J. exp. Biol. **156**, 607–610 (1991)
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SHORT COMMUNICATION

PEPTIDE POTENTIATION OF CALCIUM CHANNEL ACTIVITY CAN BE SEASONALLY VARIABLE

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Accepted 2 October 1990

Using the single-channel patch-clamp technique we have recently shown that the peptide cotransmitter proctolin can potentiate the activity of a large (38 pS in 137 mmol l^{-1} Ba^{2+}; 26 pS in 137 mmol l^{-1} Ca^{2+}), depolarization-inducible Ca^{2+} channel in the plasma membrane of the crayfish tonic flexor muscle (Bishop *et al.* 1991). Parallels between the modulatory effects of the peptide on channel activity and tension generated in the muscle suggest that this channel activation is probably partly responsible for the amplification of tension by proctolin (Bishop *et al.* 1991). Our initial studies of this phenomenon were performed during the spring and summer of 1988, using excised, inside-out patches. During that time, we found that the addition of 5×10^{-9} mol l^{-1} proctolin to the bath at least 5 min prior to seal formation (this dose gives a maximal response) caused a large increase in the proportion of patches with active channels and enhanced the single-channel open probability, P_o, by as much as 40-fold. (Ca^{2+} channels remained remarkably active in these excised patches: there was usually no obvious diminution in channel activity for the duration of the recordings; Bishop *et al.* 1991.) Identical results were obtained during the spring of 1989. In this communication we show that, during the winter months between these two periods, proctolin had virtually no effect on channel activity, indicating a seasonal variability in the potentiating effect of the peptide.

Our studies were performed on 5–7.5 cm adult crayfish, *Procambarus clarkii*, supplied by Niles Biological, Sacramento, which obtained animals from local collectors. Animals were maintained in laboratory tanks (from several days up to 2 months) before use. Tonic flexor muscles were isolated (Fig. 1A) and treated with 0.05–0.15 % collagenase IA for 20 min. Muscles were bathed in 137 mmol l^{-1} $BaCl_2$ (to enhance Ca^{2+} channel conductance), 1 mmol l^{-1} $CaCl_2$, 2.6 mmol l^{-1} $MgCl_2$ and 10.1 mmol l^{-1} Trizma (pH 7.0). Patch pipets contained 205 mmol l^{-1} sodium gluconate, 2.6 mmol l^{-1} $CaCl_2$, 2.6 mmol l^{-1} $MgCl_2$ and 10.1 mmol l^{-1} Trizma (pH 7.0). Experiments were performed at 21 °C, using the inside-out patch configuration. This configuration made it possible to identify Ca^{2+} currents easily as inward currents in depolarized patches (see Bishop *et al.*

Key words: proctolin, calcium, muscle, patch-clamp, *Procambarus clarkii*.

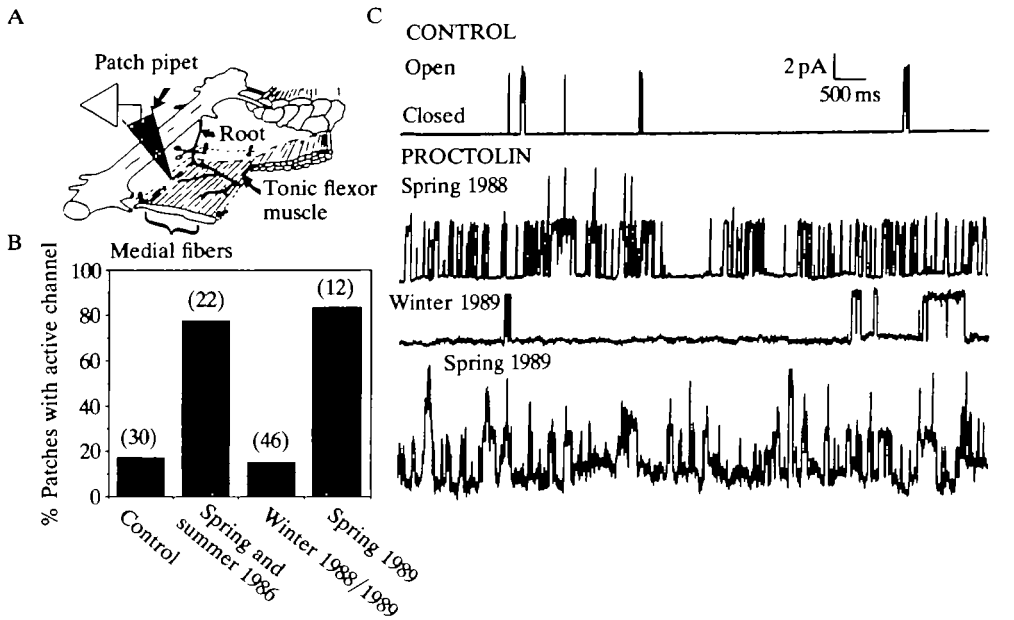

Fig. 1. (A) Tonic flexor muscle preparation from the crayfish used for single-channel patch-clamp recordings. Twelve medial muscle fibers were isolated from the third abdominal segment and treated with collagenase. Experiments were performed on inside-out patches. Current records were usually filtered at 500 Hz with an eight-pole Bessel filter, digitized at 400 μs per point and analyzed on an IBM PC AT computer using the pClamp (Axon Instruments) program. (B) Percentage of depolarized patches with active channels in control saline (filled bar) and in 5×10^{-9} mol l^{-1} proctolin in spring and summer 1988, winter 1988/1989 and spring 1989. The total number of patches examined is indicated in parentheses above each bar. Proctolin enhanced channel occurrence nearly fivefold during the spring and summer months, but had no effect in the winter. (C) Current records from patch-clamp experiments showing at least one large calcium channel in depolarized patches in the control bath and in the presence of proctolin in spring 1988, winter 1989 and spring 1989. The spring records represent *typical* channel activity recorded in the spring and summer months; the control and winter records are the *most* active examples obtained. Proctolin greatly increased the activity of the channel during the spring and summer months (in this case increasing single-channel open probability around 15-fold), but produced little enhancement in the winter. (Clamp potentials are 50 mV for all but the bottom trace, which was recorded at a clamp potential of 25 mV.)

1991). Proctolin (Sigma) was used at 5×10^{-9} mol l^{-1} and was present in the bath at least 5 min prior to seal formation. Proctolin dilutions were prepared from previously frozen samples from a common stock; a new sample was used each week. Periodically, new peptide batches were tested; these yielded similar effects. Experiments were conducted during three periods: April–September 1988 (N=22); December–March 1988/1989 (N=46); and April–May 1989 (N=12).

Patches were excised at 0 mV clamp potential and then depolarized in 20 or 50 mV steps up to 100 mV or until channel activity was seen; patches were held for

at least 30 s at each depolarizing step and for a total of at least 5 min. Patches without channel activity were usually examined for much longer periods (up to 30 min) and several series of depolarizing steps were usually tried. (Patches without channel activity were generally more stable than those with active channels.) In control experiments (performed all the year round), the 38 pS channel was present in only 5/30 (17 %) of patches (Fig. 1B); P_o, defined by [mean open time/(mean open time+mean closed time)]/n, where n is the total number of active channels (maximum number of observed channel overlaps), was always very low (<0.01). Fig. 1C (top trace) illustrates the *most* active control patch, which, at a clamp potential of 50 mV, displayed only brief and sporadic openings (P_o=0.008).

Seasonal variation in channel activity in response to proctolin was noted as a change both in the channel occurrence in depolarized patches and in the P_o. Fig. 1B compares the percentage of depolarized patches with active channels during the three periods. The channel was recorded in 77 % of patches (17/22) in spring and summer 1988, in 15 % (7/46) in winter 1988/1989 and in 83 % (10/12) in spring 1989. The percentage in winter did not differ significantly from the control level, whereas a significant fivefold increase (P<0.01, chi-square test) was seen in the two non-winter periods. P_o values for the large Ca^{2+} channel (measured at clamp potentials between 25 and 50 mV) in the two non-winter periods ranged from 0.005 to 0.30 in the presence of proctolin, with most being greater than 0.10. Typical recordings for these periods, with respective P_o values of 0.12 and 0.11, are shown in Fig. 1C (second and fourth traces). During the winter period, however, P_o ranged only from 0.005 to 0.06; Fig. 1C (third trace) illustrates the *most* active channel record. A conservative interpretation of these results shows a significant increase of three- to fourfold (P<0.01, t-test) in mean P_o during each of the two non-winter periods (mean P_o for spring and summer 1988=0.10±0.01, S.E.M.; mean P_o for spring 1989=0.11±0.01, S.E.M.) compared to winter (mean P_o=0.03±0.01, S.E.M.). (This probably represents an *underestimate* of the true current increase in the non-winter months, since multiple channels were usually present in non-winter patches and never seen in winter patches.) Together, these results indicate that, over the period of our study, proctolin potentiation was high in the spring and summer and low in the winter. (This seasonal response appeared to be confined to Ca^{2+} channel activity: no seasonal variation was detected in the activity of a 6 pS Cl^- channel that was seen at hyperpolarizing patch potentials and was present in approximately 80 % of patches during each of the three seasonal periods.)

The effect of proctolin on muscle tension was also found to vary seasonally. In experiments where tension was generated in individual muscle fibers in response to injected current, proctolin produced tension amplification 100 % of the time during April–November (N=9), but only 68 % of the time during December–March (N=19). (These results were accumulated from experiments conducted between December 1987 and June 1989.) There was no tension increase in 31 % (4/13) of the fibers examined during the 1986/1987 winter season or in 33 % of

those examined (2/6) during the 1988/1989 winter season. (The latter population of animals was the same as that used in the above single-channel study; muscle responses to tension were not examined during the 1987/1988 winter season.) Among muscle fibers that did respond to proctolin, voltage–tension curves generated in the presence of proctolin in winter (N=3) had slopes of less than half those obtained in spring (N=3). Thus, as with Ca^{2+} channel activity, an annual cycling is suggested in the tension response of the muscle to proctolin.

Together, these results raise some interesting questions concerning both the mechanism and significance of this seasonal variability. Recent results show that channel activity in winter animals can be enhanced by proctolin when 10^{-7} mol l^{-1} octopamine is also added to the bath. (Octopamine alone does not affect channel activity; Bishop *et al.* 1990.) This result indicates that the Ca^{2+} channels are still present in the winter muscle but that proctolin alone cannot activate them. Since octopamine is thought to be a circulating hormone in Crustacea (Kravitz, 1988), one possible explanation for our results is that there is a seasonal change in the circulating levels of octopamine. Of course, seasonal changes in the sensitivity of the channel to octopamine or proctolin are also possibilities that can be tested. In summary, these results make it clear that regulation of this Ca^{2+} channel is multi-faceted and indicate that the study of this system may be useful in understanding multiple controls of channel activity.

We thank Tina Law for technical assistance and Grace Hagiwara for comments. This study was supported by NIH grant 20557 and a Muscular Dystrophy Association research-aid grant to JJW and CAB.

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