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The neuropeptide, neuromedin C, activates a potassium current in mouse macrophages

Mitsuyuki Ichinose, Nobumasa Hara, Masashi Sawada and Takashi Maeno

Department of Physiology, Shimane Medical University, Izumo 693, Japan

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Neuromedin C (NmC) induced an outward current (I_o(NmC)) in macrophages. Reversal potentials were dependent on external K^+ concentrations ($[K^+]_o$) and independent of $[Cl^-]_o$. Tetraethylammonium (TEA) and quinidine effectively suppressed I_o(NmC). Charybdotoxin (ChTX) and apamin had little effect. I_o(NmC) was abolished in Ca^{2+}-free EGTA-containing solution. These results suggest that NmC activates a Ca^{2+}-dependent K^+ current ($I_{K,Ca}$) and can modulate activities in macrophages.

Macrophage; Neuropeptide; Neuromedin C; Potassium current; Tetraethylammonium; Quinidine

1. INTRODUCTION

The nervous system and immune systems are recognized to be intimately linked and involved in bidirectional communication [1,2]. Neuromedin B (NmB) and NmC isolated from the nervous system have high homology to the amphibian peptide, bombesin (BN) [3–5], and may function as neuromediators in neurotransmission and neuromodulation. BN-related peptides stimulate the uterus [3–6], and depress the activities of dorsal horn neurons [7]. The peptides also stimulate phagocytic function in phagocytic cells [8,9], and potentiate the effect of lipopolysaccharide in alveolar macrophages [10]. NmC is structurally identical to the C-terminal decapeptide of gastrin-releasing peptide (GRP), GRP_{18-27}.

Several ionic currents have been demonstrated in macrophages. There are voltage- and Ca^{2+}-gated ion channels, including 4 K^+-channels, 3 Cl^--channels and a non-selective cation channel [11]. A neurotransmitter, a hormone and inflammatory mediators change the membrane potential, that is, ATP [12,13], adrenaline [14], platelet-activating factor (PAF) [15] and complement component C5a [16], activate $I_{K,Ca}$.

2. MATERIALS AND METHODS

Macrophages were isolated from BALB/cByJJcl mice (Nihon Clea Lab., Tokyo) of either sex by intraperitoneal injection of thioglycolate medium. The cells were cultured in RPMI1640 (Nissui) containing 10% FBS (Boehringer, Mannheim, Germany) at 37°C. The recording chamber was superfused with bath solution containing (in mM): 140

Correspondence address: M. Ichinose, Department of Physiology, Shimane Medical University, Izumo 693, Japan. Fax: (81) (853) 229 304.

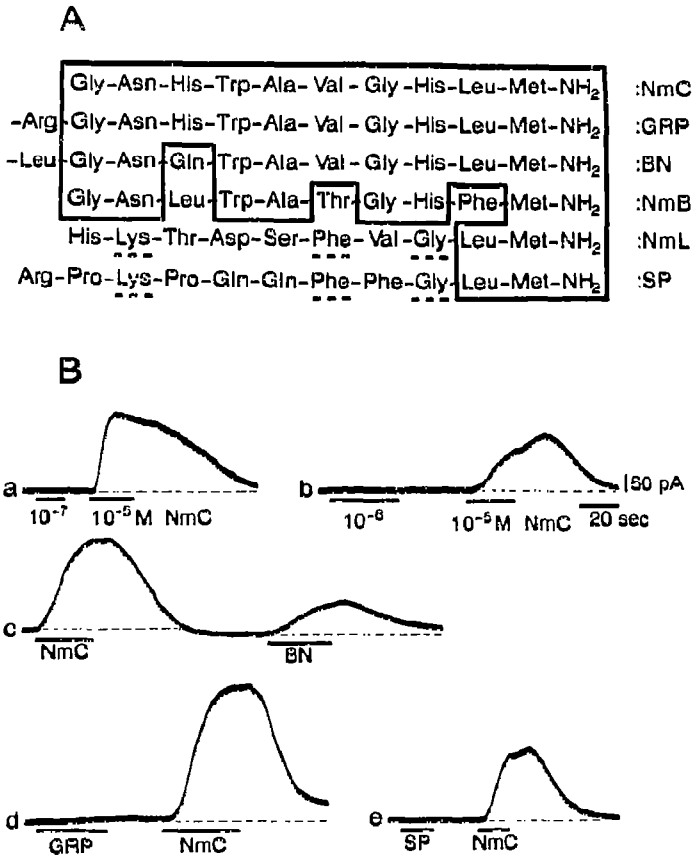

Fig. 1. (A) Amino acid sequences of NmC, GRP, BN, NmB, NmL and SP. Residues enclosed in the solid box are conserved. (B) a and b, dose dependency of outward currents to NmC in different cells; c, BN (10^{-5} M) is less effective in inducing an outward current than NmC (10^{-5} M); d, although NmC is a COOH-terminal fragment of GRP, GRP (10^{-5} M) has almost no effect; e, SP (10^{-5} M) has few homologies to NmC and did not induce an outward current. In this and subsequent figures, the duration of NmC application is indicated by bars below each record.

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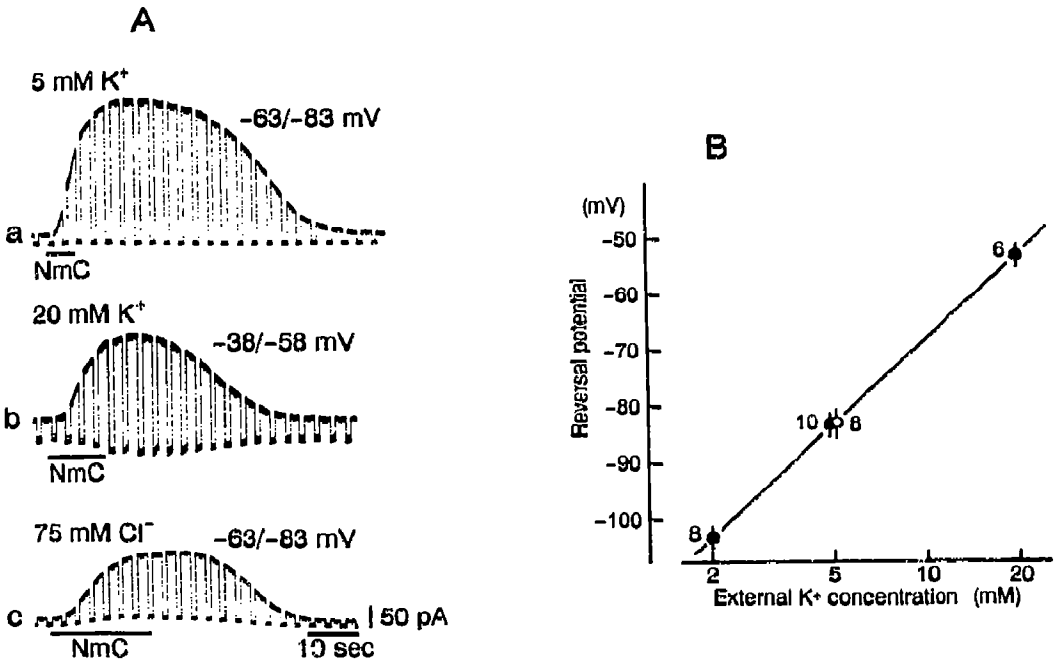

Fig. 2. Voltage dependency of outward currents to NmC. (A) The cells were voltage-clamped by command pulses (1 s duration, 3 s interval); a, current levels at voltages of −63 and −83 mV in a normal bath solution were outwardly and little shifted by NmC, respectively; b, current levels at −38 and −58 mV in 20 mM K^+ bath solution were outwardly and inwardly shifted, respectively; c, outward current at −63 and −83 mV in half the normal $[Cl^-]_o$ solution. (B) Reversal potentials at different $[K^+]_o$. Numbers at each point represent numbers of experiments. Filled circles indicate different $[K^+]_o$. Open circle, in reduced $[Cl^-]_o$ solution. Bars indicate the standard deviations of the outward current.

NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.5. Pipettes were filled with solution containing (in mM): 145 potassium aspartate, 1 $MgCl_2$, 0.1 EGTA, 10 HEPES, pH 7.4. In order to change $[K^+]_o$ or $[Cl^-]_o$, the external NaCl was replaced with KCl or sodium gluconate, respectively. Resting membrane potentials in normal bath solution and in half $[Cl^-]_o$ solution were −73.0 ± 5.7 mV (mean ± S.D., n = 35) and −77.6 ± 8.5 mV (n = 8), respectively. Whole-cell currents were recorded using a List EPC-7 amplifier (Darmstadt, Germany) at 20–23°C. NmC was applied by diffusion from a puff pipette. The liquid junction potential was +12.6 mV.

Apamin, quinidine and EGTA were purchased from Sigma, St. Louis, MO, USA; NmC and ChTX from Peptide Inst., Osaka; TEA from Wako, Osaka.

3. RESULTS AND DISCUSSION

NmC (10^{-7}, 10^{-6} and 10^{-5} M) induced I_o(NmC) in 10% (1/10 cells), 20% (3/15) and 85% (230/272) of examined cells, respectively. Structurally related peptides (Fig. 1A) had lesser effects on the induction of the outward current (Fig. 1B). GRP, BN, NmB, neuromedin L (NmL) and substance P (SP) induced outward currents in 22% (4/18 cells), 19% (5/27), 35% (7/20), 33% (2/6) and 0% (0/5) of cells, respectively. Even when BN was applied first and NmC was applied second in reverse order to Fig. 1Bc, I_o(NmC) was larger than the BN-induced outward current. Dose-dependency and structure–activity relationships suggest that there is a receptor for NmC, which is consistent with the stimulatory effect of NmC on phagocytic function [8,9] and interleukin-1 production [10].

NmC induced an outward shift of the currents at −63 mV and almost no shift at −83 mV in 5 mM K^+ solution (Fig. 2Aa), indicating that the reversal potential (E_r) of I_o(NmC) was −83.3 ± 2.1 mV (n = 10), which is close to the K^+ equilibrium potential. An increase in $[K^+]_o$ from 2 to 20 mM (Fig. 2Ab) raised E_r of I_o(NmC) from −103.3 ± 2.1 (n = 8) to −53.1 ± 2.2 (n = 6). Even if $[Cl^-]_o$ was changed to half the normal solution (75 mM), E_r was still −83.1 ± 2.4 (n = 8, Fig. 2Ac). The dependency of E_r on $[K^+]_o$ and the independency of $[Cl^-]_o$ (Fig. 2B) suggest that I_o(NmC) was carried by K^+.

The effects of $I_{K,Ca}$ blockers were examined on I_o(NmC) (Fig. 3a,b,c and d). TEA (10^{-2} M), which typically blocks large (BK) $I_{K,Ca}$ channels by 5×10^{-3} M concentration, suppressed I_o(NmC) in 11 examined cells. Quinidine (2×10^{-4} M) accelerated the recovery of I_o(NmC) in 12 examined cells. This concentration of quinidine completely abolishes the adrenaline-induced outward current [14]. ChTX (10^{-6} M), a specific channel blocker [17,18] of BK (K_D ~ 3 nM) and intermediate (IK, K_D ~ 30–100 nM) $I_{K,Ca}$, had no obvious effect on I_o(NmC) in 10 examined cells. ChTX (10^{-6} M) completely and immediately suppressed the Ca^{2+}-activated outward K^+ current induced by PAF [15]. Apamin (10^{-6} M), which specifically blocks small (SK) $I_{K,Ca}$ channel in nanomolar concentrations [17,18], also had no obvious effect on I_o(NmC) in 10 examined cells.

The effects of $[Ca^{2+}]_o$ on I_o(NmC) were examined (Fig. 3e and f). In Ca^{2+}-free solution containing EGTA,

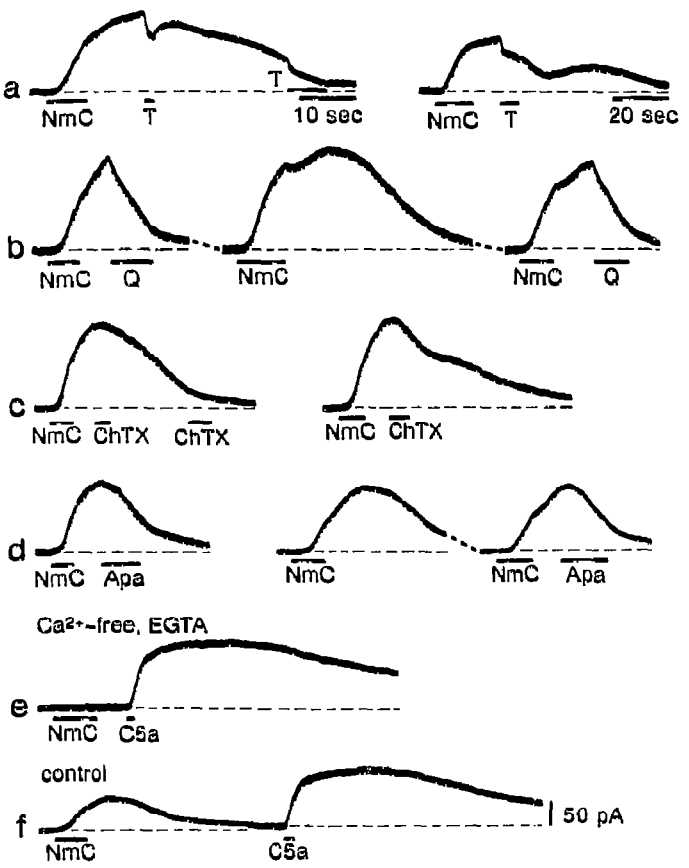

Fig. 3. Effects of K^+ channel blockers and $[Ca^{2+}]_o$. (a) TEA (10^{-2} M) suppressed the outward current. (b) Quinidine (2×10^{-4} M) accelerated recovery to baseline of the current. (c and d) ChTX (10^{-6} M) and apamin (10^{-6} M) had no obvious effect on the current. (e) NmC failed to induce the outward current 170 min after changing to Ca^{2+}-free, EGTA (1 mM)-containing solution, but C5a still induced a prolonged outward current. (f) NmC induced an outward current like C5a 50 min after changing back to Ca^{2+}-containing bath solution.

NmC did not induce any current in 14 out of 14 cells, but C5a induced an outward current in 10 out of 10 NmC-examined cells, even 200 min after changing to the Ca^{2+}-free solution. Just after changing back to the normal solution, NmC induced I_o(NmC) in 12 out of 12 cells and C5a also induced an outward current in 10 out of 10 NmC-examined cells.

The voltage dependency of I_o(NmC), E_r in different $[K^+]_o$ and $[Cl^-]_o$, and sensitivity to quinidine and TEA suggest that I_o(NmC) is a K^+ current. Because of its insensitivity to apamin and ChTX, I_o(NmC) is not SK, IK or BK. However, I_o(NmC) is $I_{k,Ca}$ because of its $[Ca^{2+}]_o$ dependency (Fig. 3e). The IK channel, which is blocked by ChTX and is insensitive to TEA, is suggested to be opened by ATP, adrenaline and PAF [12–14] in the same macrophages. C5a activates the IK channel and a Ca^{2+}-independent K^+ current [16] as shown in Fig. 3e.

Because NmC activates all steps of the phagocytic process (adherence to substrate, chemotaxis, ingestion of cells and particles, and production of superoxide anion [8,9]) present results suggest that I_o(NmC), in response to NmC, may be involved in macrophage activation. Quinine reduces $I_{K,Ca}$ channels having conductances of 218 and 32 pS, inhibits the chemiluminescence response (a parameter of phagocytosis) and inhibits LTB_4 release (a mediator of asthma) [19]. Present data directly demonstrate that NmC activates a new kind of $I_{K,Ca}$ and can modulate activities in macrophages, suggesting that the nervous system modulates immunocytes via neuropeptides.

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