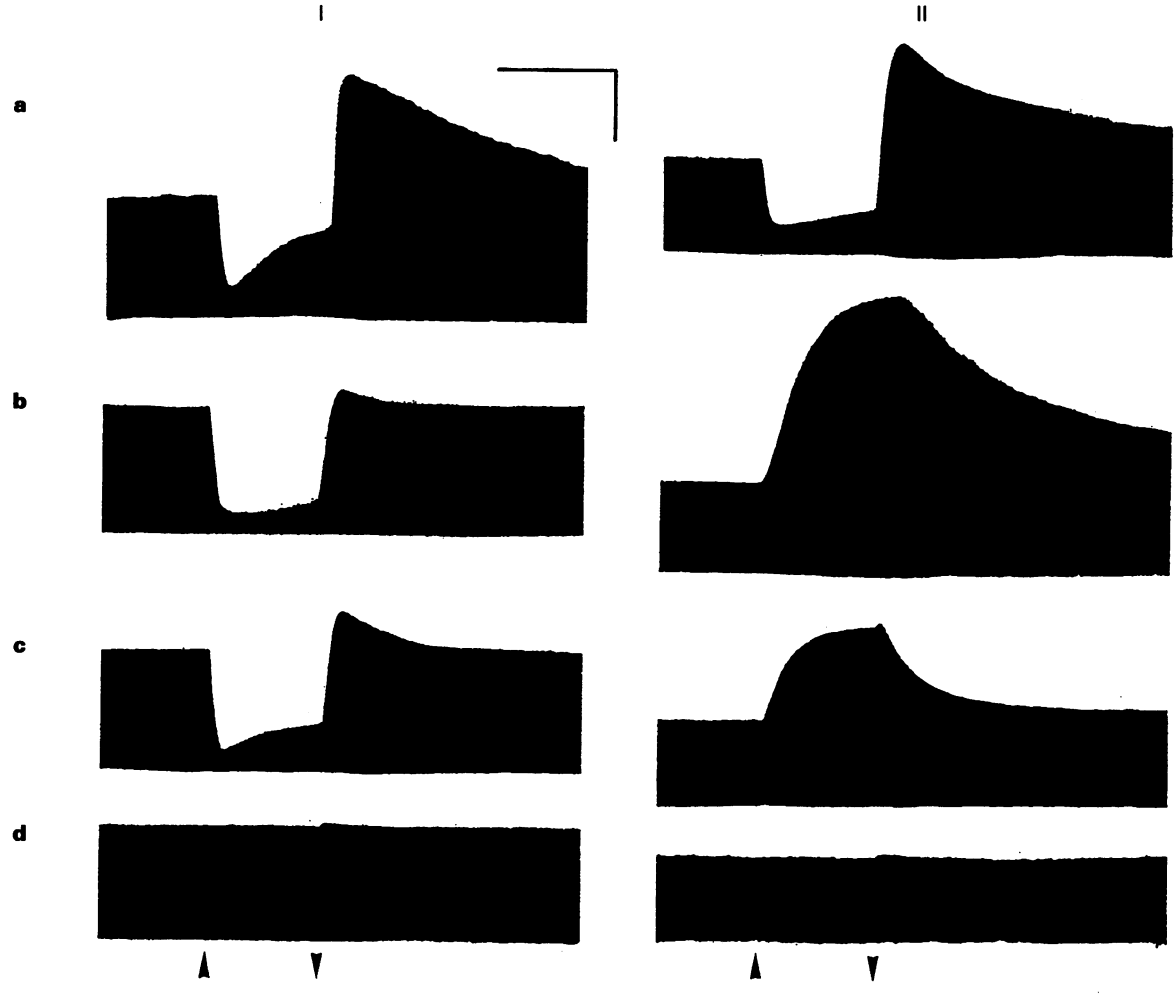

Figure 2 Effects of ω-conotoxin GVIA and ω-conotoxin MVIIC on field stimulation-induced changes of inotropic responses in left atria. Left atria were paced at 4 Hz. In each panel, field stimulations (4 pulses per pacing pulse) were delivered for 60 s in between the upward and downward arrow heads. Column I: (a) control; (b) the same atrium, 20 min after treatment with 3 μM propranolol; (c) the same preparation (after washout of propranolol) treated with ω-conotoxin GVIA 1 μM for 20 min; (d) the same preparation, 20 min after a further treatment with ω-conotoxin MVIIC 1 μM. Column II: (a) another preparation, control; (b) the same atrium, 20 min after treatment with 1 μM atropine; (c) the same preparation (after washout of atropine) treated with ω-conotoxin MVIIC 0.5 μM for 20 min; (d) the same preparation, 20 min after a further treatment with ω-conotoxin GVIA 1 μM. Note that the negative and positive inotropic responses on field stimulations were abolished when treated with both ω-conotoxins (d *vs.* a). Similar responses were obtained in 6 preparations. Calibrations: 1 g and 60 s.

Effects of ω-conotoxin MVIIC

In contrast to ω-conotoxin GVIA, ω-conotoxin MVIIC inhibited the field stimulation-induced negative and positive inotropic effects in parallel (Figure 2IIc *vs.* a and b and Figure 3). The IC_{50} values for the cholinergic and adrenergic components were 0.28 ± 0.04 μM ($n=6$) and 0.49 ± 0.05 μM ($n=6$), respectively. Interestingly, while ω-conotoxin MVIIC abolished the cholinergic component at 0.5 μM, there was a small fraction ($9 \pm 2\%$) of the adrenergic component that remained unaffected even when a 10 fold higher concentration of the toxin was used. Both the cholinergic and adrenergic components recovered $54 \pm 5\%$ ($n=10$) after washout of ω-conotoxin MVIIC (not shown).

Effects of ω-agatoxin IVA

ω-Agatoxin IVA (1 μM) had no effect on the cholinergic or the adrenergic component of field stimulation-induced inotropic responses (Figure 3). At a very high concentration (3 μM), the toxin reduced the cholinergic and adrenergic components by $37 \pm 6\%$ ($n=4$) and $21 \pm 5\%$ ($n=4$), respectively. Pretreatment with ω-agatoxin IVA (1 μM) did not reduce the inhibitory activities of either conopeptide (not shown).

Effects of nifedipine and Ni^{2+}

Unlike the peptide toxins, nifedipine (1–3 μM) and Ni^{2+} (100–200 μM) reduced the contractile strength by $73 \pm 12\%$ ($n=6$) and $26 \pm 5\%$ ($n=6$), respectively, while the field stimulation-induced negative and positive inotropic effects were not depressed (not shown, *cf.* Figure 5).

Effects of Ca^{2+} channel blockers on DMPP-induced responses

In spontaneously beating right atria, DMPP (10–30 μM) induced biphasic changes of contractions. In the initial 10–15 s, the heart rate and contractile strength decreased by $17 \pm 3\%$ and $32 \pm 5\%$ ($n=8$), respectively. After these transient negative chronotropic/inotropic effects, the heart rate and contractile strength increased, in the following 15–25 s, to a peak by $46 \pm 7\%$ and $477 \pm 24\%$ ($n=8$), respectively (Figure 4a). Thereafter, the positive chronotropic/inotropic effects declined gradually to a steady level. The negative and positive chronotropic/inotropic effects were absent in preparations pretreated with atropine (1 μM) and propranolol (3 μM) (not shown). Thus, like field stimulation, DMPP caused initial negative and late positive chronotropic/inotropic effect by way of autonomic nerve stimulation. The DMPP-responses could be

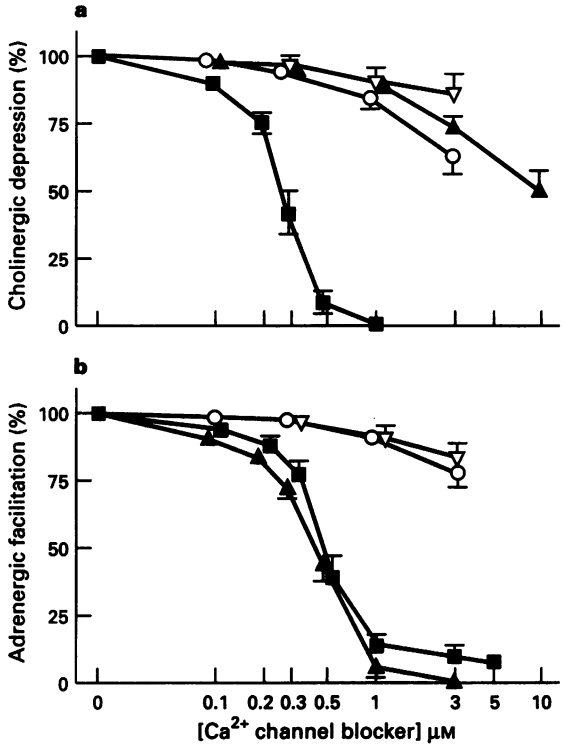

Figure 3 Effects of Ca^{2+} channel blockers on field stimulation-induced cholinergic depression and adrenergic facilitation of left atria, paced at 4 Hz. Field stimulation (4 pulses per pacing pulse) were delivered for 60–120 s. (a) Inhibition of the cholinergic depression in atria treated with 3 μM paropranolol; (b) inhibition of the adrenergic facilitation in atria treated with 1 μM atropine. In control preparations, the contractile force was reduced by 79 ± 4% (n = 10) during the maximal cholinergic depression while it was increased by 247 ± 23% (n = 11) during the maximal adrenergic facilitation. The effects of Ca^{2+} channel blockers are presented as percentages of the maximal negative or positive response relative to the *respective* control: (○) ω-agatoxin IVA; (■) ω-conotoxin MVIIC; (▲) ω-conotoxin GVIA; (▽) nifedipine. Ca^{2+} channel blockers were added cumulatively. The inhibitory effects were monitored up to 60 min. Mean ± s.e. each from 4–6 preparations; some error bars fall within symbols.

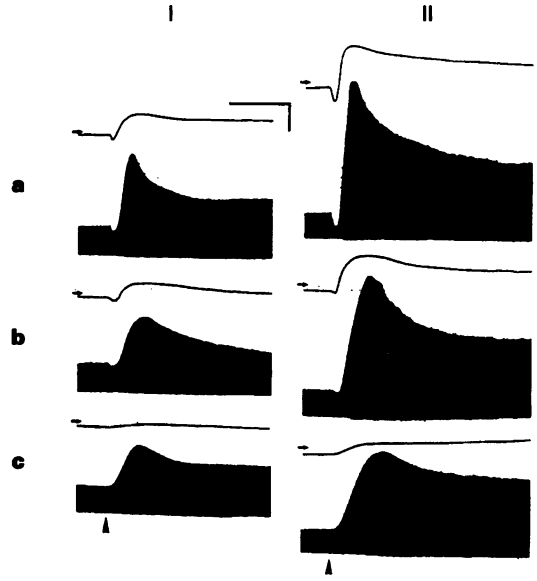

Figure 4 Effects of ω-conotoxin GVIA and ω-conotoxin MVIIC on DMPP-induced negative and positive inotropic/chronotropic responses in right atria. In each set of records, the upper trace monitored the beating rates (the horizontal arrow marks the level of 250 beats min^{-1}) and the lower traces the contractile force. The vertical arrow heads indicate application of DMPP. Column I: DMPP 10 μM: (a) control; (b) the same atrium, 20 min after treatment with ω-conotoxin GVIA 3 μM; (c) the same atrium after a further treatment with ω-conotoxin MVIIC (1 μM) for 20 min. Column II: DMPP 30 μM; (a) another preparation control; (b) the same atrium, 20 min after treatment with ω-conotoxin MVIIC 3 μM; (c) the same atrium after a further treatment with ω-conotoxin GVIA (10 μM) for 20 min. Note that ω-conotoxin MVIIC inhibited DMPP-induced negative chronotropic/inotropic effects (Ic *vs.* a; II b *vs.* a). Similar responses were obtained in 4 preparations. Calibrations: 2 g, 1 min, and 100 beats min^{-1}.

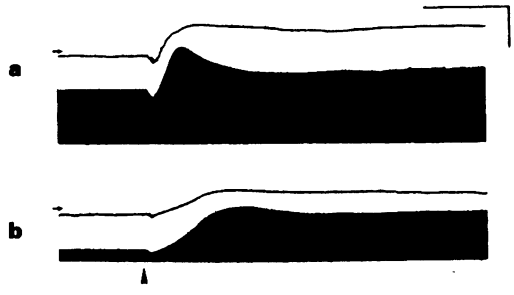

Figure 5 Effects of nifedpine on DMPP-induced negative and positive inotropic/chronotropic responses in right atria. Experiments on beating rate (upper trace) and contractile force (lower trace) as described in the legend to Figure 4. The vertical arrow head indicates application of DMPP (10 μM). (a) Control; (b) the same atrium, 20 min after treatment with nifedipine 3 μM. Note that nifedipine, while it depressed the contractile force, did not inhibit the positive inotropic effects of DMPP. Similar responses were obtained from 4 preparations. Calibrations: 2 g, 1 min, and 100 beats min^{-1}.

quantitatively reproduced, provided that the interval of drug application was longer than 20 min.

The DMPP-induced cholinergic inhibition, like that after field stimulation, was effectively inhibited by ω-conotoxin MVIIC (1-3 μM) by 93 ± 3% (n = 8, *cf.* Figure 4-IIb *vs.* a). However, unlike field stimulation, the DMPP-induced adrenergic facilitation was inhibited only to a limited extent (by 29 ± 6%, n = 8), even after high concentrations of ω-conotoxin GVIA (10 μM) and/or ω-conotoxin MVIIC (3 μM) (Figure 4-Ib *vs.* a and -I, -IIc *vs.* a). To see if the toxin-resistant adrenergic component was mediated by other types of Ca^{2+} channels, the effects of nifedipine (3 μM), Ni^{2+} (300 μM) and ω-agatoxin IVA (3 μM) were studied. None of the Ca^{2+} channel blockers inhibited the atrial responses to DMPP. Figure 5 illustrates that when the atrial contractility *per se* was depressed by nifedipine, the DMPP-induced positive inotropic effects were not reduced.

Discussion

That the biphasic inotropic responses upon field stimulation of the guinea-pig atria are due to neurotransmitter released from parasympathetic and sympathetic nerves is substantiated by the facts that the inotropic responses could be abolished by low concentrations of Na^+ channel blocker, by a β-adrenoceptor antagonist (for the positive effect) and by a muscarinic receptor antagonist (for the negative effect). Monitoring of the inotropic effects can therefore provide information on the relative release of neurotransmitters from pulse to pulse, although the magnitude of changes of release might not be linearly reflected in that of inotropic effects. Since ω-conotoxin GVIA and ω-conotoxin MVIIC did not affect the control contractile strength or the inotropic/chronotropic effects produced by direct stimulation of postsynaptic myocardial β-adrenoceptors or muscarinic receptors, the inhibition by the

conopeptides of the inotropic responses induced by field stimulation can be regarded as resulting from depression of the release of acetylcholine or noradrenaline from autonomic nerves.

Ca^{2+} channel type for sympathetic nerve

Failures of nifedipine, an L-type Ca^{2+} channel blocker (Fox *et al.*, 1987), and ω-agatoxin IVA, a blocker of P- and Q-type Ca^{2+} channels (Mintz *et al.*, 1992; Sather *et al.*, 1993), to affect the positive inotropic response to field stimulation suggests that L-, P- and Q-type Ca^{2+} channels do not play a major role in the release of noradrenaline. The slight inhibition produced by ω-agatoxin IVA (Figure 3) at concentrations 2–3 orders higher than that required for the blockage of P- and Q-type Ca^{2+} channels might be ascribed to nonspecific inhibition of other Ca^{2+} channel types. In contrast, ω-conotoxin GVIA, a toxin specific for the N-type Ca^{2+} channel (Olivera *et al.*, 1985; McCleskey *et al.*, 1987), completely inhibited this positive inotropic response with an IC_{50} value similar to that of the block of other neuronal N-type Ca^{2+} channels, suggesting that in the guinea-pig atrium the Ca^{2+} channel of the sympathetic nerve activated by nerve action potential can be categorized as N-type - the main Ca^{2+} channel subtype proposed for other sympathetic nerves (Miller, 1987; Hirning *et al.*, 1988; De Luca *et al.*, 1990; Pruneau & Angus, 1990). A block of a major fraction of the positive inotropic response by ω-conotoxin MVIIC, a blocker of OPQ subfamily and N-type Ca^{2+} channels (Hillyard *et al.*, 1992; Randall *et al.*, 1993; Sather *et al.*, 1993; Grantham *et al.*, 1994; Olivera *et al.*, 1994) can be attributed to its inhibition of the N-type Ca^{2+} channels.

In contrast to the effect induced by electrical stimulation, the positive inotropic/chronotropic responses produced by the chemical stimulant DMPP were much less sensitive to both conopeptides. It is possible that additional Ca^{2+} channels are recruited on DMPP stimulation. They could be voltage-gated and plasma membrane-bound Ca^{2+} channels of a different type, or channels of the same type in a location inaccessible to the toxins. Unlike field stimulation which causes pulsatile short term depolarization of nerve terminals, DMPP evokes transmitter release by prolonged stimulation of the nicotinic receptors located on sympathetic nerve terminals/varicosities. This mode of triggering transmitter release is analogous to that in chromaffin cells, which are anatomically homologous to sympathetic neurones and release catecholamines mainly in response to activation of nicotinic receptors. The Ca^{2+} channels involved in the catecholamine release were multiple, including L-, N- and P-type Ca^{2+} channels (Albillos *et al.*, 1994; Artalejo *et al.*, 1994; López *et al.*, 1994). It is likely that a different set of Ca^{2+} channels could be opened in response to different triggering mechanisms. It is also possible that activation of nicotinic receptors may release Ca^{2+} from internal stores directly or via second messengers.

Ca^{2+} channel type for parasympathetic nerve

Both field stimulation- and ganglion stimulant-induced negative inotropic/chronotropic responses were highly sensitive to ω-conotoxin MVIIC but not to any other peptide toxins, nifedipine and Ni^{2+}, which block T- and R-type Ca^{2+} channels at low concentrations (<100 μM, Narahashi *et al.*, 1987; Ellinor *et al.*, 1993). It may be inferred that L-, N-, P-, T- and R-type Ca^{2+} channels are not operational in the parasympathetic nerves. The affinities of ω-conotoxin MVIIC for various Ca^{2+} channels have been ranked in the order: O- (~nM) >Q- (<0.15 μM) >P- (1~10 μM), N- (0.1~10 μM) (Hillyard *et al.*, 1992; Sather *et al.*, 1993; Zhang *et al.*, 1993; Olivera *et al.*, 1994). The IC_{50} value (0.3 μM) of ω-conotoxin MVIIC against the parasympathetic nerve of guinea-pig atria seems to suggest that a Q-type channel is involved. However, this inference seems incompatible with the low sensitivity of ω-agatoxin IVA, which has an affinity for Q-type channels similar to that of ω-conotoxin MVIIC (Olivera *et al.*, 1994). It is possible that a subtype of the OPQ subfamily Ca^{2+} channels serves for the parasympathetic nerves of atria.

Among various cholinergic neurones, P-type channels have been proposed as the operational ones in neuromuscular transmission in mouse (Uchitel *et al.*, 1992; Protti & Uchitel, 1993; Hong & Chang, 1995), crayfish (Araque *et al.*, 1994) and guinea-pig (unpublished) skeletal muscles, while the N-type is used in frog motor nerve (Robitaille *et al.*, 1990; Cohen *et al.*, 1991), electric ray electric organ (Fariñas *et al.*, 1993) and rat and guinea-pig myenteric plexuses (Wessler *et al.*, 1990; Boot, 1994). In rat superior cervical ganglion (Gonzalez Burgos *et al.*, 1995) and *Aplysia* buccal ganglion (Fossier *et al.*, 1994), both N- and P-type Ca^{2+} channels seem to be involved in acetylcholine release.

In conclusion, in contrast to the myocardial L-type Ca^{2+} channel, the pharmacological characteristics of the neuronal Ca^{2+} channels of guinea-pig atria are dominated by the N type for sympathetic nerves and an OPQ subfamily for parasympathetic nerves. It may be anticipated that regulation of different types of Ca^{2+} channels have therapeutic advantages in some kinds of cardiac dysfunction.

This study was supported by a grant from the National Science Council. (NSC84-0412-B002).

References

ALBILLOS, A., ARTALEJO, A.R., LÓPEZ, M.G., GANDÍA, L., GARCÍA, A.G. & CARBONE, E. (1994). Calcium channel subtypes in cat chromaffin cells. *J. Physiol.*, **477**, 197–213.

ARAQUE, A., CLARAC, F. & BUÑO, W. (1994). P-type Ca^{2+} channels mediate excitatory and inhibitory synaptic transmitter release in crayfish muscle. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 4224–4228.

ARTALEJO, C.R., ADAMS, M.E. & FOX, A.P. (1994). Three types of Ca^{2+} channel trigger secretion with different efficacies in chromaffin cells. *Nature*, **367**, 72–76.

BERTOLINO, M. & LLINÁS, R.R. (1992). The central role of voltage-activated and receptor-operated calcium channels in neuronal cells. *Annu. Rev. Pharmacol. Toxicol.*, **32**, 399–421.

BLINKS, J.R. (1966). Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J. Pharmacol. Exp. Ther.*, **151**, 221–235.

BOOT, J.R. (1994). Differential effects of ω-conotoxin GVIA and MVIIC on nerve stimulation induced contractions of guinea-pig ileum and rat vas deferens. *Eur. J. Pharmacol.*, **258**, 155–158.

COHEN, M.W., JONES, O.T. & ANGELIDES, K.J. (1991). Distribution of Ca^{2+} channels on frog motor nerve terminals revealed by fluorescent ω-conotoxin. *J. Neurosci.*, **11**, 1032–1039.

DE LUCA, A., LI, C.G., RAND, M.J., REID, J.J., THAINA, P. & WONG-DUSTING, H.K. (1990). Effects of ω-conotoxin GVIA on autonomic neuroeffector transmission in various tissues. *Br. J. Pharmacol.*, **101**, 437–447.

DUNLAP, K., LUEBKE, J.I. & TURNER, T.J. (1995). Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends Neurosci.*, **18**, 89–98.

ELLINOR, P.T., ZHANG, J.-F., RANDALL, A.D., ZHOU, M., SCHWARZ, T.L., TSIEN, R.W. & HORNE, W.A. (1993). Functional expression of a rapidly inactivating neuronal calcium channel. *Nature*, **363**, 455–458.

FARIÑAS, I., EGEA, G., BLASI, J., CASES, C. & MARSAL, J. (1993). Calcium channel antagonist omega-conotoxin binds to intramembrane particles of isolated nerve terminals. *Neuroscience*, **54**, 745–752.

FOSSIER, P., BAUX, G. & TAUC, L. (1994). N- and P-type Ca^{2+} channels are involved in acetylcholine release at a neuroneuronal synapse: Only the N-type channel is the target of neuromodulators. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 4771–4775.

FOX, A.P., NOWYCKY, M.C. & TSIEN, R.W. (1987). Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J. Physiol.*, **394**, 149–172.

GONZALEZ BURGOS, G.R., BIALI, F.I., CHERKSEY, B.D., SUGIMORI, M., LLINÁS, R.R. & UCHITEL, O.D. (1995). Different calcium channels mediate transmitter release evoked by transient or sustained depolarization at mammalian sympathetic ganglia. *Neuroscience*, **64**, 117–123.

GRANTHAM, C.J., BOWMAN, D., BATH, C.P., BELL, D.C. & BLEAKMAN, D. (1994). ω-Conotoxin MVIIC reversibly inhibits a human N-type calcium channel and calcium influx into chick synaptosomes. *Neuropharmacology*, **33**, 255–258.

HESS, P. (1990). Calcium channels in vertebrate cells. *Annu. Rev. Neurosci.*, **13**, 337–356.

HILLYARD, D.R., MONJE, V.D., MINTZ, I.M., BEAN, B.P., NADASDI, L., RAMACHANDRAN, J., MILJANICH, G., AZIMI-ZOONOOZ, A., MCINTOSH, J.M., CRUZ, L.J., IMPERIAL, J.S. & OLIVERA, B.M. (1992). A new conus peptide ligand for mammalian presynaptic Ca^{2+} channels. *Neuron*, **9**, 69–77.

HIRNING, L.D., FOX, A.P., MCCLESKEY, E.W., OLIVERA, B.M., THAYER, S.A., MILLER, R.J. & TSIEN, R.W. (1988). Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from sympathetic neurons. *Science*, **239**, 57–61.

HOFMANN, F., BIEL, M. & FLOCKERZI, V. (1994). Molecular basis for Ca^{2+} channel diversity. *Annu. Rev. Neurosci.*, **17**, 399–418.

HONG, S.J. & CHANG, C.C. (1995). Inhibition of acetylcholine release from mouse motor nerve by a P-type calcium channel blocker, ω-agatoxin IVA. *J. Physiol.*, **482**, 283–290.

LEMOS, J.R. & NOWYCKY, M.C. (1989). Two types of calcium channels coexist in peptide-releasing vertebrate nerve terminals. *Neuron*, **2**, 1419–1426.

LINDMAR, R., LÖFFELHOLZ, K. & MUSCHOLL, E. (1968). A muscarinic mechanism inhibiting the release of noradrenaline from peripheral adrenergic nerve fibres by nicotinic agents. *Br. J. Pharmacol. Chemother.*, **32**, 280–294.

LLINÁS, R., SUGIMORI, M., HILLMAN, D.E. & CHERKSEY, B. (1992a). Distribution and functional significance of the P-type, voltage-dependent Ca^{2+} channels in the mammalian central nervous system. *Trends. Neurosci.*, **15**, 351–355.

LLINÁS, R., SUGIMORI, M. & SILVER, R.B. (1992b). Microdomains of high calcium concentration in a presynaptic terminal. *Science*, **256**, 677–679.

LÓPEZ, M.G., ALBILLOS, A., DE LA FUENTE, M.T., BORGES, R., GANDÍA, L., CARBONE, E., GARCÍA, A.G. & ARTALEJO, A.R. (1994). Localized L-type calcium channels control exocytosis in cat chromaffin cells. *Pfügers Arch.*, **427**, 348–354.

MCCLESKEY, E.W., FOX, A.P., FELDMAN, D.H., CRUZ, L.J., OLIVERA, B.M., TSIEN, R.W. & YOSHIKAMI, D. (1987). ω-Conotoxin: Direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 4327–4331.

MILLER, R.J. (1987). Multiple calcium channels and neuronal function. *Science*, **235**, 46–52.

MINTZ, I.M., VENEMA, V.J., SWIDEREK, K.M., LEE, T.D., BEAN, B.P. & ADAMS, M.E. (1992). P-type calcium channels blocked by the spider toxin ω-Aga-IVA. *Nature*, **355**, 827–829.

MOMIYAMA, A. & TAKAHASHI, T. (1994). Calcium channels responsible for potassium-induced transmitter release at rat cerebellar synapses. *J. Physiol.*, **476**, 197–202.

NARAHASHI, T., TSUNOO, A. & YOSHII, M. (1987). Characterization of two types of calcium channels in mouse neuroblastoma cells. *J. Physiol.*, **383**, 231–249.

NOWYCKY, M.C., FOX, A.P. & TSIEN, R.W. (1985). Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature*, **316**, 440–443.

OLIVERA, B.M., GRAY, W.R., ZEIKUS, R., MCINTOSH, J.M., VARGA, J., RIVIER, J., DE SANTOS, V. & CRUZ, L.J. (1985). Peptide neurotoxins from fish-hunting cone snails. *Science*, **230**, 1338–1343.

OLIVERA, B.M., MILJANICH, G.P., RAMACHANDRAN, J. & ADAMS, M.E. (1994). Calcium channel diversity and neurotransmitter release: The ω-conotoxins and ω-agatoxins. *Annu. Rev. Biochem.*, **63**, 823–867.

PROTTI, D.A. & UCHITEL, O.D. (1993). Transmitter release and presynaptic Ca^{2+} currents blocked by the spider toxin ω-Aga-IVA. *NeuroReport*, **5**, 333–336.

PRUNEAU, D. & ANGUS, J.A. (1990). Apparent vascular to cardiac sympatholytic selectivity of ω-conotoxin GVIA in the pithed rat. *Eur. J. Pharmacol.*, **184**, 127–133.

RANDALL, A.D., WENDLAND, B., SCHWEIZER, F., MILJANICH, G., ADAMS, M.E. & TSIEN, R.W. (1993). Five pharmacologically distinct high voltage-activated Ca^{2+} channels in cerebellar granule cells. *Soc. Neurosci. Abstr.*, **19**, 1478.

REGEHR, W.G. & MINTZ, I.M. (1994). Participation of multiple calcium channel types in transmission at single climbing fiber to Purkinje cell synapses. *Neuron*, **12**, 605–613.

ROBITAILLE, R., ADLER, E.M. & CHARLTON, M.P. (1990). Strategic location of calcium channels at transmitter release sites of frog neuromuscular synapses. *Neuron*, **5**, 773–779.

SATHER, W.A., TANABE, T., ZHANG, J.-F., MORI, Y., ADAMS, M.E. & TSIEN, R.W. (1993). Distinctive biophysical and pharmacological properties of class A (BI) calcium channel α_1 subunits. *Neuron*, **11**, 291–303.

TURNER, T.J., ADAMS, M.E. & DUNLAP, K. (1993). Multiple Ca^{2+} channel types coexist to regulate synaptosomal neurotransmitter release. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 9518–9522.

UCHITEL, O.D., PROTTI, D.A., SANCHEZ, V., CHERKSEY, B.D., SUGIMORI, M. & LLINÁS, R. (1992). P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 3330–3333.

WESSLER, I., DOOLEY, D.J., WERHAND, J. & SCHLEMMER, F. (1990). Differential effects of calcium channel antagonists (ω-conotoxin GVIA, nifedipine, verapamil) on the electrically-evoked release of [^{3}H]acetylcholine from the myenteric plexus, phrenic nerve and neocortex of rats. *Naunyn-Schmied Arch. Pharmacol.*, **341**, 288–294.

WESTFALL, T.C. & BRASTED, M. (1972). The mechanism of action of nicotine on adrenergic neurons in the perfused guinea-pig heart. *J. Pharmacol. Exp. Ther.*, **182**, 409–418.

WHEELER, D.B., RANDALL, A. & TSIEN, R.W. (1994). Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. *Science*, **264**, 107–111.

YAWO, H. & CHUHMA, N. (1993). Preferential inhibition of ω-conotoxin-sensitive presynaptic Ca^{2+} channels by adenosine autoreceptors. *Nature*, **365**, 256–258.

ZHANG, J.-F., RANDALL, A.D., ELLINOR, P.T., HORNE, W.A., SATHER, W.A., TANABE, T., SCHWARZ, T.L. & TSIEN, R.W. (1993). Distinctive pharmacology and kinetics of cloned neuronal Ca^{2+} channels and their possible counterparts in mammalian CNS neurons. *Neuropharmacology*, **32**, 1075–1088.

ZUCKER, R.S. (1993). Calcium and transmitter release. *J. Physiol. (Paris)*, **87**, 25–36.

(Received January 16, 1995
Revised May 9, 1995
Accepted May 17, 1995)