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NEUROSCIENCE

Suppression of neuronal excitability by the secretion of the lamprey (*Lampetra japonica*) provides a mechanism for its evolutionary stability

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Abstract Lampreys are one of the most primitive vertebrates still living today. They attach themselves to the body surface of the host fish through their sucker-like mouths and suck blood of the host for days. Recent fossil evidence has indicated that morphology of lampreys in the late Devonian period, over 360 million years ago, already possessed the present day major characteristics, suggesting the evolutionary stability of a highly specialized parasitic feeding habit. Obviously, nociceptive responses and hemostasis of the host are two major barriers to long-term feeding of the parasitic lamprey. It has been found, to counteract hemostasis of the host, that paired buccal glands of lampreys secrete antihemostatic compounds to prevent blood of the host from coagulation. However, it is not known how lampreys make the host lose nociceptive responses. Here, we prepared components of the crude extract from the buccal glands of the lampreys (*Lampetra japonica*). Then, we show that crude extract and one of its purified components reduce the firing frequency of neuronal action potentials probably through inhibiting the voltage-dependent Na^+ channels. As the voltage-gated Na^+ channels are highly conserved throughout evolution, we argue that the secretion of the lampreys could exert the similar effect on the Na^+ channels of their host fish as well. Therefore, together with its antihemostatic effect, the secretion due to its inhibitory effect on neuronal excitability might provide a mechanism for the parasitic lampreys to keep their evolutionary stability.

Keywords Lamprey · *Lampetra japonica* · Action potential · Sodium channel · CRISP · Nociceptive response

Abbreviations

AP	Action potential
BGSP-1	Buccal gland secretion protein-1
CRISP	Cysteine-rich secretory proteins
CRBGP	Cysteine-rich buccal gland protein
DRG	Dorsal root ganglion
L. japonica	*Lampetra japonica*
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel
TTX-R	Tetrodotoxin-resistant
TTX-S	Tetrodotoxin-sensitive

Introduction

Lampreys, an ancient group of jawless vertebrates, are extant representatives of the superclass Agnathans in the class of Cephalaspidomorphi [12]. As shown by paleontological analysis of the fossil in the Devonian period, lampreys are almost identical to their modern successors with well-

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developed oral disc, annular cartilages, and circumoral teeth. This means that the evolutionary stability of the highly specialized parasitic feeding habit has been preserved at least for 360 million years [14, 20]. Lampreys usually attach themselves to the body surface of host fishes through their sucker-like mouths and suck blood of the host for days [11, 30]. Therefore, parasitic lampreys have to suppress the nociceptive response and hemostasis of the host during long-term feeding. To counteract the hemostasis of the host, paired buccal glands of lampreys secrete some antihemostatic compounds to affect blood coagulation. It has been reported that the buccal gland secretion (termed lamphredin by Lennon [22]) of certain lampreys inhibits coagulation of blood [3, 11, 13, 22]. Our previous work has also shown that lamphredin from the buccal gland of *Lampetra japonica* as well as the purified buccal gland secretion protein-1 (BGSP-1) has the fibrinogenolytic activity, which facilitates the parasite to feed on the host's blood [38].

Furthermore, lampreys have to confront the nociceptive responses of the host for a successful feeding process because the fish has been shown to possess nociception to detect noxious stimuli [2, 10, 34]. The voltage-gated Na^+ channels play important functional roles in the generation of electrical excitability in excitable cells [4]. By blocking voltage-gated Na^+ channels during regional anesthesia, local anesthetic inhibits nerve conduction and action potential (AP) propagation and consequently suppresses nociceptive responses locally [17]. It has been shown that the salivary cocktail of hematophagous animals contains substances that counteract host pain [29]. For example, the saliva of the bug *Triatoma infestans* inhibits Na^+ channel activity in nerves by an unspecified anesthetic substance [8]. For lampreys, however, what makes the host lose nociceptive responses during a long-term feeding remains largely unknown. Here, we show that the secretion from buccal glands of the *L. japonica*, a species of parasitic lamprey, reduces the amplitude and firing frequency of the APs generated from neurons in both hippocampus and dorsal root ganglion (DRG) through inhibiting the voltage-dependent Na^+ channels, which might be the strategy for lampreys to counteract nociceptive responses of the host.

Materials and methods

Lamphredin of the buccal gland secretion

Lampreys (*L. japonica*) were caught at the spawning migration stage in December of 2006 in Tong River, a branch of Songhua River in Heilongjiang province of China. When the buccal glands of *L. japonica* were dissected, lamphredin was taken and immediately frozen at −80°C.

Purification

The lamphredin was diluted to ~12 mg/ml in 50 mM Tris-HCl (pH 7.4), containing 50 mM NaCl. The diluted solution was centrifuged (12,000 rpm, 4°C, 20 min), and the supernatant (380 μl) was applied to a Sephadex G-75 (Pharmacia, Sweden) column (1×40 cm) equilibrated with the Tris-HCl buffer. Samples were eluted at a flow rate of 625 μl/min and fractions (1.2 ml each) were pooled in Eppendorf tubes. Aliquots of the fractions were electrophoresed on 12% reducing sodium dodecyl sulfate-polyacrylamide gel. The apparent molecular masses of the proteins were analyzed as described previously [21]. Protein concentration was determined using a bicinchoninic acid protein assay kit (Pierce, USA).

Cell culture

Hippocampal neurons were dissociated according to a previous method [5] with slight modification. All animal protocols were approved by the animal research ethical committee (Institute of Biophysics, Chinese Academy of Sciences). Briefly, the hippocampi were dissected from neonatal Sprague-Dawley rats, and neurons were dissociated by incubation (7 min, 37°C) in Trypsin-ethylenediaminetetraacetic acid (GIBCO, USA) and triturated in Dulbecco's modified Eagle's medium (Life Technologies, USA) supplemented with 10% bovine serum (Hyclone, Logan, UT, USA). The resulting hippocampal neurons were plated at a density of 2×10^5 cells/cm^2 onto poly-L-lysine (Sigma, St. Louis, MO, USA) coated glass coverslips. The coverslips were then incubated at 37°C in a humidified atmosphere of 95% O_2 and 5% CO_2. The medium was replaced 7 h later with Neurobasal™-A Medium, B-27 (GIBCO, USA) and 0.5 mM glutamine without antibiotic solution. After 48 h, the medium was changed to Neurobasal and B27. On the other hand, DRG neurons were acutely dissociated from 3–9-day-old and adult Sprague-Dawley rats and treated with 25% trypsin in sterile Ca^{2+} and Mg^{2+}-free phosphate-buffered saline solution at 37°C for 20 min. Individual neurons were plated on glass coverslips coated with poly-L-lysine and maintained in a humidified incubator containing 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO, USA).

Electrophysiological recording

Whole-cell current-clamp and voltage-clamp recordings were performed on the hippocampal pyramidal neurons between 6 and 10 days as well as on DRG neurons within 24 h by using the EPC-9 patch-clamp amplifier (HEKA, Germany). Voltage-clamp recordings were low-pass filtered

at 2 kHz, and current-clamp recordings were low-pass filtered at 10 kHz. Pipettes were fire-polished to give a final resistance of 2–3 MΩ for whole-cell recording. Pipette and membrane capacitances were compensated automatically with the amplifier. Series resistance compensation of 50–80% was employed routinely to reduce voltage error. Offset potentials were nullified directly before formation of the seal. Transients and leakage currents were recorded and digitally subtracted offline in all experiments using averaged records with hyperpolarizing impulses (100 ms voltage steps from −80 to −120 mV) that activated no currents. A program package Pulse + Pulsefit (HEKA, Germany) was used for data acquisition and analysis. Hanks' balanced salts solution (Sigma) was taken as extracellular solution (in mM): 1.3 $CaCl_2$, 0.8 $MgSO_4$, 5.4 KCl, 0.4 KH_2PO_4, 136.9 NaCl, 0.3 Na_2PO_4, 10 D-glucose, and 4.2 $NaHCO_3$. The intracellular solution for recording K^+ currents contained (in mM) 155 KCl, 2 NaCl, 0.1 $CaCl_2$, 1 ethylene glycol tetraacetic acid (EGTA), 2 MgATP, and 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4, while it contained 150 CsCl, 0.1 $CaCl_2$, 2.5 EGTA, 2 MgATP, and 10 HEPES at pH 7.4 for recording Na^+ currents. It contained additional 100 nM tetrodotoxin (TTX) when recording the tetrodotoxin-resistant (TTX-R) Na^+ current. For current clamp, neurons were held at potentials between −70 and −60 mV with steady current injection. APs were evoked by injecting current with duration of 200 ms and amplitude increased from 0 to 200 pA in a step of 50 pA for cultured hippocampal neurons or from 500 to 800 pA in a step of 50 pA for acutely isolated DRG neurons. All experiments were performed at room temperature (22–25°C). The changes in the duration of APs were judged by the time constant of AP decay. For activation curves, the experimental data were fitted with a Boltzmann equation: $G/G_{max}=P_{max}/\{1+\exp[-(V-V_{1/2})/k]\}$, where G_{max} is the maximum conductance, P_{max} is the open probability of the channel at a given prepulse holding potential, V is the membrane potential, $V_{1/2}$ is the voltage at half-maximal activation, and k is the slope factor. All the data are presented as mean ± standard error of the mean. Statistic analysis was carried out with one-way analysis of variance.

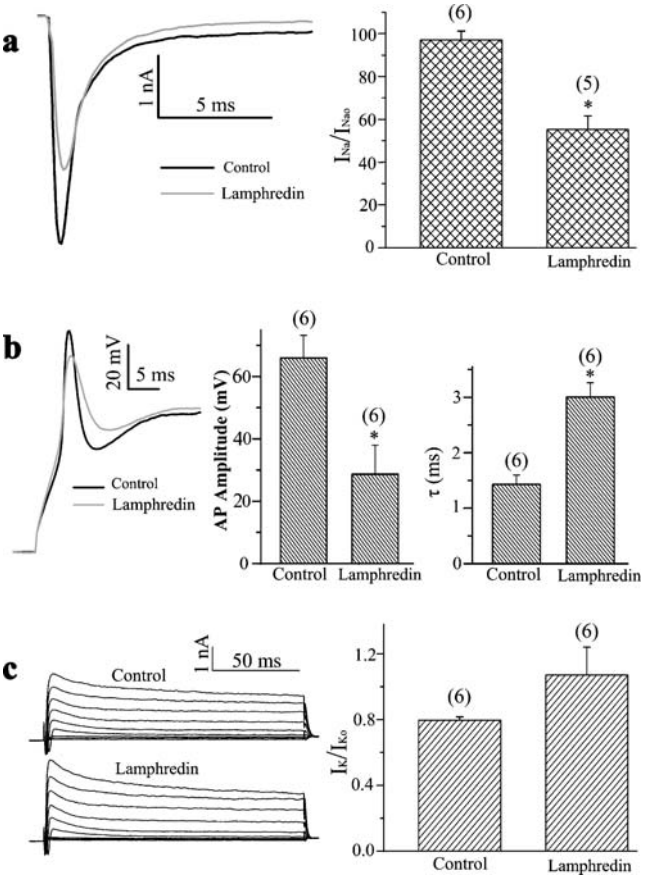

Fig. 1 Effect of lamphredin from the buccal glands of lampreys (*L. japonica*) on the excitability of hippocampal neurons. **a** Effect of lamphredin on the peak Na^+ current. *Left panel*: currents elicited by a 12-ms depolarizing step from a holding potential of −120 to −20 mV in the control (I_{Nao}) and in the presence of 1.8 mg/ml lamphredin (I_{Na}). *Right panel*: statistic data on I_{Na}/I_{Nao}. **b** Effect of lamphredin on AP. *Left panel*: representative AP in the control and in the presence of lamphredin (1.8 mg/ml). APs were aligned at the time of maximal upstroke to allow comparison of time course. *Middle and right panels*: statistics on the amplitude and decay time constant (τ) of the AP, respectively. **c** Effect of the lamphredin on the sustained K^+ currents. *Left panel*: representative K^+ current in the control (I_{Ko}) and in the presence of 1.8 mg/ml lamphredin (I_K). *Right panel*: statistic summary of I_K/I_{Ko} at a voltage of +70 mV. Times of each experiment are indicated in the parenthesis (*$P<0.05$)

Results

Effects of lamphredin on the excitability of hippocampal neurons

First, we employed rat hippocampal neurons to judge whether lamphredin could suppress Na^+ channel activity. As shown in Fig. 1a, lamphredin at a concentration of ~2 mg/ml inhibited the peak Na^+ current (n=5), whereas it had no significant effect on the K^+ current (Fig. 1c, n=6). Furthermore, lamphredin at the same concentration reduced AP amplitude and increased the AP duration in all neurons that generated a single AP. The time constant (τ) of the falling phase of AP, fitted with single exponential function, was increased from 1.4±0.2 ms (n=6) of control to 3.0±0.3 ms (n=6) in the presence of lamphredin, while the corresponding AP amplitude was reduced from 66±7 to 29±9 mV (Fig. 1b). This result indicates that the secretion of the lampreys (*L. japonica*) buccal glands contains compound(s) to suppress Na^+ channel activity.

Effects of purified components on the Na^+ current of hippocampal neurons

To find the component(s) that was (were) responsible for the suppression of Na^+ channel activity in lamphredin, two major components BGSP-1 and cysteine-rich buccal gland protein (CRBGP; corresponding to BGSP-2 of [38]) have been isolated (Supplementary Figure 1). In the presence of 5 and 12 μM CRBGP, peak Na^+ current amplitude was reduced to 70±3% (n=12, P<0.01) and 45±4% (n=10, P< 0.001) of the control, respectively (Fig. 2a, left panel). The normalized conductance, plotted against the membrane potential, showed that the extracellularly applied CRBGP did not cause significant change for the half-maximal activation voltage ($V_{1/2}$) and the slope factor (n=4–12; Fig. 2a, right panel), suggesting that CRBGP does not alter voltage-dependence of the channel. Figure 2a (middle panel) illustrates the dose-dependent inhibition of the peak Na^+ current by CRBGP. At a holding potential of −70 mV, CRBGP inhibited the peak Na^+ current with the IC_{50} value of 10.0 μM. The blockade was reversed only slightly upon washout (Fig. 2b). Extensive studies have shown that Na^+ channel blockers typically access their binding site within the channel pore from the inside [18]. This is consistent with our result, as intracellular CRBGP blocks the peak Na^+ current more strongly (Fig. 2c). In contrast, application of 12 μM BGSP-1 had little effect on the Na^+ current (Fig. 2d, e).

Effects of the purified components on the excitability of hippocampal neurons

Next, we examined the effect of CRBGP on the neuronal excitability. Application of 1 and 12 μM CRBGP resulted in a significant decrease in the AP amplitude and prolongation of the time constant of the AP (Fig. 3a). The mean time constant of AP slowed down from 2.5±0.3 (n= 13) of control to 2.9±0.4 (n=7) in the presence of 1 μM

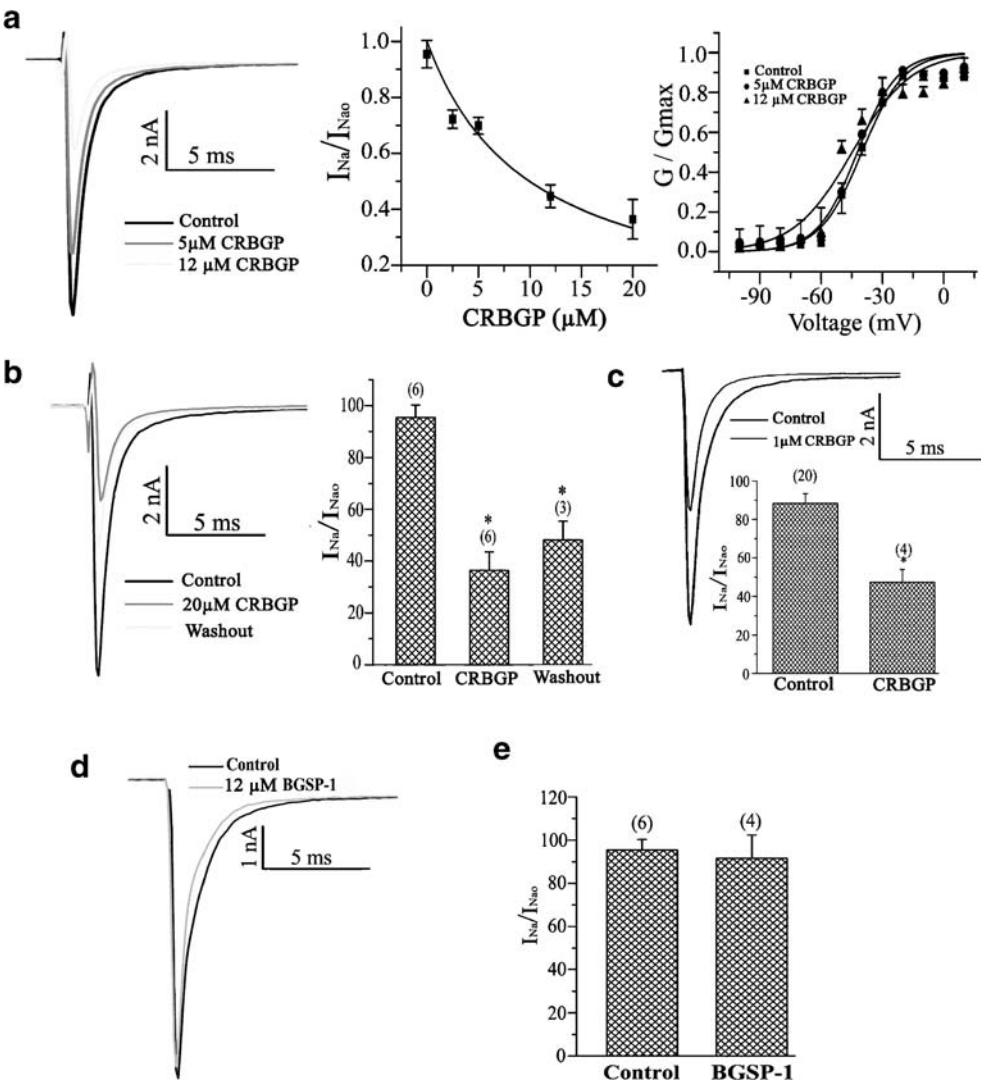

Fig. 2 Effects of CRBGP and BGSP-1 on the Na^+ currents of hippocampal neurons. **a** Effect of extracellularly applied CRBGP on Na^+ current of hippocampal neurons elicited by a voltage step from −100 to +10 mV. *Left panel*: representative Na^+ current in the control and in the presence of 0, 5, and 12 μM CRBGP. *Middle panel*: concentration–effect curve for inhibition of the peak Na^+ currents by CRBGP (1–20 μM; n= 4–12). *Right panel*: statistic summary of the effect of the CRBGP on the activation of the peak Na^+ current. **b** Current traces (*left*) and statistic summary (*right*) before, during, and after washout of 20 μM CRBGP. **c** Current traces and statistic summary for the effect of intracellularly applied CRBGP (1 μM) on the peak Na^+ current. **d** Effect of 12 μM BGSP-1 on the peak Na^+ current. **e** Statistic summary on I_{Na}/I_{Nao} in the control and in the presence of 12 μM BGSP-1

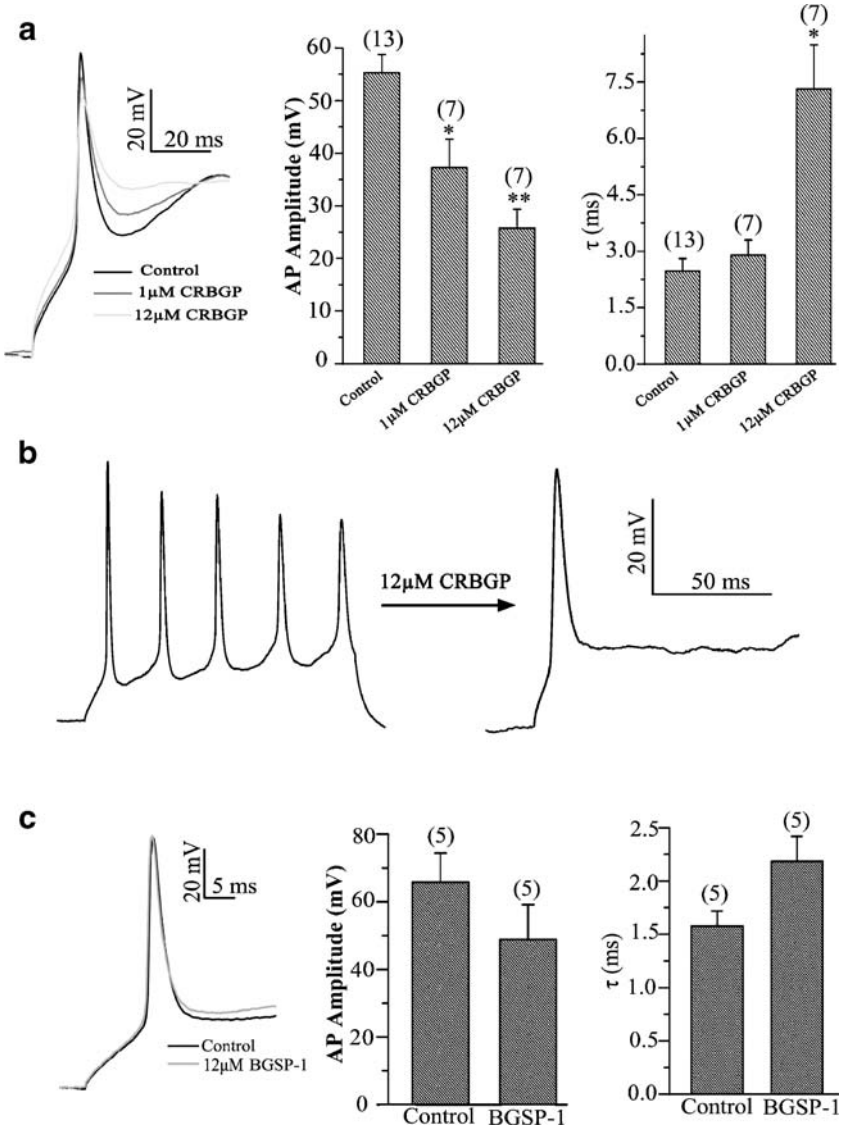

Fig. 3 Effects of the CRBGP and BGSP-1 on the AP of hippocampal neurons. **a** Representative APs in the control and in the presence of 1 and 12 μM CRBGP. *Middle and right panels*: statistics on the amplitude and decay time constant (τ) of the APs, respectively. **b** Changes in the firing patterns of the APs induced in the control and in the presence of 12 μM CRBGP (representative of seven neurons). **c** Effect of BGSP-1 on AP. *Left panel*: representative AP in the control and in the presence of 12 μM BGSP-1. *Middle and right panels*: statistic summary of effect of BGSP-1 on the amplitude and decay time constant (τ) of the AP, respectively

CRBGP and to 7.3±1.2 ms (n=7) in the presence of 12 μM CRBGP, while the corresponding AP amplitudes decreased from 55±3 to 37±5 to 26±4 mV (Fig. 3a). Besides, the number of AP firing during 200 ms period of stimulation was decreased from 3.6±0.7 (n=7) in the control condition to only once in the presence of 12 μM CRBGP for the neurons that generated multiple APs (Fig. 3b). In contrast, another purified component, BGSP-1, showed little effect on the neuronal excitability (Fig. 3c).

Effects of purified components on the K^+ current of hippocampal neurons

Voltage-gated K^+ channels play a major role in modulation of firing pattern of AP in neurons [25, 28]. Besides, crystal structure of cysteine-rich secretory protein (CRISP) showed that its cysteine-rich domain possesses a similar fold with two K^+ channel inhibitors [16, 33]. Therefore, we checked whether CRBGP could also block K^+ channels. As shown in Fig. 4, both CRBGP and BGSP-1 could block the K^+ current, which may be partly responsible for the increase in the AP duration and reduction of the firing frequency of the AP in the neurons.

Effects of purified components on the Na^+ current and AP of DRG neurons

Among the voltage-gated Na^+ channels, the TTX-R Na^+ channels (NaV1.8 and NaV1.9) that are preferentially expressed in small DRG neurons have been shown to be important in nociception [1, 7, 23, 39]. It has been shown that the TTX-R Na^+ current is more likely to be present in younger animals (3–9 days old), while the tetrodotoxin-sensitive (TTX-S) Na^+ current is more common in adult rat DRG neurons [31, 39]. Therefore, TTX-R Na^+ currents were isolated from 3–9-day-old rats by blockade of TTX-S

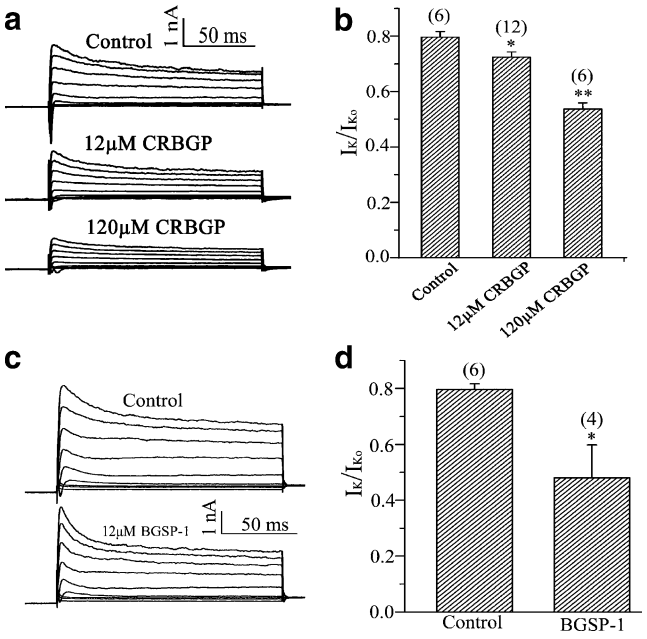

Fig. 4 Effects of the CRBGP and BGSP-1 on the K^+ currents of hippocampal neurons. **a** Recordings of K^+ current in the control (I_{Ko}) and in the presence of 0, 12, and 120 μM CRBGP (I_K), respectively. **b** Statistic summary of the effect of the CRBGP on the sustained K^+ current at a voltage of +70 mV (*P<0.05; **P<0.01). **c** Representative K^+ current in the control and in the presence of 12 μM BGSP-1. **d** Statistic summary of I_K/I_{Ko} at a voltage of +70 mV

Na^+ currents with 100 nM TTX (Fig. 5a, c), and TTX-S Na^+ currents were studied in DRG neurons from adult rat that expressed only TTX-S Na^+ currents, which were also confirmed by their fast kinetics and completely inhibited by TTX at the end of experiments (Fig. 5a, b). The amplitude of peak current is plotted as a function of test voltage for both TTX-S and TTX-R currents, and the reversal potentials obtained by extrapolation of the curves for both TTX-R and TTX-S currents are approximately +60 mV (Fig. 5a), in close agreement with the E_{Na+} for this experiment. Figure 5b showed that CRBGP suppressed the TTX-S Na^+ channel current in a dose-dependent manner (n≥5) and the currents were almost completely blocked by 100 nM TTX (n=6). Furthermore, CRBGP also suppressed the TTX-R Na^+ channel currents (n=8; Fig. 5c). This current was mainly carried out by Nav1.8 current based on its inactivation kinetics and that the CsCl-based pipette solution was not favoring recording of Nav1.9 current [7]. In contrast, the BGSP-1 has no significant effect on the Na^+ current of DRG neurons (n=5; Fig. 5d). This result suggests that the CRBGP has ability to inhibit a broad spectrum of voltage-dependent Na^+ channels.

To study its effect on the neuronal excitability, CRBGP was applied on DRG neurons that generate single spikes or long-lasting firing discharges. For single spikes, application of 12 μM CRBGP resulted in a prolongation of the time constant of the AP from 2.2±0.6 ms (n=4) of control to 6.7±1.6 ms (n=4) in the presence of 12 μM CRBGP, while the corresponding AP amplitude was not significantly changed (n=4; Fig. 6a). The reason why the CRBGP reduced the AP amplitude in hippocampal neurons (Fig. 3a) while had no effect in DRG neurons (Fig. 6a) remains unknown, even

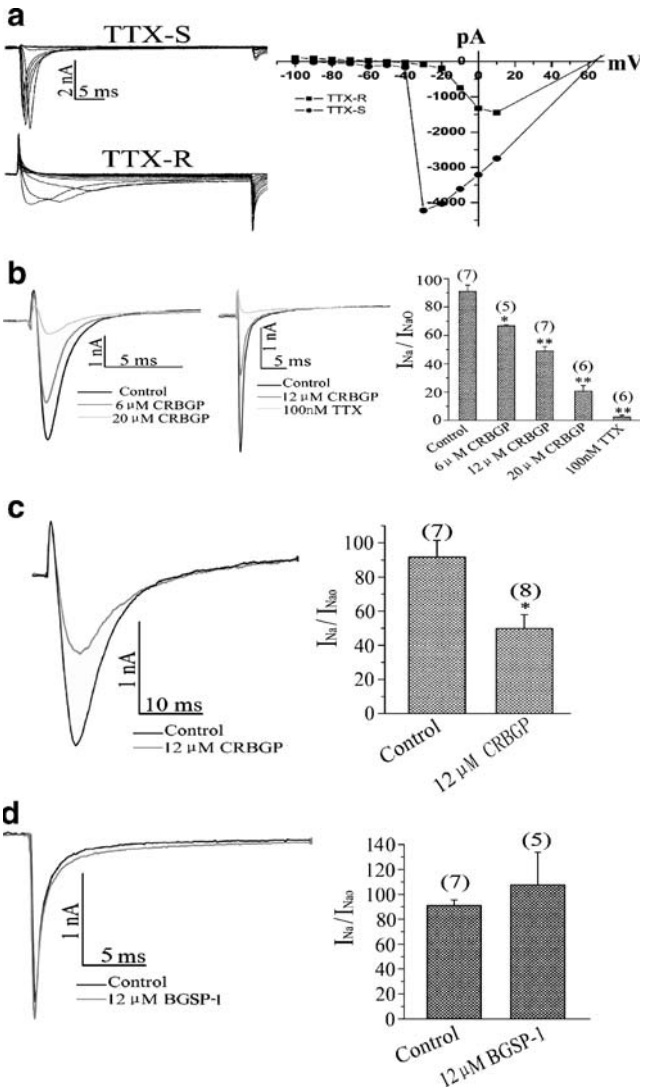

Fig. 5 Effects of CRBGP and BGSP-1 on the Na^+ currents of DRG neurons. **a** Families of TTX-S and TTX-R currents (*left*) and peak Na^+ current–voltage relationships (*right*). The TTX-R current was recorded in the presence of 100 nM TTX. The currents were elicited by a voltage step from −100 to +10 mV. **b** Effect of extracellularly applied CRBGP on TTX-S Na^+ current. *Left panel*: representative TTX-S Na^+ current in the control and in the presence of 6 and 20 μM CRBGP. *Middle panel*: representative TTX-S Na^+ current in the control, in the presence of 12 μM CRBGP and 100 nM TTX, respectively. *Right panel*: statistic summary of the dose-dependent effect of the CRBGP on the TTX-S Na^+ current. **c** Current traces and statistic summary in the control and in the presence of 12 μM CRBGP. **d** Effect of 12 μM BGSP-1 on the peak Na^+ current of DRG neurons (*P<0.05; **P<0.01). The pipette solution contained 100 nM TTX and was CsCl-based, therefore not favoring recording of Nav1.9 current [7]

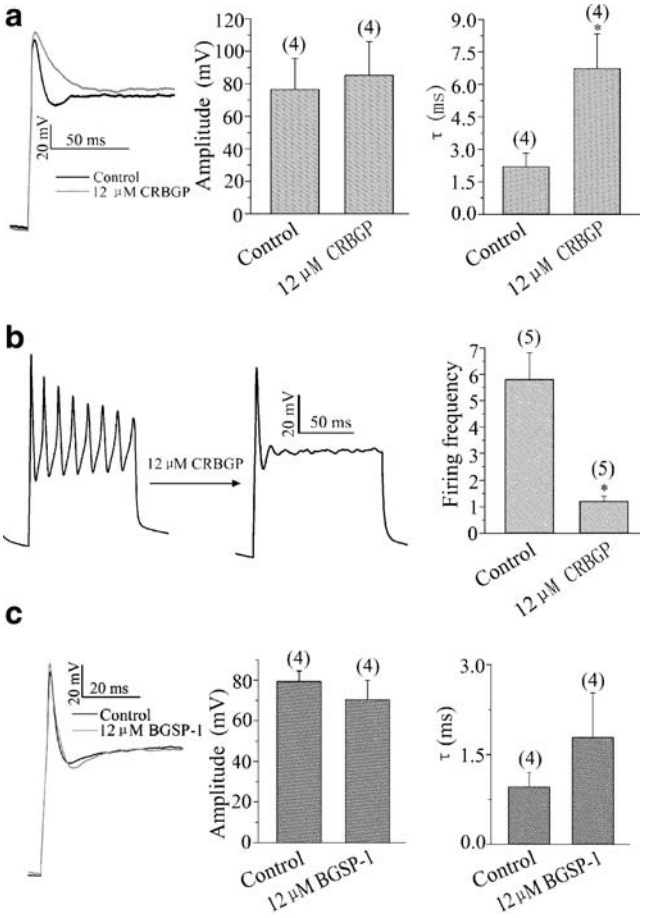

Fig. 6 Effects of CRBGP and BGSP-1 on AP of DRG neurons. **a** Representative APs in the control and in the presence of 12 μM CRBGP (*left*). *Middle and right panels*: statistics on the amplitude and decay time constant (τ) of the APs, respectively. **b** Changes in the firing patterns of the APs induced in the control (*left*) and in the presence of 12 μM CRBGP (*middle*). *Right panel*: statistics on the firing frequency of the APs. **c** Effect of BGSP-1 on AP. *Left panel*: representative AP in the control and in the presence of 12 μM BGSP-1. *Middle and right panels*: statistic summary of effects of BGSP-1 on the amplitude and decay time constant (τ) of the AP, respectively

though it reduced the Na^+ channel current in both types of the neurons. For neurons generated long-lasting firing discharges, the number of AP firing during 200 ms period of stimulation was decreased from 5.8±1.0 (n=5) under the control condition to 1.2±0.2 (n=5) in the presence of 12 μM CRBGP (Fig. 6b). Similar to that in hippocampal neurons, the other purified component, BGSP-1, showed little effect on the neuronal excitability (n=4; Fig. 6c).

Discussion

In this study, we found that CRBGP, which belongs to the CRISP family, reduced the firing frequency of neuronal APs through inhibiting the voltage-dependent Na^+ channels. The CRISPs are widely distributed in mammals, reptiles, amphibians, and secernenteas. They are members of the CRISP/antigen 5/PR-1 (CAP) superfamily. All of them share a highly conserved CAP domain [16, 32] and are characterized by 16 highly conserved cysteine residues (Supplementary Figure 1). CRISPs from lizard and snake venoms have been reported to block various ion channels, including voltage-gated Ca^{2+} channels, voltage-gated K^+ channels, and ryanodine receptors [19, 26, 33, 36, 37]. Therefore, it is not a surprise that CRBGP from lampreys could block multiple voltage-gated ion channels of the neurons. According to our previous data [38], the concentration of CRBGP in the buccal gland of *L. japonica* is about 1.7 mM (43.6 mg/ml). A buccal gland of *L. japonica* contains about 20 μl lamphredin on average (unpublished data). On the other hand, current study showed that the effective concentration of CRBGP on reduction of the AP firing frequency was at the level of micromolar. This means that the buccal gland of lamprey contains enough CRBGP to suppress neuronal excitability of the host locally during its feeding.

Voltage-gated Na^+ channels are encoded by a family of genes that have been highly conserved throughout evolution, which undoubtedly reflects the critical functional role of these proteins in regulating electrical excitability [15]. They might be evolved early in the metazoan era from an ancestral channel resembling the Ca_v3 Ca^{2+} channel family, before the separation of diploblasts and triploblasts [35]. It has been suggested that the teleost fish and mammals have conserved Na^+ channel genes in their common ancestors [24]. Furthermore, the family of sodium channel β subunit genes has also been suggested to be emerged early in vertebrate evolution, prior to the divergence of teleosts and tetrapods over 400 million years ago [6]. Besides, the functional conservation of the Na^+ channel has been suggested by the conservation of its expression patterns in teleosts and tetrapods [27]. Therefore, the voltage-gated sodium channel complexes are evolutionarily conserved entities in excitable membranes. Probably due to this conservation, lampreys are under less evolutionary pressure to improve their preying abilities and therefore could keep evolutionary stability in their feeding habit. Consistence with the notion that the voltage-gated Na^+ channel is evolutionarily conserved, CRBGP could suppress Na^+ channel current in both DRG and hippocampal neurons (Figs. 2 and 5).

Previously, Ito et al. [19] isolated and characterized the lamprey CRISP (CRBGP) independently and found that the CRBGP could suppress muscle contraction. They inferred that the suppression of muscle contraction was probably through blockade of Ca^{2+} channel, but no directly evidence (such as electrophysiological study) was given. Intracellular Ca^{2+} plays an important role in muscle contraction. Many factors could affect the intracellular Ca^{2+} concentration, and

blockade of Ca^{2+} channel is just one of the factors. On the other hand, AP has been shown to be an important factor to influence the Ca^{2+} release at the neuromuscular junction [9], and reduction of AP will cause less Ca^{2+} release, which in turn will suppress muscle contraction. Therefore, their observation is consistent with ours if we assume that the reduction of the AP firing frequency by CRBGP could cause less Ca^{2+} release at the neuromuscular junction. That is to say, suppression of muscle contraction by CRBGP might be a secondary effect of reduction of the AP firing frequency but not due to direct blockade of the Ca^{2+} channel. Of course, further studies are needed to clarify this question. We also observed that application of CRBGP increased the AP decay time. This might be due to the blockade effect of CRBGP on K^+ channel (Fig. 4) and possibly reduction of the Ca^{2+} influx carried out by Ca^{2+} channel in DRG neurons, which in turn could decrease the calcium activated potassium currents. Due to incomplete compensation, the membrane potential would be over-estimated, especially for Na^+ current under control condition. But, the main result would be held, because the effect of drug was compared at the same condition. Furthermore, the reduction of the AP firing frequency gives further evidence for the effect of the secretion on the neuronal excitability.

In summary, we have shown that the lamphredin and the purified CRBGP but not BGSP-1 are potent to reduce the neuronal excitability. Therefore, in cooperation with its antihemostatic effect, the secretion of the buccal glands offers the mechanism of a successful survival of parasitic lampreys during a long-time evolution dating back 360 million years.

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