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Short Communication

CONSTITUTION OF CALCIUM CHANNEL CURRENT IN HAMSTER SUBMANDIBULAR GANGLION NEURONS

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Abstract

The submandibular ganglion (SMG) neuron has been well established as the parasympathetic ganglion that innervates the submandibular and sublingual salivary glands. Thus this neuron plays a key role in salivary secretion. In a previous study, we reported that SMG possessed T-, L-, N-, P/Q- and R-type voltage-dependent calcium channels (VDCCs). In this study, we analyzed the contribution of the distinct subtypes of VDCCs currents (I_{Ca}) using the whole-cell configuration of the patch clamp technique in SMG neurons. In addition, we also investigated the effects of a strong voltage prepulse on the contributions of the subtypes of VDCCs. In SMG neuronal I_{Ca} without a prepulse, the mean percentages of L-, N-, P-, Q- and R-type were 39.7, 31.5, 10.6, 7.1 and 7.9%. In SMG neuronal I_{Ca} with prepulse, the mean percentages of L-, N-, P-, Q- and R-type were 37.2, 34.0, 14.0, 7.6 and 7.0%. Thus, these results showed that SMG possess multiple types of VDCCs and that N- and P-type VDCCs are facilitated by a prepulse in SMG neurons.

Key words: Voltage-dependent calcium channels—
Hamster submandibular ganglion neurons—Prepulse facilitation—
Whole-cell patch clamp recordings

INTRODUCTION

In neurons, transmembrane Ca^{2+} entry *via* voltage-dependent calcium channels (VDCCs) is of major physiological importance, because several neuronal functions, including neuronal excitability, neuronal migration, neurite outgrowth, gene expression, and neurotransmitter release, depend on this event. Biophysical and pharmacological analysis has led to the description of several classes of VDCCs, *i.e.* T-, L-, N-, P-, Q- and R-type VDCCs[26,30]. These types differ considerably in their responsiveness to neuromodulators, their distribution among various types of neurons, and their localization in different regions within individual neurons. The variety of VDCCs types provide for a multiplicity of neuronal functions.

The parasympathetic submandibular ganglion (SMG) neuron innervates the submandibular and sublingual salivary glands and thus plays a key role in salivary secretion. In a previous study, we reported that SMG possessed T- (low voltage activated) and L-, N-, P/Q- and R-type (high voltage activated) VDCCs. We have also defined readily distinguishable components of high voltage activated VDCCs current (I_{Ca}) using a series of potent VDCCs blockers. In hamster SMG neurons, the mean percentages of contribution of L-, N-, P/Q- and R-type components were 48.0, 36.1, 13.5 and 3.6%, respectively[10]. However, the full extent of P- and Q-type VDCCs diversity in SMG neurons remains incompletely understood; we have referred to them as P/Q-type VDCCs.

Prepulse facilitation is a phenomenon in which a train of depolarizations, or a long and strong depolarizing pulse, induces a form of the VDCCs that exhibits an increased opening probability in response to a given test potential that persists for several seconds after repolarization[8,18]. We previously reported that the rate of prepulse facilitation of I_{Ca} (I_{Ca} after prepulse/I_{Ca} before prepulse) was 1.2 in SMG neurons[11]. In this and subsequent descriptions, we refer to I_{Ca} before and after prepulse as '$I_{Ca}-$pp' and '$I_{Ca}+$pp', respectively. The objective of the investigations reported here was to analyze the contributions of L-, N-, P-, Q- and R-type VDCCs in $I_{Ca}-$pp and $I_{Ca}+$pp in SMG neurons.

MATERIALS AND METHODS

SMG neurons from hamsters were acutely dissociated with a modified version of the method described previously[37]. In brief, 4–6-week old male hamsters were anesthetized with pentobarbital sodium (30 mg/kg, i.p.); SMG neurons were isolated from them and maintained in Ca^{2+}-free Krebs solution of the following composition (in mM): 136 Nal, 5 KCl, 3 $MgCl_2 \cdot 6H_2O$, 10.9 glucose, 11.9 $NaHCO_3$ and 1.1 $NaH_2PO_4 \cdot 2H_2O$. The neurons were treated with collagenase type I (3 mg/ml in Ca^{2+}-free Krebs solution; Sigma, St. Louis, MO, U.S.A.) for 50 min at 37°C, followed by incubation in trypsin type I (1 mg/ml in Ca^{2+}-free Krebs solution; Sigma St. Louis, MO, U.S.A.) for an additional 10 min. The supernatant was replaced with normal Krebs solution of the following composition (in mM): 136 NaCl, 5 KCl, 2.5 $CaCl_2$, 0.5 $MgCl_2 \cdot 6H_2O$, 10.9 glucose, 11.9 $NaHCO_3$ and 1.1 $NaH_2PO_4 \cdot 2H_2O$.

Voltage-clamp recordings were conducted using the whole-cell configuration of the patch clamp technique[14]. Fabricated recording pipettes (2–3MΩ) were filled with an internal solution with the following composition (in mM): 100 CsCl, 1 $MgCl_2$, 10 HEPES, 10 BAPTA, 3.6 MgATP, 14 $Tris_2CP$, 0.1 GTP, and 50 U/ml CPK. The pH was adjusted to 7.2 with CsOH. After the formation of a giga seal, the external Krebs solution was replaced by a solution containing the following (in mM): 67 choline-Cl, 100 tetraethylammonium chloride (TEA-Cl), 5.3 KCl, 5 $CaCl_2$ and 10 HEPES in order to record I_{Ca}. The pH was adjusted to 7.4 with Tris base. Command voltage protocols were generated with a computer software pCLAMP version 8 (Axon Instruments, Union City, CA, U.S.A.) and transformed to an analogue signal using a DigiData 1200 interface (Axon Instruments, Union City, CA, U.S.A.). The command pulses were applied to the cell through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with the amplifier and a computer software pCLAMP 8 acquisition system. All experiments were performed at room temperature (24–27°C).

RESULTS

Full activation of I_{Ca} was obtained by applying a test pulse from a holding potential $= -80$ mV to a test potential $= -10$ mV (Fig. 1). An intervening strong depolarizing prepulse (100 mV, 30 msec) ended 5 msec prior to obtain I_{Ca} +pp (Fig. 2).

Specific VDCCs blockers were used to isolate each I_{Ca} component. Typical examples of sequential application of each selective VDCCs blockers on $I_{Ca}-$pp and $I_{Ca}+$pp are shown in Fig. 1B and 2B, respectively.

ω-conotoxin GVIA (ω-CgTx GVIA) blocks N-type VDCCs[36] and ω-agatoxin IVA (ω-Aga IVA) blocks both P- and Q-type channels but

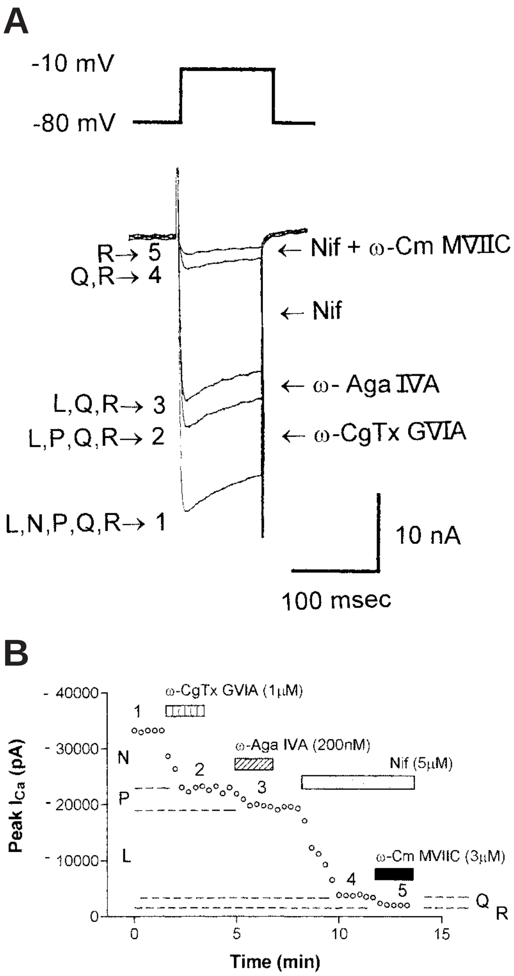

Fig. 1 Pharmacological characterization of five I_{Ca} components by sequential application of each VDCC blocker. (A) An example of the effects of VDCC blockers on whole I_{Ca} without a prepulse ($I_{Ca}-$pp). Superimposed $I_{Ca}-$pp traces at the times indicated in the time course graph (B). Current calibration, 10 nA; time calibration, 100 msec. *ω*-CgTx GVIA, *ω*-conotoxin GVIA (1μM); *ω*-Aga IVA, *ω*-agatoxin IVA (200 nM); Nif, nifedipine (5μM); *ω*-Cm MVIIC, *ω*-conotoxin MVIIC (3μM). N, *ω*-CgTx GVIA sensitive component; P, *ω*-Aga IVA sensitive component; L, Nif sensitive component; Q, *ω*-Cm MVIIC, sensitive component (after prior block of N and P); R, R-type (resistant to each blocker). (B) Time course of sequential application of each selective VDCC blocker on whole $I_{Ca}-$pp. All blockers were bath-applied during the time indicated by the horizontal bars. All recordings were obtained from the same neuron.

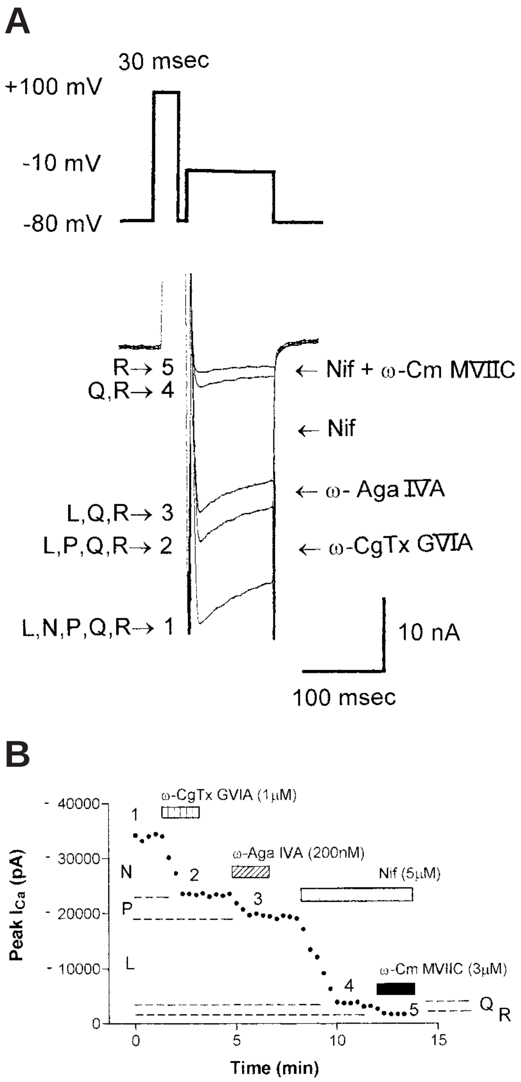

Fig. 2 Pharmacological characterization of five I_{Ca} components by sequential application of each VDCC blocker. (A) An example of the effects of VDCCs blockers on whole I_{Ca} with a prepulse ($I_{Ca}+$pp). Superimposed $I_{Ca}+$pp traces at the times indicated in the time course graph (B). Current calibration, 10 nA; time calibration, 100 msec. (B) Time course of sequential application of each selective VDCC blocker on whole $I_{Ca}+$pp. All blockers were bath-applied during the time indicated by the horizontal bars. All recordings were obtained from the same neuron.

with very different IC_{50} values of 1–20 nM and –100 nM, respectively[24,30]. In the present study, we used 200 nM *ω*-Aga IVA to isolate P-type VDCCs. Nif blocks L-type channels[5]. Furthermore, a new conus peptide, *ω*-conotoxin MVIIC (*ω*-Cm MVIIC), has been reported to block the Nif/*ω*-CgTx GVIA/*ω*-Aga IVA-insensitive VDCCs[31]. This *ω*-Cm MVIIC sen-

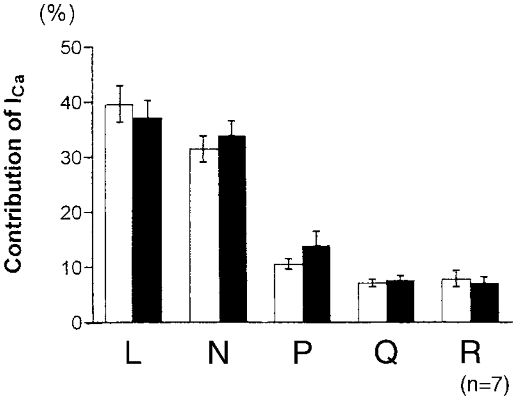

Fig. 3 Contribution of each VDCC type component to the whole I_{Ca}. Ordinate, percentages of contribution of each $I_{Ca}-pp$ (☐) and $I_{Ca}+pp$ (■) component to the whole I_{Ca}. Values in this figure are expressed as mean ± SEM.

sitive but DHP/ω-CgTx GIVA/ω-Aga IVA-insensitive VDCCs has been referred as the "Q-type" VDCCs[31)], although this ω-Cm MVIIC blocks not only Q-type but also N- and P-type VDCCs[16)]. Therefore, we applied ω-Cm MVIIC to isolate Q-type VDCCs after blocking of N-, P-type VDCCs. As reported by others[12)], the block of I_{Ca} by ω-CgTx GVIA, ω-Aga IVA and ω-Cm MVIIC was irreversible, while the block by Nif was partially reversible. Therefore, we applied both Nif and ω-Cm MVIIC together as shown in Fig. 1B and 2B. The remaining I_{Ca} in the presence of all of these blockers is termed the R-type[41)].

In this neuron, ω-CgTx GVIA blocked 30.6%, ω-Aga IVA 9.7%, Nif 48.4% and ω-Cm MVIIC 5.0% of total $I_{Ca}-pp$ (Fig. 1), and ω-CgTx GVIA blocked 30.7%, ω-Aga IVA 11.3%, Nif 47.1%, and ω-Cm MVIIC 5.8% of total $I_{Ca}+pp$ (Fig. 2).

The mean percentage contributions of the various VDCCs components to the total I_{Ca}, based on pooled data from 7 SMG neurons are shown in Fig. 3. In SMG neuronal $I_{Ca}-pp$, the mean percentage of the L-type was 39.7 ± 3.3%, the N-type was 31.5 ± 2.4%, the P-type was 10.6 ± 0.9%, the Q-type was 7.1 ± 0.7%, and the R-type was 7.9 ± 1.5%. In SMG neuronal $I_{Ca}+pp$, the mean percentage of the L-type was 37.2 ± 3.1%, the N-type was 34.0 ± 2.6%, the P-type was 14.0 ± 2.6%, the Q-type was 7.6 ± 0.9%, and the R-type was 7.0 ± 1.2% (mean ± SEM).

DISCUSSION

In summary, this article presents evidence that I_{Ca}s in SMG neurons are comprised of five components, referred to as L-, N-, P-, Q- and R-type I_{Ca}.

A particular type of I_{Ca} may have a very specific role in neuronal activity, but linking a specific type of VDCCs to a particular cellular process has proven difficult. For example, there are differing opinions regarding the identity of the channel type involved in Ca^{2+}-dependent transmitter release[17,27,29,35)]. In part, this controversy may have its source in the fact that the pharmacological properties of each type of I_{Ca} may be unique to the particular cell type under study[1,9,22,33)].

In this study, we also analyzed the effects of a prepulse on the contributions of VDCCs. Many studies have reported facilitation of an I_{Ca} by a prepulse, but the underlying mechanisms remain controversial. One common mechanism, typically observed with N- and P/Q-type VDCCs, involves a shift from the normal "willing" mode of gating, to a "reluctant" mode in which the channels can still open and close, but longer or stronger depolarization is required to open a channel[20)].

The modulation of VDCCs by a neurotransmitter mediated by G-protein coupled receptors (GPCRs) has been investigated in several neurons. Receptor-dependent activation of G-proteins leads to a modulation of I_{Ca} either through a direct interaction of G-protein $\beta\gamma$ subunits (G $\beta\gamma$) with VDCCs[21,39)] or *via* the generation of diffusible second messengers and activation of protein kinases[3)]. This modulation is initiated by G-protein activation and mediated by G $\beta\gamma$[15,19)]. G $\beta\gamma$ directly interacts with N- and P/Q-type VDCCs[7,28,40)]. Additionally, this G-protein-dependent inhibition can be reversed by a prepulse *via* the release of G $\beta\gamma$ from the VDCCs[4,6)]. Interestingly, in the present study, N- and P-type VDCCs components were slightly increased by a prepulse (Fig. 3). We suggest that these results are consistent with the evidences mentioned above.

VDCCs subtypes tend to be associated with specific cellular processes, *e.g.*, N-, P- and Q-type VDCCs are linked with neurotransmitter release[13,38)], and L-type VDCCs are implicated in neuronal growth and survival[23,25)]. However, it is not well understood why the neurons express such a wide variety of VDCCs. Certainly these different types can have different localizations within and among neurons and can undergo a wide variety of modulations[2,32,34)]. This contribution of VDCC subtypes to SMG neuron physiology may have important implications for saliva secretion. The characterization of the VDCCs in SMG neurons will allow for the future study of their modulation and their roles.

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